Occurrence of β-blocker and antihypertensive in water supply reservoir, Guarapiranga dam, São Paulo, SP, Brazil

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Abstract
This study is the first evaluation of the pharmaceuticals classes of β-blocker and antihypertensive occurrence, in the waters of the second more important producer system of potable water supply of the Metropolitan Region of São Paulo (SP, Brazil), Guarapiranga Dam. The pharmaceuticals studied were atenolol, enalapril, losartan, propranolol, and valsartan. These drugs are excreted after ingestion in its active and metabolized form and due the clandestine occupation they can be discharged directly into rivers and reservoirs used for potable water supply. The quantification of pharmaceuticals was developed using a validated method of solid phase extraction (SPE) followed by reversed phase liquid chromatography tandem mass spectrometry (LC-MS/MS). The results showed two water reservoir areas with higher pharmaceuticals concentrations. The higher concentrations of atenolol, losartan, valsartan and enalapril were 177, 114, 49 and 20 ng L^{-1}, respectively. The propranolol was not measured in any sampling spot. Atenolol and losartan were quantified in 100% of samples collected, while valsartan and enalapril were quantified in 57 % and 7 %, respectively. It is hoped that information from this study may alert competent authorities to implement improvements to the wastewater collection system and prevent discharges to aquatic environments.

Key Words: antihypertensive, β-blocker, pharmaceuticals, public supply reservoir, SPE-LC-MS/MS

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OCORRÊNCIA DE β-BLOQUEADOR E ANTI-HIPERTENSIVO NAS ÁGUAS DE RESERVATÓRIO DE ABASTECIMENTO PÚBLICO, REPRESA GUARAPIRANGA, SÃO PAULO, SP, BRASIL

Resumo

Este estudo apresenta a primeira avaliação da ocorrência de produtos farmacêuticos, pertencentes às classes terapêuticas de β-bloqueador e anti-hipertensivo, nas águas do segundo mais importante sistema produtor de água potável da Região Metropolitana de São Paulo (SP, Brasil), a Represa Guarapiranga. Os fármacos estudados foram o atenolol, enalapril, losaratana, propranolol e valsartana. Esses fármacos, após a ingestão, são excretados na sua forma ativa ou metabolizada, e muitas vezes devido à presença de habitações clandestinas são lançados diretamente nos rios e reservatórios de abastecimento público. Para a quantificação destes produtos farmacêuticos em água foi desenvolvido e validado o método de extração em fase sólida (SPE) seguido por cromatografia líquida de fase reversa acoplada a espectrometria de massas em sequência (LC-MS/MS). Os resultados obtidos mostraram que o reservatório apresenta duas áreas com concentração elevada dos fármacos. As concentrações mais elevadas dos fármacos atenolol, losartana, valsartana e enalapril foram de 177, 114, 49 e 20 ng L^{-1}, respectivamente. O propranolol não foi detectado em nenhum local de coleta e o atenolol e losartana foram quantificados em 100% das amostras coletadas. Observou-se ainda que os fármacos valsartana e enalapril apresentaram valores acima do limite de quantificação em 57% e 7% das amostras coletadas, respectivamente. Os resultados deste estudo demostram a necessidade de implementar melhorias no sistema de coleta de esgoto evitando descarte em ambientes aquáticos.

Palavras chaves: anti-hipertensivo, β-bloqueador, produtos farmacêuticos, reservatório de abastecimento público, SPE-LC-MS/MS.

Introduction

The medicine and the pharmaceutical industry advancement in the last two decades, provide the development of new pharmaceuticals for the treatment and prevention of diseases in human and veterinary medicine (Wang et al, 2011). Such process also increases the presence of a wide variety of these pharmaceuticals into aquatic environments. Actually, these are some of the great concerns among the scientific community, potable water producers and governments all over the world (Gros et al., 2009; Loss et al., 2010; Wang et al., 2011).

Published studies indicate the pharmaceutical contamination as the result of different pollution sources from human activities such as industrial wastewater, domestic sewage discharge and the improper dispose solid waste including expired medicine. The Sewage Treatment Plants (STP) with conventional treatment process not efficient to remove these classes of contaminants (Gross et al., 2009; Loss et al., 2010; Valcárcel et al., 2011). Among pharmaceuticals usually detected in the aquatic environment are the β-blockers and antihypertensives used for the treatment of cardiovascular hypertension, arrhythmia and heart failure (Gross et al., 2008). The hypertension is a chronic disease with a higher incidence among people over 60 years old. The medicine development and the sanitary condition in urban areas have increasing the life span and the population is aging, which means the increase production and use of pharmaceuticals for chronic diseases. In Brazil, among the people with chronic disease, 53.3% are hypertensive (IBGE, 2010).

