UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

PROGRAMA DE DOCTORADO EN GESTIÓN COSTERA

TESIS DOCTORAL

ESTUDIO DE LA PRESENCIA, ELIMINACIÓN E IMPACTO AMBIENTAL DE RESIDUOS FARMACÉUTICOS EN AGUAS RESIDUALES DE LA ISLA DE GRAN CANARIA

Study of the presence, removal and environmental impact of pharmaceutical residues in wastewater from island of Gran Canaria

Cristina Afonso Olivares

LAS PALMAS DE GRAN CANARIA
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UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Facultad de Ciencias del Mar

ANEXO I

Dª MARÍA ISABEL PADILLA LEÓN, SECRETARIA DE LA FACULTAD DE CIENCIAS DEL MAR, ÓRGANO RESPONSABLE DEL PROGRAMA DE DOCTORADO EN GESTIÓN COSTERA, DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA.

CERTIFICA

Que el Consejo de Doctores del Programa de Doctorado en Gestión Costera, en su sesión de fecha 25 de mayo de 2017, tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada: *"Estudio de la Presencia, Eliminación e Impacto Ambiental de Residuos Farmacéuticos en Aguas Residuales de la Isla de Gran Canaria"*, presentada por la doctoranda: **Dª Cristina Afonso Olivares** y dirigida por los Doctores D. José Juan Santana Rodríguez, Dª Zoraida Sosa Ferrera y D. José Miguel Doña Rodríguez.

Asimismo, se acordó el informar favorablemente la solicitud para optar a la Mención Internacional del Título de Doctor, por cumplir los requisitos reglamentarios.

Y para que así conste, a efectos de lo previsto en el Artº 6 del Reglamento para la elaboración, tribunal defensa y evaluación de tesis doctorales de la Universidad de Las Palmas de Gran Canaria, firmo el presente en Las Palmas de Gran Canaria, a veinticinco de mayo de dos mil diecisiete.

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

DEPARTAMENTO DE QUÍMICA

Programa de Doctorado en Gestión Costera

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Dirigida por el Prof. D. José Juan Santana Rodríguez

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El Director **La Codirectora** **El Codirector**

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La Doctoranda

Las Palmas de Gran Canaria, mayo de 2017

Agradecimientos

Estas líneas no pueden haberse escrito en un momento más idóneo, este periodo en el que me separo durante unos meses de todas aquellas personas que han contribuido de una manera u otra y me han dado un empujón para culminar esta Tesis Doctoral. A todos ellos, mil gracias.

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Gracias.

Índice general

Resumen

En apenas los últimos veinte años ha aumentado la preocupación de la comunidad científica por la presencia de nuevos contaminantes, considerados emergentes, en los diferentes compartimentos ambientales, así como, sobre sus posibles consecuencias negativas para los seres vivos. Sin embargo, su control y regulación avanza muy lentamente, por lo que se necesita seguir investigando, sobretodo, el comportamiento de aquellos grupos de compuestos que son considerados una amenaza, con el fin de obtener los suficientes datos para poder concretar las medidas necesarias.

En este sentido, los compuestos farmacéuticos, los cuales son usados de manera continua y extensa, son persistentes debido a su baja biodegradabilidad y presentan una cierta toxicidad, han sido monitorizados y detectados en muestras hidrológicas, especialmente, debido a su ruta principal de entrada después de ser consumidos y excretados por humanos y animales. Este tipo de estudios no hubiese sido

posible sin la existencia y desarrollo de metodologías analíticas cada vez más amigables con el propio medioambiente sin dejar de ser sensibles, selectivas y robustas. Sin embargo, aunque haya una gran disponibilidad de datos sobre la presencia de los fármacos en el medioambiente, todavía quedan zonas geográficas con características particulares donde no se han estudiado en profundidad.

Por todo ello, en esta Tesis Doctoral se ha llevado a cabo el desarrollo y optimización de una metodología analítica multiresiduo para la determinación simultánea de compuestos farmacéuticos pertenecientes a diferentes clases terapéuticas en muestras líquidas, que se caracteriza por ser un sistema estándar y rutinario que intenta acercarse a los principios de la química analítica verde. Esta metodología consiste en un proceso de extracción mediante la técnica de extracción en fase sólida simplificada, que se basa en la disminución de etapas de un procedimiento estándar, y en un sistema de separación, detección y determinación mediante la cromatografía líquida de alta resolución con detección por espectrometría de masas.

Dado que el principal foco de contaminación por parte de este tipo de compuestos se encuentra en las estaciones depuradoras de aguas residuales, las cuales no han sido diseñadas para eliminarlos o transformarlos, la principal aplicación de esta metodología analítica optimizada ha sido la monitorización de fármacos en muestras de agua procedente de diferentes estaciones de tratamiento de aguas residuales de la isla de Gran Canaria. El propósito principal de este estudio ha sido contribuir al repositorio sobre la presencia de estos contaminantes y calcular las eficacias de eliminación de los diferentes tratamientos de

purificación para conocer la necesidad de sistemas alternativos más avanzados. De manera adicional, se ha evaluado de forma predictiva el posible riesgo que puede causar, a los ecosistemas marinos, la descarga de las aguas tratadas con contenido en residuos farmacéuticos.

El consumo de productos farmacéuticos es necesario e inevitable, por lo que una de las alternativas para paliar el problema de contaminación causada por la descarga de este tipo de compuestos es a partir del desarrollo de sistemas avanzados de depuración de aguas. Como aplicación secundaria, la metodología multiresiduo se ha usado como herramienta de valoración para el desarrollo y optimización de diferentes procesos avanzados de oxidación, los cuales podrían ser usados como tratamientos adicionales efectivos. En este caso, los procesos de oxidación avanzados utilizados han sido la fotocatálisis con peróxido de hidrógeno y la fotocatálisis heterogénea por óxido de titanio.

Abstract

In just the last twenty years the scientific community concern have increased due to the presence of new pollutants, which are considered emerging, in the different environmental compartments, as well as, their possible negative consequences for living beigns. However, its control and regulation progress very slowly, so we need further research, especially, the behavior of those groups of compounds that are considered a threat, in order to obtain sufficient data to be able to specify the necessary measures.

In this sense, pharmaceutical compounds, which are used continuously and extensively, are persistent due to their low biodegradability and have a certain toxicity, have been monitored and detected in hydrological samples, due to their main route of entry after their consumption and excretion by humans and animals. Such studies would not have been possible without the existence and development of analytical methodologies that are increasingly friendly to the

environment, while being sensitive, selective and robust. However, although there is a high availability of data on the presence of drugs in the environment, there are still geographical areas with particular characteristics where they have not been studied in depth.

Therefore, in this thesis it has carried out the development and optimization of a multiresidue analytical methodology for simultaneous determination of pharmaceutical compounds belonging to different therapeutic classes in liquid samples, characterized by being a standard and routine system that it attempt to approach to the principles of green analytical chemistry. This methodology consists of an extraction process using the simplified solid phase extraction technique, which is based on the reduction of standard procedure stages, and subsequently system of separation, detection and determination by high performance liquid chromatography tandem mass spectrometry.

Because of the main source of contamination of these types of compounds is found in wastewater treatment plants, which have not been designed to remove or transform them, the direct application of this optimized analytical methodology has been the monitoring of drugs in water samples from different sewage treatments plants of the island of Gran Canaria. The main purpose of this study has been to contribute to the repository on the presence of these contaminants and to calculate the elimination efficiencies of different purification treatments, and thus, to know the necesity of more advanced alternative systems. In addition, it has been evaluated, in a predictive way, the possible risk produced by the discharge of treated waters with pharmaceutical residues content on marine ecosystems.

The consumption of pharmaceuticals is necessary and inevitable, so one of the alternatives to avoid the pollution problem, which is caused by the discharge of this type of compounds, is from the development of advanced systems of water purification. As a secondary application, the multiresidue methodology has been used as a evaluation tool for the development and optimization of different advanced oxidation processes, which could be used as additional effective treatments. In this case, the developed advanced oxidation processes have been the photocatalysis with hydrogen peroxide and the heterogeneous photocatalysis by oxide titanium.

Capítulo I: Introducción

I.1. Compuestos farmacéuticos

El uso de extractos naturales, generalmente de plantas, como sustancias utilizadas para la prevención y tratamiento de enfermedades se remonta a miles de años, sin embargo, no es hasta principio del siglo XX cuando la industria farmacéutica tiene su despegue con la síntesis de nuevos fármacos y las investigaciones sobre sus efectos y aplicaciones [1,2]. La introducción de los compuestos farmacéuticos manufacturados en la medicina y su continuo desarrollo ha dado lugar a la posibilidad de reducir, considerablemente, las muertes por diferentes enfermedades, además de alargar la vida media de las personas.

Aunque el objetivo principal de la introducción de nuevos compuestos farmacéuticos es beneficiar a la humanidad, en las últimas décadas ha surgido una creciente preocupación debido a su aparición a niveles traza en diferentes compartimentos medioambientales pudiendo

ocasionar un riesgo potencialmente peligroso, lo que ha originado un problema medioambiental emergente [3].

Para llevar a cabo una evaluación de la presencia de productos farmacéuticos desde un punto de vista medioambiental, sobre sus rutas de entrada, desde su origen hasta su destino, y el potencial riesgo dañino que podrían producir, hay que considerar sus características generales, las cuales son presumiblemente diferentes a los comportamientos de otros productos químicos industriales más convencionales. Algunas de estas particularidades son enumeradas a continuación [4]:

1. El compuesto neutro parental y las sales asociadas tienden a formar estados sólidos polimórficos.
2. Se introducen en el medioambiente después de su metabolización en el ser vivo (humano o animal).
3. Están formados por moléculas grandes y químicamente complejas, además poseen características físico-químicas muy diferentes entre ellas.
4. Son moléculas polares y tienen múltiples grupos ionizables repartidos.

Se deben tener muy presentes estas cualidades globales, ya que son las características propias de compuestos resistentes que hacen que potencien su persistencia en el medioambiente, quedándose a formar parte de los ciclos vitales produciendo un progresivo aumento en el riesgo dañino que podrían provocar en la dinámica y desarrollo de los ecosistemas.

I.1.1. Entrada y presencia en el medioambiente

La mayoría de los compuestos farmacéuticos son sustancias químicas complejas que han sido sintetizadas por el hombre y, por lo tanto, no debe existir niveles de fondo naturales en el medioambiente [5]. Estos compuestos son utilizados tanto en medicina humana como en veterinaria y el suministro habitual se realiza mediante vía oral, intravenosa o intramuscular. Después de la ingesta o administración, los compuestos farmacéuticos son excretados por las diferentes vías (orina o heces) llegando a las aguas residuales en forma de metabolito, mezcla de metabolitos, conjugados con un compuesto inactivador unido a la molécula o como el propio compuesto activo [6,7]. En este sentido, numerosos estudios ofrecen información acerca de los porcentajes de excreción de los compuestos farmacéuticos sin ser metabolizados, los cuales pueden llegar hasta un 90% de la dosis original [8,9], siendo principalmente desechados por la orina y parcialmente por las heces [10].

Los estudios sobre el consumo de productos farmacéuticos en los países de la Unión Europea indican que las cifras globales para algunos grupos de compuestos farmacéuticos alcanzan un rango entre 58 y 318 DDD/ 1000 hab./día (unidades de dosis diaria definida por 1000 habitantes al día) [11]. En lo que respecta a España, según los informes ofrecidos por la Agencia Española de Medicamentos y Productos Sanitarios [12], el consumo ha sido también definido y clasificado mediante las diferentes clases terapéuticas. Estos resultados, junto al porcentaje de excreción en su forma activa, sólo para una selección reducida de fármacos, se recogen en la Tabla I.1.

Tabla I.1 Consumo de una selección de fármacos y sus porcentajes de excreción del compuesto activo.

Descripción	Compuesto	Consumo (DDD/1000hab./ día)	Excreción (%)
Ansiolítico, hipnótico u antidepresivo	Lorazepam	22,0	10 [13]
	Alprazolam	16,7	10 [13]
	Diazepam	7,81	1,0 [8]
	Fluoxetina	7,84	3,0 [13]
	Paroxetina	11,0	3,0 [13]
	Sertralina	10,6	14 [13]
Antiinflamatorio	Diclofenaco	6,38	15 [13]
	Ibuprofeno	21,5	10 [13]
	Naproxeno	6,12	3,0 [13]
Antiulceroso	Omeprazol	104	30 [13]
	Pantoprazol	12,0	20 [13]
Hipolipemiante	Simvastatina	29,6	1,0 [13]
	Atorvastatina	42,0	1,0 [13]
	Bezafibrato	0,150	50 [13]
	Gemfibrozilo	1,71	2,0 [14]
Antibiótico	Amoxicilina	4,40	60 [13]
	Sulfametoxazol	0,300	30 [13]
	Eritromicina	0,100	25 [13]
	Ciprofloxacino	1,10	70 [13]
	Norfloxacino	0,300	60 [13]
Analgésico	Paracetamol	14,5	4,0 [15]
Antiepiléptico	Carbamazepina	1,23	12 [13]
Antihipertensivo	Atenolol	7,64	90 [15]
	Metoprolol	0,390	10 [15]
	Propanolol	0,640	0,5 [14]

Se observan algunos fármacos cuyo consumo es relativamente bajo, pero los porcentajes de excreción son muy altos (> 50 %), este es el caso

de compuestos como norfloxacino, ciprofloxacino o bezafibrato. En contra, otros fármacos como ibuprofeno, omeprazol, lorazepam, simvastatina o paracetamol son excretados en una mínima proporción (< 30%), sin embargo, son altamente consumidos por la población española. Además, también pueden haber compuestos con un alto consumo y altos porcentajes de excreción, como es el caso del atenolol. Tanto su consumo como los niveles de excreción determinan su mayor o menor presencia en los diferentes compartimentos ambientales que, además, vendrá condicionado por sus características fisicoquímicas intrínsecas. De esta manera, se puede llegar hacer predicciones sobre la contaminación por residuos farmacéuticos en el medioambiente directamente afectado [16].

Siguiendo con la ruta de los compuestos en estudio, una vez que han sido excretados, las aguas domésticas llegan a las estaciones depuradoras de aguas residuales (EDARs), donde los residuos farmacéuticos no son eliminados de manera eficiente, debido a que los tratamientos de depuración no están específicamente diseñados para tratar este tipo de residuos. Aunque existe una gran variedad de tratamientos de eliminación o depuración de aguas, derivados del desarrollo en la rama de tecnología del agua, éstos no son lo suficientemente eficientes para que los fármacos sean transformados en sustancias menos nocivas en su totalidad. Por lo tanto, los efluentes de las EDARs, que vierten sus aguas depuradas a ríos o directamente al mar mediante emisarios submarinos, suponen la mayor fuente de entrada de estos contaminantes a la hidrosfera [17].

De manera paralela, las estaciones depuradas son productoras de materia orgánica sólida (lodos), en los que estos compuestos pueden ser parcialmente absorbidos sobre la estructura lipídica bacteriana y la fracción grasa a través de interacciones hidrófobas, o adsorbidos a través de interacciones electrostáticas, pudiéndose unir también químicamente a proteínas bacterianas y ácidos nucleicos. Por otra parte, también pueden verse involucrados en mecanismos como de enlace de hidrógeno, intercambio iónico y complejación superficial [18].

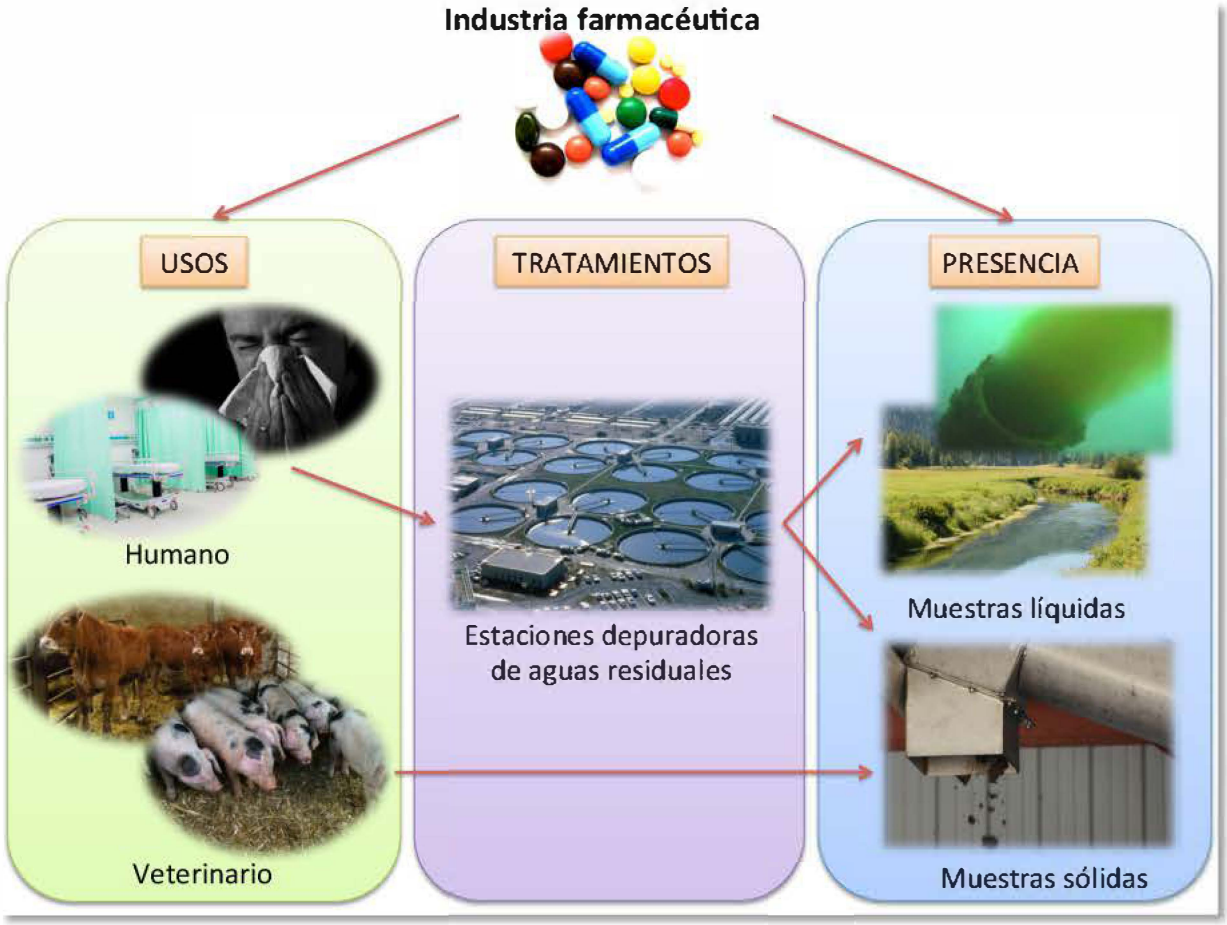

Figura I.1 Esquema de la ruta inicial de los compuestos farmacéuticos.

La actividad agrícola también da lugar a la presencia de compuestos farmacéuticos veterinarios en el estiércol, provocando así la entrada directa a las muestras sólidas en el medioambiente debido a su uso como compostaje [19]. Otras de las vías que conforman la llegada de estos contaminantes al medio se encuentra en la descarga que, de manera ocasional, puede producirse en las industrias, en los efluentes de las aguas residuales de hospitales, los cuales pueden o no tener sistemas de tratamientos previos a su liberación en las aguas superficiales, e incluso mediante el deshecho inadecuado de medicamentos no usados o caducados [20,21].

En la Figura I.1 se presenta, de manera resumida, un esquema del proceso que ocurre desde su producción hasta su presencia en muestras ambientales, tanto líquidas como sólidas. Sin embargo, el alcance del problema se extiende aún más. En la Figura I.2 se refleja la ruta secundaria de los productos farmacéuticos desde que llegan al medioambiente hasta la posibilidad de volver a retornar a los seres humanos a través de la cadena trófica.

Existen dos vías principales para que estos contaminantes puedan llegar a los productos de consumo humano ofrecidos por los recursos naturales o el sector primario: los efluentes de las aguas procedentes de estaciones de tratamiento de aguas residuales y los lodos tanto de ganadería como los que generan las propias EDARs. Por un lado, los efluentes de las EDARs pueden ser directamente vertidos a los ríos o mares afectando a la flora y fauna propia de cada lugar. Estos contaminantes son depositados en los sedimentos y adsorbidos por los ejemplares vegetales que son alimento de los organismos autóctonos y,

por tanto, llegan a introducirse en su sistema digestivo, que es el punto de partida para llegar a su sistema circulatorio, nervioso e incluso endocrino [22].

Además, las EDARs pueden proporcionar aguas depuradas que, debido a sus características, son aptas para su reutilización. El uso inicial del agua recuperada fue, principalmente, el riego en la agricultura. Hoy en día, el agua regenerada se utiliza para una amplia gama de propósitos beneficiosos, incluyendo el agua de refrigeración de plantas de energía, riego comercial y municipal, mejora de flujo y caudal de río, actividades de exploración de gas natural y aumento de suministro de agua potable [23]. Si nos centramos en algunos de sus usos, las aguas que se utilizan para regadío agrícola, comercial o municipal pueden lixiviar en los suelos llegando a filtrarse hasta los acuíferos, quedando las aguas subterráneas contaminadas. De esta manera, queda de manifiesto la presencia de fármacos en las aguas consideradas como potables.

Por último, la fertilización de suelos agrícolas con el uso de los lodos procedentes tanto de ganaderías como los generados por las EDARs, ambos con contenido de residuos farmacéuticos, pueden afectar a la vida en el propio suelo, así como ser consumidos por las plantas de los cultivos y llegar a las cosechas [24].

Dado todo el trayecto posible de estos contaminantes a través de un ecosistema, es lógico pensar que residuos de productos farmacéuticos puedan estar presentes, a diferentes escalas, tanto en todo el ciclo del agua, como en las comunidades bióticas marinas o terrestres.

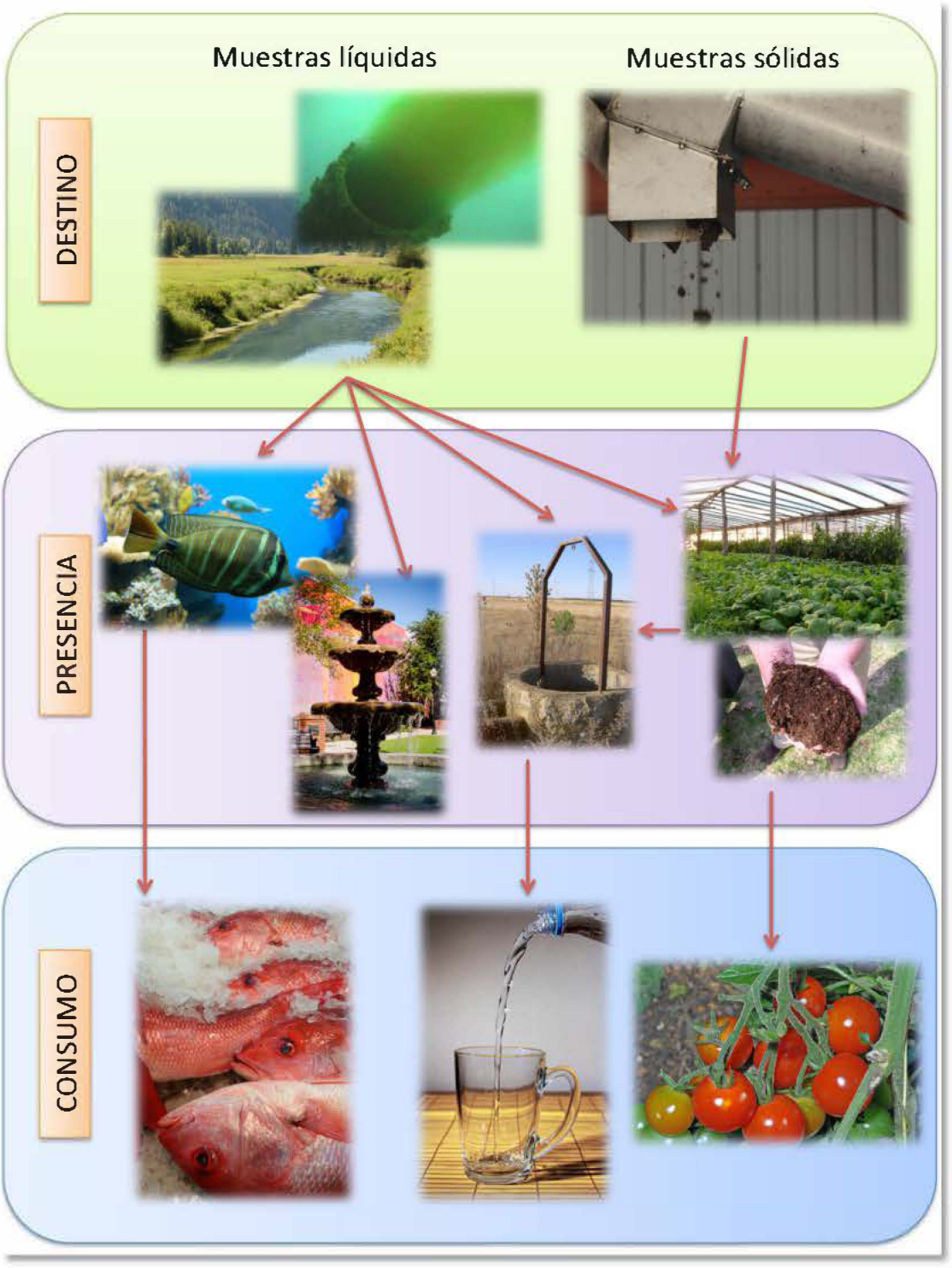

Figura I.2 Esquema de la ruta secundaria de los compuestos farmacéuticos.

Durante las últimas décadas la comunidad científica se ha encargado de dar a conocer información acerca de las concentraciones que pueden encontrarse en los diferentes compartimentos ambientales. Se han elaborado modelos multimedia de predicción de la distribución y la concentración de estos contaminantes una vez son liberados al medioambiente, los cuales consiguen predecir concentraciones que alcanzan el mismo orden que las concentraciones medidas experimentalmente [25].

Los estudios realizados sobre la aparición de estos compuestos en el medio acuático, el cual es uno de los sectores más ampliamente evaluados, revelan rangos de concentración que pueden variar desde escasos $ng \cdot L^{-1}$ en agua potable [26,27] o en muestras de agua de mar [28–30], hasta cantidades de $\mu g \cdot L^{-1}$ encontrados en muestras de aguas residuales [31,32]. En cuanto a los resultados obtenidos del análisis de muestras de sedimentos o lodos, éstos proporcionan niveles de concentración de $ng \cdot Kg^{-1}$ a $\mu g \cdot Kg^{-1}$ relativo al peso seco [33,34]. Asimismo, pero de una manera más reducida, diferentes investigadores han podido encontrar residuos farmacéuticos a niveles trazas en sistemas biológicos, tales como microorganismos, algas, moluscos, peces, e incluso, en mamíferos [29,35,36].

Para dar una visión más clara y siguiendo la dinámica de utilizar referencias procedentes de estudios o bases de datos sobre resultados obtenidos de campañas realizadas en España, en la Tabla I.2 se detalla, de manera simplificada, numerosas evaluaciones que se han presentado sobre la presencia de compuestos farmacéuticos en diferentes muestras ambientales dentro del territorio español a partir del año 2009.

La exposición de los datos se ha establecido mediante una clasificación según la naturaleza de la muestra. En primer lugar, en la Tabla I.2.1 se indican las concentraciones encontradas de una selección de diferentes estudios en muestras líquidas, las cuales se pueden desglosar en: aguas residuales y depuradas (previo y posterior a tratamientos de aguas residuales, respectivamente), aguas subterráneas, aguas superficiales (incluyendo ríos, lagos, océanos y mares), agua de regadío y agua potable. Respecto a las muestras sólidas, que incluyen lodos, sedimentos, suelos y hasta los propios cultivos, los resultados encontrados se muestran en la Tabla I.2.2. Y por último, se exponen los niveles presentes en muestras de sistemas bióticos, desde bivalvos o gasterópodos hasta ejemplares más complejos como los peces (Tabla I.2.3).

La monitorización de la presencia de compuestos farmacéuticos en muestras ambientales es un proceso que ocupa bastante tiempo, desde algunos meses hasta varios años. Además, es necesario establecer un tratamiento estadístico avanzado para conseguir resultados lo suficientemente representativos.

En el periodo revisado (2009-2017) se han obtenido hasta casi 100 estudios sólo en el territorio español; si lo extrapolásemos a nivel mundial se podría apreciar la extensión que abarca la preocupación por este problema emergente.

Tabla I.2.1 Presencia de compuestos farmacéuticos en muestras líquidas ambientales en el periodo 2009-2017 en España.

Zona geográfica	Nº de fármacos	Matriz	Rango de concentración ($ng \cdot L^{-1}$)	Ref.
Sur	24	Agua superficial	8,32 – 2050	[37]
Sureste	4	Agua depurada Agua residual	340 – 2652	[38]
Norte	12	Agua depurada Agua residual	0,15 – 6294 0,41 – 23118	[39]
Este	-	Agua superficial Agua subterránea	< 54,0	[40]
Noreste	81	Agua subterránea	1,00 – 852	[41]
Islas Baleares	27	Agua depurada Agua subterránea Agua superficial	4,00 – 38514 5,00 – 180 2,00 – 56,0	[42]
Noreste	95	Agua subterránea	0,869 – 1620	[43]
Sureste	125	Agua subterránea	5,00 – 769	[44]
Este	17	Agua regadío	< 112	[45]
Noreste	81	Agua depurada Agua subterránea	< 850	[46]
Islas Canarias	110	Agua depurada Agua subterránea	1,50 – 695	[47]
Noreste	16	Agua subterránea	0,200 – 274	[48]
Este Centro Sur	58	Agua potable	7,00 – 15,0	[49]
Noreste	81	Agua residual Agua depurada Agua subterránea	20,0 – 22012 15,0 – 7751 1,00 – 687	[50]
Sureste	83	Agua superficial	< 195	[51]
Este	59	Agua superficial	< 168	[52]
Sur	20	Agua residual Agua depurada Agua superficial	6,00 – 8600 6,00 – 3100 10,0 – 650	[53]
Noreste	81	Agua potable Agua superficial Agua superficial Agua depurada Agua residual	0,400 – 13,0 0,400 – 380 0,300 – 52,0 2,00 – 7480 5,00 – 18681	[54]

Norte Este Sur	76	Agua superficial	0,010 – 1368	[55]
Norte Este Sur	73	Agua superficial	0,110 – 469	[56]
Noreste	18	Agua depurada	2,63 – 173	[57]
Este	21	Agua potable Agua superficial Agua depurada Agua residual	1,00 – 40,0 1,00 – 830 11,0 – 753 0,600 – 4374	[58]
Noroeste	13	Agua superficial	2,80 – 171	[59]
Sur	5	Agua superficial	1,20 – 402	[60]
Noreste	10	Agua superficial	1,16 – 39,4	[61]
Noroeste	21	Agua superficial	0,500 – 118	[62]
Sur	12	Agua superficial Agua depurada Agua residual	< 121 98,0 – 736 80,0 – 3491	[63]
Noreste	57	Agua superficial	0,100 – 2930	[64]
Centro	58	Agua regadío Agua potable	2,00 – 1558	[65]
Noreste	73	Agua superficial	0,010 – 3960	[66]
Noroeste	14	Agua depurada Agua residual Agua superficial Agua potable	4,00 – 689 20,0 – 10598 10,0 – 167 11,0 – 562	[67]
Noroeste	21	Agua superficial	13,6 – 2978	[68]
Noreste	66	Agua superficial	0,100 -1500	[69]
Noreste	74	Agua superficial Agua depurada	0,500 – 1600 1,80 – 3080	[70]
Noreste	43	Agua superficial	0,420 – 1162	[71]
Noreste	74	Agua superficial	0,910 – 872	[72]
Centro	33	Agua superficial	<1,00 – 67715	[73]
Suroeste	12	Agua depurada	30,0 – 11800	[74]
Este	50	Agua superficial Agua depurada	< 2850 < 80000	[75]
Centro	24	Agua superficial	2,00 – 18000	[76]
Noreste	71	Agua superficial Agua depurada	0,030 – 224 0,040 – 1104	[77]
Sureste	16	Agua superficial Agua depurada	0,020 – 4550 0,04 – 26800	[78]
Noreste	28	Agua superficial	< 8042	[79]

Noreste	10	Agua superficial	0,270 – 2482	[80]
Noreste	29	Agua superficial	10,0 – 18740	[81,82]
Centro	7	Agua superficial	8,00 – 1160	[83]
Noreste	73	Agua depurada Agua superficial	< 2980 < 410	[84]
Centro	22	Agua superficial	0,100 – 2784	[85]
Centro	79	Agua superficial	1,00 – 9942	[86]
Noreste	73	Agua depurada Agua residual Agua superficial	2,00 – 12070 21,0 – 18410 0,300 – 219	[87]
Este	25	Agua residual	5,00 – 44258	[88]
Este	17	Agua superficial	2,20 – 17699	[89,90]
Norte Centro Sureste	66	Agua depurada Agua residual	5,00 – 32720 7,00 – 59495	[91]
Noreste	32	Agua depurada Agua residual	1,20 – 1126 3,20 – 7129	[92]
Islas Canarias	7	Agua depurada	20,0 – 12310	[93]
Este	20	Agua depurada Agua residual	10,0 – 236000 20,0 – 277000	[94,95]
Noroeste	15	Agua depurada Agua residual Agua superficial Agua potable	10,0 – 10000	[96]
Noreste	22	Agua depurada Agua residual	8,00 – 1032 20,0 – 19640	[97]
Sur	6	Agua depurada Agua residual	150 – 55000 220 – 603000	[98]
Noreste Sureste	13	Agua depurada	1,00 – 15000	[99]
Noreste	12	Agua depurada Agua residual Agua superficial	0,400 – 7015 1,50 – 40180 0,200 – 1830	[100]
Noreste	75	Agua superficial	0,110 – 4100	[101]
Sureste	69	Agua superficial	0,300 – 11204	[102]
Noreste	73	Agua superficial	0,010 – 1244	[103]
Este	17	Agua superficial Agua depurada	2,00 – 6260 8,00 – 5899	[104]
Sureste	16	Agua depurada	40,0 – 32800	[105]
Norte	7	Agua depurada Agua residual	390 – 6430 340 – 77800	[106]

Noreste	6	Agua depurada Agua residual	2,00 – 372 40,0 – 2860	[107]
Norte	6	Agua depurada Agua residual	30,0 – 910 130 – 30500	[108]
Islas Canarias	13	Agua depurada	20,0 – 34810	[109]
Sureste	14	Agua depurada	50,0 – 3373	[110]
Sureste	16	Agua depurada Agua residual	80,0 – 16100 40,0 – 84700	[111]
Noreste	49	Agua depurada Agua residual	0,500 – 29395 0,17 – 22886	[112]

Si comparamos las diferentes tablas (Tabla I.2), se puede observar que los estudios de las muestras acuosas tienen un mayor interés frente a los estudios de muestras sólidas. Este hecho se debe a que, como se ha mencionado anteriormente, el sistema hidrológico es el origen de la ruta de entrada de estos contaminantes al medioambiente.

Los datos presentados del análisis de muestras líquidas ambientales se centran, mayoritariamente, en la determinación de estos contaminantes en aguas superficiales, eligiendo como puntos de muestreos aquellos que directamente están afectados por descargas de EDARs, de esta manera se controlan los vertidos y se evalúa la situación más desfavorable en este sector. Por otro lado, un número reducido pero bastante significativo de estudios se ha encontrado sobre el análisis de muestras sólidas. En la mayoría de los casos concentran la atención en el análisis de sedimentos en ríos y mares.

Tabla I.2.2 Presencia de compuestos farmacéuticos en muestras sólidas ambientales en el periodo 2009-2017 en España.

Zona geográfica	Nº de fármacos	Matriz	Rango de concentración ($ng \cdot g^{-1}$)	Ref.
Este	17	Sedimento Suelo	< 15,1 < 8,40	[45]
Este	59	Sedimento	< 54,2	[52]
Sur	20	Sedimento	0,200 – 100	[53]
Norte Este Sur	76	Sedimento	0,020 - 127	[55]
Noreste	18	Sedimento	0,400 – 19,3	[57]
Este	21	Sedimento	2,00 – 318	[58]
Noreste	43	Sedimento	0,140 – 222	[71]
Noreste	32	Lodos	2,00 – 61,0	[92]
Este Sureste	15	Suelo	0,500 – 47,0	[113]
Noreste	11	Cultivo	0,016 – 35,5	[114]
Sur	64	Suelo	0,100 – 1,30	[115]
Sur	16	Lodos Compost	3,29 – 4105 9,19 – 974	[116]
Este	17	Sedimento Suelo	0,62 – 35,8 0,24 – 16,0	[117]
Sur	16	Lodos Compost Sedimento	3,69 – 5096 4,85 – 94,9 3,37 – 48,1	[118]
Centro Noreste	16	Lodos	3,60 – 1111	[119]
Centro	16	Lodos	2,10 – 4448	[120]
Noreste	20	Lodos	2,00 – 1958	[121]
Sur	32	Sedimento	0,100 – 88,8	[122–124]
Sur	16	Sedimento	< 52,1	[125]
Noroeste	6	Sedimento	0,170 – 25,6	[126]
Sur	13	Lodos	12,0 – 834	[127]

Por último, sólo tres estudios se presentan sobre la presencia de fármacos en muestras de sistemas bióticos. En este caso, dos de los estudios se destinan a calcular el riesgo teórico-práctico que pueden producir las concentraciones máximas de residuos farmacéuticos encontradas en ecosistemas donde se desarrollan algunos organismos, dejando de lado la evaluación de las cantidades ad/absorbidas en los mismos, dada la complejidad que supone el tratamiento de una matriz de este tipo, así como la previsión de encontrar concentraciones ínfimas.

Un considerable número de los estudios revisados se realizan con el propósito de conocer la ruta y comportamiento que siguen los compuestos farmacéuticos, por ello, se dedican a determinar su concentración, tanto en muestras acuosas como sólidas [45,52,53,55,57,58,71,92], y así, evaluar los fenómenos que se producen a nivel molecular de su predisposición a adherirse a un sistema u otro dadas sus características físico-químicas.

Tabla I.2.3 Presencia de compuestos farmacéuticos en muestras de sistemas bióticos en el periodo 2009-2017 en España.

Zona geográfica	Nº de fármacos	Matriz	Rango de concentración ($ng \cdot g^{-1}$)	Ref.
Noreste	52	Bivalvos	< 3.0	[128]
Sureste	20	Peces	0.2 – 6.3	[129]
		Bivalvos	0.1 – 3.2	
		Gasterópodos	0.4 – 2.3	
Noreste	23	Bivalvos	0.8 – 3.0	[130]

En cuanto a los niveles de concentración encontrados en cada una de las matrices, teniendo en cuenta el compuesto activo sin metabolizar, y si realizamos un análisis estadístico de los datos recabados y lo representamos en un gráfico con los máximos y el rango de medianas para cada tipo de muestra, tal y como se expone en la Figura I.3, se podría destacar la reducción paulatina a medida que vamos siguiendo el recorrido lógico de estos contaminantes en el medioambiente. Comenzando con concentraciones en el rango de 20,0 $ng \cdot L^{-1}$ (ppt) hasta 22,4 $\mu g \cdot L^{-1}$ (ppb) con un pico máximo de 60,3 $\mu g \cdot L^{-1}$ (ppb) en el punto de origen, es decir, en las muestras de aguas residuales, las cuales son procedentes de residuos domésticos, industriales y hospitalarios. Y llegando a concentraciones en el rango de 0,016 – 35,5 $ng \cdot g^{-1}$ (ppt) con un máximo de 562 $ng \cdot g^{-1}$ (ppt) en su destino final, el cual sería los organismos, agua potable y cosecha.

Existe una diferencia considerable entre las concentraciones presentes en las aguas residuales frente a las aguas depuradas y superficiales, produciéndose una disminución en el paso por cada sector. Esto es debido a un rendimiento de eliminación o transformación en subproductos tras atravesar las EDARs y, a un consecutivo efecto de dilución producido por la unión de las aguas depuradas con las grandes cantidades de agua procedentes de ríos y mares [77].

Sin embargo, las concentraciones presentes, tanto en las aguas depuradas como en aguas superficiales, siguen siendo relativamente elevadas, con unos niveles de concentración entre 0,500 $ng \cdot L^{-1}$ y 6,43 $\mu g \cdot L^{-1}$ llegando a un máximo de 236 $\mu g \cdot L^{-1}$. Si continuamos con una evaluación de las muestras líquidas, cabe destacar la extraordinaria

similitud de los resultados proporcionados por el análisis de muestras de agua subterránea y de regadío, con rangos de concentración que van desde 1,00 a 728 $ng \cdot L^{-1}$ y entre 2,00 y 835 $ng \cdot L^{-1}$, respectivamente. Esto nos podría dar una idea de que la gran parte de los compuestos farmacéuticos están siendo filtrados directamente a las aguas subterráneas, dada sus características polares, y no se están quedando retenidos en los suelos; además, puede ser corroborado debido a las mínimas concentraciones presentes en las muestras de suelos cuyos niveles alcanzan, en el peor de los casos, un máximo de 47,0 $ng \cdot g^{-1}$, cuyo rango queda establecido entre 0,240 y 12,2 $ng \cdot g^{-1}$.

En cuanto a las muestras sólidas, se aprecia una diferencia entre las fuentes de contaminación de la ruta que hemos denominado inicial (Figura I.1) con las fuentes de la ruta secundaria (Figura I.2), es decir, se ha determinado una mayor concentración de estos contaminantes en muestras de lodos y compostaje, cuyo rango oscila entre 3,29 $ng \cdot g^{-1}$ y 1,96 $\mu g \cdot g^{-1}$, frente a aquellos sectores influenciados por la ruta inicial, como son los sedimentos, suelos y el propio cultivo, cuyas concentraciones no alcanzan los $\mu g \cdot g^{-1}$ dado que su rango pasa a ser entre 0,016 – 53,1 $ng \cdot g^{-1}$.

Aunque en las tablas sólo se han aportado las concentraciones mínimas y máximas de cada uno de los estudios revisados, con el propósito de dar una visión extrema, hay que tener en cuenta la importancia de la frecuencia de detección y el rango de concentraciones donde se encuentran la mayoría del conjunto de datos, usando por ejemplo la mediana, para conocer realmente el alcance de este tipo de contaminación [76,81].

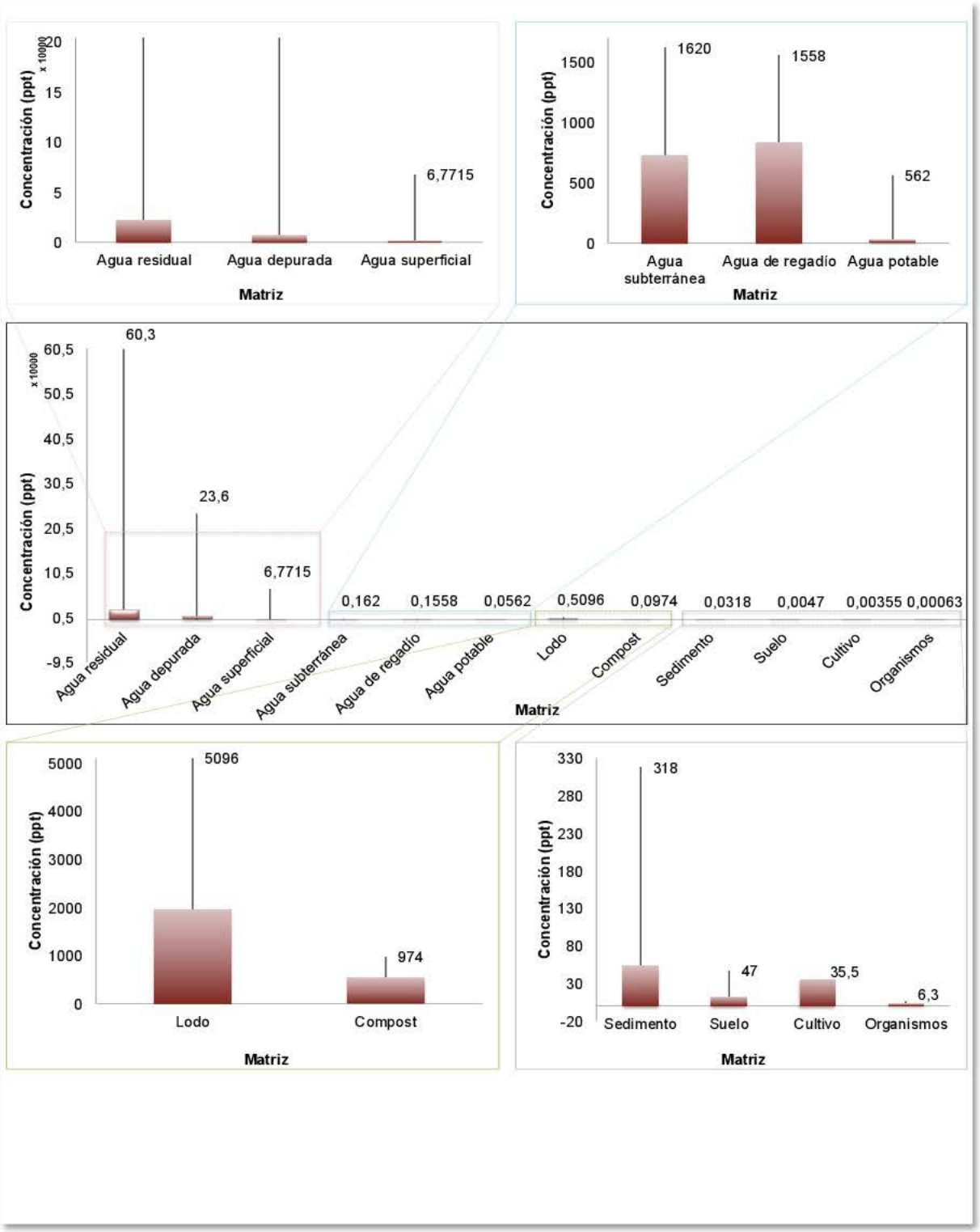

Figura I.3 Evaluación estadística mediante el rango de medianas y máximos para cada tipo de matriz

Desde una visión más geográfica, en la Figura I.4 se ha representado, mediante una simbología simple, la distribución de los estudios que se han presentado en las tablas (Tabla I.2) alrededor del territorio español durante el periodo que comprende desde el año 2009 hasta la actualidad. Se puede observar cómo la mayoría de los estudios se concentran en diferentes comunidades, tales como, Cataluña, Valencia, Madrid, Andalucía y, en menor medida, Galicia.

De esta manera más visual e intuitiva se aprecia que las mayores agrupaciones de los puntos se sitúan en estudios realizados en las diferentes cuencas de los ríos principales de España, como la del Miño, Ebro, Tajo, Júcar, Segura y Guadalquivir, los cuales reciben impacto antropogénico importante mediante flujos de descarga de industrias, EDARs y hospitales, así como de la actividad agrícola. Este escenario era muy previsible dada la evaluación descrita anteriormente, sin embargo, también juega un papel importante el hecho de que en esos lugares están instalados los laboratorios de investigación en relación al análisis químico, gestión y desarrollo medioambiental más potentes del país.

Concretamente en las Islas Canarias no se han realizado muchos estudios al respecto, sólo en la isla de Gran Canaria han sido presentadas algunas investigaciones, únicamente, en muestras líquidas. Cabe destacar que, debido a que no existen industrias farmacéuticas en Gran Canaria que puedan ocasionar contaminación de manera accidental, la presencia de estos compuestos es exclusivamente debido a la excreción después de su consumo o el deshecho inadecuado de los medicamentos caducados.

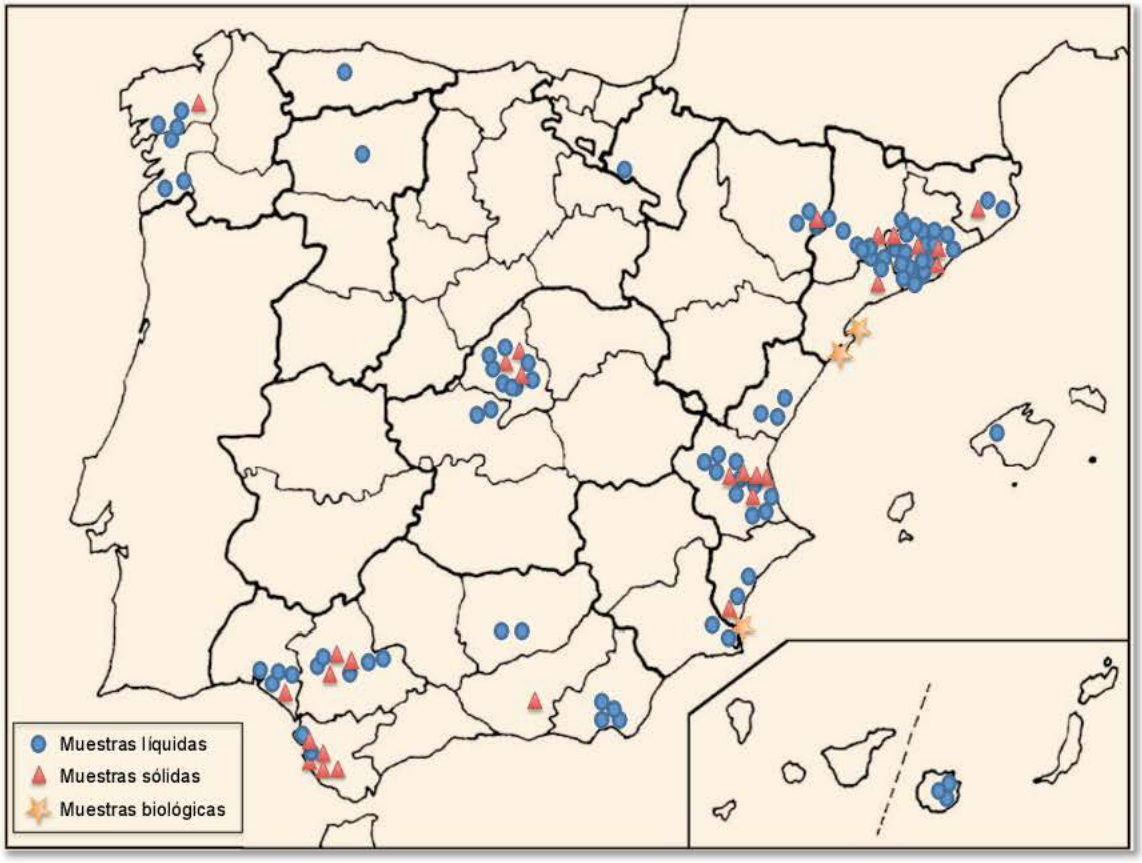

Figura I.4 Distribución de los estudios en España sobre la presencia de compuestos farmacéuticos en el medioambiente en el periodo 2009-2017.

En esta área existe una hidrología diferente a la que presenta la Península Ibérica y, por tanto, se sufre una gran escasez de recursos hídricos que hacen necesaria la búsqueda de alternativas para suplir ese déficit a través de una mayor reutilización de las aguas depuradas o la desalinización del agua de mar. En este sentido, los compuestos farmacéuticos pueden entrar indirectamente en productos alimenticios, tales como pescado o cultivos agrícolas, o afectar al agua de consumo, sin embargo, no hay estudios que puedan corroborar esta afirmación en esta zona geográfica hasta el momento.

Sabemos que las EDARs son un punto clave de la presencia de los residuos farmacéuticos en el medioambiente, por lo tanto, para poder determinar medidas a este problema emergente es esencial realizar una profunda evaluación sobre la eficacia de las EDARs, mediante el análisis de muestras de aguas residuales y depuradas alrededor de todo el territorio y no sólo en aquellos puntos cercanos a ríos, para así incluir todas las variables que puedan influir en su descarga en los diferentes compartimentos ambientales. Como consecuencia, se obtendría una base de datos nacional importante para futuras investigaciones y toma de decisiones.

I.1.2. Impacto ambiental y riesgos toxicológicos

Dado que los compuestos farmacéuticos son considerados como contaminantes, cabe esperar que produzcan un efecto toxicológico o provoquen un riesgo ambiental aún en las concentraciones a nivel de ppt a ppb en las que, como se ha mostrado en el apartado anterior, son encontradas en el medioambiente. Estos productos han sido manufacturados para poder ser introducidos en el organismo sin que alteren sus propiedades, por lo tanto, esta estabilidad, que desafortunadamente también se origina fuera de los seres vivos, junto con su uso masivo y extenso crean una profunda permanencia en el medioambiente [131].

El peligro ambiental que causa una sustancia se clasifica mediante índices basados en diferentes parámetros, los cuales han sido establecidos por la regulación REACH (cuyas siglas del inglés traducidas significa:

registro, evaluación, autorización y restricción química) [132] y son enumerados a continuación [133,134]:

1. Persistencia: cantidad de tiempo que una sustancia química permanece en el medioambiente. Una forma común de medir la persistencia ambiental se basa en su vida media, es decir, el tiempo (en días) que se requiere para que su concentración disminuya a la mitad de su valor original en un medio en concreto ($T_{½}$).
2. Bioacumulación: acumulación de una sustancia en un organismo o parte de un organismo. El factor de bioconcentración (BCF) es el dato experimental que se usa para evaluar la bioacumulación y se define como la concentración de la sustancia que ha sido absorbida por una especie, dividida por la concentración de esa sustancia en el medio en estado estacionario.
3. Toxicidad: acción de un xenobiótico sobre las reacciones o procesos bioquímicos en los organismos vivos. Este xenobiótico es una sustancia química que puede causar efectos adversos, a través de reacciones química u otra actividad a escala molecular, después de la entrada de una suficiente cantidad en el propio organismo. Este parámetro suele evaluarse en base a la toxicidad aguda o crónica y al potencial de efectos adversos (carcinógeno, disrupción endocrina, entre otros) mediante el cálculo de la concentración a la cual no se observa efecto (NOEC, por sus siglas en inglés) o mediante el cálculo de la concentración necesaria para causar el 50 o 10 % de efecto perjudicial, EC_{50} y EC_{10}, respectivamente.

Los criterios que definen los valores máximos a los que pueden llegar cada uno de los parámetros anteriormente descrito se muestran en la Tabla I.3, los cuales han sido precisados en el Anexo XIII del reglamento REACH, y así, de esta forma se puede evaluar si una sustancia es persistente, bioacumulativa o tóxica [135].

Tabla I.3 Criterios de determinación de sustancias PBT

CRITERIOS PBT	**Persistencia**	-$T_{½}$ > 60 días en agua marina -$T_{½}$ > 40 días en agua dulce y estuarios -$T_{½}$ > 180 días en sedimentos marinos -$T_{½}$ > 120 días en sedimentos de agua dulce y estuarios -$T_{½}$ > 120 días en suelo
	Bioacumulación	BFC > 2000 L Kg^{-1}
	Toxicidad	-NOEC o EC10 < 0.01 mg L^{-1} en agua dulce y marina -Clasificados como carcinogénicos, mutagénicos o tóxicos para la reproducción u otras evidencias de toxicidad crónica (Reglamento (CE) nº 1272/2008)

Estas características se encuentran íntimamente ligadas, dado que la persistencia podría intensificar los fenómenos conocidos como biomagnificación y bioacumulación y, a su vez, dado que estos compuestos permanecen activos, pueden provocar un comportamiento

tóxico no deseado debido a que estas sustancias son diseñadas para dirigirse a receptores biológicos concretos [136].

A pesar de la existencia de diferentes estudios que determinan experimentalmente de manera aislada los índices de persistencia (P), bioacumulación (B) y toxicidad (T) [137–142] de los productos farmacéuticos, otros investigadores realizan una clasificación de los mismos según sus índices PBT mediante métodos de predicción de una forma más manejable y económica, con el fin de crear listas prioritarias de estos contaminantes. Estos métodos no deben sustituir los datos experimentales pero servirían, sin embargo, de aproximación para acotar el número de compuestos farmacéuticos, entre los miles existentes, que deben ser inmediatamente controlados [143,144].

De otra manera, el impacto que se podría producir en los organismos debido a la presencia de fármacos después de su descarga en el medio suele ser estimado mediante la evaluación del riesgo ambiental, el cual es calculado usando un procedimiento que ha sido establecido en guías de diferentes agencias europeas [145].

