Developing and validating an LC-MS/MS method to quantify antidepressants in blood

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Introduction

Antidepressants are drugs which are prescribed to patients who suffer from serious depression; they minimize the symptoms and improve their overall wellbeing. However antidepressant when taken in excessive quantities can be dangerous or even lethal. This is why there is a need to identify if they have been consumed or abusively administrated in cases of suicides and homicides.
The objective of this article is to outline a quantification method for 20 antidepressants and 3 metabolites in blood. Antidepressants are generally searched in blood because this is an environment for which there are therapeutic and toxic reference concentrations. It is a very complex matrix which requires an efficient method of extraction to eliminate most interferences. The second objective of the study was to validate the chosen method to allow it to be used as a routine analysis.

Experimental methods

A method was developed to quantify the majority of antidepressants. The deuterated internal standards were added to a sample of 1mL. Sample preparation was performed by solid phase extraction on Oasis® cartridge MCX 3cc. The analysis was carried out by high performance liquid chromatography-electrospray ionization mass spectrometry (from ThermoFisher society), a specific and sensitive analytical technique. The analytes was separated by reverse-phase chromatography with a hypersil® Gold column C18 – 150 mm × 2.1 mm × 5 µm. Detection and quantification were realized in MRM mode through two transitions for each molecule.

Results

To optimize the mass spectrometry, solutions of the pure compounds at 1ng/mL were infused into the ion source. The molecular ion M+H was located in Full Scan mode. By positioning in SIM mode, it was possible to select the parent ion and thus to optimize different non-mass-dependent parameters to have the best signal. In this way, an optimal tune for the totality of these compounds was determined. Then, the mass-dependent parameters were also optimized. The parent ion selected by Q1 was fragmented by the collision cell Q2. A full scan was performed in Q3 to identify the fragments. After optimization of the Tube Lens and the collision energy, the two most intense transitions were selected. The most intense transition was used for the quantification and the second one for the confirmation (Fig 1).
Then, the HPLC chain was developed to allow the 23 compounds to be optimally separated. Liquid Chromatography separation involved a column with a gradient of acetonitrile (2 mM, pH 3) and ammonium formate. Several gradients were tested and the gradient reserved allowed eluting all the molecules in 15 minutes with a flow rate of 250µL/min (fig 2). Among the 23 molecules eluted, 17 were validated on measuring interval 50 to 1000ng/mL with a linear regression model going throw 0. Repeatability and reproducibility had variation coefficient less than 20%. Extraction efficiencies were more than 50 %. The detection limit ranged from 0.02 to 0.77ng/mL and the quantification, from 0.07 to 2.57ng/mL

Figure 1 : Infusion Spectrum of Amitriptyline

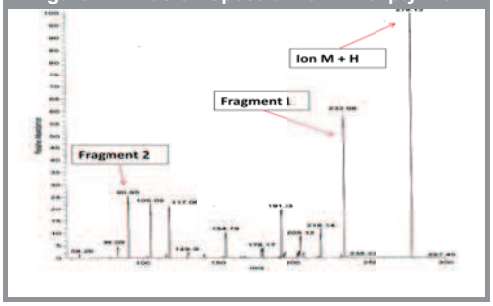
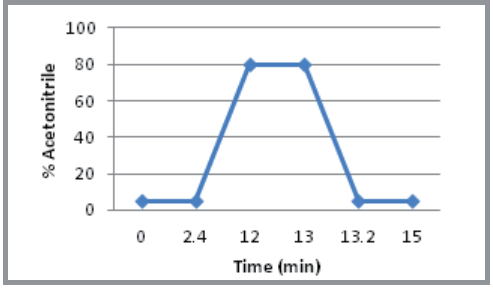


Figure 2 : Gradient Elution Chromatography



Conclusion

For the most part, antidepressants commercialized in France are detected and dosed by one main analytical method, the accuracy and the sensibility of the LC-MS/MS allow to use this analytical technique.

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