OTIMIZAÇÃO E VALIDAÇÃO DO MÉTODO PARA A DETERMINAÇÃO DE PSICOATIVOS EM ÁGUA DE RIOS POR HPLC-DAD-FLD.

OPTIMIZATION AND VALIDATION OF SPE-HPLC-DAD-FLD METHOD FOR PSYCHOACTIVE DRUGS IN ENVIRONMENTAL MATRIX

SIMÃO, L. André¹; BÖGER, Beatriz^{1*}; DOMINGUES, M. Jéssica¹; PERALTA-ZAMORA, G. Patrício²; GOMES, C. Eliane¹; WAGNER, Ricardo¹.

¹ Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Paraná (UFPR). Av. Pref. Lothário Meissner, 632 - Jardim Botânico, Curitiba-PR, Brasil. (fone: +55 41 3360-4098)

² Pós-Graduação em Química, Departamento Química, Universidade Federal do Paraná (UFPR). Rua Coronel Francisco Heráclito dos Santos, s/nº - Prédio da Engenharia Química - Jardim das Américas, Curitiba-PR, Brasil. (fone: +55 41 3361-3006)

* Autor correspondente
e-mail: beatrizboger@gmail.com

Received 23 January 2018; received in revised form 24 January 2018; accepted 30 January 2018

ABSTRACT

Recently scientific community has turned its attention to the organic micropollutants such as pharmaceuticals and hormones, because the ecotoxicological effects of chronic exposure are uncertain. These substances are neither completely degraded nor monitored in sewage treatment, which makes the aquatic environment the main disseminant, due to the usual discharge of domestic and industrial sewage. Therefore, the current work aims to develop quantitative method using HPLC-UV-DAD-FLD detection in order to determine the psychoactive drugs fluoxetine and haloperidol, potential environmental contaminants in surface water. The solid phase extraction was performed to preconcentrate of the analytes of interest in aqueous matrices, in which it was evaluated univariate for reconstitution volume and pH, and via factorial planning 2³ for the other variables. The best conditions were obtained by conditioning with 5 mL of acetonitrile and 5 mL of water pH 4.0, 100 mL of sample (pH 4.0), eluting with 5 mL of acetonitrile and reconstitution with 1 mL of water. The recovery rate was 73.7% and 87.2% for haloperidol and fluoxetine, respectively. The method was developed in real samples fortified with standards and real samples from three different points of the Belém river in Curitiba-PR.

Palavras-chave: *fluoxetine, haloperidol, validation, water.*

RESUMO

Recentemente a comunidade científica voltou sua atenção para os micropoluentes orgânicos, como produtos farmacêuticos e hormônios, pois os efeitos ecotoxicológicos da exposição crônica são incertos. Essas substâncias não são completamente degradadas e nem monitoradas no tratamento de esgoto, o que torna o meio aquático o principal disseminador, devido à descarga usual de esgotos domésticos e industriais. Portanto, o trabalho atual visa desenvolver um método quantitativo usando a detecção HPLC-UV-DAD-FLD para determinar os fármacos psicoativos de fluoxetina e haloperidol, potenciais contaminantes ambientais nas águas superficiais. Foi realizada extração em fase sólida para pré-concentrar os analitos de interesse em matrizes aquosas, na qual foi avaliada univariadamente para volume de reconstituição e pH, e via planejamento fatorial 2³ para as demais variáveis. As melhores condições foram obtidas por condicionamento com 5 mL de acetonitrila e 5 mL de água pH 4,0; 100 mL de amostra (pH 4,0), eluindo com 5 mL de acetonitrila e reconstituição com 1 mL de água. A taxa de recuperação foi de 73,7% e 87,2% para haloperidol e fluoxetina, respectivamente. Este método foi aplicado em amostras reais fortificadas com padrões e amostras reais provenientes de três pontos diferentes do rio Belém em Curitiba-PR.

