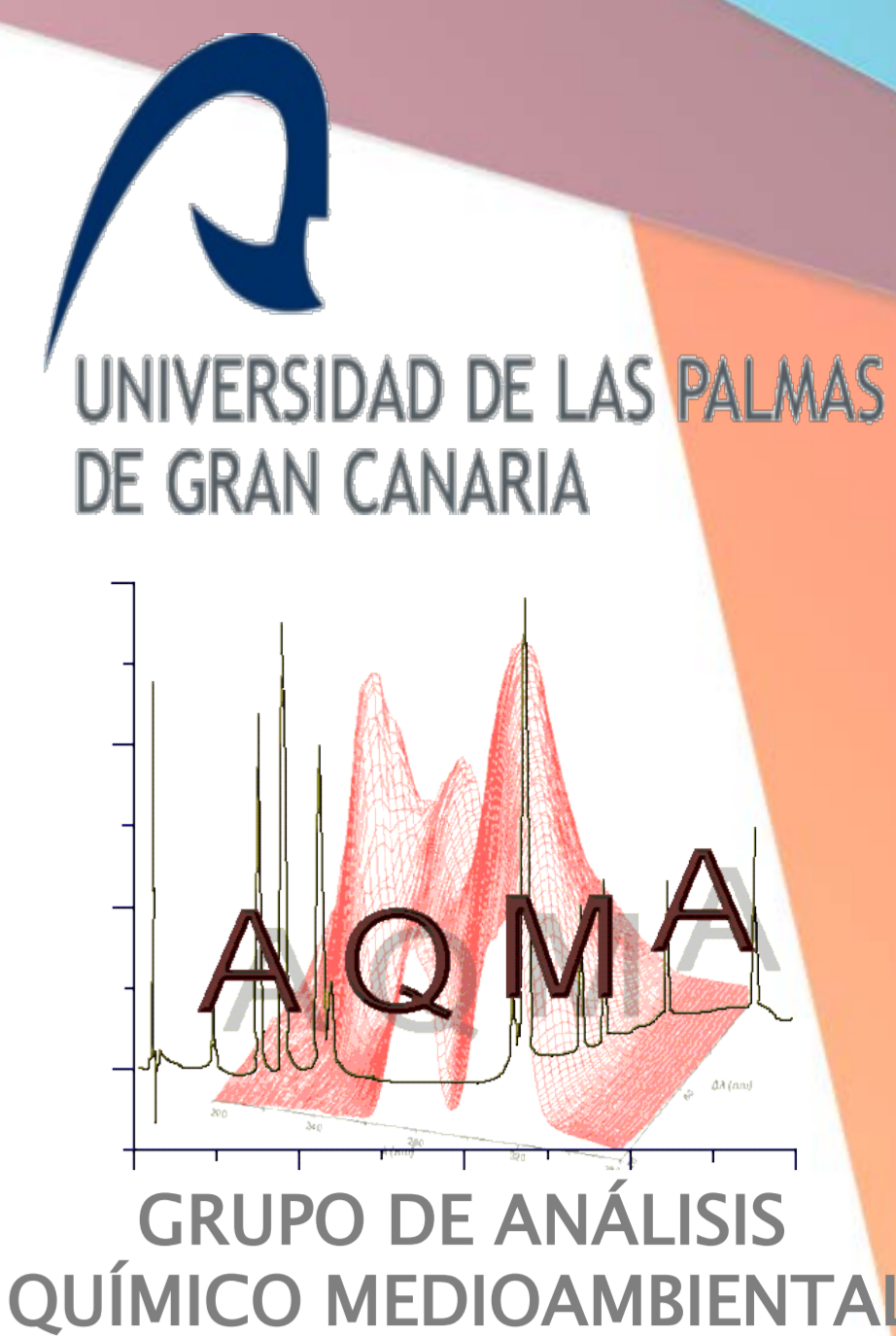


Determination of hormones in sewage from wastewater treatment plants of Gran Canaria (Spain) using an On-line Solid Phase Extraction (SPE) coupled with Ultra-High Performance Liquid Chromatography tandem Mass Spectrometry



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INTRODUCTION

Steroid hormones are an important group considered as endocrine disruptor compounds (EDCs) and their consumption has increased exponentially in last decades, due to their use in human and veterinary medicine. The introduction of hormones into the environment is continuous because the principal source of these pollutants is the wastewater treatment plants [1]. For this reason hormones can produce harmful effects into the environment, especially into the aquatic environment.

