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Supplementary Information

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

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Response surface methodology

Response surface methodology (RSM) is a multivariate tool most helpful for the optimization of analytical methods as reported in the literature.[1-3] Among other RSMs, central composite design (CCD) is very useful because it encompassed five variables levels (–α, –1, 0, 1, + α), and α is the level value of the axial part from RSM and is calculated according to equation 1:[1]

$$\alpha = 2^{(K - C_o)/4} \qquad (1)$$

The number of experiments is calculated according to equation 2:[1,3]

$$\text{No. of experiments} = 2^{K} + 2K + C_o \qquad (2)$$

where K is the number of variables and C_0 is the number of replicates of the central point. The portion 2^K comprises the factorial part of the experimental design, while $2K$ is referred to the axial part. The value of α is 1.683 for RSM with three variables, but aiming to study more the axial points, the value of α was changed to 2.

The non-linear quadratic model of CCD is fitted by a polynomial second order equation with quadratic terms according to equation 3:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i \chi_i + \sum_{i=1}^{k} \beta_{ii} \chi_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} \chi_i \chi_j + \varepsilon \qquad (3)$$

where y is the response associated to the combination of the variables levels, β_0 is a constant coefficient, β_i is the regression coefficient computed to experimental values of y, χ_i are the coded linear variables, $\chi_i\chi_j$ is the interaction between the coded variables, χ_i^2 is the coded quadratic variables and ε is the associated random error.[1,3]

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Lack of fit test by ANOVA

Table S1 shows the results of the lack of fit test applied to the calibration curve data of atenolol, metoprolol and propranolol by ANOVA. As can be seen, the results of regression (critical value < F) and lack of fit test (critical value > F) evidence the linearity of the calibration curves.

Model fitting and statistical analysis

According to the quadratic model for atenolol, the recovery (Rec) was not influenced by the studied range of variables, i.e., it showed to be independent of the changes in the physico-chemical characteristics of the sorbent and atenolol. Besides the high recoveries for atenolol, the quadratic model didn't fit well. The r^2 value for the quadratic polynomial equation (QPE, equation 4) was only 0.6015.

$$\begin{aligned} \mathrm{Rec}_{\mathrm{ATE}}\ (\%) = & -93.55 + 43.97\chi_1 - 1.67\chi_1^2 + 15.96\chi_2 - 0.34\chi_2^2 \\ & - 2.08\chi_3 + 0.002\chi_3^2 - 2.14\chi_1\chi_2 + 0.08\chi_1\chi_3 + 0.12\chi_2\chi_3 \end{aligned} \qquad (4)$$

ANOVA for metoprolol confirmed the influence of the variables evidenced in the Pareto chart of effects (see main manuscript Figure 2b). The r^2 value of QPE (equation 5) was 0.8277. The ANOVA of the propranolol recovery confirmed also the influence of the variables of the Pareto chart (see main manuscript Figure 2c) and the r^2 value of the resulting QPE equation (equation 6) was 0.9297.

$$\begin{aligned} \mathrm{Rec}_{\mathrm{MET}}\ (\%) = & -455.65 + 71.78\chi_1 - 2.89\chi_1^2 + 22.72\chi_2 - 0.96\chi_2^2 \\ & + 2.83\chi_3 - 0.02\chi_3^2 - 1.46\chi_1\chi_2 - 0.07\chi_1\chi_3 + 0.082\chi_2\chi_3 \end{aligned} \qquad (5)$$

$$\begin{aligned} \mathrm{Rec}_{\mathrm{PRO}}\ (\%) = & -843.67 + 104.27\chi_1 - 5.28\chi_1^2 + 44.24\chi_2 - 3.14\chi_2^2 \\ & + 6.40\chi_3 - 0.05\chi_3^2 - 1.05\chi_1\chi_2 + 0.004\chi_1\chi_3 + 0.23\chi_2\chi_3 \end{aligned} \qquad (6)$$

The ANOVA tables of the quadratic models from RSM of $\mathrm{Rec}_{\mathrm{ATE}}$, $\mathrm{Rec}_{\mathrm{MET}}$ and $\mathrm{Rec}_{\mathrm{PRO}}$ by the SPE procedure can be seen in Table S2.

Desirability function

The desirability profile (Figure S1) is used to help the RSM optimization process when various responses (multi-parameters) are evaluated, following the equations proposed by Derringer and Suich.[1,3-6]

Matrix effect

The complexity of the hospital wastewater can certainly affect the results.[6] Therefore, a matrix effect (ME) test over the analyte signal in HPLC-FLD was examined. For this, HWW samples were collected and filtered as described in Experimetal section.

