DCE 21 4th DOCTORAL CONGRESS IN ENGINEERING

DOCTORAL CONGRESS IN ENGINEERING

Book of Abstracts

Symposium on Environmental Engineering

U.PORTO
FEUP FACULDADE DE ENGENHARIA
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DCE 21 4th DOCTORAL CONGRESS IN ENGINEERING

Book of Abstracts

of the

4th Symposium on Environmental Engineering

Editors:
Ana Gonçalves, Joana Pesqueira,
Juliana Sá, Sara Pardilhó

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4th Symposium on Environmental Engineering

This volume contains the abstracts presented at the Symposium on Environmental Engineering, within the 4th Doctoral Congress in Engineering – DCE21, held online, between June 28th and 29th, 2021.

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OC01. Monitoring of 17β-estradiol in raw and treated samples of wastewater treatment plants

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Abstract

Nowadays, monitoring the concentration levels of estrogens in treated wastewaters of wastewater treatment plants (WWTP) is an environmental mandatory task to minimize or eliminate water pollution. The present work is divided in two main experimental stages. First, an SPE/HPLC-UV experimental methodology is optimized to detect and quantify 17β-Estradiol (E2) present in aqueous samples. The HPLC-UV operating conditions were selected by performing a screening between 10 different mobile phase compositions. A pure methanol composition was selected based in the lower retention time and the highest UV detector signal. The solid phase extraction optimization involves a three-level Box-Behnken experimental design with four factors (sample volume, sample pH, adsorbent drying time and solvent composition used for the washing step), combined with a response surface methodology. The validation of the optimized experimental methodology is done by the monitoring of estradiol in wastewater influent and effluent samples from Bragança Wastewater Treatment Plant in Portugal.

Keywords: Estradiol, wastewater, solid phase extraction, high performance liquid chromatography.

1. Introduction

Nowadays, there has been a growing concern regarding the possible consequences of exposure to estrogens through its direct or indirect consumption. The increasing utilization of estrogenic compounds, such as natural and synthetic estrogens, pharmaceuticals and pesticides has resulted in their continual occurrence in the aquatic environment. The risk that endocrine disrupting compounds (EDCs) causes to human life and wildlife, is one of the reasons why studies concerning their detection and removal from diverse aquatic environment are so important. Even at low concentration levels, EDCs can induce unhealthy changes to human lives (Vega-Morales *et al*., 2013). Estrogens are some of the most potent endocrine disrupting compounds (Racz *et al*., 2010). Monitoring the levels of estrogens is highly recommended due to its frequent detection in treated wastewaters of Wastewater Treatment Plants (WWTPs).

2. Materials and Methods

2.1. Reagents and equipment

Acetonitrile HPLC grade; Methanol HPLC grade; Ultrapure water; Ethanol HPLC grade; Hydrochloric Acid, PA; Potassium Hydroxide PA; Trifluoracetic acid PA; 17b-Estradiol analytical standard.

Chromatographic analytical column Nucleosil 100-5 C18 with a particle size diameter of 5 □m, 150 mm x 4.6 mm from Macherey-Nagel; Analytical balance ADA 210/C, ±0.0002 g, Adam Equipment; pH meter HI 2020-02 from Hanna; Chromabond HLB SPE cartridges, 60 μm; 6 mL/500 mg from Macherey-Nagel.

2.2. Experimental methodology

The present work is divided in two main experimental stages. First, an SPE/HPLC-UV (Fonseca *et al*., 2013) experimental methodology is optimized to detect and quantify 17β-Estradiol (E2) present in the aqueous samples. The HPLC-UV operating conditions were selected by performing a screening of the mobile phase composition (10 different compositions). The solid phase extraction optimization involves a three-level Box-Behnken (BBD) experimental design (Teixeira *et al*., 2019) with four factors

(sample volume, sample pH, adsorbent drying time and solvent composition in the washing step), combined with a response surface methodology. Secondly, the validation of the optimized experimental methodology is done by the monitoring of estradiol in wastewater influent and effluent samples from Bragança Wastewater Treatment Plant in Portugal.

3. Discussion

To optimize the HPLC-UV operating conditions it was performed a screening of the mobile phase composition, 10 different compositions were analyzed according to the parameters described in Table **1**.

Table 1: HPLC-UV operating conditions analyzed.

MOBILE PHASE	COMPOSITION	VOLUME	pH
1	50 ACN : 50 ULTRAPURE WATER	500 mL	7.6
2	80 ACN : 20 ULTRAPURE WATER	500 mL	5.9
3	100 ACN	500 mL	5.2
4	50 ACN : 50 ULTRAPURE WATER + 0.02 TFA	500 mL	2.5
5	80 ACN : 20 ULTRAPURE WATER + 0.02 TFA	500 mL	2.1
6	100 ACN + 0.005 TFA	500 mL	1.7
7	100 MET	500 mL	5.8
8	80 MET : 20 ULTRAPURE WATER	500 mL	6.1
9	70 MET : 30 ULTRAPURE	500 mL	6.4
10	100 MET + 0.005 TFA	500 mL	2.0

A pure methanol composition (100 MET, mobile phase 7) was selected based in the lower retention time and the highest UV detector signal. In Figure 1, is presented the chromatographic pulses of estradiol using 8 different concentrations (HPLC analysis without SPE extraction).