La forma de evaluación extensamente usada por la comunidad científica es mediante el cálculo del cociente de riesgo (RQ), el cual se define como la comparación entre la concentración máxima ambiental de contaminante esperada o medida (PEC o MEC) y la concentración sin efecto ecológico (PNEC). PNEC normalmente se extrapola dividiendo el valor EC_{50} con un factor de seguridad, por lo tanto puede ser predictivo o experimental. El cálculo de RQ se realiza para diferentes especies, dado que el efecto de una misma sustancia puede variar según las características del organismo. Para valorar si una sustancia puede producir

riesgo para una especie en concreto se utilizan los siguientes criterios: se dice que una sustancia posee un riesgo alto cuando RQ es igual o superior a la unidad ($RQ \geq 1$), riesgo medio para un rango entre 0,1 y 1,0 ($0,1 < RQ < 1,0$) y se considera que una sustancia no causa efecto para valores de RQ menores a 0,1 ($RQ \leq 0,1$) [146].

Aunque con estos métodos de predicción y con los datos experimentales nos podríamos acercar a los posibles efectos negativos que producirían en el medioambiente, el comportamiento real de estos contaminantes puede ser inesperado y desconocido llegando a producir efectos indeseados en los organismos, dada la cantidad de variables existentes que pueden desviarnos de las aproximaciones realizadas [147].

De todos modos, algunos efectos toxicológicos han sido demostrados, principalmente para los seres vivos acuáticos, y nos podría dar una idea del alcance que puede tener la presencia de fármacos en el medioambiente. Entre las situaciones más alarmantes cabe destacar la capacidad de disrupción endocrina o la resistencia bacteriana a los antibióticos. Además, la mezcla de fármacos puede agravar de una manera exponencial cualquier consecuencia toxicológica, creando efectos adictivos en la adsorción de compuestos del mismo tipo o produciendo problemas devastadores con la mezcla de fármacos de diferentes clases terapéuticas [148].

Estos efectos son merecedores de una explicación más extensa para conocer exactamente los problemas a los que nos enfrentamos, los cuales debemos remediar y evitar, por ello, se han realizado las siguientes divisiones de manera aclaratoria, proporcionando adicionalmente algunos

ejemplos de estudios realizados a diferentes especies, desde microorganismos hasta peces:

- Inhibición de procesos enzimáticos

El metabolismo se compone de todos los procesos químicos que se producen en un organismo, los cuales son catalizados por diferentes enzimas. Muchos fármacos pueden tener la capacidad de disminuir la actividad de estas enzimas, por lo tanto, son capaces de producir un desequilibrio metabólico en el organismo. Por ejemplo, Burkina y col. [149] realizan una revisión bibliográfica sobre los posibles efectos de inhibición que la presencia de fármacos en el entorno podrían producir sobre la enzima P450, la cual es encargada de metabolizar aquellas sustancias xenobióticas que entran en el organismo, como consecuencia supondría la deficiencia del poder de desintoxicación del mismo.

Otra muestra de este efecto lo ha estudiado Oliveira y col. [150], quienes evaluaron los efectos toxicológicos producidos por cuatro compuestos farmacéuticos, extensamente usados, sobre un crustáceo planctónico, *Daphnia magna*. Para ello usaron diferentes enzimas como biomarcadores, los cuales son encargados de la regulación neuronal. Los resultados indicaron que la exposición a los compuestos ensayados puede inducir alteraciones en el estado redox celular, el cual es importante para los procesos bioquímicos, lo que podría desencadenar enfermedades en el organismo, incluso carcinogénicas. Además, demostraron que dos de los compuestos (acetaminofeno y diclofenaco) tienen la capacidad de interferir sobre la neurotransmisión, mediante la inhibición de enzimas.

Efectos muy parecidos a los encontrados por Oliveira y col. fueron evaluados para una especie más compleja (anguila europea) producidos por la presencia de paracetamol. Este compuesto reveló una posible neurotoxicidad, sin embargo, esta especie tiene una alta capacidad de desintoxicación que pueden hacer frente a la presencia de paracetamol, evitando alteraciones deletéreas adicionales [151].

- Disrupción endocrina

Numerosos fármacos, los cuales han sido investigados en varios estudios, muestran capacidad de disrupción endocrina, la cual está íntimamente ligada al apartado anterior sobre la alteración de procesos metabólicos [152]. Esto supone que los contaminantes tienen la habilidad de alterar el control y regulación del sistema endocrino-reproductivo tras su entrada descontrolada en los organismos. De esta manera, se han comprobado situaciones de cambios hormonales en diferentes especies, produciendo en muchos casos una feminización, por lo que provocaría un decrecimiento de la capacidad reproductiva quedando de manifiesto una amenaza directa a la supervivencia de la especie a un relativo largo plazo [153].

Un claro ejemplo que puede ilustrar este problema es el trabajo realizado por Fernandes y col. [154], quienes hicieron un estudio in vitro sobre los efectos que, fármacos de diferentes clases terapéuticas (antiinflamatorios, antidepresivos y reguladores lipídicos), producirían sobre las enzimas encargadas de crear andrógenos en carpas macho. Los resultados revelaron que dos de los compuestos seleccionados (fluvoxamina y fluoxetina) eran los inhibidores más fuertes de las enzimas

estudiadas, las cuales influyen en la espermatogénesis y estimulan el comportamiento reproductivo y las características sexuales secundarias en los peces macho.

- Resistencia antibiótica

La aparición de bacterias con genes resistentes a los antibióticos, los cuales fueron diseñados para combatir las infecciones por dichos microorganismos, ha surgido debido a diferentes causas: (I) prescripción ineficiente, ya que la duración de los tratamientos pueden llegar a ser superior a lo necesario o la propia elección del tipo de antibiótico, (II) uso extenso en la agricultura como suplemento para el crecimiento en ganados y la prevención de infecciones y, (III) disminución de la disponibilidad y desarrollo de nuevos antibióticos [155]. Todo ello, unido a la presencia de estos contaminantes en los ecosistemas a través de las rutas que ya hemos hablado con anterioridad, supone un alto riesgo para la salud de todos los seres vivos. Cuando estos microorganismos resistentes infectan a los seres humanos pueden aumentar la mortalidad en sus huéspedes debido a la ineficacia de los antibióticos utilizados para combatir las infecciones [156]. La Organización Mundial de la salud ya contempla este hecho como una amenaza de salud pública mundial para la prevención y el tratamiento eficaz de una gama, cada vez mayor, de infecciones causadas por bacterias, parásitos, virus y hongos. Además, propone la necesidad de acciones coordinadas para minimizar la aparición y propagación de la resistencia a los antibióticos [157].

Aydin y col. nos recuerdan que los procesos de tratamiento biológico ofrecen las condiciones ideales en las que una alta diversidad de

microorganismos pueden crecer y desarrollarse. Por tanto, las aguas residuales producidas durante estos procesos, las cuales están contaminadas con antibióticos, proporcionan un escenario ideal para la proliferación de genes resistentes a los antibióticos [158]. Las concentraciones de antibióticos encontrados en el agua han permitido la formación de organismos resistentes como Aeromonas, Salmonella, Escherichia, Pseudomonas, Staphylococcus, entre otros [156].

Por otro lado, un estudio preliminar realizado por Smaldone y col. evaluó tanto la presencia como la resistencia a los antimicrobianos en especies de bacterias aisladas de diferentes peces y productos pesqueros capturados en el medio silvestre para una selección de fármacos. Los autores llegaron a la conclusión de que el riesgo de resistencia antibiótica es significativamente más grave que el propio hecho de que exista la presencia de antibióticos en peces que son consumidos por la población. Las muestras recogidas estaban contaminadas por residuos de cepas resistentes y de antibióticos que podían desempeñar el papel de propagar esa resistencia [159].

- Cóctel farmacéutico

La gran mayoría de estudios se centran en la evaluación del riesgo y la determinación de efectos toxicológicos en el medioambiente de fármacos individuales. No obstante, no se trata de una situación realista dado que la presencia de fármacos se produce mediante una mezcla de sustancias de diferentes clases terapéuticas, por lo que no existe una separación física de cada uno. De esta manera, los efectos que se han demostrado que provocan los compuestos de manera individual se

pueden ver magnificadas y agravados debido a la presencia de un cóctel de fármacos.

El impacto que puede producir la mezcla de contaminantes es mucho más difícil de evaluar, dada todas las interacciones que pueden producirse entre ellos. No se trata de una multiplicación de factores individuales, sino de un proceso más complejo. Por ello, algunos autores proponen protocolos de seguimiento en este sentido, incluyendo modelos de predicción. Sin embargo, recalcan la necesidad de seguir optimizando la metodología de evaluación para crear un criterio uniforme [160,161].

A pesar de todos los estudios realizados sobre los efectos que se producen en los organismos, muchos autores coinciden en la necesidad de estudios más profundos, sobre todo con ensayos en vivo, para conocer y ampliar el conocimiento sobre este problema. En todo caso, también existen otras publicaciones que intentan diseminar la alarma social indicando que no queda muy claro las consecuencias reales de la presencia de estos contaminantes en el medio, dado que los sistemas de predicción y análisis in vitro son cuestionables a la hora de ser transferidos a la realidad [162].

I.1.3. Reglamentación y control

Habitualmente, los estudios realizados por la comunidad científica se han enfocado a la presencia de contaminantes químicos en el medioambiente que se encontraban regulados en diferentes legislaciones, que en su mayoría se caracterizaban por ser apolares, tóxicos, persistentes y bioacumulables. Entre ellos se encuentran diferentes familias de

compuestos orgánicos prioritarios, tales como los hidrocarburos aromáticos policíclicos, los policlorobifenilos (PCBs) o las dioxinas. Sin embargo, en las últimas décadas, gracias al desarrollo de nuevos y más sensibles métodos de análisis, se ha permitido advertir de la presencia de otros contaminantes, potencialmente peligrosos, denominados globalmente como *emergentes* [163].

Los *contaminantes emergentes* son, en muchos casos, contaminantes no regulados, que pueden ser candidatos a serlo en el futuro, dependiendo de la investigación de sus potenciales efectos sobre la salud y de los datos disponibles que puedan demostrar su existencia [164]. Entre la larga lista elaborada por diferentes autores donde se clasifican este tipo de contaminantes, los productos farmacéuticos se encuentran entre ellos. Sin embargo, a pesar de los numerosos estudios que se han presentado, tanto de su presencia en el medioambiente como las posibles consecuencias negativas en los seres vivos, la reglamentación sobre el control de estos contaminantes ha evolucionado muy débilmente, de hecho, no existen niveles de concentración máximos establecidos.

Centrándonos principalmente en el mayor foco de contaminación por la que estos compuestos llegan al medioambiente, es decir, la hidrosfera, la Unión Europea ha establecido lo que se conoce como la Directiva Marco del Agua 2000/60/CE (WFD, Water Framework Directive) cuyo objetivo es lograr y asegurar un buen estado ecológico y químico de las aguas y, además, prevenir el deterioro de las aguas superficiales, continentales de transición, subterráneas y costeras. Para ello, se debe cumplir con los estándares de calidad ambiental y, de esta manera, se

unifican las actuaciones en materia de gestión de agua en la Unión Europea estableciendo criterios globales [165].

Las normas de calidad ambiental y los valores límites de emisión de algunos contaminantes fueron especificados en el Anexo IX de WFD y, específicamente, en el Anexo X se estableció la lista de sustancias prioritarias y sustancias peligrosas prioritarias. Haciendo un recorrido por la historia sobre este tema, se sabe que la primera lista de sustancias prioritarias fue establecida en la Decisión 2455/2001/CE [166], sin embargo, a través del Anexo II de la Directiva 2008/105/CE fue reemplazado el Anexo X de la WFD [167]. Años más tarde, una segunda lista fue elaborada a través de una propuesta de la Comisión Europea (COM(2011)876), la cual modificaría tanto la WFD como la Directiva 2008/105/CE [168].

La reglamentación referida a la lista de sustancias prioritaria ha seguido evolucionando y hace tan sólo cuatro años ha sido actualizada y ampliada mediante la Directiva (2013/39/UE), donde se identifica una serie de sustancias químicas emergentes, en las que se incluyen pesticidas y biocidas, productos químicos industriales y disruptores endocrinos [169]. Esta directiva también incluye el nuevo concepto conocido como la "lista de vigilancia" de sustancias (Watch List, en inglés), para las que se recopilarán datos a escala de la Unión Europea con el propósito de apoyar futuros ejercicios de priorización de sustancias. En ella se identificaron un número reducido de compuestos que deben ser incluidos en una lista inicial, específicamente, tres fármacos de uso habitual, diclofenaco, 17-beta-estradiol y 17-alfa-etinilestradiol, de los cuales se debe reunir datos

de vigilancia con el fin de facilitar la determinación de las medidas adecuadas para hacer frente al riesgo que representan [164].

Varios años más tarde se publicó la decisión de ejecución 2015/495/UE que establece la primera lista de vigilancia donde se completa el máximo de 10 compuestos que se había establecido como tope. En esta lista original se incluyen diferentes contaminantes emergentes, como son los fármacos (diclofenaco, 17-beta-estradiol, 17-alfa-etinilestradiol y antibióticos macrólidos), pesticidas (metiocarb, neonicotinoides, oxadiazón y trialato), un aditivo industrial (2,6-di-terc-Butil-4-metilfenol) y un ingrediente cosmético (4-Metoxicinamato de 2-etilhexilo); además, se proporcionan los diferentes métodos de análisis recomendados y los correspondientes límites de detección máximos aceptables [170].

Existe la necesidad de seguir desarrollando metodologías que permitan el análisis de estos contaminantes para ofrecer información acerca de las concentraciones a las que están presentes, con el objetivo de que lleguen a escalar a la lista de sustancias prioritarias una vez haya pasado un tiempo prudencial dentro de la lista de vigilancia o se recopile la suficiente información para que se consideren como tal. Además, una vez esas sustancias se prioricen, se podrían incluir nuevos contaminantes a la misma, y así seguir el ciclo del procedimiento hasta conseguir que todas aquellas sustancia potencialmente peligrosas sean controladas, dejando de ser contaminantes emergentes. Para que esto sea posible, se deben seguir todas las directrices de la Directiva Marco del Agua, incluyendo la evaluación del riesgo medioambiental mediante las diferentes guías

disponibles, tal y como se ha mencionado en la sección "Impacto ambiental y riesgos toxicológicos" [171].

En este sentido, la red de trabajo NORMAN, la cual fue financiada por la Comisión Europea en 2005 a través del proyecto NORMAN del Programa Marco 6 y se consolidó como una red autónoma permanente en 2009, tiene como objetivos [172]:

(I) mejorar el intercambio de información y la recopilación de datos sobre sustancias ambientales emergentes

(II) fomentar la validación y armonización de métodos comunes de medición y herramientas de monitoreo para que las demandas de los evaluadores de riesgo puedan ser mejor atendidas y

(III) asegurar que el conocimiento de los contaminantes emergentes se mantenga y se desarrolle estimulando proyectos interdisciplinarios coordinados sobre la investigación orientada a problemas y la transferencia de conocimientos para atender las necesidades identificadas.

Entre la amplia variedad de actividades que tienen en marcha, se encuentra la priorización de sustancias emergentes y plantean una metodología que se adapta perfectamente a las necesidades propuestas por la normativa. Entre los diferentes pasos se encuentra la categorización de las sustancias emergentes que se presentan en un esquema muy intuitivo, el cual se ha intentado reproducir en la Figura I.5.

Se definen un total de seis categorías de sustancias, las cuales se describen a continuación:

- Categoría 1: sustancias que poseen evidencias suficientes de exposición y efectos adversos a la concentración ambiental, por lo que se integra en el monitoreo de rutina y derivación de los estándar de calidad ambiental legalmente vinculante.
- Categoría 2: sustancias cuya evaluación de riesgo se basa en datos experimentales pero existen pocos datos de monitoreo, por lo que necesita estudios de detección para obtener información de la exposición actual.

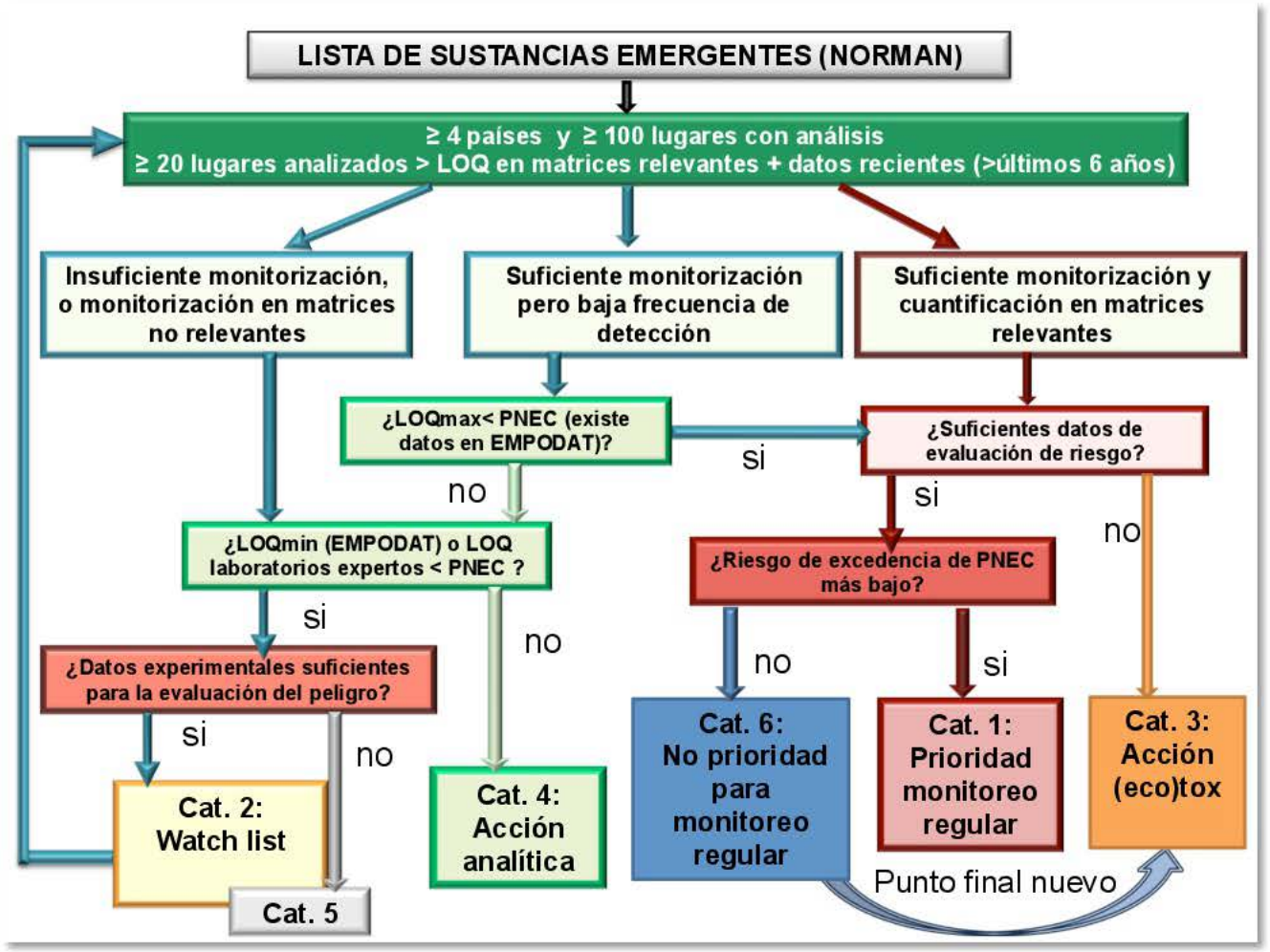

Figura I.5 Diagrama de flujo para la categorización de sustancias.

- Categoría 3: sustancias que poseen evidencias de exposición pero las evaluaciones de riesgos están basadas en la predicción de toxicidad, de manera que necesitan un estudio más riguroso del riesgo ambiental.
- Categoría 4: sustancias cuya evaluación de riesgo está realizada mediante datos experimentales, sin embargo, las capacidades analíticas no son satisfactorias, éstas necesitan ser mejoradas.
- Categoría 5: sustancias que no poseen o tienen poca información de monitoreo y, además, las evaluaciones de riesgo se basan en datos predictivos. Estas sustancias necesitan más estudios y rigurosas evaluaciones de riesgos.
- Categoría 6: sustancias cuyos datos de toxicidad son suficientes para la derivación a unos estándares de calidad ambiental y hay evidencias de que su exposición no representa un peligro para los ecosistemas. De esta manera, se puede reducir los esfuerzos sobre la monitorización de estas sustancias.

La creación de grupos de esta índole, que sean capaces tanto de clasificar las sustancias como seguir recopilando datos para la priorización de las mismas, es la tendencia actual para llevar un control de seguimiento de estos contaminantes y mejorar la reglamentación sobre el problema emergente en un futuro próximo.

I.1.4. Sistemas de eliminación

Hace algunos años la Agencia Europea de Medioambiente propuso, mediante un informe, posibles acciones para la reducción del impacto de estos contaminantes en el medioambiente, los cuales se centraban,

principalmente, en la prevención de la contaminación desde su origen [131]. Para ello sería necesario realizar un balance del ciclo de vida de estos contaminantes, desde su fabricación hasta su eliminación, además, de mejorar la gestión de los residuos mediante una mayor concienciación y mejora de información sobre el deshecho de medicamentos caducados con la ayuda de programas de acción y de estudios de investigación que faciliten información fiable tanto para la toma de decisión política, como para el conocimiento de los ciudadanos.

De manera adicional, otra de las principales soluciones para la disminución de la entrada de estos contaminantes al medio es controlar las cantidades comercializadas y prescritas por los médicos, en definitiva, reducir su uso [173]. Incluso, otra de las posibilidades sería el diseño de medicamentos más amigables con el medioambiente, lo que se conoce como, ecofarmacología. Sin embargo, se trata de unas medidas en las que, a parte de ser un procedimiento con un proceso largo, además entran en juego otros intereses, dificultando las labores de desarrollo. Por ello, debemos tener alternativas, las cuales comenzarían con evitar la entrada de estos contaminantes al medioambiente desde el foco principal, es decir, la instalación de sistemas de eliminación efectivos y diseñados específicamente para la remoción de este tipo de contaminantes en las EDARs.

Hasta ahora, los sistemas más comúnmente usados para la depuración de las aguas se basan en sistemas de tratamiento convencionales. Estos tratamientos consisten en el paso de las aguas residuales por diferentes etapas que han sido incorporadas gracias al desarrollo de la tecnología del agua. La evolución de estos tratamiento

comenzó durante el siglo XIX, con el progreso de procesos básico, y se han ido perfeccionando hasta la actualidad [174].

En general, las EDARs poseen diferentes procesos biológicos y fisicoquímicos dispuestos en diferentes tratamientos, los cuales se clasifican en pretratamiento y tratamiento primario, tratamiento secundario y, ocasionalmente, tratamiento terciario. Durante el pretratamiento y tratamiento primario, se intenta reducir el contenido de sólidos (arena, granos y sólidos sedimentables), aceites y grasas de las aguas residuales. Este paso es completamente mecánico o físico a través de sistemas de filtración y sedimentación, los cuales se les conoce como procesos de desbaste, desengrasado y desarenado. Además, se trata de sistemas de tratamiento que suelen ser común para todas las estaciones depuradoras [175].

Sin embargo, el tratamiento secundario, el cual se basa en un proceso biológico para eliminar la materia orgánica y/o nutrientes con sistemas aeróbicos o anaeróbico, pueden ser sustancialmente diferentes para cada EDAR. Existes diversos sistemas, pero el más comúnmente usado es el de lodos activados, el cual usa oxígeno disuelto para promover el crecimiento de un flóculo biológico que elimina el material orgánico y el nitrógeno, aunque también podemos encontrar los biorreactores de membrana, el reactor con película biológica de cama móvil o el biorreactor de lecho fijo [175].

De manera habitual las EDARs funcionan únicamente con los tratamientos primarios y secundarios. Sin embargo, existen muchas estaciones que han incorporado un tratamiento terciario para un mayor refinamiento y, así, elevar la calidad de las aguas depuradas a un nivel

deseado y darle una mayor reutilización. El objetivo fundamental de este tratamiento añadido es la reducción de la carga orgánica residual y otras sustancias contaminantes que no han podido ser eliminados en el tratamiento secundario, como por ejemplo, el nitrógeno, el fósforo y los nutrientes. Los procesos empleados en esta etapa son la sedimentación por coagulación-floculación y filtración, aunque también se han incorporado otros procesos como, las resinas de intercambio iónico, la adsorción en carbón activado, la ultrafiltración o la ósmosis inversa, entre otros.

En algunas estaciones depuradoras, las aguas depuradas también se desinfecta antes de ser reutilizadas o descargadas al medioambiente, siendo los sistemas típicos la cloración o la irradiación ultravioleta [175].

Dado que no existe legislación vigente sobre la regulación y control de los residuos farmacéuticos, las EDARs que actualmente se encuentran en funcionamiento no han sido diseñadas para eliminar estos contaminantes, sobretodo, aquellos compuestos que poseen un valor de persistencia muy alto. En este sentido, las eficiencias de eliminación de las EDARs varían enormemente, cuyo rango de porcentaje de eliminación puede hallarse entre prácticamente un valor nulo y hasta un 100 %. Esto es provocado por las propiedades fisicoquímicas de los diferentes compuestos, así como por las condiciones medioambientales, las características del agua residual y la configuración de los procesos de tratamiento de los que disponen [176].

En definitiva, el tipo de tratamiento usado influye en la eficacia de eliminación de estos compuestos emergentes, si bien, sí se consiguen los estándares de calidad ambiental que están legislados y son necesarios

para su descarga. Por ello, varios compuestos farmacéuticos, tal y como se demuestra en la sección "Entrada y presencia en el medioambiente", están presentes en los efluentes finales en concentraciones lo suficientemente altas para producir efectos en los ecosistemas. Entre los fármacos más comúnmente detectados se incluyen antiinflamatorios, como el ibuprofeno y el diclofenaco, antibióticos, tales como la eritromicina y oxitetraciclina, antiepiléptico como la carbamazepina y hormonas como 17-beta-estradiol, entre muchos otros [148].

Por otro lado, buscando alternativas que necesiten de un consumo energético más reducido, surgieron las depuradoras naturales, las cuales realizan el tratamiento de aguas residuales domésticas para núcleos poblacionales más pequeños. Estas depuradoras se basan en la acción combinada de la vegetación, suelo y microorganismos presentes en ambos, e incluso, en la acción de plantas y animales superiores. Se caracterizan por su baja producción de fangos, sin embargo, es necesario grandes superficies de terreno, esto es lo que limita su uso a pequeñas poblaciones que no se encuentren en zonas urbanas [177].

Existen dos técnicas de tratamiento natural de aguas residuales: mediante aplicación de agua sobre el terreno, donde se incluyen filtro verde, infiltración rápida, escorrentía superficial y lechos de turba o arena, o a partir de sistemas acuáticos, tales como, lagunajes, humedales o cultivos acuáticos [177].

Una revisión bibliográfica realizada por Verlicchi y Zambello demuestra que en estos sistemas, a pesar de que tratan un volumen menor de agua que las depuradoras convencionales, tienen presencia de residuos farmacéuticos, tanto en el flujo de entrada como en el efluente después del tratamiento. Sin embargo, se trata de tratamiento

prometedores dada su eficacia para la eliminación de estos contaminantes, que pueden llegar a rangos similares que los tratamientos convencionales para una mayoría de compuestos [178].

En todo caso, sigue existiendo la falta de sistemas más potentes para conseguir la completa eliminación o, más específicamente, completa transformación en sustancias menos tóxicas de estos compuestos y de los contaminantes emergentes en general. A pesar de que aún no haya disponible ninguna normativa que los regule, en todo caso, sería un remedio anticipado a un progresivo desarrollo negativo de este problema ambiental.

Intentando alcanzar el propósito de desarrollar tratamientos que sean más efectivos, surgieron los procesos avanzados de oxidación (PAO) como alternativas para incluirse como tratamiento terciario en las estaciones depuradoras. Dependiendo de las propiedades del agua que se desea tratar, los PAOs pueden ser empleados individualmente o de manera combinada con procesos fisicoquímicos y biológicos.

Estos procesos están basados en la intermediación de radicales para oxidar compuestos recalcitrantes, tóxicos y no biodegradables y transformarlos en diversos subproductos y, eventualmente, en productos inertes finales [179]. De otra manera, sería la oxidación de compuestos orgánicos mediante el radical hidroxilo para formar compuestos menos tóxicos seguido por la conversión completa en CO_2, agua y otros iones inorgánicos [180].

Hasta el momento, se han desarrollado numerosas técnicas diferentes de PAOs. Aunque en esta Tesis Doctoral se ha llevado a cabo una revisión bibliográfica en la que la parte introductoria habla sobre estos sistemas avanzados de oxidación (Sección I.3.1) [181], de manera

muy resumida, los PAOs más utilizados para la eliminación de compuestos farmacéuticos son la fotólisis, ozonólisis, Fenton, foto-Fenton, electro-Fenton, fotocatálisis y sonólisis [180]. El proceso que hasta ahora ha conseguido mejores resultados de mineralización de compuestos farmacéuticos es la fotocatálisis heterogénea, consiguiendo hasta la casi completa transformación de una gran cantidad de compuestos que son altamente frecuentes en el medioambiente, debido a que son difíciles de eliminar en las estaciones depuradoras convencionales y naturales, tales como naproxeno, carbamazepina, diclofenaco, acetaminofeno o ibuprofeno [182–184].

Sin embargo, estos procesos avanzados, por sí solo, no son tan eficientes, ya que necesitan ser aplicados después de los procedimientos convencionales, primarios y secundarios, que proporcionan un agua depurada con bajo contenido en materia orgánica. De esta manera, la degradación mediante los PAOs se centra en la degradación y eliminación de los contaminantes emergentes de interés y, así, se consiguen rendimientos superiores de eliminación [185].

Estos sistemas de eliminación siguen en auge permanente, por lo que necesitan de una constante optimización con el fin de alcanzar condiciones que puedan abarcar la degradación de un abanico amplio de contaminantes. Aunque la comunidad científica se esté precipitando, porque esto supondría un paso por delante de las legislaciones, es algo necesario para estar bien preparados, ya que se confía en su posible control dada toda la información actual y los avances del desarrollo científico.

I.2. Clasificación de los fármacos seleccionados

Existen miles de compuestos farmacéuticos que se encuentran en uso actualmente, tanto para la medicina humana como veterinaria, sin embargo, no todos son de interés para llevar a cabo los diferentes estudios que se presentan en esta Tesis Doctoral. Por ello, se ha realizado una selección que se basa en diferentes parámetros que se detallan a continuación:

1. Consumo en España: se ha elegido un grupo de fármacos de alto consumo según los informes elaborados y proporcionados por la Agencia Española de Medicamentos [12], los cuales nos ofrece información del consumo que se ha producido en España durante los últimos años en unidades DDD/1000 hab./día, mostrando que los fármacos como omeprazol, ranitidina, atenolol o ibuprofeno, se consumen a niveles de 4,30 a 104 DDD/1000 hab./día. En este rango de consumo también entrarían otros compuestos como: fluoxetina, naproxeno y diclofenaco.
2. Frecuencia de detección: existe una gran cantidad de artículos que especifican la frecuencia de detección de los compuestos, después de realizar un monitoreo prolongado en el tiempo, sobre los niveles de concentración en que se encuentran en los efluentes de las estaciones depuradoras o, incluso, en los sistemas directamente afectados como son los ríos. Los más frecuentemente detectados son aquellos que presentan una mayor dificultad para degradarse, son consumidos de manera desmesurada y continuada por la población y/o sus

porcentajes de excreción como forma activa son elevados. Entre ellos se encuentran compuestos como cafeína, carbamazepina, nicotina, paraxantina, ranitidina, sulfametoxazol, trimetoprim, metronidazol, diclofenaco, gemfibrozilo o ibuprofeno, entre muchos otros, que aparecen con un 100 % de frecuencia en algunos estudios [73,81].

3. Interés medioambiental: los posibles efectos toxicológicos que pueden causar estos contaminantes pueden ser clasificados a partir de sus índices PBT (Persistencia, Bioacumulación y Toxicidad) o mediante la evaluación de riesgo ambiental que pueden causar en las concentraciones a las que han sido encontrados. De esta manera, los compuestos farmacéuticos pueden ser clasificados de mayor a menor preocupación medioambiental mediante listas de prioridad. Alguno de los fármacos más destacados en este sentido son fluoxetina, bezafibrato, omeprazol, cafeína o propanolol [143,186]

Relacionado con ello, existe un estudio realizado por Sui y col. que intenta enlazar tres parámetros (consumo, eliminación en las depuradoras y efectos ecotoxicológicos), los cuales son muy similares a los que se han explicado anteriormente, para crear una lista prioritaria de fármacos. Los fármacos que se clasificaron en los primeros grupos, que coinciden con los de más alta prioridad, son compuestos tales como eritromicina, diclofenaco, ibuprofeno, sulfametoxazol, gemfibrozilo, trimetoprim, naproxeno, carbamazepina, ciprofloxacino y bezafibrato [187].

Tabla I.4 Consumo en España, porcentajes de excreción y características físico-químicas de los fármacos seleccionados.

Clase terapéutica	Compuesto	Consumo (DDD/1000 hab./día)[a]	Excreción (%)[b]	Log K_{ow}[b,c]	pK_a[b]
Antibiótico	Trimetoprim	0,300	50	0,91	7,12
	Metronidazol	-	20	-0,02	2,38
	Ofloxacino	-	65	-0,39	5,97
	Ciprofloxacino	1,10	50	0,28	6,09
	Sulfametoxazol	0,300	20	0,89	5,70
	Eritromicina	0,100	30	3,1	8,90
Antihipertensivo	Atenolol	7,64	90	0,16	9,60
	Propanolol	0,640	0,5	3,5	9,45
Anti-ulceroso	Ranitidina	4,30	70	-	-
	Omeprazol	104	30	2,2	7,40
Antidepresivo	Fluoxetina	7,84	3	4,0	-
Antiepiléptico	Carbamazepina	1,23	12	2,4	13,9
Antiinflamatorio y analgésico	Metamizol	2,68	-	-	-
	Ketoprofeno	0,060	-	2,8	-
	Naproxeno	6,12	3	3,2	4,15
	Ibuprofeno	21,5	10	4,0	5,20
	Diclofenaco	6,38	15	4,5	4,15
Regulador lipídico	Bezafibrato	0,150	50	3,6	-
	Gemfibrozilo	1,71	2	4,8	4,50
	Ácido clofíbrico	-	95	2,9	-
Estimulante	Nicotina	-	10	1,2	6,16
	Paraxantina	-	70	-	-
	Cafeína	-	1	-0,07	14,0

a) [12]

b) Datos adquiridos del banco de datos "TOXNET-Hazardous substance data Bank"

c) K_{ow}: coeficiente de partición octanol/agua

La clasificación de los compuestos farmacéuticos se establece generalmente en función de la clase terapéutica y, aquellas categorías que tienen una mayor preocupación medioambiental debido a su potencial

efecto en los ecosistemas se clasifican en: antiinflamatorios no esteroideos, analgésicos, antibióticos, betabloqueantes, antiepilépticos, reguladores lipídicos, antidepresivos, hormonas y antihistamínicos [148].

Para su estudio, en esta Tesis Doctoral han sido escogidos veintitrés compuestos farmacéuticos pertenecientes a ocho clases terapéuticas distintas y cuyo consumo en España, porcentaje de excreción y características físico-químicas, las cuales son importantes para evaluar su comportamiento, se especifican en la Tabla I.4. Además, las diferentes clases terapéuticas a las que pertenecen los fármacos seleccionados se describen a continuación:

- Antibióticos

Los antibióticos son fármacos ampliamente recetados en todo el mundo. En esta categoría pueden aparecer medicamentos tanto de uso humano como animal. La demanda de estos medicamentos ha aumentado debido a su éxito contra patógenos en seres vivos y su uso como promotores del crecimiento en la agricultura y acuicultura [156]. Incluso en los años sesenta y setenta habían reducido las complicaciones y mortalidad asociadas a enfermedades infecciosas a unos niveles insospechados [188].

En España, el consumo medio de antibióticos, sólo teniendo en cuenta el ámbito extrahospitalario, ha sido de 20.15 DHD (DDD/1000 hab./día) y en los últimos años se ha observado una estabilización en su uso, siendo los grupos de antibióticos más demandados las penicilinas, quinolonas, macrólidos y cefalosporinas [12].

Este tipo de compuesto es ampliamente consumido en los hospitales, incluso, se dice que es una de las categorías con las descargadas más altas procedentes de estas infraestructuras. Sin embargo, el uso inadecuado de este grupo terapéutico ha facilitado la aparición de organismos resistentes, por ello, tiene bastante interés a nivel medioambiental, tal y como se ha planteado en una sección anteriormente descrita [88].

Entre ellos, los compuestos seleccionados han sido: ciprofloxacino, eritromicina, metronidazol, ofloxacino, sulfametoxazol y trimetoprim.

- Antihipertensivos

La hipertensión es uno de los problemas de salud pública más importantes. Se estima que uno de cada tres adultos en todo el mundo están afectados por esta patología y el 13 % de mortalidad está asociado con la presión arterial [88]. Su importancia es cada vez mayor debido al aumento de la esperanza de vida y a la alta prevalencia de factores contribuyentes, tales como la obesidad, el sedentarismo y el estrés.

Los fármacos betabloqueantes trabajan en el corazón y el sistema circulatorio para reducir la hipertensión, aliviar el dolor en el pecho y ayudar a prevenir los ataques al corazón. El porcentaje de excreción de estos compuestos en su forma activa puede llegar a superar el 70 %, por ello su importancia medioambiental. Se trata de una de las clases terapéuticas más frecuentemente detectadas en el medio y representa el 12 % del total de datos recabados sobre presencia de fármacos [189]. Además, se ha demostrado que tienen efectos toxicológico, por ejemplo,

el propanolol produce efectos negativos en el zooplancton y organismos bentónicos [88]. Incluso, algún autor ha señalado que los betabloqueantes pertenecen a la clase de compuestos disruptores endocrinos, ya que pueden alterar los niveles de testosterona en los organismos masculinos [189].

Desde el año 1992 hasta el año 2006, el uso de hipertensivos en España se ha triplicado, pasando de 80,8 a 233 DHD. El consumo del grupo de los betabloqueantes, donde están incluidos los compuestos seleccionados de esta categoría (atenolol y propanolol), supone un 8,4% del total, donde destaca el atenolol con 7,63 DHD en 2006 [12].

- Antiulcerosos

Los antiulcerosos se utilizan en el tratamiento farmacológico de las enfermedades acidopépticas o la profilaxis de los trastornos digestivos relacionados con la secreción ácida en el estómago, en definitiva, estos fármacos actúan a través de diferentes mecanismos para reducir la acidez gástrica. En este grupo se encuentran los compuestos tales como el omeprazol y la ranitidina.

En España, el consumo de antiulcerosos ha pasado de 33,3 a 137 DHD entre los años 2000 y 2012, lo que supone un incremento de 310 % en doce años. Dentro de los diferentes grupos de antiulcerosos que existen, el omeprazol es el fármaco más utilizado, cuyo demanda se ha quintuplicado en esos doce años y representa el 76,1 % del consumo total de antiulcerosos. Sin embargo, el uso de la ranitidina ha disminuido hasta la mitad.

Dado los altos valores de consumo que presenta el omeprazol dentro de este grupo de compuestos, cabe esperar su presencia en el medioambiente en grandes cantidades, sin embargo, la detección en aguas depuradas y superficiales es muy escasa, pudiendo deberse a una alta degradación [190]. Es por ello que es interesante estudiarlo y tomarlo como punto de control. En cambio, la ranitidina es uno de los compuestos de interés debido a su presencia en diferentes sistemas acuáticos, sin embargo, estudios ecotoxicológicos no muestran riesgo alguno.

- Antidepresivos

Los antidepresivos, como la fluoxetina, ejercen efectos terapéuticos inhibiendo los transportadores de monoaminas y, de este modo, actúan sobre la recaptación de los neurotransmisores, por tanto, produce un aumento de concentración de serotonina. Terapéuticamente, es beneficioso para aquellos que sufren trastornos depresivos o de ansiedad, desórdenes de la conducta alimentaria y alteraciones del control de los impulsos [191].

Las pautas para el tratamiento farmacológico de la depresión varían de un país a otro y, también, hay una gran variación en las actuaciones de prescripción entre los médicos generales y los psiquiatras. Las altas dosis y la duración de los tratamientos son algunos de los factores que contribuyen al aumento general del consumo de antidepresivos. En algunos casos, el aumento en el uso de antidepresivos también podría estar relacionado con la ansiedad creada por la crisis económica [88].

En España, el consumo de antidepresivos ha aumentado un 200 % en trece años, pasando de un 26,5 a un 79,5 DHD (periodo desde el año 2000 hasta el 2013). De manera general, se ha observado una tendencia creciente del consumo de todos los principios activos, aunque existe la moderación del uso de algunos antidepresivos en el año 2005, produciendo una disminución para diferentes principios activos. Esto puede tener relación con las recomendaciones que se hicieron en ese periodo sobre el uso de algunos fármacos en niños y adolescentes. En la actualidad, sólo la fluoxetina cuenta con autorización para su uso en niños y adolescentes con depresión moderada.

Se han realizado estudios en los que la presencia de fluoxetina ha producido una disminución de la respuesta agresiva de los peces ante la amenaza de otro predador o reduce la capacidad para capturar presas, entre muchos otros efectos. Sin embargo, las concentraciones a las que se producían estos efectos están muy lejos de los valores encontrados en el medioambiente [191]. A pesar de ello, no deja de ser uno de los grupos de compuestos que deben seguir siendo controlados y evaluados por la comunidad científica.

- Antiepilépticos

Los fármacos antiepilépticos previenen la estimulación rápida y repetitiva del cerebro que provoca la actividad convulsiva tal como en la epilepsia. Estos se utilizan para la prevención de convulsiones y complicaciones asociadas [192]. En nuestro caso, el compuesto seleccionado es la carbamazepina, aunque este compuesto también podría incluirse en la clase terapéutica anterior, los antidepresivos, dada

sus diversas aplicaciones en desórdenes psiquiátricos y alcoholismo crónico.

La epilepsia afecta a menos del 1 % de la población española. De esta manera, el consumo de antiepilépticos se ha duplicado, desde 5,11 a 10,8 DHD, en el periodo comprendido desde 1992 hasta 2006. La oferta de los principios activos también ha aumentado, existiendo una mayor variedad de ellos. Sin embargo, la carbamazepina, uno de los antiepilépticos más usados, ha mantenido su nivel de consumo.

La carbamazepina tiene una baja biodegradabilidad, por lo tanto es difícil de eliminar en las estaciones depuradoras y, como resultado, existe una alta persistencia, tanto en el medio acuoso como en el sólido, debido a su capacidad de adsorción, revelando también su capacidad de bioacumularse. Su alta persistencia la muestra debido a que se trata de uno de los compuestos más frecuentemente detectados y en unos niveles altos de concentración [193]. En cuanto a sus efectos toxicológicos, una mayoría de estudios de toxicidad aguda a partir de concentraciones de ensayo han deducido que la carbamazepina es proclive a producir daños en los organismos, por lo tanto, la presencia de este compuesto en el medio representa una amenaza real [194].

- Antiinflamatorio y analgésicos

Este tipo de fármacos es usado para aliviar el dolor y disminuir la inflamación presente en la mayoría de enfermedades, por ello, son extensamente usados en todo el mundo [91]. Además, se trata de un grupo de compuestos que puede ser adquirido sin preinscripción médica, con lo que conduce a un mayor consumo, estimándose en cientos de

toneladas anuales en los países desarrollados [195]. En esta categoría, aunque se haya denominado como un conjunto, se pueden separar los antiinflamatorios no esteroideos (NSAID, por sus siglas en inglés), tales como ketoprofeno, naproxeno, ibuprofeno y diclofenaco, y analgésicos como el metamizol.

El ibuprofeno es el analgésico más popular suponiendo el 43,9% del uso del total de NSAIDs, empleado por millones de personas al día como remedio para el dolor de cabeza, reducir síntomas de fiebre o dolores óseos y articulares crónicos. En segundo lugar en la escala de consumo se encuentra el diclofenaco, que tiene propiedades antiinflamatorias analgésicas y antipiréticas. En cuanto al ketoprofeno es usado para tratar los dolores como la artritis, al igual que el naproxeno, que también es usado para aliviar el dolor y para tratar la inflamación provocada por la artritis y tendinitis, entre otros. Por último, el metamizol es un anlagésico, antipirético y espasmolítico muy potente.

Atendiendo particularmente al grupo de los analgésicos, en España el consumo ha aumentado en un 75 % desde 1992 hasta 2006, pasando de un consumo de 5,75 a 18,7 DHD. A pesar de la alta demanda de analgésicos, el uso de los antiinflamatorios no esteroideos es aún mayor, dada la prevalencia de cuadros clínicos que pueden ser tratados con estos fármacos. En este caso, el consumo en 2012 se estableció en 49,0 DHD con un aumento del 26,5 % desde el año 2000, sin embargo, se ha visto una tendencia a la disminución de su consumo a partir del año 2009 [12].

Esta clase de compuestos se han encontrado en los diferentes sistemas acuáticos superando el nivel de los $\mu g \cdot L^{-1}$, rango de concentración en el cual podría causar toxicidad crónica en los organismos

acuáticos o efectos indeseados de salud en humanos tras una exposición prolongada [195]. Se ha demostrado, por ejemplo, que los residuos de diclofenaco tienen la posibilidad de afectar negativamente a varios tejidos de la trucha marrón en concentraciones cercanas a las encontradas regularmente en las aguas superficiales [88].

- Reguladores lipídicos

Una de las clases de fármacos más comúnmente detectadas en los medios acuáticos son los reguladores lipídicos, particularmente los fármacos de la familia de los fibratos, como el bezafibrato, gemfibrozilo o ácido clofíbrico (metabolito del clofibrato), compuestos que han sido seleccionados en nuestro caso [191].

Los reguladores lipídicos, conocidos como los hipolipemiantes, son un grupo de fármacos que, por diferentes mecanismos, mejoran el perfil lipídico disminuyendo el riesgo de sufrir un evento cardiovascular en pacientes con elevado riesgo de padecer esta patología. Según la Organización Mundial de la Salud (OMS), en 2008 las enfermedades cardiovasculares fueron la primera causa de muerte precoz en el mundo y se espera un progresivo incremento de su incidencia durante las próximas décadas debido al incremento de las tasas de obesidad y diabetes. En España, el consumo de hipolipemiantes ha incrementado desde 18,9 hasta 102 DHD entre los años 2000 y 2012, lo que supone un 442 % de aumento.

Aunque la familia de los fibratos ha experimentado una disminución en España, de entre un 95-99 %, e incluso algunos compuestos como el clofibrato, de donde proviene el ácido clofíbrico que es el metabolito activo excretado, ha dejado de ser comercializado, el estudio de estos

compuestos es interesante debido a que se han encontrado trazas de estos contaminantes en agua potable. Además, evaluaciones sobre sus efectos nocivos han demostrado que el ácido clofíbrico perjudica al sistema reproductivo mediante diferentes factores que incluyen un recuento de esperma reducido, espermatogénesis alterada y concentración de andrógenos disminuida. De manera similar, la exposición a gemfibrozilo reduce la testosterona en más del 50 % en los testículos de peces dorados [191].

- Estimulantes

Los compuestos estimulantes del sistema nervioso central son sustancias que aumentan la excitabilidad en diversas regiones del cerebro o médula espinal. Entre ellos, se encuentran la cafeína, la paraxantina (metabolito principal de la cafeína) y la nicotina. Los principales efectos producidos por el consumo de estos fármacos es un aumento de la función motora, disminución de la fatiga, mejora de la capacidad de concentración, incremento de energía y motivación y favorece el estado anímico [196].

La cafeína es un alcaloide de la xantina que se encuentra en el té, el cacao, el chocolate, las bebidas energéticas y otros alimentos derivados. Sin embargo, el café tiene el contenido más alto de cafeína con respecto a otros productos dietéticos y, según el Instituto Nacional de Salud Pública, el café es una de las bebidas más consumidas en todo el mundo. Además, está presente en un gran número de prescripciones debido a sus propiedades diuréticas y beneficios asociados con mejoras en el estado de alerta, la capacidad de aprendizaje y el rendimiento del ejercicio. Un 80 %

de este compuesto es metabolizado como paraxantina después de su ingesta. Por otro lado, la nicotina es un alcaloide que está presente principalmente en el tabaco [197].

Debido al consumo mundial de café y tabaco, o alimentos y bebidas que contienen estos estimulantes, tanto los compuestos activos como sus metabolitos se introducen continuamente en el medio acuático, pudiendo servir de indicadores para los científicos sobre el destino de los contaminantes. Aunque no se han apreciado efectos letales en los organismos, se pueden producir efectos sinérgicos con otros compuestos farmacéuticos, debido a las altas concentraciones presentes en el medio, lo que conllevaría a riesgos potenciales de forma más intensa [198].

I.3. Metodologías analíticas

Uno de los principales enfoques de la comunidad científica se centra en dar a conocer la importancia de determinar y cuantificar las concentraciones de residuos farmacéuticos en las diferentes matrices ambientales. Esto es debido a las implicaciones que está produciendo la presencia de residuos farmacéuticos en los diferentes compartimentos ambientales, especialmente en las muestras hidrológicas, dentro del ámbito de los riesgo toxicológicos e impacto ambiental y, por consiguiente, en el marco legislativo.

La evaluación, seguimiento y control de los contaminantes en estudio no sería posible sin la existencia de las metodologías analíticas avanzadas. Es casi impensable que hace tan sólo algunas décadas no se conocía de la presencia de los residuos farmacéuticos en muestras medioambientales, hasta que los sistemas de análisis y tratamiento de

muestras evolucionaron hasta alcanzar límites de detección en los niveles de concentración en los que estos contaminantes se encuentran.

Tabla I.5 Los doce principios de la química analítica verde por Galuszka y col. y su regla mnemotécnica [199]

Principio	Regla mnemotécnica	Expresión
Aplicar técnicas analíticas directas para evitar el tratamiento de muestras	**S**	**S**elect direct analytical technique
Integrar procesos y operaciones analíticas para ahorra energía y uso de reactivos	**I**	**I**ntegrate analytical processes and operations
Evitar la generación de un gran volumen de residuos analíticos y proporcionar una gestión adecuada de ellos	**G**	**G**enerate as little waste as possible and treat it properly
Minimizar uso de energía	**N**	**N**ever waste energy
Seleccionar métodos automatizados y miniaturizados	**I**	**I**mplement automation and miniaturization of methods
Preferencia por reactivos obtenidos a partir de fuentes renovables	**F**	**F**avor reagent obtained for renewable source
Aumentar la seguridad del operador	**I**	**I**ncrease safety for operator
Se deben realizar mediciones in situ	**C**	**C**arry out *in-situ* measurements
Evitar la derivatización	**A**	**A**void derivatization
Usar tamaño y número mínimo de muestras	**N**	**N**ote that the sample number and size should be minimal
Elegir métodos de múltiples analitos o múltiples parámetros frente a los métodos que utilizan uno	**C**	**C**hoose multi-analyte or multi-parameter method
Eliminar o reemplazar reactivos tóxicos	**E**	**E**liminate or replace toxic reagent

Con motivo de este rápido progreso experimentado por la química analítica actual, una de las principales tareas que se ha llevado a cabo es la de establecer guías claras y concisas sobre los principios de la química analítica verde, los cuales pretenden ir en concordancia con la propia

química verde y la ingeniería verde y sean útiles para las prácticas de laboratorio. De esta manera, en un trabajo realizado por Galuszka y col. se han establecido los doce principios de la química analítica verde que se han intentado describir en la Tabla I.5, junto a la regla mnemotécnica que se ha determinado mediante la palabra "SIGNIFICANCE" [199].

De forma general, los principales objetivos de la química analítica verde son obtener nuevas tecnologías analíticas o modificar un método antiguo para incorporar procedimientos que utilizan productos químicos menos peligrosos y/o en menor cantidad. En los últimos dos o tres años, se han incluido en las bases de datos diferentes artículos donde se recopilan los trabajos e ideas en esta línea de investigación [200–202].

Tal y como se ha visto en la Figura I.5 de la sección I.1.3, el hecho de tener unas metodologías de detección bien definidas es esencial para llegar a unos límites de detección y cuantificación necesarios para llevar a cabo una valoración y categorización de la sustancia a la que nos enfrentamos. Es muy probable que el problema que se plantea en esta Tesis Doctoral sobre la continua entrada, presencia y permanencia de los productos farmacéuticos en el medioambiente se presente como un panorama incompleto, dado que las metodologías actuales de detección no han abarcado los miles de fármacos que se encuentran en el mercado y, además, no existe una metodología estandarizada, por lo tanto, los límites de detección son muy variables [148].

Por ese motivo, numerosos trabajos son presentados año tras año sobre metodologías analíticas empleadas, de manera exclusiva, para el análisis de fármacos en muestras líquidas ambientales. Cada uno de las metodologías presentan sus particularidades, sin embargo, de manera

común el procedimiento llevado a cabo comienza desde una preparación de la muestra, mediante pretratamientos y procesos de extracción, hasta su determinación y cuantificación a través de técnicas de separación acoplados a sistemas de detección.

- Técnicas de separación

Para llevar a cabo un análisis para la determinación y detección de fármacos presentes en muestras líquidas ambientales, dado que los compuestos que se desean estudiar se encuentran en un mezcla dentro de una matriz compleja, se deben aplicar previamente procesos de separación, además de utilizar sistemas de detección adecuados para poder identificar y cuantificar cada uno de los compuestos.

El sistema de separación que se ha utilizado para los compuestos farmacéuticas es la cromatografía, tanto líquida (LC) [203] como gaseosa (GC) [204], además, también ha sido usada la electroforesis capilar (CE) [205], que a pesar de ser una técnica muy barata y menos compleja, no llega a alcanzar la sensibilidad de la cromatografía líquida o gaseosa.

La cromatografía de gases es usada, preferiblemente, para compuestos no-polares y volátiles. Sin embargo, mediante un proceso de derivatización se ha conseguido utilizar para la determinación de fármacos. La GC presenta una alta selectividad y resolución, buena exactitud y precisión, amplio rango dinámico y alta sensibilidad. Incluso recientemente, GC × GC se ha introducido en el análisis ambiental, proporcionando una mejor separación e identificación de los analitos en muestras complejas.

A pesar de ello, la derivatización es uno de los pasos que se deben evitar para ir en concordancia con la química analítica verde; por ello y por ser una técnica más rápida se prefiere la cromatografía líquida. Sin embargo, el principal inconveniente del análisis de fármacos por LC en muestras ambientales es el efecto matriz (el fenómeno de supresión iónica) que puede reducir la sensibilidad, linealidad, exactitud y precisión del método [206].

Para seguir con el desarrollo de métodos más amigables con el medioambiente, se han modificado las técnicas de cromatografía líquida para minimizar su impacto. Por ello, los avances han llegado a reducir los tiempos de análisis a partir de la modificación de las columnas cromatográficas y, por consiguiente, se ha hecho necesario la mejora de la instrumentación, lo que se conoce actualmente como la cromatografía líquida de ultra resolución (UHPLC) [200].

De forma adicional, para cualquiera de las técnicas cromatográficas, es necesario tener detectores sensibles acoplados, que permitan realizar una detección y cuantificación adecuada de los analitos de interés. Las técnicas de detección usadas van desde los sistemas ópticos como el diodo de Array (DAD) o fluorescencia (FD) [203,207] hasta la espectrometría de masas con sus diferentes sistemas de ionización (MS) [208].

- Técnicas de preparación y extracción

A pesar de saltarnos el primer principio de la química analítica verde, tal y como se describe en la Tabla I.5, los compuestos

farmacéuticos se presentan en muestras ambientales a unas concentraciones que no son capaces de ser detectadas directamente, incluso mediante los equipos más avanzados. Por ello, es necesario un proceso previo de preparación y preconcentración de la muestra.

Además, el efecto matriz en LC puede verse disminuido gracias a una extracción selectiva de analitos o a un proceso de limpieza adecuado [206].

El procedimiento de preparación de muestras es una de las partes más importantes del análisis de compuestos orgánicos en matrices ambientales. El primer paso en la preparación de la muestra, a parte de los procesos de conservación, mediante la acidificación de la muestra, es la filtración de un volumen apropiado para evitar ineficiencias de la extracción debido a la presencia de sólidos en suspensión. El tamaño de poro de los filtros a utilizar puede variar desde < 1 µm, por ejemplo a través de filtros de fibra de vidrio, hasta 0,20 µm. El uso de unos u otros dependerá de las necesidades, tanto de la técnica de extracción como de los sistemas de detección.

La siguiente etapa es la extracción de los productos farmacéuticos de la muestra en un pequeño volumen de disolvente. Esto puede realizarse mediante varias técnicas, siendo la más común la extracción en fase sólida (SPE) [209]. Otras técnicas de extracción que se han aplicado, siempre para ir en la dirección de la química analítica verde, incluyen técnicas de microextracción [210,211].

