CIÊNCIA e NATURA

UFSM

Ci. e Nat., Santa Maria v.42, e11, p. 1-20, 2020

DOI: 10.5902/2179460X42691

ISSN 2179-460X

Química

Submissão 05/03/20 Aprovação 08/05/20 Publicação 11/05/20

Remotion of the 17α-Ethinylestradiol Hormone (EE2) by Biosorbent (Arachis hypogaea) in Aqueous Solutions: Validation of Analytical Methodology and Adsorption Study

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ABSTRACT

This study describes the application of peanut shells, a biodegradable agroindustrial residue as a biosorbent, in the 17α-ethinylestradiol (EE2) removal in water matrices. An analytical method was developed and validated from the high-performance liquid chromatographic technique with fluorescence detection (HPLC-FLD) in a λ_{Ex}= 230 nm and λ_{Em}= 310 nm in the determination of EE2. The evaluated parameters were: selectivity, linearity (R^2 = 0.9984 and R = 0.9992), precision (Variation coefficient = 2.90% and 2.95% for the first and second analyst, respectively), accuracy (recovery rate = 100.2 – 110.4%), limit of detection (3.4 – 5.0 μg L^{-1}), limit of quantification (10.0 – 11.3 μg L^{-1}) and robustness (recovery rate = 98.7 – 115.5%). The chromatographic analysis conditions were: mobile phase (water 2:3 acetonitrile), mobile phase flow (0.5 mL min^{-1}), injection volume (10 μL), column temperature (45ºC) and analysis time (10 min). An experiment planning (Box-Behnken Model) was carried out for the investigation and verification of the adsorptive capacity of the peanut shells, of which 3 parameters were evaluated (stirring rate, pH and adsorbent mass) on 3 levels. Optimum experimental condition (2 g of adsorbent, pH = 6 and stirring at 500 rpm) with a removal rate of 90% in 24 hours of the process.

Keywords: 17α-ethinylestradiol; Endocrine disruptors; Emerging pollutants; Validation of analytical methodology; Box-Behnken; Removal; Biosorbents peanut shells

RESUMO

Este estudo descreve a aplicação de cascas de amendoim, um resíduo agroindustrial biodegradável como biossorvente, na remoção do 17α-etinilestradiol (EE2) em matrizes de água. Foi desenvolvido e validado um método analítico a partir da técnica cromatográfica líquida de alta eficiência com detecção de fluorescência (CLAE-DFL) a um λ_{Ex}= 230 nm e λ_{Em}= 310 nm na determinação do EE2. Os parâmetros avaliados foram: seletividade, linearidade (R^2 = 0.9984 e R = 0.9992), precisão (Coeficiente de variação = 2.90% e 2.95% para o primeiro e segundo analista, respectivamente), exatidão (taxa de recuperação = 100.2 – 110.4%), limite de detecção (3.4 – 5.0 μg L^{-1}), limite de quantificação (10.0 – 11.3 μg L^{-1}) e robustez (taxa de recuperação = 98.7 – 115.5%). As condições cromatográficas de análise foram: fase móvel (água 2:3 acetonitrila), vazão da fase móvel (0.5 mL min^{-1}), volume de injeção (10 μL), temperatura da coluna (45ºC) e tempo de análise (10 min). Um planejamento de experimento (Modelo

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Box-Behnken) foi realizado para a investigação e verificação da capacidade adsortiva das cascas de amendoim, do qual foram avaliados 3 parâmetros (velocidade de agitação, pH e massa adsorvente) em 3 níveis. Condição experimental ótima (2 g de adsorvente, pH = 6 e agitação de 500 rpm) com uma taxa de remoção de 90% em 24 horas de processo.
Palavras chave: 17α-etinilestradiol; Interferentes endócrinos; Poluentes emergentes; Validação de metodologia analítica; Box-Behnken; Remoção; Biossorventes; Cascas de amendoim

1. INTRODUCTION

Contamination of water bodies by numerous substances is a worldwide challenge, despite of the large volume of water on the planet. Among these pollutants, there is a class of compounds called emerging contaminants (ECs) capable of causing serious damage even in small amounts (ng L^{-1} to μg L^{-1}) (CHOINA *et al*., 2013; CANDIDO *et al*., 2016). ECs refer to any chemical compound present in a range of commercial compounds like medicines, veterinary use, food packaging, hygiene products, nutritional supplements, diagnostic agents, agrochemicals, etc., or any micro-organism that can be found in environmental and biological matrices that are normally not monitored or do not have the corresponding regulatory legislation yet, but which pose a potential risk to human health and the environment (SILVA and COLLINS, 2011; DEBLONDE; COSSU-LEGUILLE; HARTEMANN, 2011; JURADO *et al*., 2012; LUO *et al*., 2014; BRAZ *et al*., 2014; BIRCH *et al*., 2015). These ECs include not only compounds or substances in active or past production but also their metabolites and other transformation products and chemical by products generated during their production and use (PATIÑO *et al*., 2015).

Among the ECs there are many substances known as endocrine disrupters (EDs). These compounds raise special concern due to their ability to interfere in the endocrine system of organisms by blocking or minimizing the normal effect of a particular hormone that affects synthesis or metabolism (BARREIROS *et al*., 2016; MEI *et al*., 2011; GIULIVO *et al*., 2016; DIAMANTI-KANDARAKIS *et al*., 2009; NOHYNEK *et al*., 2013; SODRÉ *et al*., 2007).

Synthetic estrogen 17α-ethinylestradiol (EE2), target substance of this study, derived from natural estrogen 17β-estradiol (E2) is considered a potent endocrine disruptor and is used in the production of pharmaceuticals, mainly in contraceptive

pills formulations (contraceptives) and hormone replacement therapies in menopause (SODRÉ *et al*., 2007; CHOINA *et al*., 2010; FERNANDES *et al*., 2011; SILVA; OTERO; ESTEVES, 2012; HAN *et al*., 2013; ARIS; SHAMSUDDIN; PRAVEENA, 2014; LEONARD *et al*., 2017). Often this pollutant is excreted in individuals' urine and feces who take medications reaching the water bodies (LEONARD *et al*., 2017; IFELEBUEGU, 2012).

