# ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY DETECTION (UHPLC-MS/MS) FOR THE DETERMINATION OF FIFTEEN HORMONAL COMPOUNDS IN WASTEWATER FROM WASTEWATER TREATMENT PLANTS OF GRAN CANARIA (SPAIN)

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## INTRODUCTION:

From all of emerging pollutants, the hormonal residues constitute a group of great interest due to they are considered as endocrine disruptor compounds (EDCs). These compounds are defined as chemical substances capable of altering the natural hormonal equilibrium producing harmful effects in animals, humans and their progeny [1]. The hormones are used in human and veterinary medicine, and their consumption has increased exponentially, which produces a continuous introduction of them into the environment [2].

In this study, the determination of a group of fifteen natural and synthetic hormones (five estrogens, three androgens, four progestogens and three corticosteroids), is presented. The extraction and preconcentration method chosen has been Solid Phase Extraction (SPE) which has been optimized and combined with ultra-high performance liquid chromatography coupled to mass spectrometry detection (UHPLC-MS/MS). The hormonal compounds have been studied in wastewater samples from wastewater treatment plants in Gran Canaria (Spain), with different water treatment methods.

## EXPERIMENTAL PROCEDURE:

### Chromatographic conditions:

| | |
|---|---|
| Instrument | Waters ACQUITY UPLC with TQ Detector |
| Column | ACQUITY UHPLC BEH Waters $C_{18}$ |
| Injection volume | 10 µL |
| Flow rate | 0.3 mL·min$^{-1}$ |
| Mobil phases | A: Water (0.1% v/v $NH_3$ + 15 mM $CH_3COONH_4$)<br>B: Methanol |

### Gradient used:

| Time (min) | % (A) | % (B) |
|---|---|---|
| 0.00 | 80 | 20 |
| 1.50 | 40 | 60 |
| 2.75 | 25 | 75 |
| 3.75 | 25 | 75 |
| 6.00 | 80 | 20 |
| 6.50 | 80 | 20 |

### Optimum SPE conditions:

| | |
|---|---|
| Cartridge | SepPak $C_{18}$ 6cc (Waters) |
| Sample volume | 250 mL |
| Sample pH | 8 |
| Ionic strenght | 0% NaCl |
| Wash step | 5 mL Milli-Q water |
| Desorption solvent | Methanol |
| Desorption volume | 2 mL (1 mL·min$^{-1}$) |

## RESULTS:

### Analytical parameters:

| Compound | RT[a] (min) | LOD[b] (ng·L$^{-1}$) | RSD[c] (%) (25 ng·L$^{-1}$) n=6 | RSD[c] (%) (150 ng·L$^{-1}$) n=6 | Recovery (%) (25 ng·L$^{-1}$) n=3 | Recovery (%) (150 ng·L$^{-1}$) n=3 |
|---|---|---|---|---|---|---|
| Estriol | 2.12 | 1.35 | 5.12 | 6.50 | 106.6 ± 8.2 | 99.0 ± 1.8 |
| Prednisone | 2.29 | 0.27 | 12.9 | 4.79 | 121.3 ± 5.3 | 105.0 ± 3.3 |
| Cortisone | 2.32 | 0.05 | 4.55 | 4.50 | 118.5 ± 1.2 | 103.2 ± 6.3 |
| Prednisolone | 2.45 | 0.18 | 6.90 | 4.56 | 112.9 ± 5.6 | 99.2 ± 3.1 |
| Boldenone | 2.87 | 0.17 | 5.67 | 4.47 | 117.2 ± 3.5 | 112.7 ± 10.8 |
| Nandrolone | 2.93 | 1.00 | 3.83 | 3.81 | 119.4 ± 4.6 | 102.2 ± 5.7 |
| Norethisterone | 2.95 | 0.22 | 7.08 | 4.37 | 113.0 ± 1.8 | 99.3 ± 5.1 |
| 17β-estradiol | 2.97 | 1.63 | 12.4 | 6.57 | 120.4 ± 8.5 | 103.0 ± 7.8 |
| Estrone | 2.99 | 0.35 | 3.51 | 4.87 | 122.2 ± 9.9 | 109.3 ± 2.5 |
| 17α-ethinylestradiol | 3.00 | 2.95 | 6.84 | 6.63 | -- | 103.0 ± 10.6 |
| Diethylstilbestrol | 3.02 | 0.16 | 9.15 | 5.79 | 98.8 ± 14.2 | 80.4 ± 12.0 |
| Testosterone | 3.15 | 0.04 | 6.33 | 3.82 | 118.7 ± 3.3 | 103.3 ± 5.4 |
| Norgestrel | 3.29 | 0.32 | 5.14 | 3.68 | 114.9 ± 2.5 | 103.4 ± 4.4 |
| Megestrol acetate | 3.54 | 0.22 | 8.69 | 4.98 | 107.4 ± 1.2 | 99.4 ± 3.4 |
| Progesterone | 3.71 | 0.21 | 7.41 | 4.59 | 115.9 ± 4.0 | 100.6 ± 4.3 |