Keywords: *fluoxetina; haloperidol; validação; água.*

INTRODUCTION

Some years ago the contamination of water reached only two forms of contamination: microbiological and industrial waste. Today, industrialized nations are facing another type of pollution - the contamination of the water with pharmaceuticals, cosmetics and personal hygiene which are called emerging contaminants. In Brazil, the pharmaceutical industry had its expansion in 1940 somewhat; however the big environmental concern is not necessarily the volume of production of a drug, but its persistence in the environment. Biological activity such as high toxicity and high potential for effects on biological functions like reproduction, and existing data on their biodegradation and destination in the environment are still insufficient. The pollution of water by drugs is being recognized as an environmental concern, because the pollution caused by them is a complex phenomenon. The consumption of drugs is substantial. In most developed countries, the production and use of drugs are increasing annually. It is interesting to note that the consumption of the drugs used is sometimes as high as that of pesticides and other organic compounds (Kot-Wasik et al., 2007).

These contaminants have been in the environment for a long time, but their importance and presence are now being elucidated. The increasing interest in the determination of these contaminants is due to the fact that they are not part of legislation that regulates water quality and therefore may be candidates for future legislation depending on the research on their toxicity and potential effects on the environment and health (Suchara et al., 2007).

After administration, the drugs are absorbed, distributed, partially biotransformed and finally excreted in its original form either as dissolved metabolites in feces or urine. This process occurs both in humans and in animals in which veterinary drugs are used. Other forms of drug contamination are through waste from the pharmaceutical industry, aquaculture, the disposal of unused or overdue drugs and hospitals (Kot-Wasik et al. 2007; Tambosi et al. 2010).

Drugs are not degraded at sewage treatment plants (STP) and are released into water places resulting in contamination of rivers, lakes, estuaries and rarely into groundwater. The sludge generated in the purification process is launched into agricultural fields and can cause

soil contamination, surface water and percolation to groundwater. The same can occur with substances of veterinary use. In addition, veterinary drugs can enter aquatic systems through the application of organic fertilizer in fields and subsequent levitation or also by direct application in aquaculture (Ruhoy and Daughton, 2008; Glassmeyer, 2009).

It is known that the analytical techniques for quantification of drugs are well established and registered in government agencies, for example, ANVISA. The official methodologies are used both to control the quality of the raw material and to control the finished product in the market (Brasil, 2003). However, quantification of drugs in aqueous environment at trace levels (in order to get environmental water samples) is a newer field.

Fluoxetine, an antidepressant, has already been detected in sewage and surface waters in many countries at concentrations ranging from 0.013 to 5.85 $\mu\text{g L}^{-1}$ (Metcalf et al., 2003; Deblonde et al., 2011). Studies have also shown that this drug has ecotoxicological effects on fish and crustaceans, as well as being a potential endocrine disrupter (Borrely et al., 2012; Kimura et al., 2003). Haloperidol, an antipsychotic, is also prevalent in sewage treatment plants at relatively low concentrations, and the literature has noted that its waste is also toxic to aquatic organisms (Petkovska and Dimitrovska, 2008; Trautwein and Kummerer, 2012).

Based on what was discussed above, the main objective of this study was to develop and validate the analytical method for the determination of Fluoxetine (FX) and Haloperidol (HL) in water samples and to optimize solid phase extraction conditions in order to increase the sensitivity of the method.

MATERIALS AND METHODS

1 Reagents

The standards used were fluoxetine, 99.9% pure (USP) and haloperidol, 99.6% pure (USP). Solvents used were methanol and acetonitrile HPLC grade (99.99%, J.T. Baker, Philipsburg, NJ, USA) and ultra pure water (18.2 $\text{M}\Omega\text{ cm}$, Millipore-Simplicity UV, Bedford, MA, USA). All solutions were prepared using previously calibrated and clean analytical glassware.

2 Equipment

Chromatographic determinations were performed on a high-performance liquid chromatography (Agilent 1260 Infinity) equipped with DAD (Diode Array Detector) and fluorescence detector, automatic injector, C18 XBridge column from Water-BEH (2.1 x 100 mm, with a particle size of 5 μm) and OpenLabEZChrom Elite software for data acquisition and processing. The mobile phases were degassed in an ultrasound Ultracleaner 1400 (Unique).