Due to inefficiency and inability to remove this substance from conventional water and sewage treatment plants, many other proposals have been published in the scientific literature (CLOUZOT *et al*., 2010). Although several treatment technologies have been suggested for the EDs removal, such as ozonation, advanced oxidation and UV radiation, membrane technologies, photocatalysis, electrochemical and enzymatic treatment, etc., their high operating and maintenance costs make them unsustainable solutions and not preferable (PAPAEVANGELOU *et al*., 2016; LIU; KANJO; MIZUTANI, 2009; ROSAL *et al*., 2010; AQUINO; BRANDT; CHERNICHARO, 2013; DIAGBOYA and DIKIO, 2018).

Aware of the serious consequences that these pollutants can cause, new measures and processes of effluents decontamination need to be developed. Cellulose based materials are being designed to remove EDs by adsorption, a new alternative for the removal of micropollutants in surface waters. Cellulose is the most abundant natural biopolymer, and can be obtained directly from agricultural waste, chemically modified and mixed with other polymers or manufactured on a nanoscale (TAPIA-OROZCO *et al*., 2016; SAXENA; GARG; JANA, 2012). However, the selection of biosorbents depends on several factors such as: cost/benefit ratio, versatility, robustness, adsorption capacity, market availability, final destination after use, adverse reactions, etc. (GONÇALVES, 2013). In the literature we can find works that use many kinds of biomass as biosorbents for water decontamination (GONÇALVES, 2013; BHARATHI and RAMESH, 2013; GRASSI *et al*., 2012; TANYILDIZI, 2011; DENIZ and KARAMAN, 2011; SHARMA; KUMAR; JOSEPH, 2008; SEIXAS; GIMENES; FERNANDES-MACHADO, 2016).

In this work, a new HPLC-FLD method was developed, optimized and validated for the estrogen 17α-ethinylestradiol (EE2) determination in aqueous solution. The

adsorption tests were carried out from a planning of experiments, using the Box-Behnken statistical model in the adjustment of parameters and experimental conditions (pH, adsorbent mass and stirring rate), optimized and validated with the analysis of variance (ANOVA). As will be shown, the advantages of this method are simplicity of operation, cost/benefit, high removal and environmental advantage that fit the requirements of green chemistry.

2. MATERIALS AND METHODS

2.1 Reagents and Solutions

All reagents used for the stock solutions preparation were of high purity with spectroscopic grade or HPLC grade. Ultra-pure deionized water (resistivity of 18.2 MΩ – cm 25°C) was obtained from a purification system Direct-Q (Millipore®). The solvents methanol, acetonitrile (ACN), isopropanol were purchased from Sigma – Aldrich®. For the filtration of water and ACN were used cellulose and nylon ester membranes of 0.45 µm porosity that were acquired from Millipore® and Supelco Analytical, respectively. The 17α-ethinylestradiol (EE2) standard hormone was purchased from Fluka Analytical® with a purity greater than 99%.

The EE2 standard stock solution (100 mg L^{-1}), was prepared in methanol and for the preparation of the EE2 working standard solution (10 mg L^{-1}), an aliquot of 10.0 mL was transferred of the standard stock solution for a volumetric flask of 100.0 mL, completing the volume with methanol. The solutions were stored in a polyethylene bottle at 4°C.

2.2 Instrumentation

All EE2 measurements were performed on an Infinity 1260 high efficiency liquid chromatograph (Agilent Technologies®, Germany), consisting of a quaternary pump, an automatic injector, a column heating module and a molecular fluorescence detector. The chromatographic column used was Eclipse Plus C8 (4.6 mm x 150 mm – 5 µm), also from Agilent Technologies. The chromatographic conditions used were as follows: mobile phase (MP), isocratic mode – ACN:water (3:2), previously filtered on

cellulose and nylon ester membranes of 0.45 µm porosity and deaerated in a digital ultrasonic bath (Modelo SoniClean 2 – Sanders Medical), mobile phase flow – 0.5 mL min^{-1}, injection volume – 10 µL, chromatographic column temperature – 45°C, fluorescence detector wavelength (FLD) – 230 nm (excitation) and 310 nm (emission).

2.3. Analytical Methodology Validation

The analytical methodology used was evaluated from the main merit figures described in the paper by RIBANI *et al*. (2004) and some validation protocols such as INMETRO (2003) and ANVISA (2003). They were: selectivity, linearity, precision, accuracy, detection limit, quantification limit and robustness.

The selectivity was evaluated by comparing the analyte in a sample of ACN:water (3:2) enriched with 100 µg L^{-1} and a sample of only ACN:water (3:2) to measure if any interferent present in the matrix elutes at the same EE2 retention time.

The linearity was verified in the concentration range studied: 5, 8, 10, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350 and 400 µg L^{-1}; all being prepared in ACN:water (3:1).

The accuracy was determined from 10 solutions of concentration 100 µg L^{-1} in ACN:water (3:2). This procedure was performed by two analysts on different days to ensure the repeatability of the method. Being statistically evaluated, test of hypothesis F and t-Student to levels of 95% of reliability. Accuracy was verified by analytical recovery trials due to non-availability of certified reference material of water contaminated with EE2. Thus, solutions in ACN:water (3:2) fortified by the analyte were prepared in triplicates at three levels (120, 100 and 80 µg L^{-1}).

