

ACREDITACIÓN A LA ETAPA DE INVESTIGACIÓN
DOCTORADO EN GESTIÓN COSTERA

OPTIMIZACIÓN Y APLICACIÓN DE UN MÉTODO BASADO EN SPE-LC-MS/MS PARA LA DETERMINACIÓN DE MULTIRESIDUOS DE COMPUESTOS FARMACÉUTICOS EN AGUAS DEPURADAS

Doctorando: Cristina Afonso Olivares

Directores: Dra. Dña. Zoraida Sosa Ferrera

Dr. D. José Juan Santana Rodríguez



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- INTRODUCCIÓN
- OBJETIVOS
- PROCESO EXPERIMENTAL
- RESULTADOS Y DISCUSIÓN
- CONCLUSIONES
- DIFUSIÓN CIENTÍFICA



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INTRODUCCIÓN

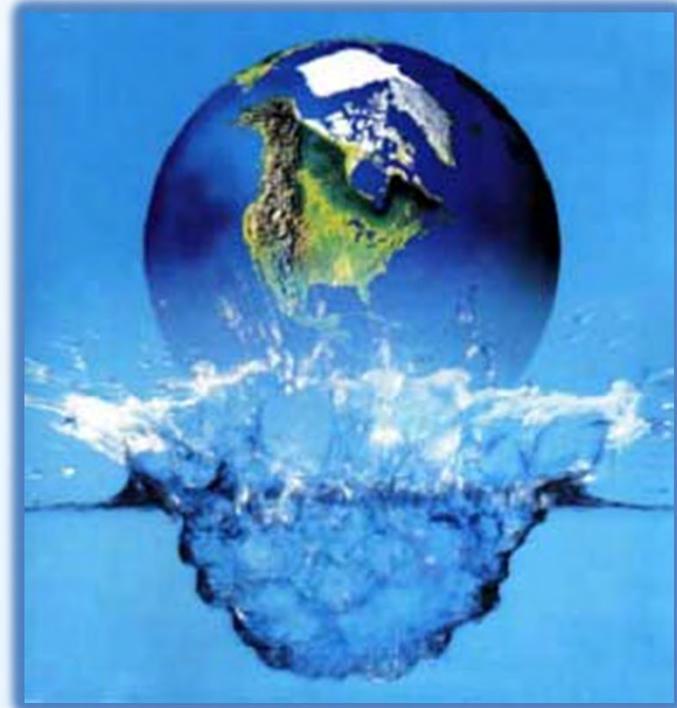
Crecimiento económico y globalización.



Existencia de problemas ambientales.



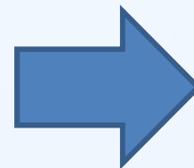
Contaminación de las **aguas** (recurso fundamental para asegurar la buena calidad de vida).



INTRODUCCIÓN

Sustancias químicas peligrosas

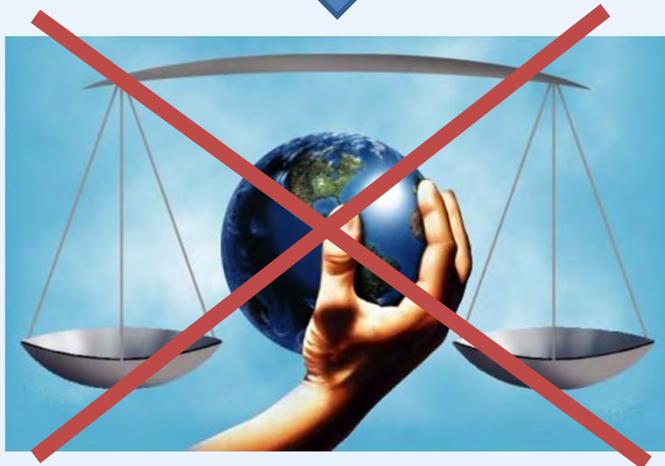
- Plaguicidas
- Compuestos orgánicos volátiles
- PCBs
- Alteradores endocrinos