The matrix effect was calculated according to equation 7:[7]

$$\text{ME}\ (\%) = \frac{A_{std} - (A_a - A_b)}{A_{std}} 100 \qquad (7)$$

where ME is the matrix effect, A_{std} is the peak area of the standard analyte, A_a is the peak area of the HWW matrix spiked and A_b is the peak area of the matrix without spike.

The matrix effect found for Rec_{ATE} was below 10% for HWW samples from all the three points of collection, while the matrix effect over Rec_{MET} was 15% for the 'Emergence', and below 5% for the 'HUSM general' and 'Receiving waters' samples. The matrix effect over Rec_{PRO} was around 15% for samples from all three collecting points. These effects were considered in the determination of ATE, MET and PRO in the HWW (Figure S2).

Estimation of the breakthrough volume

This evaluation serves to determine the sorbent capacity on retaining the analyte without significant losses in terms of recovery.[8,9] This estimation was carried out at different concentrations and volumes varying from 4 to 1000 mL in aqueous solutions and from 4 to 200 mL in HWW samples. Finally, the concentrations of 100 and 200 µg L^{-1} for the aqueous solution and HWW samples were fixed, respectively. The experiments were carried out in quadruplicate, with and without spiking, in order to check possible interference by β-blockers present in HWW samples.

The breakthrough of the sorbent used (C18 ec, 200 mg:3 mL) was estimated for different volumes. As can be seen in Figure S3a, even for high volumes of aqueous solution (ultrapure water) the recoveries were > 80%.

For HWW samples, the estimation of breakthrough is critical due to the high organic load. Figure S3b shows the breakthrough for 'Emergence' samples and Rec_{ATE} was > 90% even by varying the volume (4-200 mL), while Rec_{MET} and Rec_{PRO} decreased all the way. Surprisingly, beyond 100 mL volume Rec_{MET} increased again.

Rec_{ATE} was also > 90% for HWW samples, 'HUSM general' and 'Receiving waters' (Figure S3c and S3d), while Rec_{MET} decreased by varying the volume (4-200 mL) for 'HUSM general' sample. An irregular variation was found for Rec_{MET} of the 'Receiving waters' sample. Rec_{PRO} decreased varying the volume in all cases; this can be related to the fact that PRO shows higher hydrophobicity, i.e., high log K_{ow} and log K_d, resulting in low recoveries by the SPE procedure.

Considering these results, the determination of ATE, MET and PRO was performed in HWW samples of 100 mL that corresponds to an enrichment factor of 50 times.

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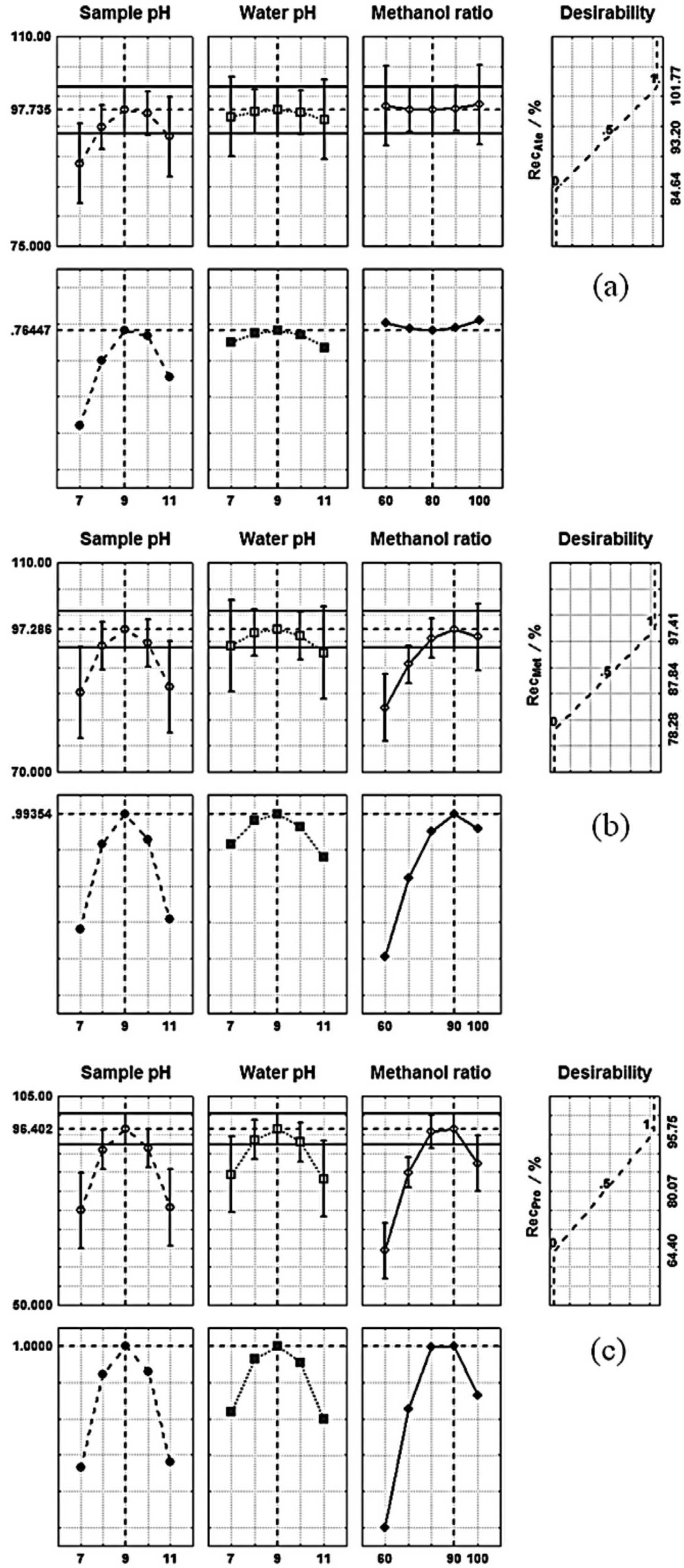