To obtain the limit of detection (LD) and limit of quantification (LQ), the method based on parameters of the analytical regression curve, expressed by Equations $LD=3.3x(S_y/S)$ e $LQ=10x(S_y/S)$, where s_y is the estimate of the standard deviation of the response and S is the angular coefficient of the analytical curve, and by the signal to noise (S/R) ratio, expressed by the S/R ratio equal to 3.0 for the LD and the S/R ratio equal to 10.0 for the LQ.

The robustness was evaluated from the variation of ±5% of the FM composition, the chromatographic column temperature, the FM flow rate and the sample injection volume.

2.4 Adsorption Experiments

2.4.1 Biosorbent

Samples of peanut shells as biosorbent obtained in the municipality of Itajubá, MG, were used in the natural form for the adsorption experiments without any additional chemical or physical treatment.

2.4.2 Adsorption process

The adsorption experiments were performed at room temperature (approximately 20°C) using 150 mL beakers containing the biosorbent and 100 mL of EE2 solution, to which a contraceptive pill was macerated and transferred to a 100 mL volumetric flask, completing the volume with deionized water. The experiments were brought to constant stirring for 6 hours.

Aliquots of 1.0 mL were collected from the solutions at times 1, 2, 3, 4, 5 and 6 hours, including at time zero hours (before shaking) for preliminary testing. All aliquots were filtered through two filtering processes, first by a qualitative filter paper in a glass funnel for removal of larger particles and impurities, and the second by a syringe (10.0 mL - Art Glass) coupled by a filter (Allcrom) of 0.45 µm porosity for the removal of minor particulates. The samples were stored in vial and stored in a refrigerator at 4°C for further HPLC analysis.

Determination of the EE2 removal rate in solution by the biosorbent was obtained according to Equation 1 (CARDOSO *et al*., 2011):

$$\%Removal = 100 \times \frac{C_0 - C_f}{C_0} \quad \text{(Eq. 1)}$$

in which C_0 (µg L^{-1}) is the EE2 initial concentration placed in solution; C_f (µg L^{-1}) is the EE2 concentration remaining in solution after the adsorption process.

The EE2 adsorbed amount by the biosorbent was obtained according to Equation 2 (DOGAN *et al*., 2006):

$$q_t = \frac{V(C_0 - C_t)}{m} \text{ (Eq. 2)}$$

where q_t is the EE2 amount retained in the adsorbent at time t (μg L^{-1}), C_t (μg L^{-1}) is the EE2 concentration remaining in solution at time t, V (L) is the volume of solution and m (g) is the biosorbent mass.

After this study, the adsorption variables were optimized using an experimental design to obtain the highest EE2 removal rate, however, in this study the reaction kinetics, isotherm and thermodynamics were not evaluated, only the efficiency of the removal rate of peanut shells was evaluated.

2.5 Adsorption Experiments optimization

2.5.1. Experiment Planning

To verify the adsorption efficiency of the biosorbent, an experiment planning was carried out Box-Behnken (BBD) model, for three factors (stirring rate - 300, 400 and 500 rpm, pH - 4, 6 and 8, and adsorbent mass - 0.5, 1 and 2 g) and three levels (-1,0 e 1), totaling 15 experiments (Table 1).

Table 1 - Box-Behnken model matrix for three coded factors

Experiment	Agitation	Adsorvent Mass	pH
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

Each independent experiment of Table 1 was performed acordding to **item 2.4.2**, however, before contact of the EE2 solution with the biosorbents, a filtration of the qualitative paper solution was performed to remove the remaining insoluble particles from the pill and solutions of HCl and NaOH 0.1 mol L^{-1} were used to adjust the pH of the solutions from a pHmeter (Model mPA-210 – MS Tecnopon®). The solutions were then measured to 100.0 mL and the measured peanut shells were added to the solution of the hormone where it remained under constant agitation for 6 hours, however, at this stage the aliquots were collected only at time zero and 6 hours.

3. Results and Discussion

3.1. Analytical Methodology Validation

Under optimized conditions, the validation procedure of the analytical method was performed according to the merit figures proposed in the methodology.

The selectivity was confirmed by comparing the chromatograms obtained from the analyte at the concentration of 100 μg L^{-1} (Figure 1-A), and whites of the method: the mobile phase (Figure 1-B) and the peanut shells in aqueous solution for 24h (Figure 1-C), that is, matrices free of the analyte of interest. The blanks were performed under the same chromatographic conditions of EE2.

Figure 1 - Chromatogram of EE2 at the concentration of 100 μg L−1 (A), mobile phase chromatogram (B) and chromatogram of the peanut shells in aqueous solution for 24h (C)

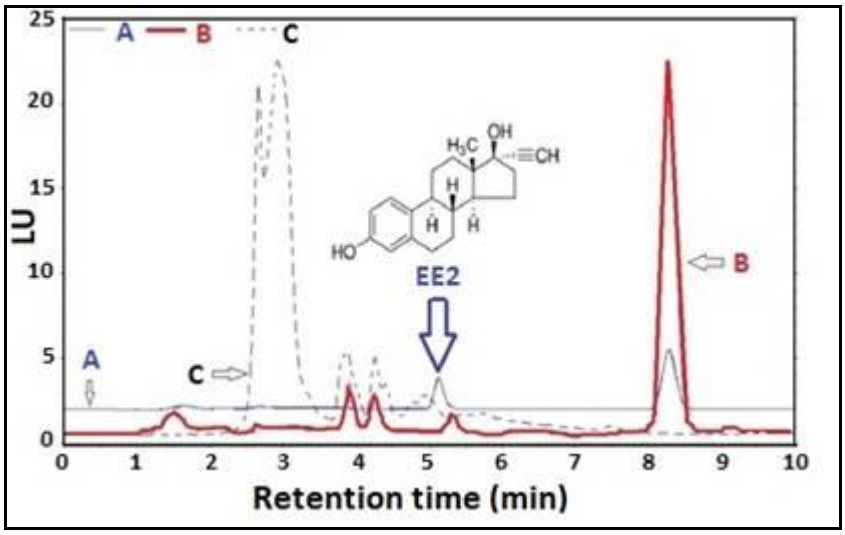


Figure 1 shows that the method was selective for the EE2 hormone, however, the "white" chromatograms presented peaks close to the T_R (retention time) region of 5.20 minutes of the EE2, however, no interferences were eluted in the T_R of the compound, mainly T_R of 4.95 and 5.35 minutes; and they did not interfere with its determination.