Medidas de evaluación,
reducción y control de riesgo

LEGISLACIÓN

Ej: DMA 2000/60/CE

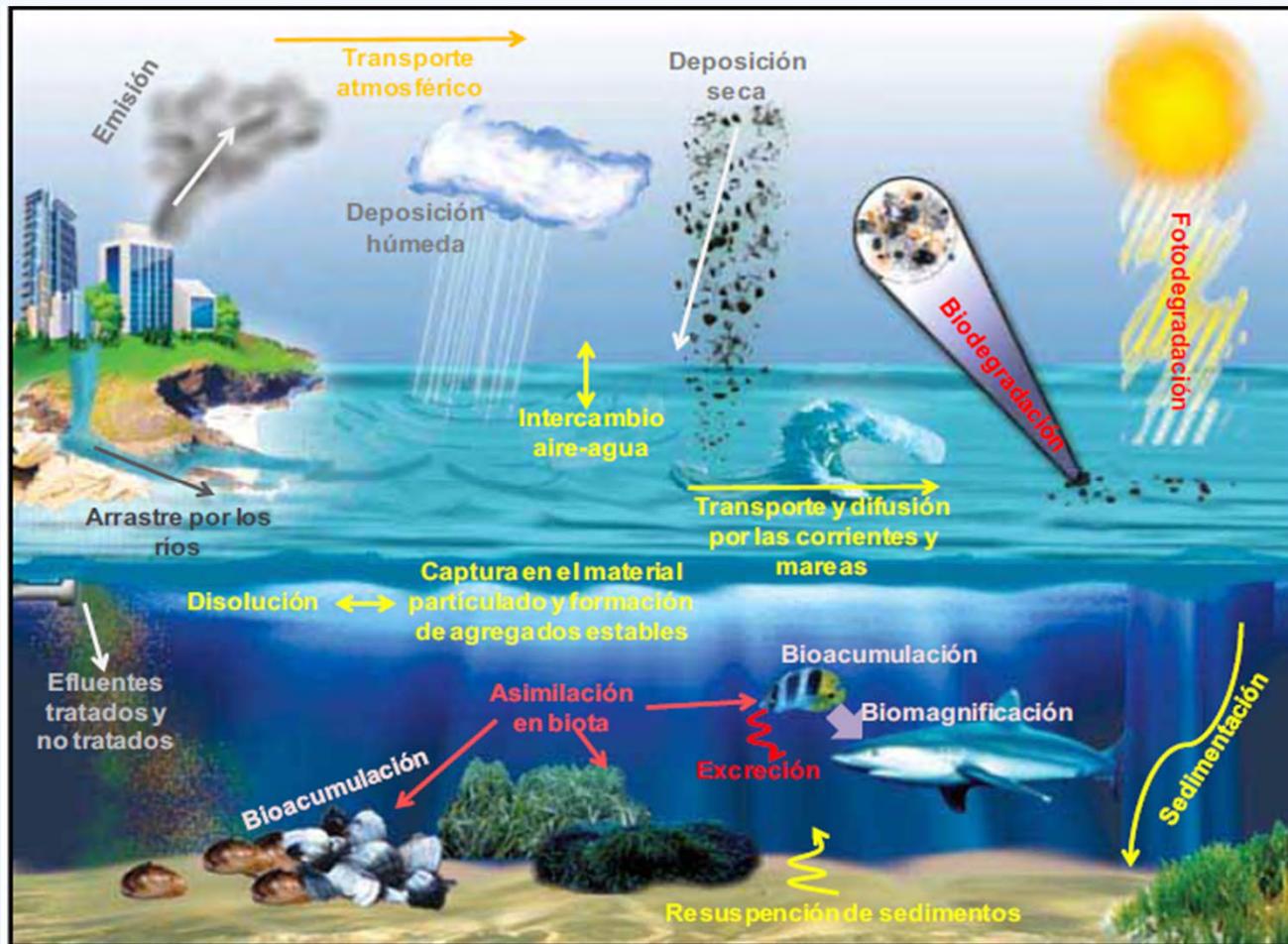


Contaminantes emergentes



RESIDUOS FARMACÉUTICOS

INTRODUCCIÓN



FUENTES DE dispersión

- Excreción ambientales
- Eliminación
- ~~Incorrecta~~ control
- Residuos complejas durante producción
- Concentraciones $\text{ng} \cdot \text{L}^{-1}$

INTRODUCCIÓN

- RESIDUOS FARMACÉUTICOS

Application	Compound	Application	Compound
	Diclofenac	Antiepileptic	Carbamazepine
	Ibuprofen	Antidepressant	Fluoxetine
Anti-inflammatory	Naproxen	Antibiotic	Ofloxacin
	Metamizole	Antibiotic	Ciprofloxacin
	Caffeine	Antibiotic	Erythromycin
Stimulant	Paraxanthine	Antibiotic	Trimethoprim
	Propanolol	Antibiotic	Sulfamethoxazole
Antihypertensive	Atenolol	Antibiotic	Metronidazole
	Gemfibrozil	Antibiotic	Omeprazol
Lipid regulator	Clofibric acid	Antibiotic	Ranitidine
	Bezafibrate		