3 Solid phase extraction

The extraction of psychoactive drugs was performed using Phenomenex polymer cartridges (Strata X, 200 mg, 3 mL) using a Waters manifold system (WAT200608) with vacuum pump (Tecnal TE-0581). The parameters were evaluated (the sample pH, concentration and volume of sample elution solvent volume and reconstitution in water) to assess the best recovery samples. For pH evaluation, which was varied in 4.00, 6.50 and 8.00, triplicates were performed, conditioning the cartridges with 5.00 mL of methanol and 5.00 mL of water at the respective pHs, 200.0 mL of sample fortified with 1.15 $\mu\text{g L}^{-1}$ FX and 1.18 $\mu\text{g L}^{-1}$ HL. Elution was performed with 5.00 mL of methanol and the eluates were dried under heating at 40 $^{\circ}\text{C}$ and N_2 flow and then reconstituted with 1.00 mL of ultrapure water. The evaluation of the concentration, sample volume and elution solvent were performed via factorial design 2^3 with triplicate of the center point at optimized pH, equal to 4.00. The values of the planning levels were defined based on the literature (Amaral, 2014). All experiments were conducted SPE with a flow rate of 3.0 mL min^{-1} .

After optimization of pH, concentration, sample volume and elution solvent was evaluated univariate in duplicate the volume of reconstitution in ultra pure water, being evaluated 0.250, 0.50 and 1.00 mL. The reconstituted samples were analyzed in the respective chromatographic methods described in section 2.4.

4 Development and validation of the method

The optimization of the chromatographic conditions of analysis as well as the analytical curves were established based on information described in the literature (Brasil, 2010). From this information an isocratic elution mode was applied for the simultaneous determination of

Fluoxetine (FX) and Haloperidol (HL) with mobile phase consisting of an acetonitrile:phosphate buffer 0.01 mol L^{-1} pH 3.50 (75:25, v:v) mixture. Column temperature was maintained at 30 $^{\circ}\text{C}$. The wavelengths monitored were FX for fluorescence with excitation at 230 nm and emission at 290 nm and HL absorption at 254 nm DAD.

4.1 Preparation of the analytical curve and linearity

After determining the ideal chromatographic conditions for FX and HL analysis, the analytical curve was built. The concentration ranges for the construction of analytical curves using HPLC-DAD-FLD were evaluated in triplicate between 10.0 and 1500 $\mu\text{g L}^{-1}$ for FX and HL.

4.2 Limit of detection and quantification

The LOD and LOQ can be calculated in three different ways: visual method, signal to noise ratio method and the method based on parameters of the calibration curve (Ribani et al., 2004). In this study the limits for determination method were based on the parameters of the analytical curve.

4.3 Precision

It was evaluated through the repeatability and intermediate precision, where in the first the degree of agreement between the results of successive measurements within a short period of time was evaluated by the same analyst, same instrumentation and same method. The second is the agreement between the results from the same laboratory, but obtained on different days, with analysts or different equipment (Brazil, 2003; ICH, 2005). The determinations were performed in nine solutions of the sample in concentrations of three levels of concentrations (high, medium and low) found within the linear range used under the same working conditions. Relative standard deviation was considered acceptable, according to the ANVISA resolution (Brasil, 2003).

4.4 Accuracy

The accuracy of an analytical method is the proximity of the results obtained by the method under study to the true value, using an experimental procedure for the same sample for repeated times (Brasil, 2003). The determination of accuracy of the method was based on the

degree of recovery of FX and HL expressed as a percentage of fortified solutions at three concentration levels, considering the linear range of the calibration curve, if performing at least five determinations per concentration. ANVISA also recommends that the recovery percentage be close to 100% for drugs, however, it also allows for smaller values since the recovery is exact and accurate. For environmental samples there are no protocols that guide the recovery value, so the recovery values were oriented in the literature.