HPLC-FLD showed good linearity for EE2 in the concentration range of 5-400 µg L^{-1} com R^2 superior than 0.998; offering a lower uncertainty in the angular and linear coefficients of the estimated equation. In addition to obtaining a correlation coefficient (R) above 0.999; it was possible to choose this estimated line (Y=28,851.85x + 81,843.05) as a mathematical model for the determination of the analyte because the correlation between the area and the concentration of the hormone was very strong. Therefore, with R^2 and R highs, the model can explain more than 99.8% of the variability of the data.

Precision was determined by comparing the analyte concentration values of the real and estimated analytical curve. The method presented good repeatability (n = 10), with a coefficient of variation (CV) of 2.90% for the first analyst and 2.95 for the second analyst. In statistical terms, the F and t-Student hypothesis tests were used at a 5% probability level or at a 95% confidence level. According to the results, the F-test calculated for the hormone was smaller than the tabulated F-test, predicting that, for a 95% confidence level, the standard deviations estimated by the analysts are statistically similar, ie, there are no differences between their results. For the t-Student test, the t calculated for the EE2 was also lower than the t-table, which indicates that for a 95% confidence level, the averages estimated by the analysts are statistically similar, and there was no significant difference between the results.

The accuracy study was performed in triplicate and at three concentration levels (80, 100 and 120 µg L^{-1}) of the analyte. The EE2 recovery rate (RR) was 100.27-110.43%, while the relative error (RE) value was less than 11%, and the CV value was in the range of 0.41-1.00%. Therefore, the method presented good accuracy because the results obtained are below the values described as acceptable for these concentration

levels, which are 40-120% for RR and up to 20% for CV values for the determination of micropollutants (CARDOSO *et al*., 2011; DOGAN *et al*., 2006; BRITO *et al*., 2003).

The LD and LQ for the analyte was 3.40 and 11.32 μg L^{-1}, respectively, based on the parameters of the analytical regression curve, and 5.0 and 10.0 μg L^{-1}, respectively, by the signal/noise ratio method (3:1). Both methodologies presented close LD and LQ and can then be used in all circumstances, however, the method based on analytical curve parameters is statistically more reliable for analytical methods using separation techniques such as chromatographic, since the noise measurement can be subjective, which can directly affect the signal-to-noise ratio and consequently the boundary result (RIBANI *et al*., 2004).

The robustness of the methodology was evaluated by the variation of ±5% of the following chromatographic conditions: composition of the mobile phase, temperature of the chromatographic column, flow of the mobile phase and volume of injection of the sample. It was observed that the robustness of the method for EE2 RR was 98.64-115.47% with an RE of less than 16% and a CV of 0.13-0.93%. The results of the variations of the chromatographic conditions did not present significant changes in the values of recovery and CV stipulated in the literature (ROSAL *et al*., 2010; AQUINO; BRANDT; CHERNICHARO, 2013; DIAGBOYA and DIKIO, 2018), therefore, the method presented to be robust even against the small variations of the chromatographic parameters.

3.2 Optimization of Adsorption Experiments

As described in item 2.5.1, an experiment planning (Box-Behnken Model) was used to optimize the parameters studied. This model allows to evaluate the factors in three levels (low, medium and high) and it does not have points of vertices, that is, when all the levels of the factors are in an extreme, therefore its choice.

The factors stirring rate, pH and adsorbent mass were varied at three levels, resulting in 15 experiments. Statistica 7® software was used to estimate the coefficients (a, b, c, d, e, f, g, h, i, j) of the second-order or quadratic polynomial regression equation (Equation 3).

$$y = a + bx_1 + cx_2 + dx_3 + ex_1^2 + fx_2^2 + gx_3^2 + hx_1x_2 + ix_1x_3 + jx_2x_3 \ (Eq.3)$$

Table 2 expresses the experimental responses (removal rate) and predicted by the Box-Behnken model from the 15 randomized trials.

Table 2 - Experimental and predicted BBD responses for 15 experiments.

Experiment	X_1	X_2	X_3	EE2 Removal (%)	
				Experimental	Predicted
1	-1	-1	0	40.520	37.958
2	1	-1	0	51.872	47.994
3	-1	1	0	48.256	52.680
4	1	1	0	64.627	62.715
5	-1	0	-1	47.516	46.339
6	1	0	-1	55.815	56.374
7	-1	0	1	56.076	50.043
8	1	0	1	60.195	60.078
9	0	-1	-1	43.397	43.542
10	0	1	-1	57.758	53.427
11	0	-1	1	39.500	42.410
12	0	1	1	63.535	61.968
13	0	0	0	53.126	53.209
14	0	0	0	54.319	53.209
15	0	0	0	52.181	53.209

In Table 2, experiments 13, 14 and 15 show the replicates at the central point (variables coded at 0), while the remaining experiments are the midpoints of the 12 edges of the cube (variables coded between -1 and +1). The experimental responses ranged from 39.5 to 64.6% (removal rate of EE2) and by the model were in the range of 37.9 to 62.7%.