The present study aims to evaluate the occurrence of β-blockers and antihypertensives in water reservoir Guarapiranga using solid phase extraction (SPE) followed by LC-MS/MS. The Guarapiranga is an urban water reservoir considered the second largest system responsible to produce drinking water for 4 million people of the Metropolitan Region of São Paulo (MPSP), State of Sao Paulo, Brazil, (Whately and Cunha, 2006, Fontana *et al*., 2014).

Methodology

Study area

The Guarapiranga water reservoir after conventional water treatment supplies with drinkable water 95% of the resident population in the districts of Santo Amaro, Morumbi, Pinheiros, Butantã and 5% in the region of Taboão da

Serra, with flow rate greater than 12 m^3/s (Whately and Cunha, 2006). Table 1 describes the geographic coordinates (GPS) of the choosing 14 sampling sites. The GU000-14 is the site nearest of the water collection to the Water Treatment Plant-Alto da Boa Vista (WTP-ABV). Water samples were collected in September 2012, using Van Dorn bottles and the samples were transferred to amber glass vials for conditioning and transport. At laboratory, samples were filtered under vacuum with a 0.45 µm membrane and stored in a refrigerator. The collected samples were submitted to SPE to concentrate and purify the organic compounds, it was performed within 7 days of collection.

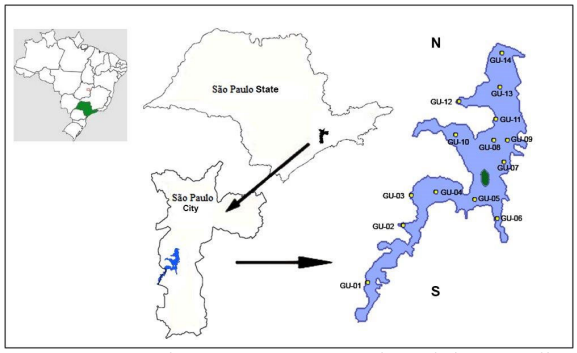

Figure 1. Guarapiranga water reservoir and the sampling sites.

Table 1. Geographical coordinate of the sampling sites in Guarapiranga Dam.

Sampling	Sampling site (Fig 1)	Geographical Coordinate	
GU000-01	GU-01	23°46'49.6"S	46°47'22.0"W
GU000-02	GU-02	23°45'29.5"S	46°46'18.7"W
GU000-03	GU-03	23°44'52.2"S	46°46'13.6"W
GU106-04	GU-04	23°44'44.6"S	46°45'25.8"W
GU000-05	GU-05	23°44'57.5"S	46°44'24.2"W
GU107-06	GU-06	23°45'01.2 S	46°43'61.5"W
GU108-07	GU-07	23°43'64.7"S	46°43'42.3"W
GU000-08	GU-08	23°42'96.9"S	46°43'61.2"W
GU109-09	GU-09	23°43'04.6"S	46°43'34.0"W
GU105-10	GU-10	23°42'89.9"S	46°44'68.7"W
GU104-11	GU-11	23°42'53.4"S	46°43'44.9"W
GU103-12	GU-12	23°41'88.5"S	46°44'67.3"W
GU102-13	GU-13	23°41'58.0"S	46°43'57.3"W
*GU000-14	GU-14	23°40'78.2 S	46°43'55.9"W

** Sampling sites of the Water Supply Company of the State of Sao Paulo (SABESP).*

Chemical and reagent

In this study, acetonitrile (ACN), methanol (MeOH) and isopropanol analytical grade LC/MS were purchased from JT Baker. The formic acid and ammonium acetate analytical grade PA from Sigma-Aldrich were used. The ultrapure water with low conductance was purified on "EasyPure" Barnstead system and the analytical standards of atenolol, enalapril, propranolol, losartan and valsartan were purchased from Sigma-Aldrich. The atenolol, enalapril, losartan, and valsartan was dissolved in MeOH/water (1:1, v/v), the propranolol in ACN / water (1:1, v/v) to concentration of 200 mg L^{-1}. The analytical curves were obtained with 8 solutions as a result of standards mixture in the concentration in the range of 5 to 50 mg L^{-1} for enalapril, propranolol and valsartan and in the range of 5 to 200 mg L^{-1} for atenolol and losartan.