Centrándonos en la extracción en fase sólida, se trata de una técnica exhaustiva con altas cualidades en cuestión de reproducibilidad, aplicabilidad y facilidad de manejo, capaz de conseguir buena purificación

y concentración de muestras. Además, existe una gran variedad de adsorbentes que hace que la técnica pueda ser bastante variable a la vez que selectiva. Los polímeros de balance hidrófilo-lipófilos y las fases de base de sílice, con una fuerte hidrofobicidad, son los materiales más ampliamente utilizados para la preconcentración y extracción de compuestos farmacéuticos. Pero además, otros adsorbentes han sido también usados como las fases poliméricas de modo mixto de intercambio catiónico fuerte o resinas de poliestireno-divinilbenceno modificada con grupos carboxilo [206], incluyendo además, los polímeros impresos molecularmente (MIP), los cuales se usan para extraer grupos de compuestos con estructuras similares o características físico-químicas muy parecidas [212].

Un aspecto interesante de los procedimientos de SPE es su automatización, que puede mejorar la precisión y la velocidad de análisis. Este sistema automatizado puede permitir la inyección directa de muestras, conduciendo automáticamente los pasos propios de SPE, requiriendo menos tiempo y menores cantidades de disolvente, mejorando la reproducibilidad, reduciendo los límites de detección y disminuyendo los riesgos para la salud durante el análisis [206].

En el siguiente apartado de esta introducción se detallan las metodologías analíticas que se han empleado durante los últimos años para la determinación, en muestras de aguas depuradas, de compuestos farmacéuticos, incluyendo las técnicas de preparación, los sistemas de extracción/preconcentración y las técnicas de determinación. Además, se incluye una descripción de los procesos avanzados de oxidación que se han utilizados para su eliminación.

I.3.1. Herramientas analíticas empleadas para determinar compuestos farmacéuticos en aguas residuales después de la aplicación de un proceso avanzado de oxidación.

Como ya se ha comentado, una gran cantidad de diferentes sustancias químicas llegan al medioambiente después de pasar por las estaciones de tratamiento de aguas residuales sin ser eliminadas. Esto se debe a la ineficiencia de los procesos convencionales de eliminación y a la falta de regulaciones gubernamentales.

Diferentes procesos de oxidación avanzados, especialmente fotoquímicos, se han propuesto como complemento a los tratamientos tradicionales para la eliminación de contaminantes, mostrando ventajas significativas sobre el uso de métodos convencionales. Este trabajo tiene como objetivo revisar las metodologías analíticas empleadas para el análisis de compuestos farmacéuticos presentes en aguas residuales en estudios en los que se aplican procesos avanzados de oxidación.

A pesar de esta particularidad sobre los tratamientos avanzados de oxidación, que ha supuesto una forma de acotar la búsqueda, este trabajo describe las metodologías analíticas para la extracción, detección y cuantificación de compuestos farmacéuticos en muestras de aguas residuales en general, dado que no interviene tanto el sistema de tratamiento de depuración previo sino que depende del tipo de matriz y las características de los compuestos estudiados.

De esta manera, los procedimientos analíticos que se utilizan extensamente y de manera habitual en el análisis de productos farmacéuticos en muestras líquidas ambientales se basan en un proceso previo de extracción mediante la técnica de extracción en fase sólida

(SPE), seguidos de una detección y cuantificación usando la cromatografía líquida con diferentes sistemas de detección, donde la espectrometría de masas se lleva toda la atención por ser unos de los detectores más sensible, robusto y selectivo. Adicionalmente, se incluyen algunas particularidades en la preparación de la muestra debido a la interacción de los procesos avanzados de oxidación, como puede ser la inactivación del catalizador antes de llevar a cabo el procedimiento analítico.

La principal conclusión que se ha podido obtener de este trabajo es que el empleo de la metodología analítica apropiada es esencial para obtener una idea de la efectividad del tratamiento. Sin embargo, actualmente, la información proporcionada por muchos autores acerca de la metodología analítica utilizada es escasa. La estimación exacta de las eficiencias de eliminación está estrechamente ligada a una buena comprensión de las limitaciones analíticas relacionadas con las características particulares de los compuestos y, también, a las interferencias que podrían afectar la cuantificación o identificación de los analitos en estudio. Por lo tanto, se deben hacer esfuerzos para aclarar y ampliar los procedimientos de preparación y determinación elegidos.

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GLOBAL POLLUTION PROBLEMS, TRENDS IN DETECTION AND PROTECTION

Analytical tools employed to determine pharmaceutical compounds in wastewaters after application of advanced oxidation processes

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Abstract Today, the presence of contaminants in the environment is a topic of interest for society in general and for the scientific community in particular. A very large amount of different chemical substances reaches the environment after passing through wastewater treatment plants without being eliminated. This is due to the inefficiency of conventional removal processes and the lack of government regulations. The list of compounds entering treatment plants is gradually becoming longer and more varied because most of these compounds come from pharmaceuticals, hormones or personal care products, which are increasingly used by modern society. As a result of this increase in compound variety, to address these emerging pollutants, the development of new and more efficient removal technologies is needed. Different advanced oxidation processes (AOPs), especially photochemical AOPs, have been proposed as supplements to traditional treatments for the elimination of pollutants, showing significant advantages over the use of conventional methods alone. This work aims to review the analytical methodologies employed for the analysis of pharmaceutical compounds from wastewater in studies in which advanced oxidation processes are applied. Due to the low concentrations of these substances in wastewater, mass spectrometry detectors are usually chosen to meet the low detection limits and identification power required. Specifically, time-of-flight detectors are required to analyse the by-products.

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Keywords Advanced oxidation processes · Pharmaceutical compounds · Wastewater · Sample preparation · Determination methods

Abbreviations

AC	Activated carbon
AOPs	Advanced oxidation processes
API	Atmospheric pressure ionisation
CNF	Carbon nanofiber
E1	Estrone
E2	17-beta-estradiol
EAOPs	Electrochemical AOPs
EDCs	Endocrine disruptor compounds
EE2	17-alpha-ethinylestradiol
ESI	Electrospray ionisation
GC	Gas chromatography
HS	Head space
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
MTBSTFA	*N*-(*t*-butyldimetylsilyl)-*N*-methyltrifluoroacetamid
NSAIDs	Non-steroidal anti-inflammatory drugs
DAD	Diode array detector
POPs	Persistent organic pollutants
PPCPs	Pharmaceutical and personal care products
SBSE	Stir-bar sorptive extraction
SPE	Solid-phase extraction
TAP	Thermally activated persulfate
TOF	Time-of-flight
UHPLC	Ultra-high performance LC
US	Ultrasonic

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UV	Ultraviolet
UV-vis	UV-visible
WWTPs	Wastewater treatment plants

Introduction

The quality of the water supply is essential to maintaining the lifestyle of modern society. The increase in population, accumulation of people in large and industrialised cities and growing use of chemical substances in our ordinary life demand that we pay more attention to water purification and reuse. It is estimated that almost one billion people around the world do not have access to safe water resources and that 200 million people die every year because of infections caused by water (Amin et al. 2014). In addition to well-known persistent organic pollutants (POPs), over the last few decades, the scientific community has focused on so-called emerging contaminants. This group of compounds includes different families of analytes from sources such as pharmaceuticals, personal care products, hormones, detergents and flame retardants. These have not been studied in depth. Therefore, there is not enough information about their long-term consequences in the environment. Current wastewater treatment plants (WWTPs) are designed to control a wide range of substances, such as particulates, carbonaceous substances, nutrients and pathogens, but are not specifically designed to eliminate other pollutants. As a consequence, the emerging contaminants pass through the treatment processes without being eliminated and may end up in the aquatic environment via marine outfalls or sludge spreading on lands, threatening both wildlife and the drinking water industry (Bolong et al. 2009). The occurrence of emerging contaminants in the aquatic environment has frequently been associated with short-term and long-term toxicity, endocrine-disrupting effects, development of antibiotic resistance by micro-organisms (Fent et al. 2006), bioaccumulation and carcinogenicity (Trapido et al. 2014).

Specifically, the occurrence and fate of pharmaceutically active compounds in aquatic media have been recognised over the last decade as a serious environmental problem in most developed countries (Valavanidis et al. 2014). To date, there are no discharge guidelines, and only a few countries or regions have adopted regulations for a small number of compounds (Luo et al. 2014). The Directive 2013/39/EU promotes preventive action and the development of innovative treatment technologies and a watch list of substances has been established by the European Commission to be monitored according to the available information of matrices that should be investigated as well as the respective methods of analysis (Decision 2015/495, 20 March 2015). The watch list includes pharmaceutical compounds, such as the non-steroidal anti-inflammatory drug (NSAID) diclofenac, the synthetic hormone 17-alpha-ethinylestradiol (EE2), the natural hormones estrone (E1) and 17-beta-estradiol (E2) as well as and the macrolid antibiotics erythromycin, clarithromycin and azithromycin (Barbosa et al. 2016).

To improve the quality of wastewater before being discharged or reused, different purification methods have been applied. WWTPs generally employ a primary treatment (removal of suspended solids), a secondary treatment (removal of dissolved and suspended biological matter, typically performed by indigenous, water-borne micro-organisms in a managed habitat) (Ajobo and Abioye 2014) and an optional tertiary treatment, which are commonly used to produce higher quality discharged water for certain purposes, such as water reuse; however, these treatments are always associated with high cost (Luo et al. 2014). Secondary (activated sludge) or tertiary treatment processes (activated carbon, nanofiltration and reverse osmosis membrane) are often not effective at treating complex polluted waters containing pharmaceuticals, personal care products, surfactants or industrial additives (Amin et al. 2014) or at removing some recalcitrant compounds, such as the carcinogenic azo dyestuffs generated by the textile, paper, food, cosmetic and pharmaceutical industries (Thennarasu and Sivasamy 2015).

Because of these limitations, advanced treatment technologies have been proposed, with the most promising being membrane filtration and advanced oxidation processes, including several modifications with UV applications (H_2O_2/UV, ozone/UV, ozone/H_2O_2/UV, H_2O_2/Fe^{2+}/UV and TiO_2/UV). The membrane filtration process is very effective at solid-liquid separation and the removal of organic and inorganic materials. Its most important application is desalination by reverse osmosis, but microfiltration and ultrafiltration could be useful for the disinfection of resistant micro-organisms.

Advanced oxidation processes (AOPs) were defined in 1987 as water treatment technologies that are performed at room temperature and normal pressure and are based on the in situ generation of a powerful oxidising agent at a sufficient concentration to effectively decontaminate water (Glaze 1987). The ·OH radical is one of the strongest oxidising species, and it is able to accelerate the rates of contaminant oxidation. Usually, the combination of ozone (O_3), hydrogen peroxide (H_2O_2), titanium dioxide (TiO_2), UV radiation, ultrasound and/or high electron beam irradiation accelerates the generation of ·OH radicals. The main advantages of the implementation of AOPs over solo conventional treatment processes are as follows: (a) they have a higher effectiveness at removing resistant organic compounds, (b) they almost completely mineralise organic contaminants into carbon dioxide, (c) they only have a minor susceptibility to the presence of toxic chemicals, (d) they produce a minor amount of harmful by-products and (e) they have a better microbial disinfection (Zhou and Smith 2002).

Pharmaceuticals are commonly present at trace concentrations ranging from a few nanogrammes per litre to several

micrograms per litre, which makes their analysis difficult using conventional procedures and creates challenges for purification processes (Luo et al. 2014). The complexity of the matrix often implies the need to apply a previous treatment of the sample to purify and pre-concentrate it before analysis. To clean and pre-concentrate the sample, the most commonly used preparative technique is solid-phase extraction, while several mass spectrometry detectors with different ionisation sources are usually preferred for detection, taking into account the low concentration levels of the analytes. However, reviewing the literature of the analytical methodologies employed for the evaluation of AOPs is often difficult because authors typically pay more attention to the removal and clean-up technique, while they only briefly describe the analytical procedure.

In the last few years, different general reviews regarding AOPs applied to remove emerging pollutants have been published (Wols and Hofman-Caris 2012; Trapido et al. 2014; Oturan and Aaron 2014; Buthiyappan et al. 2015; Ribeiro et al. 2015; Sathishkumar et al. 2016). There have also been reviews devoted to describing AOP techniques applied to specific families of emerging compounds, such as gasoline additives (Levchuk et al. 2014), cytostatics (Zhang et al. 2013), alkylphenols (Priac et al. 2014), organic dyes (Martínez-Huitle and Brillas 2009; Brillas and Martínez-Huitle 2015) or pharmaceutical compounds (Feng et al. 2013; Rivera-Utrilla et al. 2013; Kanakaraju et al. 2014; Mohapatra et al. 2014).

Nevertheless, there are few publications that have focused on elimination procedures and not on the analytical methods used to evaluate them. For this reason, we review the recent analytical procedures, including determination and sample preparation, published between 2010 and 2015 that have been employed to test advanced oxidation processes for the removal of pharmaceutical compounds from wastewater samples.

Advanced oxidation processes

There are different categories and classifications of AOPs depending on the author. For example, we can distinguish between several processes based on the in situ formation of ·OH radicals by the means of chemical, photochemical, sonochemical or electrochemical reactions (Babuponnusami and Muthukumar 2014). In addition to the Fenton method, a chemical AOP in which a mixture of a soluble iron (II) salt and H_2O_2, known as Fenton's reagent, which is the oldest and most-used AOP, other photochemical, sonochemical and electrochemical processes are increasingly being developed because of their better performance (Oturan and Aaron 2014). Meanwhile, Fernández-Castro et al. (2015) grouped AOPs into the following categories: (i) Fenton processes that include conventional Fenton, Fenton-like and photo-Fenton processes; (ii) photolytic and photocatalytic systems; (iii) electrochemical technologies that take electro-oxidation, photo-electro-oxidation and photo-electrocatalytic processes, and electrical discharges into consideration; (iv) technologies based on ultrasound, such as sonolysis and sonocatalysis, and hydrodynamic cavitation; and (v) γ-radiolysis and heavy ions.

The most widely discussed AOPs for wastewater treatment are ultraviolet (UV), H_2O_2/UV, ozone/UV, ozone/H_2O_2, ozone/H_2O_2/UV, photocatalytic oxidation, Fenton and photo-Fenton reactions. It seems that the combination of the Fenton reaction with UV radiation results in better degradation of organic contaminants compared with the typical Fenton reaction (Buthiyappan et al. 2015). Adopting the classification of Babuponnusami and Muthukumar (2014), Fig. 1 shows the most representative AOPs, which will be described in the following sections.

Chemical AOPs

The Fenton method has been applied to the oxidation and degradation of organic pollutants as early as the mid-1960s. This oxidation in the presence of ferrous or ferric ions with hydrogen peroxide is a very simple and flexible method that produces hydroxyl radicals without any special reactants or apparatus. Iron is a non-toxic, relatively inexpensive and very abundant element, while hydrogen peroxide is easy to handle and environmentally safe (Mohapatra et al. 2014). Moreover, this procedure has no need for energy input, but has some disadvantages. Its efficiency depends on various factors (temperature, pH, H_2O_2 and catalyst concentrations), and the accumulation of iron sludge must be removed at the end of the treatment (Oturan and Aaron 2014).

Other types of chemical AOPs are ozonation and peroxonation. Ozonation is a widely employed and investigated technique because it is known to be highly effective. It is commonly used as a disinfecting agent in WWTPs. However, the combination of ozonation with hydrogen peroxide, known as peroxonation (O_3/H_2O_2), is especially convenient because it improves the degradation of many organic pollutants. Unlike H_2O_2, which reacts very slowly with the ozone molecule in aqueous solution, its conjugate base (HO_2^-) can rapidly react with molecular ozone to generate hydroxyl radicals (Klavarioti et al. 2009).

In general, this combined oxidation process usually has a higher reaction efficiency than an individual oxidation process because of the enhanced generation of hydroxyl radicals (Mohapatra et al. 2014).

Photochemical AOPs

Photochemical approaches appear to overcome some of the limitations of existing chemical AOPs, as they are generally

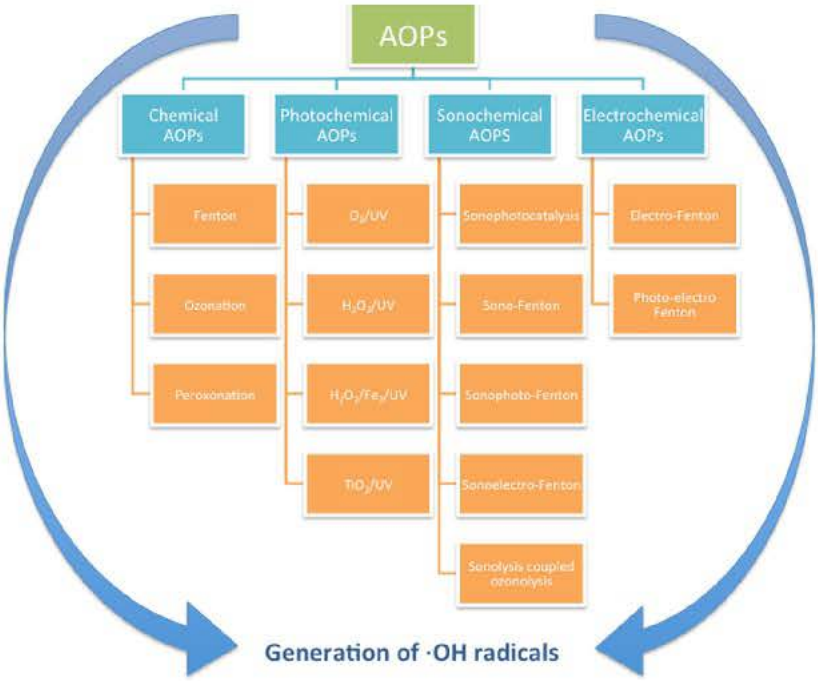

Fig. 1 Classification of the most commonly employed AOPs

simpler, cleaner, relatively cheaper (dependent upon the use of radiation) and are also more efficient because their combination with light irradiation enhances the generation of hydroxyl radicals (Huber et al. 2003). Moreover, photochemical approaches are an efficient and sustainable alternative for the degradation of recalcitrant contaminants compared with the use of UV alone (Buthiyappan et al., 2015). The most used photochemical AOPs are O_3 photolysis (O_3/UV), H_2O_2 photolysis (H_2O_2/UV), the photo-Fenton process (H_2O_2/Fe^{2+}/UV) and heterogeneous photocatalysis (TiO_2/UV). H_2O_2, unlike ozone, has low molar absorption in the wavelength range of 200 to 300 nm. The Fenton process can also be improved by irradiation at wavelengths greater than 300 nm, accelerating the degradation of organic pollutants. In addition, it has been recently demonstrated that the UV-vis/ferrioxalate/H_2O_2 combination is more efficient than the photo-Fenton reaction for the degradation of organic pollutants because the irradiation of ferrioxalate in acidic solution generates carbon dioxide and ferrous ions (Fe^{2+}), either free or combined with oxalate, which in combination with H_2O_2 provides a continuous source of Fenton's reagent (Oturan and Aaron 2014). Heterogeneous photocatalysis (most often, TiO_2/UV) is a promising technology. However, there are very few real applications for this technology, despite its effectiveness in the partial or full mineralisation of recalcitrant pollutants. Heterogeneous photocatalysis consists of the catalysis of photochemical reactions on the surface of a catalyst, usually a semiconductor and involves simultaneous oxidation and reduction reactions. These reactions occur through oxidation–reduction processes, generating HO· radicals by water dissociation (da Silva et al. 2015). Titanium dioxide (TiO_2) is the most frequently used photo-catalyst because it is inexpensive, non-toxic, and chemically resistant (Badawy et al. 2014).

Sonochemical AOPs

Sonolysis is considered to be a safe, clean and versatile technique (Nejumal et al. 2014). There are several combinations of AOPs that use the sonolysis technique, such as sonophotocatalysis, the sono-Fenton technique, the sonophoto-Fenton technique, the sonoelectro-Fenton technique or sonolysis coupled with ozonolysis (Sathishkumar et al. 2016). These techniques stand out from other AOPs that require intensive chemical and energy inputs for acceptable removal efficiencies. Moreover, ultrasound waves have the ability to be perfectly transmitted through opaque systems, unlike those of ultraviolet light (Ince et al. 2001). One drawback of ultrasonic systems is that they are extremely sensitive and vulnerable to operational parameters, which cannot be controlled without good knowledge and understanding of the physical and chemical phenomena involved (Ince et al. 2001).

Recently, the combination of ultrasound with the Fenton reaction has been developed, resulting in a very promising approach for decontamination purposes. However, for its application at the industrial level in real-time wastewater treatment plants, it is still necessary to demonstrate its economic and commercial feasibility because most experimental

workups until now have been performed at the laboratory scale using artificial systems (Oturan and Aaron 2014).

Electrochemical AOPs

Electrochemical technologies for the elimination of organic contaminants from wastewater show advantages, such as high energy efficiency, amenability to automation, ease of use (simple equipment), safety (mild conditions) and versatility. Among these, electrochemical advanced oxidation processes (EAOPs) have received great attention, and combined methods with fewer harmful effects (often referred to as process-integrated environmental protection) have been developed (Brillas and Martínez-Huitle 2015). This type of AOP generates ·OH radicals by applying a potential or current density to an electrochemical cell containing one or more pairs of electrodes instead of using chemical reagents (da Silva et al. 2015). Pollutants are adsorbed on the anode surface and are then destroyed through anodic electron exchange (direct oxidation) or are degraded in the bulk liquid with the mediation of the electroactive species, which act as intermediaries for the transfer of electrons between the electrode and organic compounds (indirect oxidation) (Homem and Santos 2011). The electro-Fenton process, which requires a lower Fe^{2+} concentration than the conventional Fenton process, is among the most eco-friendly electrochemical AOPs. Basically, it is an electrically assisted Fenton process. Moreover, the efficiency of the electro-Fenton process can be increased by applying UV radiation, and this particular process is called the photo-electro-Fenton process (Ribeiro et al. 2015).

Analytical methodologies

Over the last 6 years, there have been an increasing number of publications related to the removal of emerging pollutants, particularly pharmaceuticals, from wastewater samples using AOPs. Figure 2 shows the ratio of studies containing the keywords "pharmaceuticals," "advanced oxidation process (AOPs)" and "wastewater or sewage" in the title or abstract, as determined from the Scopus database. The reviews that have been published have generally focused on describing the varieties of techniques used to degrade these groups of compounds (Ikehata et al. 2006; Esplugas et al. 2007; Ikehata et al. 2008), and no attention has been paid to explaining the correct use of the analytical methodologies.

For this reason, in the following sections, we will describe the analytical procedures that have been employed by authors to probe the validity of their advanced oxidation processes applied to degrade pharmaceutical compounds in wastewater samples. Table 1 summarises the publications in the selected time period (2010–2015), which are classified by the type of AOP, target pharmaceutical compounds and different steps of

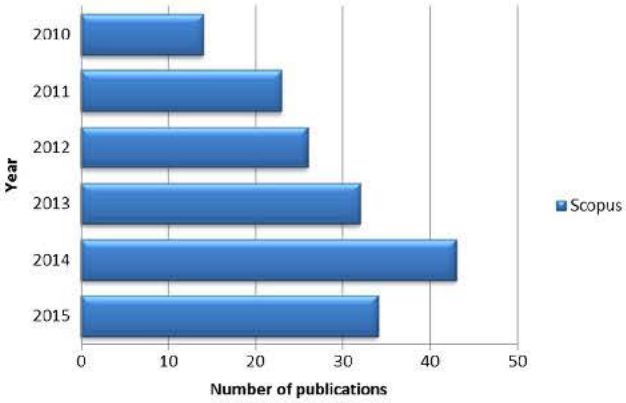

Fig. 2 Number of publications per year from 2010 to 2015 from the Scopus database

the analytical methodology. These methodologies include both sample preparation and determination procedures. The detection and quantification systems are used with a greater or lesser degree of sensitivity depending on the amount and concentration of contaminants as well as the type of sample that requires analysis. However, in many cases, sample preparation is necessary after applying AOPs and before the determination procedure, either because of the low concentration or to stop the oxidative activity.

Regarding the origin of the employed samples in these works, most do not use real water from WWTPs to validate their procedures; instead, they use artificially prepared samples. Generally, we found lab-scale experiments (Trovó et al. 2011; Razavi et al. 2011; Palo et al. 2012). Pilot-scale (Gerrity et al. 2010; Álvarez et al. 2011; Köhler et al. 2012) and full-scale experiments (Reungoat et al. 2010; Abdelmelek et al. 2011) are less frequently employed. For example, Badawy et al. (2014) employed a simulated hospital wastewater sample prepared by mixing five pharmaceutical compounds, while Espejo et al. took wastewater from the first sedimentation unit (primary effluent) of a WWTP that were then were spiked with nine selected pharmaceuticals (Espejo et al. 2014a, b). Hey et al. (2014) and Romero et al. (2014) used real water to validate their optimised methodologies, collecting samples from four municipal WWTPs in Sweden and the secondary clarifier of a wastewater treatment plant (WWTP) from Spain, respectively. Miralles-Cuevas et al. (2014a) dissolved the target compounds in effluent wastewater from the secondary biological treatment supplied at the municipal WWTP for their pilot-scale experiments. James et al. (2014) also carried out micropollutant removal experiments using a pilot plant that treated 600 m^3 day^{-1} of final effluent from a WWTP with a conventional activated sludge process. More complete studies provide results obtained by both pilot and full-scale experiments. For example, Gerrity et al. (2012) used eight wastewaters to evaluate the ability of pilot- and full-scale systems to oxidise 18 organic contaminants, mainly pharmaceutical compounds.

Table 1 The overview of AOPs and the analytical methodologies used for pharmaceutical compounds in aqueous samples from 2010 to 2015

AOPs	Compounds	Sample preparation	Determination	LOD/LOQ	Ref.
Electrochemical: electro-Fenton	Caffeine	LLE, derivatisation	LC-DAD, GC-MS (by-products)		Ganzenko et al. (2015)
Chemical: ozonation Photochemical: photolysis, photolytic ozonation, photocatalysis, photocatalytic ozonation	Ibuprofen, naproxen, tramadol, azithromycin, clarithromycin, erythromycin, sulfamethoxazole, trimethoprim, fluticasone propionate, montelukast, warfarin, clopidogrel, metoprolol, propranolol, hydrochlorothiazide, atorvastatin, bezafibrate, simvastatin, carbamazepine, citalopram, fluoxetine, norfluoxetine, venlafaxine, diphenhydramine, E2, EE2, E1, clofibric acid	SPE	LC-DAD, UHPLC-MS/MS		Moreira et al. (2015)
Chemical: thermally activated persulfate (TAP)	Naproxen	SPE: recovery 98 %	LC-DAD-MS-ESI Ion trap LC-DAD-MS-ESI Ion trap or GC-MS Ion trap (by-products)		Ghauch et al. (2015)
Sono-electrochemical	Ibuprofen		Spectrophotometry, LC-MS/MS (by-products)		Tran et al. (2015)
Photochemical: gamma-irradiation/ozonation	Paracetamol	SPE, derivatisation	GC-MS Ion trap		Torun et al. (2015)
Photochemical: UV/H_2O_2 Chemical: ozonation	Naproxen, trimethoprim, ketoprofen, sulfamethoxazole, diclofenac, clarithromycin, gemfibrozil, carbamazepine, diazepam, lorazepam, atenolol	Catalase enzyme, SPE	LC-MS/MS-ESI	LOD: 5 ng L^{-1}	Justo et al. (2015)
Photochemical: UV/H_2O_2	Diclofenac, fluoxetine, iohexal, iopamidol, iopromide, simazine, sulfamethoxazole, ibuprofen, naproxen, atenolol, carbamazepine, gemfibrozil, primidone, trimethoprim, clofibric acid, ditiazem.	SPE: recovery, 61.2–145.1 %	LC-MS/MS	LOD, 0.1–13.1 ng L^{-1}	Yu et al. (2015)
Photochemical: photolysis, photocatalysis	Carbamazepine		Spectrophotometer, LC-MS/MS-ESI Ion Trap (by-products)	LOD, 0.2 μg L^{-1}	Carabin et (al. 2015)
Photochemical: UV/$TiSiO_4$ (titanium silicone oxide), UV/H_2O_2/O_2, UV/H_2O_2/$TiSiO_4$	Balsalazide		Spectrophotometer		Sikarwar and Jain (2015)
Chemical: ferrous ion-activated persulfate; peroxide-activated persulfate; base-activated persulfate	Levofloxacin		LC-DAD		Epold and Dulova (2015)
Photochemical: UV/H_2O_2	Atenolol, bezafibrate, carbamazepine, clofibric acid, cyclophosphamide, diatiazoic acid, diclofenac, erythromycin, fluoxetine, furosemide, gemfibrozil, ifosfamide, ketoprofen, metoprolol, metronidazole, naproxen, paroxentine, phenazone, prednisolone, propranolol, sotalol,	Filtered	UHPLC-MS/MS	LOD, 0.01–0.025 μg L^{-1}	Wols et al. (2015)

Table 1 (continued)

AOPs	Compounds	Sample preparation	Determination	LOD/LOQ	Ref.
	sulfachloropyridine, sulfadiazine, sulfamethoxazole, sulfaquinoxolin, trimethoprim, venlafaxine				
Chemical: ozonation, O_3/H_2O_2 Photochemical: UV/O_3, UV/O_3/H_2O_2	Berberine hydrochloride		Spectrophotometer, CG-MS spectra (degradation process)		Qin et al. (2015)
Sonochemical: Fenton/US, sonolysis Chemical: Fenton	Amantadine	Filtered, SPE	GC-MS ion trap		Zeng et al. (2015)
Sonophotochemical: US/UV/H_2O_2	Salicylic acid, chloramphenicol, paracetamol, diclofenac		Spectrophotometer		Ghafoori et al. (2015)
Sonochemical: Fenton/US, sonolysis, US/Fenton/TiO_2, US/TiO_2, US/CCl_4 Chemical: Fenton	Acetaminophen, naproxen	Filtered	LC-DAD		Im et al. (2015)
Photochemical: UV/H_2O_2	Iopromide, iopamidol	Filtered	LC-MS-ESI-Q-TOF		Singh et al. (2015)
Electrochemical: anodic oxidation with a boron-doped diamond (BDD) anode	Sulfadiazone, hydrochlorothiazide, trimethoprim, ranitidine, ibuprofen, norfloxacin, lincomycin, sertraline, gemfibrozil, acetaminophen, roxithromycin, tramadol, metoprolol, citalopram, diatriazole, diclofenac, carbamazepine, phenytoin, caffeine, enrofloxacin, venlafaxine, iopromide	SPE	UHPLC-QTrap-MS-ESI	LOQ, 1.5–74.3 ng L^{-1}	Garcia-Segura et al. (2015)
Chemical: cobalt (II) activation of oxone	Caffeine		LC-DAD		Yunleiyu Guo et al. (2015)
Photochemical: heterogeneous photocatalysis (Ag/TiO_2)	Chloramphenicol, paracetamol, salycylic acid, sulfamethoxazole, diclofenac		LC		Badawy et al. (2014)
Photochemical: UV/H_2O_2, UV/H_2O_2/TiO_2	Metoprolol		LC-DAD, LC-ESI-MS, and LC-MS-TOF (by-products)		Romero et al. (2014)
Sonochemical: sonolysis	Atenolol		LC-DAD, LC-ESI-Q-TOF (by- products)		Nejumal et al. (2014)
Photochemical: nanofiltration + solar photo-Fenton	Carbamazepine, flumequine, ibuprofen, ofloxacin, sulfamethoxazole	SPE: recovery 80 %	UHPLC-DAD	LOD, 0.06–2 μg L^{-1}	Miralles-Cuevas et al. (2014b)
Photochemical: UV/H_2O_2	E1, E2, EE2		LC-MS/MS		James et al. (2014)
Chemical: ozonation (O_3/H_2O_2)	Clomipramine, sulfamethoxazole repaglinide, EE2, fexofenadine, codeine, naproxen, diltiazem, eprosartan, atracurium, carbamazepine, trimethoprim, rosuvastatin, hydroxyzine, orphenadrine, cilazapril, haloperidol, beclomethasone, diclofenac, citalopram, tramadol, irbesartan, risperidone, sertraline, bisoprolol, metoprolol, venlafaxine,	SPE: recovery 25.2–129 %	LC-MS/MS	LOQ, 0.1–10 ng L^{-1}	Hey et al. (2014)

Table 1 (continued)

AOPs	Compounds	Sample preparation	Determination	LOD/LOQ	Ref.
	biperiden, maprotiline, amitriptyline, fluoxetine, bupropion, oxazepam, levonorgestrel, memantine, fluconazole, flutamide, ketoprofen, ibuprofen				
Photochemical: photocatalytic ozonation (Espejo et al. 2014b), O_3/UVA/Fe(III), O_3/UVA/Fe_3O_4 (Espejo et al. 2014a)	Acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hidrochlorothiazole, ketorolac, metoprolol, sulfamethozaxole		LC-DAD	LOD, 2 μg L^{-1}	Espejo et al. (2014b) Espejo et al. (2014a)
Photochemical: TiO_2/UVA, TiO_2/UVC, H_2O_2/UVC	Acetaminophen, caffeine, carbamazepine, cimetidine, propranolol, sulfamethoxazole		LC-DAD		Choi et al. (2014)
Photochemical: UV/H_2O_2 (OH·), Rose Bengal (RB)/H_2O_2 (O_2)	Ranitidine, cimetidine		Spectrophotometry		Brame et al. (2014)
Photochemical: photo-initiated, photo-induced (UV/H_2O_2)	Sulfamethazine		LC, LC-MS-ESI-TOF (by-products)	LOD, 0.170 mg L^{-1}	Batista et al. (2014)
Chemical: induced cavitation/H_2O_2	Clofibric acid, ibuprofen, naproxen, ketoprofen, carbamazepine, diclofenac	Filtered; SPE: recovery 81–95 %; derivatisation	GC-MS	LOD, 0.4–3.7 ng L^{-1}	Zupanc et al. (2014)
Electrochemical: electro-Fenton, electro-Fenton/UV	Salicylic acid	LLE, derivatisation	GC-HS-MS		George et al. (2014)
Photochemical: solar photo-Fenton	Carbamazepine, flumequine, ibuprofen, ofloxacin, sulfamethoxazole	SPE: recovery 90 %	LC-DAD	LOD, 0.06–2 μg L^{-1}	Miralles-Cuevas et al. (2014a)
Sonophotochemical: US/UV/H_2O_2	Salicylic acid, chloramphenicol, paracetamol, diclofenac		Spectrophotometer		Mowla et al. (2014)
Chemical: ozonation, O_3/H_2O_2	Carbamazepine, clarithromycin, diclofenac, furosemide, lidocaine, meferamic acid, ranitidine, sotalol, sulfapyridine, sulfamethoxazole, atenolol, metoprolol, tramadol, venlafaxine, bezafibrate, gabapentin, oxazepam, primidone, valsartan, fluconazole, iopromide, levertiracetam	Filtered, SPE	LC-MS/MS	LOQ, 1–7000 ng L^{-1}	Lee et al. (2014)
Photochemical: photolysis, photo-Fenton, UV/H_2O_2, UV/H_2O_2/Fenton	Ibuprofen, sulfamethoxazole, diclofenac		LC-DAD		Trapido et al. (2014)
Chemical: ozonation Electrochemical: electrolysis, electro-peroxone	Ibuprofen	SPE	LC-DAD, UHPLC-ESI-MS/MS, and UHPLC-Q-TOF-MS (ESI) (by-products)		Li et al. (2014)
Chemical: ozonation Photochemical: UV/O_3	Ketoprofen		LC-DAD-MS (ESI)	LOD, $2.5 \cdot 10^{-8}$ mol d-m^{-3}	Illés et al. (2014)
Sonochemical: sonolysis	Diclofenac, carbamazepine, amoxicillin		Spectrophotometer, LC-MS/MS (ESI)	LOQ, 1 ng L^{-1}	Secondes et al. (2014)
Photochemical: UV/H_2O_2	Atenolol, bezafibrate, carbamazepine, clenbuterol , clindamycin, clofibric acid, cortisol, cortisone, cyclophosphamide, diatrizoic acid,		UHPLC-MS/MS	LOD, 0.01–0.025 μg L^{-1}	Wols et al. (2013)

Table 1 (continued)

AOPs	Compounds	Sample preparation	Determination	LOD/LOQ	Ref.
	diclofenac, erythromycin, fluoxetine, furosemide, gemfibrozil, ifosfamide, ketoprofen, lincomycin, metformin, metoprolol, metronidazole, naproxen, niacin, paracetamol, paroxetine, penicillin, pentoxifylline, phenazone, pindolol, prednisolone, propranolol, salbutamol, sotalol, sulfachloropyridazine, sulfadiazine, sulfamethoxazole, sulfaquinoxalin, terbutaline, tramadol, trimethoprim, venlafaxine				
Photochemical: UV/H_2O_2	Naproxen, carbamazepine, diclofenac, gemfibrozil, ibuprofen, caffeine, 2,4-D, 2,4-DCP, mecoprop		LC-DAD		Shu et al. (2013)
Chemical: ozonation Sonochemical: O_3/US	Acetaminophen, 4-aminoantipyrine, atorvastatin, bezafibrate, ciprofloxacin, clarithromycin, clindamycin, diclofenac, enalapril, erythromycin, gemfibrozil, ibuprofen, ketoprofen, lincomycin, lorazepam, naproxen, ofloxacin, salicylic acid, sulfamethazine, sulfamethoxazole, venlafaxine, valsartan, irbesartan, furosemide, carbamazepine, gabapentin	SPE	UHPL-ESI-MS/MS	LOQ, 0.2–170 ng L^{-1}	Ibáñez et al., (2013)
Chemical: ozonation Photochemical: black-light /TiO_2, AC/TiO_2	Acetaminophen, norfloxacin, metoprolol, caffeine, antipyrine, sulfamethoxazole, ketorolac, hydroxybiphenyl, diclofenac		LC-DAD, LC-MS-ESI-TOF		Encinas et al. (2013)
Chemical: ozonation, ceramic honeycomb monoliths coated with carbon nanofibers (CNF)	Bezafibrate, erythromycin,		LC-DAD		Derrouiche et al. (2013)
Chemical: ozonation Photochemical: UV, UV/H_2O_2	Carbamazepine, ciprofloxacin, diclofenac, metoprolol, sulfamethoxazole	Filtered	LC-QTrap-MS		Eyser et al. (2013)
Photo-electrochemical: Ti/TiO_2	Carbamazepine		Spectrophotometer, LC-MS/MS ion trap (ESI; by-products)		Daghrir et al. (2013)
Photochemical: UV, UV/TiO_2	Ciprofloxacin		UPLC-MS/MS (ESI)		Vasquez et al. (2013)
Sonochemical	Acetaminophen, atenolol, atrazine, carbamazepine, diclofenac, metoprolol, caffeine, iopromide, erythromycin, fluoxetine, trimethoprim, propranolol, sulfamethoxazole, ibuprofen, naproxen, gemfibrozil, triclosan		Spectrophotometer, LC-QTrap-MS/MS (ESI)		Naddeo et al. (2013)
Chemical: ozonation	Fluoxetine, norfluoxetine, paraxantine, sertraline, citalopram, fluvoxamine, venlafaxine, amitriptyline, nortriptyline, carbamazepine	SPE	LC-MS/MS (ESI), LC-Q-TOF-MS/MS (ESI; by-products)		Lajeunesse et al. (2013)
Chemical: ozonation, O_3/H_2O_2	Acetylsalicylic acid, sulfadiazine, sulfamethoxazole, amoxicillin, atenolol, azithromycin, bendroflumethiazide, bezafibrate,	SPE	LC-MS/MS		Nielsen et al. (2013)

Table 1 (continued)

AOPs	Compounds	Sample preparation	Determination	LOD/LOQ	Ref.
	bisoprolol, capecitabine, carbamazepine, cefuroxime, chloramphenicol, ciprofloxacin, citalopram, clarithromycin, clindamycin, clofribic acid, cyclophosphamide, diclofenac, erythromycin, erythromycin, fenofibrat, fenofibrinsaure, furosemide, gemcitabine, ibuprofen, ifosfamide, ketoprofen, megastrol, metoprolol, metronidazol, naproxen, ofloxacin, oxcarbazepine, paracetamol, phenanzon, propranolol, roxithromycin, simvastatin, sotalol, sulfadiazine, sulfametazine, sulfamethizole, sulfamethoxazole, tamoxifen, tramadol, trimethoprim, venlafaxin, E1, E2, EE2, amidotrizoeacid, iohexol, iomeprol, iopamidol, iopromide, ioversol.				
Photochemical: photolysis, UV/H_2O_2, photo-Fenton Chemical: Fenton/H_2O_2	Amitriptyline hydrochloride, methyl salicylate, 2-phenoxyethanol		LC-DAD		Real et al. (2012)
Chemical: Fenton/H_2O_2	Triclosan	SBSE	LC-DAD, GC-MS-TOF (by-products)	LOD, 0.05–0.001 mg L^{-1}	Munoz et al. (2012)
Chemical: O_3/H_2O_2 Photochemical: UV/H_2O_2	Equilenin, fenoterol, tetracycline, triclosan, E2, methicillin, metformin, sulfamethoxazole. Gemfibrozil, clofibric acid, iomeprol		LC-DAD	LOD, 0.007–0.16 μg L^{-1}	Jin et al. (2012)
Photochemical: UV photolysis, UV/H_2O_2	Mestranol, progesterone, estrone, estriol, E2, EE2		UHPLC-DAD-FD	LOD, 50–100 μg L^{-1}	Pereira et al. (2012)
Chemical: ozonation	Phenytoin, atenolol, meprobamate, atrazine, naproxen, carbamazepine, primidone, diclofenac, sulfamethoxazole, gemfibrozil, triclosan, ibuprofen, trimethoprim	SPE: recovery 88–118 %	LC-QTrap-MS	LOD, 10–25 ng L^{-1}	Gerrity et al. (2012)
Photochemical: UV photolysis, UV-H_2O_2	Acetyl-sulfamethoxazole, ciprofloxacin, clarithromycin, erythromycin, sulfamethoxazole, diclofenac, lidocaine, naproxen, carbamazepine, atenolol, cyclophosphamide, ifosfamide, iodixanol, iohexol	SPE	LC-MS/MS		Köhler et al. (2012)
Chemical: ozonation	Carbamazepine		LC-DAD		Palo et al. (2012)
Chemical: ozonation Sonochemical: sonolysis, US/O_3	Diclofenac		Spectrophotometer		Naddeo et al. (2012)
Chemical: O_3/H_2O_2, Fenton Photochemical: photolysis, UV/O_3, UV/O_3/H_2O_2, UV/H_2O_2	Ibuprofen, sulfamethoxazole		LC-DAD		Epold et al. (2012)
Photochemical: UV/H_2O_2	Carbamazepine		LC-QTrap-MS (by-products)		Keen et al. (2012)
Photochemical: UV/O_3/TiO_2	Diclofenac		LC-DAD (by-products)		Aguinaco et al.

Table 1 (continued)

AOPs	Compounds	Sample preparation	Determination	LOD/LOQ	Ref.
					(2012)
Chemical: O_3, O_3/H_2O_2	Atenolol, trimethoprim, carbamazepine, atrazine, phenytoin, primidone, meprobamate	SPE: recovery 88–118 %	LC-QTrap-MS/MS	LOD, 10–25 ng L^{-1}	Pisarenko et al. (2012)
Chemical: O_3 Photochemical: UV/O_3/TiO_2	Diclofenac, sulfamethoxazole, caffeine		LC-DAD	LOD, 100 μg L^{-1}	Beltrán et al. (2012)
Photochemical: UV/chlorine, UV/HOCl, UV/ClO_2	Sulfamethoxazole, carbamazepine, diclofenac, benzotriazole, tolyltriazole, iopamidole, EE2	SPE	LC-MS/MS	LOD:1–10 ng L^{-1}	Sichel et al. (2011)
Photochemical: heterogeneous photocatalysis (TiO_2)	Metoprolol, propranolol	Filtered samples	LC-DAD, ESI-MS and LC-MS-TOF (by-products)		Romero et al. (2011)
Photochemical: solar photo-Fenton	Acetaminophen, antipyrine, atrazine, caffeine, carbamazepine, diclofenac, flumequine, ibuprofen, ketorolac, ofloxacin, progesterone, sulfamethoxazole, triclosan	SPE	UHPLC-DAD	LOD, 0.6–5.0 μg L^{-1}	Klamerth et al. (2010, 2011)
Chemical: sulphate radical oxidation	Carbamazepine	2 mL quenched with 100 μL of an aqueous solution of $NaNO_2$ (10 M)	LC-DAD-FD, LC-ESI-MS/MS ion trap (by-products)		Matta et al. (2011)
Chemical: ozonation	Sulfasimethoxine, sulfamethoxazole, erythromycin, lincomycin, ciprofloxacin, levofloxacin, doxycycline, tetracycline, trimethoprim, carbamazepine, primidone, iopromide, ibuprofen, acetaminophen, diclofenac, triclosan, EE2, caffeine	SPE: recovery 36–184 %, derivatisation	GC-MS, LC-MS/MS	LOD, 10–5000 ng L^{-1}	Yang et al. (2011)
Chemical: ozonation Photochemical: UV/TiO_2	Sulfamethoxypyridazine	Centrifugation	LC-DAD, LC-API-MS (by-products)		Chuang et al. (2011)
Photochemical: UV/O_3/H_2O_2	Ciprofloxacin, trimethoprim, cyclophosphamide	Lyophilisation	LC-DAD-MS (ESI)		Lester et al. (2011)
Photochemical: UV/Fenton Sonochemical: US	Penicillin		LC-DAD		Saghafinia et al. (2011)
Photochemical: photolysis, UV/H_2O_2	Sulfamethoxazole, sulfamethazine, sulfadiazine, trimethoprim, diclofenac	Catalase, filtered	LC-DAD		Baeza and Knappe (2011)
Chemical: ozonation, O_3/H_2O_2, AC/O_3 Photochemical: photolysis	Paracetamol, diclofenac, sulfamethoxazole, ketorolac, metoprolol		LC-DAD		Álvarez et al. (2011)
Photochemical: solar photo-Fenton	Diclofenac	Catalase	LC-DAD	LOD, 0.14 mg L^{-1}	Trovó and Nogueira (2011)
Chemical: O_3/H_2O_2	Caffeine, ciprofloxacin, clofibric acid, nicotine, sulfamethoxazole, azythromycin, cotnine, loratidine, salicilic acid	SPE	LC-QTrap-MS/MS		Rodríguez et al. (2011)
Photochemical: UV/H_2O_2 Electrochemical: electron pulse radiolysis	Gemfibrozil, naproxen, carbamazepine, ofloxacin, erythromycin, trimethoprim, venlafaxine, atenolol, metoprolol, caffeine, nalidixic acid, iohexol, sulfamethoxazole, atorvastatin,	Filtered, SPE	UHPLC-MS/MS (ESI)	LOD, 0.01–2.0 μg L^{-1}	Abdelmelek et al. (2011)

Table 1 (continued)

AOPs	Compounds	Sample preparation	Determination	LOD/LOQ	Ref.
	lavastatin, enrofloxacin, sulfamethazine, sulfamethizole, sulfamesazine, cimetidine, famotidine, ranitidine, iopamidol, iomeprol, iopromide				
Electrochemical: electron pulse radiolysis	Fluvastatin, lovastatin, pravastatin, simvastatin		LC-DAD, LC-MS (ESI) (by-products)		Razavi et al. (2011)
Chemical: O_3, O_3/H_2O_2	Acetaminophen, atenolol, caffeine, carbamazepine, diclofenac, iopromide, naproxen, cefaclor, sulfamethoxazole, dilantin, ibuprofen	SPE	LC-API-MS		Sarp et al. (2011)
Photochemical: UV/Fenton	Amoxicillin	Catalase, filtered	LC-TOF-MS (ESI)	LOD, 5 μg L^{-1}	Trovó et al. (2011)
Photochemical: UV/H_2O_2	Meprobamate, carbamazepine, dilantin, atenolol, primidone, trimethoprim		LC-MS	LOD, 10 ng L^{-1}	Rosario-Ortiz et al. (2010)
Chemical: O_3 Photochemical: UV/H_2O_2, UV/O_3	Carbamazepine, clofibric acid, diazepam, diclofenac	SPE	LC-QTrap-MS (ESI)	LOD, 0.001–0.002 ng L^{-1}	José et al. (2010)
Chemical: O_3 Photochemical: UV/H_2O_2, UV/O_3	Acetaminophen, antipyrine, diclofenac, ethenzamide, fenoprofen, indomethazine, isopropylantipyrine, ketoprofen, mefanomic acid, naproxen, atenolol, disopyramide, metoprolol, propranolol, bezafibrate, prenzepine, caffeine, diltrazem, dipyridamole, azithromycin, chloratetracycline, clarithromycin, erythromycin, levofloxacin, lincomycin, nalidixic acid, norfloxacin, sulfadimethoxine, sulfamethoxazole, tetracycline, trimethoprim, carbamazepine, primidone, cyclophosphamide, sulpiride, theophylline	Filtered, SPE	LC-MS/MS (ESI)	LOD, 0.02–6.18 μg L^{-1}	Kim and Tanaka (2010)
Chemical: TiO_2	Fluoroquinolone, norfloxacin, levofloxacin, lomefloxacin	Filtered	LC-ESI-MS/MS (by-products)		An et al. (2010)
Electrochemical: non-thermal plasma (NTP)	Meprobramate, dilantin, primidone, carbamazepine, atenolol, trimethoprim, atrazine	SPE	LC-MS/MS	LOD, 10–25 ng L^{-1}	Gerrity et al. (2010)

Determination

To optimise and check the validity of advanced oxidation technologies, the most frequently used determination systems have been both liquid and gas chromatography (LC and GC) coupled with different detectors (see the relative percentage in Fig. 3), which the scientific community employs to analyse emerging pollutants in aqueous samples, regardless of their origin (Wille et al. 2012). However, traditional optical methods, such as UV-vis spectroscopy, have also been used in a few studies. Regardless, the use of either system is not dependent on the choice of AOP, but rather on the types and amounts of the compounds. The matrix also has a role in the selection of the detection technique.

In general, spectrophotometry is applied to determine one or a few pollutants that have a relatively high concentration (higher than real samples). For example, Tran et al. (2015) used ibuprofen to develop a sono-electrochemical procedure that removes up to 90 % of the compounds from wastewater samples. Brame et al. (2014) optimised different photochemical AOPs to degrade two pharmaceutical compounds (ranitidine and cimetidine). The anti-inflammatory drug balsalazide was photodegraded after applying two processes in a study by Sikarwar and Jain (2015). Moreover, Mowla et al. (2014) and

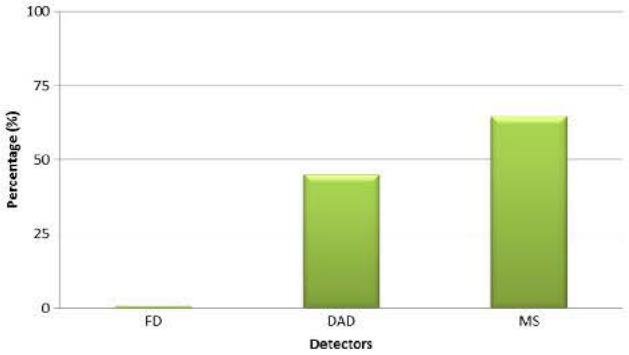

Fig. 3 Relative percentages of the reviewed works concerning the use of different detectors coupled to chromatographic systems

Ghafoori et al. (2015) used sonophotolysis to remove the same groups of drugs. All of the studies used spectrophotometry to follow the degradation of the target compounds, and it was necessary to use spiked samples in the micro- to milligrammes per litre range to calculate the degradation kinetics because of the high limit of detection of this method and the produced spectral interferences by transformation intermediates, which may absorb radiation in the same spectral region as the target compound. Despite this assumption, spectrophotometric methods provide a quick and indicative determination of degradation (Naddeo et al. 2013).

In contrast, chromatographic systems can be useful to simultaneously control a large number of compounds at lower concentration levels (in the range of ng L^{-1} to μg L^{-1}). LC or GC can be used, depending on the type of target compound and the characteristics of the required analysis. GC has been used to evaluate the performance of chemical, photochemical and electrochemical AOPs to degrade different pharmaceuticals and their by-products (Yang et al. 2011; Munoz et al. 2012; George et al. 2014; Zupanc et al. 2014; Ganzenko et al. 2015; Torun et al. 2015; Ghauch et al. 2015). However, in recent years, LC has been the most commonly applied methodology for removing pharmaceuticals because degradation is carried out in aqueous media. Thus, an important advantage of this technique is the possibility of directly injecting samples without any preparation of the sample.

Another important point under this heading is the use of different detectors coupled to chromatographic systems (see the relative percentage in Fig. 3). Optical detectors, such as diode array detectors (DADs), and different mass analysers have been used to verify the efficiencies of developed AOPs. Generally, the differences between the diverse types of detectors are in the limit of detection (LOD) and also in the ability to follow the parent compounds and by-products. In this way, Encinas et al. (2013) treated a water solution of pharmaceuticals by chemical (ozonation) and photochemical (activated carbon (AC)/TiO_2 or black-light/TiO_2) AOPs, merging the LC-DAD and LC-MS-ESI-TOF methods to determine the high and low concentrations, respectively. In addition, LC-DAD has been applied by different authors to determine the identity of parent compounds and, in the same studies, a LC-MS/MS ion trap, LC-MS-TOF, LC-MS-ESI-TOF or LC-API-MS was used to determine the by-products of the target pharmaceutical compounds after varying treatments. For example, UV/H_2O_2 was used to remove up to 73 % of total sulfamethazine (Batista et al. 2014), to perform complete mineralisation of carbamazepine by sulphate radical oxidation (Matta et al. 2011), and to sonochemically oxidise degrade atenolol between 90 and 100 % (Nejumal et al. 2014). Heterogeneous photocatalysis, UV/H_2O_2 and their combinations were used with a high efficiency in Milli-Q water (above 94 %), but with a very low efficiency in real water (Romero et al. 2011, 2014). The study of Li et al. (2014) carried out an electro-phenton process to degrade ibuprofen using three different pieces of analytical equipment: a LC-DAD to quantify the parent and phenolic intermediates as well as an UHPLC-Q-TOF-MS and UHPLC-ESI-MS/MS to identify the by-products and major aromatic intermediates, respectively.

Generally, in order to carry out a suitable determination of intermediates, only a restricted number of analytes is used. This can provide a better approximation of the degradation pathway of the target compound without potential interference. Mass detection is necessary for this type of determination, and the most commonly used detector is a MS-TOF or its variations (Munoz et al. 2012; Batista et al. 2014; Nejumal et al. 2014) because this method provides excellent automatic screening. In some studies, the identification of the provisional structure is compared with authentic standards (Tran et al. 2015), but this procedure is rarely executed. In addition, other determination procedures, such as ion-exclusion LC or ionic chromatography, are also employed to analyse carboxylic acids or different cations or anions produced during the degradation of the parent compound, with the objective of following the intermediates (Ganzenko et al. 2015).

Several authors have combined both traditional optical and chromatography methods in the same studies to monitor the target compounds. The purpose of this type of analytical procedure can be: (1) to quantify higher and lower levels of concentrations; for example, Secondes et al. (2014) used a spectrophotometer and LC-MS after a sonochemical AOP to determine pharmaceuticals in the milli- to nanogrammes per litre range, respectively; (2) to quantify the parent compound using a spectrophotometer as well as its products of degradation using MS detectors coupled with chromatography systems (LC or GC) (Daghrir et al. 2013; Qin et al. 2015; Carabin et al. 2015); and (3) to optimise a few compounds using spectrophotometric analysis and validate the optimal conditions of the AOP with a greater amount of pharmaceuticals by mass spectrometric determination. In this sense, Naddeo et al. (2013) used a spectrophotometer as an analytical method to quantify two compounds to develop a sonochemical treatment, which was used to remove 23

pharmaceutical compounds (diclofenac and carbamazepine) and was evaluated by LC-QTrap-ESI-MS/MS.

Regarding LODs, values on the order of microgrammes per litre have been achieved using LC-DAD by certain authors (Klamerth et al. 2010, 2011; Jin et al. 2012; Espejo et al. 2014a, b). However, better LODs on the order of ng L^{-1} were obtained by MS in other studies of pharmaceuticals after diverse photochemical AOPs (Rosario-Ortiz et al. 2010; Sichel et al. 2011; Hey et al. 2014). In particular, if we consider the effectiveness of the treatment, these results could emphasise that the authors who used optical detectors expressed the results in terms of complete degradation; however, it is still possible to assign a numerical value when the study was carried out using an MS detector. In this case, transformations can be achieved anywhere in the range of 0 to 100 %.