An on-line solid phase extraction process coupled with ultra-high performance liquid chromatography following by tandem mass spectrometry detection (UHPLC-MS/MS) has been optimized and applied to determine fourteen natural and synthetic hormones of different types (Table 1).

EXPERIMENTAL:

Compounds studied:

Estrogens:	Diethylstilbestrol (DES)	17 β -estradiol (E2)	Estrone (E1)	Estriol (E3)
Progestogens:	Norethisterone (NORET)	Megestrol Acetate (MGA)	Norgestrel (NRG)	Progesterone (PRG)
Androgens:	Testosterone (TES)	Nandrolone (NAN)	Boldenone (BOL)	
Glucorticoids:	Prednisolone (PRDL)	Prednisone (PRD)	Cortisone (COR)	

Table 1. Compounds studied

Chromatographic and extraction conditions:

Time (min)	BSM			QSM					
	Flow (mL·min ⁻¹)	A (%)	B (%)	Flow (mL·min ⁻¹)	A2 (%)	B2 (%)	C (%)	D (%)	
0.00	0.300	80	20	2.000	100	0	0	0	Loading phase
0.50	0.300	80	20	2.000	0	100	0	0	
3.80	0.300	80	20	2.000	0	100	0	0	Weak wash step
4.10	0.300	80	20	2.000	0	0	0	100	
7.00	0.300	0	100	2.000	100	0	0	0	Strong wash step
8.00	0.300	0	100	2.000	100	0	0	0	
10.50	0.300	80	20	2.000	100	0	0	0	
									Re-equilibration time

Table 2. Extraction conditions and chromatographic gradient

- Column: ACQUITY UPLC BEH Waters C18 (50 x 2.1 mm, 1.7 μ m)
- Mobile phases: A: Water + 0.1% NH₃ and B: Methanol
A2: Water + 0.05% acetic acid
B2 & C: Water and Methanol without additives
D: Mixture 1:1:1 of methanol:acetone:hexane

Extraction and detection parameters:

An Acquity UPLC system was used. It consists in a quaternary pump for the extraction process, a binary pump for the chromatographic separation and a triple quadrupole detector (TQD)



Acquity UPLC system

On-line SPE conditions:

- SPE sorbent: Two Oasis HLB columns (20 μ m, 2.1x30mm) working in parallel.
- Sample volume: 2000 mL of filtered by 0.22 μ m wastewater
- Sample pH: Basic pH (pH \approx 10)



Oasis HLB columns

MS/MS detection conditions:

- Capillary voltage: 3.5 kV (ESI+) and -2.5 kV (ESI-)
- Source temperature: 150°C
- Desolvation temperature: 500°C
- Desolvation gas: Nitrogen at 1000 L·hr⁻¹
- Collision gas: Argon at 0.15 mL·min⁻¹

RESULTS:

