

Multivariate Optimization of Analytical Methodology and a First Attempt to an Environmental Risk Assessment of β -Blockers in Hospital Wastewater

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O presente estudo avalia, preliminarmente, o risco da exposição ambiental a β -bloqueadores em efluente de um hospital universitário (Hospital Universitário de Santa Maria (HUSM), Rio Grande do Sul). Propranolol mostrou o maior quociente de risco (0,56). A ocorrência de β -bloqueadores foi avaliada com auxílio de metodologia de SPE-HPLC-FLD (extração por fase sólida-cromatografia líquida de alta eficiência com detecção por fluorescência) otimizando-se multivariadamente as variáveis pH da amostra, pH da água e a razão entre metanol:acetonitrila:ácido fórmico. As concentrações médias de atenolol, metoprolol e propranolol medidas durante o período de uma semana foram de $2,45 \pm 1,14$, $4,67 \pm 1,63$ e $0,70 \pm 0,88 \mu\text{g L}^{-1}$ no efluente do pronto atendimento, $0,95 \pm 0,68$, $0,70 \pm 0,33$ e $0,315 \pm 0,62 \mu\text{g L}^{-1}$ no efluente geral do hospital e $1,26 \pm 0,47$, $1,27 \pm 0,35$ e $0,56 \pm 0,47 \mu\text{g L}^{-1}$ no córrego receptor, respectivamente. Propranolol mostrou razão MEC/PNEC > 1 (MEC: concentração ambiental mensurada e PNEC: concentração predita que não causa efeito), exigindo maior atenção em termos de toxicidade. A ocorrência de β -bloqueadores e o risco ambiental associado demonstram a necessidade de um sistema mais eficiente de tratamento do efluente hospitalar.

This preliminary study evaluated an assessment of the risks arising from environmental exposure to β -blockers from wastewater of an university hospital (University Hospital of Santa Maria (HUSM), Rio Grande do Sul State, Brazil). Propranolol showed the highest risk quotient (0.56). The occurrence of β -blockers was evaluated using an analytical procedure SPE-HPLC-FLD (solid phase extraction-high performance liquid chromatography with fluorescence detection), optimizing the variables sample pH, water pH, and methanol:acetonitrile:formic acid ratio of the elution stage. The average concentrations of atenolol, metoprolol and propranolol for a sampling period of one week were 2.45 ± 1.14 , 4.67 ± 1.63 and $0.70 \pm 0.88 \mu\text{g L}^{-1}$ in the 'Emergence' sewage; 0.95 ± 0.68 , 0.70 ± 0.33 and $0.315 \pm 0.62 \mu\text{g L}^{-1}$ in the 'HUSM general' sewage and 1.26 ± 0.47 , 1.27 ± 0.35 and $0.56 \pm 0.47 \mu\text{g L}^{-1}$ in the 'Receiving waters', the receptor stream of the effluents, respectively. Propranolol showed an MEC/PNEC ratio > 1, and thus requires more attention in terms of toxicity. The occurrence of β -blockers and the associated environmental risks demonstrate the need of a more efficient treatment system for the hospital wastewater.

Keywords: risk assessment, hospital wastewater, β -blockers, response surface methodology, SPE-HPLC-FLD

Introduction

In the last decade, the presence of pharmaceuticals in the environment has become a subject of increasing concern.¹⁻⁴ Studies have shown that these substances occur in water and wastewater at levels ranging from ng L^{-1} up to $\mu\text{g L}^{-1}$. Hospital wastewater (HWW) is one of the main sources of environmental pollution by pharmaceuticals.

The determination of pharmaceutical levels in wastewater from Brazilian hospitals is almost non-existent and, owing to the inadequacy of sewage treatment systems, this subject is of great environmental importance.

β -Blockers belong to a group of drugs used in the therapy of cardiovascular diseases such as hypertension and cardiac arrhythmias, and are widely used both in hospitals and domestically.¹ Atenolol (ATE), metoprolol (MET) and propranolol (PRO) are the most widely used.⁵

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As a result, β -blockers and metabolites have been found in trace levels in sewage and surface waters.⁶⁻¹³

The use of β -blockers varies considerably among different countries, e.g., in United Kingdom the use *per capita* achieves up to 3.2 g *per year*, and, in Switzerland and Finland, 1.1 and 0.1 g *per year*, respectively.⁵ No data referring to the use and occurrence of β -blockers in Brazil was found in the literature.