Because of analytical limitations, Pereira et al. (2012) spiked water with an initial concentration in the milligrammes per litre range. This is a higher concentration than is typically found in environmental samples and was necessary to follow the degradation of compounds using direct injection into an UHPLC-DAD (ultra-high performance liquid chromatography-DAD) after applying UV photolysis and UV/H_2O_2, which produced a high removal efficiency. This problem can be solved during sample preparation by using preconcentration techniques.

Encinas et al. (2013) and Tran et al. (2015) comment on the influence of matrix effects on the efficiency of AOPs when low initial concentrations of the emerging contaminants are used. In light of these effects, it is very important to use an analytical method that has a suitable performance to follow this type of pollutant at the same concentration level as the environmental samples and to ensure that these treatments are successful under real conditions. Regardless, publications generally focus on the details of data optimisation for advanced oxidation techniques and ignore the importance of analytical methodologies, such as the study of Badawy et al. (2014), which did not provide a sufficient number of parameters.

Sample preparation

Sample preparation is geared toward two main objectives, to stop any oxidation process that may affect the instrumentation and to improve the limit of detection by concentrating and cleaning the samples. Therefore, this important step improves the quality of the determination procedure, and in some cases, it is essential to perform the analysis.

If chemical catalysts suspended in the solution are used as oxidants, one should be especially careful with the use of direct injection, and the particles should be removed or inactivated before analysis. For this reason, Romero et al. (2011) filtered the samples with a 0.45-μm polyethersulphone membrane filter to remove the suspended TiO_2 catalyst before LC analysis, although a centrifugation step can also be used. In contrast, Matta et al. (2011) performed treatment using a sulphate radical and then quenched a 2-mL sample with 100 μL of an aqueous solution of $NaNO_2$ (10 M) before injecting it onto an LC column. A catalase solution is often employed to quench the reaction and guarantee the absence of hydrogen peroxide (catalase consumes residual H_2O_2) (Trovó et al. 2011; Trovó and Nogueira 2011; Baeza and Knappe 2011; Keen et al. 2012; Justo et al. 2015). In Gerrity et al. (2010), the residual oxidants in each sample were quenched with calcium thiosulfate.

The disadvantage of using gas chromatography to analyse pharmaceuticals is the necessity of an additional step for sample preparation. This procedure requires a chemical reaction, which increases the selectivity, namely, derivatisation (Olariu et al. 2010). For this, Torun et al. (2015) and Zupanc et al. (2014) used trimethylsilane and *N*-(*t*-butyldimetylsilyl)-*N*-methyltrifluoroacetamid (MTBSTFA), respectively, to derivatise before their respective determinations.

The pre-concentration and extraction techniques applied in this field range from more conventional techniques, such as liquid-liquid extraction (LLE), to the latest micro-extraction procedures. LLE coupled to GC-MS was used by George et al. (2014) and Ganzenko et al. (2015) to follow the degradation of salicylic acid and caffeine (with derivatisation), respectively, after electro-Fenton processing. In contrast, Munoz et al. (2012) analysed the by-products of triclosan using SBSE and GC-MS-TOF after a Fenton/H_2O_2 procedure with a LOD below 1 ng L^{-1}.

However, within the realm of conventional techniques, solid-phase extraction (SPE) is the most commonly used pre-treatment to clean and concentrate the sample to take advantage of the levels of detection that are capable of being measured by the instrumentation. This technique, coupled with a detection system, achieves LODs on the order of nanogrammes per litre or a few microgrammes per litre. In many publications in which the authors use SPE followed by LC (with different detectors) with spiked samples, chemical and photochemical AOPs were developed to remove EDCs and PPCPs (Klamerth et al. 2010, 2011; Sichel et al. 2011; Ibáñez et al. 2013; Hey et al. 2014; Miralles-Cuevas et al. 2014b). However, Moreira et al. (2015) used the same methodology to degrade different micro-pollutants in non-spiked urban wastewater using a large variety of AOPs. In all cases, nearly complete degradation was obtained.

It is necessary to have adequate knowledge of the possible matrix effects generated from the use of AOPs before applying them in a full-scale treatment. The use of a validated analytical methodology (in terms of both sample preparation and the determination procedure) also offers a closer approximation to the real removal rates of AOPs. These percentages can be confusing for methodologies with high detection limits. Non-identification of a compound may not correspond to

100 % removal. Therefore, it is advisable to describe the effectiveness of the AOP relative to the analytical technique used. This means proposing different scales, depending on the sensitivity of the method. One option is the use of either a qualitative scale (low, medium or high/complete degradation) for less sensitive methods or a quantitative scale (providing a numerical percentage) for more accurate methodologies.

Overall, there is little information regarding the analytical procedures used. Thus, this is an unresolved issue for future research.

Future trends

The inability of conventional wastewater treatments to completely degrade emerging pollutants is well known. Alternative treatments, such as different AOPs, have appeared as improvements to conventional procedures, but generally, they are limited because they require a large economic investment and have very few real applications. Therefore, the main advantage of AOPs, which cause the complete mineralisation of pollutants, can only be obtained with very long contact times, causing very high operative costs, and, in practice, AOP are almost never used. Consequently, there is an increasing need for alternative wastewater treatment processes that have high removal efficiencies and a reasonable cost (Zhang et al. 2014). Moreover, because the determination of pharmaceutical compounds and other emerging compounds in complex matrices, such as wastewater, may be difficult, the chemical additives employed in some AOPs may add further difficulty to the analysis. Thus, the development of advanced procedures without the requirement of using additives would facilitate the determination of pollutants using simple analytical methods. Additionally, the use of aquatic plant-based systems is gaining attention and has been recommended for wastewater purification for small communities (<2000 population equivalent) (Herrera-Melián et al. 2015). However, although advanced procedures have been studied to remove conventional pollutants, their application to emerging compounds is still scarce.

Regarding the preparation and determination procedures, to evaluate the efficiency of AOPs to remove pharmaceutical compounds, it is essential to apply the most sensitive and specific techniques because these pollutants are found at very low concentrations. Conventional extraction and preconcentration techniques are often not able to meet the purification and the detection limits required for these types of samples. The current trend in analytical chemistry is toward automatised techniques, such as on-line SPE coupled to LC, which avoids manual errors and allows complete injection of the sample, instead of a portion of a few millilitres, such as in conventional SPE. On the other hand, the availability of a very selective detector is imperative to follow the degradation of the pollutants and determine their by-products. In the future, all studies involving the degradation of pharmaceutical and other emerging compounds should include an analysis of these transformation products because the negative effects of these products could be more important that those generated by the original substance.

Conclusions

Different AOPs and their combinations have seen rapid development, resulting in a very promising approach for the treatment of different wastewaters. Nevertheless, the majority of assays have been carried out at the laboratory scale; thus, their applicability has not been sufficiently demonstrated in real urban wastewater treatment plants. Moreover, very few studies have been conducted to evaluate the economic feasibility of this novel technology.

In any study based on the use of AOPs, it is relevant to note that employing the proper analytical methodology is essential to obtain an idea of the effectiveness of the treatment. However, currently, the information provided by many authors regarding the analytical methodology is scarce. The accurate estimation of elimination rates is closely linked with a good understanding of the analytical limitations related to the particular characteristics of the compounds and also to the interferences that could affect the identification or quantification of the target analytes. Thus, efforts should be made to clarify and expand the chosen preparation and determination procedures.

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I.4. Análisis multiresiduo

Con el objetivo de alcanzar los principios de la química analítica verde, así como abarcar la mayor cantidad de fármacos posibles, sobre todo aquellos que tienen mayor prioridad ambiental, los investigadores orientan su investigación en optimizar metodologías analíticas que puedan llegar a ser análisis de rutina con las que, a partir de un único análisis, se pueda controlar un abanico amplio de productos farmacéuticos, incluso, de diferentes clases terapéuticas con características físico-químicas distintas, lo que se conoce como metodologías multiresiduo [213].

Este tipo de metodologías, en un caso ideal, debe cumplir varios criterios [213]:

(I) la preparación y preconcentración de la muestra se debe realizar en un solo paso, aunque los analitos posean diferentes propiedades,

(II) los límites de detección y cuantificación deben ser lo suficientemente bajos para cada analito,

(III) la detección debe ser específica de la sustancia, es decir, que sea selectiva y

(IV) deben ser de fácil aplicación a diferentes matrices.

Para poder acercarnos a estos ideales es necesario una minuciosa optimización de todos aquellos parámetros que intervienen durante todo el procedimiento analítico, desde el almacenamiento de la muestra, pasando por la preparación y extracción, hasta las variables que afectan a la separación cromatográfica y detección. Sin embargo, el análisis simultáneo de compuestos de diferentes grupos con características fisicoquímicas muy distintas, requiere un compromiso en la selección de

condiciones experimentales que, en algunos casos, no son las mejores condiciones para todos los analitos estudiados, sino para una mayoría de ellos.

Hoy en día, las metodologías analíticas multiresiduo se están convirtiendo en las herramientas preferidas y necesarias frente al análisis de un solo grupo, ya que proporcionan un conocimiento más amplio sobre la presencia de contaminantes en los medios acuáticos, necesario para un estudio más detenido de su eliminación, transformación y destino final en el medioambiente. [214].

Las técnicas adecuadas para llevar a cabo este tipo de análisis deben caracterizarse por una alta sensibilidad y precisión, robustez analítica y que, operacionalmente, sean rápidas. Queda claro que la técnica de extracción preferida es SPE debido a su alta precisión y robustez analítica, mientras que los sistemas de detección y determinación generalmente son LC-MS, LC-MS/MS y GC-MS, debido a su alta selectividad, además de permitir todas las variaciones de sus sistemas de ionización. Sin embargo, estos sistemas no son los únicos utilizados para llevar a cabo un análisis multiresiduo, ya que se trata de instrumentos bastante caros y sofisticados y que requieren de altos costos de mantenimiento y personal técnico. Por estas razones, no están ampliamente disponibles en todos los laboratorios y, por ello, algunos autores han intentado conseguir un método lo suficientemente sensible, económico y de fácil aplicación mediante el uso de LC con detección simultánea mediante DAD y FD [203].

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Capítulo II: Objetivos

Es un hecho que existe la presencia de una gran variedad de compuestos farmacéuticos en los diferentes compartimentos ambientales, siendo el sistema hidrológico el más estudiado por la comunidad científica, dado que se trata del punto de partida de su entrada directa al medioambiente debido a que los sistemas actuales de depuración de las aguas no están preparados para la eliminación o degradación de estos compuestos en particular. Sin embargo, el estudio de la aparición de estos contaminantes, los cuales se encuentran a niveles traza, no hubiese sido posible sin la existencia y desarrollo de metodologías analíticas cada vez más amigables con el propio medioambiente, sin dejar de ser sensibles, selectivas y robustas.

Estas metodologías analíticas son capaces de determinar compuestos farmacéuticos de diferentes clases terapéuticas en un solo análisis. Por tanto, se pueden monitorizar los niveles de contaminación de aquellas sustancias que poseen un alto interés medioambiental, debido a

su frecuencia de aparición en el medio y a sus potenciales efectos ecotoxicológicos, de manera conjunta mediante análisis rutinarios.

A pesar de la gran cantidad de estudios existentes sobre esta clase de contaminantes, la legislación para el control de su vertido ha avanzado muy levemente. Con lo cual, se debe seguir investigando sobre este problema emergente con el fin de obtener los suficientes datos para poder concretar las medidas necesarias y, además, ofrecer sistemas de análisis estándar para su determinación, tanto para contribuir al repositorio, en este caso de los estudios del territorio español, como para evaluar sistemas alternativos de eliminación. Son los temas que se corresponden con las tendencias actuales.

En este sentido, los objetivos generales que se presentan en esta Tesis Doctoral se especifican a continuación:

a. Establecer las condiciones cromatográficas necesarias para separar detectar y determinar, mediante la cromatografía líquida de alta resolución acoplada a un detector de espectrometría de masas de triple cuadrupolo con ionización mediante electrospray (LC-ESI-MS), todos los compuestos farmacéuticos seleccionados en un solo análisis.

b. Optimizar las variables que influyen en la extracción de los compuestos farmacéuticos bajo estudio en muestras líquidas, haciendo uso de la técnica de extracción en fase sólida (SPE).

c. Ejecutar un muestreo temporal de muestras líquidas procedentes de estaciones depuradoras de aguas residuales (EDARs) con

diferentes sistemas de tratamiento, situadas en la isla de Gran Canaria (España).

d. Evaluar la presencia de los compuestos farmacéuticos en estudio en aguas residuales y depuradas de la isla de Gran Canaria (España).

e. Calcular y analizar el riesgo ambiental que podría llegar a producir en diferentes organismos, los máximos niveles de concentración encontrados en las muestras analizadas de los efluentes estudiados.

f. Determinar la eficacia de eliminación de los compuestos farmacéuticos estudiados, tanto en el proceso completo como por etapas de tratamiento, de las estaciones depuradoras de aguas residuales evaluadas.

g. Aplicar la metodología analítica optimizada para determinar la eficacia de un proceso avanzado de oxidación (PAO) que consiste en la aplicación de energía ultravioleta junto a la adición de peróxido de hidrógeno (UV/H_2O_2) en suspensión, para la transformación de los compuestos farmacéuticos seleccionados en muestras de agua depurada enriquecidas.

h. Aplicar la metodología analítica optimizada para determinar la eficacia de la fotocatálisis heterogénea, utilizando un reactor fotocatalítico con óxido de titanio (TiO_2) inmovilizado en espumas de alúmina, para la transformación de los compuestos farmacéuticos seleccionados en muestras de agua depurada enriquecidas.

Capítulo III: Parte experimental y Resultados

Como ya se ha indicado, la monitorización de la presencia de residuos farmacéuticos en el medioambiente es de gran importancia, siendo numerosos los estudios encaminados hacia su determinación. Sin embargo, los métodos tradicionales usados con este fin requieren grandes cantidades de disolventes orgánicos y generan grandes volúmenes de residuos. Por ello, las tendencias en la Química Analítica actual van orientadas hacia la utilización de procedimientos que sean más amigables con el medioambiente, que impliquen una reducción del volumen de disolventes orgánicos y que generen menos residuos.

Como punto de partida, es sumamente importante la determinación de residuos farmacéuticos en las diferentes zonas geográficas para conocer el alcance de este problema ambiental. De esta manera, se podría predecir el riesgo que supone sobre los organismos a través de los niveles de concentración encontrados en su hábitat. De forma adicional, la

monitorización de muestras procedentes de estaciones depuradoras de aguas residuales nos proporciona información sobre la efectividad de eliminación de los tratamientos empleados y la necesidad de desarrollar y aplicar sistemas avanzados de eliminación para resolver los problemas derivados de la presencia medioambiental de estos contaminantes.

De acuerdo a ello, los trabajos que se presentan a continuación, los cuales conforman la parte experimental y resultados de esta Tesis Doctoral, se centran, por una parte, en el desarrollo de una metodología analítica multiresiduo para la determinación de residuos farmacéuticos en muestras líquidas procedentes de estaciones depuradoras de aguas residuales. Y, por otra, se presentan las aplicaciones de dicha metodología, las cuales van dirigidas a la monitorización de la presencia de fármacos en muestras líquidas ambientales para poder contribuir a aumentar la base de datos sobre este problema. Además, se valora el riesgo ambiental debido a la descarga de agua depurada con contenido de residuos farmacéuticos, dado el incompleto rendimiento de los tratamientos convencionales de depuración empleados.

Proporcionada la inevitable presencia de estos contaminantes en los efluentes de las estaciones depuradoras, adicionalmente, se presentan algunos trabajos donde se aplica la metodología multiresiduo para evaluar la eficiencia de diferentes sistemas avanzados de oxidación como mejoras de tratamientos de aguas, con el propósito de reducir, más eficazmente, los niveles de fármacos.

III.1. Procedimiento de extracción en fase sólida simplificada combinada con cromatografía líquida de alta resolución con detección por espectrometría de masas para la evaluación multiresiduo de compuestos farmacéuticos en muestras líquidas ambientales.

En este sentido y como bloque principal de la parte experimental de esta Tesis Doctoral, se ha llevado a cabo la optimización de una metodología analítica multiresiduo para la extracción y determinación de los compuestos farmacéuticos en estudio en muestras de aguas residuales. Este procedimiento sirve de punto de partida para evaluar la contaminación que se está produciendo en el foco principal de entrada al medioambiente de los residuos farmacéuticos, como son las estaciones depuradoras de aguas residuales y, además, usarlo como herramienta para proponer alternativas de tratamientos avanzados.

En este artículo se presenta el desarrollo, optimización y aplicación de una metodología analítica para la determinación multiresiduo de veintitrés compuestos farmacéuticos de uso común que aparecen en las aguas residuales. La metodología está constituida por un procedimiento de extracción en fase sólida (SPE) simplificada y la posterior determinación y cuantificación mediante cromatografía líquida de alta resolución acoplada a un detector de espectrometría de masas de triple cuadrupolo con ionización electrospray (LC-ESI-MS/MS). Con este desarrollo metodológico se intenta mejorar los métodos establecidos previamente y seguir acercándonos, aún más, a los doce principios de la química analítica verde.

Mediante el procedimiento de extracción en fase sólida simplificada se ha logrado una disminución del tiempo de análisis y de la

cantidad de disolvente orgánico utilizado. Con ello, reducimos el procedimiento de extracción a tres únicos pasos, eliminando los dos primeros del protocolo habitual (acondicionamiento y equilibrado) gracias a las características que ofrecen los cartuchos poliméricos de N-vinilpirrolidona-divinilbenceno.

Se estudiaron las condiciones óptimas de las variables que afectan a la SPE, como pueden ser el pH, fuerza iónica, volumen de muestra y volumen y tipo de eluyente. Todas estas variables se validaron mediante la aplicación a diferentes clases de aguas residuales y depuradas.

Los resultados de la validación analítica revelan que en el 82,6 % de los casos se alcanzaron recuperaciones cuya mediana eran superior al 70 % para todos los compuestos en cada muestra. En cuanto a las desviaciones estándar relativas (RSDs) fueron inferiores al 14,4 % y 22,0 % para la repetitividad intra- e inter-día, respectivamente. Mientras que los límites de detección (MDLs) y los límites de cuantificación del método (MQLs) oscilaron entre 0,011 y 188 $ng \cdot L^{-1}$ y entre 0,033 y 628 $ng \cdot L^{-1}$, respectivamente.

La aplicabilidad del método se comprobó en muestras reales procedentes de estaciones de tratamiento de aguas residuales con diferentes sistemas de tratamientos, una con tratamiento natural mediante humedales y otra convencional de lodos activos y posterior tratamiento de osmosis inversa. Los resultados obtenidos indicaron rangos de concentración encontrados de 0,013 a 91,5 $\mu g \cdot L^{-1}$ y de 0,004 a 49,1 $\mu g \cdot L^{-1}$, respectivamente. Dieciocho de los veintitrés fármacos estudiados se detectaron en todas las muestras de ambas EDARs. Ácido clofíbrico y eritromicina fueron los únicos compuestos que no se

detectaron en ninguna de las muestras. Por otro lado, los compuestos metronidazol, sulfametoxazol y trimetoprim sólo aparecían en la estación depuradora con tratamiento convencional.

En definitiva, el método optimizado es adecuado para la determinación de residuos farmacéuticos en muestras líquidas procedentes de estaciones depuradoras de aguas residuales.

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Simplified solid-phase extraction procedure combined with liquid chromatography tandem–mass spectrometry for multiresidue assessment of pharmaceutical compounds in environmental liquid samples☆

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ABSTRACT

To follow the twelve "green analytical chemistry" (GAC) principles, it is necessary to continuously develop analytical extraction and determination methodologies to assess the presence of micropollutants, such as pharmaceuticals, in environmental samples.

A reduction in the analysis time and solvent quantity, which is one of the GAC principles, has been achieved through a simplified solid-phase extraction (SPE) procedure combined with high-performance liquid chromatography-tandem mass spectrometry (LC–MS/MS) for the determination of twenty-three pharmaceuticals in liquid environmental samples using *N*-vinylpyrrolidone-divinylbenzene copolymer (OASIS HLB) cartridges. The optimal SPE conditions were studied. In these optimized conditions, 82.6% of the data have a median recovery above 70% for all compounds in each sample. The relative standard deviations (RSDs) were below 14.4% and 22.0% for intra- and inter-day repeatability, respectively. Method detection limits (MDLs) and method quantification limits (MQLs) ranged from 0.011 to 188 ng L^{-1} and from 0.033 to 628 ng L^{-1}, respectively. The applicability of the method was evaluated in real samples from natural and conventional wastewater treatment plants (WWTPs), and results were obtained in concentration ranges from 0.013 to 91.5 μg L^{-1} and from 0.004 to 49.1 μg L^{-1}, respectively.

1. Introduction

Wastewater treatment plants (WWTPs) were developed in the nineteenth century with the objective of improving the quality of effluents based on certain standard parameters, such as suspended solids, biodegradable organics, pathogenic bacteria and nutrients among others. The overall purpose was to avoid a source of environmental pollution and to successfully reuse water without human health problems [1,2]. However, with industrial development and scientific advancements over time, the appearance of different microcontaminants or so-called emerging contaminants, which WWTPs have not been designed to remove, has produced a significant issue [3,4].

Emerging contaminants have been widely studied by the scientific community in recent decades [5–7] for their presence in the environment (liquid, solid and organism samples) and their negative toxicological effects [8–10]. Despite extensive studies about their occurrence, no specific normative standard is available that controls and regulates these types of pollutants. Because of this, more definitive studies regarding their risks are needed [11,12].

The current long list of emerging pollutants contains pharmaceutical residues. These compounds come from a variety of therapeutic classes with different chemical structures and therefore different physicochemical characteristics and applications. However, only certain compounds, such as a non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac), hormones (17-alpha-ethinylestradiol (EE2), estrone (E1) and 17-beta-estradiol (E2)) and macrolide antibiotics (eritromicin, claritromicin and

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azitromicin), have been included in a watch list by the European Commission (Decision 2015/495, March 20, 2015) and are monitored [13].

Generally, pharmaceuticals are polar or semi-polar, contain acidic and basic functional groups and are stable and difficult to degrade in liquid samples [14,15]. With advances in analytical techniques, multiresidue methods have been developed to assess a large number of compounds in a single analysis in spite of their different behaviours [16].

While developing a valid method for extraction, identification and quantification of these contaminants, a compromise between the adherence to the twelve "green analytical chemistry" (GAC) principles [17] and the accuracy, reproducibility and ease of use of analytical methods is needed. However, this task is arduous due to the presence of a wide variety of pharmaceutical compounds and their occurrence in the concentration range from ng L^{-1} to μg L^{-1} in the environment [18,19].

Multiresidue determination methods of pharmaceuticals from environmental liquid samples are normally performed using liquid chromatography-tandem mass spectrometry with different ionization sources [20,21]. Moreover, solid-phase extraction (SPE) using sorbents such as N-vinylpyrrolidone-divinylbenzene copolymer, octadecyl-bonded end-capped silica or N-vinylpyrrolidone-divinylbenzene copolymer-SO_3H and others is the preferred sampling technique due to the necessity of cleaning and preconcentration processes for these types of samples [22–25]. Moreover, molecularly imprinted polymers (MIPs) are typically used to extract a group of compounds with similar structures or very similar physicochemical characteristics [26]. However, multi-template MIPs have been developed to analyse a limited selection of pharmaceuticals solely related to the acid structure [27].

Standard SPE uses a protocol with five steps that include conditioning, equilibration, loading, washing and elution. A large amount of time and, in many cases, a large quantity of solvent are consumed during the first steps. However, the Oasis HLB cartridges have a sorbent with a hydrophilic-lipophilic balanced copolymer structure that can be used in a simplified three-step system, which removes the first two steps (conditioning and equilibration) from the standard protocol. As a result, we have reduced the sample preparation time by up to 40% and used up to 70% less solvent. Accordingly, this technique is close to the principles of "green chemistry" sought by the scientific community [28,29].

In this sense, the aim of this work has been the development of a three-step SPE procedure followed by LC–MS/MS for the analysis of twenty-three pharmaceuticals in liquid environmental samples. The applicability of the proposed method was evaluated in several samples, which have been given different treatments, from both natural and conventional WWTPs.

2. Experimental

2.1. Materials

The representative pharmaceutical compounds belonging to different therapeutic classes that were used are listed as follows: diclofenac (DCLF), ketoprofen (KPF), ibuprofen (IBU), naproxen (NPX), metamizole (MTZ), nicotine (NICO), caffeine (CAFF), paraxanthine (PRX), propranolol (PNL), atenolol (ATE), carbamazepine (CBZ), fluoxetine (FLX), ofloxacin (OFLO), ciprofloxacin (CIPRO), erythromycin (ERY), trimethoprim (TRIM), sulfamethoxazole (STX), metronidazole (MDZ), omeprazole (OME), ranitidine (RND), gemfibrozil (GMF), clofibric acid (CLOF) and bezafibrate (BZF). All compounds were purchased from Sigma-Aldrich (Madrid, Spain) with purities above 97%. Three internal standards (IS), atenolol d7 (Ad7), sulfamethoxazole d4 (Sd4) and ibuprofen d3 (Id3), were acquired from Toronto Research Chemicals Inc. (Toronto, Canada), Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Sigma-Aldrich (Madrid, Spain), respectively. Stock solutions of each analyte were prepared by dissolving the compound in methanol (1000 mg L^{-1}), and the solutions were stored in glass-stoppered bottles at −20 °C prior to use. Both standard and internal standard working solutions were prepared daily at a concentration of 1 mg L^{-1} in Milli-Q water and 10 mg L^{-1} in methanol, respectively.

LC–MS quality methanol and water, obtained from Scharlab S.L. (Barcelona, Spain), were used to prepare the mobile phase for LC–MS/MS. All of the reagents that were used to adjust the pH of the mobile phase and the samples or increase the ionic strength were obtained from Panreac (Barcelona, Spain). Ultra-high purity water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) water purification system and used for the washing step of the solid-phase extraction and for preparing aqueous standard solutions.

2.2. Environmental liquid samples

Water samples from in an urban WWTP (titled WWTP1) located in Gran Canaria (Spain) were taken from the following four different points: influent (Point 1.1), after the microfiltration process and before the reverse osmosis treatment (Point 1.2), reverse osmosis concentrate (Point 1.3) and treated water after reverse osmosis treatment (Point 1.4). WWTP1 was designed to treat 18,000 m^3 day^{-1}. In addition, water samples were collected from a second wastewater treatment plant (WWTP2) located in the same area, which is based on a natural process of purification to treat sewage from a population of 500 inhabitants. The sampling points were: after a bar screen (Point 2.1), after a subsequent treatment (in which it is injected with effective microorganisms) (Point 2.2), from a vertical filter (Point 2.3) and, finally, from a horizontal flow (Point 2.4). Both of the last treatments have gravel substrates in the presence of plants.

The samples were collected in amber glass bottles that had been pre-rinsed with a small amount of methanol and generous amount of deionized water. Then, samples were filtered using 0.65 μm polyvinylidene fluoride (PVDF) membrane filters from Merck Millipore (Cork, Ireland), acidified to pH 3 with formic acid and stored in the dark in a refrigerator. The samples were extracted within 48 h.

2.3. Extraction and determination procedures

2.3.1. Simplified SPE procedure

A manifold SPE system from Varian with a capacity for 12 simultaneous extractions was used for the extraction step. The optimized sample volume at an optimal pH was passed through an Oasis HLB (6 cm^3, 500 mg) polymeric cartridge from Waters (Barcelona, Spain). Subsequently, 5 mL of Milli-Q water was used as a wash step. The cartridge loading in these last two steps was executed at a flow rate of 5 mL min^{-1}. Thereafter, the cartridges were dried under vacuum for approximately 15 min and then were eluted with 5 mL of methanol at 1 mL min^{-1}. Finally, the extract was evaporated under a gentle nitrogen stream and reconstituted with 1 mL of an internal standard solution (a mixture of the three IS in Milli-Q water at 50 μg L^{-1}). The final extract was filtered through a 0.20 μm syringe filter.

2.3.2. Liquid chromatography tandem-mass spectrometry

The final extract was analysed and quantified by a liquid chromatography-tandem mass spectrometry (LC–MS/MS) Varian system (Varian Inc., Madrid, Spain) consisting of a 212-LC binary gradient chromatography pump fitted with a Prostar 410 HPLC autosampler and a 320-MS system (triple quadrupole) equipped with an electrospray ionization (ESI) interface.

Table 1
Classification and detection parameters of the target compounds.

Therapeutic class	Compound	Retention time (min)	Mw[a]	Precursor ion (*m*/*z*)	Cone voltage (V)	Fragment ions (collision potencial, V)	Ion mode
Antibiotics	TRIM	9.70	290.3	291.1	64	230.0 (19), 122.9 (21)	ESI +
	MDZ	13.0	171.1	172.0	40	127.9 (10), 81.90 (21)	ESI +
	OFLO	12.6	361.4	362.1	52	318.1 (14), 261.0 (22)	ESI +
	CIPRO	13.7	331.3	332.1	52	313.9 (19), 230.8 (36)	ESI +
	STX	22.3	253.3	254.0	44	155.9 (11), 91.90 (23)	ESI +
	ERY	26.2	733.9	734.5	48	576.3 (13), 158.0 (21)	ESI +
Antidepressant	FLX	27.4	309.3	310.0	30	44.00 (6.5), 148.0 (6.5)	ESI +
Antiepileptic	CBZ	33.3	236.3	237.1	40	194.0 (13), 192.0 (17)	ESI +
Antihypertensive	ATE	3.90	266.3	267.0	52	145.0 (23), 190.0 (16)	ESI +
	PNL	19.5	259.3	260.2	48	116.1 (13), 183.1 (12)	ESI +
Anti-inflammatory	MTZ	9.10	311.3	218.1	36	56.00 (11), 97.00 (8.0)	ESI +
	KPF	37.6	254.0	255.0	52	209.0 (10), 104.9 (18)	ESI +
	NPX	38.1	230.3	231.0	40	185.0 (9.0), 170.0 (20)	ESI +
	IBU	42.0	206.3	204.7	40	160.8 (6.5)	ESI −
	DCLF	43.2	296.1	295.9	32	214.0 (30), 250.0 (11)	ESI +
Antiulcer	RND	4.30	314.4	315.0	44	175.9 (11), 129.8 (20)	ESI +
	OME	31.1	345.4	346.0	32	198.0 (6.0), 135.9 (26)	ESI +
Lipid regulator	BZF	42.5	361.8	359.8	64	273.7 (15), 153.5 (28)	ESI −
	GMF	43.6	250.3	248.9	30	120.7 (12)	ESI −
	CLOF	44.2	214.6	213.0	32	85.00 (10), 127.0 (13)	ESI −
Stimulant	NICO	2.30	162.2	163.0	30	130.0 (18), 84.00 (17)	ESI +
	PRX	16.0	180.2	181.0	44	124.0 (14), 96.10 (19)	ESI +
	CAFF	19.7	194.2	195.0	56	138.0 (18)	ESI +
IS	Ad7	3.80	273.4	274.2	72	144.9 (20), 123.0 (14)	ESI +
	Sd4	22.2	257.3	258.0	44	160.0 (11), 95.90 (22)	ESI +
	Id3	42.0	209.3	207.8	40	163.8 (7.0)	ESI −

[a] Molecular weight.

The stationary-phase column was a 3.0 mm × 100 mm, 3.5 μm particle SunFireTM C18. The mobile-phase gradient consisted of water (containing 0.015% formic acid) and methanol at 90:10 (v/v) for 1 min, then was changed to 60:40 (v/v) for 20 min and 10:90 (v/v) for 19 min and finally returned to its initial condition for 3 min. An equilibration time of 4 min was employed. The injection volume was 10 μL and the flow rate was 200 μL min^{-1}.

The ionization of target compounds in the ESI source was achieved using a source and desolvation temperature of 60 °C and 250 °C, respectively. Nitrogen was used as both a drying and nebulizer gas at 30 psi and 65 psi, respectively. The capillary voltage was set to 5.0 kV in the positive mode (ESI+) and −4.5 kV in negative mode (ESI−), while the shield voltage was maintained at −600/600 V (ESI+/ESI−). Collision-induced dissociation (CID) was conducted with argon as the collision gas at a fixed pressure of 2.00 psi.

The optimization of multiple reaction monitoring (MRM) parameters, such as precursor and product ions, cone voltage and collision gas energy, was carried out by directly injecting 1 mg L^{-1} in a methanol standard solution of each individual compound into the MS detector at a flow rate of 10 μL min^{-1}. The optimal parameters are displayed in Table 1. The precursor ions for most compounds were $[M\text{-}H]^-$ in negative ion mode (ESI-) and $[M\text{-}H]^+$ in positive ion mode (ESI+) except for metamizole, which undergoes a hydrolysis process [30].The system and data management were controlled by MS Varian LC/MS Workstation Version 6.9 SP1 software.

3. Results and discussion

3.1. Optimization of the simplified SPE procedure

The selection of optimal conditions for the extraction of target compounds using a simplified SPE, which is based on a standard SPE protocol without the two initial steps, is necessary because different parameters, such as the cartridge type, sample volume, sample pH or ionic strength may affect the yield of the procedure.

In this study, the cartridge type was not assessed. The OASIS HLB cartridges were chosen to perform the extraction procedure because the features offered by the sorbent were required for a simplified SPE. Furthermore, the variables that have a greater influence on the extraction efficiency (pH, sample volume and ionic strength) were studied with an experimental design. A 2^3 factorial experimental design (three variables at two levels) was developed. To compare the results of the different assays, triplicates of the spiked samples (the addition of 125 mL of 10 mg L^{-1} standard solution) were used. Moreover, blanks were assiduously used to check for carryover.

3.1.1. Factorial design

An initial assessment to verify the contribution of each variable (both individually and collectively) was accomplished using a 2^3 factorial design, and every test was randomized to avoid a possible carryover effect. The three different variables were chosen and evaluated at two relatively extreme levels: sample volume (100 and 500 mL), sample pH (3 and 11) and ionic strength (0 and 10% (v/v) of NaCl). The choice of both pH values was a complicated task due to the variety of characteristics of the target compounds; however, we have focused on levels that represent the majority of these pharmaceuticals in a neutral state to achieve the best performance of the extraction process.

Pareto charts were built for each pharmaceutical to observe their diverse behaviours. In Fig. 1, four representative effects on the extraction of several compounds are shown: the strong effect of the combination of two variables (Fig. 1a), the strong effect of each independent variable (Fig. 1b), a combination of both effects at the same time (Fig. 1c) and the absence of influence of both the independent variables and their combinations on the extraction of the compound (Fig. 1d). The first behaviour (Fig. 1a) that occurred for the extraction of most target compounds, where the ionic strength and sample pH were the variables, had the greatest effect on the extraction. In addition, it was observed that the pH parameter has a greater influence on the extraction recovery for a large majority of compounds. For only a few compounds, no relationships existed between the variables and no dependence occurred within them. In this sense, it was necessary to further optimize the ionic strength and pH parameters. The bivariate correlations of the sample

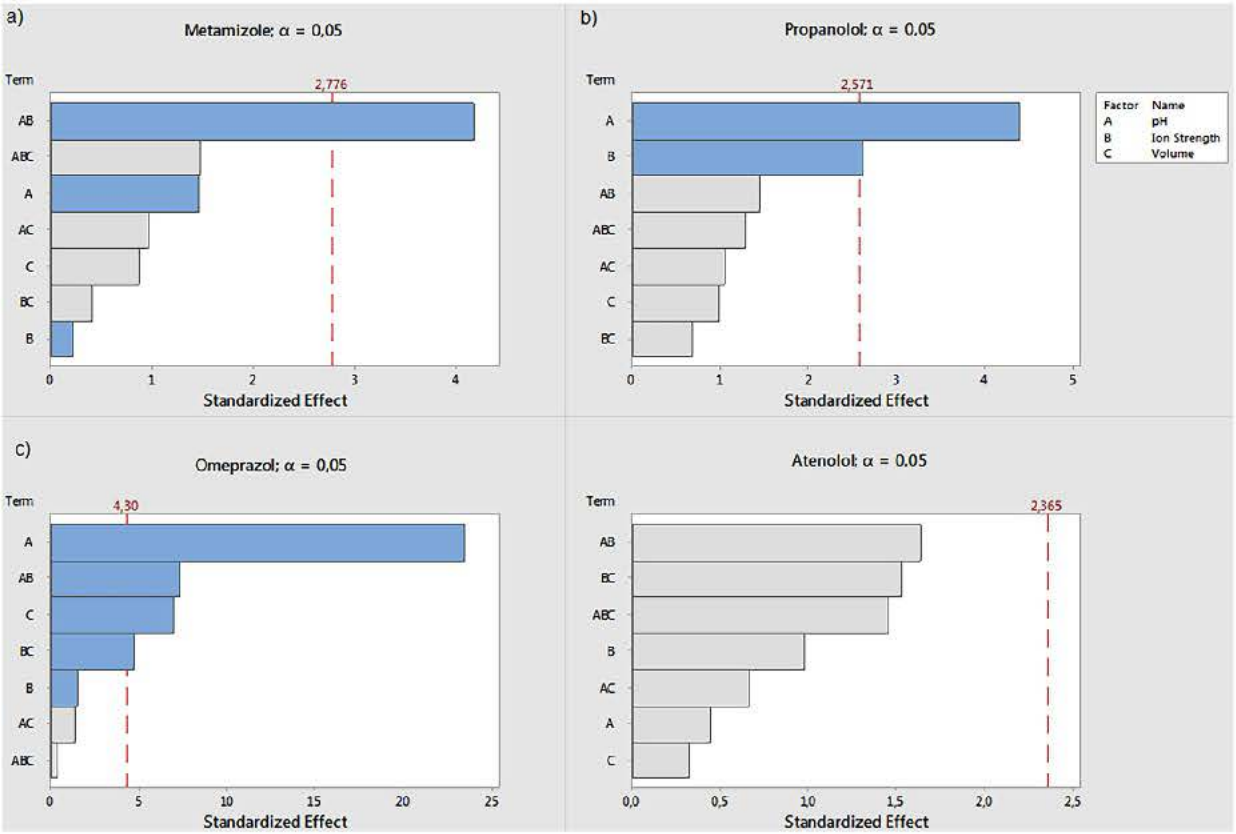

Fig. 1. Pareto charts of the standardized effects for different behaviours of certain pharmaceuticals.

volumes show an average value of −0.365, therefore, there is a very weak dependence of this variable in the extraction process. Moreover, the ratio is negative in all cases, so a smaller sample volume provides better results. Taking this last statement into account, we decided to fix 250 mL as an intermediate value for the sample volume to obtain a good preconcentration factor. After studying the significance of each variable and the interaction between them, the variables that most affect the process were individually assessed due to the difficulty of achieving a compromise between parameters for target compounds that have different physical and chemical properties.

3.1.2. *pH of sample*

The adsorption of each compound by the sorbent largely depends on sample pH. For this reason, this parameter has been optimized using a wide range of values in order to achieve a better process efficiency. Five different pH values (3, 5, 7, 9 and 11) were evaluated. A decrease in signal was shown at pH 3 for most compounds. Some compounds, such as NICO and ATE, obtained a recovery less than 10%. Moreover, pH 9, an intermediate pH between 7 and 11, did not offer satisfactory results. Therefore, the recoveries from three pH values with the best responses (5, 7 and 11) are plotted in Fig. 2.

The results shown at these three levels are highly similar for each compound. The compounds with neutral molecules at a lower pH (less than 6), such as MDZ and PRX, offer a greater extraction efficiency (higher than 80%) at pH 5. The majority of the compounds with an intermediate pK_a (less than 10), such as ATE, CAFF and TRIM, have recoveries above 50% at pH 7, and only five compounds have recoveries less than 40% at this pH. Some reviews, such us that presented by Kruve et al. [31], propose acceptable recovery between 70 and 120%. However, due to the different characteristics of the compounds in this multiresidue method, it is necessary amplify this range. In this sense, we have established acceptable recoveries between 40 and 120% in this optimization stage according to other authors and guidance documents, where suitable recovery percentages as a function of the analyte concentration have been defined [32–34]. Although the behaviour at pH 11 was similar to that of pH 7, pH 7 was chosen as the optimum value for further studies because it offers less number of compounds with efficiencies below 40%.

3.1.3. *Ionic strength*

The other parameter that may influence in the extraction efficiency is the ionic strength. In the present study, we have evaluated the ionic strengths of 0%, 5% and 10% NaCl solutions. In Fig. 3, the recoveries from the various salt percentages of show three types of behaviour: (i) some compounds, such as CAFF, IBU, NICO or GMF, maintain similar recoveries and are independent of salt percentage; (ii) some compounds show an increasing extraction efficiency when the ionic strength is also increased, as in the case of ERY, where the maximum efficiency is obtained with 10% NaCl, or in the case of OFLO, where the maximum is reached at an intermediate value of 5%; and (iii) other compounds (ATE, MTZ, OME and TRIM) are weakly affected by the addition of salt.

Therefore, because of the different physicochemical characteristics of the target compounds, a compromise of 0% NaCl was chosen as the optimal ionic strength. This selection was based on LC–MS/MS maintenance issues. The addition of salt in the sample, which is not totally removed in the sample preparation procedure, produces equipment malfunction and worsens the accuracy of the method.

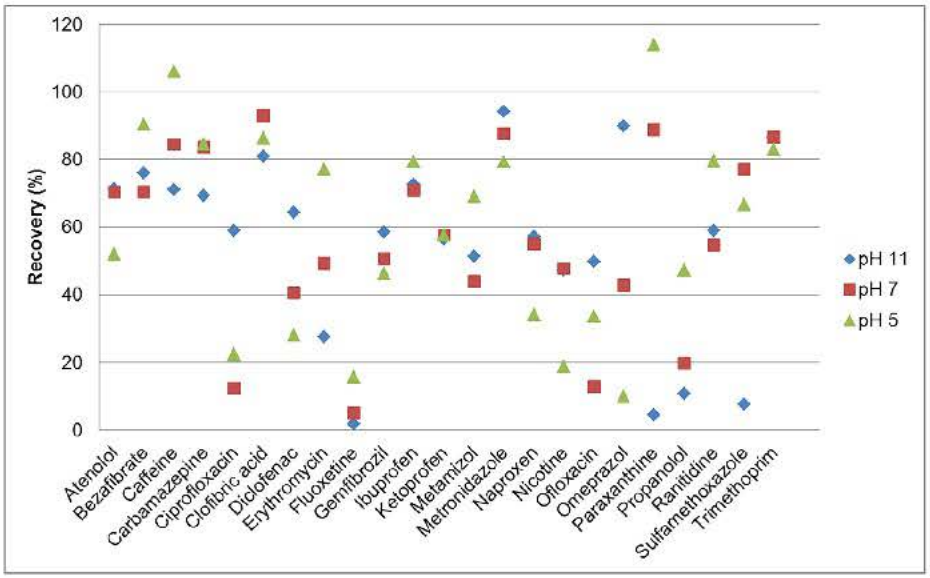

Fig. 2. Achieved recoveries as a function of pH.

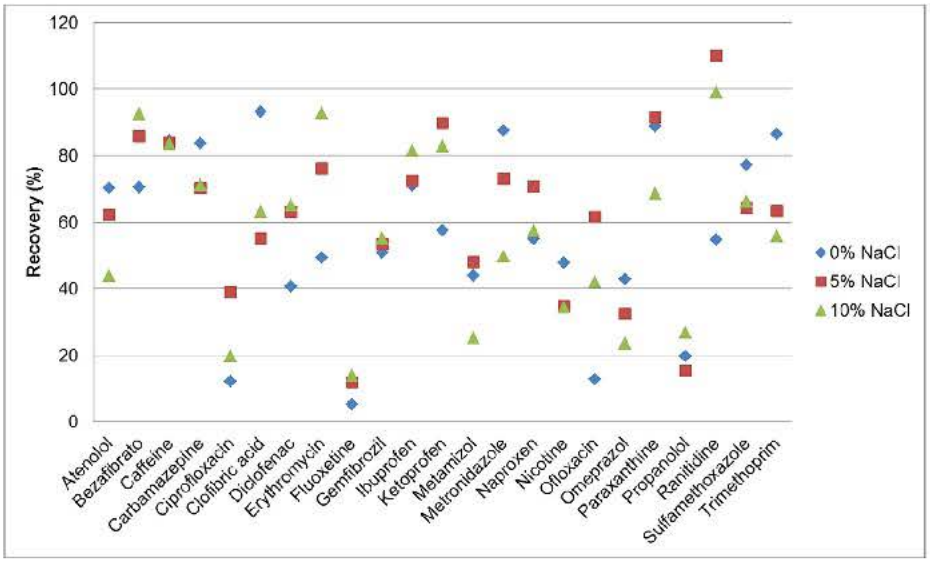

Fig. 3. Comparative study using various levels of ionic strength.

3.1.4. Type of solvent

The efficiency of the extraction can be affected by the type of eluent. Therefore, we have assessed the effect of methanol and acetonitrile (5 mL) in the elution step. As shown in Fig. 4, methanol was the optimal solvent to extract the target compounds.

In summary, the optimum extraction process directly begins with a charge step where 250 mL of the sample at pH 7 and 0% NaCl was used; subsequently, 5 mL of water was added as a wash step, and finally, the elution step with 5 mL of methanol was utilized, therefore, it has been removed conditioning and equilibration steps. In this sense, this extraction process aims to follow the GAC principles implemented for pharmaceutical analysis. With this method, the use of hazardous solvents and sample preparation steps are reduced, which simplifies the entire extraction process.

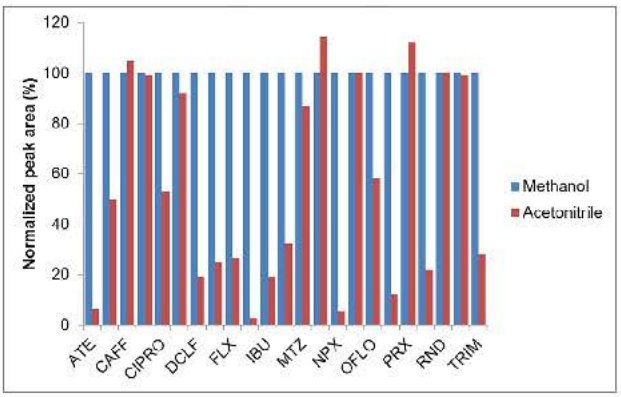

Fig. 4. Effect of eluent type in the extraction efficiency of the SPE method.

Table 2
Linearity and analytical parameters of the optimized SPE-LC–MS/MS method from Milli-Q water samples at initial concentrations of 200 and 800 ng L^{-1}.

Compound	Linearity range (μg L^{-1})	Regression coefficient	Recovery[a] (%)		MDL[b] (ng L^{-1})	MQL[c] (ng L^{-1})	RSD Intra-day[d] (%)		RSD Inter-day[d] (%)	
			200 ng L^{-1}	800 ng L^{-1}			200 ng L^{-1}	800 ng L^{-1}	200 ng L^{-1}	800 ng L^{-1}
ATE	0.5–1000	0.9955	99.60 ± 7.15	94.30 ± 7.22	0.469	1.56	10.1	5.48	15.9	15.6
BZF	1.0–1000	0.9948	76.97 ± 7.47	80.79 ± 4.98	0.853	2.84	6.23	6.94	16.3	18.8
CAFF	1.0–30,000	0.9934	75.99 ± 2.60	67.50 ± 2.41	2.35	7.84	11.4	7.92	13.3	9.04
CBZ	0.5–1000	0.9968	86.83 ± 4.41	80.76 ± 0.63	0.200	0.668	8.22	14.0	12.5	8.38
CIPRO	10–1000	0.9940	52.45 ± 19.82	16.34 ± 2.81	33.3	111	9.20	5.96	19.9	14.7
CLOF	0.5–1000	0.9904	50.61 ± 4.30	60.30 ± 4.07	0.278	0.928	8.37	7.05	9.49	18.2
DCLF	1.0–1000	0.9933	56.98 ± 4.07	71.26 ± 7.66	1.93	6.43	8.15	11.1	18.1	15.0
ERY	5.0–1000	0.9941	52.86 ± 5.47	39.73 ± 3.70	4.17	13.9	10.3	9.31	15.9	11.5
FLX	0.5–1000	0.9947	23.47 ± 0.31	12.56 ± 2.65	0.259	0.864	7.41	10.5	14.2	21.2
GMF	0.5–1000	0.9910	61.05 ± 6.13	62.12 ± 2.64	0.400	1.33	12.7	10.9	17.6	19.8
IBU	5.0–10000	0.9986	57.76 ± 3.96	87.83 ± 5.74	8.33	27.8	7.64	13.3	9.40	14.7
KPF	1.0–1000	0.9928	79.83 ± 3.32	96.37 ± 2.94	1.32	4.39	8.04	9.14	19.7	11.7
MTZ	1.0–5000	0.9917	24.87 ± 2.10	38.75 ± 7.75	3.43	11.4	12.2	6.20	14.0	16.7
MDZ	1.0–1000	0.9910	41.74 ± 3.66	51.21 ± 4.66	1.54	5.14	12.4	6.85	22.0	14.1
NPX	5.0–5000	0.9960	58.93 ± 2.68	82.03 ± 1.46	5.45	18.8	9.84	8.81	16.9	14.2
NICO	5.0–5000	0.9994	79.16 ± 16.77	103.7 ± 4.2	20.7	69.0	5.61	9.40	12.0	19.1
OFLO	10–1000	0.9928	20.07 ± 10.99	13.82 ± 7.89	35.3	118	8.87	12.1	15.3	20.1
OME	0.5–1000	0.9902	83.19 ± 6.84	74.33 ± 14.19	0.227	0.758	6.94	7.23	18.3	19.1
PRX	5.0–10000	0.9908	123.6 ± 3.97	117.8 ± 13.9	9.09	30.3	14.4	7.41	17.8	16.4
PNL	5.0–1000	0.9996	19.88 ± 2.42	38.37 ± 1.65	6.82	22.7	9.75	8.53	12.2	21.1
RND	5.0–1000	0.9904	39.49 ± 2.64	50.85 ± 5.76	5.17	17.2	12.5	14.4	12.7	15.6
STX	1.0–1000	0.9993	85.42 ± 2.01	77.08 ± 1.99	0.721	2.404	5.65	4.92	15.9	8.49
TRIM	0.5–1000	0.9944	75.43 ± 17.99	119.13 ± 1.43	0.350	1.167	11.6	6.12	16.8	19.0

[a] n = 3.
[b] Method detection limit.
[c] Method quantification limit.
[d] RSD: relative standard deviation (n = 6).

Table 3
Recoveries and their relative standard deviations (n = 3) from different kind of wastewater samples at two initial concentration levels (200 and 800 ng L^{-1}).

Compound	Effluent-Osmosis		Effluent-Rejection Osmosis		Effluent-Microfiltration		Influent	
	200 ng L^{-1}	800 ng L^{-1}	200 ng L^{-1}	800 ng L^{-1}	200 ng L^{-1}	800 ng L^{-1}	200 ng L^{-1}	800 ng L^{-1}
ATE	97.67 ± 5.67	102.6 ± 13.6	84.16 ± 4.73	101.4 ± 7.2	110.0 ± 6.9	105.5 ± 4.0	91.70 ± 2.87	75.38 ± 11.59
BZF	99.35 ± 1.29	101.6 ± 0.8	91.24 ± 2.72	89.81 ± 3.88	88.65 ± 3.84	113.8 ± 5.1	78.88 ± 9.58	86.59 ± 11.63
CAFF	50.25 ± 3.91	96.50 ± 0.57	84.95 ± 1.29	91.35 ± 9.09	114.3 ± 4.9	113.1 ± 6.3	123.6 ± 4.9	91.28 ± 7.26
CBZ	110.8 ± 14.2	108.1 ± 4.1	93.78 ± 0.56	108.0 ± 5.0	116.4 ± 3.2	110.8 ± 11.0	121.8 ± 5.1	103.3 ± 8.3
CIPRO	45.80 ± 5.96	23.97 ± 2.54	62.30 ± 6.08	62.29 ± 10.85	77.29 ± 6.20	62.80 ± 3.07	125.6 ± 12.5	74.28 ± 5.80
CLOF	96.75 ± 2.04	101.0 ± 5.6	105.5 ± 5.4	124.3 ± 10.4	103.7 ± 10.0	109.4 ± 5.5	92.60 ± 11.84	94.12 ± 13.73
DCLF	98.66 ± 4.20	96.04 ± 8.37	126.6 ± 17.1	112.5 ± 16.7	120.8 ± 16.4	97.50 ± 7.01	102.9 ± 9.3	84.95 ± 9.78
ERY	87.43 ± 0.87	18.62 ± 1.41	102.6 ± 3.0	94.74 ± 3.99	104.6 ± 28.6	125.5 ± 1.3	125.1 ± 6.5	99.94 ± 9.29
FLX	30.29 ± 5.75	23.30 ± 2.34	60.28 ± 15.62	51.13 ± 8.96	114.1 ± 23.1	110.7 ± 20.8	123.3 ± 16.8	128.2 ± 6.8
GMF	100.3 ± 8.4	90.85 ± 8.51	95.50 ± 16.89	103.1 ± 32.5	93.41 ± 5.59	98.72 ± 6.11	96.10 ± 14.26	97.93 ± 11.70
IBU	93.16 ± 6.94	100.2 ± 4.8	110.2 ± 5.1	95.29 ± 0.67	92.03 ± 2.76	97.51 ± 2.34	89.17 ± 5.51	86.57 ± 13.39
KPF	99.72 ± 3.20	103.9 ± 9.4	110.7 ± 3.5	106.3 ± 0.9	116.1 ± 5.8	112.8 ± 7.2	90.06 ± 7.18	100.2 ± 13.0
MTZ	28.00 ± 0.37	30.88 ± 3.00	95.47 ± 7.84	51.81 ± 0.63	105.8 ± 5.6	94.73 ± 5.39	86.71 ± 7.77	79.78 ± 15.80
MDZ	101.7 ± 9.3	108.8 ± 16.8	88.66 ± 15.12	101.9 ± 12.3	109.4 ± 5.0	98.24 ± 10.54	86.23 ± 4.14	75.58 ± 17.24
NPX	97.21 ± 7.15	94.87 ± 7.99	109.7 ± 9.5	100.2 ± 4.02	101.9 ± 7.2	103.6 ± 11.9	98.84 ± 6.18	103.7 ± 10.5
NICO	106.9 ± 2.0	79.92 ± 5.58	75.12 ± 11.59	103.3 ± 12.7	101.7 ± 5.4	103.6 ± 0.9	79.41 ± 6.43	73.37 ± 14.31
OFLO	58.83 ± 6.46	25.18 ± 3.00	122.3 ± 6.6	86.69 ± 9.71	81.39 ± 3.12	97.24 ± 4.67	122.3 ± 11.9	79.80 ± 16.97
OME	72.22 ± 15.55	39.27 ± 4.89	107.3 ± 3.0	104.8 ± 15.6	94.80 ± 15.46	129.6 ± 3.9	24.49 ± 9.43	20.70 ± 1.04
PRX	118.9 ± 17.5	108.5 ± 9.8	107.3 ± 12.3	110.3 ± 11.3	107.1 ± 2.6	89.13 ± 9.29	91.13 ± 2.27	75.12 ± 19.12
PNL	38.02 ± 4.99	9.453 ± 0.187	57.72 ± 3.83	57.44 ± 3.80	101.1 ± 20.0	115.1 ± 16.8	131.9 ± 16.3	106.0 ± 25.9
RND	76.13 ± 9.78	88.28 ± 14.33	88.34 ± 4.96	71.44 ± 2.95	89.39 ± 10.15	76.80 ± 8.75	129.6 ± 8.9	67.94 ± 2.17
STX	105.6 ± 9.2	87.63 ± 4.55	105.0 ± 6.6	88.36 ± 1.91	106.8 ± 10.3	109.6 ± 4.5	90.77 ± 4.30	82.21 ± 3.89
TRIM	122.4 ± 23.0	95.36 ± 12.60	112.3 ± 11.3	75.44 ± 1.10	110.6 ± 2.5	101.7 ± 5.0	86.48 ± 1.31	71.78 ± 2.94

3.2. Analytical parameters

The precision, accuracy and selectivity of the SPE-LC–MS/MS method (as shown in Tables 2–4, respectively) were evaluated for linearity, recovery, repeatability (intra- and inter-day), method detection limit (MDL) and method quantification limit (MQL).

External calibration curves were prepared in the range between 0.5 and 30000 μg L^{-1} (the range of each compound is shown in Table 2). Three internal standards (Ad7, Sd4 and Id3) at a fixed concentration of 50 μg L^{-1} were added to each calibration level. Whenever possible, the relationship between the peak areas and the concentrations of the compounds and internal standards was used to calculate the linearity. Determination coefficients (r^2) higher than 0.9902 were obtained.