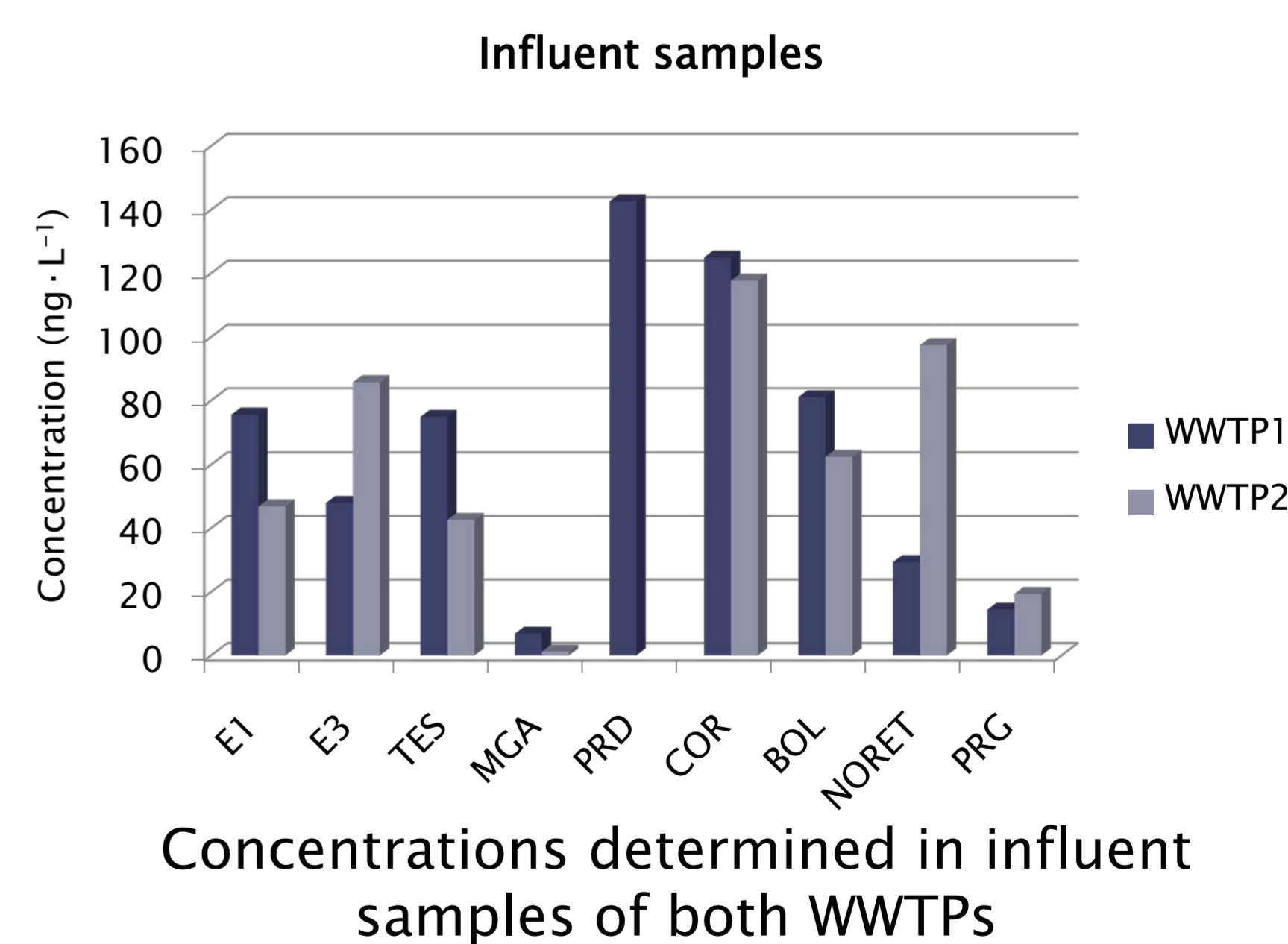
Analytical parameters

Compound	Detection limit (ng·L ⁻¹)	100 ng·L ⁻¹		500 ng·L ⁻¹	
		Recovery (%) n=6	RSD* (%) n=6	Recovery (%) n=6	RSD* (%) n=6
Diethylstilbestrol	13.2	44.3	7.3	42.3	14.7
17 β -estradiol	8.5	88.8	26.4	104.0	7.0
Estrone	4.1	75.1	15.1	81.6	8.8
Estriol	4.5	76.8	5.2	69.7	17.1
Norgestrel	1.6	34.5	8.6	36.7	11.6
Testosterone	1.0	43.1	6.9	48.3	3.7
Megestrol acet.	1.2	138.7	6.8	154.4	10.8
Prednisone	9.2	61.7	11.5	60.7	5.0
Prednisolone	6.1	95.2	9.4	100.0	8.7
Cortisone	2.1	69.5	7.3	66.3	3.2
Boldenone	0.7	61.1	4.5	67.5	2.7
Norethisterone	2.3	42.7	2.9	44.3	3.3
Nandrolone	4.1	59.0	9.6	59.6	3.3
Progesterone	0.5	43.4	10.7	43.7	10.3

Table 3. Analytical parameters * Relative standard deviation

Real samples

Sewage samples were collected from two WWTPs located in Gran Canaria (Spain). WWTP1 presents a membrane bioreactor technique while WWTP2 has a treatment with activated sludge



Concentrations determined in influent samples of both WWTPs

CONCLUSIONS

In accordance with the obtained results, the on-line SPE-UHPLC-MS/MS procedure is easy, selective and sensitive, with low detection and quantification limits. The application in real samples from WWTPs was satisfactory.

REFERENCES

- [1] T. Vega-Morales, Z. Sosa-Ferrera, J.J. Santana-Rodríguez. J. Hazard. Mater. 183 (2010) 701-711.