There are many methods for the determination of β -blocker levels in the environment, such as liquid chromatography tandem mass spectrometry (LC-MS), which has a number of advantages when compared to the HPLC-UV (high performance liquid chromatography with UV detection) and HPLC-FLD (high performance liquid chromatography with fluorescence detection) methods.^{12,14}

Hospital wastewater is a complex matrix that interferes with the analysis of micro-contaminants. The problem of eliminating interference has to be addressed, and the most commonly applied technique for clean-up and enrichment is solid phase extraction (SPE).¹⁵

Chemometrics is a tool that aims to develop analytical methodologies, which reduces the number of experiments, as well as the consumption of time and material. The multivariate method based on response surface methodology (RSM) includes, among other advantages, the ability to carry out screening on a large number of variables.¹⁶⁻¹⁸ The widely used sequential univariate method of optimization involves conducting a large number of experiments and is unable to establish multiple interactions between the studied parameters. Thus, systematic planning and optimization are essential.^{16,19,20}

There have been few studies on the presence of pharmaceuticals and metabolites in wastewater from Brazilian hospitals and, until now, no satisfactory risk assessment has been conducted.^{21,22}

Apart from their more general use in Brazil, to the best of our knowledge, β -blockers have not been investigated until now. Although current in developed countries,^{23,24} the employment of risk assessment tools to evaluate the widespread disposal of drugs in hospital effluents in developing countries is also a new breakthrough.

The aim of this study was thus to evaluate the occurrence of β -blockers in HWW and to assess their inherent risk to the regional environment by optimizing the SPE methodology in regard to enrichment and clean-up.

Experimental

Reagents

Atenolol (CAS Nr. 29122-68-7), metoprolol tartrate (CAS Nr. 56392-17-7) and propranolol hydrochloride

(CAS Nr. 318-98-9) (all > 99% purity) were purchased from Sigma-Aldrich (Deisenhofen, Germany). The organic solvents (acetonitrile, methanol and hexane) were of HPLC grade and purchased from JT Baker (Mexico City, Mexico). Formic acid, sodium formiate and the inorganic reagents used were of analytical grade. The buffers and aqueous solutions were prepared in ultrapure water (Direct-Q 3 UV ultrapure, 18.2 M Ω cm).

Hospital wastewater sampling

The University Hospital of Santa Maria (HUSM) at the Federal University of Santa Maria (UFSM) is the most important health institution in the central region of Rio Grande do Sul State, Brazil. It provides 302 beds and covers approximately 112 towns and cities (around 3 million inhabitants).

The three main points of the HUSM sewage treatment system (Figure 1) that were chosen to evaluate the β -blockers emission were designated as (a) 'Emergence', which comprises the emergency and south side of the HUSM, (b) 'HUSM general', the main part of the HWW current, and (c) 'Receiving waters', the receptor stream of the effluents. The average flow of HUSM wastewater (HWW) is about 190 m³ *per day* and sewage treatment is undertaken through a septic tank-anaerobic filter.²¹

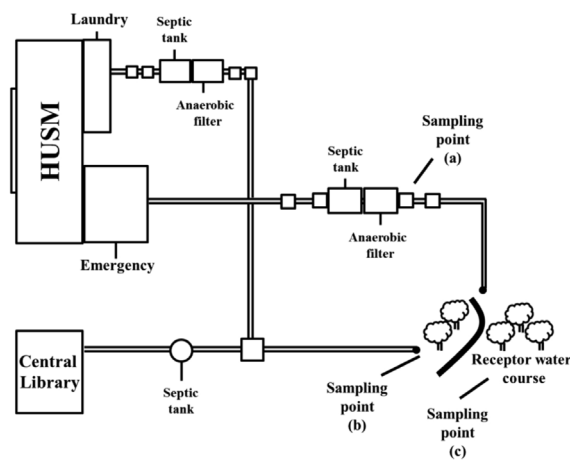


Figure 1. Scheme of the HUSM wastewater treatment system.

The composite samples were collected every hour, starting at 8 a.m. and finishing at 8 p.m., for a period of one week (from February 14th to 20th, 2011). The collected samples were filtered (cellulose, 26 μ m) and stored in amber flasks at 4-8 °C in the dark. The preservation was carried out according to the recommendations of Vanderford *et al.*²⁵

Before the SPE stage, the samples were filtered again, this time through a 0.45 μ m cellulose nitrate filter (Sartorius, Göttingen, Germany) and the pH was adjusted

to the optimized conditions. The analysis was performed in quadruplicate, in spiked and non-spiked samples.

