Optimization of an on-line solid phase extraction (SPE) coupled with UHPLC-MS/MS, for the determination of hormonal compounds in sewage from wastewater treatment plants of Gran Canaria (Spain)

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INTRODUCTION:

Steroid hormones are an important group inside of the emerging pollutants, and are considered as endocrine disruptor compounds (EDCs) due to their capacity of altering the natural hormonal equilibrium, producing harmful effects in animals, humans and their progeny. These changes are more noticeable in the marine environment [1]. The consumption of steroid hormones has increased exponentially in last decades, due to their use in human and veterinary medicine and the principal source of these pollutants are the wastewater treatment plants. Several studies have determined the presence of this type of compounds in wastewater samples [2].

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

EXPERIMENTAL:

Compounds studied:

Estrogens	Androgens	Progestogens	Corticosteroids
Diethylstilbestrol (DES)	Boldenone (BOL)	Norethisterone (NOR)	Prednisone (PRD)
17β-estradiol (E2)	Nandrolone (NAN)	Norgestrel (NRG)	Cortisone (COR)
Estrone (E1)	Testosterone (TES)	Megestrol Acetate (MGA)	Prednisolone (PRDL)
Estriol (E3)		Progesterone (PRO)	

Table 1: Compounds studied

Extraction and chromatographic conditions:

Time (min)	BSM Flow (mL·min^{-1})	BSM A (%)	BSM B (%)	QSM Flow (mL·min^{-1})	QSM A2 (%)	QSM B2 (%)	QSM C (%)	QSM 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	Weak wash step
3.80	0.300	80	20	2.000	0	100	0	0	
4.10	0.300	80	20	2.000	0	0	0	100	Strong wash of the cartridges
7.00	0.300	0	100	2.000	100	0	0	0	Re-equilibration time
8.00	0.300	0	100	2.000	100	0	0	0	
10.50	0.300	80	20	2.000	100	0	0	0	

- Column: ACQUITY UPLC BEH Waters C18 (50 x 2.1 mm, 1.7 µm)
- Mobile phases: A: Water + 0.1% NH_3 and B: Methanol

Table 2: Gradient used for extraction process and chromatographic separation

On-line SPE optimization:

i) Cartridge used

Two Oasis HLB columns (20 µm, 2.1x30mm) working in parallel.
Flow rate: 2 mL·min^{-1}.
Mobile phases consist of:
A2: water (0.05% acetic acid)
B2: water without additives
C: methanol without additives
D: methanol:acetone:hexane

ii) pH of the sample

pH studied:
→ pH = 3.5 (using acetic acid)
→ pH = 5.7 (without additives)
→ **pH = 10.4 (using NH_3)**

iii) Load phase and sample volume

Load phases studied:
→ **pH = 3.4 (Water + 0.05% CH_3COOH)**
→ pH = 5.8 (Water)
→ pH = 8.1 (Water + 0.1% NH_3 + 0.1M CH_3COONH_4)
→ pH = 10.1 (Water 0.1% NH_3)

Sample volume: 1, **2**, 3, 4 and 5 **mL of wastewater**

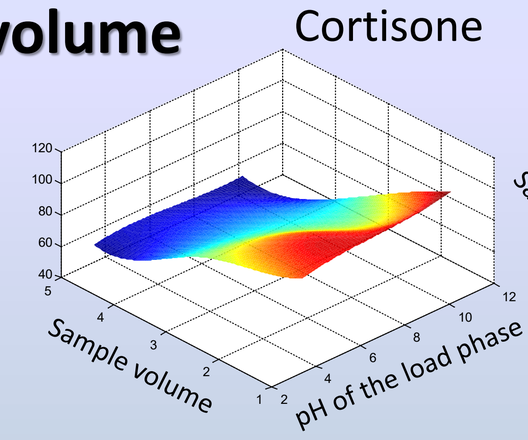

Figure 1. Response surface of estriol and cortisone in load phase and sample volume optimization

iv) Wash step

Solvents studied:
→ **Water and MeOH without additives**
→ Water and MeOH with 0.1% NH_3

% of organic solvent:
→ **0% of MeOH**
→ 10% of MeOH
→ 20% of MeOH
→ 30% of MeOH
→ 40% of MeOH

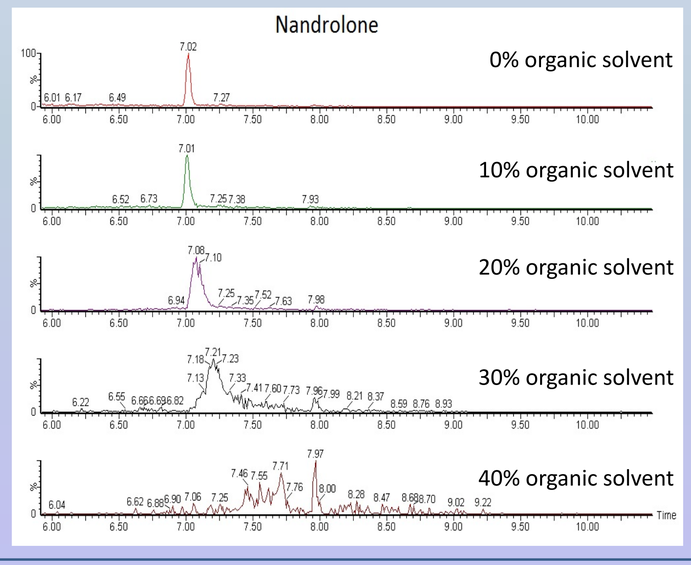

Figure 2. Chromatographic peaks of nandrolone with different proportions of water:methanol in the wash step optimization

RESULTS:

Analytical parameters:

Compound	Detection limit (ng·L^{-1})	100 ng·L^{-1} Recovery (%) n=6	100 ng·L^{-1} RSD* (%) n=6	500 ng·L^{-1} Recovery (%) n=6	500 ng·L^{-1} RSD* (%) n=6
Diethylstilbestrol	13.2	44.3	7.3	42.3	14.7
17β-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 of the method. * Relative standard deviation

Real samples:

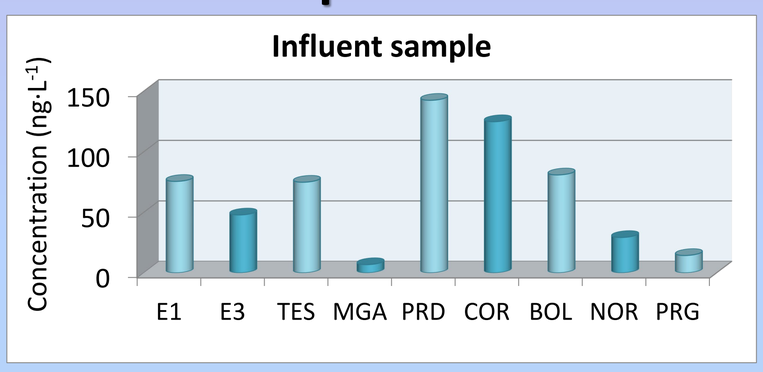

Figure 3. Concentrations detected in influent sample

Wastewater samples were collected from a WWTP located in Gran Canaria (Spain)
WWTP had a novel membrane bioreactor system (MBR). The samples were taken from the influent of the plant.

CONCLUSIONS

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

REFERENCES

[1] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli. Science. **313** (2006) 1072-1077
[2] P.B. Fayad, M. Prévost, S. Sauvé. Talanta. **115** (2013) 349-360