5 Real sample analysis

Samples of natural water were collected in the source, 5 Km from the source and at the outfall of the Belém River, in the city of Curitiba – PR (Figure 1), in 1.0 L amber bottles, previously conditioned with 1 mL of methanol in order to promote a previous disinfection and thus avoid possible biological degradation. These collections were carried out on four different dates. After collection, the samples were filtered (GF3 55 mm filter and 0.45 μm pore), fortified ($100\text{ }\mu\text{g L}^{-1}$ with standard solution of the two analytes) and acidified with 2% hydrochloric acid 2mol L^{-1} to pH 4.

Figure 1. Location of the watershed of the river Belém.

The analysis were performed in triplicates as well as the control analysis (blank tests). Samples were then preconcentrated using the solid phase extraction method, which was performed using Strata X polymer cartridges (200 mg, 3mL, Phenomenex) with the help of a Waters manifold vacuum pump system. The amount of the analytes in each real sample analyzed was estimated using the constructed analytical curve.

RESULTS AND DISCUSSION

1 Solid phase extraction

For the optimization of pH, its effect was evaluated in the recovery of all the analytes. For pH 6.5 and 8.0 it was not possible to observe elution bands for the analytes. Only in pH 4.00 it was possible to observe the elution of the analytes, with good recovery rates of 71.2% HL and 95.8% FX. The pH reduction in the extraction process, as well as in the chromatography, guarantees the prevalence of an analyte species in the environment, which should preferably be ionized, in order to improve the interaction of the analytes with the solid phase of the cartridges and thus raise the rate recovery. For optimization of the elution solvent, sample volume and analyte concentration variables a planning 2^3 was used as already mentioned. In order to evaluate which assay presented the best conditions for preconcentration of the analytes and the influence of the factors studied in the response, the calculation of the effects of the planning was performed. As all third-order effects were found to be significant in the preconcentration of the analytes studied, these factors cannot be evaluated.

Based on these considerations, the chosen working conditions were $1.15\text{ }\mu\text{g L}^{-1}$ FX concentration, and $1.18\text{ }\mu\text{g L}^{-1}$ HL, 100.0 mL sample volume and acetonitrile as the elution solvent, keeping the pH equal to 4.00 as previously optimized.

In the optimization of the reconstitution volume, the volumes of 1.00, 0.500 and 0.250 mL of ultrapure water were univariate evaluated. The recovery was more significant for reconstitution with 1.00 mL for all analytes, despite reducing the preconcentration factor due to higher dilution, and it was 87.2% FX and 73.7% HL. Reconstitution with 0.500 mL and 0.250 mL provided lower recoveries for the two analytes (66.0% and 52.3% for FX and 42.0% and 28.0% for HL, respectively).

Therefore the best conditions for preconcentration, considering the best recoveries for the analytes were obtained by conditioning with 5.00 mL of acetonitrile and 5.00 mL of water pH 4.00, flow 3 mL min^{-1} , 100 mL of sample at pH 4.00, elution with 5.00 mL of acetonitrile and reconstitution with 1.00 mL of water.

2 Optimization of analysis conditions by HPLC-DAD-FLD

In order to optimize the chromatographic separation, different volumes of injection, mobile phase and flow ratios were tested, reaching 15

μL , mobile acetonitrile phase: 0.01 mol L^{-1} phosphate buffer pH 3.50 (75:25, v:v) and 0.4 mL min^{-1} flow rate for the monitoring of FX and HL. All analyzes were performed at 30°C .

Figure 2 shows the chromatograms obtained in the external standardization of FX and HL under the best monitoring conditions by FLD and UV-DAD, with fluorescence detection λ_{exc} at 230 nm and λ_{emi} at 290 nm and UV-DAD at 254 nm. There was a good separation of the chromatographic peaks FX and HL, and the retention times were 6.98 minutes for the HL by DAD at 254 nm and 24.98 minutes for the FX fluorescence at 230/290 nm which is in agreement with the literature (Brasil, 2010).