Sample preparation and solid phase extraction procedure

ATE, MET and PRO were accurately weighted and diluted to make a 100 mg L⁻¹ stock solution with ultrapure water (resistivity: 18.2 MΩ cm) and stored at 4-8 °C. All the solutions for optimization and analytical determination were prepared from these stock solutions.

SPE was performed with a Chromabond® Manifold vacuum system and Chromabond® C18 ec cartridge (45 µm, 60 Å), 200 mg:3 mL from Macherey-Nagel.

The general SPE procedure is shown in Figure 2. By means of SPE optimization, the concentration of β-blockers was set to 200 µg L⁻¹, which was high enough to evaluate possible losses in the loading and washing stages.

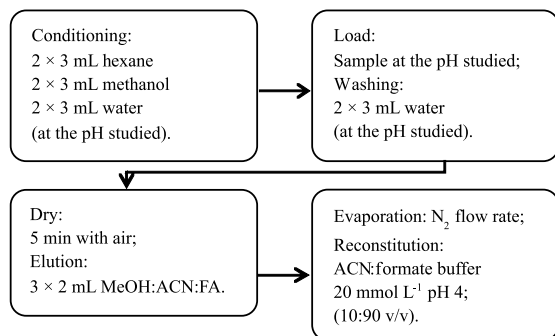


Figure 2. General SPE procedure adopted.

Chromatographic conditions

The HPLC-DAD-FLD device employed was a Shimadzu Prominence equipped with a quaternary bomb (LC-20AT), a degasser (DGU-20A5), an auto-sampler (SIL-20AC), a diode array detector (DAD, SPD-M20A), a fluorescence detector (FLD, RF-10AXL) and interface communication (CBM-20A). The chromatograms were acquired and analyzed with the aid of LC-Solution software (Shimadzu, Tokyo, Japan). The flow rate used was 1 mL min⁻¹ and the injection volume was 50 µL. Detection was carried out in FLD with excitation (λ_{ex}) and emission (λ_{em}) wavelengths of 230 and 312 nm, respectively. DAD was used qualitatively to evaluate the UV-Vis spectra and the peak purity of the analytes.

Chromatographic separation was carried out using a reversed phase column C18 ec (RP18 CC 125-4 mm Nucleodur 100-5) and a guard column (RP18 CC 8-4 mm Nucleodur 100-5), both from Macherey-Nagel (Düren, Germany).

The determination of ATE, MET and PRO in aqueous solution and in HWW were performed using as the mobile

phase (A) formate buffer 0.02 mol L⁻¹ pH 4 and (B) acetonitrile as follows: 0-1 min isocratic flow with 5% of B, 1-4 min linear gradient flow of 5-40% B; 4-10 min isocratic flow at 40% B; 10-11 min linear gradient flow of 40-10% B; 11-15 min isocratic flow of 5% B (equilibration phase).

Response surface methodology (RSM)

Once the main parameters had been established, the most suitable experimental design was defined. Response surface methodology is a valuable chemometric tool because it makes use of a central composite design (CCD), which includes five levels of variables ($-\alpha$, -1 , 0 , 1 , $+\alpha$). Further details can be found in the Supplementary Information (SI) section.

The CCD independent variables chosen for the SPE procedure were: (i) water pH for the conditioning-washing stages, which interferes through adsorption of the analytes,²⁶ (ii) sample pH, which confers polarity (or neutrality) to the analyte and (iii) the methanol ratio of the ternary mixture methanol-acetonitrile-formic acid (MeOH:ACN:FA) in the elution step. The chosen dependent parameter, recovery (Rec), was determined by equation 1:

$$\text{Rec (\%)} = \frac{B_A}{B_B} 100 \quad (1)$$

where B_A is the analytical peak area measured after the SPE procedure and B_B is the analytical peak area before SPE. All experiments were performed in triplicate and in random order.