[a] Retention time [b] Detection limit [c] Relative Standard Deviation

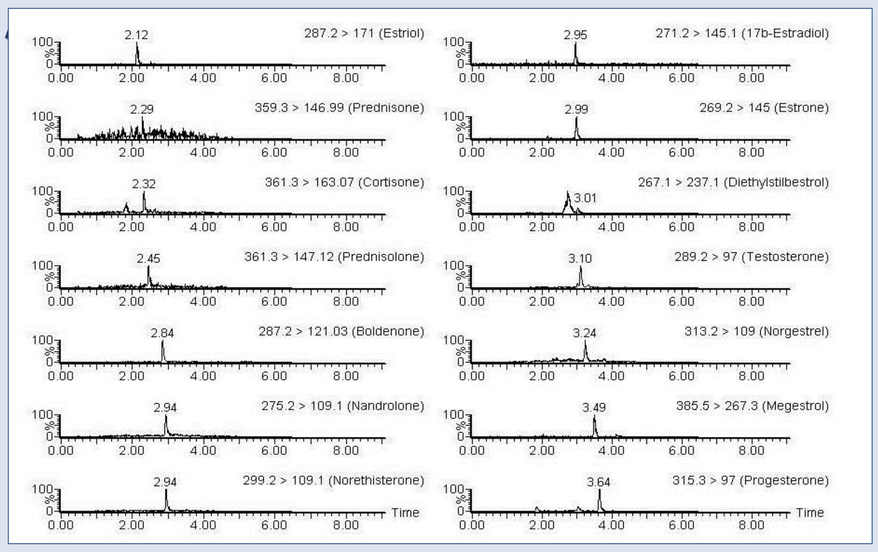


Figure 1: *Chromatogram of spiked wastewater sample (200 ng·L$^{-1}$ of each analyte) using SPE-UHPLC method*

- Wastewater samples were collected in two WWTPs located in Gran Canaria (Spain)
- Samples from both WWTPs were taken from the influents and effluents of the plants, but only in influent samples hormones were detected.

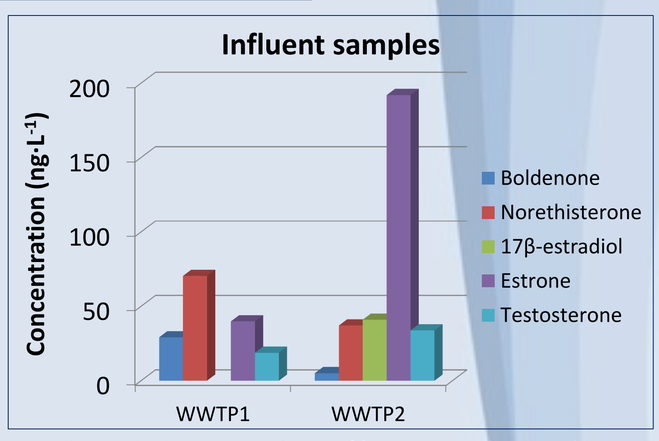


Figure 2: Concentrations of hormones detected in influent samples.

## CONCLUSIONS:

An analytical method for the simultaneous extraction, preconcentration and determination of fifteen hormones in wastewater matrices has been optimized and developed. The limits of detection reached were between 0.04 – 1.63 ng·L$^{-1}$. In addition, the method presented high recoveries, up to 80%, for the majority of compounds and RSD lower than 13%. The application of the method to samples from two different WWTPs showed that the concentrations of hormones found, only in influent samples, ranged from 5 to 192 ng·L$^{-1}$.

## REFERENCES:

[1] A. Kortenkamp, Environ. Health Perspect. **115** (2007) 98-105.
[2] C.G. Campbell, S.E. Borglin, F.B. Green, A. Grayson, E. Wozei, W.T. Stringfellow, *Chemosphere* **65** (2006) 1265-1280

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