The intra- and inter-day repeatability of the method was evaluated using six spiked Milli-Q water samples with target compounds at the same two initial concentration levels (200 and 800 ng L^{-1}) per day. The elapsed time after the inter-day variability was 48 h, while the intra-day variability was executed at the same time. As is shown in Table 2, both repeatability values were suitable, and, in all cases, the relative standard deviations were below 14.4% and 22.0% for intra- and inter-day repeatability, respectively.

Five different water matrices (Milli-Q water, water effluent from the tertiary treatment, rejection from the tertiary treatment, water

effluent from the secondary treatment and water influent samples) were used to study the recoveries of the extraction method. Spiked samples before the SPE procedure were compared with the spiked final extracts of wastewater samples after the drying process, with the exception of the Milli-Q water sample where the direct injection of the standard solution was employed. This comparison was performed at two initial concentration levels (200 and 800 ng L^{-1}) in triplicate. The results are shown in Tables 2 and 3. It was found that 82.6% of the data have a median recovery above 70% for all compounds in each sample, while the remaining results have a median recovery between 55.7% and 65.8%. Several differences exist between the matrices. A yield increase was produced for several compounds using wastewater samples in comparison with Milli-Q water. This fact could be due to the presence of a slightly greater ionic strength. In contrast, a considerable decrease of recovery percentage was achieved for some compounds, such as OMP and PRX, in the influent sample, which is a complex matrix with a high organic content.

The method detection and quantification limits (MDLs and MQLs), defined as the concentration that produces a signal to noise ratio of 3 and 10, respectively, were calculated for the same five sample types for each compound (Tables 2 and 4). The MDLs and MQLs ranged from 0.011 to 188 ng L^{-1} and from 0.3 to 628 ng L^{-1}, respectively, in all liquid samples. However, it was observed that the MDL only exceeds 100 ng L^{-1} in a few cases (CIPRO, CAFF, IBU, NPX and PRX).

The obtained limits are appropriate for the analysis of the twenty-three pharmaceuticals from sewage samples and are very near the range of limits achieved by other studies. For example, Petrović et al. [35] developed an SPE procedure and an ultra-high-performance liquid chromatography method coupled to mass spectrometry with a hybrid triple quadrupole–linear ion trap (UPLC–QqLIT–MS/MS) investigate a large list of pharmaceutical compounds (twenty-three) from water samples, and their limits of detection ranged from 0.2 to 26 ng L^{-1} for effluent wastewater. In addition, Zhao et al. [24] studied nine residual pharmaceuticals from influent and effluent wastewater samples using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QToF- MS) after an SPE procedure. Despite the use of a different detection system, the limits of detection were higher than the limits reached in this study (0.05–1.34 ng mL^{-1}).

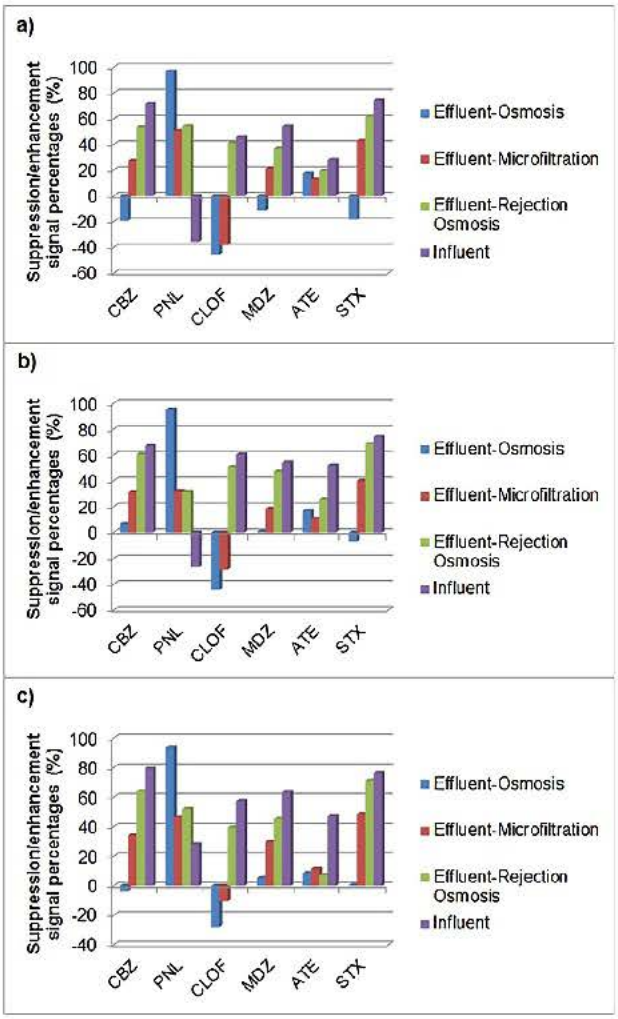

Fig. 5. Signal suppressions/enhancements for a group of target pharmaceuticals in different matrices. a) 200 ng L^{-1} b) 800 ng L^{-1} c) 2000 ng L^{-1}.

3.3. Matrix effect

When electrospray is used as the ion source of a mass spectrometer detector, it may produce signal suppression in the analysis of complex matrices due to the interferences extracted along with the target compounds. To evaluate this phenomenon in four different matrices (effluent from the tertiary treatment, rejection from the tertiary treatment, effluent from the secondary treatment and influent samples), the following algorithm (Vieno et al. [36]) was used (Eq. (1)).

$$100 - \left(\frac{(A_{sp} - A_{usp}) \cdot 100}{A_s} \right) \quad (1)$$

where A_s corresponds to the peak area of the analyte in the pure standard solution, A_{sp} corresponds to the peak area in the spiked matrix extract (contaminated extract after drying) and A_{usp} corresponds to the matrix extract of a real sample. The matrix effect was evaluated at three initial concentration levels (200, 800 and 2000 ng L^{-1}).

The signal suppression/enhancement was too inconsistent for most of the compounds between different matrices, which have characteristics that hinder or stimulate the ionization. The percentages range from an enhancement of above 100% to a suppression up to 96%. In this sense, to minimize the different matrix effects of the samples, the use of internal standards or internal calibrations is necessary.

In general, the signal suppressions/enhancements were higher at low concentration levels; however, the behaviours of the compounds were widely constant in the whole range of analytical interest. The signal suppressions/enhancements from the three initial concentrations of a representative group of pharmaceuticals are plotted in Fig. 5. As shown, some target pharmaceuticals in few of the matrices, such as ATE or MDZ, indicate moderate suppression/enhancement signals (less than 20%), where the matrix effect is not considered because it may be due to analytical deviation. However, other compounds are affected by the matrix; for example, a negative matrix effect was increased for CBZ or STX when the amount of organic matter in the sample was higher. In contrast, a slight increase in the positive matrix effect for PNL was indicated when the organic matter was increased.

3.4. Analysis of wastewater samples

The applicability of the method was tested using the optimized method to determine the target pharmaceutical compounds in real wastewater samples from two WWTPs (1 and 2) located on the

Table 4
Method detection and quantification limits (ng L^{-1}) in different types of wastewater samples.

Compound	Effluent-Osmosis		Effluent-Rejection Osmosis		Effluent-Microfiltration		Influent	
	MDL[a]	MQL[b]	MDL[a]	MQL[b]	MDL[a]	MQL[b]	MDL[a]	MQL[b]
ATE	9.56	31.9	7.81	26.0	3.76	12.5	2.39	7.96
BZF	0.226	0.472	1.33	4.44	0.653	2.23	1.21	4.05
CAFF	1.65	5.51	31.4	104	29.3	97.6	188	628
CBZ	0.117	0.391	0.663	2.21	0.421	1.40	0.266	0.886
CIPRO	155	517	40.6	135	174	581	27.6	92.3
CLOF	0.162	0.486	0.402	1.34	0.327	1.03	0.641	2.11
DCLF	11.0	36.6	7.89	26.3	3.00	10.0	3.51	11.7
ERY	2.01	6.53	4.44	14.8	1.32	4.42	1.14	3.76
FLX	0.011	0.033	0.042	0.141	1.25	4.19	0.096	0.321
GMF	0.991	3.30	10.8	35.9	3.09	10.3	2.85	9.50
IBU	15.7	52.4	20.1	67.1	8.61	28.7	105	352
KPF	1.26	4.20	20.9	69.6	10.1	33.7	18.1	60.2
MTZ	7.71	25.6	4.14	13.8	3.43	11.5	3.25	10.8
MDZ	2.53	8.44	1.08	3.60	0.716	2.50	0.816	2.72
NPX	2.49	8.29	105	352	20.6	68.6	58.5	195
NICO	68.1	227	6.54	21.8	10.0	33.4	6.93	23.1
OFLO	29.7	98.8	20.7	68.9	26.6	88.8	24.3	81.1
OME	0.313	0.936	0.809	2.70	0.084	0.279	33.1	110
PRX	50.2	167	48.2	160	11.4	38.0	187	625
PNL	12.5	41.7	2.35	7.84	3.65	12.2	9.46	31.5
RND	3.22	10.6	6.10	20.2	3.92	13.2	4.06	13.5
STX	0.480	1.60	2.81	9.37	3.42	11.4	4.21	14.0
TRIM	6.33	21.1	3.34	11.1	2.23	7.44	2.99	9.96

[a] Method detection limit.
[b] Method quantification limit.

Table 5
Concentrations (μg L^{-1}) determined in wastewater samples (n = 3) at different points in WWTP1 and WWTP2.

Compound	WWTP1				WWTP2			
	Point 1.1	Point 1.2	Point 1.3	Point 1.4	Point 2.1	Point 2.2	Point 2.3	Point 2.4
ATE	1.91 ± 0.14	0.676 ± 0.063	0.939 ± 0.068	0.217 ± 0.019	2.72 ± 0.22	2.14 ± 0.22	1.99 ± 0.12	0.131 ± 0.018
BZF	0.610 ± 0.072	0.108 ± 0.018	0.197 ± 0.009	nd[b]	0.013 ± 0.002	0.393 ± 0.035	0.423 ± 0.017	0.025 ± 0.004
CAFF	49.1 ± 2.47	1.26 ± 0.05	1.92 ± 0.99	0.099 ± 0.014	70.7 ± 5.5	67.1 ± 3.9	91.5 ± 3.1	9.97 ± 0.35
CBZ	0.265 ± 0.035	0.571 ± 0.006	1.05 ± 0.68	0.084 ± 0.002	2.07 ± 0.18	0.737 ± 0.003	0.857 ± 0.068	0.930 ± 0.037
CIPRO	0.646 ± 0.085	<MQL[a]	0.352 ± 0.054	<MQL	1.84 ± 0.46	0.832 ± 0.075	nd[b]	nd
CLOF	nd	nd	nd	nd	nd	nd	nd	nd
DCLF	0.292 ± 0.043	0.350 ± 0.053	0.515 ± 0.052	nd	0.295 ± 0.021	0.638 ± 0.057	0.839 ± 0.064	1.03 ± 0.09
ERY	nd	nd	nd	nd	nd	nd	nd	nd
FLX	0.277 ± 0.007	0.057 ± 0.010	0.095 ± 0.017	0.005 ± 0.001	0.135 ± 0.015	0.106 ± 0.018	0.113 ± 0.014	0.085 ± 0.003
GMF	1.08 ± 0.16	1.94 ± 0.05	3.16 ± 0.43	0.125 ± 0.010	2.31 ± 0.22	1.73 ± 0.12	1.64 ± 0.16	2.15 ± 0.11
IBU	18.2 ± 1.0	0.004 ± 0.003	0.167 ± 0.011	nd	8.46 ± 1.20	17.5 ± 0.4	22.6 ± 0.3	26.6 ± 2.3
KPF	2.43 ± 0.28	1.09 ± 0.08	1.82 ± 0.24	0.074 ± 0.007	0.260 ± 0.035	2.29 ± 0.065	1.93 ± 0.21	1.30 ± 0.05
MTZ	4.81 ± 0.35	nd	nd	nd	15.5 ± 1.3	15.0 ± 1.3	15.1 ± 1.7	14.0 ± 2.2
MDZ	0.044 ± 0.003	0.033 ± 0.002	0.059 ± 0.001	0.043 ± 0.006	nd	nd	nd	nd
NPX	8.66 ± 0.43	0.405 ± 0.046	0.915 ± 0.035	0.038 ± 0.009	3.47 ± 0.64	9.58 ± 0.86	10.5 ± 1.1	3.31 ± 0.20
NICO	10.2 ± 1.4	0.230 ± 0.015	0.298 ± 0.040	0.409 ± 0.116	13.0 ± 0.5	16.4 ± 1.5	9.89 ± 0.67	0.091 ± 0.020
OFLO	1.16 ± 0.15	0.346 ± 0.058	0.751 ± 0.052	0.128 ± 0.025	0.183 ± 0.022	0.177 ± 0.001	nd	nd
OME	nd	0.023 ± 0.003	0.064 ± 0.014	nd	0.144 ± 0.011	0.058 ± 0.003	0.050 ± 0.009	0.119 ± 0.012
PRX	24.7 ± 1.2	0.148 ± 0.032	0.820 ± 0.004	0.819 ± 0.322	21.0 ± 2.3	19.7 ± 1.6	23.0 ± 2.2	8.03 ± 1.49
PNL	0.378 ± 0.053	0.151 ± 0.007	0.205 ± 0.017	nd	0.300 ± 0.030	nd	nd	nd
RND	0.721 ± 0.045	nd	nd	nd	0.857 ± 0.042	0.172 ± 0.034	<MQL	nd
STX	0.783 ± 0.119	0.023 ± 0.003	0.022 ± 0.002	0.085 ± 0.002	nd	nd	nd	nd
TRIM	0.319 ± 0.029	0.152 ± 0.012	0.183 ± 0.001	0.063 ± 0.002	nd	nd	nd	nd

[a] Below method quantification limit.
[b] Not detected.

island of Gran Canaria (Spain). Both WWTPs employ completely different treatments to purify the wastewater. WWTP1 is in an urban environment with a conventional activated sludge system coupled to a tertiary treatment (reverse osmosis) system and is designed to treat 18,000 m^3 day^{-1} of domestic and industrial wastewaters. WWTP2 is in a rural environment and is based on a natural purification process designed to treat domestic sewage from a population of 500 inhabitants.

Four points in both WWTP1 and WWTP2 were chosen as follows: influent (Point 1.1), after the microfiltration process and before the reverse osmosis treatment (Point 1.2), reverse osmosis concentrate (Point 1.3) and the treated water after reverse osmosis (Point 1.4) from WWTP1 and after a bar screen (Point 2.1), after an injection treatment of effective microorganisms (Point 2.2), from a vertical filter (Point 2.3) and horizontal flow (Point 2.4), which had a gravel substrate in the presence of plants, from WWTP2.

Table 5 shows the occurrence of target compounds in each studied point. Even though the feed water of both wastewater treatment plants comes from different sources, a comparison of both influents indicates similar concentration ranges: 0.004–49.1 μg L^{-1} and 0.013–91.5 μg L^{-1} for WWTP1 and WWTP2, respectively. Notably, the concentrations of some compounds such as CAFF and MTZ are significantly higher in WWTP2 than WWTP1, which could be due to a further dilution of these compounds in WWTP1.

In contrast, there are differences in the results from the effluents, which show a difference in the yield of the treatment processes. The concentration ranges are 4.74–819 ng L^{-1} and 0.025–26.6 μg L^{-1} for the advanced and natural treatments, respectively. Nevertheless, if we compare the conventional activated sludge process (Point 1.2) and natural treatment (Point 2.4), we may conclude that the latter is a promising treatment.

In each WWTP assessment, the results show a successive decrease in the detected concentration of all target compounds in Points 1.1, 1.2 and 1.4; however, the range of concentration in Point 1.3 is higher that in Point 1.2 because the sample is concentrated by this process. In contrast, WWTP2 does not have a standard behaviour of progressive reduction. For example, some compounds, such as GMF and MTZ, maintain their concentration levels throughout the process. For others, an increase in the levels occurs during the treatment process, as in the case of IBU. These irregular behaviours could be due to residence times, but the assessment was not possible because the scarcity of samples.

More extensive monitoring studies are necessary to give a more accurate view. Nevertheless, the number of samples is sufficient to evaluate the applicability of the method.

4. Conclusions

A simplified solid-phase extraction combined with the LC–MS/MS method was optimized and successfully applied to wastewater samples for the determination of twenty-three commonly used pharmaceutical compounds.

In accordance with the GAC principles, the established method was demonstrated to be suitable to reduce the time and amount of solvent used to analyse pharmaceuticals in wastewater samples. Moreover, the simplified SPE-LC–MS/MS method has satisfactory median recovery percentages, mostly above 70%, and their relative standard deviations were below 14.4% and 22.0% for intra- and inter-day repeatability, respectively. The MDLs and MQLs ranged from 0.011 to 188 ng L^{-1} and from 0.033 to 628 ng L^{-1}, respectively, which are appropriate for the analysis of target compounds that appear in environmental liquid samples.

Eighteen pharmaceuticals were detected in all samples from both WWTPs, which ranged from 0.004 to 91.5 μg L^{-1}. CLOF and ERY were not found in any sample, while MDZ, STX and TRIM only appear in WWTP1. A reduction in concentration levels was assessed through the treatment process in WWTP1; however, an irregular behaviour in the concentration level was observed in WWTP2. More extensive monitoring in future studies is required to obtain more specific conclusions.

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III.2. Aplicaciones

Tras la optimización de un método multiresiduo de extracción, detección y determinación de residuos farmacéuticos en muestras líquidas ambientales, a continuación se presentan los trabajos donde se ha podido aplicar directa o indirectamente dicha metodología.

Un primer trabajo proporciona la monitorización de fármacos en muestras procedentes de estaciones depuradoras de aguas residuales, como punto de partida para conocer el alcance del problema en la isla de Gran Canaria, analizando incluso el riesgo que podría producir su descarga al medioambiente.

De forma complementaria y tras conocer el déficit de eficacia de eliminación de fármacos con sistemas convencionales de tratamiento de aguas residuales, otros dos trabajos son dirigidos al uso de la metodología analítica como herramienta para el desarrollo de dos sistemas alternativos de eliminación de fármacos basado en los procesos avanzados de oxidación.

III.2.1. Presencia e impacto ambiental de residuos farmacéuticos en plantas de tratamiento de aguas residuales convencional y natural de la isla de Gran Canaria (España).

La presencia y el destino de residuos farmacéuticos en muestras ambientales son de gran interés debido a los posibles riesgos toxicológicos que pueden producir. La fuente principal de entrada de estos contaminantes en el medioambiente son las estaciones depuradoras de aguas residuales (EDARS).

Aunque en España se ha proporcionado un gran número de estudios sobre la presencia y el posible riesgo de los compuestos farmacéuticos en muestras líquidas procedentes de EDARs, en Canarias existe una falta de información respecto a esta cuestión. Por otra parte, la hidrología en esta región es totalmente diferente a la encontrada en la Península Ibérica, contando con una gran escasez de recursos hídricos que hace esencial la reutilización del agua depurada y la desalinización de agua de mar. Por lo tanto, es realmente interesante la recolección, de forma sistemática y amplia, de datos sobre la presencia de productos farmacéuticos en esta área.

Por ello, como aplicación directa del método analítico multiresiduo optimizado, basado en una técnica simplificada de extracción en fase sólida (SPE) acoplada a la cromatografía líquida con espectrometría de masas (LC-MS/MS) para la extracción y determinación de veintitrés compuestos farmacéuticos de diferentes clases terapéuticas de uso común, se ha efectuado un monitoreo de manera quincenal durante un periodo de seis meses para establecer la presencia de estos compuestos en muestras líquidas procedentes de dos EDARs localizadas en la isla de Gran Canaria. Una de las EDARs posee un tratamiento convencional basado en un sistema de lodos activo seguido de un sistema de ósmosis inversa, mientras que la otra está caracterizada por el uso de un tratamiento natural mediante humedales. También se ha evaluado la eficacia de esos tratamientos en la eliminación de compuestos farmacéuticos, para conocer si existe la necesidad de aplicar nuevos procedimientos de purificación más avanzados.

Además, otro de los objetivos de este trabajo ha sido la evaluación predictiva del impacto ambiental que puede llegar a producir la presencia de estos compuestos en los efluentes de las EDARs sobre diferentes organismos acuáticos (algas, dafnias y peces), mediante el cálculo del cociente de riesgo.

Con los estudios realizados, hemos comprobado que los niveles de concentración se encuentran en el intervalo de 0,004 ± 0,001 a 59,2 ± 11,7 $\mu g \cdot L^{-1}$ para la EDAR convencional, mientras que para el caso de la EDAR natural de humedales se encontró entre 0,018 ± 0,001 y 148 ± 14,7 $\mu g \cdot L^{-1}$.

En general, los porcentajes de eliminación en ambas EDARs indican una degradación evidente para una gran mayoría de los compuestos objeto de estudio. Aunque los niveles de concentración en ambas EDARs fueron similares, la EDAR convencional ofreció una mejor eficiencia, con una mediana de eliminación del 99,7 %. A pesar de ello, el tratamiento natural es capaz de reducir de manera importante los niveles de fármacos, alcanzando un porcentaje medio de eliminación de todo el proceso del 89,6 %. Por lo tanto, este tipo de tratamiento puede ser utilizado para el tratamiento de aguas residuales para poblaciones pequeñas en áreas rurales con un amplio espacio para construir humedales y, así, reducir el consumo energético.

En cuanto a la evaluación del impacto ambiental, la mayoría de los compuestos estudiados no presentan un riesgo significativo porque sus valores expresados como cociente de riesgo (RQ) fueron inferiores a 0,1. Sin embargo, gemfibrozilo, ibuprofeno y ofloxacino podrían producir un riesgo potencial para todos los organismos seleccionados, con valores de RQ por encima de la unidad (> 1). Además, otros compuestos, como

ciprofloxacino, ácido clofíbrico, diclofenaco, eritromicina, propanolol y sulfametoxazole, podrían producir impactos medianos o altos para al menos uno de los niveles tróficos inferiores (dafnias y/o algas), cuyos RQ oscilaron entre 0,102 y 27,0.

Sin duda, según los resultados obtenidos en este trabajo, las investigaciones futuras deben enfocarse en la mejora de la eficiencia de eliminación para alguno de los residuos farmacéuticos, por ejemplo, mediante tratamientos terciarios adicionales basados en procesos avanzados de oxidación, en el caso de las EDARs convencionales, o mejorando la distribución del proceso y la evaluación de nuevos sustratos para las EDARs naturales.

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Occurrence and environmental impact of pharmaceutical residues from conventional and natural wastewater treatment plants in Gran Canaria (Spain)

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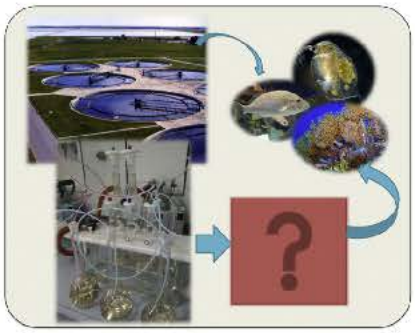
HIGHLIGHTS

- Twenty-three pharmaceuticals were monitoring in sewage from WWTPs.
- Simplified SPE-LC-MS/MS was employed to analyse pharmaceuticals from sewage samples.
- Removal efficiencies of pharmaceuticals from two different WWTPs were evaluated.
- Environmental risk assessment of pharmaceuticals was determined.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence and fate of pharmaceutical residues in environmental samples are of great interest. There is a vast number of studies published regarding their input, presence, effects and risks in ecosystems. Moreover, it has been demonstrated that the primary source of input of these contaminants in the environment is from Wastewater Treatment Plants (WWTPs). It is therefore essential to evaluate the efficiency of commonly used treatments and the necessity of applying novel purification processes in order to eliminate or reduce the concentration of pharmaceuticals from wastewater or from the effluent of WWTPs.

The aim of this work was to quantify twenty-three pharmaceutical compounds in the aqueous phase at different stages of a conventional and a natural WWTP situated in Gran Canaria (Spain). The results indicate concentration levels in the range of 0.004 ± 0.001 to $59.2 \pm 11.7\ \mu g\ L^{-1}$ and 0.018 ± 0.001 to $148 \pm 14.7\ \mu g\ L^{-1}$ from conventional and natural WWTPs, respectively. Better efficiency was, however, offered by the conventional WWTP with a removal median of 99.7%. In addition, the impact on different aquatic organisms (algae, daphnids and fish) was assessed in terms of risk quotients. The results reveal a possible highly harmful effect towards organisms by gemfibrozil, ibuprofen and ofloxacin.

1. Introduction

Advances in the pharmaceutical industry have been demonstrated due to the speed of scientific progress. <100 years have elapsed from

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large-scale manufacturing of pharmaceutical compounds to the first studies regarding to their recognition as environmental micropollutants (Halling-Sørensen et al., 1998; Pearson, 2007).

As commonly known, there is a wide variety of therapeutic classes that are used in both human and veterinary medicine to treat or prevent diseases, as well as husbandry growth promoters in agriculture. In this sense, the main pollution sources of these compounds to the environment is through industrial effluent, hospital effluent, septic tank and wastewater treatment plants (WWTPs) (Li, 2014).

Extensive studies provide information regarding the presence of these micropollutants in different environmental compartments (hydrosphere, soil and biota) (aus der Beek et al., 2016; Petrovic et al., 2004). Regarding the assessment of occurrence in the aquatic system, which is more broadly studied, it has reported that concentrations range from a few ng L^{-1} in drinking/tap water ("WHO, 2012") or seawater samples (Alygizakis et al., 2016; Arpin-Pont et al., 2016; Borecka et al., 2015), to μg L^{-1} in wastewater samples (Balakrishna et al., 2017; Guerra et al., 2014), while results obtained from sediment or sludge samples reveal concentration levels between ng kg^{-1} to μg kg^{-1} (dry weight) (Lees et al., 2016; Verlicchi and Zambello, 2015). Moreover, although in fewer publications, trace level concentrations have been observed in microorganisms, fish, mollusc or mammals (Arpin-Pont et al., 2016; Bottoni and Caroli, 2015; Roig and D'Aco, 2015).

Given this information, it is evident that the input of these pollutants into the environment is through bioconcentration, bioaccumulation and biomagnification processes, due to the inactivation resistance character of these pollutants (Fabbri, 2015; Zenker et al., 2014). Indeed, the removal or transformation of pharmaceuticals and their metabolites by WWTPs is partial and, in some cases, is very inefficient (Kaplan, 2013; Petrović et al., 2003; Wang and Wang, 2016). In this sense, the knowledge of concentration levels is necessary as a starting point to investigate other more advanced treatments, which could increase the removal rates and, thus, reduce the environmental risk.

Despite the massive amount of studies on the occurrence of pharmaceuticals in environmental compartments, less information exists regarding their actual hazards. This issue is a current concern for the scientific community and such information is gradually increasing. Some research even discloses the negative effects that pharmaceutical pollutants produce in organisms. It has been demonstrated that the continuous exposure to trace levels of certain pharmaceuticals can cause unexpected consequences and undesirable effects on ecosystems (Aubertheau et al., 2017). For example, Maranho et al. (2015), has assessed the effects produced in marine polychaetes (benthic biota), which are considered as a suitable bioindicator, by the exposure to sediment spiked with different pharmaceuticals in the concentration range of 0.01 to 500 ng g^{-1}. The conclusions of that study show that the pharmaceuticals have potential effects on organism health, survival, inflammation process and reproduction. Several studies have demonstrated the damaging impact on fish fecundity due to the endocrine-disrupting potential of some pharmaceuticals (reviewed by Overturf et al., 2015). Furthermore, the massive use of antibiotics has generated other major concerns, that is, the acceleration of antibiotic resistance in different bacteria (Ventola, 2015). Other studies have reported that sewage sludge contains antibiotics, antibiotic-resistant bacteria and antibiotic-resistance genes, all which arrive in the environment through land application of antibiotics (Bondarczuk et al., 2016). In the context of the European Water Framework (Directive 2000/60/EC of the European Parliament and of the Council of 23 October, 2000), studies of the impact of wastewater management on water resources is essential to formulate recommendations on emerging issue, such as, pharmaceuticals.

To perform a predictable evaluation of possible negative consequences, an environmental risk assessment (ERA) is normally established in term of risk quotients (RQs), as a comparison between measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) in different organisms. According to the criteria used by Ashfaq et al. (2017), an RQ with a value <0.1 indicates minimum risk to the organisms, the range between 0.1 and 1 implies medium risk and an RQ of 1 implies high risk. Many authors have used this kind of evaluation; for example, RQs of various pharmaceuticals in environmental liquid samples have been calculated for algae, crustaceans and fish, and their results show a potential risk for some pharmaceutical compounds (Ashfaq et al., 2017; Pereira et al., 2015; Zhao et al., 2016).

In Spain it is well known the issue of the presence and the possible risk caused by pharmaceutical compounds, due to the large number of studies that provide results in liquid samples from WWTPs. Regarding Canary Islands, there is lack information, only a few studies about the occurrence of a reduced number of pharmaceuticals from WWTPs (Afonso-Olivares et al., 2012; Guedes-Alonso et al., 2013; Montesdeoca-Esponda et al., 2015) have been reported. However, in no case, a multiresidue method to determine much more compounds in a single analysis has been employed. Moreover, the hydrology in this region is totally different to the encountered in Iberian Peninsula and it is essential the reuse of the reclaimed water and the desalination of seawater because of their scarcity of water resources. Thus, it is really interesting the collection of new data about the occurrence of pharmaceuticals in this area, and also, the investigation of the better treatment systems to avoid the pollution due to this kind of contaminants.

In this work, a simplified SPE multiresidue procedure, which is based on only three steps protocol (without conditioning and equilibration steps compared to the standard process), coupled with LC-MS/MS (Afonso-Olivares et al., 2017) was employed to detect and quantify twenty-three pharmaceutical compounds from wastewater samples. A fortnightly sampling from downstream and discharge from different control points during six months was carried out in two WWTPs, one of them with conventional treatment based in an activated sludge process and the other with natural treatment by constructed wetland, both located in Gran Canaria island (Spain). The purposes of this research were, on the one hand, to evaluate the occurrence of pharmaceuticals from WWTPs which existence is only due to human and veterinary uses or improper disposal because there is not any pharmaceutical industry in the geographical area studied and, on the other hand, to evaluate the wastewater treatments' efficiencies and the environmental risk that the presence of pharmaceuticals can produce to the environment.

2. Experimental

2.1. Materials

The representative pharmaceutical compounds belonging to different therapeutic classes, shown in Table 1, were purchased from Sigma-Aldrich (Madrid, Spain) with purities above 97%. The internal standards (IS) atenolol-d_7 (Ad7), ibuprofen-d_3 (Id3) and sulfamethoxazole-d_4 (Sd4) were acquired from Toronto Research Chemicals Inc. (Toronto, Canada), Sigma-Aldrich (Madrid, Spain) and Dr. Ehrenstorfer GmbH (Augsburg, Germany), respectively.

Working solutions were prepared daily at a concentration of 1 mg L^{-1} of pharmaceuticalsin Milli-Q water and 10 mg L^{-1} of IS in methanol, respectively, from stock solutions of each analyte. Stock solutions were prepared by dissolving the compound in methanol (1000 mg L^{-1}) and were stored in glass-stoppered bottles at −20 °C prior to use.

Methanol and water with LC-MS quality, obtained from Scharlab S.L. (Barcelona, Spain), were used to prepare the mobile phase for LC-MS/MS. The reagents used to adjust the pH of the mobile phase and the samples were purchased from Panreac (Barcelona, Spain). Ultra-high purity water, generated by a Milli-Q (Millipore, Bedford, MA, USA) water purification system, was used for the washing step of the solid-phase extraction and for preparing aqueous standard solutions.

Table 1
Classification of target compounds.

Therapeutic class	Compound	Abbreviation
Antibiotics	Trimethoprim	TRIM
	Metronidazole	MDZ
	Ofloxacin	OFLO
	Ciprofloxacin	CIPRO
	Sulfamethoxazole	STX
	Erythromycin	ERY
Antihypertensive	Atenolol	ATE
	Propanolol	PNL
Antiulcer	Ranitidine	RND
	Omeprazole	OME
Antidepressant	Fluoxetine	FLX
Antiepileptic	Carbamazepine	CBZ
Anti-inflammatory	Metamizole	MTZ
	Ketoprofen	KPF
	Naproxen	NPX
	Ibuprofen	IBU
	Diclofenac	DCLF
Lipid regulator	Bezafibrate	BZF
	Gemfibrozil	GMF
	Clofibric acid	CLOF
Stimulant	Nicotine	NICO
	Paraxanthine	PRX
	Caffeine	CAFF
IS	Atenolol d7	Ad7
	Sulfamethoxazole d4	Sd4
	Ibuprofen d3	Id3

2.2. Samples collection

The samples for the determination of pharmaceutical residues were collected from two WWTPs (WWTP1 and WWTP2) located in the southeast on the island of Gran Canaria (Spain) fortnightly during six months.

WWTP1 has a conventional active sludge process coupled with reverse osmosis as a tertiary treatment and was designed to treat 18,000 m^3 day^{-1} of sewage from a high density urban area, while WWTP2 is based on a natural process of purification to treat sewage from a rural area with a population of 500 inhabitants (Fig. 1).

Samples were taken from the following four different points in WWTP1 according to different treatments: influent (Point A), after the microfiltration process and before the reverse osmosis treatment (Point B), reverse osmosis concentrate (Point C) and treated water after reverse osmosis treatment (Point D). In addition, four other sampling points were selected from WWTP2: after a bar screen (Point E), after a subsequent treatment, in which the wastewater is injected with effective microorganisms (Point F), from a vertical flow wetland (Point G) and, finally, from a horizontal flow wetland (Point H). Both of the last treatments have gravel substrates in the presence of pruned branches and plants, respectively. A scheme of the sampling points from both WWTPs is shown in Fig. 1.

Pre-rinsed amber glass bottles of 1 L were used to collect the samples. Collected samples were then filtered through 0.65 μm polyvinylidene fluoride (PVDF) membrane filters from Merck Millipore (Cork, Ireland), acidified to pH 3 with formic acid and stored at 4 °C. The samples were extracted within 48 h.

2.3. Analytical method

For the assessment of target pharmaceuticals, we employed a methodology previously established by our research group that is based on a simplified solid phase extraction (SPE). This method consist on a procedure with three steps as a result of elimination of both conditioning and equilibration steps from the standard protocol of SPE thanks to the characteristics of used cartridge, and subsequent analysis by liquid chromatography tandem-mass spectrometry (LC-MS/MS) (Afonso-Olivares et al., 2017).

The employed analytical methodology provides method detection limits (MDLs) and method quantification limits (MQLs) ranged from 0.011 to 188 ng L^{-1} and from 0.033 to 628 ng L^{-1}, respectively, for water samples from different nature. Moreover, relative standard deviations (RSDs) of the method were below 14.4% for intra-day repeatability and 22.0% for inter-day repeatability.

2.3.1. Simplified SPE procedure

Briefly, 250 mL of liquid sample at pH 7 was passed through an Oasis HLB (6 cm^3, 500 mg) polymeric cartridge from Waters (Barcelona, Spain) using a manifold SPE system from Varian (Varian Inc., Madrid, Spain) with a capacity for 12 simultaneous extractions. Subsequently, 5 mL of Milli-Q water was used as a wash step. The cartridge charging in these last two steps was accomplished at a flow rate of 5 mL min^{-1}. Afterward, the cartridges were dried under vacuum for approximately 15 min and then were eluted with 5 mL of methanol at 1 mL min^{-1}. Blanks were run to assess any carryover during SPE. Finally, the extract was evaporated under a gentle nitrogen stream and reconstituted with 1 mL of an internal standard solution (a mixture of the three IS in Milli-Q water at 50 μg L^{-1}). The final extract was filtered through a 0.20 μm syringe filter to perform LC-MS/MS analysis.

2.3.2. Liquid chromatography tandem-mass spectrometry

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (Varian Inc., Madrid, Spain) consisting of a 212-LC binary gradient chromatography pump fitted with a Prostar 410 HPLC autosampler and a 320-MS system (triple quadrupole) equipped with an electrospray ionization (ESI) interface was used to analyse and quantify the final extract.

The stationary-phase was a 3.0 mm × 100 mm, 3.5 μm particle SunFireTM C18 column (Waters, Barcelona, Spain). The mobile phase gradient began with water (containing 0.015% formic acid) and methanol at 90:10 (v/v) for 1 min, then was changed to 60:40 (v/v) for 20 min and 10:90 (v/v) for 19 min and finally returned to its initial condition for 3 min. An equilibration time of 4 min was employed. The injection volume was 10 μL, and the flow rate was 200 μL min^{-1}.

2.4. Environmental risk assessment

RQs have been used to evaluate the environmental risk that the occurrence of these compounds from effluents could produce in the organisms after a discharge into the environment. The calculation of RQs was carried out by comparing the maximum measured environmental concentration (MEC) of the target pharmaceutical in liquid samples with the predicted no-effect concentration (PNEC) in different organisms (algae, daphnids and fish). The PNEC values for most of the pharmaceuticals were acquired from literature. Normally, PNEC is extrapolated through dividing EC_{50} values by an assessment factor, in this case, typically 1000 (Ashfaq et al., 2017; Ginebreda et al., 2010; Yamamoto et al., 2007).

3. Results and discussion

A total of 48 samples from four different sampling points for each WWTP were collected biweekly during the six months from May to November, coinciding with the end of spring through summer and the three months of autumn. Two WWTPs were evaluated in this study. WWTP1 has a conventional active sludge process with a subsequent microfiltration coupled with reverse osmosis as tertiary treatment, while WWTP2 has natural process of purification based on vertical and horizontal flow wetlands.

The results obtained from the determination of target compounds in samples are shown in Tables 2 and 3, where the frequency of detection and the median concentration of all analysed samples are given.

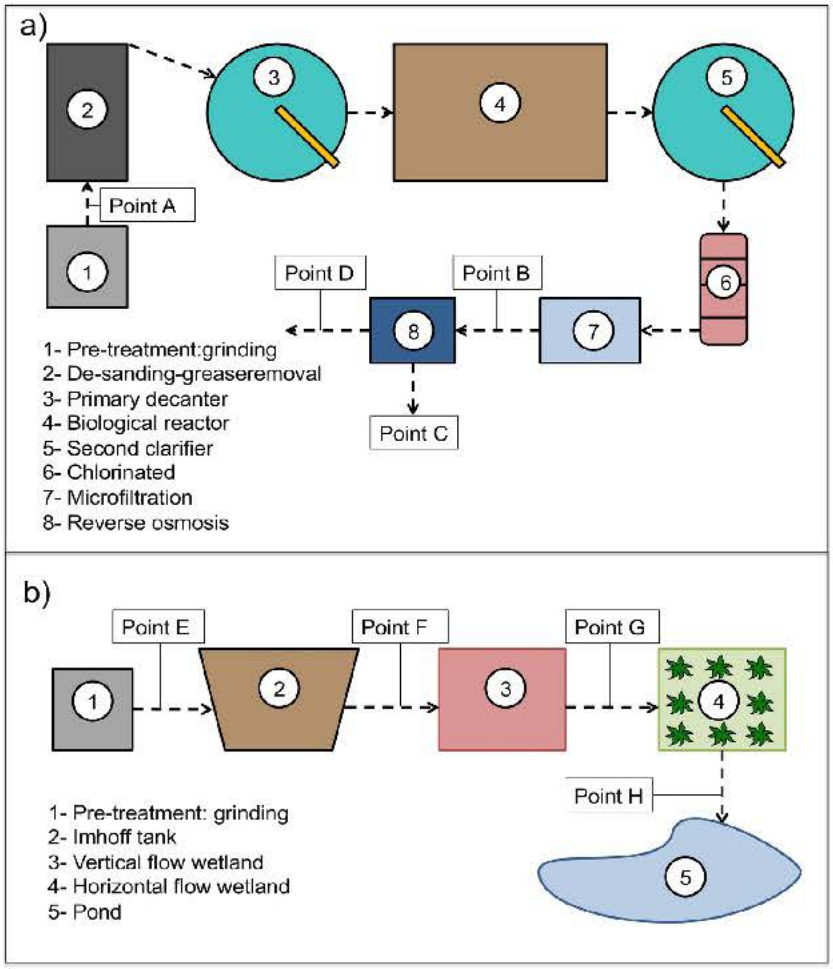

Fig. 1. Scheme of the treatments and sampling points from a) WWTP1 and b) WWTP2.

3.1. Conventional WWTP

Municipal WWTPs with conventional activated sludge process are specially designed to reduce soluble organic pollutants, suspended solids and flocculated matter in order to achieve high-quality effluent before environmental discharge. It is an ancient technique that is widely used in developed and underdeveloped countries for the treatment of wastewater. The treatment system is not, however, sufficiently efficient for the removal of microcontaminants due to their nature and low concentrations. The incorporation of additional tertiary treatment, such as, reverse osmosis, is, however, a viable option to remove these pollutants (Margot et al., 2015; Melvin and Leusch, 2016; Rodriguez-Mozaz et al., 2015).

The median of determined pharmaceutical concentrations in WWTP1 were in the range of 0.004 ± 0.001 to 59.2 ± 11.7 μg L^{-1} (Table 2). All target compounds with high percentages of occurrence frequency, except CLOF, ERY and FLX, were determined at Point A because the feed water comes from an area with a high density of population and industrial sites. The results in Table 2 show a successive decrease in the detected concentration (removal median > 99%, see Table 4) and a reduction of the occurrence frequency of all pharmaceuticals from Point A to Point D; however, the range of concentration in Point C was higher than in Point B because the sample was concentrated by this process. Half of the results in the influent samples reported a concentration over 1 μg L^{-1}. This level was not, however, exceeded in the first treatment process (Point B). Therefore, there was a significant reduction with a removal median up to 88% (more detail in Table 4) of all target compounds. In addition, there was evidence of several behaviours of the compounds during the purification process; for example, CBZ kept its occurrence and concentration levels during the secondary treatment of active sludge due to its high persistence and resistance to degradation (Deng et al., 2013). A decrease in CBZ was found only in the tertiary reverse osmosis treatment. On the other hand, RND was totally removed in the secondary treatment.

The characteristics of the samples and the levels of concentration play a vital role in the quantification procedure because these influence the limits of detection. For this reason, the detection of OME in Point C or a missing signal for CIPRO in Point B were observed.

The results were also compared in terms of seasonal fluctuations in the observed concentrations of pharmaceuticals in the analysed water samples. The monitoring of the seasonal variation in the concentration of 23 pharmaceutical compounds in WWTP1 did not, however, show any considerable variation, neither for the influent nor the effluents. This fact may be because the selected pharmaceuticals are highly consumed throughout the year and the temperature did not have any influence on the improvement of the yield.

3.2. Natural WWTP

Constructed wetlands in the middle of nature areas are often used as decentralized wastewater treatment systems for small communities. It is possible that such WWTPs may contribute efficiently to decreasing the pharmaceutical load to the environment.

The median of concentrations values between 0.018 ± 0.001 and 148 ± 14.7 μg L^{-1} was been found in WWTP2. As shown in Table 3,

Table 2
Occurrence, range of concentrations (median ± standard deviation), and detection frequency of target compounds in WWTP1.

Compound	Point A		Point B		Point C		Point D	
	Occurrence ($\mu g\ L^{-1}$)	Detection frequency (%)	Occurrence ($\mu g\ L^{-1}$)	Detection frequency (%)	Occurrence ($\mu g\ L^{-1}$)	Detection frequency (%)	Occurrence ($\mu g\ L^{-1}$)	Detection frequency (%)
ATE	0.940–2.12 (1.27 ± 0.342)	100	0.070–0.512 (0.170 ± 0.133)	100	0.213–1.09 (0.390 ± 0.265)	100	0.008–0.073 (0.039 ± 0.027)	41.7
BZF	0.068–0.832 (0.253 ± 0.212)	91.7	0.008–0.026 (0.017 ± 0.012)	16.7	0.014–0.193 (0.069 ± 0.047)	91.7	nd[a]	–
CAFF	36.6–59.1 (49.1 ± 7.72)	100	0.040–3.92 (0.288 ± 1.31)	66.7	0.154–8.24 (0.598 ± 2.59)	100	0.017–0.178 (0.098 ± 0.114)	16.7
CBZ	0.284–0.763 (0.443 ± 0.164)	100	0.143–0.517 (0.412 ± 0.112)	100	0.329–1.29 (0.965 ± 0.252)	100	0.011–0.026 (0.016 ± 0.007)	25.0
CIPRO	1.12–4.22 (1.97 ± 1.05)	100	>MDL[b]	–	0.071–0.242 (0.143 ± 0.055)	91.7	0.059–0.089 (0.065 ± 0.010)	66.7
CLOF	0.005	8.33	0.007–0.019 (0.013 ± 0.009)	16.7	0.007–0.033 (0.020 ± 0.011)	33.3	0.004	8.33
DCLF	0.113–0.453 (0.207 ± 0.127)	91.7	0.025–0.997 (0.086 ± 0.302)	83.3	0.100–1.95 (0.420 ± 0.550)	100	nd	–
ERY	0.051–0.102 (0.076 ± 0.036)	16.7	0.035–0.189 (0.039 ± 0.076)	33.3	0.036–0.540 (0.052 ± 0.186)	58.3	nd	–
FLX	0.165–0.207 (0.205 ± 0.024)	16.7	0.063–0.086 (0.075 ± 0.009)	41.7	0.082–0.101 (0.085 ± 0.009)	33.3	0.063	8.33
GMF	1.28–9.73 (5.41 ± 2.45)	100	0.276–1.48 (0.788 ± 0.355)	100	0.560–4.42 (1.60 ± 1.30)	100	0.008–0.138 (0.021 ± 0.049)	75.0
IBU	16.1–28.0 (20.8 ± 3.20)	100	0.029–1.81 (0.078 ± 0.876)	33.3	0.025–5.37 (0.087 ± 1.75)	75.0	0.021–0.150 (0.071 ± 0.055)	50.0
KPF	0.663–1.23 (0.991 ± 0.169)	100	0.378–1.13 (0.616 ± 0.248)	100	0.449–1.16 (0.773 ± 0.241)	100.0	0.152–0.257 (0.178 ± 0.037)	100
MTZ	5.92–12.3 (8.98 ± 1.87)	100	0.180–0.334 (0.257 ± 0.108)	16.7	nd	–	nd	–
MDZ	0.028–0.836 (0.168 ± 0.209)	100	0.002–0.068 (0.006 ± 0.037)	25.0	0.017–0.154 (0.037 ± 0.049)	66.7	nd	–
NPX	1.45–3.40 (2.51 ± 0.605)	100	0.077–0.433 (0.144 ± 0.093)	100	1.02	8.33	0.050–0.185 (0.111 ± 0.043)	100
NICO	9.55–20.9 (15.3 ± 3.91)	100	0.043–0.868 (0.097 ± 0.241)	91.7	0.076–0.720 (0.227 ± 0.179)	100	<MQL[c]	–
OFLO	0.746–2.28 (1.15 ± 0.409)	100	0.084–0.254 (0.134 ± 0.052)	100	0.121–1.01 (0.454 ± 0.275)	100	<MQL	–
OME	nd	–	nd	–	0.017	8.33	nd	–
PRX	11.0–20.9 (15.8 ± 3.24)	100	0.034–2.50 (0.331 ± 0.797)	100	0.132–4.33 (0.805 ± 1.34)	100	0.999	8.33
PNL	0.198–0.695 (0.425 ± 0.149)	83.3	0.059–0.149 (0.087 ± 0.028)	91.7	0.041–0.421 (0.263 ± 0.107)	83.3	<MQL	–
RND	0.966–2.43 (1.52 ± 0.457)	100	nd	–	nd	–	nd	–
STX	0.245–1.15 (0.748 ± 0.350)	100	0.150	8.33	0.005–0.341 (0.019 ± 0.121)	58.3	nd	–
TRIM	0.060–0.452 (0.201 ± 0.121)	100	0.029–0.143 (0.056 ± 0.032)	100	0.056–0.398 (0.165 ± 0.093)	100	0.031	8.33

[a] No detected.
[b] Method detection limit.
[c] Method quantification limit.

Table 3
Occurrence, range of concentrations (median ± relative standard deviation), and detection frequency of target compounds in WWTP2.

Compound	Point E		Point F		Point G		Point H	
	Occurrence (μg L^{-1})	Detection frequency (%)	Occurrence (μg L^{-1})	Detection frequency (%)	Occurrence (μg L^{-1})	Detection frequency (%)	Occurrence (μg L^{-1})	Detection frequency (%)
ATE	0.033–2.28 (0.384 ± 0.631)	100	0.607–2.05 (0.864 ± 0.426)	100	0.334–0.843 (0.551 ± 0.168)	100	0.029–0.211 (0.056 ± 0.059)	100
BZF	nd[a]	–	0.084–0.486 (0.173 ± 0.211)	25.0	0.018–0.628 (0.156 ± 0.288)	33.3	0.142–0.260 (0.173 ± 0.061)	25.0
CAFF	14.0–145 (52.7 ± 47.2)	100	23.9–68.0 (45.9 ± 18.7)	100	0.844–1.89 (1.58 ± 0.420)	100	0.019–3.26 (0.317 ± 0.969)	100
CBZ	0.281–3.03 (0.755 ± 0.942)	100	0.324–1.04 (0.970 ± 0.105)	16.7	0.571–1.34 (0.823 ± 0.243)	100	0.553–1.77 (0.946 ± 0.345)	100
CIPRO	0.094–2.25 (0.264 ± 0.635)	100	0.128–0.484 (0.181 ± 0.126)	100	>MDL[b]	–	>MDL	–
CLOF	nd	–	nd	–	nd	–	nd	–
DCLF	0.045–1.65 (0.429 ± 0.618)	75.0	0.178–3.91 (0.504 ± 1.12)	100	0.066–2.16 (0.951 ± 0.594)	100	0.460–2.24 (1.19 ± 0.603)	100
ERY	nd	–	0.034	8.33	nd	–	nd	–
FLX	0.077–0.155 (0.116 ± 0.055)	16.7	0.078–0.095 (0.078 ± 0.010)	25.0	nd	–	0.068–0.072 (0.070 ± 0.003)	16.7
GMF	0.126–45.2 (4.14 ± 12.43)	100	1.46–12.5 (7.17 ± 3.04)	100	5.09–14.8 (10.3 ± 2.80)	100	5.83–20.1 (11.2 ± 4.17)	100
IBU	1.15–56.3 (18.3 ± 19.3)	91.7	4.31–27.5 (16.6 ± 6.89)	91.7	10.8–27.4 (17.9 ± 5.13)	91.7	10.0–21.7 (17.2 ± 3.52)	91.7
KPF	0.116–24.3 (0.333 ± 6.89)	100	0.233–1.09 (0.469 ± 0.249)	100	0.336–0.780 (0.546 ± 0.141)	100	0.335–1.17 (0.629 ± 0.250)	100
MTZ	1.68–69.1 (11.8 ± 18.8)	91.7	3.01–8.60 (6.19 ± 1.61)	100	0.343–8.31 (4.44 ± 2.16)	100	0.240–3.81 (0.723 ± 1.10)	83.3
MDZ	1.05	8.33	nd	–	nd	–	nd	–
NPX	0.144–5.14 (0.738 ± 1.44)	100	0.341–1.32 (0.730 ± 0.327)	100	0.294–1.64 (0.618 ± 0.412)	100	0.072–0.872 (0.261 ± 0.223)	100
NICO	1.66–28.8 (10.1 ± 7.40)	100	2.25–13.5 (5.69 ± 3.16)	100	0.529–4.18 (2.01 ± 1.31)	100	0.033–0.218 (0.113 ± 0.065)	75.0
OFLO	0.043–0.374 (0.104 ± 0.109)	100	0.050–0.248 (0.107 ± 0.074)	33.3	>MQL[c]	–	>MQL	–
OME	nd	–	nd	–	nd	–	nd	–
PRX	4.73–20.2 (16.3 ± 4.14)	100	1.11–10.5 (6.57 ± 2.90)	100	0.060–4.66 (0.304 ± 1.34)	91.7	0.013–0.072 (0.071 ± 0.322)	58.3
PNL	0.054–0.46 (0.213 ± 0.189)	50.0	0.115	8.33	nd	–	nd	–
RND	0.027–5.46 (0.494 ± 2.29)	91.7	0.020–0.257 (0.091 ± 0.073)	75.0	nd	–	nd	–
STX	0.019	8.33	0.028–0.225 (0.043 ± 0.092)	41.7	0.018–1.29 (0.149 ± 0.600)	33.3	0.429–1.52 (0.977 ± 0.775)	16.7
TRIM	0.006–0.139 (0.072 ± 0.094)	16.7	0.029	8.33	nd	–	nd	–

[a] No detected.
[b] Method detection limit.
[c] Method quantification limit.

Table 4
Removal percentages of different treatment process from both WWTPs.

Compound	WWTP1				WWTP2			
	Process 1.1	Process 1.2	Process 1.3	Treatment 1	Process 2.1	Process 2.2	Process 2.3	Treatment 2
ATE	86.6	77.1	−129	96.9	−125	36.2	89.8	85.4
BZF	93.3	>99.9	−306	>99.9	−100	9.83	−10.9	−100
CAFF	99.4	66.0	−109	99.8	12.9	96.6	79.9	99.4
CBZ	7.00	96.1	−134	96.4	−28.5	15.1	−14.9	−25.3
CIPRO	>99.9	–	–	96.7	>99.9	>99.9	–	>99.9
CLOF	−160	69.2	−53.8	20.0	–	–	–	–
DCLF	58.4	>99.9	−388	>99.9	−17.5	−88.7	−25.1	−177.4
ERY	48.7	>99.9	−33.3	>99.9	−100	>99.9	–	–
FLX	63.4	16.0	−13.3	69.3	32.8	>99.9	−100	39.7
GMF	85.4	97.3	−103	99.6	−73.2	−43.6	−8.74	−170
IBU	99.6	8.97	−11.5	99.7	9.29	−7.83	3.91	6.01
KPF	37.8	71.1	−25.5	82.0	−40.8	−16.4	−15.2	−88.9
MTZ	97.1	>99.9	>99.9	>99.9	47.5	28.3	83.7	93.9
MDZ	96.4	>99.9	−517	>99.9	>99.9	–	–	>99.9
NPX	94.3	22.9	−608	95.6	1.08	15.3	57.8	64.6
NICO	99.4	>99.9	−134	>99.9	43.7	64.7	94.4	>99.9
OFLO	88.3	>99.9	−239	>99.9	−2.88	100	–	>99.9
OME	–	–	−100	–	–	–	–	–
PRX	97.9	−201.8	−143	93.7	59.7	95.4	76.6	99.6
PNL	79.5	>99.9	−202	>99.9	46.0	>99.9	–	>99.9
RND	>99.9	–	–	>99.9	>99.9	>99.9	–	>99.9
STX	79.9	>99.9	87.3	>99.9	−126	−246	−555.7	−5042
TRIM	72.1	44.6	−195	84.6	59.7	>99.9	–	>99.9
Median	87,48	96,7	−129	99,7	9,29	50,4	−2.41	89,6

WWTP2 had a standard behaviour of a lesser progressive reduction. For example, some compounds, such as CBZ and IBU, maintained their concentration levels throughout the process. For others, such as DCLF and GMF, a slight increase in the levels occurred during the treatment process. Although only seven compounds exceeded a concentration of 1 μg L^{-1} in Point E, these compounds were adequately removed during the treatment except GMF and IBU, whose levels of concentration were practically unaltered until almost the last point of the purification procedure.

As with the WWTP1, seasonal fluctuation was been evaluated. The results obtained did not show any significant change during the period of sampling. There were, however occasionally some unusual concentrations values during the period of sampling, such as with CAFF, GMF, KPF and MTZ, with the maximum higher than average values present in the influent of WWTP2. This fact may be due to a variable flow of feed water.

Despite the irregular behaviour of the system of sewage treatment in WWTP2 (due to low human control of the procedure), some compounds were adequately removed with a significant reduction of the concentration. Therefore, the natural WWTP could be a purification treatment to reduce the levels of these micropollutants for small populations in rural areas with an extensive space to build wetlands, thus energetic consumption is reduced versus conventional WWTPs. However, future research is needed to improve the system.

In summary, the concentration ranges oscillated between 0.004 ± 0.001 to 59.2 ± 11.7 μg L^{-1} from WWTP1 and between 0.018 ± 0.001 to 148 ± 14.7 μg L^{-1} from WWTP2. The highest concentration pharmaceutical pollutant in the influent from both WWTPs was CAFF, which is from commonly consumed beverages (Sui et al., 2015). These levels of concentrations are typical of those found in sewage samples from other studies (Luo et al., 2014).

Moreover, all target compounds were detected in the most sampling points. Exceptions include OME, which was found only in point C from WWTP1, and CLOF, which was not detected in any samples from WWTP2.