Results and Discussion

Some figures of merit of the validated HPLC-DAD-FLD method can be observed in Table 1.²⁷ The linear curve (in 8 levels), together with the evaluation of the correlation coefficient (r^2), was also matched by an analysis of variance (ANOVA) with statistical significance of 0.05 (see SI section).²⁸ The F -test (Fischer) value was higher than the critical value, while the lack of fit test was below the critical value. The limits of detection (LOD) and quantification (LOQ) were estimated according to equations 2 and 3:²⁹

$$\text{LOD} = C_s \frac{3}{S/N} \quad (2)$$

$$\text{LOD} = C_s \frac{10}{S/N} \quad (3)$$

where C_s is the amount or concentration of injected analyte and the S/N is the signal:noise ratio.²⁹

Table 1. Figures of merit of the HPLC-FLD method (n = 6)

			Atenolol	Metoprolol	Propranolol
Linearity ^a / (µg L ⁻¹)			10-300	10-300	10-300
r ²			0.9994	0.9993	0.9994
LOD / (µg L ⁻¹)			1.79	2.20	2.38
LOQ / (µg L ⁻¹)			5.96	7.35	7.94
Intra-day	10 µg L ⁻¹	Precision (RSD / %)	6.59	6.62	10.09
		Accuracy (RE / %)	3.81	4.45	6.40
	100 µg L ⁻¹	Precision (RSD / %)	4.24	3.94	2.33
		Accuracy (RE / %)	2.83	0.12	3.17
	250 µg L ⁻¹	Precision (RSD / %)	2.02	1.50	1.25
		Accuracy (RE / %)	0.56	1.90	3.66
	10 µg L ⁻¹	Precision (RSD / %)	6.03	8.20	15.81
		Accuracy (RE / %)	26.71	15.68	14.32
Inter-day	150 µg L ⁻¹	Precision (RSD / %)	1.66	1.87	1.37
		Accuracy (RE / %)	3.38	4.01	2.08
	300 µg L ⁻¹	Precision (RSD / %)	0.51	0.62	2.46
		Accuracy (RE / %)	0.49	1.62	0.58

^a8 levels; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation; RE: relative error; r²: correlation coefficient.

Solid phase extraction (SPE)

The first optimization approach for the SPE procedure required previous knowledge of the interaction between the target molecule and the sorbent characteristics, such as neutrality, the octanol-water partition coefficient (log K_{ow}), the solid-water distribution coefficient (log K_d), etc.²⁰

As these β -blockers have acidity constants (pKa) of around 9 and, hence, show neutral characteristics in pH > pKa, there is a potential for interaction with non-polar sorbent, such as C18 ec.^{5,30} For this reason, the pH of the analyte solutions was investigated, as well as the water used in the conditioning-washing stage from neutral up to alkaline pH.

The independent variables, their respective levels and the analyte and the recoveries (%) of ATE (Rec_{ATE}), MET (Rec_{MET}) and PRO (Rec_{PRO}) can be seen in Table 2. The recoveries with the relative standard deviations for ATE, MET and PRO always remained below 10%.

Model fitting and statistical analysis

The variables with an influence on the recovery of ATE, MET and PRO by the SPE procedure can be seen in Figure 3.

The studied variables showed no influence on the recovery of ATE with 95% confidence. In contrast, for MET, the quadratic variable (Q) of sample pH and the linear

variable (L) of the methanol ratio played an important role. The recovery of PRO was influenced by the sample pH (Q), the methanol ratio (L + Q) and the water pH (L + Q) in the conditioning-washing stage.

ANOVA was employed to determine which relevant variables exerted an influence on recovery by comparing the sources of variation with *F*-tests and *p* probabilities for 95% confidence.¹⁶ More information is available in the SI section.

Response surface for the SPE of atenolol, metoprolol and propranolol

When the recoveries (%) of ATE, MET and PRO were examined (Figure 4), it was evident that the optimal condition for both sample pH and water pH was pH 9, and that the most favourable elutropical strength of the MeOH:ACN:FA mixture was obtained using the ratio 90:9.9:0.1.

Under these optimized conditions, the obtained recoveries were 97.4 ± 3.2 , 95.4 ± 2.1 and $82.6 \pm 2.4\%$ for ATE, MET and PRO, respectively (n = 3). When the desirability profiles of ATE, MET and PRO were observed (see SI section), on the basis of the proposed mathematical models, the recoveries were estimated to be 97.7, 97.2 and 96.4% for ATE, MET and PRO, respectively.