Diferentes propiedades físicas y químicas

Procesos analíticos complejos

MÉTODOS MULTIRESIDUOS



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OBJETIVOS

- Determinación de residuos farmacéuticos, más relevantes en el medioambiente, en aguas depuradas:
 - Optimización de las variables que afectan a la extracción en fase sólida (SPE)
 - Optimización de las variables que afectan a la determinación (LC-MS/MS)
 - Aplicación a muestras reales

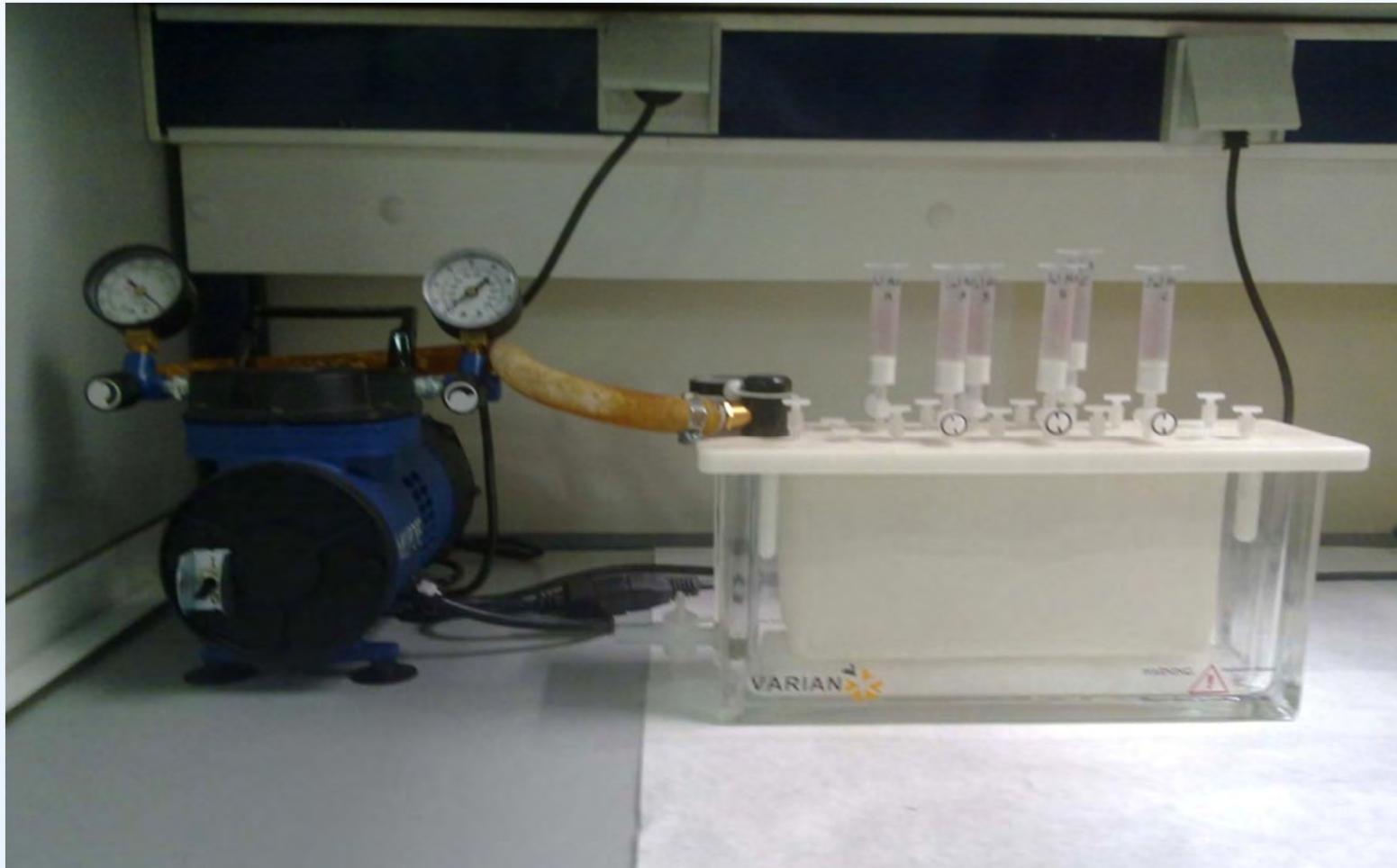




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PROCESO EXPERIMENTAL



LC-MS/MS

SPE

PROCESO EXPERIMENTAL

PROCEDIMIENTO SPE

- 1.- Acondicionamiento
- 2.- Paso de la muestra
- 3.- Retención de analitos
en el adsorbente
- 4.- Paso de lavado
- 5.- Paso de elución



