COMPARISON OF SORBENTS IN ON-LINE SPE COUPLED TO UHPLC-MS/MS FOR THE DETERMINATION OF HORMONES IN WATER SAMPLES

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INTRODUCTION

Endocrine disrupting compounds (EDCs) are a wide group of compounds that can affect the endocrine system of organisms, producing different problems, like changes in fertility or feminization [1]. These changes are linked with the effluent discharges of wastewater treatment plants, because these are the principal way of EDCs into the environment. Steroid hormones have been measured in environmental waters at very low concentrations, so it is necessary to develop extraction and preconcentration methodologies to achieve these concentrations. Solid phase extraction (SPE) has been used widely in recent years to separate and preconcentrate hormones from environmental water samples [2] and the use of on-line SPE presents many advantages like less sample handling or shorter time analysis.

In this study, a optimization of two different sorbents used in on-line SPE coupled with ultra-high performance liquid chromatography following by tandem mass spectrometry detection (on line SPE-UHPLC-MS/MS) has been developed to determine several natural and synthetic hormones (estrogens, androgens, progestogens and corticosteroids) in effluent wastewater samples.

EXPERIMENTAL

Compounds studied:

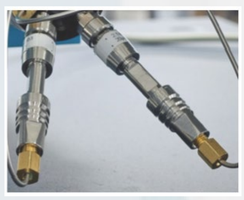
Estrogens:	Diethylstilbestrol	17β-estradiol	Estrone	Estriol
Progestogens:	Norethisterone	Megestrol Acetate	Norgestrel	Progesterone
Androgens:	Testosterone	Nandrolone	Boldenone	
Glucorticoids:	Prednisolone	Prednisone	Cortisone	

Extraction conditions and gradient used:

Time (min)	Binary Solvent Manager Flow (mL·min^{-1})	A1 (%)	B1 (%)	Quaternary Solvent Manager Flow (mL·min^{-1}) OASIS HLB	XBrigde C18	A2 (%)	B2 (%)	C (%)	D (%)	
0.00	0.300	80	20	2.000	2.000	100	0	0	0	Loading phase
0.50	0.300	80	20	2.000	0.010	0	100	0	0	Weak wash step
3.80	0.300	80	20	2.000	0.010	0	100	0	0	
4.10	0.300	80	20	2.000	2.000	0	0	0	100	Strong wash of the cartridges
7.00	0.300	0	100	2.000	2.000	100	0	0	0	Re-equilibration time
8.00	0.300	0	100	2.000	2.000	100	0	0	0	
10.50	0.300	80	20	2.000	2.000	100	0	0	0	

- Column: ACQUITY UPLC BEH Waters C18 (50 x 2.1 mm, 1.7 µm)
- Mobile phases: A1: Water + 0.1% NH_3 and B1: Methanol

On-line SPE optimization:



On-line SPE columns

- **SPE columns:**
  - Oasis HLB (20 µm, 2.1x30mm)
  - XBridge C_{18} (10 µm, 2.1x30mm)
- **Sample volume:** 1000 to 5000 of wastewater filtered by 0.22 µm
- **Load phase:**
  - pH = 3.4 (Water + 0.05% CH_3COOH)
  - pH = 5.8 (Water)
  - pH = 10.1 (Water 0.1% NH_3)
- **Wash step:**
  - Water without additives
  - Water with 0.1% NH_3

  ✚ Different concentrations of methanol
- **Sample pH:**
  - pH = 3.5
  - pH = 5.5 - 7
  - pH = 10.4

RESULTS

Optimum extraction conditions

OASIS HLB column:
- **Sample volume:** 2000 µL of wastewater
- **Load phase:** pH = 3.4 (Water + 0.05% CH_3COOH)
- **Wash step:** Water without additives
- **Sample pH:** pH = 10.4

Recoveries:
- **Estrogens:** 42 – 104%
- **Progestogens:** 48 – 68%
- **Androgens:** 36 – 150%
- **Glucorticoids:** 60 – 100%

XBridge C_{18}
- **Sample volume:** 2000 µL of wastewater
- **Load phase:** pH = 10.4 (Water + 0.1% NH_3)
- **Wash step:** No wash step
- **Sample pH:** pH = 10.4

Recoveries:
- **Estrogens:** < 15%
- **Progestogens:** 9 – 57%
- **Androgens:** 11 – 26%
- **Glucorticoids:** 13%
- Estriol, prednisone and prednisolone are not extracted with these cartridges

CONCLUSIONS

The optimization of two different sorbents used in on-line SPE coupled to UHPLC-MS/MS has been studied. OASIS SPE columns are better for the extraction of steroid hormones of wastewater with aceptable recoveries that ranged from 40 to 100% for the most of compounds under study.

XBridge SPE columns are not so efficient for the extraction. In fact, some analytes as estriol, prednisone and prednisolone are not extracted with this type of cartridges. The recoveries for XBridge columns are below 40% for the most of compounds.

REFERENCES

[1] C. Wang, R.P. Croll. Aquaculture **238** (2004) 483–498

[2] R. Guedes-Alonso, Z. Sosa-Ferrera, J. J. Santana-Rodríguez. Journal of Analytical Methods in Chemistry **Article ID 210653** (2014) 1–8.

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