Despite the fact that the r² value for the ATE and PRO models was < 0.9, the resulting models gave an accurate

Table 2. Experimental design based on central composite design (CCD) of the solid phase extraction of atenolol, metoprolol and propranolol and the recoveries (Rec) obtained

Coded	Variables	Levels				
		−2	−1	0	+1	+2
χ_1	Sample pH	7	8	9	10	11
χ_2	Water pH	7	8	9	10	11
χ_3	Methanol ratio ^a	60	70	80	90	100
Experiment	Sample pH	Water pH	Methanol ratio	Rec _{ATE} / %	Rec _{MET} / %	Rec _{PRO} / %
1	8	8	70	98.7	88.9	83.9
2	8	8	90	91.4	95.6	91.6
3	8	10	70	98.2	85.3	73.5
4	8	10	90	101.8	95.7	89.4
5	10	8	70	97.7	91.9	82.5
6	10	8	90	99.9	96.3	89.4
7	10	10	70	94.9	82.9	66.9
8	10	10	90	95.5	90.2	83.9
9	7	9	80	84.6	78.3	69.5
10	11	9	80	94.8	88.1	78.8
11	9	7	80	96.1	90.9	84.7
12	9	11	80	93.9	90.9	80.7
13	9	9	60	96.6	81.9	64.4
14	9	9	100	97.7	94.5	86.5
15	9	9	80	99.2	95.1	95.7
16	9	9	80	94.7	91.2	95.4
17	9	9	80	98.9	97.4	95.0
18	9	9	80	95.5	96.9	95.7

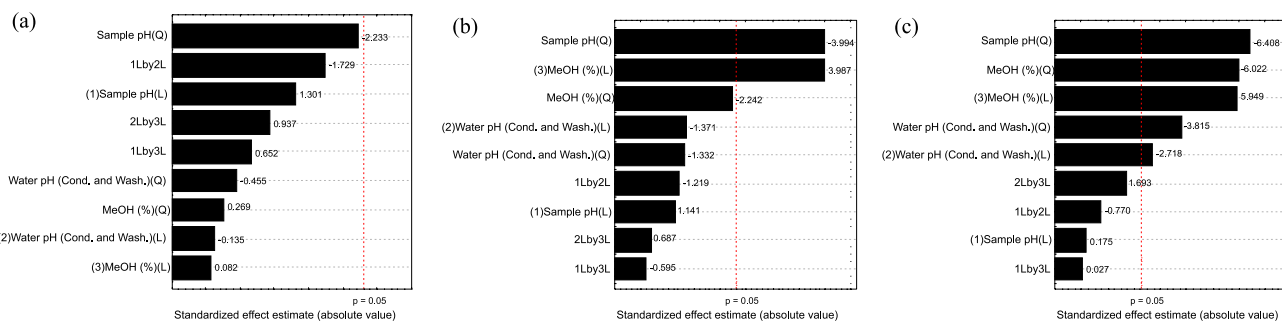
^aPercentage of MeOH in MeOH:ACN:FA.

prediction of the observed recoveries. The study of the matrix effect and breakthrough volume can be seen in the SI section.

Occurrence of β -blockers in hospital wastewater

The discharge of β -blockers through the HUSM effluent was evaluated with the aid of composite samples

collected over a period of one week. Figure 5 shows the measured environmental concentrations (MEC) at the three collection points of the HWW samples. In the case of the ‘Emergence’ and ‘HUSM general’ points, the samples were collected after passing through the septic tank-anaerobic filter assembly, and the MEC values correspond to the amounts of β -blockers released directly to the environment.

**Figure 3.** Pareto chart of effects (absolute values) of the solid phase extraction of (a) atenolol, (b) metoprolol and (c) propranolol. The vertical line defines the level of 95% of confidence, (L) mean linear variable and (Q) quadratic variable.