PROCESO EXPERIMENTAL

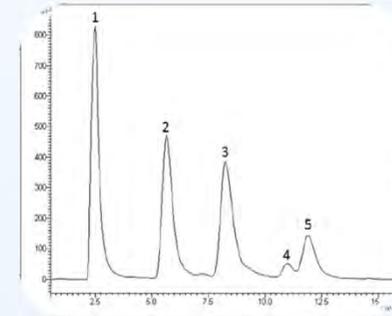
Optimización de la
determinación



Optimización de la
extracción



Detector MS



Separación cromatográfica



SPE

- Cartucho
- Volumen de muestra
- pH
- Fuerza iónica
- Volumen de eluyente
- Paso de lavado

Parámetros analíticos



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RESULTADOS Y DISCUSIÓN

Condiciones de ionización LC-MS/MS

Compound	Precursor ion (m/z)	Cone V	Fragment ions (collision potencial)	Ion mode
Nicotine	163	30	130(18.5) ^a , 84 (17)	ESI +
Atenolol	267	52	145 (23.5) ^a , 190 (16.5)	ESI +
Ranitidine	315.0	44	175.9 (11) ^a , 129.8 (20)	ESI +
Trimethoprim	291.1	64	230 (19) ^a , 122.9 (21)	ESI +
Metamizole	218	30	56 (12.5) ^a , 97 (11.5)	ESI +
Ofloxacin	362.1	52	318.1 (14.5) ^a , 261.0 (22.5)	ESI +
Ciprofloxacin	332.1	52	313.9 (19.0) ^a , 230.8 (36.0)	ESI +
Metronidazole	172	40	127.9 (10.0) ^a , 81.9 (21.0)	ESI +
Paraxanthine	181	40	124 (17) ^a	ESI +
Propranolol	260.2	48	116.1 (13) ^a , 183.1 (12)	ESI +
Caffeine	195	56	138 (18) ^a	ESI +
Sulfamethoxazole	254	44	155.9 (11.5) ^a , 91.9 (23)	ESI +
Erythromycin	734.5	48	576.3 (11) ^a , 157.8 (22.5)	ESI +
Fluoxetine	310	30	44 (6.5) ^a , 148 (5.5)	ESI +
Omeprazole	346	32	198.0 (7) ^a , 135.8 (27.5)	ESI +
Carbamazepine	237.1	40	194 (13.5) ^a , 192 (17)	ESI +
Ketoprofen	255.1	52	209 (10) ^a , 104.9 (18.5)	ESI +
Naproxen	231.2	36	153.1 (28.5) ^a , 170 (22)	ESI +
Ibuprofen	204.7	40	160.8 (6.5) ^a , 158.5 (6.0)	ESI -
Bezafibrate	359.8	64	273.7 (15.5) ^a , 153.5 (28.5)	ESI -
Diclofenac	295.9	32	214.0 (30) ^a , 250.0 (11.0)	ESI +
Gemfibrozil	251	30	128.9 (8.0) ^a , 233 (5.0)	ESI +
Clofibric acid	213	32	85 (10) ^a , 127 (13.5)	ESI -