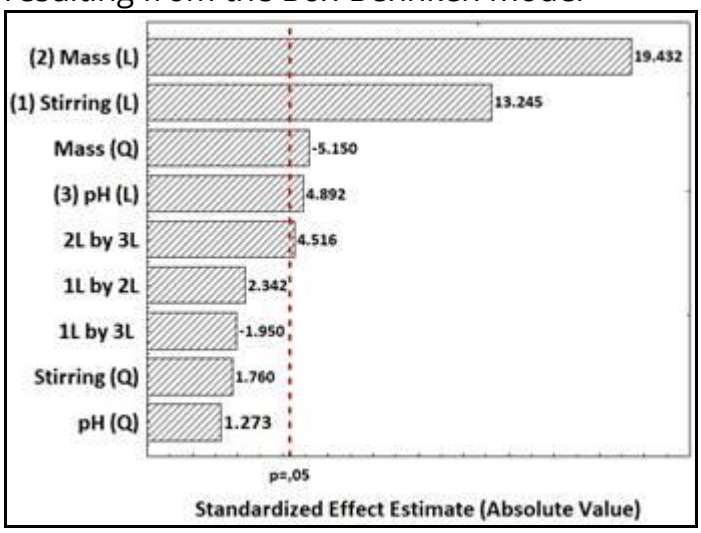
The coefficients determined from the regression analysis of the experimental data are shown in Equation 4.

$$\begin{aligned} Y = {} & 53.208_{\pm 0.619} + 5.017_{\pm 0.379}X_1 + 7.361_{\pm 0.379}X_2 + 1.852_{\pm 0.379}X_3 + 0.982_{\pm 0.558}X_1^2 \\ & - 2.872_{\pm 0.558}X_2^2 + 0.710_{\pm 0.558}X_3^2 + 1.255_{\pm 0.536}X_1X_2 - 1.045_{\pm 0.536}X_1X_3 \\ & + 2.418_{\pm 0.536}X_2X_3 \ (Eq.4) \end{aligned}$$

where Y is the removal efficiency (%) of EE2 by the biosorbent, X_1 is the stirring rate of the process, X_2 is the adsorbent mass and X_3 is the pH in water.

For the adjustment of the regression model proposed in Equation 4, it was necessary to verify the significant parameters. Pareto diagram was used to express the variables that were statistically important at the 95% confidence level (Figure 2), determine the possible significant parameters.

Figure 2 - Pareto diagram as a function of the values of the t-Student test statistic resulting from the Box-Behnken model



By the Pareto diagram, the adsorbent mass, stirring speed, pH, quadratic term of adsorbent mass and the interaction of second (pH/adsorbentn mass) were significant (p = 0.05) and included in the mathematical model, whereas, the parameters of quadratic terms (stirring speed and pH), second degree interactions (stirring speed/adsorbent mass and stirring speed/pH), were not significant and excluded from the model.

Thus, the proposed model for the studied variables was as follows (Equation 5):

$$Y = 53.208_{\pm 0.619} + 5.017_{\pm 0.379}X_1 + 7.361_{\pm 0.379}X_2 + 1.852_{\pm 0.379}X_3 - 2.872_{\pm 0.558}X_2^2 + 2.418_{\pm 0.536}X_2X_3 \quad (Eq.5)$$

Predicted values calculated from the proposed model optimized for the studied variables presented a good agreement with the experimental values, shown in the Table 3.

The model proposed in the optimization of variables was validated by applying the analysis of variance (ANOVA). An ANOVA table was built with the following

parameters: QSR (Quadratic Sum of Regression), QSres (Quadratic sum of the residues), TQS (Total Quadratic Sum), QSep (Pure error quadratic sum), QSfaj (Quadratic sum of the lack of adjustment). Table 3 shows the results of ANOVA in a summarized and succinct way.

Table 3 - ANOVA for fitting the proposed model

Parameter	**SS**	**DF**	**Mean Square (MS)**	**Test F_{calc}**	**F_{tab} (95%)**
Regression	728.71	5	145.74	11.41	3.48
Residue	114.94	9	12.77		
Total	843.65	14			
Pure Error	2.29	2	1.15		
Lack of fit	112.64	7	16.09	14.01	19.35
Explained Variation (R^2)	0.864	R	0.929		
Maximum Explainable Variation (R^2 maximum)	0.997	R maximum	0.998		

From the data obtained in Table 3, it was verified that the value of QSres (114.94) shows the amount of information that is not being modeled by the proposed model (regression coefficients) of which are related to QSep (2.30) and QSfaj (112.64). The QSep is the parameter associated with the replicas at the central point, a value of great importance that gives an idea of what the intrinsic error of the measures is. Whereas, QSfaj is related to the inability of the model to conform to the experimental values obtained.

To verify the quality and reliability of the model, a comparison of the calculated variances (MQ) was performed using the F test. Based on the ratio between the MQR and MQres, the proposed model was statistically significant, that is, the model was able to predict adequately the response since the calculated F of the regression is greater than the F tabulated at a confidence level of 95% for 5 and 9 degrees of freedom, that is, the two variances (MQR and MQres) are statistically different.

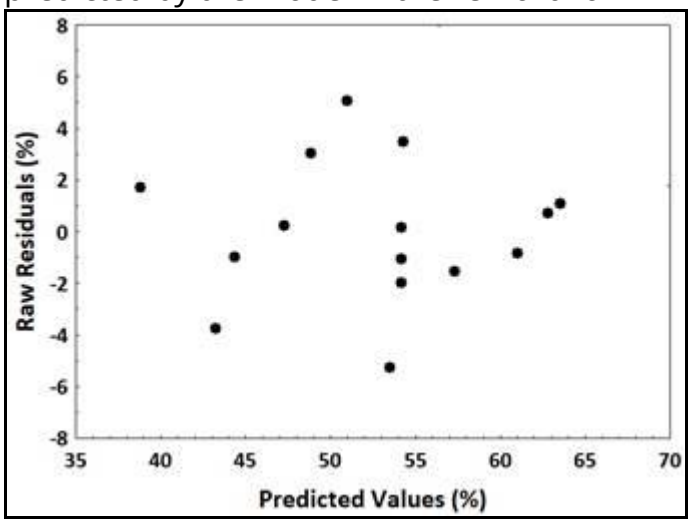
In order to gauge the fit of the model, in this case it was necessary to compare the variances MQfaj and MQep also by the calculated F. OF test is less than the F table

at a 95% confidence level for 7 and 2 degrees of freedom, or better, the two variances are considered statistically equal. A perfect condition for the model, since its inability to fit is confused with the intrinsic error of the replicates of the central point, affirming that it did not present lack of adjustment.