Solid phase extraction

The solid phase extraction (SPE) was performed based on the study of Barrek et al. (2009) with some modifications. The StrataX SPE cartridge (Phenomenex, 200 mg, 3 mL) was used. The cartridge was preconditioned with 3 mL of ACN, 3 mL of MeOH/Isopropanol/ACN (1:1:1, v/v /v) and 4 mL of water. The sample volume of 250 mL was loaded onto the cartridge at a flow rate of 5 mL min^{-1}. The washing procedure was carried out with 3 mL of water, followed by the passage of air for 10 minutes. The elution was carried out with 3 mL of MeOH/Isopropanol/ACN (1:1:1, v/v/v) and 3 mL of ACN. The obtained extracts were evaporated until dryness under a gentle air stream at 40 °C, reconstituted with 0.25 mL of ACN/H_2O (5:95, v/v) and analyzed by LC-ESI-MS/MS.

LC-MS/MS

The chromatographic separations of pharmaceuticals presents in 10µL of the extracted samples were performed on reversed phase column Eclipse XDB-C18, 4.6x50 mm, 1.8 µm, Agilent (USA) using the Agilent HPLC (Agilent Technologies, Waldbronn, AL) compound for quaternary pump, degasser (model 1260), column oven at 25 °C and automatic injector (model 1290). A binary mobile phase gradient with water and 0.1% formic acid (solvent A) and ACN (solvent B) was used at a flow rate of 0.7 mL min^{-1}. The elution started with 95% of solvent A, decreasing linearly to 5% in 5 minutes. This condition was held for 1 minute and returned linearly to the initial condition within 2 minutes, which was held for 1 minute before the next injection.

The detection and quantification of pharmaceuticals were performed by a 3200 QTRAP (quadrupole-ion trap) MS/MS system (Sciex, Toronto, CA) using a turbo ion spray source positive mode. The optimization of compound dependent MS parameters for each product ion was performed by flow injection analysis of

1.0 mg L^{-1} individual standard solutions with flow rate of 10 ml min^{-1}. The parameters dependent of ionization source adopted in this study were: ESI voltage of 5500 V, source temperature 650 °C, nebulizer gas at 45 a.u., heating gas to 65 a.u. and interface gas 20 a.u. The quantification of pharmaceuticals by LC-MS/MS was performed in "Multiple Reaction Monitoring" (MRM), where mass analyzers Q1 and Q3 select the precursor and product ions, respectively, defining a specific transition m/z. In this method the two most intense product ions were chosen, the first more intense used in the quantification and the second for confirmation. The data acquisition was performed with Analyst ® 1.5.2 software.

Validation of the analytical procedure

Statistical validation of the analytical method was performed by evaluating the selectivity, linearity, detection limit (MDL) and quantitation limit (MQL), precision and recovery following the guidelines of INMETRO (2003).The evaluation of the validation parameters was performed with the calibration curve prepared by the standard mixture solution in the matrix. The standard mixture solution to the analytical curve was prepared by extractions of 4 aliquots of 250 mL of the GU106-04 sample, and the obtained extract were divided into nine equal parts, one for determining the matrix blank and the others to the standard solutions addition on increasing levels of 8 concentrations. In previous assays, the water sample GU106-04 showed less contaminants. All solutions were evaporated to dryness, reconstituted with 0.25 mL of ACN: H_2O (5:95, v/v) and analyzed by LC-ESI-MS/MS 7 times.

Results and discussion

Performance of analytical procedure

The selectivity of the analytical method was confirmed accordingly with the guidelines of the European Commission (2002). The quantitative analyzes were performed in MRM mode and two more intense transitions were monitoring for each compound. Other criteria suggested by López-Serna et al. (2011) have also been followed: the relative intensity of the ion and retention time of the product should not vary by more than 20% (in the present study was ≤ 6%) and 2% (this study was ≤ 0.7%), respectively, compared with the reference solution. The linearity was evaluated according with determination coefficient (r^2) of the linear regression obtained with 5 experimental points and each point result of at least 3 analyzes. According INMETRO (2003), the method can be considered linear if correlation coefficient is greater than 0.90. In this study, for all compounds r^2 was greater than 0.99.