The assessment of the occurrence of pharmaceutical in WWTPs from Gran Canaria island is a useful way to corroborate the continuous arrival of these compounds to environmental aquatic matrices without any accidental interference due to the fact that the unique input of pharmaceutical residues into sewage is through the excretion after human or veterinary consumption or an improper disposal. In addition there is no pharmaceutical industry in this region that can disturb the results.

3.3. Removal efficiency

The removal percentages of the different treatment processes from both selected WWTPs are shown in Table 4. This parameter was calculated using the median values of concentration of all target compounds as representative values without consider the retention time, due to the fact that it is only few minutes for the conventional treatment and variable for the natural process. For these reason, the collection of sample was done fortnightly in order to appreciate any extreme variation that should be taken into account.

The yields from WWTP1 were assessed comparing the concentrations from influent with activated sludge and subsequent microfiltration procedure (Process 1.1), the reverse osmosis with microfiltration samples (Process 1.2) and the reverse osmosis concentrate with the microfiltration samples (Process 1.3). The removal generated between influent samples and Point F (Process 2.1), samples from Point F and vertical flow wetland (Process 2.2) and, both vertical and horizontal flow wetland treatment (Process 2.3) were also assessed for WWTP2. In addition, the efficiency from the whole process taking into account the influent and the final effluent from both WWTPs (Treatment 1 and 2) was calculated (Fig. 2).

In general, the median recoveries from the whole treatment indicate an evident degradation for a large majority of target compounds in both WWTPs. When comparing both kinds of wastewater treatment, however, WWTP1 provided a better yield for removal of the target pharmaceuticals.

During the wastewater treatment in WWTP1, most of the pharmaceuticals were at least partially removed from the influent. Removal efficiency of targets ranged from 20% for CLOF to above 99.9% for BFZ, DCLF, ERY, MTZ, MDZ, NICO, OFLO, PNL, RND and STX. CAFF, GMF and IBU were also removed quite efficiently, with removal percentages between 99.6 and 99.8%. High levels of elimination of most pharmaceuticals during wastewater treatment is consistent with the results found in other works (Liu et al., 2017; Luo et al., 2014; Wang and Wang, 2016).

A lack of degradation was shown in WWTP2 for the most compounds during the Process 2.1. This behaviour changed in the Process 2.2, where yields were better, achieving practically the complete elimination of

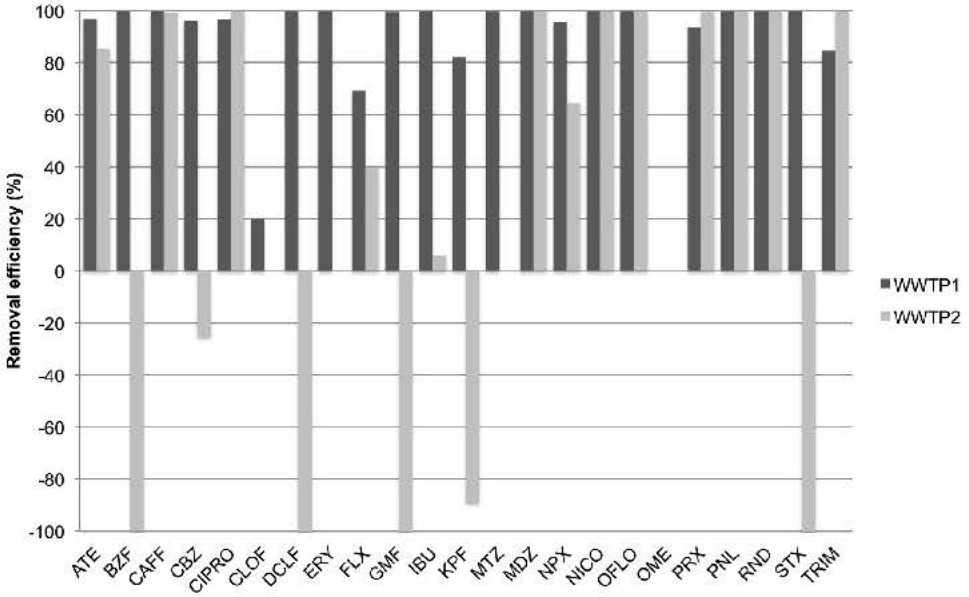

Fig. 2. Removal efficiencies of the whole process for each target pharmaceuticals from both WWTPs. Outliers: DCLF, GMF and STX.

CIPRO, ERY, FLX, PNL, RND and TRIM. The removal percentages only increased for a limit number of compounds in the Process 2.3. (ATE, CAFF, MTZ, NICO and PRX). The final result of the whole process was, however, acceptable, with removal percentages above 80% for most compounds, except for those whose elimination effectiveness rate reaches, in the better case, only 40%, such as, CBZ, FLX and IBU. The removal efficiencies of DCLF, GMF, KPF, BZF and STX during the whole process were negative. This may be explained because of the concentrating effect produced by possible variation in water flow due to contact with the vegetation and the substrates and the evapotranspiration in wetland (Verlicchi and Zambello, 2014). Despite the irregular behaviours, we posit that the natural WWTP is a promising treatment with a median removal percentage up to 90%, and the results are comparable with other studies (Li et al., 2014; Verlicchi and Zambello, 2014). However, it is necessary to improve the treatment distribution of the natural process and investigate new substrates.

Those compounds that offer high resistance to degradation in both WWTPs are CBZ, DCLF and KPF. These pollutants require a tertiary treatment for removal from the effluent, such as reverse osmosis; however, they are transferred to the reverse osmosis concentrate. This fact was observed in the results from WWTP1, where the removal percentages are in the range 7.00% to 58.4% for these three compounds in Process 1.1. Then, percentages above 70% are achieved with the tertiary treatment; however, the compounds appear in the reverse osmosis concentrate with high negative removal percentages. Therefore, a concentration of the analytes occurs in this process because all target compounds migrate from the microfiltration to the reverse osmosis concentrate in a lower volume of water.

Despite the large removal percentage, except for CLOF, in WWTP1, it is a necessity to incorporate an additional advanced treatment process after the effluent from microfiltration, as proposed in other study (Afonso-Olivares et al., 2016), to avoid pollution produced by the reverse osmosis concentrate, which is directly discharged to the environment.

The proposed removal efficiencies were calculated in the liquid samples. However, it is necessary evaluate the occurrence of these compounds in other matrices to know the removal mechanisms more deeply because different processes may be occurring. For example, the sorption mechanisms in sludge for conventional treatment or in plants and substrates in natural WWTPs, as well as, the presence of metabolites in the liquid matrix itself.

Although the sorption process cannot be evaluated due to the lack of data, an approximation of the behaviour of the compounds can be made based on their physicochemical characteristics. In this way, the sorption process is difficult to produce because the log Kow values of the target compounds are below 5 (Verlicchi and Zambello, 2015).

However, it could be indicated that the major degradation mechanism for this type of compounds, both in the treatment of active sludge and in the natural sewage treatment plant, is biodegradation (Tiwari et al., 2017). Although compounds like CBZ, DCLF, FLX and KPF have a high resistance, other pharmaceuticals like CAFF, CIPRO, MTZ, MDZ, NICO, PRX and RND offer an easy degradation.

3.4. Environmental risk assessment

The effects of pharmaceuticals on ecosystems, particularly aquatic systems, have been mainly assessed by determining their acute toxicity on organisms. Many such compounds are mutagenic or genotoxic. The results of every ecotoxicological effect of every pharmaceutical occurring in trace levels on living organisms, however, remain unknown. Real world exposures may generate unpredictable consequences.

Taking into account that the direct source of emerging pollutants in the aquatic environment is produced by the discharge of the effluent water from WWTPs and, accordingly, the physicochemical properties of these compounds as well as stability, resistance to biodegradation, or solubility in water can influence the removal of these compounds during treatment processes in WWTPs, there is a potential risk that these pollutants can be transferred indirectly to living organisms. In this study, we evaluated the environmental risk that the occurrence of the target compounds could produce in different organisms (algae, daphnids and fish). This is only an approach of the possible environmental risk on some species. However, the combination of the analytical methods to identify the compounds and the bioassays, which directly signal the effects on aquatic organisms, must be necessary to obtain significant results (Papa et al., 2016). Moreover, the different quality parameter of discharged water into the environment, as well as, other kind of contaminated matrices can influence in the effect that it produce on the environment ecosystems (Bai et al., 2017). Therefore, the predictable data can be different, even, more optimistic than reality.

Risk quotients (MEC/PNEC) were used for the assessment as the relation between maximum measured environmental concentrations (MEC)

of target pharmaceuticals found in the effluents of the selected WWTPs with the predicted no-effect concentration (PNEC) (Bouissou-Schurtz et al., 2014).

The maximum MEC from different effluents (both samples of reverse osmosis and reverse osmosis concentrate from WWTP1 and horizontal flow wetland samples from WWTP2) for each target compound of all analysed samples and the PNEC values for three different organisms are shown in Table 5 along with the results of RQ. Following the criteria used by Ashfaq et al., 2017, most of the studied compounds do not present a significant risk because their RQ values were between <0.001 to 0.077. GMF, IBU and OFLO could, however, produce a potential risk for all selected organisms, with an RQ values above unity (>1), reaching up to the 50.5 RQ value produced by OFLO to algae. In addition, other compounds, such as, CIPRO, CLOF, DCLF, ERY, PNL and STX, could produce medium or high impacts for at least one of the lower trophic levels (daphnids and/or algae), which RQs range from 0.102 to 27.0.

A full comparison with other studies would be an arduous task because the occurrence concentration of pharmaceuticals depends of many factors, such as the fraction of a population that consumes these compounds and the given treatment of the WWTP. Some similarities can, however, be observed. Ji et al., 2016 proposed a priority list of pharmaceuticals based on ecological risks and availability of relevant information, which included compounds such as OFLO or CIPRO that present high risk in this study. Pereira et al., 2015 is in accord in that their and our results regarding CIPRO have an RQ value above 1 solely for algae. In addition, compounds such as IBU and GEM were also suspected to produce elevated environmental risk in an overview by Hernando et al., 2006. Even the results shown by Ashfaq et al., 2017 exhibit a high risk for six pharmaceuticals, among which are NPX, DCLF, IBU, OFLO and CIPRO that have been assessed in this study, where only NPX has not demonstrated an important risk.

Undoubtedly, according to the results obtained in this work, future investigations should be focused to improve the removal efficiency of some of the pharmaceutical residue, for example, through including an additional tertiary based on advanced oxidation process for the conventional WWTPs or improving the distribution of the process and the assessment of other substrates for natural treatment.

4. Conclusions

The evaluation of the occurrence, removal and environmental risk of twenty-three pharmaceuticals from different therapeutic classes in liquid samples from a conventional WWTP with an active sludge system coupled with a reverse osmosis tertiary treatment and a natural WWTP with both vertical and horizontal flow wetland treatments have been studied.

All target compounds studied, except OME, were detected from most sampling points in the range 0.004 ± 0.001 to 59.2 ± 11.7 μg L^{-1} from WWTP1 and 0.018 ± 0.001 to 148 ± 14.7 μg L^{-1} from WWTP2. Conventional treatment provided a better yield for the removal target pharmaceuticals thanks to the tertiary treatment, achieving 99.7% of median removal from the entire process. Respect to natural WWTP, removal percentages above 80% were achieved. Only a 40% was obtained for CBZ, FLX and IBU.

These compounds present environmental risk for many species as it has been demonstrated in different studies. Thus, the concern regarding pharmaceuticals in the environment and their removal by WWTPs is increasing and it is necessary to perform more extensive research. Nevertheless, with the help of these kinds of estimations, future research could focus on the prioritization of pharmaceuticals.

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Table 5
Environmental risk assessment produced by maximal pharmaceutical concentration from treated water in aquatic species.

Species	Compound	MEC (μg L^{-1})	PNEC (μg L^{-1})	RQ	Compound	MEC (μg L^{-1})	PNEC (μg L^{-1})	RQ	Compound	MEC (μg L^{-1})	PNEC (μg L^{-1})	RQ
Algae	ATE	1.09	78.0[c]	0,014	FLX	0.101	0.800[a]	0,126	OFLO	1.01	0.020[b]	50,5
Daphnids			83.0[c]	0,013			0.510[a]	0,198			1.44[b]	0,701
Fish			1500[c]	0,001			1.70[a]	0,059			0.530[b]	1,91
Algae	BZF	0.260	18.0[a]	0,014	GMF	20.1	4.00[a]	5,02	OME	0.017	MV	–
Daphnids			30,0[a]	0,009			10,4[a]	1,93			MV	–
Fish			6.00[a]	0,043			0.90[a]	22,3			MV	–
Algae	CAFF	8.24	MV	–	IBU	21.7	4.00[a]	5,42	PRX	4.33	MV	–
Daphnids			MV	–			9.02[a]	2,41			MV	–
Fish			MV	–			5.00[a]	4,34			MV	–
Algae	CBZ	1.77	85.0[a]	0,021	KPF	1.17	164[a]	0,007	PNL	0.421	5.50[c]	0,077
Daphnids			76.3[a]	0,023			248[a]	0,005			2.30[c]	0,183
Fish			35.4[a]	0,050			32.0[a]	0,037			30.0[c]	0,014
Algae	CIPRO	0.242	0.010[b]	24,2	MTZ	3.81	MV	–	RND	–	66.0[a]	–
Daphnids			3415[b]	<0.001			MV	–			63.0[a]	–
Fish			7285[b]	<0.001			MV	–			1076[a]	–
Algae	CLOF	0.033	192[a]	<0.001	MDZ	0.154	MV	–	STX	1.52	0.120[a]	12,7
Daphnids			0.110[a]	0,300			MV	–			25.2[a]	0,060
Fish			53.0[a]	0,001			MV	–			562[a]	0,003
Algae	DCLF	2.24	14.5[a]	0,154	NPX	1.02	22.0[a]	0,046	TRIM	0.398	16.0[a]	0,025
Daphnids			22.0[a]	0,102			15.0[a]	0,068			121[a]	0,003
Fish			532[a]	0,004			34.0[a]	0,030			795[a]	0,001
Algae	ERY	0.540	4.30[a]	0,126	NICO	0.720	MV	–				
Daphnids			0.020[a]	27,0			MV	–				
Fish			61.5[a]	0,009			MV	–				

MV: Missing value.
[a] Values acquired from Ginebreda et al. (Ginebreda et al., 2010).
[b] Values acquired from Ashfaq et al. (Ashfaq et al., 2017).
[c] Values acquired from Yamamoto et al. (Yamamoto et al., 2007).

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III.2.2. Estimación de los parámetros cinéticos y la dosis UV necesaria para eliminar veintitrés compuestos farmacéuticos de aguas residuales urbanas pre-tratadas mediante UV/H_2O_2

A partir de la colaboración entre nuestro grupo de investigación, Grupo de Análisis Químico Medioambiental (AQMA), y el Grupo de Fotocatálisis y Espectroscopía Aplicada al Medioambiente (FEAM), ambos pertenecientes al Instituto Universitario de Estudios Ambientales y Recursos Naturales (i-UNAT), se ha utilizado una metodología analítica multiresiduo para el desarrollo de procesos avanzados de oxidación para la purificación de aguas residuales, optimizando todas aquellas variables que intervienen en el tratamiento mediante la evaluación de la eliminación de los veintitrés compuestos farmacéuticos objeto de estudio en esta Tesis Doctoral.

Los procesos que se han podido desarrollar en este trabajo consisten, por un lado, en la fotólisis mediante luz ultravioleta (UV) y, por otro lado, en la fotocatálisis a partir de la adición de peróxido de hidrógeno (UV/H_2O_2), mientras que la metodología analítica aplicada está basada, como es habitual, en una extracción en fase sólida (SPE) previa a la detección y determinación por cromatografía líquida de alta resolución con detección por espectrometría de masas de triple cuadrupolo (LC-MS/MS).

En definitiva, en este trabajo se investigó la efectividad de UV y UV/H_2O_2 para degradar veintitrés compuestos farmacéuticos presentes en el efluente del tratamiento secundario tras pasar por un sistema de microfiltración de una planta de tratamiento de aguas residuales urbanas. La constante cinética aparente y las dosis UV requeridas para la

eliminación efectiva de los compuestos estudiados se calcularon utilizando un reactor fotoquímico hecho a medida con una capacidad de 25 litros y una irradiancia de 15,47 $W \cdot m^{-2}$, cuya dosificación óptima de H_2O_2 determinada fue de 20 $mg \cdot L^{-1}$.

Los resultados revelan que los compuestos antiinflamatorios, tales como metamizol, diclofenaco y ketoprofeno, se eliminaron en su mayor parte por fotólisis. Sin embargo, la cafeína y su metabolito paraxantina, fueron dos de los compuestos que mostraron mayor resistencia a la oxidación por los radicales hidroxilo generados en este proceso.

Además, se calcularon las constantes cinéticas aparentes y las dosis de UV necesarias para eliminar cada producto farmacéutico del agua residual tratada. Ciproflacina, diclofenaco, ketoprofeno y metamizol se degradaron con dosis de UV inferiores a 100 $mJ \cdot cm^2$. Sin embargo, dosis de UV de 2369 y 4318 $mJ \cdot cm^2$ fueron necesarias para reducir la cafeína y su metabolito, paraxantina, a una décima de sus valores iniciales. También se monitorizó la mineralización de carbono orgánico y la absorbancia a 254 nm del agua depurada y, aunque no se observó ningún cambio en la concentración de carbono orgánico disuelto después del tratamiento con UV/H_2O_2, la absorbancia a 254 nm disminuyó en un 43 %.

De esta manera, se concluye que la combinación de UV y H_2O_2 para eliminar productos farmacéuticos diferentes en muestras de aguas residuales da resultados satisfactorios. Aunque algunos compuestos muestran una alta sensibilidad a la fotólisis, la irradiación únicamente por UV es insuficiente para degradar todos los compuestos estudiados. Gracias a la adición de una cantidad óptima de H_2O_2 es posible alcanzar una tasa media de eliminación del 93 %, pero se requiere de un tiempo de

residencia de al menos 75 min para eliminar más del 90 % de todos los productos farmacéuticos estudiados.

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Estimation of kinetic parameters and UV doses necessary to remove twenty-three pharmaceuticals from pre-treated urban wastewater by UV/H_2O_2

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ABSTRACT

In this work, the effectiveness of UV and UV/H_2O_2 to degrade twenty-three pharmaceutical compounds present in microfiltered secondary effluent of an urban wastewater treatment plant was investigated. Apparent kinetic constant and UV doses required for effective removal of the studied compounds were calculated using a custom-made photochemical reactor with a capacity of 25 Liters and an irradiance of 15.47 W m^{-2}. An optimal H_2O_2 dosage of 20 mg L^{-1} was determined. Antiinflammatory compounds, such as metamizole, diclofenac and ketoprofen, were mostly removed by photolysis. Caffeine and its metabolite paraxanthine were the compounds which displayed most resistance to oxidization by hydroxyl radicals generated in this process. The apparent kinetic constants and UV doses necessary to remove each pharmaceutical from the treated wastewater were calculated. Ciprofloxacin, diclofenac, ketoprofen and metamizole were degraded with UV doses lower than 100 mJ cm^{-2}. However, UV doses of 2369 and 4318 mJ cm^{-2} were necessary to reduce caffeine and its metabolite paraxanthine to one tenth of their initial values. The mineralization of organic carbon and absorbance at 254 nm of the recycled water were also monitored. Though no change in dissolved organic carbon concentration was observed after UV/H_2O_2 treatment, UV absorbance at 254 nm decreased by 43%.

1. Introduction

The occurrence of micropollutants in the aquatic environment has become an important concern in recent years. Most of these compounds reach rivers, lakes and seas through the discharge of effluents from wastewater treatment plants (WWTPs) [1]. At the present time, most WWTPs around the world are ineffective at removing these compounds from treated water. Consequently, a large number of micropollutants are discharged on a daily basis into aquatic environments. One of the most important goals for wastewater treatment is to obtain treated water without the presence of these compounds. Micropollutants, also known as emerging contaminants, consist of a vast and expanding array of anthropogenic as well as natural substances. Pharmaceutical compounds are one of the most important groups of the various types of emerging contaminants [2]. The impact of these substances on human health and the ecosystem is a major concern and has been extensively investigated in the last years [3,4]. Though pharmaceutical compounds are commonly found in aquatic environments at concentration levels in the μg L^{-1} or ng L^{-1} range [5], the accumulation of these residues produces ecotoxicological effects that are difficult to predict. Many of these pollutants have the potential to affect the health of humans and animals species whether, for example, through interference with the endocrine system by disrupting the function of hormones or as a result of the spread of pathogens resistant to antibiotics [6–8]. In addition, concentration and bioaccumulation of target compounds have been determined in samples of different organisms [9]. Only three of the large number of pharmaceuticals (17β-estradiol, 17α-ethinylestradiol and diclofenac) are in the process of being included in the first European Union "Watch List" [10]. Meanwhile in the US, the EPA (Environmental Protection Agency) has

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proposed the inclusion in the Contaminant Candidate List (CCL) of other pharmaceutical compounds such as carbamazepine and chloramphenicol [11]. However, maximum concentrations of these pollutants have not been fixed for the time being.

In Spain, WWTPs mostly employ primary and secondary treatment processes [12]. Several studies have shown that advanced technologies, such as membrane bioreactors (MBR), nanofiltration/ultrafiltration, reverse osmosis (RO), adsorption on activated carbon, ozonation and advanced oxidation processes (AOPs), can provide effective and viable solutions in wastewater treatment to ensure better elimination of emerging pollutants and water purification [13–15]. RO membranes have been shown to provide a cost-effective water purification solution for the elimination of pharmaceuticals from recycled water [16]. The use and development of ozonation and AOPs for the removal of emerging contaminants from aqueous samples is becoming increasingly widespread [17]. Different kinds of AOPs (heterogeneous photocatalysis, Fenton, photo-Fenton, sonolysis, UV/H_2O_2, wet air oxidation and electrolysis) have been developed with positive results, obtaining the complete removal of the contaminants [18].

One of the main disadvantage of the RO treatment method is that the resulting concentrate, usually comprising 20–50% of the influent, contains a high concentration of these pollutants which normally are discharged without further treatment [19]. In a previous study, the pharmaceuticals found in RO concentrate revealed an average concentration factor of 3- to 4-fold that of the municipal effluent [20]. Treatment of secondary effluent before RO through ozonation and AOPs could avoid the generation of such high pollutant concentrations, eliminating the problem in both the RO concentrate and the recovered water. Ozonation is the most commonly applied AOP in studies at laboratory, pilot scale [21] and even full scale system [22]. A previous study with twenty-four selected micropollutants indicated that compounds with deactivated aromatic rings such as phthalate and halo-substituted compounds show moderate to very low reactivity toward ozone. However, their high reactivity toward hydroxyl radicals confirmed that hydroxyl radicals are relatively non-selective oxidants [23]. Justo et al. [24] proved that UV/H_2O_2 removes pharmaceuticals from RO concentrate more effectively than ozonation process. Though numerous articles have been published on the application of UV/H_2O_2, the only calculations of the degradation kinetic parameters and UV dose estimations for each pharmaceutical present in treated wastewaters were performed by Kim et al. [25] and Wols et al. [26]. However, these authors used a highly polished treated wastewater with low levels of dissolved organic carbon. In addition, most of the pharmaceuticals selected by Kim et al. [25] were analgesics and antibiotics. Some of the studied compounds in the present work such as caffeine and its metabolite paraxanthine, ciprofloxacin, ibuprofen, metamizole, nicotine, norfloxacin, omeprazole, ranitidine were not studied in neither of the above referred works.

The aim of this work was to calculate the degradation kinetic parameters by UV/H_2O_2 and the UV doses necessary to transform, from treated wastewater, twenty-three pharmaceuticals. The target compounds, 21 active ingredients and 2 metabolites, have been chosen based on three properties: (I) these pharmaceuticals are classified in more commonly detected therapeutic groups in environmental liquid samples [17], such as, anti-inflammatories and analgesics (diclofenac, ketoprofen, ibuprofen, naproxen, metamizole), antidepressants (fluoxetine), antiepileptic (carbamazepine), antiulcer drugs and antihistamines (omeprazole and ranitidine), lipid regulator (gemfibrozil, clofibric acid and bezafibrate), β-blockers (propranolol and atenolol), antibiotics (ofloxacin, ciprofloxacin, erythromycin, trimethoprim, sulfamethoxazole and metronidazole) and others (nicotine, caffeine and paraxanthine); (II) according to reports by Spanish Agency for Medicines and Health Products, many target active ingredients are the most consumed pharmaceuticals in Spain in the last years, from 0.06 defined daily dose value (DDD/population per day) of ketoprofen up to 104.2 DDD/population per day of omeprazole [27], therefore, (III) these compounds have been detected in effluents from wastewater treatment plants with traditional treatments located around of Spain [28–30]. Moreover, many of active ingredients instead of metabolites has been chosen due to the fact that the pharmaceuticals after ingestion are excreted without transformation with a high percentage, reaching 90% in some cases [31,32]. However, in this study has been used two metabolites, paraxanthine and clofibric acid, because these compounds derived from active substances which are highly metabolized, caffeine and clofibrate, respectively.

Specifically, the study was performed with water from a WWTP with high conductivity (1344 μS/cm) and dissolved organic carbon (DOC) (20.4 mg L^{-1}). A continuous, custom-made photochemical reactor with a capacity of 25 Liters was used for this purpose.

2. Experimental

2.1. Materials

Twenty-three pharmaceutical compounds belonging to different therapeutic classes, including anti-inflammatories (diclofenac, ketoprofen, ibuprofen, naproxen and metamizole), stimulants (nicotine, caffeine and paraxanthine), antihypertensives (propranolol and atenolol), antiepileptics (carbamazepine), antidepressants (fluoxetine), antibiotics (ofloxacin, ciprofloxacin, erythromycin, trimethoprim, sulfamethoxazole and metronidazole), antiulcer (omeprazole and ranitidine) and lipid regulators (gemfibrozil, clofibric acid, bezafibrate) were purchased from Sigma-Aldrich (Madrid, Spain). Test water was prepared by spiking 1 μg L^{-1} of the twenty-three pharmaceuticals from stock solution into treated water. Methanol and water (99.9%), with LC–MS quality, were used to prepare the mobile phase for LC–MS/MS analysis. All of the solvents and formic acid that were used to adjust the pH of the mobile phase for the different analytical processes were obtained from Scharlab S.L. (Barcelona, Spain). Ultrapure water used for conditioning the process of solid-phase extraction and for preparing aqueous standard solutions was obtained from a Milli-Q (Millipore, Bedford, MA, USA) water purification system. Hydrogen peroxide 30% from Scharlab S.L. (Barcelona, Spain) was used for decontamination reactions as oxidizing substance. Reagents used for water characterization such as sodium hydroxide or methanesulfonic acid were purchased from Fluka (Madrid, Spain).

2.2. Water samples

Water samples were taken after the microfiltration process and before the RO treatment of an urban WWTP located in Gran Canaria (Spain). The samples were collected in amber glass bottles that had been previously rinsed with methanol and deionized water and then stored in the dark at a temperature of 4 °C. The samples were not filtered in order to simulate real conditions before AOP application. To ensure a minimum concentration of each compound of 1 μg L^{-1}, this amount was added to the water before carrying out the experiments.

2.3. Custom-made photochemical reactor

A cylindrical photochemical reactor with dimensions of 0.36 × 0.30 m and a capacity of 25 L was used for this study (Fig. 1). The construction material is polished stainless steel (304)

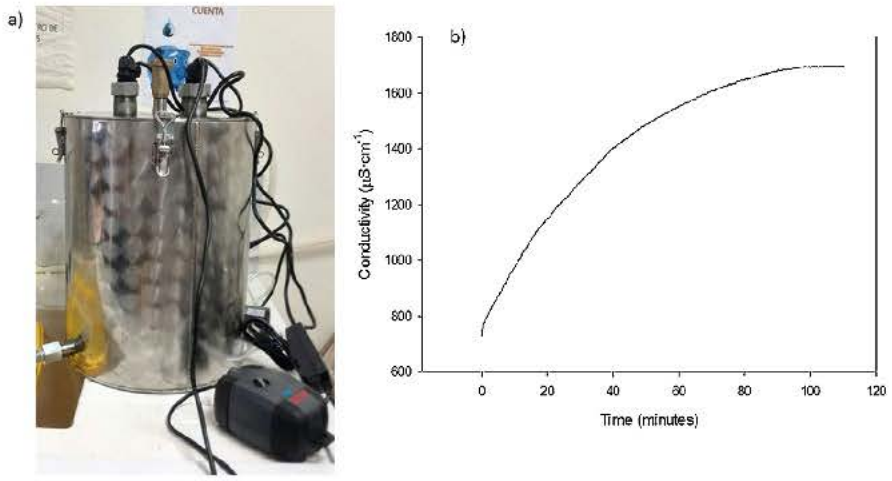

Fig. 1. a) Image of the 25 L custom-made photochemical reactor and b) Reactor tracer test with 0.01 M NaCl.

with a thickness of 3 mm. The reactor contains a lower inlet for the flow of contaminated water, an outlet on the top for overflow and a lower inlet for the aeration process. Both the water outlet and inlet have an internal diameter of 8 mm and a ball valve. The cover has four equidistant holes 25 mm in diameter in which the quartz sleeve tubes for the UV-C lamps are arranged and held by O-rings. The four low pressure mercury 14 W UV-C lamps are 29 cm long and were obtained from UV Superstore, Winder, GA, US. Irradiance at the center of the reactor at 26 cm from the base is 15.47 W m^{-2}.

The reactor also has an aeration system that works during the reaction consisting of a micro-perforated hose located at the lower end which is connected through the air inlet of the reactor to a 3.5 W compressor pump. Air flow is adjustable in the range of 50–100 L h^{-1}. During the course of the reactions maximum air flow was 100 L h^{-1}. This system was used in order to create a turbulent flow favoring proper homogenization within the reactor, thereby ensuring that the UV-C radiation reached the whole treated volume.

A peristaltic Selecta Percom NM pump was used to drive the flow into the reactor with an electronic speed control in the range of 20–200 rpm. Pump capacity ranges between 1080 and 73800 mL h^{-1}.

Tracer testing was performed in the photochemical reactor to determine the proper operation of the reactor. A 0.01 M NaCl solution was used for this purpose. As can be seen in the results (Fig. 1), conductivity increased gradually with time until stabilizing after 95 min at 1695 µS cm^{-1}. No sudden change in conductivity over time at the outlet was observed, indicating that correct homogenization was obtained inside the reactor with no preferential flows or dead zones.

2.4. Water characterization

2.4.1. Physicochemical parameters

Concentrations of anions and cations from wastewater samples were determined by ionic chromatography. DIONEX equipment was used with a GP50 gradient pump, ED50 electrochemical detector and IonPac AS11-HC (4 × 250 mm) column for anion determination or IonPac CS12A (2 × 250 mm) column for cation determination. The equipment also included an AS40 Autosampler. Data acquisition was performed by the program Chromeleon. The mobile phase used for anion determination consisted of water/60 mM of NaOH 50:50% (v/v), with a flow rate of 1.5 mL min^{-1}. Suppression current was 112 mA. For cation measurement, 20 mM of methanesulfonic acid was used as mobile phase, with a flow rate of 1 mL min^{-1} and suppression current of 59 mA.

A Shimadzu TOC-L analyzer with an ASI-L Autosampler were used to characterize the wastewater samples by measuring dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). A TN-100 EUTECH turbidimeter was used to determine sample turbidity.

A Thermo-Heλios double beam spectrophotometer was used for assessment of substances that absorb light at 254 nm. Quartz optical cells were used with a length of 10 mm and measurements were taken at a wavelength of 254 nm. Transmittance of the water at 254 nm was also measured.

The same spectrophotometer was also used to monitor the hydrogen peroxide concentrations by reaction with potassium oxalate and titanium in acid medium. The measurements were made at a wavelength of 400 nm.

2.4.2. Pharmaceuticals analysis

SPE procedure combined with LC–MS/MS was developed for the determination of the pharmaceuticals after the reaction process. Briefly, an SPE Supelco Visiprep™ system with capacity for 12 simultaneous extractions was used for the extraction step. 250 mL of water sample at pH 9 was passed through an Oasis HLB polymeric cartridge. Subsequently, 2 mL of methanol was used as an extracting dissolvent. The methanol extract was analyzed by LC–MS/MS with a 320 MS LC/MS/MS Varian System equipped with an electrospray ionization (ESI) interface.

Ionization in the ESI source was achieved using nitrogen as a nebulizer and drying gas with a pressure of 65 psi and 30 psi, respectively, whereas that the housing and desolvation temperatures were 60 °C and 250 °C, respectively. Capillary voltage was set to 5.0 kV in positive mode (ESI+) and −4.5 kV in negative mode (ESI−). Shield voltage was maintained at −600/600 V (ESI+/ESI−). Collision-induced dissociation (CID) was conducted with argon as the collision gas at a fixed pressure of 2.00 psi.

The standard solutions of each individual target compound were injected into the MS detector to carry out the optimization of mass spectrometer parameters, such as, cone voltage, collision gas energy and fragment ions. These parameters are displayed in Table 1. The system and the data management were controlled by MS Varian LC/MS Workstation Version 6.9 SP1 software.

Table 1
Mass spectrometer parameters for the determination of target analytes.

No.	Compound	Precursor ion (m/z)	Cone V	Fragment ions (collision potential)	Ion mode
1	Atenolol	267	52	145 (23.5)[a], 190 (16.5)	ESI +
2	Bezafibrate	359.8	64	273.7 (15.5)[a], 153.5 (28.5)	ESI −
3	Caffeine	195	56	138 (18)[a]	ESI +
4	Carbamazepine	237.1	40	194 (13.5)[a], 192 (17)	ESI +
5	Ciprofloxacin	332.1	52	313.9 (19.0)[a], 230.8 (36.0)	ESI +
6	Clofibric acid	213	32	85 (10)[a], 127 (13.5)	ESI −
7	Diclofenac	295.9	32	214.0 (30)[a], 250.0 (11.0)	ESI +
8	Erythromycin	734.5	48	576.3 (11)[a], 157.8 (22.5)	ESI +
9	Fluoxetine	310	30	44 (6.5)[a], 148 (5.5)	ESI +
10	Gemfibrozil	248.9	30	120.7 (12.5)[a]	ESI −
11	Ibuprofen	204.7	40	160.8 (6.5)[a], 158.5 (6.0)	ESI −
12	Ketoprofen	255.1	52	209 (10)[a], 104.9 (18.5)	ESI +
13	Metamizole	218	30	56 (12.5)[a], 97 (11.5)	ESI +
14	Metronidazole	172	40	127.9 (10.0)[a], 81.9 (21.0)	ESI +
15	Naproxen	231.2	36	153.1 (28.5)[a], 170 (22)	ESI +
16	Nicotine	163	30	130(18.5)[a], 84 (17)	ESI +
17	Ofloxacin	362.1	52	318.1 (14.5)[a], 261.0 (22.5)	ESI +
18	Omeprazole	346	32	198.0 (7)[a], 135.8 (27.5)	ESI +
19	Paraxanthine	181	40	124 (17)[a]	ESI +
20	Propranolol	260.2	48	116.1 (13)[a], 183.1 (12)	ESI +
21	Ranitidine	315.0	44	175.9 (11)[a], 129.8 (20)	ESI +
22	Sulfamethoxazole	254	44	155.9 (11.5)[a], 91.9 (23)	ESI +
23	Trimethoprim	291.1	64	230 (19)[a], 122.9 (21)	ESI +

[a] Fragment ion used for quantitation (MRM).

The stationary-phase column was a 3.0 mm × 100 mm, 3.5 μm particle SunFireTM C18. The mobile phase consisted of water (containing 0.015% formic acid)/methanol (90:10) (v/v) for 1 min, it was then changed for 20 min to 60:40 (v/v), for 19 min to 10:90 (v/v) and finally for 3 min it was returned to its initial condition. The injection volume and the flow rate were 10 μL and 200 μL min^{-1}, respectively.

3. Results and discussions

3.1. Water characterization

An analysis of various physicochemical parameters from urban WWTP water was carried out before starting the experimental study. The results obtained are shown in Table 2. The concentrations of the pharmaceuticals present in the treated water were also determined and can be seen in Table 3. Samples were extracted and analyzed in triplicate using the optimized conditions described above. Quantification was performed by internal additions, obtaining a calibration line with a correlation coefficient of 0.9901. At the same time, in Table 3 we can observe the recovery rates, which are influenced by the efficiency of extraction process and the matrix effect. Most of the compounds under study were found in concentrations ranging from 0.01 to 0.69 μg L^{-1}. Only four compounds (ciprofloxacin, ranitidine, metamizole and propranolol) were below detection limits.

3.2. Photodegradation of pharmaceuticals by UV

The reaction methodology was carried out without adding hydrogen peroxide to study the contribution of only UV-C to the UV/H_2O_2 process. The reaction time was 45 min. The results seen in Table 4 show a complete or almost complete degradation of ten target compounds (metamizole, ketoprofen, omeprazole, sulfamethoxazole, ranitidine, propranolol, ofloxacin, nicotine, diclofenac and clofibric acid), photolysis of this series of compounds occurred at rates above 90%. However, UV energy at 254 nm was insufficient to degrade other compounds such as caffeine, carbamazepine, gemfibrozil, naproxen, paraxanthine and trimethoprim, whose degradation rates were below 20%.

3.3. Photodegradation of pharmaceuticals by UV/H_2O_2

3.3.1. Optimization of hydrogen peroxide dose

Different concentrations of H_2O_2 (5, 15, 20 and 25 mg L^{-1}) were tested to optimize the decontamination of pharmaceuticals by hydrogen peroxide and UV-C. The concentrations and percentages of degradation of each compound after the addition of the tested H_2O_2 concentrations are shown in Table 5.

It can be seen for most compounds that the percentage degradation rose with increasing concentration of H_2O_2, supplied at the start of the reaction. In some cases, degradation occurred completely with only 5 mg L^{-1} concentration, mostly due to the photolysis contribution. While an average degradation rate of 63.3% is observed when employing only photolysis, this value is increased to 75.8% after the addition of 5 mg L^{-1} of H_2O_2. The average degradation rate for higher concentrations was above 90%. It should be noted that the antibiotic ofloxacin remained practically unchanged at the different H_2O_2 doses. As the results with 15, 20 and 25 mg L^{-1} were fairly similar in most cases, metronidazole was taken as a model compound because significant differences in degradation of this compound were observed at the different concentrations of H_2O_2. Although the results showed that 25 mg L^{-1} was the best concentration, the chosen optimum concentration was 20 mg L^{-1}, since removal rates approaching >99% for all compounds were achieved with less peroxide. Justo et al. [24] investigated the optimal H_2O_2 dose to degrade eleven pharmaceuticals from osmosis concentrate (DOC = 27.6 mg C L^{-1}).

Table 2
Analysis of different water parameters from tested treated wastewater.

Parameter		Amount	Parameter		Amount
pH		7.97	Transmittance (%)		55
Conductivity (μS/cm)		1344	Abs at 254 nm (cm^{-1})		0.26
Turbidity (NTU)		1.08	DOC (mg L^{-1})		20.42
			DIC (mg L^{-1})		35.25
Anions (mg·L^{-1})	Cl^-	344.09	Cations (mg·L^{-1})	Na^+	234.22
	NO_2^-	8.91		NH_4^+	6.60
	SO_4^{2-}	66.24		K^+	35.30
	NO_3^-	22.26		Mg^{2+}	14.71
	PO_4^{3-}	97.50		Ca^{2+}	40.58

Table 3
Initial concentration of pharmaceuticals in microfiltered water before and after spiking pharmaceuticals (1 μg·L^{-1}) and analytical parameters of method (limit of detection and quantification, recovery and reproducibility).

Compound	LOD (ng L^{-1})	LOQ (ng L^{-1})	Recovery (%) (n = 3)	RSD[a] (%) (n = 6)	Post-MF[b] conc (ng L^{-1})	Post-Spiked conc (ng L^{-1})
Atenolol	12.3	41.0	85.7	19.7	330	1330
Bezafibrate	1.99	6.63	68.8	14.2	110	1110
Caffeine	5.38	17.9	37.6	10.5	410	1410
Carbamazepine	1.17	3.90	31.3	14.7	170	1170
Ciprofloxacin	19.1	63.8	68.6	6.70	<LOD	1000
Clofibric acid	0.39	1.30	76.4	17.7	20	1020
Diclofenac	0.19	0.63	73.6	18.6	690	1690
Erythromycin	0.21	0.69	57.7	19.1	300	1300
Fluoxetine	0.16	0.53	30.6	19.9	110	1110
Gemfibrozil	2.34	7.79	83.0	9.12	570	1570
Ibuprofen	67.9	180	117	8.46	190	1190
Ketoprofen	2.94	9.79	53.4	9.37	540	1540
Metamizole	23.5	78.3	140	19.6	<LOD	1000
Metronidazole	5.50	18.3	119	14.7	630	1630
Naproxen	0.72	2.40	95.6	17.6	660	1660
Nicotine	30.8	103	29.4	10.6	112	1120
Ofloxacin	28.4	94.8	32.3	17.6	140	1140
Omeprazole	0.72	2.40	48.7	10.8	10	1010
Paraxanthine	35.3	117	93.1	2.44	680	1680
Propranolol	10.4	34.7	56.3	15.5	<LOD	1000
Ranitidine	6.26	20.9	58.7	19.3	<LOD	1000
Sulfamethoxazole	0.58	1.93	106	12.7	130	1130
Trimethoprim	4.29	14.3	66.6	6.82	100	1100

[a] RSD: Relative standard deviation (n = 6).
[b] MF: microfiltered water.

Table 4
Degradation of pharmaceutical compounds by photolysis with UV-C after 45 min of reaction.

Compound	C/C$_0$[a]	Degraded%	Compound	C/C$_0$	Degraded%
Atenolol	0.70	29.57	**Metamizole**	0.00	>99.0
Bezafibrate	0.18	82.02	**Metronidazole**	0.31	68.94
Caffeine	0.89	10.99	**Naproxen**	1.00	0.00
Carbamazepine	0.81	18.90	**Nicotine**	0.05	94.73
Ciprofloxacin	0.00	>99.0	**Ofloxacin**	0.29	71.00
Clofibric acid	0.00	>99.0	**Omeprazole**	0.01	98.52
Diclofenac	0.00	>99.0	**Paraxanthine**	1.00	0.00
Erythromycin	0.67	32.87	**Propranolol**	0.20	>99.0
Fluoxetine	0.16	83.57	**Ranitidine**	0.00	>99.0
Gemfibrozil	1.00	0.00	**Sulfamethoxazole**	0.00	>99.0
Ibuprofen	0.36	63.59	**Trimethoprim**	0.81	18.80
Ketoprofen	0.00	>99.0	**Mean**	0.37	63.3

[a] Ratio between final concentration (C) and initial concentration (C$_0$).

They found that 19.8 mg H_2O_2 were necessary for higher than 90% removal of the assayed compounds, except trimethoprim which exhibited a lower degradation rate. In the present work, the degradation rates of other studied pharmaceuticals, like erythromycin and the above-mentioned ofloxacin and metronidazole, were below that of trimethoprim using MF-water.

3.3.2. Batch reaction and kinetic study

Batch-mode reactions with 25 L of MF-wastewater sample were carried out for the kinetic study of degradation of the pharmaceuticals. Fig. 2 shows the changes in relative residual concentrations versus reaction time for pharmaceuticals which were mostly degraded by photolysis and for pharmaceuticals which were slowly degraded by UV/H_2O_2.

Diclofenac, ketoprofen, ciprofloxacin, metamizol and omeprazol were almost completely degraded during the first five minutes of reaction. However, a much longer reaction time was required for removal of propranolol, atenolol, carbamazepine, caffeine and its metabolite paraxanthine, as can be seen in Fig. 2b.

As can be observed in Fig. 3, the average percentages of removed pharmaceuticals rose significantly during the first 45 min, attaining an average degradation rate for all compounds of 86.76%. After 75 min, the corresponding value was 94.27%. Though the complete or almost complete degradation of many pharmaceuticals was attained during the first 45 min, >99% removal rate was observed for most of them after 75 min. Therefore, this time (75 min) was taken as the residence time for the subsequent reaction in continuous mode.

The H_2O_2 concentration was also monitored and observed to decrease over time in the discontinuous reaction (Fig. 3). Average remaining concentration of H_2O_2 at the end of sample collection (75 min) was 10.5 mg L^{-1}.

Subsequently, the apparent rate constant (K_{ap}) for each compound was determined. The values were adjusted to a first order kinetic (Eq. (1)). Calculations were also performed to determine the time required for a 90% removal rate (t_{90}, Eq. (2)) and the required UV dose for this (UV_{90}, Eq. (3)).

$$\ln\frac{C_0}{C} = K_{ap}\cdot t \tag{1}$$

$$C = C_0(1-X); X = 90\% \rightarrow t_{90} = \frac{\ln(1-0.9)}{-K_{ap}} \tag{2}$$

$$UV_{90} = \frac{P_{irradiated}\,(15.47W\cdot m^{-2})}{Transmittance(55\%)}\cdot t_{90} \tag{3}$$

According to the results shown in Table 6, compounds with high sensitivity to photolysis as diclofenac or ketoprofen, needed a UV_{90} dose lower than 60 mJ cm^{-2} to be reduced to a tenth of their initial concentration. Contrastingly, caffeine and its derivative paraxanthine were the most resistant to degradation by the UV-C/H_2O_2 process. Fourteen of the twenty-three studied pharmaceuticals needed a UV_{90} dose lower than 1000 mJ cm^{-2} to be degraded. At the other extreme, caffeine and paraxanthine were the most recalcitrant compounds requiring UV_{90} doses of 2369 and 4318 mJ cm^{-2}, respectively.

Table 5
Pharmaceutical removal at different H_2O_2 doses in the UV/H_2O_2 process.

H_2O_2 dose (mg L^{-1})	5		15		20		25	
Compound	C/C_0[a]	Degraded%	C/C_0	Degraded%	C/C_0	Degraded%	C/C_0	Degraded%
Atenolol	0.66	34	0.11	89	0.09	91	0.00	>99
Bezafibrate	0.19	81	0.01	99	0.02	98	0.00	>99
Caffeine	0.74	26	0.12	88	0.15	81	0.13	87
Carbamazepine	0.48	52	0.02	98	0.07	93	0.00	>99
Ciprofloxacin	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Clofibric acid	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Diclofenac	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Erythromycin	0.66	34	0.30	70	0.24	76	0.00	>99
Fluoxetine	0.02	98	0.08	92	0.02	98	0.00	>99
Gemfibrozil	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Ibuprofen	0.33	67	0.00	>99	0.00	>99	0.00	>99
Ketoprofen	0.03	97	0.06	94	0.08	92	0.05	95
Metamizol	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Metronidazole	0.30	70	0.21	79	0.08	92	0.00	>99
Naproxen	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Nicotine	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Ofloxacin	0.30	70	0.26	74	0.24	76	0.21	79
Omeprazol	0.02	98	0.00	>99	0.00	>99	0.00	>99
Paraxanthine	1.00	0	0.14	86	0.11	89	0.00	>99
Propranolol	0.18	82	0.00	>99	0.00	>99	0.00	>99
Ranitidine	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Sulfamethoxazole	0.00	>99	0.00	>99	0.00	>99	0.00	>99
Trimethoprim	0.65	35	0.14	86	0.17	83	0.04	96
Mean	0.24	75.8	0.06	93.7	0.05	94.3	0.02	98.1

[a] Ratio between final concentration (C) and initial concentration (C_0).

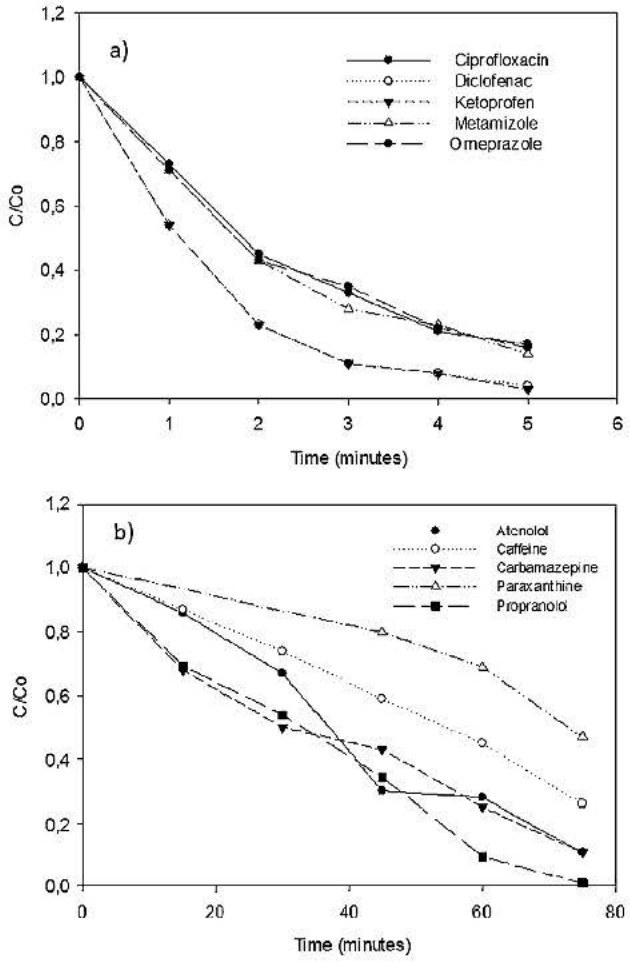

Fig. 2. Plots of relative residual concentrations of a) ciprofloxacin, diclofenac, ketoprofen, metamizol, omeprazol and b) propranolol, paraxanthine, carbamazepine, atenolol and caffeine versus reaction time.

Kim et al. [25] previously calculated the UV dose necessary to remove 30 pharmaceuticals and personal care products (PPCPs) from treated water from secondary effluent with low DOC. Although these authors only mention that the initial concentrations of the thirty PPCPs in tested water ranged from 5 $\mu g\,L^{-1}$ (clembuterol) to 119 $\mu g\,L^{-1}$ (propranolol), it was nevertheless decided to compare the UV_{90} results reported in that work with those obtained in our experiments. The results of this comparison are shown in Table 7.

In our experiments lower UV_{90} doses were required to remove pharmaceuticals with a high degradation rate by photolysis. Contrastingly, higher values were required to remove propranolol and carbamazepine. The variations in the results may be due to differences in the ratio H_2O_2 doses and DOC, with values of 0.97 for the present work and 2.05 for that of Kim et al. [25]. Rosario-Ortiz et al. [33] investigated the percentage degradation of carbamazepine, atenolol and trimethoprim at a UV dose of 700 mJ cm^{-2} with the addition of 20 mg L^{-1} of H_2O_2 using a treated water with similar transmittance at 254 nm but with a lower concentration of DOC.

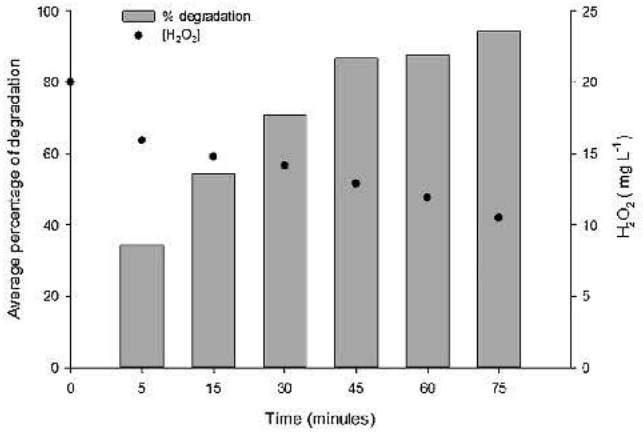

Fig. 3. Average percentage of degradation and H_2O_2 remaining concentration at different reaction times.

Table 6
Results of t_{90}, K_{ap} and UV_{90} for each compound.

Compound	t_{90} (min)	K_{ap}x10(min^{-1})	UV_{90} ($mJ\,cm^{-2}$)
Atenolol	79.7	0.289	1345
Bezafibrate	81.9	0.281	1383
Caffeine	140.4	0.164	2369
Carbamazepine	81.7	0.282	1378
Ciprofloxacin	6.2	3.610	105
Clofibric acid	35.6	0.647	601
Diclofenac	3.6	6.450	60
Erythromycin	99.7	0.231	1682
Fluoxetine	22.8	1.010	385
Gemfibrozil	49.2	0.468	830
Ibuprofen	72.0	0.320	1214
Ketoprofen	3.4	6.790	57
Metamizol	5.9	3.900	100
Metronidazole	42.6	0.540	720
Naproxen	15.0	1.540	252
Nicotine	28.7	0.802	485
Ofloxacin	37.1	0.620	627
Omeprazol	6.2	3.720	105
Paraxanthine	255.8	0.090	4318
Propranolol	64.1	0.359	1082
Ranitidine	17.71	1.300	299
Sulfamethoxazole	16.45	1.400	278
Trimethoprim	91.01	0.253	1536

Table 7
Comparative results of UV_{90} doses.

Pharmaceutical	UV_{90} dose ($mJ\,cm^{-2}$) [present study]	UV_{90} dose ($mJ\,cm^{-2}$) [25]
Diclofenac	60	113
Naproxen	252	434
Ketoprofen	57	45
Sulfamethoxazole	278	314
Propranolol	1082	515
Carbamazepine	1378	605

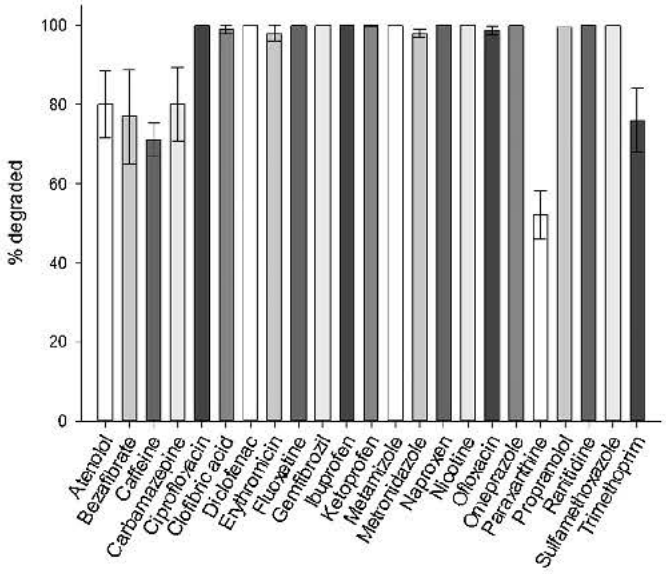

Fig. 4. Average percentage degradation of pharmaceuticals in continuous reaction.

The degradation rates reported by these authors were 44% for atenolol, 50% for carbamazepine and 39% for trimethoprim. In our experiments, the degradation rates of these compounds at the same UV dose were 47%, 46% and 41%, respectively.

The effect of the composition of Effluent Organic Matter (EfOM) on the performance of the process was previously reported by Rosario-Ortiz et al. [34]. They determined the absolute second-order rate constants for the reaction between the hydroxyl radical (kEfOM-HO•) and non-isolated EfOM collected at different wastewater and reclamation sites. They observed that the value of kEfOM-HO• is directly related to the aromaticity of the EfOM (measured as specific absorbance at 254 nm). Dong et al. [35] also investigated the reactivity of EfOM with hydroxyl radical, but in this case, as a function of the subcomponents of EfOM . They observed that the kEfOM-HO• values decreased as the apparent molecular weight (AMW) of the fraction increased. Therefore, soluble microbial products (SMP) with AMW <1 kDa are higher •OH scavengers than polysaccharides with high molecular weight. Consequently, different values of DOC may give rise to similar •OH scavenging effects. The scavenging contribution of water components in three different wastewater effluents was studied by Rosario-Ortiz et al. [33]. They found that the •OH scavenging contribution of effluent organic matter in wastewater ranged from 76 to 93% of the overall •OH scavenging rate.

In addition, considering the sulfate, chloride and nitrate ions present in a wastewater sample, the overall scavenging factor is around 18%, higher than those obtained when only DOC, H_2O_2 and alkalinity are considered. Yang et al. [36] evaluated the importance of different inorganic ions for altering the UV/H_2O_2 AOP performance in the absence of EfOM. Experiments conducted with each of the ionic constituents of RO brine confirmed that the most responsible anions for radical •OH scavenging were carbonates and bromides.

3.3.3. Continuous reaction

A study was also made of pharmaceutical removal in continuous mode in the custom-made photochemical reactor. A flow rate of 333 mL min^{-1} and residence time of 75 min were employed for this part of the experiment. The samples were collected for 4 h to ensure regularity of the results.

The average percentage degradation rates of the pharmaceuticals were obtained in continuous reaction and are shown in Fig. 4 together with their deviations from the mean. The results were reasonably constant with allowable deviations. Significantly, the degradation rates for continuous and discontinuous reaction were very similar.