Finally, when evaluating the amount of information SQR can explain in relation to SQT, it was found to be quite satisfactory, since more than 86% of the SQ of the whole system or information is explained by the regression and almost 14% were modeled or explained by the model. Now, for a maximum percentage of information that the model could explain, a SQep equal to zero was required, that is, a standard deviation of the null center point; so the model could explain more than 99% of the SQ of the information.

Figure 3 shows the behavior of the residues left by the adjusted model.

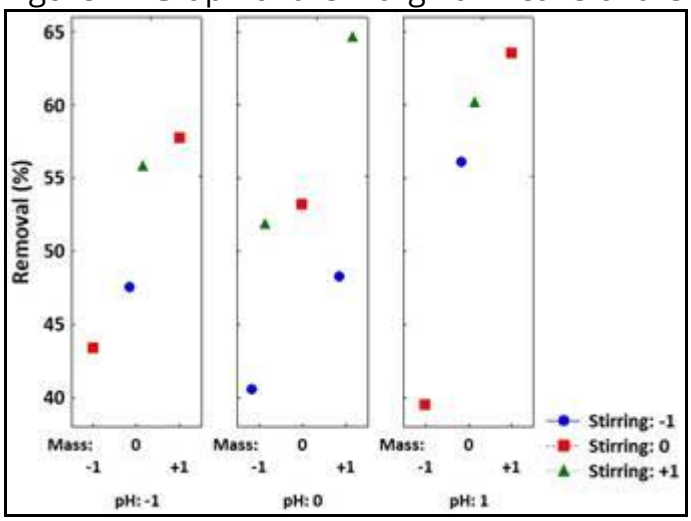
Figure 3 - Values of residues left between the experimental values and those predicted by the model in the removal of EE2



These residues are the differences between the experimental values and those predicted by the model in the removal of EE2. There is a good agreement between the experimental and predicted responses due to the random fluctuation of the residuals around the zero value. Therefore, the mathematical model was appropriate for this experimental domain, reinforced by the results of ANOVA.

The study of the combined effect of the three variables on the response is shown in Figure 4.

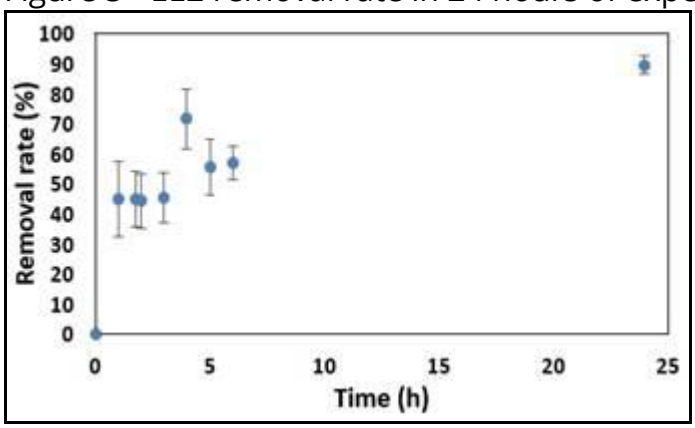
Figure 4 - Graph of the marginal means of the experiments



By Figure 4, it was possible to find the maximum analytical signal in the studied region. The optimum condition found for the EE2 removal process was stirring speed (+1), adsorbent mass (+1) and pH (0).

In view of these observations, a triplicate experiment was carried out with the following optimum conditions: stirring speed at 500 rpm, adsorbent mass of 2 grams and pH = 6 solution. the result obtained in removing EE2 for 24 hours is shown in Figure 5.

Figure 5 - EE2 removal rate in 24 hours of experiment



In preliminary tests, the interval from zero to 6 hours showed a good EE2 removal rate, so the choice of times 0, 1, 1.75, 2, 3, 4, 5 and 6 hours for sample collection, as can be seen in the Figure 5. In zero hour, the initial concentration of EE2 was 263.24 μg L^{-1}, after 6 hours of adsorption, 57% removal of EE2 obtained. The 24 hours time was chosen so that the adsorption equilibrium was reached completely

and after that time, the total removal of EE2 was quite satisfactory, approximately 90%, having a standard deviation (SD) values in the range of 3.08-12.57 and CV values between 3.43-27.88%, considered acceptable (BRITO *et al*., 2003; GONZÁLEZ; HERRADOR; ASUERO, 2010; LIZ *et al*., 2017).

The rate of EE2 removal found in this study was considered significant when compared to other studies involving adsorption of EE2 applying different adsorbent materials. FERNANDES *et al*. (2011), for example, evaluated the removal rate of EE2 in aqueous solutions employing peat decomposed as adsorbent material and obtained a maximum removal of approximately 55% using an initial EE2 concentration of 0.10 mg L^{-1} and varying the adsorbent mass by 50 to 200 mg, and the adsorption process operates under magnetic stirring for 36 hours. On the other hand, CLARA *et al*. (2004) evaluated adsorption of EE2 in activated and inactivated sludge, EE2 in 1.0 mg L^{-1} and the adsorbents at concentrations of 1 to 7.0 g L^{-1}, under magnetic stirring for 24 hours; in which high adsorption affinity to the adsorbent was present, even at very high initial concentrations, from the results obtained from the parameters of partition coefficient or distribution, organic matter and the organic carbon content of the sorbent. While, RUDDER *et al*. (2004) studied the EE2 removal in water by an advanced treatment; using three bioreactors upstream of 2 liters capacity with sand, granulated activated carbon and MnO_2 granules, obtaining 17.3%, 99.8% and 81.7% EE2 removal, respectively, varying the initial EE2 concentration by 5,000 to 20,000 ng L^{-1}. In this way, the good biosorption capacity of the peanut shells studied here is noted.

