Quantification Method for Benzodiazepines in Human Plasma by LC-MS/MS

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Introduction

The increase of toxicological analyses requests for living human beings, leads to constant improvements of results and their clinical interpretation. Benzodiazepines are the main xenobiotics involved in the cases of acute poisoning, followed by antidepressants, aspirin, paracetamol and alcohol. They are often found in doping analyses, chemical submission, driving under the influence cases and drug addiction. It is important to be able to quantify the presence of these medicines within the blood, so as to adapt a patient treatment posology or to identify any abnormal rates. In the laboratory, benzodiazepines qualitative detection was obtained with an immuno-enzymologic screening method. However, this technique was not specific to all benzodiazepines; some substances were not recognized with the available reactive product. Thus, a new analysis method was developed and tuned, to quantify the benzodiazepines present in the studied patients plasma.

Experimental Conditions

The analyses were performed by high-performance liquid chromatography – tandem mass spectrometry. The chromatographic system was an Agilent Technologies® 1200 Series, equipped with 2 pumps (binary and isocratic). Separation was obtained with a phenylhexyl column (50 x 2 mm x 5 µm), a mobile phase containing 97% of methanol and 0.1% acetic acid, at a flow rate of 350 µl/min and 5 min chromatographic run. Detection was carried out by an Applied Biosystems ® API 3200 mass spectrometer equipped with an electrospray source (positive ionization) and a triple-quadrupole analyzer. The analysis mode for MS/MS was based on the multiple reactions monitoring (MRM) method. All the calibrators (diazepam, flunitrazepam, lorazepam, midazolam, oxazepam and prazepam) and the patients' samples were prepared using 2 extraction methods (precipitation and liquid/liquid extraction) according to the laboratory protocol, in order to compare the specificity and the sensitivity of these two methods. The chosen method was validated according to the laboratory protocol, which included the determination of: linearity, repeatability, reproducibility, accuracy and extraction output.

Results and discussion

Bar charts, in Figure 1, show the observed signal differences for low concentration samples extracted by precipitation or liquid/liquid extraction methods. The latter is the most appropriate technique because it provides a better answer, as well as a better sensitivity and specificity at low concentrations. Indeed, for all the benzodiazepines, the measured signal was significantly increased when samples were treated by liquid/liquid extraction. In addition, this method permitted a very good extraction output (in the range of 80%), corresponding to the performances required by the laboratory. We determined experimentally the linear range of 5 µg/l to 1000 µg/l and the limit of quantification of 10 µg/l of the method.

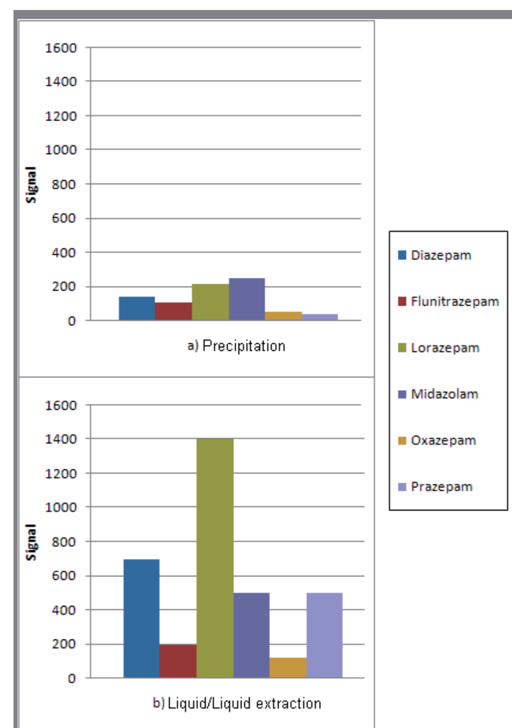

Figure 1 : Comparison of two extractions (C = 5 µg/l)
a) Precipitation with methanol
b) Liquid/liquid extraction with diethylether

However, the fragmentation could not be reproduced, as the ionization may be significantly reduced by the phenomenon of ion suppression.

Conclusion

Several medicines or drugs classes, relevant to clinical and forensic toxicology or doping controls, were analyzed in urine and/or blood. The simultaneous detection of these molecules was performed using liquid chromatography, coupled with a mass spectrometer in a single stage or in tandem (LC-MS, LC-MS/MS). Both techniques were excellent supplements to gas chromatography-mass spectrometry (GC-MS), particularly for the detection of more polar, thermolabile and/or low-dosed drugs.

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