RESULTADOS Y DISCUSIÓN

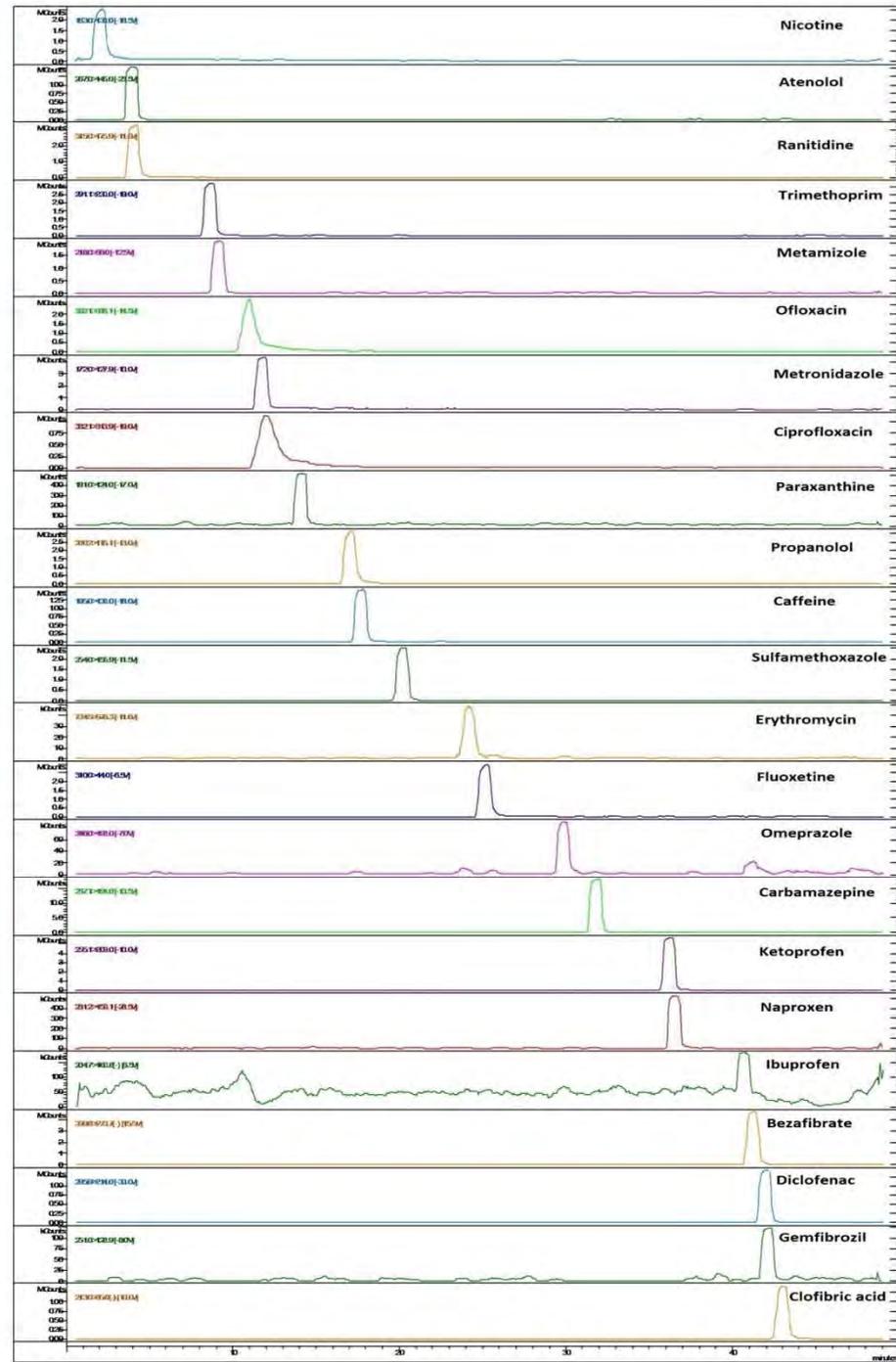
Condiciones cromatográficas

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

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



RESULTADOS Y DISCUSIÓN

Parámetros del proceso de extracción

- **Cartucho** → relación entre variables → 2^3 ✓ **Oasis HLB**
✓ **250 mL de muestra**
- **pH/fuerza iónica** → 3^2 ✓ **pH 9**



