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Optimization of solid phase extraction of β-blockers from hospital effluent by Response Surface Methodology

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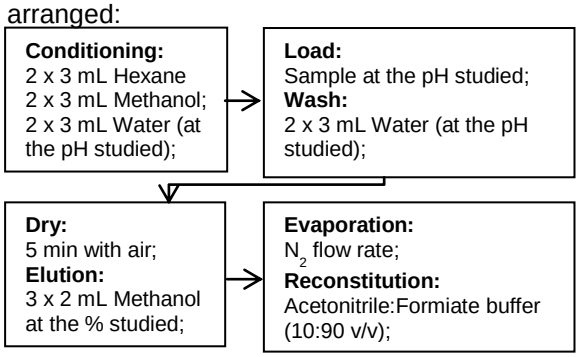
After administration and excretion pharmaceuticals and their metabolites end up in the sewage and superficial waters, where they can be found at trace levels[1]. Their analysis needs sample pretreatment.

Solid phase extraction (SPE) is a suitable procedure process involving depending on several parameters such as: flow rate, pH, composition and quantity of solvents (used for conditioning and elution). Other important parameters are the physical-chemical characteristics of the target substances (polarity, solubility, pKa, etc.).

To find the best conditions of extraction, optimization is usually required. For that, an experimental design was employed. The model chosen was a Central Composite Design (CCD) and the variables studied were: pH of water (7-11) in the conditioning and washing steps, sample pH (7-11) in the load step and the % of acidified methanol (60-100%) in the elution step. Thus, the combination of the levels led to 16 experiments.

The experiments were carried out with vacuum manifold and cartridge sorbent Chromabond® C18 ec 200 mg/3 mL (45 µm, 60 Aº). For this, the following general scheme was arranged:



The optimization and efficiency of the extraction procedure was evaluated by calculating absolute recovery values. In order to analyze the results, a method by HPLC-FLD was developed. Separation was performed on a HPLC Prominence Shimadzu with detector RF-10AXL (λex: 230 nm, λem: 312 nm). A Macherey-Nagel column Nucleodur 100-5 C18 ec (CC 125 mm x 4 4 mm d.i., 5 µm) equipped with a guard cartridge C18ec Nucleodur 100-5 (CC 8 x 4 mm d.i.,5 µm) was used with gradient elution (mobile phase A: formiate buffer 20 mmol pH 4,2 and B: acetonitrile: 0-1 min isocratic) with 5% of B, 1-4 min linear gradient (lg) of 5-40% B; 4-10 min isocratic at 40% B; 10-11 min lg of 40-10% B; 11-15 min isocratic of 5% B (equilibration step). Flow rate used was 1 mL min^{-1}, injection volume 20 µL.

The evaluation of the results by analysis of variance (ANOVA) led to the optimized conditions: water pH 9 for conditioning and washing steps; sample pH 9 in the load step; for elution, the best condition was 90% methanol. By using these conditions the obtained recovery was 97.4, 95.4 and 82.6% for Atenolol (Ate), Metoprolol (Met) and Propranolol (Pro), respectively (n=3). A breakthrough assay with different volumes (50-1000 mL) was performed without any perceptive loss of efficiency.

The optimized SPE procedure was used for determination of the studied β-Blockers in samples collected at different points of the sewage system of the university hospital (HUSM).The results can be seen at Table 1.

Table 1.Concentration of β-blockers at different points of the sewage system of HUSM (*n*= 4).

Sewage point	Atenolol µg L^{-1}	Metoprolol µg L^{-1}	Propranolol µg L^{-1}
Emergence clinic	4.67 ± 0.04	1.55 ± 0.13	1.37 ± 0.38
HUSM/main	0.46 ±0.099	0.271 ± 0.001	0.030 ± 0.003
Water receptor	1.88 ± 0.05	0.70 ± 0.11	0.30 ± 0.02

Using the CCD it was possible to find easily the optimal conditions for the extraction of the β-blockers from the hospital effluent samples.

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References

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