CONCLUSION

A validated and optimized methodology based on high performance liquid chromatography with fluorescence detection (HPLC-FLD) was developed for the determination of 17α-ethinylestradiol in aqueous samples.

The chromatographic method provides good selectivity, linearity with R^2>0.998 and R>0.999, precision with good repeatability for n = 10 and CV<2.95%, accuracy with a RR <110%, RE <11% and CV <1%, low detection limits (<5 μg L^{-1}) and quantification

(<12 μg L^{-1}), and an excellent robustness with a RR <116%, RE<16% and CV<1%. In addition, this methodology allows the quantification of EE2 in the presence of residues of peanut shells.

The conditions for the adsorption process were optimized using the Box-Benhken model, one of the most used mathematical models for multivariate optimization of experimental conditions. Based on an incomplete factorial design of three-level experiments, it was possible to find the most efficient experimental condition, which was: stirring rate (coded at +1 = 500 rpm), mass of adsorbent (coded at +1 = 2 g) and pH of the solution (coded at 0 = 8). In addition to being validated by analysis of variance (ANOVA), where it did not present a lack of adjustment and obtained a good quality and reliability since more than 86% of the QS of the information is explained by the regression.

Approximately 90% of synthetic hormone removal was achieved within 24 hours of the adsorption process. The biosorbent presented a great and sustainable alternative for adsorption processes.

ACKNOWLEDGMENTS

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal do Nível Superior (CAPES) for a fellowship and Rede Mineira de Química (RQ-MG) and Multicentric Chemistry Postgraduate Program for financial support.

REFERENCES

ANVISA. Agência Nacional de Vigilância Sanitária. **Resolução - RE nº 899/2003 [cited 2016 oct 12]**. Available from:
http:// http://portal.anvisa.gov.br/documents/10181/2718376/RE_899_2003_COMP.pdf.

AQUINO, S. F.; BRANDT, E. M. F.; CHERNICHARO, C. A. L. Removal of pharmaceuticals and endocrine disrupters in sewage treatment plants: literature review. **Engenharia Sanitária Ambiental**. 2013;18(3):187-204.

ARIS, A. Z.; SHAMSUDDIN, A. S.; PRAVEENA, S. M. Occurrence of 17 α-ethynylestradiol (EE2) in the environment and effect on exposed biota: a review. **Environment International**. 2014;69:104-119.

BARREIROS, L. *et al*. Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices — A review. **Microchemical Journal**. 2016;126:243-262.

BHARATHI, K. S.; RAMESH, S. T. Removal of dyes using agricultural waste as low-cost adsorbents: a review. **Applied Water Science**. 2013;3:773-790.

BIRCH, G. F. *et al*. Emerging contaminants (pharmaceutical, personal care products, a food additive and pesticides) in waters of Sidney estuary, Australia. **Marine Pollution Bulletin**. 2015;97(1-2):56-66.

BRAZ, F. S. *et al*. Photocatalytic Degradation of Ibuprofen Using TiO_2 and Ecotoxicological Assessment of Degradation Intermediates against *Daphnia similis*. **Journal of Environmental Protection**. 2014;5:620-626.

BRITO, N. M. *et al*. Validação de métodos analíticos: Estratégia e discussão. **Pesticidas**: Revista de Ecotoxicologia e Meio Ambiente. 2003;13:129-146.

CANDIDO, J. P. *et al*. Ibuprofen removal by heterogeneous photocatalysis and ecotoxicological evaluation of the treated solutions. **Environmental Science and Pollution Research**. 2016;23:19911-19920.

CARDOSO, N. F. *et al*. Application of cupuaçu shell as biosorbent for the removal of textile dyes from aqueous solution. **Journal of Environmental Management**. 2011;92(4):1237-1247.

CHOINA, J. *et al*. Removal of hazardous harmaceutical from water by photocatalytic treatment. **Central European Journal of Chemistry**. 2010;8:1288-1297.

CHOINA, J. *et al*. Photocatalytic decomposition of pharmaceutical ibuprofen pollutions in water over titania catalyst. **Applied Catalysis B**: Environmental. 2013;129:589-598.

CLARA, M. *et al*. Adsorption of bisphenol-A, 17β-estradiole and 17α-ethinylestradiole to sewage sludge. **Chemosphere**. 2004;56(9):843-851.

CLOUZOT, L. *et al*. Membrane bioreactors for 17α-ethinylestradiol removal. **Journal of Membrane Science**. 2010;362(1-2):81-85.

DEBLONDE, T.; COSSU-LEGUILLE, C.; HARTEMANN, P. Emerging pollutants in wastewater: A review of the literature. **International Journal of Hygiene and Environmental Health**. 2011;214(6):442-448.

DENIZ, F.; KARAMAN, S. Removal of an azo-metal complex textile dye from colored aqueous solutions using an agro-residue. **Microchemical Journal**. 2011;99(2):296-302.

DIAGBOYA, P. N. E.; DIKIO, E. D. Silica-based mesoporous materials; emerging designer adsorbents for aqueous pollutants removal and water treatment. **Microporous and Mesoporous Materials**. 2018;266:252-267.

DIAMANTI-KANDARAKIS, E. *et al*. Endocrine-disrupting chemicals: an endocrine society scientific statement. **Endocrine Reviews**. 2009;30(4):293-342.