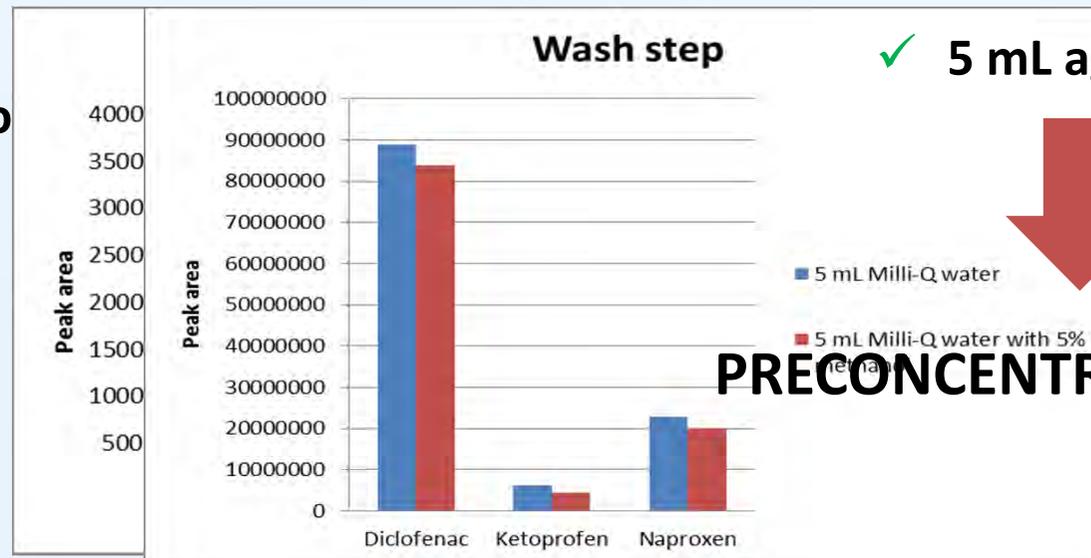
↑ [sal] Oasis HLB Fase reversa → mayor eficiencia extracción
↑ [sal] → interacciones electrostáticas con las moléculas polares = menor eficiencia de extracción

ExtraBond ECX-Intercambio iónico

RESULTADOS Y DISCUSIÓN

Parámetros del proceso de extracción

- **Cartucho** → relación entre variables → 2^3
 - ✓ Oasis HLB
 - ✓ 250 mL de muestra
 - ✓ pH 9
 - ✓ 15% NaCl
 - ✓ 2 mL MeOH
 - ✓ 5 mL agua Milli-Q
- **pH/fuerza iónica** → 3^2
- **Volumen de eluyente**
- **Paso de lavado**



PRECONCENTRACIÓN=125

RESULTADOS Y DISCUSIÓN

Parámetros analíticos

Nº	Compound	LDR ^a (µg L ⁻¹)	r ²	50 µg L ⁻¹		LOD ^b (ng L ⁻¹)	LOQ ^c (ng L ⁻¹)
				RSD (%)	Recovery (%)		
1	Nicotine	1-300	0.9971	10.6	29.4	30.8	103
2	Atenolol	1-300	0.9994	19.7	85.7	12.3	41.0
3	Ranitidine	1-300	0.9913	19.3	58.7	6.26	20.9
4	Trimethoprim	5-300	0.9959	6.82	66.6	4.29	14.3
5	Metamizole	50-300	0.9919	19.6	147	23.5	78.3
6	Ofloxacin	5-300	0.9980	17.6	32.3	28.4	94.8
7	Metronidazole	5-300	0.9988	14.7	119	5.50	18.3
8	Ciprofloxacin	5-300	0.9909	6.70	68.6	19.1	63.8
9	Paraxanthine	50-300	0.9948	2.44	93.1	35.3	117
10	Propranolol	5-300	0.9958	15.5	56.3	10.4	34.7
11	Caffeine	5-300	0.9986	10.5	37.6	5.38	17.9
12	Sulfamethoxazole	5-300	0.9979	12.7	106	0.58	1.93
13	Erythromycin	1-300	0.9982	19.1	57.7	0.21	0.69
14	Fluoxetine	1-300	0.9982	19.9	30.6	0.16	0.53
15	Omeprazol	1-300	0.9971	10.8	48.7	0.72	2.40
16	Carbamazepine	1-300	0.9905	14.7	31.3	1.17	3.90
17	Ketoprofen	1-300	0.9953	9.37	53.4	2.94	9.79
18	Naproxen	1-300	0.9983	17.6	95.6	0.72	2.40
19	Ibuprofen	50-300	0.9901	8.46	117	67.9	226
20	Bezafibrate	1-300	0.9988	14.2	68.8	1.99	6.63
21	Diclofenac	1-300	0.9950	18.6	73.6	0.19	0.63
22	Gemfibrozil	5-300	0.9942	9.12	83.0	2.34	7.79
23	Clofibric acid	5-300	0.9969	17.7	76.4	0.39	1.30

