Determination and quantification of clonidine, antihypertensive drug in plasma by GC-MS

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Introduction

The Desgenettes military toxicology and pharmacology laboratory uses GC-MS for screening narcotics such as amphetaminics, cannabinoids, opiates and cocainics.
GC-MS is also used for the development of a method to identify and quantify clonidine in plasma. Clonidine is a drug commercialized as Catapressan®, a potent drug used in the treatment of arterial hypertension.
This drug is present in very low therapeutic concentration in plasma (<2ng/ L). Manipulations are time consuming and quantification of clonidine is achieved by the method of internal standardization using moxonidine, a compound with a similar structure to clonidine. Sample preparation is very laborious (separation, purification, derivatization, clean up…)

Experimental conditions

Before injection, samples are extracted by Solid Phase Extraction (SPE), derived overnight and washed several times.
Clonidine and moxonidine are observed in chromatograms obtained with a HP-5MS column (30 m x 0.25 µm x 0.25 mm) on a gas chromatography coupled with a mass spectrometer (GC-MS).

For GC-MS, Helium was used as carrier gas and injection is in splitless pulse at 100 kPA.
The oven temperature program was chosen as follows:
- 70°C during one minute
- 70°C to 200°C (25°C/min)
- 200 to 300°C (10°C/min)
- 300°C (1min)

A simple quadruple in SIM is used for the detection. Clonidine is identified by two ions target m/z: 354 and 356. Moxonidine by two other ions ions: 366 and 401.

Results and discussion

Figure 1 shows clonidine at 13.1 minutes and internal standard at 13.3 minutes. In addition, we find that the observed concentrations are in the range of hundreds of pg/mL.
To increase the equipment sensitivity, we played on a function of the ChemStation software: the voltage applied to the detector. As the chromatogram is clean we used a voltage of + 1000V.
Then the chromatographic peaks were integrated for different concentrations. The calibration line was plotted: area ratio (clonidine moxonidine) depending on the concentration of clonidine (Figure 2).

Figure 1 : Chromatogram of Clonidine and Monxonidine in GC-MS (Helium, HP-5MS, gradient temperature, SIM)

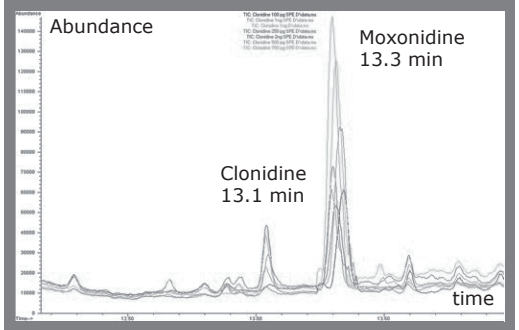
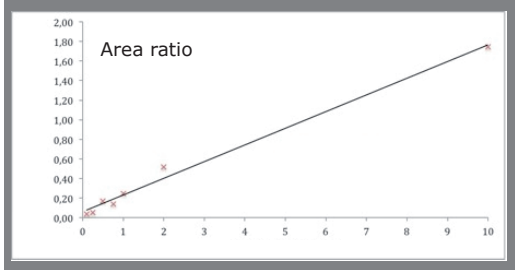


Figure 2 : Calibration of clonidine in plasma



Concentration (ng/mL)

Conclusion

Next step will consist into the method validation. We have already demonstrated that the method is repeatable.
However, the following must be tested :
- Linearity study
- Calibration function study (homogeneous variance, Student's and Fisher's test),
- Fidelity study (reproducibility),
- Accuracy

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