The remaining concentration of H_2O_2 (10.37 ± 0.99 mg L^{-1}) was the same in both the continuous and discontinuous reaction. No major changes in TOC were observed with the continuous reaction, remaining steady at 20.42 ± 1.06 mg L^{-1}. Finally, absorbance at 254 nm of the water at the outlet remained at 0.15 ± 0.01. Absorbance therefore decreased in a 43%. A linear correlation between the percent removal of UV254 and the percent removal of pharmaceuticals during UV/H_2O_2 treatment of three different wastewater effluents was previously observed by Baeza and Knappe [37]. In this study, a 25% reduction in UV254 resulted in approximately 80–90% removal of carbamazepine and atenolol. In our case, the aforementioned percentages of removal were reached with a 43% reduction in UV254 probably due to the different composition of the treated effluent.

Despite the intermediates produced by UV/H_2O_2 oxidation of some pharmaceuticals may become more toxic than parent compounds [38,39], the few existing studies about the toxicity assessment of UV/H_2O_2 process performed on real wastewater did not reveal an increase of the toxic effects in the treated effluent. Vom Eyser et al. [40] investigated the toxicity assessment of oxidative transformation by UV/H_2O_2 of carbamazepine, ciprofloxacin, diclofenac, metoprolol and sulfamethoxazole. They did not observe an increase in the genotoxicity and cytotoxicity of water sample after UV/H_2O_2 process. Rozas et al. [41] neither

detect toxicity on Daphnia Manga after UV/H_2O_2 treatment of a mixture of atrazine, carbamazepine, diclofenac and triclosan at higher UV dose (1000 mJ cm^{-2}). Moreover, Justo et al. [42] observed that this oxidative process also decreased the dioxin-like activity from the treated reverse osmosis brine.

4. Conclusions

The combination of UV-C and H_2O_2 in a custom-made photochemical reactor to remove twenty-three different pharmaceuticals from wastewater samples gave satisfactory results. Metamizole, ketoprofen, omeprazole, sulfamethoxazole, ranitidine, propranolol, ofloxacin, nicotine, diclofenac and clofibric acid displayed high sensitivity to photolysis. However, UV irradiation alone was insufficient to degrade all the studied compounds. An average removal rate of 93% was possible with the optimum concentration of H_2O_2 (20 mg L^{-1}). After optimization, a kinetic study of each compound was performed in the reactor and a residence time of at least 75 min was required to remove more than 90% of all the studied pharmaceuticals. Calculations of the apparent rate constant and UV_{90} showed that a high dose of UV is required in this kind of water to remove compounds such as caffeine and its metabolite paraxanthine. A 43% reduction in absorbance at 254 nm was obtained at the end of the reaction. However, TOC remained unchanged after removal of the pharmaceuticals.

A follow-up treatment with activated carbon is proposed to completely remove all pharmaceuticals from treated effluents.

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III.2.3. Eliminación de 23 fármacos en agua usando TiO_2 inmovilizado en filtros de espumas cerámica.

Fruto de una segunda colaboración con el Grupo de Fotocatálisis y Espectroscopía Aplicada al Medioambiente (FEAM) se ha usado una metodología analítica multiresiduo como herramienta para desarrollar y optimizar otro proceso avanzado de oxidación, basado en la fotocatálisis heterogénea, usado para la eliminación o reducción de principios activos de residuos farmacéuticos presentes en muestras de agua.

La aplicabilidad y eficacia de la fotocatálisis heterogénea con dióxido de titanio (TiO_2) para degradar contaminantes emergentes, como los productos farmacéuticos, en aguas residuales ha sido investigado por numerosos autores. Sin embargo, la mayoría de los estudios están enfocados en optimizar sus parámetros para degradar simultáneamente sólo un número reducido de compuestos. Además, otra limitación de la mayoría de los estudios que se han publicado es que la eficacia de esta técnica de oxidación avanzada sólo se comprueba cuando se utiliza el fotocatalizador en forma de polvo y sin tener en cuenta las dificultades y rentabilidad de la separación de las nanopartículas de TiO_2 del agua tratada y reciclado del propio fotocatalizador. Por lo tanto, la inmovilización del fotocatalizador sobre un soporte adecuado es vital para minimizar la descarga de nanopartículas de TiO_2 en el medioambiente y posibilitar su mayor reutilización.

En este sentido, este trabajo se llevó a cabo con el objetivo de desarrollar un sistema fotocatalítico eficiente que pudiera ser utilizado para eliminar contaminantes persistentes, no biodegradables y tóxicos presentes en bajas concentraciones en aguas. Para este propósito, se

sintetizó un TiO_2 altamente fotoactivo mediante un método simple y económico y se apoyó sobre una espuma cerámica formada por macroporos en forma de red, con una porosidad abierta de aproximadamente 90 %, lo que le confirió una alta superficie específica y baja resistencia a la circulación.

La eliminación de 23 productos farmacéuticos de aguas residuales se evaluó utilizando un reactor fotocatalítico con TiO_2 inmovilizado en un filtro de espuma cerámica. El método desarrollado para soportar el fotocatalizador en las espumas de alúmina se basó en el proceso de recubrimiento por inmersión (dip-coating) a escala de laboratorio. La morfología superficial y la textura del TiO_2 sobre la espuma fue monitorizada mediante microscopio electrónico de barrido (SEM) y, también, se analizó el fotocatalizador por difracción de rayos X (XRD) para comprobar su actividad fotocatalítica. Por otra parte, se evaluó la desactivación del catalizador después de seis ciclos de tratamiento y se demostró que, aunque la fotoactividad del catalizador disminuye lentamente después del primer ciclo, la degradación del contaminante continúa.

En definitiva, las pruebas sobre el sistema fotoactivo soportado indicaron que éste es adecuado para procesos continuos sin que suponga una pérdida del material. Asimismo, no se observaron diferencias significativas en la fotoactividad entre TiO_2 en suspensión o cuando se soportó sobre el sistema de alúmina. En consecuencia, el reactor a escala de laboratorio diseñado con este proceso avanzado de oxidación es un método eficaz y eficiente para separar las nanopartículas de TiO_2 del agua

tratada. Además, el reciclado y la reutilización del fotocatalizador también podrían ser posibles.

Los resultados de los experimentos fotocatalíticos aplicados a la eliminación de productos farmacéuticos presentes en agua mostraron que los compuestos con carácter ácido se degradan más rápidamente que los productos farmacéuticos con carácter básico. Además, la influencia de la materia orgánica en diferentes tipos de agua en la eliminación del compuesto se probó en el fotorreactor en condiciones óptimas con el fin de evaluar el efecto matriz del agua. Se pudo observar que el proceso era severamente inhibido en las aguas procedentes de efluentes de estaciones depuradoras con contenido en materia orgánica y una cierta alcalinidad. En todo caso, se trata de una técnica altamente eficaz para la degradación de compuestos farmacéuticos presentes en agua potable, cuya degradación es casi completa después de 45 min de reacción, o en aguas residuales recuperadas altamente tratadas. Por ejemplo, en aguas regeneradas mediante tratamiento de ósmosis inversa se obtiene una degradación completa tras 90 min de reacción.

REMOVAL OF PHARMACEUTICALS FROM RECLAIMED WATERS USING TiO_2 IMMOBILIZED ON CERAMIC FOAMS

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ABSTRACT

In this work, the removal of persistent pharmaceuticals from reclaimed water was evaluated using a photocatalytic reactor with TiO_2 immobilized on ceramic foam. A facile up-scaling dip-coating method has been developed to support a lab-made TiO_2 on commercial ceramic foam with a macropore density of 20 ppi. A temperature of 973K was required to obtain the maximum performance of the synthesized photocatalyst. The photocatalytic properties of the novel TiO_2 ceramic foam was compared with the TiO_2 in slurry or immobilized on borosilicate glass. Results on phenol degradation and mineralization showed that the photoactivity of ceramic foam is comparable to the slurry system. Catalyst deactivation after six cycles of treatment was also assessed. It was observed that though catalyst photoactivity decreased slowly after the first cycle, contaminant degradation continued. Photocatalytic degradation of twenty-three persistent pharmaceuticals at initial concentration of 1 $\mu g \cdot L^{-1}$ was successfully achieved. The results showed that the acidic pharmaceutical compounds (diclofenac,

ketoprofen, ibuprofen, naproxen, gemfibrozil, clofibric acid, bezafibrate, ofloxacine, ciprofloxacine) are degraded faster than basic compounds (carbamazepine, metronidazol and caffeine). In addition, the water matrix effect on compound removal was also investigated. The photocatalytic process was severely inhibited in reclaimed effluents containing background organic matter and alkalinity. However, this technology can be applied in the elimination of micropollutants present in recycled effluents subjected to more refined purification processes or in freshwater.

Keywords: Immobilized TiO_2, ceramic foam, reclaimed water, micropollutants, pharmaceuticals.

1. INTRODUCTION

The presence of a wide variety of pharmaceuticals and personal care products (PPCPs) in aqueous environments has been frequently reported since the findings of Ternes 1998 [1] and Daughton and Ternes 1999 [2]. The ubiquity of these compounds is due to they are not efficiently removed in conventional wastewater treatment plants and are released into surface waters resulting in adverse effects on associated aquatic ecosystems [3]. For this reason, the scientific community has focused on the development of new treatment systems to effectively remove these compounds which are found in wastewater at concentrations of between 10^{-6} and 10^{-9} g L^{-1} [4]. Adsorption on activated carbon, membrane filtration technologies and advanced oxidation processes are some of the types of treatment used to remove these substances from depurated water at wastewater treatment plants [5].

Advanced oxidation processes (AOPs) are based on the production of highly reactive oxidizing species, such as hydroxyl radicals, or other reactive oxygen species (ROS), such as superoxide radical anions, hydroperoxyl radicals, or singlet and triplet oxygen [6]. AOPs are able to non-selectively degrade organic pollutants. Among AOPs, combining UV light with hydrogen peroxide (H_2O_2) and Fenton-type processes have successfully been applied to mineralize or convert pharmaceuticals to less harmful subproducts [6,7]. The applicability and effectiveness of heterogeneous photooxidation using titanium dioxide (TiO_2/hν) to degrade these contaminants from wastewater have also been investigated by various authors [8,9]. A recent article reviewed studies carried out on a variety of TiO_2 photocatalytic treatments, with diverse modifications, used to remove a long list of selected pharmaceuticals [10]. However, the majority of these studies focused on the optimization of operational parameters to simultaneously degrade just a few compounds. Studies considering a large number of pollutants with different physical-chemical characteristics and under realistic conditions are scarce. Only Choi et al. [11] performed a comparative study of the efficacy of TiO_2-UVA to degrade pharmaceuticals from different real water matrices. Another limitation of most of the studies that have been published is that the effectiveness of TiO_2 in removing pharmaceuticals from reclaimed water is only tested when using the photocatalyst in powder form and without taking into consideration the difficulties and cost-effectiveness of downstream separation of the nano-size TiO_2 from the treated water and recycling of the photocatalyst, especially in urban wastewaters [12]. Therefore, immobilization of the photocatalyst onto a proper support is vital to minimize the discharge of TiO_2 nanoparticles into the environment. A proper support needs to be resistant to oxidizing environments and have a large surface area in order to enhance the contaminant/photocatalyst contact [13,14]. In the field of organic micropollutant

degradation, the most commonly tested support has been glass with diverse configurations. Borges et al. 2015 [15] showed that the use of packed glass spheres was an effective method to remove acetaminophen from water. In their study, the benefits were shown of using the TiO_2 coating on glass spheres compared to suspended material. Glass materials have also been used as photocatalyst support to degrade pesticides, PPCPs or endocrine disrupting compounds (EDCs) with good results [16–18]. However, the surface area of glass materials is low. Other structured materials with a larger surface area, such as quartz fiber filters, porous titanium sheets [19] or ceramic membranes [20] have also been tested on micropollutant degradation. The results showed that neutral compounds are poorly removed when quartz filter or porous titanium sheets are used. In the case of ceramic membranes, the need to use an increased TiO_2 load to have an acceptable activity results in blockage of the membrane porous structure.

Several authors reported that ceramic or metal foams with macroporous structure are suitable as photocatalysts supports to remove different organic compounds dissolved in water [14,21–33]. However, none of the previous studies have evaluated the photocatalytic capacity of a TiO_2-ceramic foam system in the treatment of real waters.

Another of the fundamental requirements of a workable water-based photocatalytic reaction system for a realistic application is that it should include a high performance TiO_2 coating. Previous investigation observed that thick TiO_2 coatings are very efficient to remove micropollutans from water [29,34]. It is also important to note that the immobilization method of the photocatalyst to the support should be simple and inexpensive. Some methods of preparation have been proposed to obtain functional thick TiO_2 coatings on ceramic supports, including spraying of commercial TiO_2 [26],

dip-coating of commercial or precipited TiO_2 nanopowder or direct sol-gel synthesis [14,32]. Normally the immobilized TiO_2-ceramic foams that obtain higher photoactivity are those that use commercial Aeroxide® P25 as photocatalyst. However, previous studies have demonstrated that photocatalysts with larger particle size than commercial P25 and polyhedral morphology are more effective to degrade pollutants with moderate or low adsorption on TiO_2 surface [35–38].

In this sense, this work was carried out with the aim of developing an efficient photocatalytic system that can be used to eliminate persistent, non-biodegradable pharmaceuticals present at low concentrations in waters. For this purpose, a highly photoactive bare TiO_2 was synthesized by a simple and inexpensive method and supported on ceramic foam formed by reticulated macropores, with an open porosity of about 90% giving it a high specific surface area and low resistance to throughflow. The novel photocatalytic system has also been evaluated in terms of stability and reproducibility.

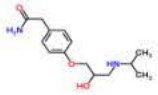
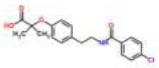
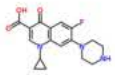
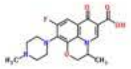
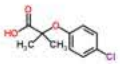
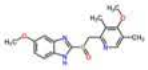
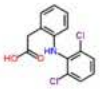
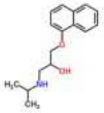
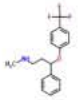
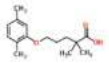
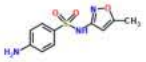
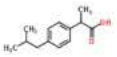
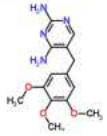
2. MATERIALS AND METHODS

2.1. Chemicals

Phenol (99%) and the pharmaceutical compounds (99%) (Table 1) used in this study were purchased from Sigma-Aldrich. Stock solutions of pharmaceuticals were prepared in methanol at a concentration of 1 $g \cdot L^{-1}$.

Methanol and water (LC-MS grade) were used to prepare the mobile phase for LC-MS/MS. Solvents and formic acid required to adjust the pH of the mobile phase were obtained from Panreac Química. Acetonitrile HPLC grade (≥99.8%, Panreac Química was used in the HPLC/DAD analysis.

Table 1. List of selected pharmaceuticals

No.	Compound	Molecular structure	No.	Compound	Molecular structure
1	Atenolol		**13**	Metamizole	
2	Bezafibrate		**14**	Metronidazole	
3	Caffeine		**15**	Naproxen	
4	Carbamazepine		**16**	Nicotine	
5	Ciprofloxacin		**17**	Ofloxacin	
6	Clofibric acid		**18**	Omeprazole	
7	Diclofenac		**19**	Paraxanthine	
8	Erythromycin		**20**	Propranolol	
9	Fluoxetine		**21**	Ranitidine	
10	Gemfibrozil		**22**	Sulfamethoxazole	
11	Ibuprofen		**23**	Trimethoprim	
12	Ketoprofen				

For catalyst preparation, ethanol (≥99%, Panreac Química), isopropanol (≥99%, Panreac), titanium (IV) n-butoxide (97%, Sigma-Aldrich) and titanium (IV)

isopropoxide (97%, Sigma-Aldrich) and ultrapure deionized water (18 MΩ·cm), prepared with a Millipore system, were used to synthesize the lab-made TiO_2. Commercial Evonik Aeroxide® P25 was used as reference.

Commercial VUKOPOR® A foam was purchased in Lanik® Foam Ceramics (Czech Republic) (Figure 1). Ceramic pieces of 50 mm x 50 mm x 20 mm were used except for the continuous-flow reactor for which pieces of 50 mm x 20 mm x 20 mm The chemical composition of is Al2O3 (85%), SiO2 (14.5%) and MgO (0.8%), with a specific surface area of 0.202 $m^2 \cdot g^{-1}$.

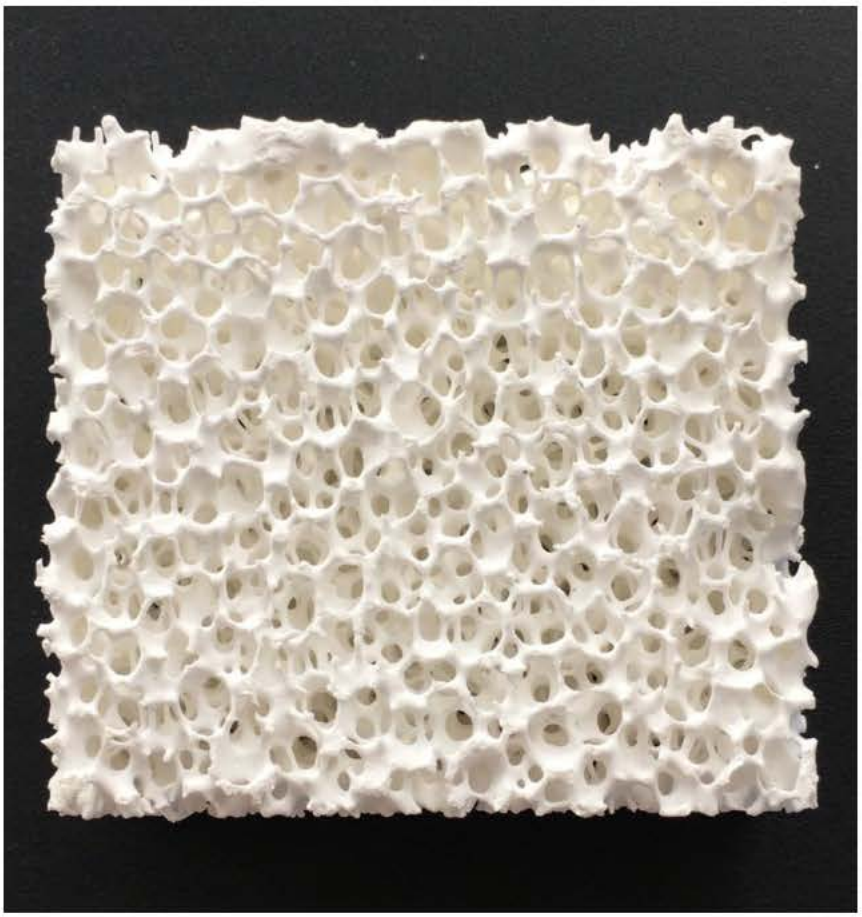


Figure 1. Image of Vukopor® A (20 ppi) foam.

2.2. Optimization of TiO_2 synthesis

Different TiO_2 nanoparticles were synthesized by the precipitation method using titanium (IV) butoxide or isopropoxide as Ti precursors. Also, ethanol and isopropanol were tested as solvents. The necessary amount of titanium precursor (titanium (IV) butoxide (TTB) or titanium (IV) isopropoxide (TTIP)) to obtain a molar ratio of 50:3.5 were added to 40 mL of ethanol or isopropanol and stirred for 10 minutes. Subsequently, the mixture was added drop by drop to a dilution consisting of 15 mL of water and 40 mL of appropriate solvent in each case. The synthesis was then stirred continuously for 30 minutes. Finally, it was dried for 24 hours and each assayed sample was calcined to 923, 973 and 1023 K.

2.3. Dip-coating process

Each alumina foam piece was immersed in the synthesis of the amorphous TiO_2 precipitate previously selected as the most photoactive. The fresh synthetized TiO_2 was subjected to different treatments in order to improve the suspension stability (Table 2). Then, the alumina piece was dipped at a speed of 29 $mm \cdot s^{-1}$. The immersed alumina was maintained during 4 min and then removed at 1.5 $mm \cdot s^{-1}$. This cycle was repeated four times. Subsequently, it was calcined to 973 K with a ramp of 303 $K \cdot h^{-1}$. At the same time, the TiO_2-foam was also prepared with commercial P25 using the same method, employing a suspension in ethanol of 40 $g \cdot L^{-1}$ and calcining to 723 K.

Table 2. Treatments performed to stabilize the suspension of TiO_2 on ceramic foam.

Foam	Disaggregation treatment	Thermal treatment (K)
Fresh synthesis (40 g·L^{-1})	--	973
Fresh synthesis (40 g·L^{-1})	Sonication pH 3	973
Fresh synthesis (40 g·L^{-1})	Sonication pH 11	973
Fresh synthesis (40 g·L^{-1})	Ball milling (30 min)	973

2.4. Photocatalytic experimental set-up

Photocatalytic activity experiments with slurry or immobilized TiO_2 were carried out in a continuous-flow borosilicate reactor with recirculation vessel. TiO_2 was deposited on borosilicate glass tube or alumina foam placed within the reactor (Figure 2). The volume of treated water was 300 cm^3 and the recirculation flow was 200 L·h^{-1}. The internal volumes of alumina or glass/slurry reactor were 84 and 112 cm^3, respectively. The photoreactor was irradiated by eight Philips CLEO fluorescent tubes (60 W power, maximum emission spectrum at 365 nm and irradiance flux of 9 mW·cm^{-2}).

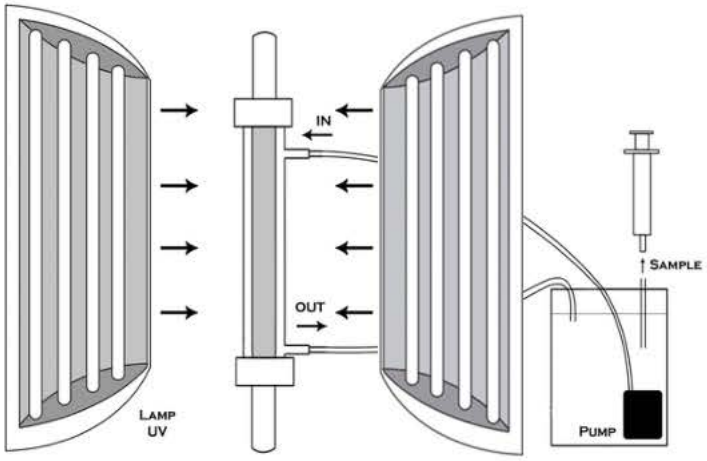


Figure 2. Photocatalytic system with TiO_2 supported on alumina foam or borosilicate tube.

A different UVA/TiO_2-ceramic foam reactor was used for photocatalytic experiments when low concentrations of pollutants were used. The volume of treated water was 1.5 L and the initial concentrations of pharmaceuticals and phenol were 1 $\mu g \cdot L^{-1}$ and 1 $mg \cdot L^{-1}$, respectively. The UVA/TiO_2-foam reactor was equipped with one immersed fluorescent tube and twelve fluorescent tubes surrounding the reactor. The reactor design is shown in Figure 3.

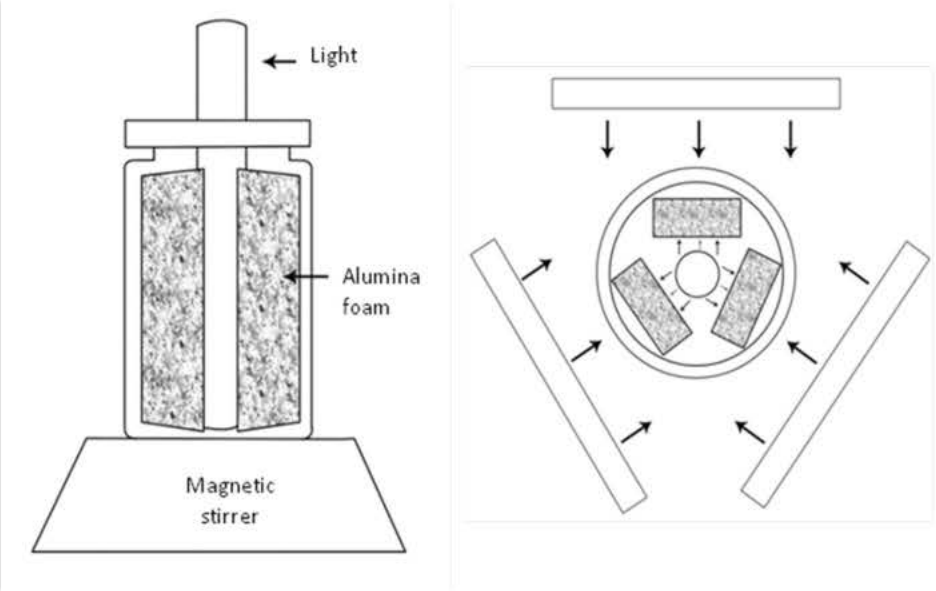


Figure 3. Lab-made photocatalytic reactor with TiO_2-ceramic foam

2.5. Analytical Methods

For extraction and concentration of the selected pharmaceuticals a solid-phase extraction method (SPE) was performed [39].

Variation in pharmaceuticals concentration was monitored using a LC-MS/MS system (triple quadrupole) equipped with an electrospray ionization (ESI) interface. The analytical method used for the pharmaceuticals determination was previously reported [40].

Phenol concentration was measured using an HPLC Varian Prostar system with a UV-Vis diode array detector ($\lambda = 270$ nm). A Supelco Discovery C_{18} column (250 mm × 4.6 mm, 5 µm) and 70:30 water-acetonitrile (v/v) were used as stationary and mobile phases, respectively. The limit of quantification reached for phenol was 0.5 $mg \cdot L^{-1}$.

Dissolved Organic Carbon (DOC) from the treated water samples was measured with a TOC-V_{SCN} analyser (Shimadzu).

Ion Chromatography was used to measure ion concentrations in reclaimed waters. For this purpose, a DIONEX Ionic Chromatograph equipped with a GP50 gradient pump and ED50 electrochemical detector was used. Anions and cations were separated with a column IonPac AS11-HC (4 × 250 mm) and an IonPac CS12A column (2 x 250 mm), respectively. Aqueous NaOH (30 mM) for anions or 20 mM metasulphonic acid were also used as eluents at a constant flow rate of 1 ml / min.

X-ray diffraction (XRD Bruker D8 Advance) with a monochromatized source of Cu-Kα1 radiation (λ = 1.5406 nm) at 1.6 kW (40 KV, 40 mA) was used the identification of crystalline phases. Crystal sizes were estimated from line broadening of the corresponding X-ray diffraction peaks using the Scherrer equation.

The morphology of the bare ceramic foam and the TiO_2 coating were observed by Field emission scanning electron microscopy (Hitachi S-5200 microscope) at a accelerated voltage of 5.0 kV. Additionally, images of a transmission electron microscopy (JEOL 2100F) operating at 100 kV was obtained to observe the morphology of the primary TiO_2 particles.

Table 3. Water samples characterization

Parameter	MF effluent	RO effluent
pH	7.97 ± 0.02	7.53 ± 0.02
Conductivity (µS/cm)	1369 ± 10	339 ± 8
DOC ($mg \cdot L^{-1}$)	14.8 ± 0.5	1.8 ± 0.5
%Transmittance UV365	77 ± 1	99 ± 0.5
Turbidity (NTU)	1.08 ± 0.07	0
HCO^{3-} ($mg \cdot L^{-1}$)	174.1 ± 0.5	20 ± 0.8
Cl^{-} ($mg \cdot L^{-1}$)	344 ± 2	77 ± 1
NO_2^{-} ($mg \cdot L^{-1}$)	8.91 ± 0.06	1.77 ± 0.03
SO_4^{2-} ($mg \cdot L^{-1}$)	66.2 ± 0.3	0.92 ± 0.04
NO^{3-} ($mg \cdot L^{-1}$)	22.26 ± 0.08	0
PO_4^{3-} ($mg \cdot L^{-1}$)	97.50 ± 0.05	26.01 ± 0.04
Na^{+} ($mg \cdot L^{-1}$)	234.22 ± 0.05	37.29 ± 0.07
K^{+} ($mg \cdot L^{-1}$)	35.30 ± 0.04	7.85 ± 0.02
Mg^{2+} ($mg \cdot L^{-1}$)	14.71 ± 0.04	6.96 ± 0.03
Ca^{2+} ($mg \cdot L^{-1}$)	40.58 ± 0.09	4.74 ± 0.01

2.6. Reclaimed water characterization

Water samples were obtained from a municipal wastewater treatment plant after a biological treatment with activated sludge. The MF effluent was obtained after subsequent filtration of the depurated water with a microfiltration (MF) system. RO effluent was treated by additional reverse osmosis (RO) process. Both effluents were vacuum-filtered through 1.2 µm and 0.45 µm cellulose acetate membranes immediately

upon receipt and stored at 277 K prior to use in the experiments. The water quality parameters, including pH, dissolved organic carbon (DOC), conductivity, % transmittance at 365 nm and anion and cation concentrations, are listed in Table 3.

3. RESULTS AND DISCUSSION

3.1. Optimization of TiO_2 synthesis

Percentages of anatase and rutile phase from different synthesized samples are shown in Table 4. The respective sizes of the different phases found were also determined. The use of TTB as precursor provides a catalyst with a higher proportion of anatase phase and a larger particle size of the anatase crystallite than with the use of TTIP as precursor, independently of the calcination temperature employed. In addition, the use of isopropanol as solvent produces a higher presence of rutile phase of the samples at 973 and 1023 K.

The sample based on TTB and ethanol and calcined at 973 K showed the highest photocatalytic activity. The percentage of degraded phenol was 82.5% after 30 minutes of reaction when using this sample. The corresponding value when using the commercial P25 was just 45%. It should also be noted that a greater percentage of rutile is obtained at 973 K using isopropanol as solvent, regardless of the precursor used.

3.2 Dip-coating process

The amount of supported TiO_2 and attachment to the ceramic substrate was evaluated as a function of the number of cycles. As can be seen in Table 5, the amount

of deposited mass increases with the number of cycles. However no differences in phenol degradation were observed above a deposited mass of 370 mg.

Table 4. Characterization of TiO_2 synthesis

Precursor	Solvent	Calcination Temperature (K)	Anatase Crystallite size (nm)	% Anatase	% phenol degraded after 30 minutes*
		923	43.90	85.0	72.01
	Ethanol	973	47.35	88.0	82.46
Titanium		1023	51.22	51.0	79.30
Butoxide		923	32.39	75.0	67.59
	2-Propanol	973	48.51	62.0	66.34
		1023	50.89	42.0	55.42
		923	42.66	75.0	68.03
	Ethanol	973	44.46	70.9	65.48
Titanium		1023	50.83	47.5	59.48
Isopropoxide		923	32.39	75.0	59.40
	2-Propanol	973	42.62	62.4	70.66
		1023	50.77	22.5	57.77
Evonik P25	---	---	22	80	45.09

* 50 $mg \cdot L^{-1}$ of initial phenol

Table 5. Assessment of dip-coating process as a function of number of cycles

Cycles	Deposited mass (mg)	Degraded phenol after 30 min (%)	Turbidity test[1] (NTU)	Ultrasonic test[2] (%)
2	243.60	63.00	0.90	32.00
4	290.70	68.00	0.93	45.00
6	326.60	71.00	1.34	50.00
8	370.40	75.00	1.40	55.00
10	450.80	75.00	1.67	60.00
12	555.00	74.00	1.89	65.00

[1]Results after the phenol degradation assays (initial concentration: 10 $mg \cdot L^{-1}$)

[2]Catalyst detached after 2 minutes of sonication

The results of the adhesion tests indicate that as the number of cycles increases, a higher release from the catalyst occurs. It was determined that the optimal number of cycles was four since turbidity was below 1 NTU after stirring and less than 50% of the catalyst became detached after ultrasonic application.

The effect of each treatment described in Table 2 on the stability of the suspension was also evaluated in terms of the amount of deposited mass, photoactivity in phenol degradation and catalyst adhesion to the ceramic substrate. The results are shown in Figure 4.

The modification of the pH to 3 or 11 for stabilization of the TiO_2 particles which had been broken up through application of an ultrasonic treatment did not result in any improvement in adhesion of this material to the substrate. However, a better adhesion to the ceramic foam is produced if the synthesis is ground for 30 minutes using

a planetary mill prior to the dip-coating process. In terms of TiO_2 deposited mass, the results are similar whether or not the original pH is modified from 4.7 to 3. However, if the pH of the synthesized catalyst suspension is increased to 11 or the planetary mill used, then less catalyst mass is deposited.

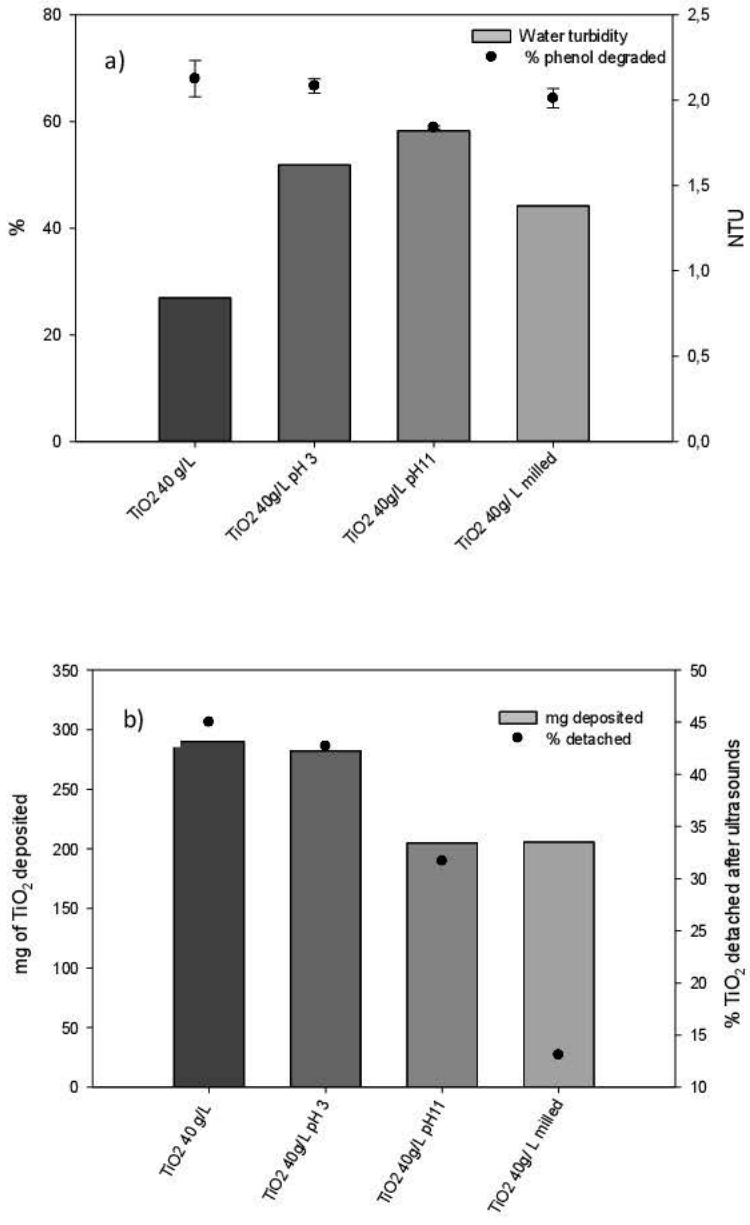


Figure 4. a) Percentage of degraded phenol and water turbidity (NTU) and b) TiO_2 deposited mass (mg) and percentage detached after ultrasound treatment.

There are no significant differences in the results for photoactivity in phenol degradation. Moreover, the adhesion of TiO_2 after the stirring in water is very similar in all treatments (between 0.8 and 1.8 NTU). Nevertheless, the grinding treatment of the fresh synthesis significantly improved adhesion of this material to the ceramic substrate. Only 13% of photocatalyst is detached after ultrasonic treatment of the piece. Nonetheless, as no differences were observed in the turbidity test, the subsequent studies of characterization and photoactivity were carried out with the deposited TiO_2 after four cycles of immersion-emersion and without treatment prior to the dip-coating process.

The photochemical reactor with twelve blocks of foam arranged in three columns (Figure 3) was used to carry out the photocatalytic treatment of the water containing pharmaceuticals. The mass of deposited catalyst on each foam and the final average turbidity values are detailed in Table 6. The total mass of catalyst and the reaction volume used were 2.19 g and 1.5 L, respectively.

Table 6. Adhesion test and mass of TiO_2 deposited on ceramic foam unit

Foam unit (50x50 mm)	**Catalyst load per unit**	**Adhesion test**	
		Initial turbidity	**Final turbidity**
12	0.274 (±0.09)	0.93 (±0.01)	2.14 (±0.24)

3.3 Structural characterization

The XRD analysis of the VUKOPOR® foam is shown in Figure 5. α-Al_2O_3 (corundum), $Al_6Si_2O_{13}$ (mullite) and SiO_2 (silica) were determined by XRD analysis of the pure foam (Fig. 4a).

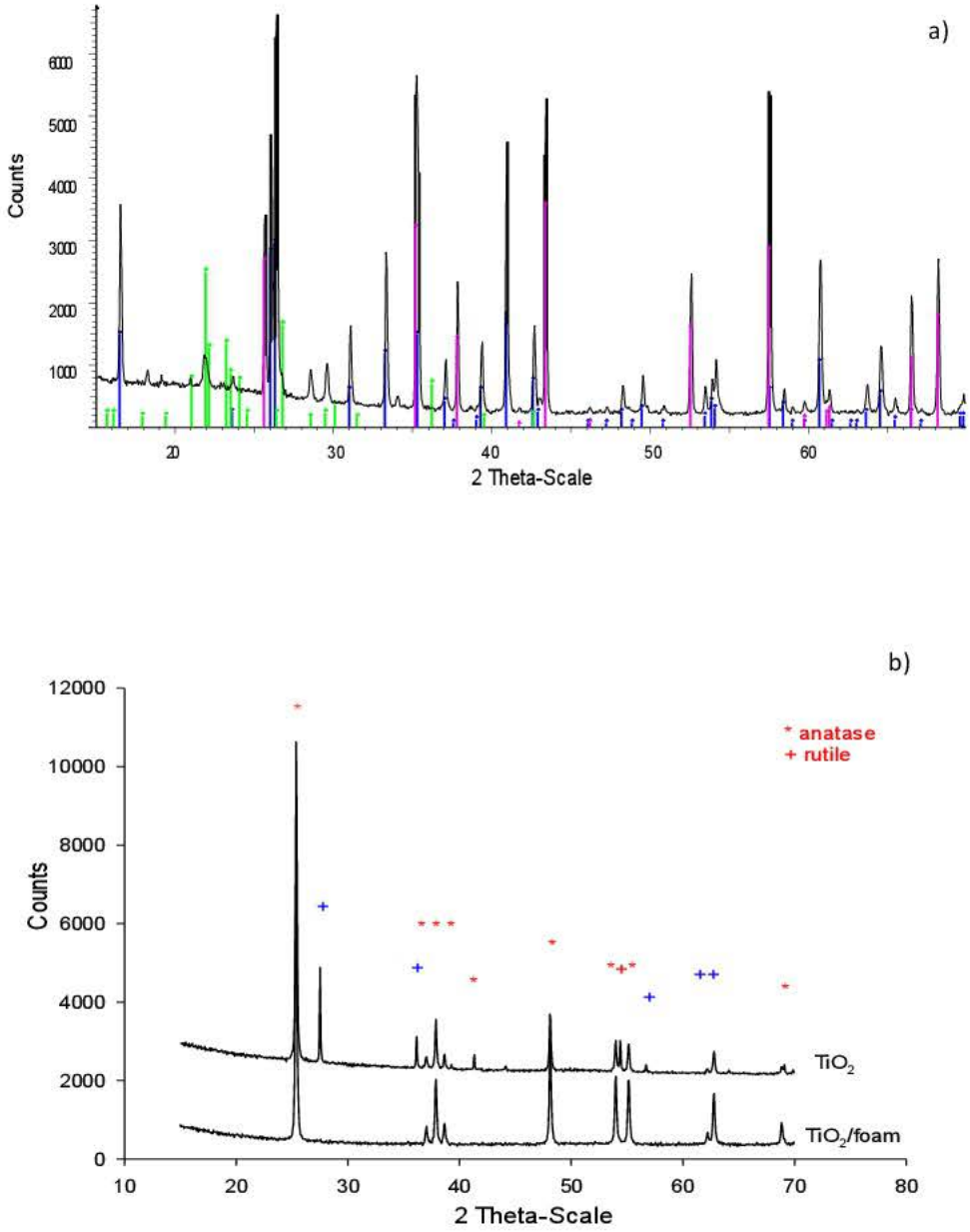


Figure 5. XRD analysis of a) VUKOPOR® foam. ● SiO_2 ▲ α-Al_2O_3 ■ $Al_6Si_2O_{13}$ and b) TiO_2 on foam and powder material calcined at 973 K

The formation of mullite is due to the fusion of silica and alumina at high temperatures [41]. The proportions of each phase, based on peak intensity, are

corundum (38%), mullite (53%) and silica (9%). The XRD analysis of calcined TiO_2 at 973 K on the ceramic foam is shown in Figure 4b, where it is also compared with XRD analysis of powder TiO_2 calcined at 973 K in a porcelain vessel. Stabilization of the anatase phase is observed in the TiO_2-ceramic system, obtaining TiO_2 with 100% anatase phase at 973 K. The obtained size of particle is, in this case, 43 nm, a value slightly lower than the powder catalyst.

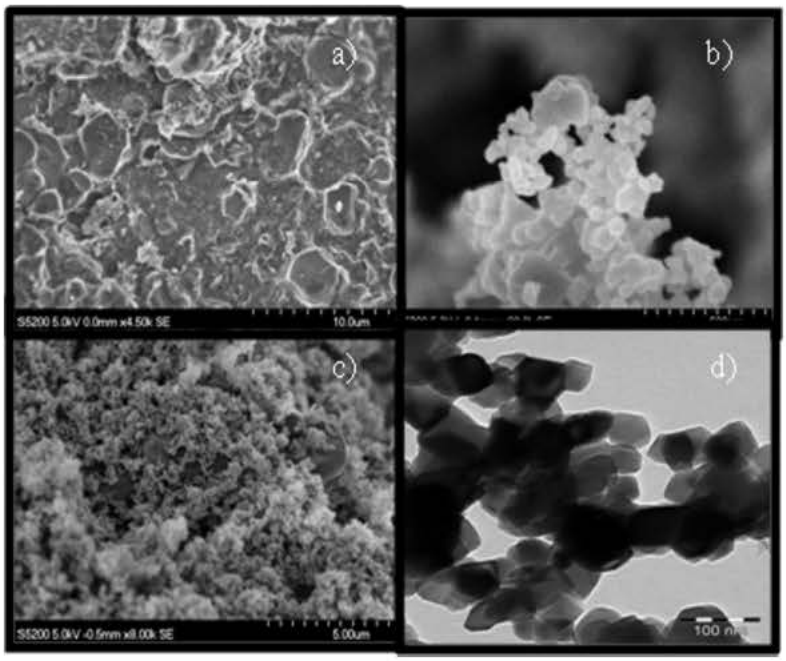


Figure 6. SEM images of a) microstructure of the surface of the VUKOPOR® ceramic foam, b) TiO_2 particles on alumina foam c) TiO_2 coating on alumina foam surface, and d) TEM image of deposited TiO_2.

An SEM analysis was also performed (Fig. 6), with the image showing a very rough surface of the VUKOPOR® foam consisting of grains with highly variable sizes whose diameters range up to 10 mm (Fig. 5a). The foam surface with deposited TiO_2 was also analyzed (Fig. 5c) and shows a homogeneous distribution in the particle size of

anatase crystallite SEM (Fig. 5b). Finally, a TEM image of TiO_2 particles (Fig. 5d) shows faceted particles with an average size of 50 nm. These results for average size are similar to those obtained in the XRD analysis (43 nm).

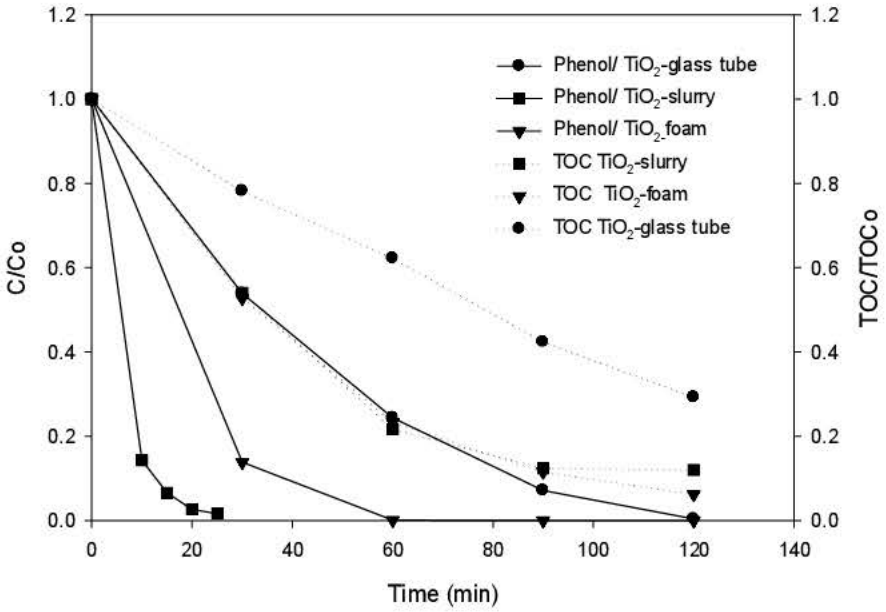


Figure 7. Evolution of phenol photodegradation (solid line) and TOC reduction (broken line) during a reaction run when using TiO_2 slurry ($1 g \cdot L^{-1}$) and TiO_2/ceramic foam or TiO_2/glass tube as immobilized system

3.4 Photocatalytic test

In order to compare the photocatalytic efficiency of the supported system with the activity of TiO_2 as slurry, photocatalytic phenol degradation and mineralization of TiO_2-coated foam and suspensions of powders containing equivalent mass concentrations of TiO_2 were measured under the same conditions. In addition, photoactivity of synthetized TiO_2 supported on a glass tube was also measured. Figure 7

shows the photocatalytic activities of TiO_2 in slurry and immobilized on glass tube or alumina foam.

Comparing the results of immobilized TiO_2, the ceramic foam system shows a higher rate of phenol degradation (10 $mg \cdot L^{-1}$) and mineralization than the glass tube support.

The degradation rate of phenol is higher when the photocatalyst is used in slurry. Apparent degradation constants were 0.175 and 0.030 min^{-1}, respectively. However, the percentages of mineralization of this compound, which were attained after 120 minutes using the two systems, are quite similar, with mineralization rate constants of 0.025 min^{-1} for the slurry and 0.024 min^{-1} for the TiO_2-ceramic foam system. Additionally, a comparison of the ceramic foam coated with the commercial P25 catalyst was performed. Phenol mineralization with deposited P25 was 0.010 min^{-1}. Vargova et al [14] deposited commercial P25 and lab-made precipited anatase power on macroporous reticulated Al_2O_3 foams. Phenol was completely degraded by thick P25 coating. However, the phenol mineralization was lower when TiO_2/Al_2O_3 foam prepared from precipitated anatase powder was used. Plesch et al [33] also studied the photocatalytic mineralization of phenol through the use of the previous TiO_2 immobilized systems. Only the presence of Zr (IV) as dopant increased significantly the photoactivity of the bare precipited anatase TiO_2. . Elatmani et al [21] also compared the mineralization capacity of P25 in slurry and immobilized on aluminum foam or cellulosic tissue. In this case, the photoactivity of the photocatalyst in slurry was higher than when it was immobilized on the aluminum foam.

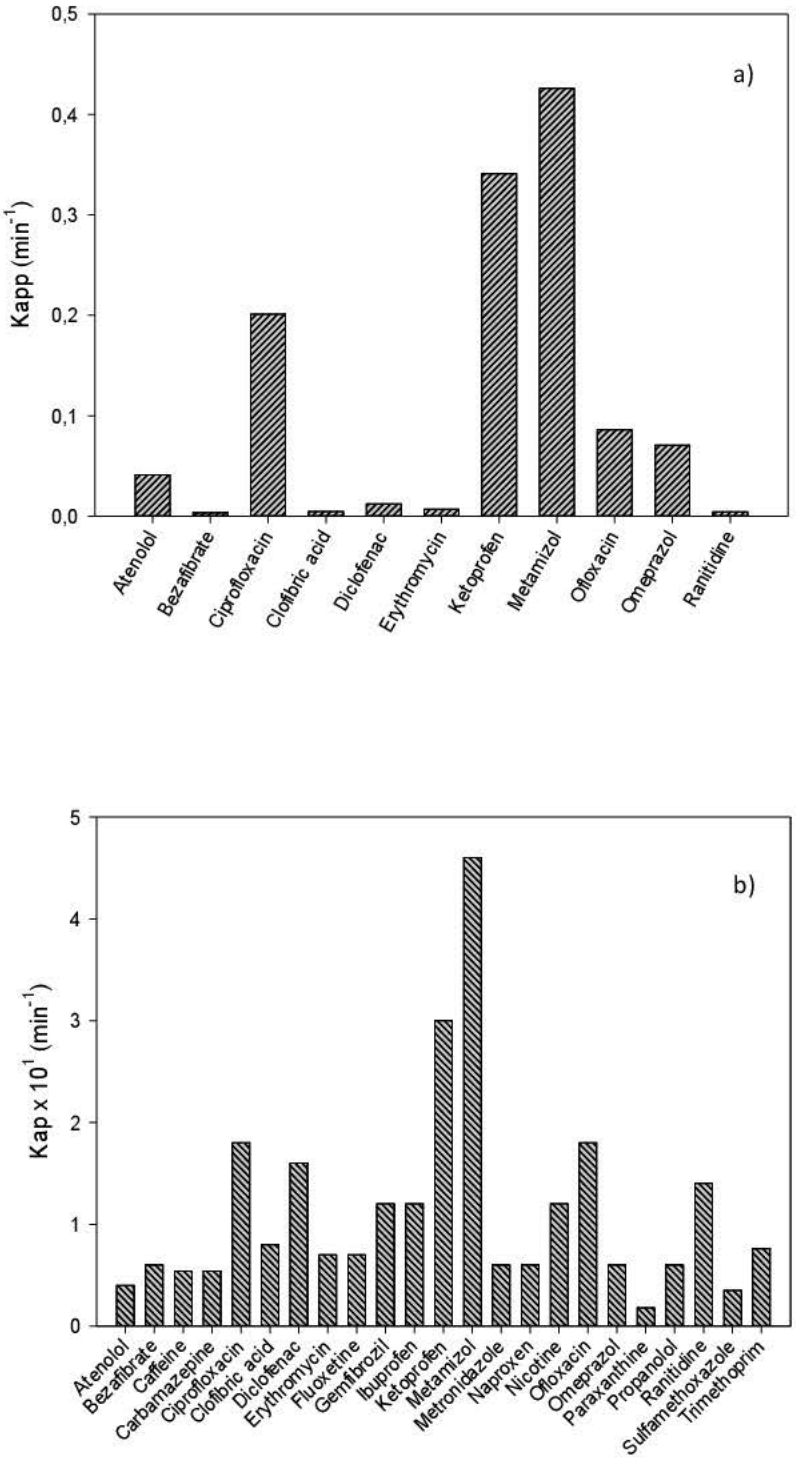


Figure 8. Kinetic degradation rate constants of 1 $\mu g \cdot L^{-1}$ of each target pharmaceutical compound calculated by means of a) UVA and b) UVA/TiO_2

Once the efficiency of the TiO_2-ceramic system to degrade and mineralize phenol had been measured, the photocatalytic degradation of 1 $\mu g \cdot L^{-1}$ of each target pharmaceutical compound was also evaluated. Some of the selected pharmaceuticals can be directly degraded by UVA light. The degradation rate constants of the compounds by means of UVA or UVA/TiO_2 are shown in Figure 8.

Eleven of the 23 target pharmaceutical compounds undergo photolysis in the presence of UVA light. Ketoprofen, metamizol and ciprofloxacin are degraded completely after 10 minutes of exposure to UVA light. The apparent degradation rate constants range between 0.018 and 0.46 min^{-1}. Paraxanthine, a derivative of caffeine, is the compound with the lowest degradation rate by photolysis with supported TiO_2 on ceramic foam. Metamizol, ketoprofen and ciprofloxacin have the same degradation rate constant through UVA and UVA/TiO_2. In general, the compounds with acid character are degraded faster than the pharmaceuticals with basic character. The isoelectric point of the photocatalyst is 4.85. The reaction pH was adjusted to 8. At that pH value, the TiO_2 surface and acidic pharmaceuticals (diclofenac, ketoprofen, ibuprofen, naproxen, gemfibrozil, clofibric acid, bezafibrate, ofloxacine, ciprofloxacine) are negatively charged as well as the TiO_2. Although the adsorption of acidic compounds is not favored, the synthetized TiO_2 is very efficient at degrading poorly adsorbed molecules, as seen above in the phenol degradation studies. The low degradation rates of caffeine and paraxanthine could be due to the degradation mechanism by direct hole oxidation not being favored in the synthesized TiO_2. The reduced photoactivity of TiO_2 samples with similar morphology in the degradation of strongly adsorbed compounds has been previously reported [42].

Finally, the effects of the water matrix on the pharmaceutical degradation rate were evaluated using a microfiltered effluent (MF) and another effluent treated with reverse osmosis (RO) in a Wastewater Treatment Plant (WWTP) (Fig. 9).

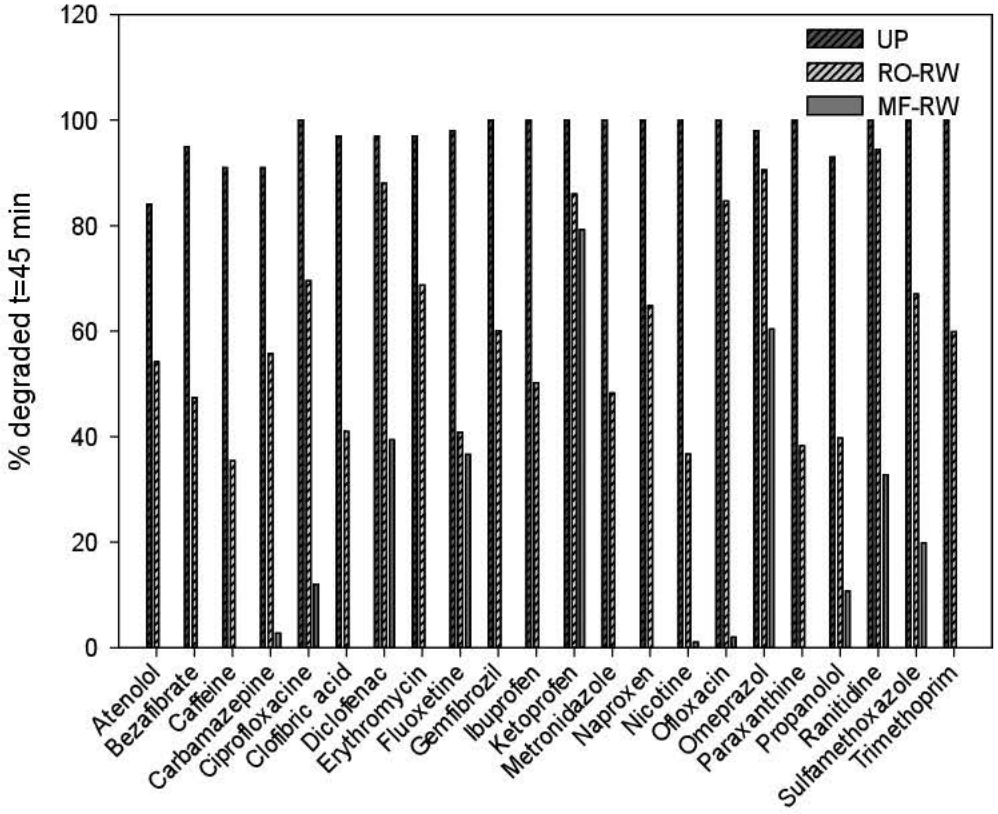


Figure 9. Percentage of pharmaceuticals degraded using a TiO_2/foam photocatalytic system after 45 min in ultrapure water (UP), reverse osmosis reclaimed water (RO-RW) and microfiltered reclaimed water (MF-RW)

The results show a low degradation percentage in MF reclaimed waters containing background organic matter and alkalinity. In this case, the TiO_2 process was severely inhibited due to factors such as blocking of the adsorption of organic molecules

and radical OH scavenging effects of natural organic material (NOM) or bicarbonates present in the effluent. This negative effect was also observed by Choi et al. [11]. Contrastingly, a near total degradation of the pharmaceuticals took place in the UP water after 45 min of reaction. The pharmaceuticals spiked in RO reclaimed water effluents were completely degraded after 90 minutes.