Figure 2. Chromatogram for FX (A) and HL (B) standard obtained by isocratic elution (acetonitrile: 0.01 mol L^{-1} phosphate buffer pH 3.5, 75:25, v:v) and detection by fluorescence at 230/290 nm and DAD at 254 nm.

The FX retention time was high because in the test with another drug (carbamazepine) these two analytes co-eluted in mobile phase compositions in which the acetonitrile ratio was greater than 25%, causing large distortions of the monitored fluoxetine elution band by fluorescence at 230/290 nm due to the interaction between these two drugs, taking into account that the CP presents no fluorescence.

3 Linearity of the analytical curve

Figure 3 is the evaluation of the linearity of the methods, from the analytical curves for each

component. Analyzing the merit parameters for the respective standard curves (Table 1) it is possible to realize a good linearity for all the compounds evaluated, they showed a correlation coefficient (R) exceeding 0.990 in the evaluated concentration ranges as recommended by ANVISA (Brazil, 2003; INMETRO, 2010).

Figure 3. Analytical curves for FX and HL.

The sensitivity of the method was evaluated through the angular coefficients of the line equations obtained.

Table 1. Parameters of regression of the analytical curves obtained by HPLC for FX using 230/290 nm FLD and for HL using UV-DAD 254 nm and main parameters of merit.

Parameters	FX	HL
Linear Coefficient (a)	-7151.43	-1798.00
Angular Coefficient (b)	1263.77	102.19
Correlation Coefficient (R)	0.9974	0.9991
LOD ($\mu\text{g L}^{-1}$)	0.27	0.14
LOQ ($\mu\text{g L}^{-1}$)	0.81	0.42

4 Limits of detection and quantification

According to ANVISA (Brasil, 2003) the limits of detection and quantification can be calculated based on parameters of the analytical curve, the ratio between the estimate of the standard deviation of the linear coefficient and the slope of the regression curve multiplied by 3, in the case of LOD and by 10 when LOQ.

Applying this criterion, LOD obtained was of 0.27 and 0.14; and LOQ 0.81 and 0.42 for FX and HL respectively. The values of LOD and LOQ obtained for the analytes are close to that described in the literature (Khetan et al., 2007, Pavlović et al., 2007).

INMETRO (2010) determines a maximum coefficient of variation of 20%. ANVISA

recommends that the coefficients of variation should not exceed 5% for the determination of drugs in medicines (BRASIL 2003). However, for the determination of these compounds in more complex matrices, such as serum, blood or plasma, a value of up to 15% is accepted. Thus, according to the determined values, the method used is accurate, since all compounds studied have a relative standard deviation of less than 14%.

5 Accuracy tests

In this work, the accuracy aimed to evaluate the SPE efficiency in water samples. In the recovery studies, water samples were fortified at three concentration levels (50, 500, 1000 $\mu\text{g L}^{-1}$) in triplicate. After application of the solid phase extraction process, the samples were evaluated in the chromatographic method mentioned. The average of the lowest recovery rates obtained were $87.2\% \pm 9.3\%$ and 73.7% for FX $\pm 8.1\%$ for LH.

6 Robustness

The robustness parameters evaluated (flow, temperature, pH of the phosphate buffer solution and different batches of manufacturers) showed that the method can withstand small and deliberate variations without significant changes in residue recovery and chromatographic efficiency.

The values of the relative standard deviations of the areas found for both FX and HL in the working concentration are within the variation of the method itself. Therefore, it can be affirmed that the changes provoked did not interfere in the recovery of the method.

The variable that most interferes with the retention of analytes is the flow, but this variation is almost in the same proportion for all analytes, with no significant loss in chromatographic efficiency, with a small change in efficiency due to the change in the number of theoretical plates, by the change in retention time. The other variables (temperature, pH of the phosphate buffer solution and different batches of manufacturers) showed no significant changes in the chromatograms.