The MDL value was calculated using the standard deviation of 7 measurements from the point of lowest concentration of the calibration curve in matrix, multiplied by the value of 2.447 (Student's t, for 6 degrees of freedom and 95% confidence). The MDLs obtained for atenolol, enalapril, losartan, propranolol and valsartan were 0.6, 3.0, 0.7, 1.3 and 1.4 ng L^{-1}, respectively. The MQL value was calculated by the average of 7 measurements from the point of lowest concentration of the calibration curve in matrix, with addition of 5 times the value of the standard deviation of the average. The MQLs obtained for atenolol, enalapril, losartan, propranolol and valsartan were 6.9, 9.2, 6.1, 7.2 and 7.7 ng L^{-1}, respectively.

The precision of the method was evaluated by the coefficient of variation (CV%). In this study, the CVs obtained for atenolol, enalapril, losartan, valsartan and propranolol were 4, 12, 2, 5 and 3%, respectively, according Horvwitz et al. (2006) the analytical method can be considered accurate (%CV <20%). The recovery method was calculated by analysis of GU106-04 samples spiked with standard mixture solution on the average concentration of the working range for each compound. The spiked samples were subjected to the proposed procedure and the determined concentration was compared to the expected value. The recovery values obtained between 77% and 96% were acceptable for the analysis of residue (Ribani et al. 2004).

Application of the methodology

The developed analytical procedure was used to analyze 14 sampling sites collected from the water reservoir Guarapiranga. Considering the 5 drugs analyzed, only propranolol was not detected in any water sample. The atenolol and losartan were quantified in 100% of collected samples, while valsartan and enalapril were quantified in 57% and 7% of the collected samples, respectively.

The obtained results were summarized in Table 2. The values were obtained using the mean concentration from 3 extractions on triplicate analysis followed by estimates of the expanded uncertainties, which were calculated according to the guidelines of EURACHEM (2012).

Table 2. Concentrations of atenolol, enalapril, losartan, propranolol and valsartan obtained for the water samples from 14 sites collected of the Guarapiranga Dam, in September 2012.

Samplig site	Atenolol Conc. (ng L^{-1})	Enalapril Conc. (ng L^{-1})	Losartan Conc. (ng L^{-1})	Propranolol Conc. (ng L^{-1})	Valsartan Conc. (ng L^{-1})
G000_01	23 ± 1	< LDM	7 ± 1	<LDM	<LQM
G000_02	17 ± 1	< LDM	10 ± 1	<LDM	<LQM
G000_03	22 ± 1	< LDM	22 ± 3	<LDM	<LQM
G106_04	22±1	< LDM	34 ± 4	<LDM	<LQM
G000_05	22 ± 1	< LDM	29 ± 4	<LDM	<LQM
G107_06	44 ± 2	< LDM	62 ± 8	<LDM	14 ± 1
G108_07	52 ± 2	< LDM	59 ± 7	<LDM	16 ± 1
G000_08	18 ± 1	< LDM	34 ± 4	<LDM	10.1 ± 0.8
G109_09	23 ± 1	< LDM	46 ± 6	<LDM	11.8 ± 0.7
G105_10	17 ± 1	< LDM	24 ± 3	<LDM	<LQM
G104_11	14 ± 1	< LDM	53 ± 7	<LDM	11 ± 1
G103_12	177 ± 5	20±3	114 ± 8	<LQM	49 ± 2
G102_13	15 ± 1	< LDM	53 ± 7	<LDM	12 ± 1
G000_14	11.0 ± 0.5	< LDM	45 ± 5	<LDM	11 ± 1

MDL=method detection limit; MQL=method quantitation limit

At Figure 2 are showed two sampling sites with higher pharmaceuticals concentration (GU107-06 to GU108-07 and GU103-12). The concentration of atenolol (177 ng L^{-1}), enalapril (20 ng L^{-1}), losartan (114 ng L^{-1}) and valsartan (49 ng L^{-1}) were higher in GU103 -12 (Figure 2), which corresponds to the site of dense human activity and high urban growing. Despite the GU103-12 be closer to the water collection for WTP-ABV, in GU000-14, the atenolol concentration was the smallest and concentration of losartan and valsartan were in the intermediate range of the analyzed samples. This demonstrates that there was an important dilution factor from one sampling site to another due the water dam circulation resulting in pharmaceuticals dilution. The intermediate concentrations of these pharmaceuticals were obtained for GU107-06 and GU108-07. The highest pharmaceuticals concentrations in this area are probably due to the proximity of Parelheiros River, a tributary of Guarapiranga, which receives the waters of the Billings Dam also environmental impacted (Almeida and Weber, 2005, Wathely and Cunha, 2006).