Figure S1. Profiles for predicted values and the desirability function for SPE of (a) atenolol, (b) metoprolol e (c) propranolol. Dashed line indicated current values after optimization.

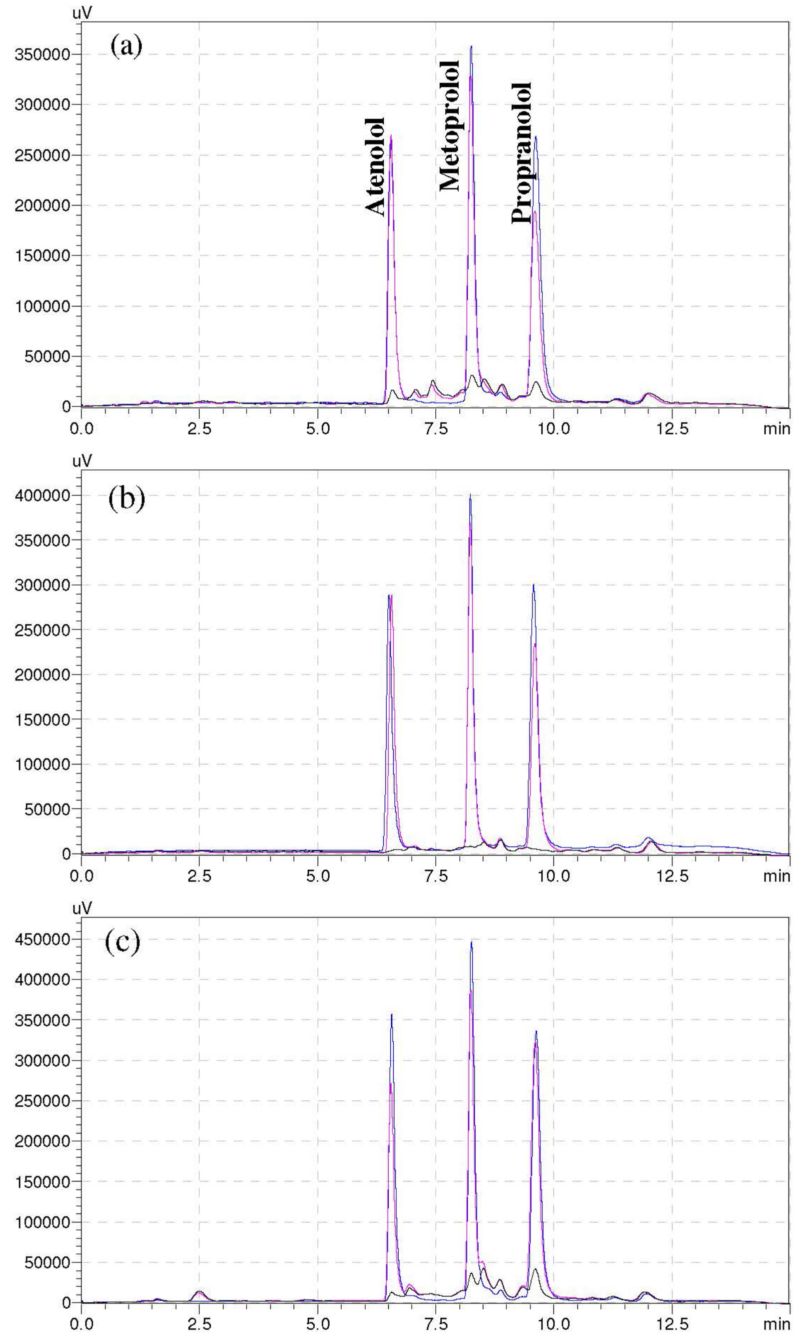

Figure S2. HPLC-FLD chromatograms showing the matrix effect on the peak signals of ATE, MET e PRO: (a) 'Emergence', (b) 'HUSM general' and (c) 'Receiving waters', and (—) HWW without spike, (—) HWW with spike, and (—) 200 μg L^{-1} standard solutions of atenolol, metoprolol and propranolol.