7 Recovery analysis in real sample

From the results obtained by pre-

concentration, followed by HPLC analysis in the methods, it was possible to evaluate recoveries of analytes in real samples fortified. After applying the extraction process in solid phase the quantity of analyte in each sample was estimated using real-chromatographic analytical curves. For the fortified and analyzed river water samples recovery rates and relative standard deviations were $86.50 \pm 14.2\%$ for FX and $55.25 \pm 4.59\%$ for HL.

8 Analysis of the Belém River - Curitiba \ PR

After the development of the methodology and validation of the method, a monitoring of the occurrence of these compounds was carried out in the river and in the previously mentioned sites in the city of Curitiba-PR. A total of 16 samples were analyzed, in each day of collection it was sampled three different aqueous matrices (headspring, 5Km from the source and). Samples were collected in the year 2015 in four different dates: the first on May 12, the second on June 1, the third on the 6th of July and the fourth on October 20. All samples were filtered after the collection, concentrated and analyzed according to the procedures described above. In no sample the analytes in question were present in concentrations measurable by the proposed method.

CONCLUSIONS

After analysis of obtained results the developed method presented a good linearity for the determination of the analytes ($R > 0.997$), and good limits of detection (LOD) and quantification (LOQ). In addition, solid phase extraction of the analytes of interest in aqueous matrices was feasible, obtaining good recoveries for most analytes, ranging from 73.7% to 87.2% for HL and FX. Currently it is growing concern about pollution, particularly that of water. With the increase of the consumption of controlled medicines, it is necessary to develop techniques that can quantify this type of pollution in the water. The determination of drug residues, which are widely used today in the therapy of diseases such as depression, anxiety, psychoses among others, these aqueous matrices show the importance of such monitoring and show the increasing concern about the quality of water that is treated before being returned to the rivers.

Taking into account that the Belém river is strictly urban and much waste is dumped in this river, it has not been observed presence of these

drugs in its waters yet, but due to the importance to the prevention and surveillance of health risks of these substances and from the ecotoxicological point of view, it is essential to verify their occurrences in surface aquatic systems, even if it is not yet contemplated in Brazilian environmental legislation. Further research is needed in this area and other future studies will contribute to the establishment of legal parameters of drug residues in aqueous matrices.

ACKNOWLEDGMENTS

We would gratefully like to thank the Federal University of Paraná (UFPR), and the financial support of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Araucária and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their support.