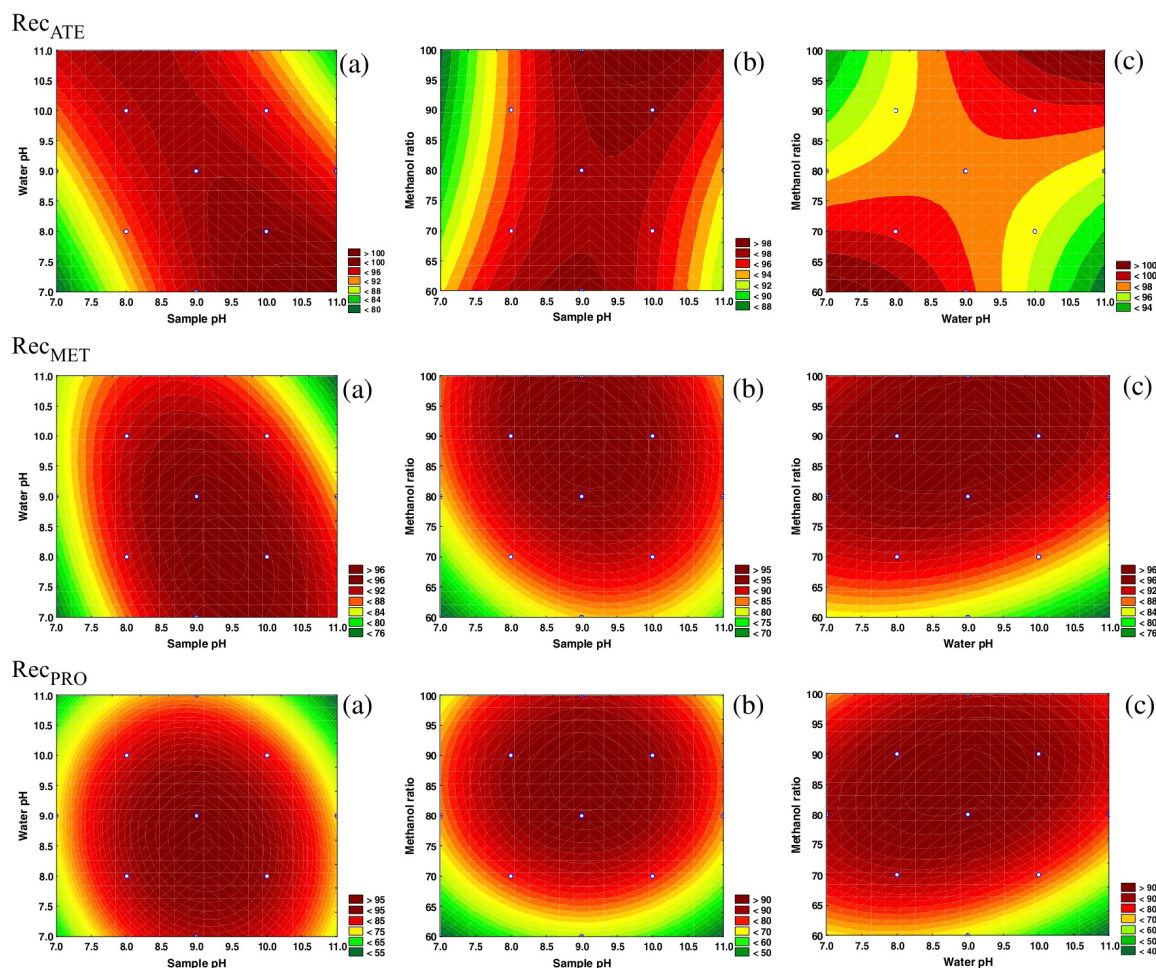


Figure 4. Response surface related to the recovery (Rec, %) of atenolol, metoprolol and propranolol by solid phase extraction: (a) water pH vs. sample pH, (b) methanol ratio vs. sample pH and (c) methanol ratio vs. water pH.

Figure 5a shows the MEC values of β -blockers for the 'Emergence' point. The weekly variation in the ATE concentration was from 1.37 to 4.48 $\mu\text{g L}^{-1}$, while the measurements for MET were in the range of 1.37 to 9.93 $\mu\text{g L}^{-1}$, and the lowest concentration was shown for PRO, from > LOD to 1.63 $\mu\text{g L}^{-1}$.

Figure 5b shows the occurrence of β -blockers at the 'HUSM general' point, where ATE was measured from

0.19 to 1.49 $\mu\text{g L}^{-1}$, MET from > LOD to 2.37 $\mu\text{g L}^{-1}$ and PRO from > LOD to 1.37 $\mu\text{g L}^{-1}$.

The 'Receiving waters', where the HWWs are discharged, can be regarded as an open air sewer. The MEC values of this sampling point can be seen in Figure 5c, in which ATE was measured in the range from 0.75 to 1.88 $\mu\text{g L}^{-1}$, MET from 0.73 to 2.81 $\mu\text{g L}^{-1}$ and PRO in the range from 0.15 to 1.44 $\mu\text{g L}^{-1}$.

