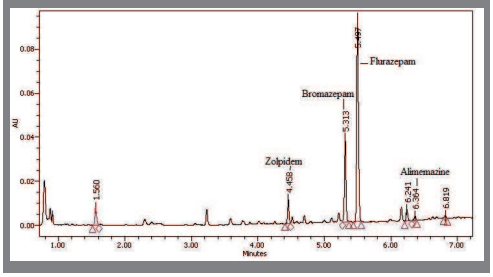
Drug assays in biological samples using UPLC

Amélie LAGARDE

Introduction

The pharmacology laboratory of Desgenettes Hospital performs assays by chromatographic methods in the fields of pharmacology (e.g. bio-equivalence) and toxicology for both the hospital and for forensic analysis. One of the analysis performed was the toxicological screening which allows drugs to be identified and to be quantified in biological samples. The case study concerned a 73 year-old man who was found dead in the bathtub of a physiotherapist. He was treated with an anxiolytic called Lexomil® (whose active ingredient is bromazepam). The analyses required were toxicological screening in the blood and in urine.

Figure 1 : Chromatogram in blood at 254nm



Material and methods

LC separation was performed using a C18 BEH column and detection wavelength was 254nm. The characteristics of the column were:

- Length 150mm,
- Intern diameter 2.1mm
- Particle diameter 1.7µm.

The chromatographic method was developed in gradient mode. The internal standard used for the quantification was the flurazepam. The analytical procedure for the screening was:

a. Liquid-liquid extraction with a toxitube
b. Evaporation under nitrogen flow at 50°c
c. Resumption with 60µL of mobile phase
d. Recuperate the 30 µL supernatant
e. Injection of 5 µL in UPLC

Results and discussion

The UPLC chromatogram made in blood is presented in figure 1. The bromazepam from treatment was found in the analysis, but in the blood there was zolpidem, a hypnotic related to benzodiazepines and alimemazine, as well as an antihistamine. The concentrations of these drugs were 258ng/mL, 130ng/mL and 16ng/mL respectively. In the urine, apart from bromazepam, only zolpidem, and traces of paracetamol were detected. Following this discovery, the dosages of zolpidem, bromazepam and alimemazin were undertaken to know their concentrations in the blood and whether they were close to the threshold of toxicity. Bromazepam was present with a quantity of 147ng/mL and zolpidem, with a concentration of 130ng/mL.
According to table 1, only therapeutic concentrations were detected, therefore the possibility of drug poisoning was eliminated.

Table 1 : Therapeutic, toxic and lethal concentrations for paracetamol, zolpidem, bromazepam and alimemazin

Molecules	Therapeutic concentrations (ng/mL)	Toxic concentrations (µg/mL)	Lethal concentrations (µg/mL)
Paracetamol	2500 to 25000	100 to 150	200 to 300
Zolpidem	30 to 270	0.5	> to 0.5
Bromazepam	50 to 200	0.3 to 0.4	1 to 2
Alimemazin	1 to 400	0.5	> to 0.5

Fédération de Biologie Clinique
Hôpital d'Instruction des Armées Desgenettes

Laboratory of toxicology
HIA Desgenettes
108 Boulevard Pinel, 69998 Lyon