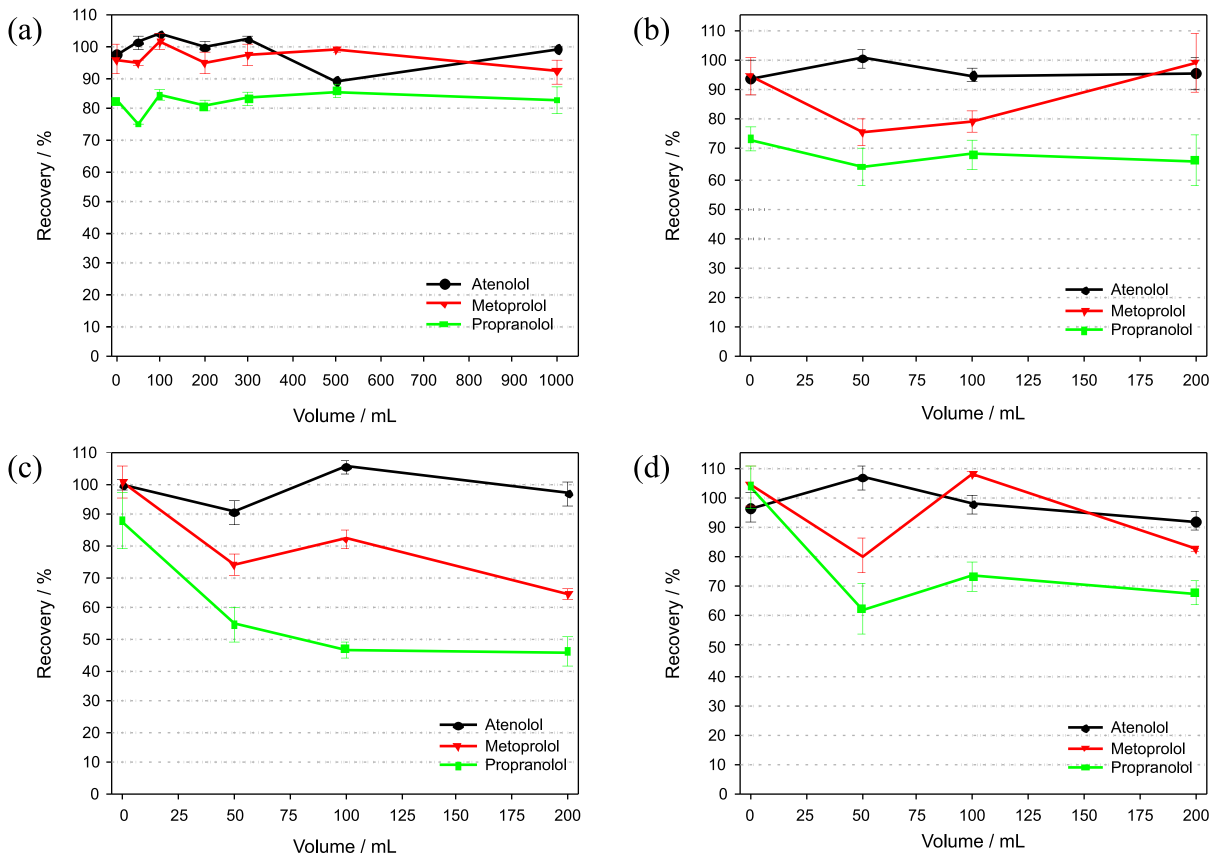

Figure S3. Estimation of the breakthrough volume for the C18 ec sorbent (200 mg:3 mL) used in the SPE procedure for atenolol, metoprolol and propranolol for (a) ultra-pure water, (b) 'Emergence', (c) 'HUSM general' and (d) 'Receiving waters'. SPE conditions: sample pH and water pH both 9, and the MeOH:ACN:FA ratio 90:9.9:0.1 in the elution step.