RESULTADOS Y DISCUSIÓN

Efecto matriz

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

Nº	Compound	Matrix effect (%)	Nº	Compound	Matrix effect (%)
1	Nicotine	15.40	13	Erythromycin	54.86
2	Atenolol	39.74	14	Fluoxetine	80.91
3	Ranitidine	74.99	15	Omeprazol	27.78
4	Trimethoprim	-154.3	16	Carbamazepine	33.76
5	Metamizole	-85.30	17	Ketoprofen	-1.624
6	Ofloxacin	-23.71	18	Naproxen	72.82
7	Metronidazole	66.99	19	Ibuprofen	60.10
8	Ciprofloxacin	56.13	20	Bezafibrate	21.11
9	Paraxanthine	-119.8	21	Diclofenac	25.46
10	Propranolol	96.24	22	Gemfibrozil	60.37
11	Caffeine	47.97	23	Clofibric acid	34.93
12	Sulfamethoxazole	48.79			

RESULTADOS Y DISCUSIÓN

Evaluación de compuestos farmacéuticos en agua depurada

Nº	Compound	WWTP1 (ng L ⁻¹)	WWTP2 (ng L ⁻¹)
1	Nicotine	nd ^b	nd
2	Atenolol	246.9 ± 11.5	147.1 ± 0.7
3	Ranitidine	nd	234.3 ± 38.1
4	Trimethoprim	202.9 ± 25.3	69.54 ± 1.07
5	Metamizole	nd	nd
6	Ofloxacin	253.7 ± 21.3	202.6 ± 8.6
7	Metronidazole	nd	nd
8	Ciprofloxacin	453.9 ± 30.9	416.8 ± 13.0
9	Paraxanthine	nd	nd
10	Propranolol	nd	nd
11	Caffeine	nd	nd
12	Sulfamethoxazole	106.8 ± 5.7	205.1 ± 5.3
13	Erythromycin	nd	nd
14	Fluoxetine	287.3 ± 38.5	312.6 ± 35.7
15	Omeprazol	nd	nd
16	Carbamazepine	444.0 ± 43.4	185.1 ± 31.9
17	Ketoprofen	189.9 ± 6.5	nd
18	Naproxen	615.9 ± 49.0	318.5 ± 51.6
19	Ibuprofen	645.9 ± 102.7	443.7 ± 82.0
20	Bezafibrate	nd	nd
21	Diclofenac	nd	35.37 ± 4.48
22	Gemfibrozil	nd	nd
23	Clofibric acid	nd	nd



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CONCLUSIONES

- ✓ Se ha optimizado la separación cromatográfica de los compuestos farmacéuticos mediante LC-MS/MS, en un tiempo adecuado de análisis.
- ✓ Se ha optimizado el proceso de extracción y preconcentración (SPE)
- ✓ Se ha desarrollado un método analítico válido para el análisis de 23 compuestos farmacéuticos basado en SPE seguido de LC-MS/MS.
 - Método optimizado sensible, reproducible y aplicable a muestras reales
 - Límites de detección alcanzados adecuados y comparables.



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Analysis of anti-inflammatory, analgesic, stimulant and antidepressant drugs in purified water from wastewater treatment plants using SPE-LC tandem mass spectrometry

Cristina Afonso-Olivares^a, Zoraida Sosa-Ferreira^a & José J. Santana-Rodríguez^a

^aDepartamento de Química, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

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Article

Assessment of the Presence of Pharmaceutical Compounds in Seawater Samples from Coastal Area of Gran Canaria Island (Spain)

Cristina Afonso-Olivares, M^a Esther Torres-Padrón, Zoraida Sosa-Ferreira and José Juan Santana-Rodríguez *

Departamento de Química, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria 35017, Spain; E-Mails: cristina.afonso102@alu.ulpgc.es (C.A.-O.); mtorres@dqui.ulpgc.es (M.E.T.-P.); zsosa@dqui.ulpgc.es (Z.S.-F.)

* Author to whom correspondence should be addressed; E-Mail: jsantana@dqui.ulpgc.es; Tel.: +34-928-452-915; Fax: +34-928-452-922.