REFERENCES

1. AMARAL, B.; ARAUJO, J.A.; ZAMORA, P.G.P.; NAGATA, N. Simultaneous determination of atrazine and metabolites (dia and dea) in natural water by multivariate electronic spectroscopy. **Microchemical Journal**, Brazil, v. 117, p. 262-267, 2014.
2. BORRELY, S.I.; CAMINADA, S.M.L.; PONEZI, A.N.; SANTOS, D.R.; SILVA, V.H.O. Contaminação das águas por resíduos de medicamentos: ênfase ao cloridrato de fluoxetina. **O Mundo da Saúde**, São Paulo, v. 36, n. 4, p. 556-563, 2012.
3. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA – ANVISA (Brasil). Resolução nº 899, de 29 maio de 2003. Determina a publicação do Guia para Validação de Métodos Analíticos e Bioanalíticos. **Diário Oficial da União** 02 fev. de 2003. <http://portal.anvisa.gov.br/documents/33836/349509/Consolidado%2Bde%2Bnormas%2BCO BIO.pdf/3122249b-48cb-47aa-be78-76f3129a62ba>. Accessed 30 march 2017.
4. FARMACOPEIA BRASILEIRA (Brasil), volume 2 / Agência Nacional de Vigilância Sanitária. Brasília: ANVISA, p. 546, 2010. http://www.anvisa.gov.br/hotsite/cd_farmacopeia/pdf/volume2.pdf. Accessed 30 march 2017.
5. DEBLONDE, T.; COSSU-LEGUILLE, C.; HARTEMANN, P. Emerging pollutants in wastewater: A review of the literature. **International Journal of Hygiene and Environmental Health**, France, v. 214, p. 442-448, 2011.
6. GLASSMEYER, S. T.; HINCHEY, E. K.; BOEHME, S.; DAUGHTON, C. G.; RUHOY, I. S.; CONERLY, O.; DANIELS, R. L.; LAUER, L.; MCCARTHY, M.; NETTESHEIM, T. G.; SYKES, K.; THOMPSON, V. G. Disposal practices for unwanted residential medications in the United States. **Environment International**, United States, v. 35, n. 3, p. 566-72, 2009.
7. INMETRO (Instituto Nacional de Metrologia, Normalização e Qualidade Industrial). DOQ-CGCRE-008. **Orientações sobre validação de métodos analíticos**, INMETRO. Rio de Janeiro. 2010. http://www.inmetro.gov.br/Sidoq/Arquivos/Cgcre/DOQ/DOQ-Cgcre-8_04.pdf. Accessed 30 march 2017.
8. INTERNATIONAL CONFERENCE ON HARMONISATION (ICH). **Validation of Analytical Procedures: Text and Methodology Q2(R1)**. November 2005. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf. Accessed 30 march 2017.
9. KHETAN, S. K.; COLLINS, T. J. Human Pharmaceuticals in the Aquatic Environment: A Challenge to Green Chemistry. **Chemical Reviews**, Pennsylvania, v. 107, p. 2319-2364, 2007.
10. KIMURA, K.; AMY, G.; DREWES, J.; HEBERER, T.; KIM, T.; WATANABE, Y. Rejection of organic micropollutants (disinfection by-products, endocrine disrupting compounds, and pharmaceutically active compounds) by NF/RO membranes. **Journal of Membrane Science**, v. 227, n. 1-2, p. 113-121, 2003.
11. KOT-WASIK, A.; DĘBSKA, J.; NAMIEŚNIK, J. Analytical techniques in studies of the environmental fate of pharmaceuticals and personal-care products. **Trends in Analytical Chemistry**, Poland, v. 26, p. 557-568, 2007.
12. METCALFE, C. D.; MIAO, X.-S.; KOENIG,

B. G.; STRUGER, J. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower great lakes, Canada. **Environmental Toxicology and Chemistry**, v. 22, n. 12, p. 2881-2889, 2003.

13. PAVLOVIĆ, D. M.; BABIĆ, S.; HORVAT, A. J.; KAŠTELAN-MACAN, M. Sample preparation in analysis of pharmaceuticals. **Trends in Analytical Chemistry**, Croatia, v. 26, p. 1062-1075, 2007.

14. PETKOVSKA, R.; DIMITROVSKA, A. Use of chemometrics for development and validation of an RP-HPLC method for simultaneous determination of haloperidol and related compounds. **Acta Pharmaceutica**, v. 58, p. 243–256, 2008.

15. RIBANI, M.; BOTTOLI, C. B. G.; COLLINS, C. H.; JARDIM, I.; MELO, L. F. C. Validation for chromatographic and eletrophoretic methods. **Química Nova**, São Paulo, v. 27, n. 5, p. 771-780, 2004.

16. RUHOY, I. S.; DAUGHTON, C. G. Beyond the medicine cabinet: An analysis of where and why medications accumulate. **Environment International**, Las Vegas, v. 34, n. 9, p. 1157-69, 2008.

17. SUCHARA, E. A.; BUDZIAK, D.; MARTENDAL, E.; COSTA, L. L.; CARASEK, E. A combination of statistical and analytical evaluation methods as a new optimization strategy for the quantification of pharmaceutical residues in sewage effluent. **Analytica Chimica Acta**, Brazil, v. 613, p. 169–176, 2008.

18. TAMBOSI, J. L. Recent research data on the removal of pharmaceuticals from sewage treatment plants (STP). **Química Nova**, São Paulo, v. 33, p. 1-10, 2010.

19. TRAUTWEIN, C.; KÜMMERER, K. Degradation of the tricyclic antipsychotic drug chlorpromazine under environmental conditions, identification of its main aquatic biotic and abiotic transformation products by LC-MSn and their effects on environmental bacteria. **Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences**, Germany, v. 889-890, p. 24-38, 2012.