Table S1. Lack of fit test of the HPLC-FLD calibration curve method by ANOVA

Source	SS	dof	MS	*F*	Critical value
Atenolol					
Regression	4.19×10^{13}	1	4.19×10^{13}	31849.58	4.493998
Lack of fit	1.56×10^{10}	6	2.60×10^{9}	1.974217	2.741311
Pure error	2.11×10^{10}	16	1.32×10^{9}		
Total	4.19×10^{13}	23			
Metoprolol					
Source	SS	dof	MS	*F*	Critical value
Regression	5.01×10^{13}	1	5.01×10^{13}	6040.641	4.493998
Lack of fit	6.99×10^{10}	6	1.17×10^{10}	1.405744	2.741311
Pure error	1.33×10^{11}	16	8.30×10^{9}		
Total	5.03×10^{13}	23			
Propranolol					
Source	SS	dof	MS	*F*	Critical value
Regression	5.77×10^{13}	1	5.77×10^{13}	9132.41	4.493998
Lack of fit	1.54×10^{10}	6	2.57×10^{9}	0.407683	2.741311
Pure error	1.01×10^{11}	16	6.31×10^{9}		
Total	5.78×10^{13}	23			

SS: sum of squares; dof: degree of freedom; MS: medium square; *F*: Fischer test.

Table S2. ANOVA of the quadratic model for Rec_{ATE}, Rec_{MET} e Rec_{PRO} of the SPE procedure

Rec_{ATE}	SS	dof	MS	*F*	p
(1) Sample pH (L)	20.6570	1	20.65703	1.693802	0.229327
Sample pH (Q)	60.8980	1	60.89804	4.993422	0.055895
(2) Water pH (L)	0.2209	1	0.22090	0.018113	0.896265
Water pH (Q)	2.5322	1	2.53223	0.207634	0.660742
(3) Methanol ratio (L)	0.0812	1	0.08122	0.006660	0.936962
Methanol ratio (Q)	0.8777	1	0.87774	0.071971	0.795276
1 L by 2 L	36.4658	1	36.46580	2.990065	0.122034
1 L by 3 L	5.1842	1	5.18420	0.425086	0.532691
2 L by 3 L	10.7185	1	10.71845	0.878875	0.375945
Error	97.5652	8	12.19565		
Total SS	244.8090	17			
Rec_{MET}	SS	dof	MS	*F*	p
(1) Sample pH (L)	14.8803	1	14.8803	1.30294	0.286691
Sample pH (Q)	182.2819	1	182.2819	15.96081	0.003977
(2) Water pH (L)	21.4601	1	21.4601	1.87907	0.207663
Water pH (Q)	20.2831	1	20.2831	1.77601	0.219346
(3) Methanol ratio (L)	181.5083	1	181.5083	15.89307	0.004024
Methanol ratio (Q)	57.4670	1	57.4670	5.03187	0.055151
1L by 2 L	16.9653	1	16.9653	1.48550	0.257633
1L by 3 L	4.0470	1	4.0470	0.35436	0.568108
2L by 3 L	5.3956	1	5.3956	0.47245	0.511295
Error	91.3647	8	11.4206		
Total SS	530.3134	17			
Rec_{PRO}	SS	dof	MS	*F*	p
(1) Sample pH (L)	0.456	1	0.4556	0.03080	0.865054
Sample pH (Q)	607.401	1	607.4010	41.05660	0.000207
(2) Water pH (L)	109.307	1	109.3070	7.38849	0.026323
Water pH (Q)	215.295	1	215.2950	14.55263	0.005128
(3) Methanol ratio (L)	523.723	1	523.7232	35.40049	0.000342
Methanol ratio (Q)	536.500	1	536.4998	36.26412	0.000316
1 L by 2 L	8.778	1	8.7780	0.59334	0.463267
1 L by 3 L	0.011	1	0.0113	0.00076	0.978676
2 L by 3 L	42.412	1	42.4121	2.86680	0.128879
Error	118.354	8	14.7942		
Total SS	1683.204	17			

Rec: recovery; SS: sum of squares; dof: degree of freedom; MS: medium square; *F*: Fischer test; p: probability.