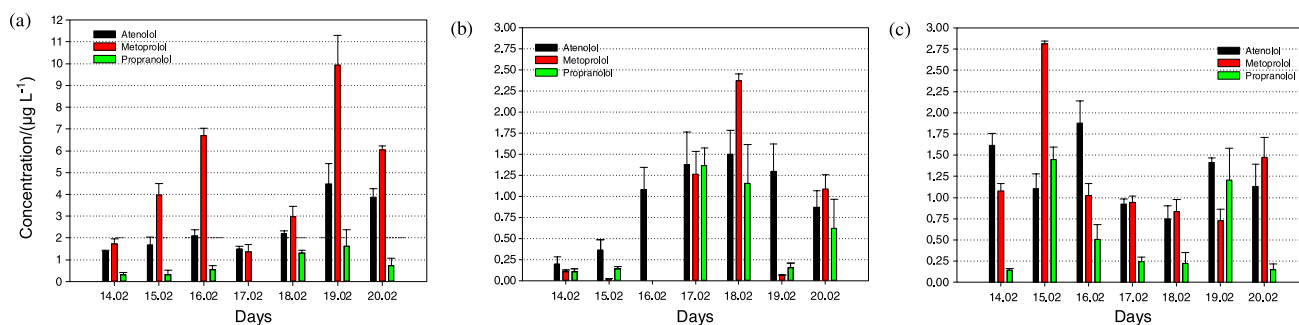


Figure 5. Measured environmental concentration (MEC) of atenolol, metoprolol and propranolol released by the HUSM in a period of a week: (a) 'Emergence', (b) 'HUSM general' and (c) 'Receiving waters'.

In contrast, the average concentrations of ATE, MET and PRO in the sampling period (one week, $n = 7$) were 2.45 ± 1.14 , 4.67 ± 1.63 and $0.70 \pm 0.88 \mu\text{g L}^{-1}$ in the 'Emergence' sewage, 0.95 ± 0.68 , 0.70 ± 0.33 and $0.315 \pm 0.62 \mu\text{g L}^{-1}$ in the 'HUSM general' sewage and 1.26 ± 0.47 , 1.27 ± 0.35 and $0.56 \pm 0.47 \mu\text{g L}^{-1}$ in the 'Receiving waters', respectively.

The MEC values for HWW corresponded closely to those reported in a previous review of hospital effluents, where there was an average concentration of β -blockers of $5.9 \mu\text{g L}^{-1}$ and, in addition, the MEC values of pharmaceuticals that were 2-150 times higher than those in wastewater treatment plants.³¹

It should be pointed out that, in developed countries, HWW is collected and then treated in municipal wastewater treatment plants, while in this study the MEC values were calculated on the basis of the HWW that was directly discharged into the environment.

First attempt at a risk assessment

A preliminary risk assessment (RA) which compared the predicted environment concentration (PEC) and the MEC values was carried out to evaluate the risk of releasing β -blockers into the regional environment. This was done using toxicity data reported in the literature such as predicted no-effect concentration (PNEC).³²

According to Escher *et al.*,³³ the RA for a specific hospital can be estimated by the PEC value given in equation 4:

$$\text{PEC}_{\text{HWW}} = \frac{A E}{V_{\text{HWW}} 365} \quad (4)$$

where PEC_{HWW} is the PEC in the HWW (g L^{-1}), A is the amount of pharmaceuticals used in the hospital (g), E is the excreted fraction of the pharmaceutical in the urine and faeces and V_{HWW} is the volume of HWW released *per day* (L per day).

For the prediction of the surface water ($\text{PEC}_{\text{Receiv. waters}}$), such as a river or water receptor, a dilution factor (D) must

be added to equation 4. In this case, a factor of 10 was assumed, resulting in equation 5:

$$\text{PEC}_{\text{Receiv. waters}} = \frac{A E}{V_{\text{HWW}} 365 D} \quad (5)$$

The annual consumption of β -blockers was obtained from the HUSM administration data. As previously described, the HWW flow rate was estimated to be $190 \text{ m}^3 \text{ per day}$.³⁴ A worst case scenario was assumed for the PEC calculations.^{35,36}

Other important RA parameter is the risk quotient (RQ), which is estimated by the PEC/PNEC and MEC/PNEC ratios.³³ For this study, excretions of 90, 5 and 10% were assumed for ATE, MET and PRO, respectively.³⁷

The theoretical PEC and RQ values estimated for the HWW can be observed in Table 3. All the three β -blockers showed PECs above $0.01 \mu\text{g L}^{-1}$ (a point worth noting) but PRO was the only β -blocker with a medium theoretical risk level ($0.1 \leq \text{QR} < 1.0$).³⁸