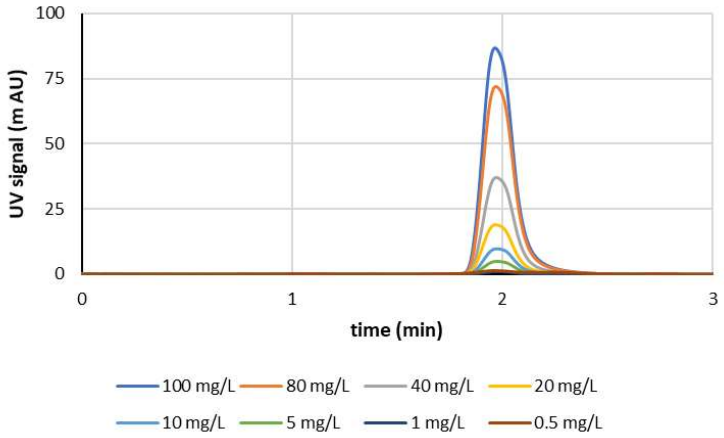

Figure 1: HPLC-UV chromatographic pulses of estradiol using 8 different estradiol concentrations between 0.5 and 100 mg/L.

For the purpose of the optimization of SPE conditions, a three-level Box-Behnken experimental design with four factors, as presented in **Table 2**. This methodology involves a total of 27 experiments. The maximization of the experimental HPLC chromatographic area for estradiol was defined as the objective function.

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

FACTORS	LEVELS
Sample Volume	500, 1000 and 1500 (mL)
Sample pH	2, 5 and 8
Adsorbent drying time	10, 35 and 60 (min)
Solvent composition in washing	0, 5 and 10 (%)

After the SPE procedure was completed, the samples were analyzed in the HPLC-UV system. The highest response was obtained with experiment "Run 20", that has the following parameters: a sample volume of 500 mL, a pH value of 2, 60 minutes for the adsorbent drying time and a 10% methanol added to ultrapure water in washing. **Figure 2** presents the surface response methodology (RSM) obtained with the BBD methodology.

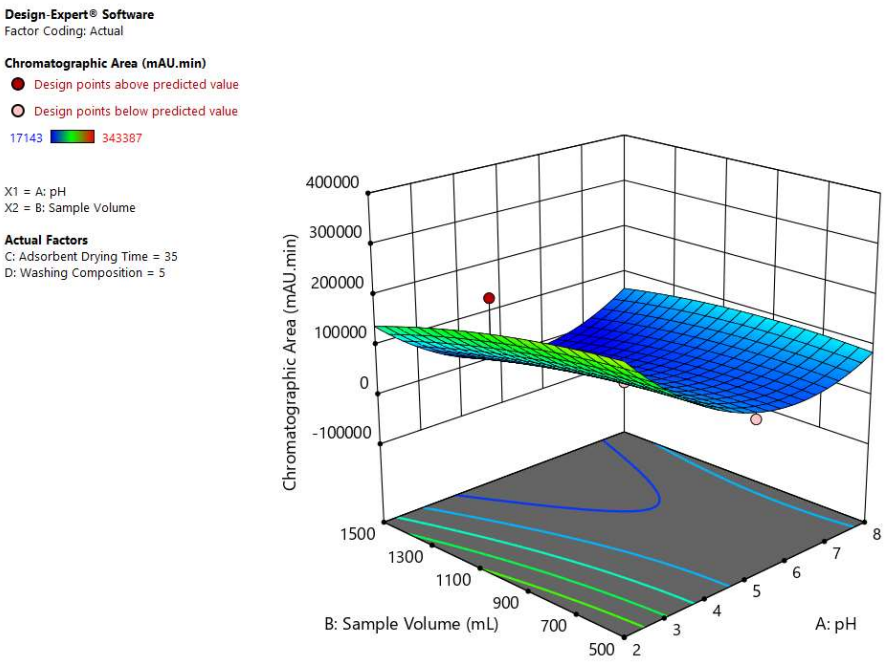

Figure 2: Response surface regarding the influence of pH (A) and sample volume (B) on the chromatographic area.

4. Conclusions

Mobile phase consisting of 100% methanol resulted in the best conditions to operate the HPLC-UV system, once it is the one that present a lower retention time. Regarding the SPE conditions, the maximum area (and also higher recovery) 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. Results for methodology validation using real WWTP samples will be also presented.

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