OPTIMIZATION OF A SOLID-PHASE EXTRACTION PROCEDURE COMBINED WITH ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND FLUORESCENCE DETECTION TO DETERMINE ESTROGENS IN WASTEWATER SAMPLES

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INTRODUCTION

In recent years, the ecosystem problems, the increasing of water demand and the continuous discovery of new emerging pollutants have attracted the attention of scientific community. This is the reason why many researches have been focused on developing new methodologies to determine all substances with environmental interest.

Within this broad group of compounds, the use of estrogenic compounds has increased exponentially nowadays. (1,2).

In this research, a solid phase extraction (SPE) procedure was optimized and coupled to ultra-high performance liquid chromatography with fluorescence detection (UHPLC-FD) for the determination of a group of four estrogens: estriol, β-estradiol, 17β-estradiol glucuronide and 17α-ethinyl estradiol. All parameters involved in solid-phase extraction were optimized, such as, type of SPE cartridge, sample volume, pH and ionic strength of sample, wash step and desorption volume. The results were evaluated to obtain optimum extraction conditions.

EXPERIMENTAL

Chromatographic separation and Fluorescence detection

An UHPLC system with fluorescence detector from Waters (Madrid, Spain) and a C_{18} column were used. Analytes separation was carried out employing this gradient: starts at 55:45 (water/methanol) during 3 minutes, it changed to 50:50 (v/v) and stayed for 2.5 min. Finally, came back to initial conditions in 1 minute, and stayed for 1.5 minutes. Therefore, the analysis took 9 minutes at a flow of 0.5 $mL \cdot min^{-1}$. Fluoresce detector was operated at an excitation and emission wavelength of 280 and 310 nm respectively.

Ident. nº	Compound	λ_{ex}	λ_{em}
1	Estriol	280 nm	310 nm
2	17β-estradiol glucuronide		
3	17β-estradiol		
4	17α-ethinyl estradiol		

Table 1: Id. number of compounds and excitation and emission wavelengths

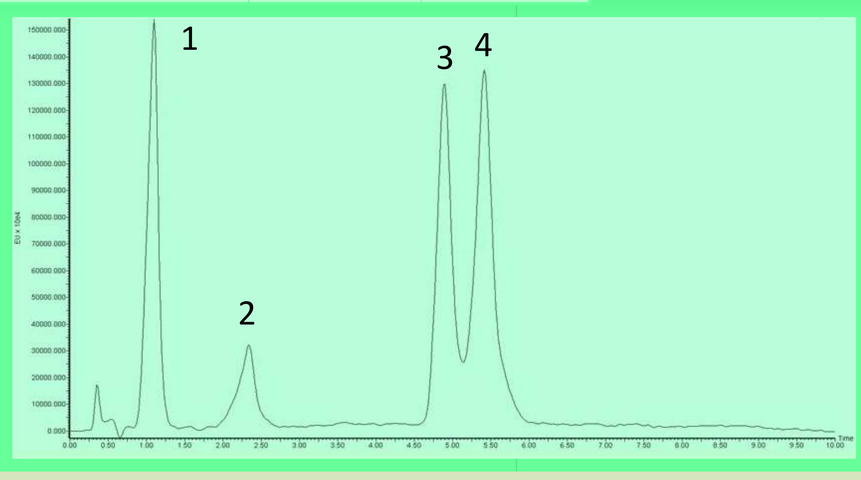

Fig. 1: Chromatogram of a spiked deionized water sample (250 $ng \cdot mL^{-1}$ for each analyte) using optimized SPE method

RESULTS

From the results obtained in the study of different cartridges, we can observe that the better signals are found for SepPak C18 cartridge (figure 2).

The pH, ionic strength and sample volume were optimized using a 2^3 design (three variables, two levels), because that ionic strength and sample volume present the higher correlations, a 3^2 experimental design was done. Figure 3 shows the response surface obtained for the estriol, where can be observe that the best conditions were 0% of NaCl and 250 mL of sample volume.

Finally, the desorption volume and the wash-step were fixed at 2 mL of methanol in one step and 5 mL of Milli-Q water without methanol respectively. In these conditions we achieved a preconcentration factor of 125.