The RQ assessment (MEC/PNEC) for β -blockers at the HWW sampling points can be seen in Figures 6a-6c. MET showed $\text{RQ} > 1.0$ on only one single day of collection at the 'Emergence' point, while, as a result of its low toxicity, ATE showed no risk to the environment in terms of MEC values. Despite its low concentration in HWW, PRO represents a major risk when compared to ATE and MET due to its high toxicity and pseudopersistence.^{32,37,38}

Conclusions

The SPE-HPLC-FLD methodology employed that was optimized with the aid of RSM and henceforward validated was found to be suitable for the determination of β -blocker levels in HWW and similar effluents.

The determination of ATE, MET and PRO in HWW samples at the HUSM confirmed the occurrence of these drugs in trace levels ($\mu\text{g L}^{-1}$) at the three different sampling points evaluated, 'Emergence', 'HUSM general' and 'Receiving waters' during the one week sampling period.

Table 3. Predicted environmental concentration (PEC) and risk quotient (RQ) of the β -blockers in the HUSM wastewater

β -Blocker	Excreted unchanged / %	Annual consumption in the HUSM / (g per year) ^b	$\text{PEC}_{\text{HWW}} / (\mu\text{g L}^{-1})^d$	$\text{PEC}_{\text{Receiv. waters}} / (\mu\text{g L}^{-1})^d$	PNEC / ($\mu\text{g L}^{-1}$) ^c	RQ (PEC/PNEC) ^d
Atenolol	90 ^a	117	1.52	0.152	310	4.9×10^{-3}
Metoprolol	5 ^a	185	0.13	0.013	7.90	16.4×10^{-3}
Propranolol	10 ^a	284	0.41	0.041	0.73	0.56

^aReference 22; ^bHUSM data (year 2007); ^creference 31; ^dpresent study.

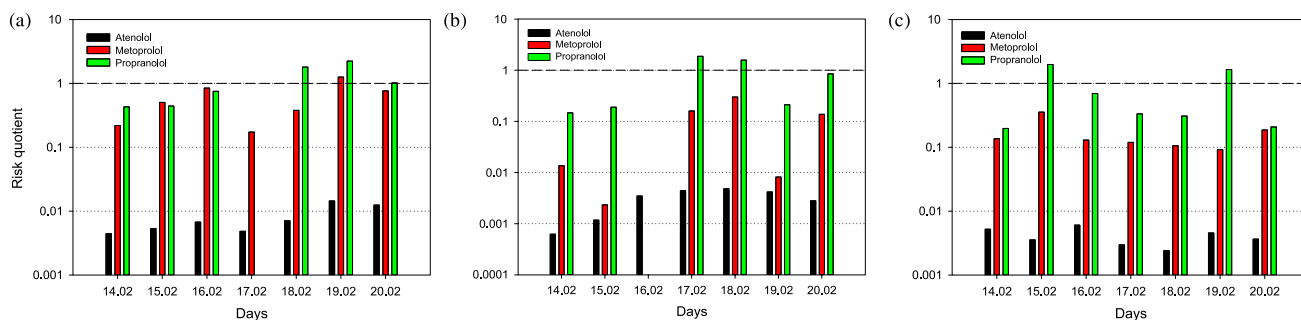


Figure 6. Risk quotient (RQ) of the β -blockers atenolol, metoprolol and propranolol released into hospital wastewater: (a) 'Emergence', (b) 'HUSM general' and (c) 'Receiving waters'.

The theoretical RA (PEC/PNEC) showed that PRO is the β -blocker with the highest RQ (0.56, medium risk). This result should not be underestimated when compared to RA of real (MEC/PNEC) exposure to β -blockers in the HUSM effluent. Although it was measured only in low concentrations, PRO showed the highest environmental risk ($RQ > 1.0$), which means that more attention should be paid to its ecotoxicological effects.

These results emphasize the need for a more efficient treatment for hospital effluents as a means of avoiding the discharge of micro-contaminants into the environment and the unknown environmental risks that this may cause.

As far as we are aware, this is the first research study that has addressed the problem of the occurrence of β -blockers in hospital effluents and attempted to undertake an environmental risk assessment of their effects in a developing country.

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Supplementary Information

Supplementary material (Tables S1-S2, Figures S1-S3) is available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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