Identification and quantitative analyses of citalopram in serum using UPLC®.

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Introduction

The Laboratory of Pharmacology and Toxicology of the military hospital Desgenettes uses UPLC® separation techniques like toxicological screening which allows the identification and quantification of molecules such as psychotropics. The screening method uses ammonium acetate buffer at pH 3.8. There is co-elution of some molecules with flurazepam, used as internal standard. These molecules are clozapine, citalopram and mianserin. The optimization of the method had led to the modification of pH to 6.4. This caused a change in selectivity, and finally permitted the separation of the different molecules. Calibration ranges are made at pH 6.4 and now allow the determination of clozapine, citalopram and mianserin. This case study is about an external quality control of the SFTA (French Society of Analytical Toxicology). The laboratory must identify and measure citalopram in serum.

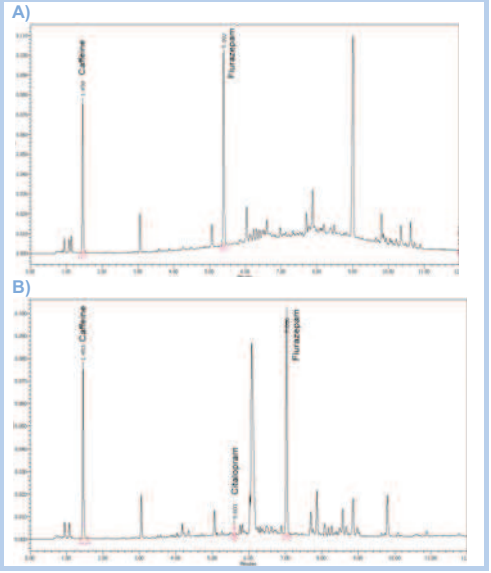
Experimental conditions

Citalopram and flurazepam were separated using a 1.7µm, 150×2.1 mm, Acquity BEH column on a UPLC® Waters system. The detection system was a DAD detector (wavelength: 240 and 254 nm). This separation was operated with two different ammonium acetate buffer's pH: 3.8 and 6.4 and was carried out in gradient mode with a maximal flow-rate of 0.45 ml/min. The mobile phase consisted of 85% buffer and 15% acetonitrile.

Results and discussion

UPLC® chromatograms are presented in figures A and B. The chromatogram A was performed at pH 3.8. The retention times of citalopram and flurazepam were respectively 5.44 and 5.47 min. Co-elution between citalopram and the internal standard prevented the identification and dosage of citalopram. The chromatogram B was performed at pH 6.4. The retention times of citalopram and flurazepam are now 5.54 and 6.99 mn. A change in selectivity was clearly visible. A calibration was performed on blood overloaded with citalopram. A Seroplex ® tablet was used and the range extended from 20 ng/mL to 1000 ng/mL. The regression was: Y = 0.001593X. The interpretation of toxicological screening quality control provided a citalopram concentration of 263.5 ng/mL with a bias of 5.4% to a target value of 250 ng/mL. As the optimization of the screening method was successful, a validation protocol at pH 6.4 to UPLC® with a study of the precision, accuracy, linearity, robustness can be considered.

Figure : Separation of citalopram and flurazepam on column C18 Acquity BEH UPLC®, 1.7 µm, 150×2.1 mm; mobile phase A1: ammonium acetate buffer; mobile phase B1: acetonitrile; analysis time: 15 mn.
Chromatogram A: Buffer's pH: 3.8.
Chromatogram B: Buffer's pH: 6.4.



Conclusion

Increasing the pH of ammonium acetate buffer from 3.8 to 6.4 allows the separation of citalopram and the internal standard. The realization of the calibration range leads to quantification of citalopram. It is now possible to provide the concentration of the latter, contained in the serum of external quality control of the SFTA. This optimization also applies for mianserin and clozapine molecules which co-eluted with flurazepam and that now can be quantified in the same manner as citalopram.

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