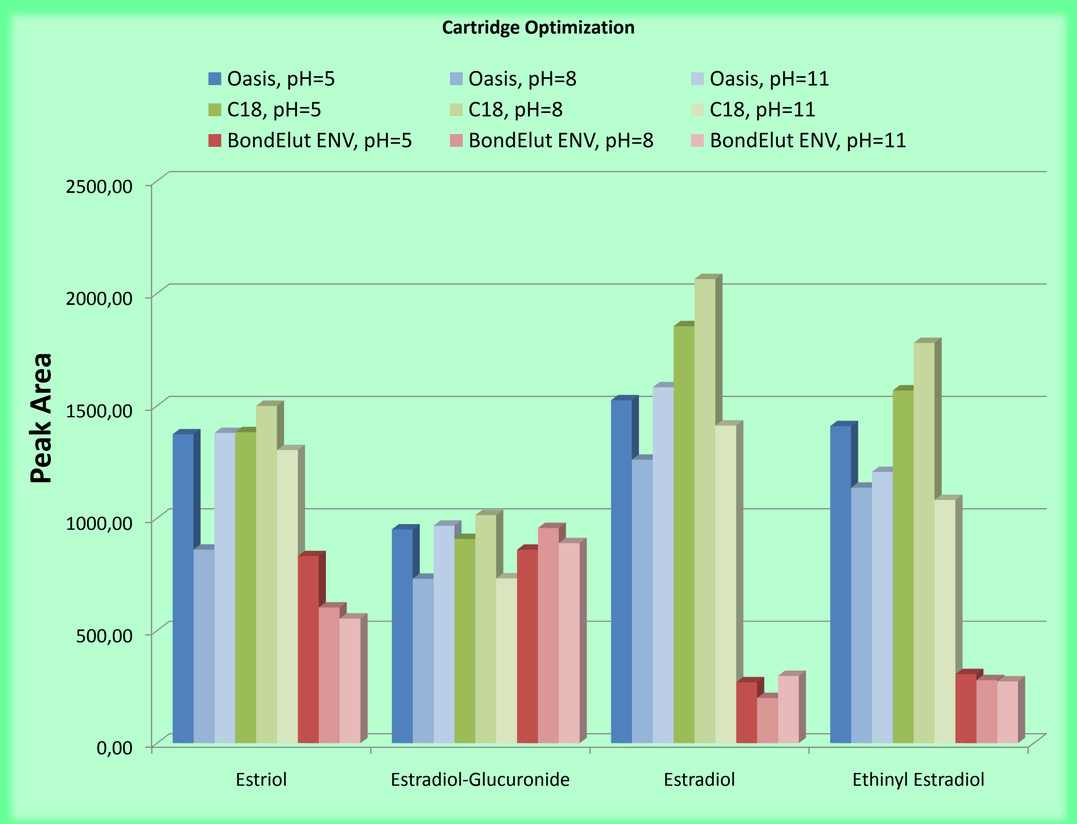

Fig. 2: Optimization of SPE cartridges at different pH.

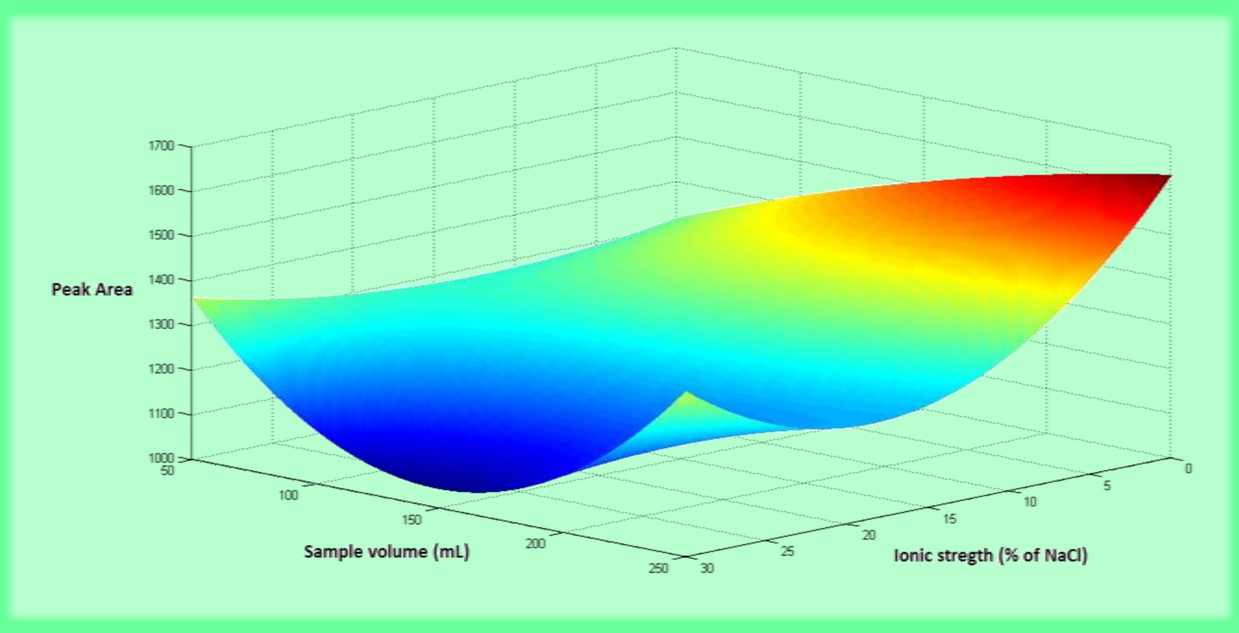

Fig. 3: Effect of ionic strength and sample volume on the SPE extraction of Estriol.

CONCLUSIONS

In accordance with the obtained results, the optimum conditions for SPE procedure were: SepPak C18 cartridge, 250 mL of sample at pH=8 and 0% of NaCl, desorption with 2 mL of methanol in one step and wash step with 5 mL of Milli-Q water.

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REFERENCES

1. Falconer, I.R; Chapman, H.F; Moore, M.R; Ranmuthugala, G. 2006. Environmental Toxicology, 21(2): 181-191.
2. Vega-Morales, T; Sosa-Ferrera, Z; Santana-Rodríguez, J.J. 2010. Journal of Hazardous Materials, 183: 701-711.