3.5. Reuse Test

The possibility of reusing the TiO_2/ceramic filter was evaluated. The value of the phenol degradation kinetic rate constant was measured in successive cycles of treatment. The results are shown in Figure 10.

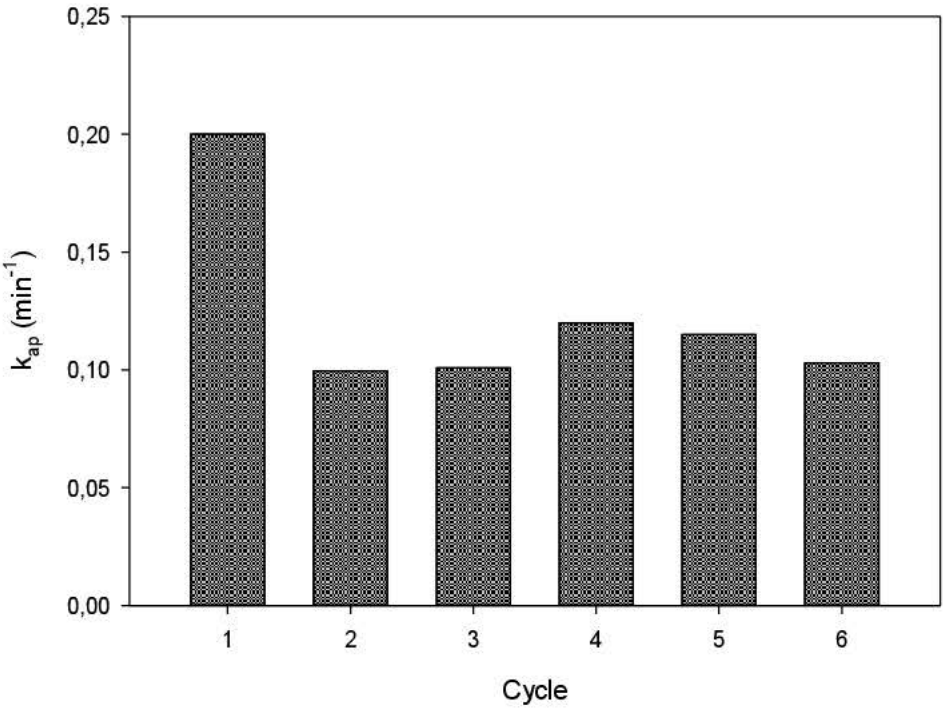


Figure 10. Phenol photodegradation kinetic rate constant at different treatment cycles

The phenol photodegradation rate falls slightly lower after the first run, but remains virtually unaltered in subsequent cycles. In addition, no loss of TiO_2 from alumina foam was observed, so catalyst deposition by the dip-coating method seems to provide adequate mechanical resistance.

4. CONCLUSIONS

In this study, a non-expensive dip-coating method was developed to deposit a highly photoactive synthesized TiO_2. This was carried out by using a simple precipitate sol-gel synthesis route on alumina foam. The adhesion to substrate tests showed that the supported photoactive system is suitable for continuous processes without loss of photoactive material. Moreover, no significant differences in photoactivity were observed between TiO_2 in slurry or when supported on the alumina system. Consequently, use of the lab-scale reactor designed for this advanced oxidation process is an effective and efficient method which avoids the need to separate nano-size TiO_2 from treated waters. In addition, recycling and reuse of the photocatalyst might also be possible.

Ceramic foam supported TiO_2 could be applied in the degradation of micropollutants, such as pharmaceutical compounds present in drinking water or highly treated reclaimed wastewaters. In waters from effluents containing background organic matter and alkalinity the TiO_2 process was severely inhibited, as shown by the results of the matrix effect evaluation.

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Capítulo IV: Conclusiones

Del conjunto de trabajos que conforman la presente Tesis Doctoral se pueden extraer las siguientes conclusiones:

a) Del desarrollo y la optimización de la metodología de análisis multiresiduo:

a.1. El método simplificado de extracción en fase sólida (SPE) optimizado en esta Tesis Doctoral ofreció una reducción tanto del tiempo de preparación de la muestra como de la cantidad de disolvente orgánico empleado, acercándonos de esta forma a los principios de la química analítica verde.

a.2. La técnica basada en la cromatografía líquida con detección por espectrometría de masas de triple cuadrupolo con ionización electrospray (LC-ESI-MS/MS) permitió, en un único análisis, la separación, detección y determinación de los residuos farmacéuticos

seleccionados en los extractos de las matrices líquidas estudiadas. A pesar del efecto matriz producido en este tipo de ionización, el uso de patrones internos y la correcta evaluación cuantitativa hace posible evaluar los niveles de concentración de dichos compuestos en las muestras analizadas.

a.3. Los parámetros analíticos proporcionados por la metodología analítica optimizada para la determinación de residuos farmacéuticos en muestras líquidas ofrece una mediana de recuperaciones del 70 % con desviaciones estándar relativas (RSDs) inferiores al 14,4 % y 22,0 % para la repetitividad intra- e inter-día, respectivamente. Mientras que los límites de detección (MDL) y los límites de cuantificación del método (MQLs) oscilaron entre 0,011 y 188 $ng \cdot L^{-1}$ y entre 0,033 y 628 $ng \cdot L^{-1}$, respectivamente.

De acuerdo a lo expuesto anteriormente, podemos concluir que la metodología de análisis multiresiduo desarrollada, basada en la combinación de la técnica simplifica de extracción en fase sólida con la determinación mediante la cromatografía líquida con detección por espectrometría de masas de triple cuadrupolo para la determinación de residuos farmacéuticos en muestras líquidas, se muestra como un procedimiento rutinario eficaz de análisis suficientemente sensible para monitorizar, mediante un solo análisis, los niveles de concentración presentes en muestras reales del medioambiente acuático.

b) De la aplicación de la metodología a muestras reales:

b.1. El método analítico optimizado se aplicó a la detección y determinación de fármacos en muestras líquidas procedentes de las diferentes etapas de tratamiento de dos EDARs situadas en la isla de Gran Canaria, con sistemas de depuración diferentes. Una de las EDARs posee un tratamiento convencional basado en un sistema de lodos activo y, seguidamente, un sistema de ósmosis inversa, mientras que la otra EDAR evaluada tiene un tratamiento natural mediante humedales.

b.2. A partir del monitoreo llevado a cabo durante seis meses de manera quincenal de muestras de agua procedentes de las diferentes etapas de tratamiento de las dos EDARs evaluadas, se han encontrado niveles de concentración en el rango de 0,004 ± 0,001 a 148 ± 14,7 $\mu g \cdot L^{-1}$.

b.3. La gran mayoría de compuestos farmacéuticos seleccionados fueron detectados frecuentemente en las diferentes etapas de tratamiento durante todo el intervalo temporal de estudio, siendo la cafeína el compuesto presente con mayores niveles de concentración a la entrada de las estaciones depuradoras estudiadas.

b.4. Se trata de la primera aplicación de una metodología multiresiduo para la determinación simultánea de una lista de fármacos en muestras procedentes de una zona geográfica con características hidrográficas diferentes a las encontradas en cualquier otra área de

España. Ello supone una contribución adicional e importante al repositorio de los datos sobre este problema ambiental.

b.5. La evaluación de la presencia de productos farmacéuticos en las EDARs de la isla de Gran Canaria es una forma útil de corroborar que la entrada principal de estos compuestos en matrices acuáticas medioambientales se produce a través de la excreción después de su uso o por una eliminación inadecuada, debido a que no existe ninguna industria farmacéutica en esta región que pueda perturbar los resultados.

c) *De la evaluación de la eficiencia de eliminación de diferentes tratamientos en activo en las EDARs:*

c.1. De los resultados obtenidos se observa que ambos tratamientos mostraron una evidente eliminación de una gran mayoría de los compuestos farmacéuticos durante el transcurso de las diferentes etapas, resultando el tratamiento convencional ser el más efectivo con una mediana de eliminación del 99,7 %.

c.2. En la EDAR convencional, la eliminación se mostró progresiva a medida que transcurrían las diferentes etapas de eliminación. Se comprobó que el tratamiento terciario de ósmosis inversa es un sistema clave para reducir los niveles de concentración de alguno de los compuestos más persistentes, sin embargo, no se trata de una eliminación o degradación sino de una transferencia hacia el agua de rechazo del sistema, produciendo un aumento de concentración en

esa etapa. Dado este hecho, se hace necesaria la introducción de nuevos sistemas de tratamiento avanzados.

c.3. En la EDAR natural, el comportamiento de los contaminantes fue más irregular, a pesar de ello, se alcanzaban una mediana de eliminación de hasta el 90 %. De esta forma, se puede concluir que es un sistema aceptable para núcleos poblacionales pequeños.

c.4. Para conocer, de manera específica, los mecanismos de eliminación de estos compuestos es necesario la adquisición de más datos acerca de la presencia de estos compuestos en otras matrices (por ejemplo matrices sólidas) o de sus metabolitos. De esta manera se puede conocer si existen procesos de adsorción o si, por el contrario, se está produciendo una transformación.

d) *Del cálculo del riesgo medioambiental:*

d.1. Los cocientes de riesgo calculados de manera predictiva para diferentes organismos acuáticos sobre las aguas con contenido en fármacos, que pueden ser descargadas directamente al medioambiente, revelaron que algunos compuestos como gemfibrozilo, ibuprofeno y ofloxacino podrían producir un riesgo importante en cualquiera de los organismos que se eligieron como indicadores. Otros compuestos como ciprofloxacino, ácido clofíbrico, diclofenaco, eritromicina, propanolol o sulfametoxazol podrían producir un impacto medio o alto en al menos uno de los niveles

tróficos más bajos. Sin embargo, la mayoría de los compuestos no presentaban un riesgo significativo.

d.2. El cálculo del riesgo ambiental está basado en predicciones, por lo que no se descarta que exista un riesgo real en los organismos que se encuentran en contacto con la descarga de aguas depuradas contaminadas con residuos farmacéuticos. Incluso estos cálculos no proporcionan información sobre el riesgo producido por la acción de la mezcla de los diferentes fármacos. De esta manera, el cálculo del impacto ambiental mediante el cociente de riesgo sólo nos ofrece una forma de escoger aquellos contaminantes que deberían ser vigilados más detenidamente a partir de ensayos *in vivo*.

Todo lo expuesto anteriormente nos lleva a concluir que existe la necesidad de tratamientos de aguas residuales adicionales o alternativos a los que se encuentran activos habitualmente en las EDARs. De esta forma se podría evitar la descarga de estos contaminantes al medioambiente, ya que se trata de un riesgo para los ecosistemas, sobretodo, en la zona geográfica donde se han realizado estos estudios donde la reutilización de las aguas regeneradas y el sistema acuático marino son indispensables como fuentes de recursos hídricos.

e) Del desarrollo y optimización de procesos avanzados de oxidación:

e.1. La combinación de UV y H_2O_2 para eliminar productos farmacéuticos diferentes de muestras de aguas residuales ofrece

resultados satisfactorios, alcanzando una tasa media de eliminación del 93 %, requiriendo de un tiempo de residencia de al menos 75 min para eliminar más del 90 % de todos los productos farmacéuticos estudiados.

e.2. Aunque algunos compuestos muestran una alta sensibilidad a la fotólisis, la irradiación únicamente por UV es insuficiente para degradar todos los compuestos bajo estudio.

e.3. El uso de un reactor fotocatalítico con TiO_2 inmovilizado en un filtro de espuma cerámica se trata de una técnica altamente eficaz para la degradación de compuestos farmacéuticos presentes en agua potable, cuya degradación es casi completa después de 45 min de reacción, o para aguas regeneradas, en las cuales la degradación se completa tras 90 min de reacción.

e.4. Ambos sistemas avanzados de oxidación que han sido evaluados, haciendo uso de la metodología analítica multiresiduo, resultan ser una alternativa para incluirlos como tratamientos adicionales en las EDARs debido a su capacidad de eliminación de compuestos farmacéuticos. Sin embargo, es indispensable el uso de tratamientos convencionales o naturales para evitar reducción de las eficiencias del tratamiento de depuración debido a "efectos matrices" producidos por altas cantidades en contenido orgánico.

Conclusions

The following conclusions can be obtained from the whole of the work presented in this Doctoral Thesis.

a) About the development and the optimization of the multiresidue analytical methodology:

a.1. The simplified solid phase extraction (SPE) method that has been optimized in this Doctoral Thesis offered a reduction both the sample preparation time and the amount of organic solvent used. At this way, it allows us to approach the principles of green analytical chemistry.

a.2. High performance liquid chromatography tandem triple quadrupole mass spectrometry allowed, in a single analysis, the separation, detection and determination of selected pharmaceutical residues in the extracts of the liquid matrices under study. In spite of the matrix effect produced in this type of ionization, the use of

internal standards and the correct quantitative evaluation make possible the assessment of the concentration levels of these compounds in the analyzed samples.

a.3. Analytical parameters achieved by the optimized analytical methodology to determine the pharmaceutical residues in liquid samples offer a recovery median of 70 % with relative standard deviations below to 14.4 % and 22.0 % for intra- and inter-day repeatability, respectively. In addition, method detection limits (MDLs) and method quantification limits (MQLs) were in the range 0.011 to 188 $ng \cdot L^{-1}$ and 0.033 to 628 $ng \cdot L^{-1}$, respectively.

According to the above, we can conclude that the multiresidue analytical methodology, based on the combination of the simplified solid phase extraction technique with determination by liquid chromatography tandem triple quadrupole mass spectrometry for the assesment of pharmaceutical residues in liquid samples, is shown as a routine procedure with a sensitive enough to monitoring the levels of concentration in real samples of the aquatic environment through a single analysis.

b) About the application of analytical methodology to real samples:

b.1. The optimized analytical method was applied for the detection and determination of drugs in the liquid samples from the different treatment stages of two EDARs located on the island of Gran Canaria,

whith different purification systems. One of WWTPs has a conventional treatment based on an active sludge system and, subsequently, reverse osmosis system, while another of the evaluated WWTPs has a natural treatment through constructed wetlands.

b.2. Based on the biweekly monitoring carried out of water samples from the different stages of treatment of two WWTPs located on the island of Gran Canaria for six months, it has been detected concentration levels in the range of 0.004 ± 0.001 to 148 ± 14.7 $\mu g \cdot L^{-1}$.

b.3. Most selected pharmaceutical compounds were frequently detected at different stages of treatment throughout the temporal interval of the study, and caffeine presented the higher concentration levels at influent of wastewater treatment plants.

b.4. This is the first application of a multiresidue methodology for the simultaneous determination of a list of drugs in liquid samples from a geographical area with different hydrographic characteristics from those found in any other area of Spain. It supposes an additional and important contribution to the repository on this environmental problem.

b.5. The evaluation of the presence of pharmaceutical products in the WWTPs of the island of Gran Canaria is a useful way of corroborating that the main entry of these compounds in environmental aquatic matrices occurs through excretion after use or by inappropriate

disposal, because there are not any pharmaceutical industries in this region that could disrupt the results.

c) *About the assessment of removal efficiencies of different active treatments from wastewater treatment plants (WWTPs):*

c.1. From the obtained results, it is observed that both treatments showed an evident elimination of a large majority of pharmaceutical compounds through different stages, providing the conventional treatment to be more effective with a median elimination of 99.7 %.

c.2. In the conventional WWTP, the elimination was progressive through the different elimination stages. Is has proved that the tertiary treatment of reverse osmosis is a key system for reducing the concentration levels of some of the more persistent compounds, however, it is not a matter of elimination or degradation but of a transfer to the water of rejection of the system, which produces an increased of concentration in this stage. Given this fact, the introduction of new advanced treatment systems is necessary.

c.3. In the natural WWTP, the behavior of the contaminants was more irregular. Despite this fact, a median elimination of up to 90 % was reached. In this way, it can be concluded that it is an acceptable system for small population.

c.4. In order to know more specifically the mechanisms of elimination of these compounds it is necessary to acquire more data about the

presence of these compounds in other matrices (for examples solid matrix) or its metabolites. In this way, it is possible to know if there are adsorption processes or, on the contrary, if a transformation is taking place.

d) *About the estimation of environmental risk:*

d.1. Predicted risk quotient for different aquatic organisms in contact with drug containing waters, which can be discharged directly into the environment, revealed that some compounds, such as, gemfibrozil, ibuprofen and ofloxacin could produce a significant risk in any of the organisms chosen as indicators. Other compounds, such as, ciprofloxacin, clofibric acid, diclofenac, erythromycin, propanolol or sulfamethoxazole could produce a medium or high impact in at least one of the lower trophic levels. However, most compounds did not present a significant risk.

d.2. The calculation of the environmental risk is based only on predictions, so a real risk is not ruled out in organisms that are in contact with the discharge of purified water contaminated with pharmaceutical residues. Even these calculations do not provide information on the risk produced by the action of the mixture of different drugs. In this way, the calculation of the environmental impact through the risk quotient only offers us a way to choose those pollutants that should be monitored more closely from *in vivo* tests.

All of the above leads us to conclude that it is neccesary the development of additional or alternative wastewater treatments to those that are usually active in the WWTPs. At this way, it is possible to prevent the discharge of these contaminants to the environment, specially in the geographical area where these studies have been carried out, where reuse of regenerated waters and the marine aquatic system are indispensable as sources of water resources.

e) *About the development and optimization of advanced oxidation processes:*

e.1. The combination of UV and H_2O_2 to remove different pharmaceuticals from wastewater samples provides satisfactory results, reaching an average elimination rate of 93 % and requiring a residence time of at least 75 min to eliminate more than 90 % of all target pharmaceuticals.

e.2. Although some compounds show a high sensitivity to photolysis, only UV irradiation is insufficient to degrade all compounds under study.

e.3. The use of a photocatalytic reactor with TiO_2 immobilized on a ceramic foam filter is a highly efficient technique for the degradation of pharmaceutical compounds present in potable water, whose degradation is almost complete after 45 min of reaction or for

regenerated water, in which the degradation is complete after 90 min of reaction.

e.4. Both advanced oxidation processes, which have been evaluated using multiresidue analytical methodology, are an alternative to include them as additional treatments in WWTPs due to their capacity to eliminate pharmaceutical compounds. However, it is essential to use conventional or natural treatments to prevent reduction efficiencies purification treatment due to "matrix effects" caused by high amounts in organic content.

Anexos

I. Acrónimos

AQMA: Análisis Químico Medioambiental

BCF: Bioconcentration factor

CE: Capillary electrophoresis

DAD: Diode Array detector

DDD: Dosis diaria definida

DHD: DDD/1000 habitantes por día

EC_{10}: Effective concentration to 10 %

EC_{50}: Half maximal effective concentration

EDAR: Estación depuradora de agua residual

ESI: Electrospray ionization

FD: Fluorescence detector

FEAM: Fotocatálisis y Espectroscopía Aplicada al Medioambiente

GC: Gas chromatography

i-UNAT:	Instituto de estudios ambientales y recursos naturales
LC:	Liquid chromatography
MDL:	Method determination limit
MEC:	Measured environmental concentration
MIP:	Molecularly imprinted polymer
MQL:	Method quantification limit
MS:	Mass spectrometry
NOEC:	No Observed Effect Concentration
NSAID:	Nonsteroidal anti-inflammatory drug
OMS:	Organización Mundial de la Salud
PAO:	Proceso avanzado de oxidación
PBT:	Persistencia, bioacumulación y toxicidad
PCB:	Polychlorinated biphenyl
PEC:	Predicted environmental concentration
PNEC:	Predicted no-effect concentration
REACH:	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ:	Risk quotient
RSD:	Relative standard deviation
SEM:	Scanning electron microscope
SPE:	Solid phase extraction
UHPLC:	Ultra high performance liquid chromatography
UV:	Ultraviolet
WFD:	Water framework directive
WWTP:	Wastewater treatment plant
XRD:	X-ray diffraction

II. Lista de publicaciones de la Tesis Doctoral

1) **Autores:** C. Afonso-Olivares, C. Fernández-Rodríguez, R. Ojeda-González, Z. Sosa-Ferrera, J. J. Santana-Rodríguez, J.M. Doña-Rodríguez

   Título: "Estimation of kinetic parameters and UV doses necessary to remove twenty-three pharmaceuticals from pre-treated urban wastewater by UV/H_2O_2"

   Revista: *Journal of Photochemistry and Photobiology A: Chemistry.*

   Volumen: 329

   Páginas: 130 – 138

   Fecha: 2016

2) **Autores (p.o. de firma):** C. Afonso-Olivares, S. Montesdeoca-Esponda, Z. Sosa-Ferrera, J. J. Santana-Rodríguez.

   Título: "Analytical tools employed to determine pharmaceutical compounds in wastewaters after application of advanced oxidation processes"

   Revista: *Environmental Science and Pollution Research*

   Volumen: 23 (24)

   Páginas: 24476 – 24494

   Fecha: 2016

3) **Autores (p.o. de firma):** C. Afonso-Olivares, T. Cadková, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, L. Nováková.

Título: "Simplfied solid-phase extraction procedure combined with liquid chromatography tandem–mass spectrometry for multiresidue assessment of pharmaceutical compounds in environmental liquid samples"

Revista: *Journal of Chromatography A*

Volumen: 1487

Páginas: 54 – 63

Fecha: 2017

4) **Autores (p.o. de firma):** C. Afonso-Olivares, Z. Sosa-Ferrera, J.J. Santana-Rodríguez.

Título: "Occurrence and environmental impact of pharmaceutical residues from conventional and natural wastewater treatment plants in Gran Canaria (Spain)"

Revista: *Science of the Total Environment*

Volumen: 599-600

Páginas: 934-943

Fecha: 2017

5) **Autores (p.o. de firma):** C. Fernández-Rodríguez, C. Afonso-Olivares, D. Garzón-Sousa, R.J. Ojeda-González, J. Araña, J.J. Santana-Rodríguez and J.M. Doña Rodríguez

Título: "Removal of pharmaceuticals from reclaimed waters using TiO_2 immobilized on ceramic foams"

Revista: *(en preparación para su envío a revista)*

Volumen:

Páginas:

Fecha: 2017

III. Comunicaciones presentadas a congresos

1) **Autores:** C. Afonso-Olivares, Z. Sosa Ferrera, C. Fernández-Rodríguez, J.M. Doña-Rodríguez, J.J. Santana Rodríguez.

   Título: "Optimization of a solid phase extraction combined with liquid chromatography tandem mass spectrometry procedure to simultaneous determination of pharmaceuticals compounds in water samples"

   Tipo de participación: Póster

   Congreso: 39th International Symposium on High-Performance-Liquid-Phase Separations and Related Techniques (HPLC 2013)

   Lugar de celebración: Ámsterdam (Holanda)

   Fecha: Junio 2013

2) **Autores:** C. Fernández Rodríguez, D. Garzón Sousa, C. Afonso Olivares, J.M. Doña Rodríguez, J. Araña, M.Z. Sosa Ferrera and J.J. Santana Rodríguez.

   Título: "Removal of pharmaceuticals from reclaimed water using a photocatalytic reactor with TiO_2 supported on alumina foams"

   Tipo de participación: Póster

   Congreso: 8^{th} European Meeting on Solar Chemistry and Photocatalysis: Environmental Applications (SPEA 8)

   Lugar de celebración: Thessaloniki (Grecia)

   Fecha: Junio 2014

3) **Autores:** C. Fernández Rodríguez, C. Afonso Olivares, D. Garzón Sousa, J.M. Doña Rodríguez, D. Fernández Hevía, T. de la Torre, J. Malfeito, Z. Sosa Ferrera and J.J. Santana Rodríguez.

Título: "Removal of pharmaceutical compounds from finish waste water treatment plant by using a photocatalytic reactor with TiO_2 immobilized on alumina filters"

Tipo de participación: Póster

Congreso: IWA World Water Congress & Exhibition

Lugar de celebración: Lisboa (Portugal)

Fecha: Septiembre 2014

4) **Autores:** C. Afonso Olivares, C. Fernández-Rodríguez, R.J. Ojeda González, J.J. Santana-Rodríguez, J.M. Doña Rodríguez.

Título: "Removal of pharmaceuticals from wastewater by UV/H_2O_2"

Tipo de participación: Póster

Congreso: International Meeting on Environmental and Pharmaceutical Analysis

Lugar de celebración: Las Palmas de Gran Canaria (España)

Fecha: Diciembre 2014

5) **Autores:** C. Fernández-Rodríguez, C. Afonso-Olivares, A. Martín González, R.J. Ojeda González, O. Domínguez Santana, J.J. Santana-Rodríguez, P. Susial, J.M. Doña Rodríguez.

Título: "Eliminación de microcontaminantes orgánicos mediante tratamientos avanzados para un uso potable indirecto de aguas regeneradas"

Tipo de participación: Oral

Congreso: II Workshop "Estudio, aprovechamiento y gestión del agua en terrenos e islas volcánicas"

Lugar de celebración: Las Palmas de Gran Canaria (España)

Fecha: Enero 2015

6) **Autores:** C. Afonso-Olivares, Z. Sosa-Ferrera, J.J. Santana-Rodríguez

Título: "A simplified solid phase extraction procedure combined with liquid chromatography tandem mass spectrometry to assess pharmaceuticals in liquid samples"

Tipo de participación: Póster

Congreso: 31ST International Symposium on Chromatography (ISC2016)

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7) **Autores:** J.M. Doña Rodríguez, C. Fernández-Rodríguez, D. Garzón-Souda, C. Afonso-Olivares, J. Araña, Z. Sosa-Ferrera, J.J. Santana-Rodríguez.

Título: "Removal of pharmaceuticals from reclaimed waters using a photocatalytic reactor with tio2 supported on alumina foams"

Tipo de participación: Oral

Congreso: Vth Euro-Mediterranean Workshop on Water, Sediments, Catalysis and Environment

Lugar de celebración: Fez (Marruecos)

Fecha: Octubre 2016

8) **Autores:** C. Afonso-Olivares, Z. Sosa-Ferrera, J.J. Santana-Rodríguez

Título: "Applying a simplified solid phase extraction combined with liquid chromatography tandem mass spectrometry to asses pharmaceuticals from wastewater"

Tipo de participación: Póster

Congreso: International Conference on Water: From Pollution to Purification (ICW2016)

Lugar celebración: Kottayam, Kerala (India)

Fecha: Diciembre 2016

OPTIMIZATION OF A SOLID PHASE EXTRACTION COMBINATED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY PROCEDURE TO SIMULTANEOUS DETERMINATION OF PHARMACEUTICALS COMPOUNDS IN WATER SAMPLES

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INTRODUCTION

The presence of pharmaceutical compounds in wastewater is one of the challenges in environmental monitoring, they are part of the so-called emerging contaminants. Control of these pollutants is particularly difficult due to the wide dispersion of emission sources ranging from household waste, hospital and industrial to discharges from farming and ranching [1]. These are subject to a constant discharge and also customary purification systems are not designed to remove, so that their levels in the medium can easily achieve high values [2]. The simultaneous detection of different classes of these pollutants, which require complex analytical processes, has become a major issue. Therefore, a multi-residue method permits analysis of a wide range of contaminants of different properties in a single run [3].

In this study, we develop a Solid Phase Extraction procedure combined with High Performance Liquid Chromatography tandem Mass Spectrometry (SPE-LC-MS/MS) for the determination of a multi-residue pharmaceutical compounds of different therapeutic classes. The parameters involved in SPE process and LC-MS/MS are optimized. The developed method could be applied like analytical method to determine the effectiveness of treatments for removing organic pollutants from wastewaters.

EXPERIMENTAL PROCEDURE

CHROMATOGRAPHY CONDITIONS

Chromatographic conditions	
Instrument	LC system from Varian with 320 MS mass spectrometry
Column	SunFireTM C_{18}
Inyection volume	10 µL
Flow rate	200 µL·min^{-1}
Mobil phase	A: water (0.015% formic acid)
	B: methanol

Gradient used		
Time (min)	% (A)	%(B)
0:0	95	5
1:0	95	5
21:0	60	40
40:0	10	90
43:0	10	90
46:0	95	5

RESULTS

OPTIMIZATION OF SOLID PHASE EXTRACTION

We studied the parameters that affect to the SPE procedure. The pH and ionic strength were optimized using a experimental design 3^2 (two variables, three levels). In Figure 1 we can observe that exist a different behaviour of the compounds.

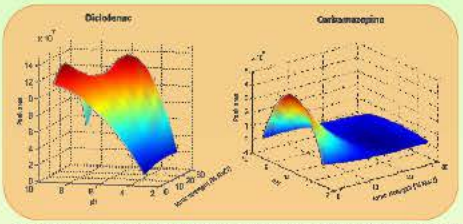

Figure 1: Response surface of the effect of ionic strength and pH on the SPE extraction for diclofenac and carbamazepine

Optimal conditions for SPE procedure:

Optimized variables			
Cartridge	Oasis HLB	Sample volume	250 mL
pH	9	Wash step	5 mL of Milli-Q water
Ionic strenght	15% NaCl	Desorption volume	2 mL of methanol
PRECONCENTRATION FACTOR = 125			

ANALYTICAL PARAMETERS

Compound	R_t	r^2	Recovery (%)	LOD (ng·L^{-1})[a]	RSD (%) (n=6) 50 µg·L^{-1}
Nicotine	2.03	0.9971	29.4	30.8	10.6
Atenolol	3.90	0.9994	85.7	12.3	19.7
Ranitidine	4.00	0.9913	58.7	6.26	19.3
Trimethoprim	8.60	0.9959	66.6	4.29	6.82
Metamizole	9.07	0.9919	147	23.5	19.6
Ofloxacin	11.13	0.9980	32.3	28.4	17.6
Metronidazole	11.75	0.9988	119	5.50	14.7
Ciprofloxacin	12.21	0.9909	68.6	19.1	6.70
Paraxanthine	14.01	0.9948	93.1	35.3	2.44
Propanolol	17.05	0.9958	56.3	10.4	15.5
Caffeine	17.70	0.9986	37.6	5.38	10.5
Sulfamethoxazole	20.14	0.9979	105	0.58	12.7
Erythromycin	24.20	0.9982	57.7	0.21	19.1
Fluoxetine	25.10	0.9982	30.6	0.16	19.9
Omeprazol	29.89	0.9971	48.7	0.72	10.8
Carbamazepine	31.78	0.9905	31.3	1.17	14.7
Ketoprofen	36.15	0.9953	53.4	2.94	9.37
Naproxen	36.38	0.9983	95.6	0.72	17.6
Ibuprofen	40.71	0.9901	117	67.9	8.46
Bezafibrate	41.05	0.9988	68.8	1.99	14.2
Diclofenac	41.94	0.9950	73.6	0.19	18.6
Gemfibrozil	42.00	0.9942	83.0	2.34	9.12
Clofibric acid	43.05	0.9969	76.4	0.39	17.7

a) Limit of detection

Table 1: Analytical parameters for SPE procedure combined with LC-MS/MS.

CONCLUSIONS

We have developed an analytical method for the evaluation of 23 pharmaceutical compounds that consists of SPE as extraction step and, subsequently, the detection was made by high performance liquid chromatography with mass spectrometry detection (LC-MS/MS).

The limits of detection that were achieved with the method were appropriate for the detection of pharmaceutical compounds in real samples.

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ACKNOWLEDGMENTS

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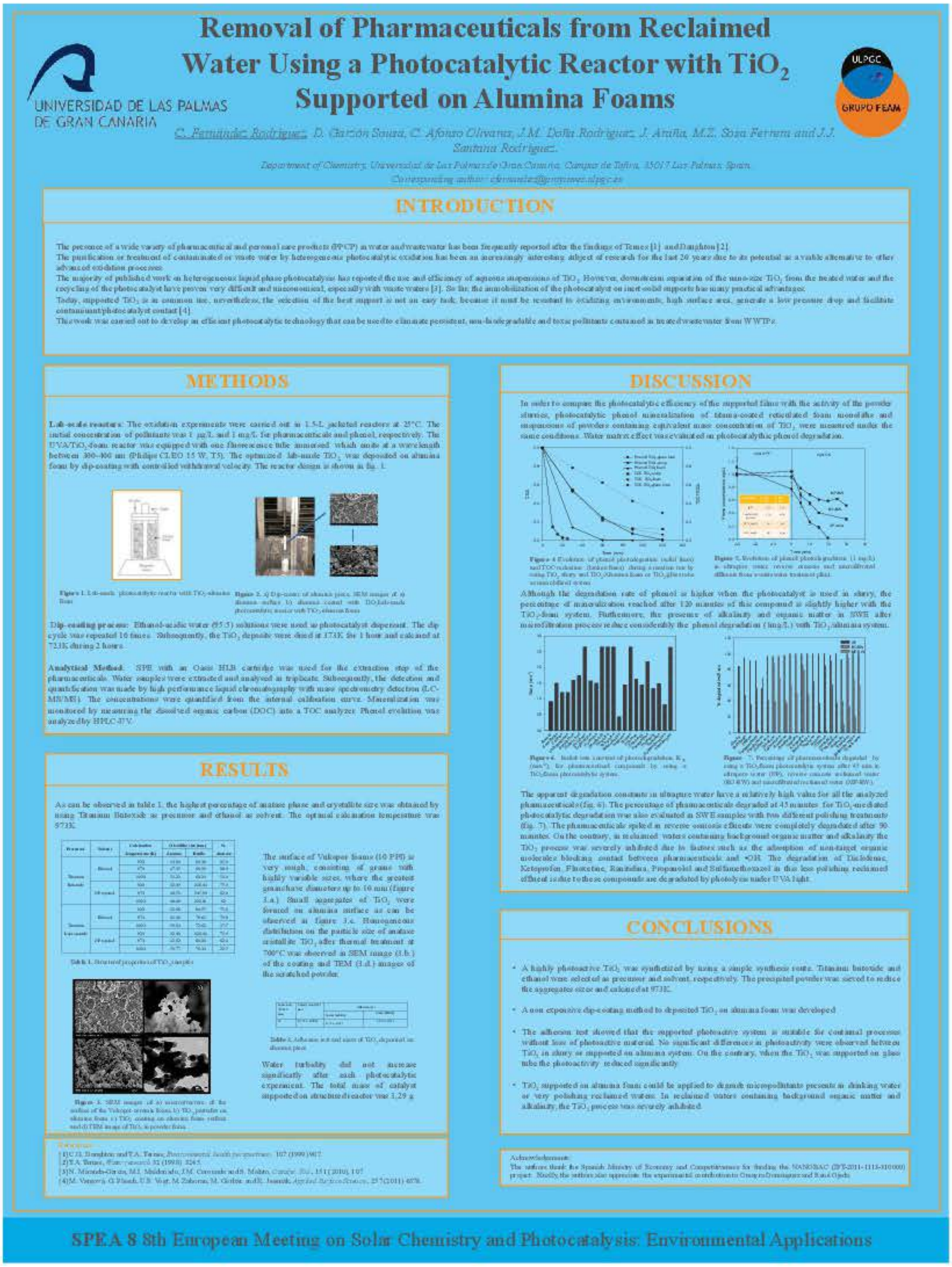
39th International Symposium on High-Performance-Liquid-Phase Separations and Related Techniques (HPLC 2013)

Ámsterdam (Holanda) 2013



8th European Meeting on Solar Chemistry and Photocatalysis: Environmental Applications (SPEA 8)

Thessaloniki (Grecia) 2014

Removal of Pharmaceuticals in MBR Permeate using a photocatalytic reactor With TiO_2/Al_2O_3 filter

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the international water association

acciona

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INTRODUCTION

The presence of a wide variety of pharmaceutical and personal care products (PPCP) in water and wastewater has been frequently reported after the findings of Ternes [1] and Daughton [2].
The purification or treatment of contaminated or waste water by heterogeneous photocatalytic oxidation has been an increasingly interesting subject of research for the last 20 years due to its potential as a viable alternative to other advanced oxidation processes. The majority of published work on heterogeneous liquid phase photocatalysis has reported the use and efficiency of aqueous suspensions of TiO_2. However, downstream separation of the nano-size TiO_2 from the treated water and the recycling of the photocatalyst have proven very difficult and uneconomical, especially with waste waters [3]. So far, the immobilization of the photocatalyst on inert-solid supports has many practical advantages.
Today, supported TiO_2 is in common use, nevertheless, the selection of the best support is not an easy task, because it must be resistant to oxidizing environments, high surface area, generate a low pressure drop and facilitate contaminant/photocatalyst contact [4].
This work was carried out to develop an efficient photocatalytic technology that can be used to eliminate persistent, non-biodegradable and toxic pollutants contained in treated wastewater from WWTPs.

METHODS

Lab-scale reactors: The oxidation experiments were carried out in 1.5-L jacketed reactors at 25ºC. The initial concentration of pollutants was 1 µg/L and 1 mg/L for pharmaceuticals and phenol, respectively. The UVA/TiO_2-foam reactor was equipped with one fluorescence tube immersed which emits at a wavelength between 300-400 nm (Philips CLEO 15 W, T5). The optimized lab-made TiO_2 was deposited on alumina foam by dip-coating with controlled withdrawal velocity. The reactor design is shown in fig. 1.

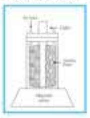

Figure 1. Lab-made photocatalytic reactor with TiO_2-alumina foam.

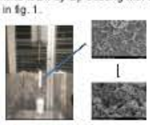

Figure 2. a) Dip-coater of alumina piece, SEM images of a) alumina surface b) alumina coated with TiO_2 Lab-made photocatalytic reactor with TiO_2-alumina foam.

Dip-coating process: Ethanol-acidic water (95:5) solutions were used as photocatalyst dispersant. The dip cycle was repeated 10 times. Subsequently, the TiO_2 deposits were dried at 373K for 1 hour and calcined at 723K during 2 hours.

Analytical Method: SPE with an Oasis HLB cartridge was used for the extraction step of the pharmaceuticals. Water samples were extracted and analysed in triplicate. Subsequently, the detection and quantification was made by high performance liquid chromatography with mass spectrometry detection (LC-MS/MS). The concentrations were quantified from the internal calibration curve. Mineralization was monitored by measuring the dissolved organic carbon (DOC) into a TOC analyzer. Phenol evolution was analyzed by HPLC-UV.

RESULTS

As can be observed in table 1, the highest percentage of anatase phase and crystallite size was obtained by using Titanium Butoxide as precursor and ethanol as solvent. The optimal calcination temperature was 973K.

Table 1. Structural properties of TiO_2 samples

Precursor	Solvent	Calcination Temperature (K)	Crystallite size (nm)		% anatase
			Anatase	Rutile	
Titanium butoxide	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]
Titanium isopropoxide	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]

The surface of Vukopor foams (10 PPI) is very rough, consisting of grains with highly variable sizes, where the greatest grains have diameters up to 10 mm (figure 3.a.). Small aggregates of TiO_2 were formed on alumina surface as can be observed in figure 3.c. Homogeneous distribution on the particle size of anatase cristallite TiO_2 after thermal treatment at 700ºC was observed in SEM image (3.b.) of the coating and TEM (3.d.) images of the scratched powder.

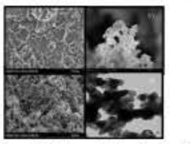

Figure 3. SEM images of a) microstructure of the surface of the Vukopor ceramic foam, b) TiO_2 particles on alumina foam c) TiO_2 coating on alumina foam surface and d) TEM image of TiO_2 in powder form.

Table 2. Adhesion test and mass of TiO_2 deposited on alumina piece

Foam unit (50x50 mm)	Catalyst load per unit	Adhesion test	
		Initial turbidity	Final turbidity
12	0.274 (±0.088)	0.71 (±0.11)	1.99 (±0.24)

Water turbidity did not increase significatly after each photocatalytic experiment. The total mass of catalyst supported on structured reactor was 3,28 g.

DISCUSSION

In order to compare the photocatalytic efficiency of the supported films with the activity of the powder slurries, photocatalytic phenol mineralization of titania-coated reticulated foam monoliths and suspensions of powders containing equivalent mass concentration of TiO_2 were measured under the same conditions.

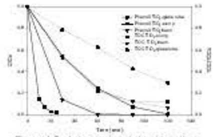

Figure 4 Evolution of phenol photodegration (solid lines) and TOC reduction (broken lines) during a reaction run by using TiO_2 slurry and TiO_2/Alumina foam or TiO_2/glass tube as immobilized system.

Although the degradation rate of phenol is higher when the photocatalyst is used in slurry, the percentage of mineralization reached after 120 minutes of this compound is slightly higher with the TiO_2-foam system

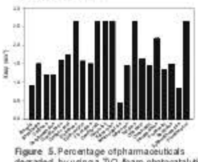

Figure 5. Percentage of pharmaceuticals degraded by using a TiO_2/foam photocatalytic system after 45 min in ultrapure water (UP), reverse osmosis reclaimed water (RO-RW) and MBR reclaimed water (MBR-RW).

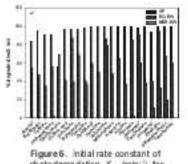

Figure 6. Initial rate constant of photodegradation, K_{ap} (min^{-1}), for pharmaceutical compounds by using a TiO_2/filter photocatalytic system.

The apparent degradation constants in ultrapure water have a relatively high value for all the analyzed pharmaceuticals (fig. 5). The percentage of pharmaceuticals degraded at 45 minutes for TiO_2-mediated photocatalytic degradation was also evaluated in SWE samples with two different polishing treatments (fig. 6). The pharmaceuticals spiked in reverse osmosis efluents were completely degradated after 90 minutes. On the contrary, in reclaimed waters containing background organic matter and alkalinity the TiO_2 process was severely inhibited due to factors such as the adsorption of non-target organic molecules blocking contact between pharmaceuticals and •OH.

CONCLUSIONS

- A highly photoactive TiO_2 was synthetized by using a simple precipited synthesis route.
- A non expensive dip-coating method to deposited TiO_2 on alumina foam was developed. The adhesion to substrate test showed that the supported photoactive system is suitable for continual processes without loss of photoactive material.
- No significant differences in photoactivity were observed between TiO_2 in slurry or supported on alumina system.
- TiO_2 supported on alumina foam could be applied to degrade micropollutants presents in drinking water or very polishing reclaimed waters. In reclaimed waters containing background organic matter and alkalinity, the TiO_2 process was severely inhibited.
- A continuously aerated tank with TiO_2/Al_2O_3 filters with a capacity of 25 L has been fabricated to further investigations.

Figure 7. Continuously aerated tank with TiO_2/Al_2O_3 filters.

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REMOVAL OF PHARMACEUTICALS FROM WASTEWATERS BY UV/H_2O_2

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INTRODUCTION

Since decades, a large list of emerging compounds has been created that includes the pharmaceutical compounds. This occurrence has been produced due to the continuous presence of these contaminants in the different environmental compartments and their negative effects on different organisms, such as endocrine disruption and antibiotic resistance. Municipal Wastewater Treatment Plants (WWTPs) discharge daily these compounds to the aquatic bodies since, in the most cases, they were not designed for removing this kind of compounds [1]. Different studies have demonstrated the limitations of using only conventional treatments to decompose pharmaceuticals [2]. Therefore, the use of advanced treatments is needed, such as reverse osmosis, membrane bioreactor, advanced oxidation processes, etc. [3].

The aim of this work is to evaluate the effectiveness of UV/H_2O_2, as advanced treatment, on the elimination of twenty three pharmaceuticals, normally present at concentration in the order of ng L^{-1} in urban wastewaters. For the analysis, Solid Phase Extraction (SPE) and Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) has been used. Moreover, a continuous photochemical reactor with a capacity of 25 L has been designed and manufactured, in this work. The optimization of H_2O_2 concentration necessary to remove the pharmaceuticals from the studied wastewater has been also performed. Furthermore, the kinetic constants and UV_{90} dose has been determined for each studied pharmaceuticals. Finally, energy cost of the process was also calculated.

EXPERIMENTAL PROCEDURE

Photochemical reactor: The oxidation experiments were carried out in a photochemical reactor with a effective volume of 25 L and equipped with four low-pressure mercury lamps with a total output powder of 56 W . UV intensity measured in the central part of reactor was 15,47 W/m^2. During UV process, tested water was aerated at an air flow rate of 100 L/h. The reactor design is shown in fig. 1.

Experimental procedure: 1 µg/L of pharmaceuticals was additionally added to microfiltrated (MF) water obtained from SWTP sited in Gran Canary, after secondary treatment. Physico-chemical parameters of MF water are depicted on table 1.

Table 1: concentration detected in the microfiltered water from the secondary treatment in urban WWTP.

Parameter	MF Effluent
Conductivity (µS/cm)	1369
DOC mg/L	14,8
HCO_3^- mg/L	174,1
UV254 absorbance	0,233
NO_2-mg/L	8,91
$SO4^{2-}$ mg/L	66,2
NO_3- mg/L	22,26
PO_4^{3-} mg/L	97,50
Na^+ mg/L	234,22
K^+ mg/L	35,30
Mg^{2+} mg/L	14,71
Ca^{2+} mg/L	40,58

Figure 1. Photochemical reactor designed and manufactured in this work.

Analytical Method: SPE with an Oasis HLB cartridge was used for the extraction step of the pharmaceuticals. Water samples were extracted and analysed in triplicate. Subsequently, the detection and quantification was made by high performance liquid chromatography with mass spectrometry detection (LC-MS/MS).

Chormatographic conditions	
Instrument	LC system from Varian with 320 MS mass spectrometry
Column	SunFireTM C_{18}
Injection volume	10 µL
Flow rate	200 µL·min^{-1}
Mobil phase	A: water (0.015% formic acid)
	B: methanol

RESULTS

Table 2: concentration detected in the microfiltered water from the secondary treatment in urban WWTP.

Compound	Concentration (µg/L)	Compounds	Concentration (µg/L)
Atenolol	0.33	Metamizole	>LOD
Bezafibrate	0.11	Metronidazole	0.63
Caffeine	0.41	Naproxen	0.66
Carbamazepine	0.17	Nicotine	0.12
Ciprofloxacin	>LOD[a]	Ofloxacin	0.14
Clofibric acid	0.02	Omeprazol	0.01
Diclofenac	0.69	Paraxanthine	0.68
Erythromycin	0.30	Propanolol	>LOD
Fluoxetine	0.11	Ranitidine	>LOD
Gemfibrozil	0.57	Sulfamethoxazole	0.13

a LOD: Limit of Detection

Table 3: Percentage of pharmaceuticals degraded at different initial H_2O_2 concentrations.

H_2O_2 (mg/L)	Average degradation (%)	Remove Metronidazole (%)
5	70	0
15	94	79
20	93	92
25	97	100

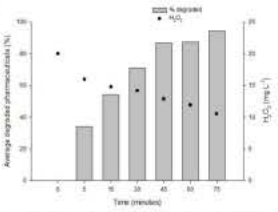

Figure 2. Evolution of average degraded pharmaceuticals in function of reaction time

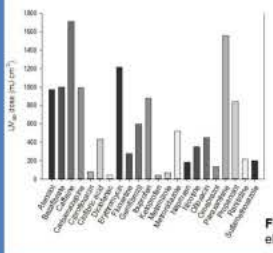

Figure 3. UV_{90} dose necessary to eliminate the studied pharmaceuticals.

Energy cost of the process: The energy cost of the process of H_2O_2/UV to eliminate the studied pharmaceuticals was 0.67 €/m3.

CONCLUSIONS

- UVC/H_2O_2 appears to be a treatment very efficient to remove pharmaceuticals present in the studied WWTP wastewater.
- The concentration of H_2O_2 for the removal of over 90% of all pharmaceutical was optimized to 20 mg·L^{-1}.
- The photochemical reactor designed presented a proper homogenization, having no preferential flows.
- Kinetic studies of elimination of the studied pharmaceuticals showed that the residence time should be at least 75 minutes to obtain a degradation greater than 90%.
- High UV_{90} dose is necessary to eliminate caffeine and its metabolite paraxanthine.
- The energy cost of the process of H_2O_2/UV to eliminate the 90% of studied pharmaceuticals amounts to 0.67 €/m^3.

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International Meeting on Environmental and Pharmaceutical Analysis

Las Palmas de Gran Canaria (España) 2014

II Workshop "Estudio, aprovechamiento y gestión del agua en terrenos e islas volcánicas"

Las Palmas de Gran Canaria (España) 2015

A SIMPLIFIED SOLID PHASE EXTRACTION PROCEDURE COMBINED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY TO ASSESS PHARMACEUTICALS IN LIQUID SAMPLES

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INTRODUCTION

Direct analytical technique, to avoid sample treatment, is within twelve "Green Analytical Chemistry" (GAC) principles [1]. However, the existence of micropollutants, such as pharmaceuticals, which are compounds in the range from $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$ [2], does not allow to follow this goal due to the necessity of a pre-concentration technique before to analysis by the modern analytical equipments.

Solid phase extraction (SPE) is the most commonly extraction technique employed. The standard protocol uses five steps (conditioned, equilibrated, load, wash step and elution), therefore, a relative elevated consumption of time and quantity of solvent is required. However, thanks to the characteristic of hydrophilic-lipophilic balanced copolymer sorbents, it could be used with a simplified system in three steps (without two first steps of standard SPE). As a result, we can obtain a reduction of consumed time and the quantity of solvent according to another GAC principle [3].

In this sense, we use N-vinylpyrrolidone-divinylbenzene copolymer (OASIS HLB) cartridges in a simplified SPE procedure, combined with Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) in order to determine twenty-three residual pharmaceuticals in liquid environmental samples.

SPE PROCEDURE

- Conditioned ✗
- Equilibrated ✗
- Load
  - 250 mL, pH 7
- Wash step
  - 5 mL Milli-q water
- Elution
  - 5 mL methanol

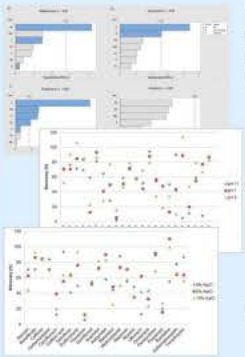
EXPERIMENTAL PROCEDURE

Chromatographic conditions

Chormatographic conditions	
Instrument	LC system from Varian with 320 MS mass spectrometry
Column	SunFireTM C_{18}
Injection volume	10 µL
Flow rate	200 $\mu L \cdot min^{-1}$
Mobil phase	A: water (0.015% formic acid)
	B: methanol

Gradient used		
Time (min)	% (A)	%(B)
0:0	95	5
1:0	95	5
21:0	60	40
40:0	10	90
43:0	10	90
46:0	95	5

SPE Optimization



- ❑ 2^3 factorial experimental design
  - pH (3 and 11)
  - Sample volume (100-500 mL)
  - Ionic strenght (0-10% NaCl)
- ❑ Evaluation of pH
- ❑ Influence of ionic strength

RESULTS

Table 1. Analytical parameters for SPE-LC-MS/MS

Compound	Recovery (%)	Intra-RSD[a] (%)	Inter-RSD[b] (%)	LOD[c] (ng L^{-1})
Atenolol	98.63	10.09	15.91	0.5
Bezafibrate	89.23	6.23	16.26	0.9
Caffeine	91.31	11.42	13.32	2.4
Carbamazepine	108.1	8.22	12.47	0.2
Ciprofloxacin	62.29	9.20	19.88	33.3
Clofibric acid	98.87	8.37	9.49	0.3
Diclofenac	98.08	8.15	18.11	1.9
Erythromycin	97.34	10.35	15.87	4.2
Fluoxetine	55.70	7.41	14.21	0.3
Gemfibrozil	95.80	12.71	17.56	0.4
Ibuprofen	92.59	7.64	9.40	8.3
Ketoprofen	102.1	8.04	19.72	1.3
Metamizole	65.79	12.18	14.02	3.4
Metronidazole	93.45	12.4	22.04	1.5
Naproxen	99.51	9.84	16.90	5.5
Nicotine	90.81	5.61	12.01	20.7
Ofloxacin	80.59	8.87	15.35	35.3
Omeprazole	78.76	6.94	18.32	0.2
Paraxanthine	107.9	14.38	17.75	9.1
Propanolol	57.58	9.75	12.19	6.8
Ranitidine	76.46	12.49	12.72	5.2
Sulfamethoxazole	89.56	5.65	15.95	0.7
Trimethoprim	98.54	11.64	16.75	0.4

a. Intra-day Relative Standard Deviation (n=6).
b. Inter-day Relative Standard Deviation (n=6).
c. Limits of Detection are calculated as signal-to-noise ratio of 3.

Table 2. Evaluation of target compounds in wastewater samples from two WWTPs. Minimum and maximum concentrations ($ng \, L^{-1}$)

Sampling points	WWTP1		WWTP2	
	[Min]	[Max]	[Min]	[Max]
Point 1	44.36 ± 3.51	49099.16 ± 2468.66	12.95 ± 1.68	70685.59 ± 5522.58
Point 2	22.79 ± 3.05	1259.38 ± 54.69	58.28 ± 2.76	67151.70 ± 3874.19
Point 3	21.56 ± 1.28	1924.89 ± 99.14	50.46 ± 8.82	91532.85 ± 3072.77
Point 4	4.74 ± 0.95	819.27 ± 321.69	25.19 ± 4.09	26578.62 ± 2299.33

CONCLUSIONS

A simplified SPE coupled with LC-MS/MS was optimized and successfully applied to real wastewater samples for the determination of twenty three further used pharmaceutical compounds.

The proposed method proved to be suitable for reducing the extraction time and the amount of solvent used for the analysis of pharmaceuticals and, in this sense, it is close to the "Green Analytical Chemistry" (GAC) principles.

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Cork (Irlanda) 2016

V[th] Euro-Mediterranean Workshop on Water, Sediments, Catalysis and Environment. EST- Fès, October 14 [th] - 16 [th], 2014

Removal of pharmaceuticals from reclaimed waters using a photocatalytic reactor with TiO_2 supported on alumina foams.

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V[th] Euro-Mediterranean Workshop on Water, Sediments, Catalysis and Environment

Fez (Marruecos) 2016

APPLYING A SIMPLIFIED SOLID PHASE EXTRACTION COMBINED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY TO ASSES PHARMACEUTICALS FROM WASTEWATER

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INTRODUCTION

Wastewater Treatment Plants (WWTPs) were developed to avoid a source of environmental pollution and to reuse water successfully without human health problems[1]. However, WWTPs have not been designed to remove emerging contaminants, such as pharmaceuticals, which produce a significant issue[2,3]. There is growing concern about the removal of emerging pollutants by natural, conventional and advanced treatments. Moreover, assessment of these compounds, using methods that pursuing twelve "Green Analytical Chemistry" (GAC) principles, is being a challenge.

This work aims to apply a simplified Solid Phase Extraction (SPE) procedure combined with High Performance Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) for evaluate the presence of pharmaceutical residues in wastewater samples, with which is achieved a reduction in analysis time and the quantity of solvent. The process was applied to assess two WWTPs with different treatments (natural and conventional) from Gran Canaria (Spain).

EXPERIMENTAL

Determination procedure

Chormatographic conditions	
Instrument	LC system from Varian with 320 MS mass spectrometry
Column	SunFireTM C_{18}
Injection volume	10 µL
Flow rate	200 µL·min^{-1}
Mobil phase	A: water (0.015% formic acid)
	B: methanol

The gradient starts with 95% of A for 1 min, then it changed to 60:40 (A:B) (v/v) for 20 min, 10:90 (A:B) (v/v) for 19 min, and finally returned to its initial condition for 3 minutes. An equilibration time of 4 min was employed.

Extraction procedure

RESULTS

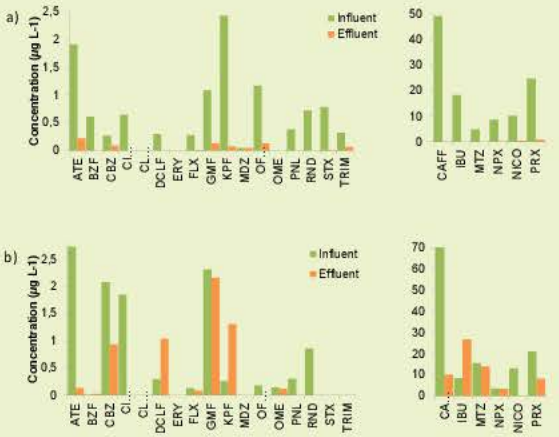

Figure 1. Evaluation of target compounds in wastewater samples from two WWTPs. a) WWTP-1 is urban, conventional activated sludge system coupled reverse osmosis. b) WWTP-2 is rural, natural process of purification to treat domestic sewage.

CONCLUSIONS

A simplified SPE combined with LC-MS/MS method was applied to wastewater samples for the determination of twenty-three commonly used pharmaceutical compounds. Eighteen pharmaceuticals were detected in all samples from both WWTPs. The different treatments were compared and a reduction of concentration levels through the treatment process from WWTP-1 was assessed, however, an irregular behaviour was shown by WWTP-2.

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