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Abstract: This study presents the evaluation of seven pharmaceutical compounds belonging to different commonly used therapeutic classes in seawater samples from coastal areas of Gran Canaria Island. The target compounds include atenolol (antihypertensive), acetaminophen (analgesic), norfloxacin and ciprofloxacin (antibiotics), carbamazepine (antiepileptic) and ketoprofen and diclofenac (anti-inflammatory). Solid phase extraction (SPE) was used for the extraction and preconcentration of the samples, and liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the determination of the compounds. Under optimal conditions, the recoveries obtained were in the range of 78.3% to 98.2%, and the relative standard deviations were less than 11.8%. The detection and quantification limits of the method were in the ranges of 0.1–2.8 and 0.3–9.3 ng L⁻¹, respectively. The developed method was applied to evaluate the presence of these pharmaceutical compounds in seawater from four outfalls in Gran Canaria Island (Spain) during one year. Ciprofloxacin and norfloxacin were found in a large number of samples in a concentration range of 9.0–3551.7 ng L⁻¹. Low levels of diclofenac, acetaminophen and ketoprofen were found sporadically.

Keywords: pharmaceutical compounds; SPE; LC-MS/MS; seawater; outfall

Gauden-Abramo et al. SpringerPlus 2013, 2:21
<http://www.springerplus.com/content/2/1/21>

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RESEARCH

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An assessment of the concentrations of pharmaceutical compounds in wastewater treatment plants on the island of Gran Canaria (Spain)

Rayco Guedes-Abramo, Cristina Afonso-Olivares, Sarah Montenegro-Espinoza, Zoraida Sosa-Ferreira and José Juan Santana-Rodríguez*

Abstract

An assessment of the concentrations of thirteen different therapeutic pharmaceutical compounds was conducted on water samples obtained from different wastewater treatment plants (WWTPs) using solid phase extraction and high- and ultra-high performance liquid chromatography with mass spectrometry detection (HPLC-MS/MS and UHPLC-MS/MS), was carried out. The target compounds included ketoprofen and naproxen (anti-inflammatory), bezafibrate (lipid regulating), carbamazepine (antiepileptic), metamizole (analgesic), acetaminophen (analgesic), paracetamol (analgesic), fluoxetine (antidepressant), and levofloxacin, norfloxacin, ciprofloxacin and sarafloxacin (fluoroquinolone antibiotic). The relative standard deviations (obtained in method) were below 11%, while the detection and quantification limits were in the range of 0.3–97.4 ng L⁻¹ and 1.1–324.0 ng L⁻¹, respectively. The water samples were collected from two different WWTPs located on the island of Gran Canaria in Spain over a period of one year. The first WWTP (Sotomoro de WWTP) used conventional activated sludge for the treatment of wastewater, while the other plant (Sotomoro de WWTP) employed a membrane bioreactor system for wastewater treatment. Most of the pharmaceutical compounds detected in the study during the sampling period, were found to have concentrations ranging between 0.02 and 34.81 µg L⁻¹.

Keywords: Pharmaceutical compounds; Solid phase extraction; Liquid chromatography; Mass spectrometry; Wastewater

Introduction

Many modern pollution problems are a result of the intermittent or continuous release of chemical substances into the environment. Their presence is one of the main emerging issues that the organization committed to public and environmental health have to address (Hernandez et al. 2006). Pharmaceutical compounds within this group of pollutants have raised increasing concerns over the last two decades because their effects on the environment are unknown. Thousands of tons of pharmaceuticals are used every year, in both human and veterinary medicine, and are

released to the environment through metabolic excretion and improper disposal techniques. These compounds are not completely degraded at the wastewater treatment plants, and many of them are discharged into the environment through many sources and pathways (Wick et al. 2009). These pharmaceutical compounds are objects of evaluation for their potential effects on aquatic organisms (Sunderman et al. 2004) and non-target species (Fent et al. 2006). The monitoring of these pharmaceuticals is therefore required to provide a greater knowledge with respect to their occurrence, their distribution in the environment and what effects they have on organisms when these organisms are exposed to low levels of pharmaceutical compounds (Pal et al. 2010).

*Correspondence: jsantana@dqui.ulpgc.es
Departamento de Química, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria 35017, Spain

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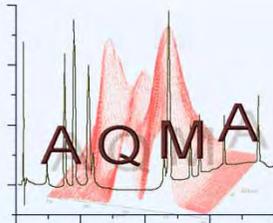
NANOBAC

Producción de fotocatalizadores nanoestructurados por procesos de bajo coste y alta productividad para descontaminación de aguas residuales



AGRADECIMIENTOS

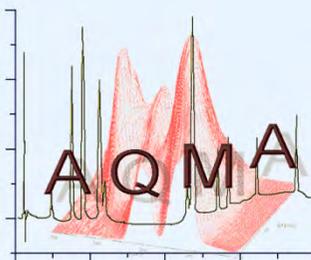
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