OPTIMIZATION OF ESTRADIOL MONITORING IN RAW AND TREATED WASTEWATER SAMPLES BY RESPONSE SURFACE METHODOLOGY

Ana Queiroz[1,2], Nathalia S. Foureaux[1,3], Eduardo V. Carneiro[3], António E. Ribeiro[1,2], Paulo Brito[1,2]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[2]Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[3]Fundação Mineira de Educação e Cultura (FUMEC), Rua Cobre, nº 200, Cruzeiro, Belo Horizonte – MG, 30310-190, Brazil
E-mail: amqueiroz@ipb.pt

INTRODUCTION & MAIN OBJECTIVE

The ever-increasing use of endocrine disruptors compounds (EDCs), through pharmaceuticals such as synthetic estrogens, both in humans as well as in animals, are raising its concentration in the environment. Estradiol, also designed as 17β-Estradiol (see Fig. 1), belongs to the pharmaceutical class of steroid estrogens and was included in the "Watch List" since 2013 the Directive 2013/39/EU due to its potential risk to human health and environment. The low removal efficiency of estrogens by the conventional wastewater treatment plants (WWTPs), becomes a major source of their release into different aquatic matrices. Therefore, the occurrence and, more importantly, the destination of these compounds are matters of utmost importance towards a better public health.

The aim of this work is the optimization of solid phase extraction/high performance liquid chromatography (SPE/HPLC) using the response surface methodology (RSM) to detect and quantify 17β-Estradiol in WWTPs effluents.

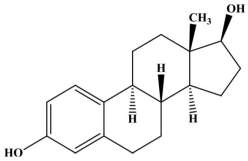


Fig. 1. Molecular structure of 17β-Estradiol.

EXPERIMENTAL METHODOLOGY

1. Optimization of the **quantification method** (HPLC-UV)
2. Optimization of the **extraction/concentration method** (SPE)
3. Determination of the **method quality parameters** (LD, LQ, …)
4. **Method validation** with real water matrice samples

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Table 1. HPLC-UV main operating conditions.

PARAMETER	CONDITION
Column	Nucleosil 100-5 C18, dp = 5 µm, 150 mm x 4.6 mm from Macherey-Nagel
Injection Volume	20 µL
Wavelength	281 nm
Flow-rate	1 mL/min

SCREENING OF MOBILE PHASE COMPOSITION AND CALIBRATION CURVE

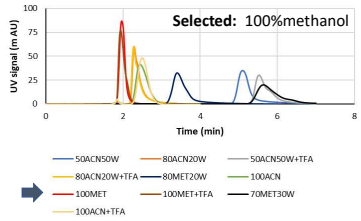


Fig. 2. HPLC-UV chromatographic pulses of estradiol using 10 different mobile phase compositions.

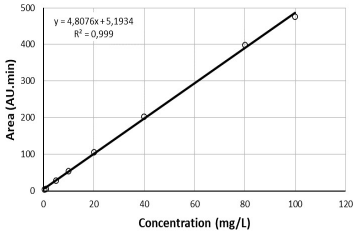


Fig. 3. HPLC-UV calibration curve for estradiol quantification.

OPTIMIZATION OF SOLID PHASE EXTRACTION CONDITIONS

Table 2. Experimental planning using the **three-level Box-Behnken experimental design**.

PARAMETERS	LEVELS		
	-1	0	+1
Sample Volume (mL)	500	1000	1500
Sample pH	2	5	8
Adsorbent drying time (min)	10	35	60
Solvent composition in washing (%)	0	5	10

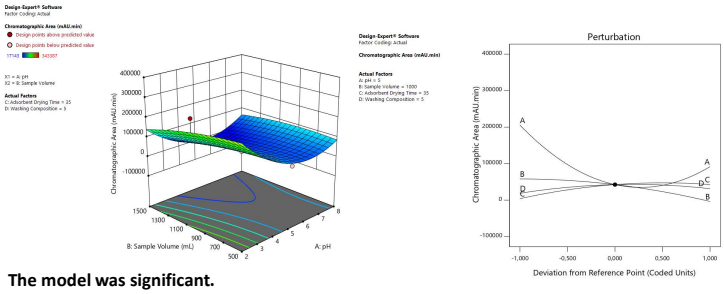
General equation for the quadratic mathematical model relating all four parameters and their response.

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{j<i} \beta_{ji} X_j X_i$$

$$Y = 42648.93 - 57050.4\,A - 30819.5\,B + 19334.8\,C + 6310\,D + 1.06E{+}05\,A^2 - 15386.7\,B^2 - 19258.2\,C^2 - 17487.2\,D^2 + 19510.6\,AB - 32558.9\,AC - 9030.4\,AD - 33256.3\,BC - 1417\,BD + 765.3\,CD$$

(A – pH value; B – sample volume; C – adsorbent drying time; D – washing composition)

Response surface regarding the influence of pH (A) and sample volume (B) on the chromatographic area and Perturbation analysis.



The model was significant.
pH is the more significative parameter.

WWTPs samples preparation and analysis by SPE/HPLC

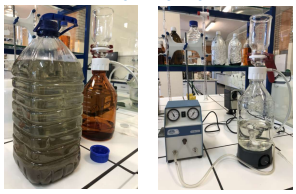


Fig. 4. Sample before and after vacuum filtration.

Table 3. Average concentration for all the three type of WWTP samples.

SAMPLE COLLECTING POINT	SAMPLE LOCATION POINT	AVERAGE CONCENTRATION (mg/L) ± SD
1	INLET	6.61 ± 0.54
2	AERATION TANK	2.44 ± 0.53
3	OUTLET	0.89 ± 0.18

CONCLUSIONS

- Mobile phase of 100% methanol resulted in the best conditions to operate the HPLC-UV system (lower retention time) and lower dispersion;
- Regarding the SPE conditions, the maximum area, and also higher recovery of estradiol is obtained using a sample with a pH value of 2, a sample volume of 500 mL, using 60 min for the adsorbent drying time and a 10% methanol added to ultrapure water in washing.
- Estradiol was detected in all the three types of WWTPs samples.
- WWTP treatment needs a complementary treatment for hormones degradation / removal.

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