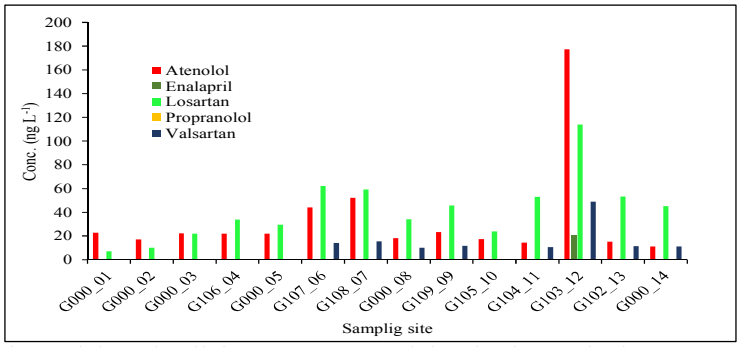

Figure 2. Occurrence of atenolol, enalapril, losartan, propranolol and valsartan in the waters of Guarapiranga Dam.

Atenolol was also detected in the aquatic environment from various countries. In Billings Dam, Brazil, Almeida and Weber (2005) found a concentration range of 0.9 to 16.4 ng L^{-1}; in Han River, Korea, Yoon (2010) detected the concentration of 2.4 to 150 ng L^{-1} and in Ebro River, Spain, López-Serna, et al. (2011) it was found an average of 1031 ng L^{-1}. Therefore, the values obtained in this study in the range of 11 to 177 ng L^{-1}) are in agreement with those related in the literature for the surface water samples. In this study, enalapril was quantified only on GU103-12 sampling site (20 ng L^{-1}) with a concentration higher than reported by Gros et al. (2009) in the waters of the Ebro River (<0.3 ng L^{-1}). However, it is far below the values reported by the same author, for the affluent (293-

1268 ng L^{-1}) and the effluent (0.7-1041 ng L^{-1}) of Sewage Treatment Plant (STP). These results demonstrate the poor or no elimination of enalapril by the STP.

Gros et al. (2009) also reported the results obtained, in urban STP located in Catalonia, Spain, for atenolol in influent (104-1160 ng L^{-1}) and in effluent (120-1310 ng L^{-1}) and of propranolol in influent (1-130 ng L^{-1}) and in effluent (2 - 130 ng L^{-1}). These results demonstrate that the atenolol, enalapril, and propranolol are poor or even not removed during the conventional wastewater treatment, considering the similar concentrations were detected in both, the influent and effluent wastewater.

Conclusion

The application of validated SPE-LC-MS/MS method allowed the assessment and distribution of β-blockers and anti-hypertensive pharmaceuticals in water reservoir Guarapiranga of the MRSP. Two sampling sites showed higher concentrations of the analyzed pharmaceuticals, one is located in dense urban area and other is located near the Parelheiros River mouth, which receives indirectly the water of the Billings Dam, also environmental impacted. The occurrence of pharmaceuticals in aquatic environments will become increasingly frequent and at higher concentrations, since the world's population is aging which means increased consumption of antihypertensive and b-blockers combined with its low removal of existing STP. In this study, the presence of pharmaceuticals in Guarapiranga is concern due the clandestine occupation and precarious sanitary sanitation, with direct disposal of sewage in waters used for potable water supply after conventional water treatment. This information may assist the public policies to improve the sewage system collection and treatment, in order to avoid direct release of such pharmaceuticals into water reservoirs. Additionally, give grants to government authorities to encourage more studies on urban area occupancy to save and protect the water reserve areas with green restoration mostly for those located in dense urban areas. In addition, some improvements in water treatment process are need accordingly with this new pharmaceuticals contaminants control. Also for to set the maximum allowable limits for pharmaceuticals on drinkable water, which not covered yet by environmental enforcement law.

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