DOGAN, M. *et al*. Adsorption kinetics of maxilon blue GRL onto sepiolite from aqueous solutions. **Chemical Engineering Journal**. 2006;124:89-101.

FERNANDES, N. A. *et al*. Remoção dos hormônios 17β-estradiol e 17α-etinilestradiol de soluções aquosas empregando Turfa Decomposta como material adsorvente. **Química Nova**. 2011;34(9):1516-1533.

GIULIVO, M. *et al*. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. **Environmental Research**. 2016;151:251-264.

GONÇALVES, J. R. A. C. Descontaminação e monitoramento de águas e solos na região amazônica utilizando materiais adsorventes alternativos, visando a remoção de metais pesados tóxicos e pesticidas. **Inclusão Social**. 2013;6(2):105-113.

GONZÁLEZ, A. G.; HERRADOR, M. A.; ASUERO, A. G. Intra-laboratory assessment of method accuracy (trueness and precision) by using validation standards. **Talanta**. 2010;82(5):1995-1998.

GRASSI, M. *et al*. Removal of emerging contaminants from water and wastewater by adsorption process. **Briefs Green Chemical Sustain**. 2012;45:15-37.

HAN, J. *et al*. Adsorption of ethinylestradiol (EE2) on polyamide 612: Molecular modeling and effects of water chemistry. **Water Research**. 2013;47(7):2273-2284.

IFELEBUEGU, A. O. Removal of steriod hormones by activated carbon adsorption-kinetic and thermodynamic studies. **Journal of Environmental Protection**. 2012;3(6):469-475.

INMETRO. Instituto Nacional de Metrologia, Normalização e Qualidade Industrial. **Orientações sobre validação de métodos de ensaios químicos DOQ-CGCRE-008**. Revisão: 01-Março/2003 [cited 2016 oct 12]. Available from: http://www.inmetro.gov.br/Sidoq/Arquivos/CGCRE/DOQ/DOQ-CGCRE-8_01.pdf.

JURADO, A. *et al*. Emerging organic contaminants in groundwater in Spain: A review of sources, recente occurrence and fate in a European context. **Science of the Total Environmental**. 2012;440:82-94.

LEONARD, J. A. *et al*. Extending the toxicity-testing paradigm for freshwater mussels: Assessing chronic reproductive effects of the synthetic estrogen 17α-ethinylestradiol on the unionid mussel Elliptio complanata. **Comparative Biochemistry and Physiology C**. 2017;191:14-25.

LIU, Z. H.; KANJO, Y.; MIZUTANI, S. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment – physical means, biodegradation, and chemical advanced oxidation: a review. **Science of the Total Environmental**. 2009;407(2):731-748.

LIZ, M. V. *et al*. Sensitive strogens determination in wastewater samples by HPLC and fluorescence detection. **Journal of the Brazilian Chemical Society**. 2017;28(8):1453-1460.

LUO, Y. *et al*. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the Total Environmental. 2014;473-474:619-641.

MEI, S. *et al*. Determination of trace bisphenol A in complex samples using selective molecularly imprinted solid-phase extraction coupled with capillary electrophoresis. Microchemical Journal. 2011;98(1):150-155.

NOHYNEK, G. J. *et al*. Endocrine disruption: fact or urban legend?. **Toxicology Letters**. 2013;223(3):295-305.

PAPAEVANGELOU, V. A. *et al*. Removal of endocrine disrupting chemicals in HSF and VF pilot-scale constructed wetlands. **Chemical Engineering Journal**. 2016;294:146-156.

PATIÑO, Y. *et al*. Adsorption of emerging pollutants on functionalized multiwall carbon Nanotubes. **Chemosphere**. 2015;136:174-180.

RIBANI, M. *et al*. Validação em métodos cromatográficos e eletroforéticos. **Química Nova**. 2004;27(5):771-780.

ROSAL, R. *et al*. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. **Water Research**. 2010;44(2):578-588.

RUDDER, J. *et al*. Advanced water treatment with manganese oxide for the removal of 17a-ethynylestradiol (EE2). **Water Research**. 2004;38(1):184-192.

SAXENA, S.; GARG, S.; JANA, A. K. Synthesis of cellulose based polymers for sorption of azo dyes from aqueous solution. **Journal of Environmental Research and Development**. 2012;6:424-431.

SEIXAS, F. L.; GIMENES, M. L.; FERNANDES-MACHADO, N. R. C. Tratamento da vinhaça por adsorção em carvão de bagaço de cana-de-açúcar. **Química Nova**. 2016;39(2):172-179.

SHARMA, R. K.; KUMAR, A.; JOSEPH, P. E. Removal of atrazine from water by low cost adsorbents derived from agricultural and industrial wastes. **Bulletin of Environmental Contamination and Toxicology**. 2008;80(5):461-464.

SILVA, C. G. A.; COLLINS, C. H. Aplicações de cromatografia líquida de alta eficiência para o estudo de poluentes orgânicos emergentes. **Química Nova**. 2011;34(4):665-676.

SILVA, C. P.; OTERO, M.; ESTEVES, V. Processes for the elimination of estrogenic steroid hormones from water: a review. **Environmental Pollution**. 2012;165:38-58.

SODRÉ, F. F. *et al*. Ocorrência de interferentes endócrinos e produtos farmacêuticos em águas superficiais da região de Campinas (SP, Brasil). **Journal of the Brazilian Society of Ecotoxicology**. 2007;2(2):187-196.

TANYILDIZI, M. S. Modeling of adsorption isotherms and kinetics of reactive dye from aqueous solution by peanut hull. **Chemical Engineering Journal**. 2011;168(3):1234-1240.

TAPIA-OROZCO, N. *et al*. Removal strategies for endocrine disrupting chemicals using cellulose-based materials as adsorbents: A review. **Journal of Environmental Chemical Engineering**. 2016;4(3):3122-3142.