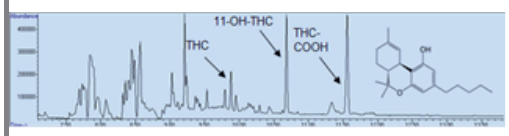
Development of a method for the determination of cannabinoids in hairs by GC-MS

Antoine BOUVET

Introduction

Hair is a very interesting matrix for the determination of drugs because it offers the possibility to detect their consumption dating back to several months, against a few hours in blood or serum and from a few days to a few weeks in urine. Cannabis is the most consumed drug in France and is often detected during roadside checks. The most important active ingredient is THC, which causes the psychoactive effects of the plant. That is why when measuring cannabinoids, molecule to be identified is THC. The concentration of two other compounds was determined, 11-hydroxy-tetrahydrocannabinol (11-OH-THC) and 11-nor-Δ9-tetrahydrocannabinol-carboxylic acid (THC-COOH), which are the metabolites of THC formed in the body.

Figure: Example of chromatogram and view of the THC molecule.



GC-MS : Agilent
Colum DB 5MS (length : 30 m, internal diameter : 0.25 mm and thickness film : 0.25 µm)
Vector gas : helium

Material and method

To begin, hairs are washed in two baths of dichloromethane to remove impurities. After drying, they are crushed and weighed. The analytes are added at the desired concentration, and their deuterated analogues, as internal standards, as well. A solution of sodium hydroxide (1 mol.L-1) allows the digestion of hairs. To facilitate the process of digestion tubes are heated at 80°C for 20 mn. After neutralization by hydrochloric acid, 400 µL of a buffer at pH 5.9, 4.5 mL of hexane and 500 µL of ethyl acetate are added. After stirring and centrifugation, samples are transferred into silanized glass tubes. After evaporating to dryness by heating at 30°C under nitrogen, all the hydroxide and carboxylic acid moieties are methylated, by reaction with methyl iodide in the presence of TMAH as a base, in DMSO. After 2 mn, the reaction is stopped by HCl and the analytes are extracted in isooctane.

Results and discussion

Four parameters have been studied: inter sample contamination, repeatability, reproducibility, and limit of quantification. The study of cross contamination sample showed contamination below 1 % for each of the molecules, which is acceptable. For the toxicology laboratory of the University Hospital of Dijon, the maximum coefficient of variation allowed for repeatability and reproducibility is 20 % for low values and 15 % for high values. The results for repeatability meet these requirements.
The reproducibility results are not conform for the lowest concentration, for 11-OH-THC and THC-COOH. This is in accordance with their quantification limits, which are higher than the lowest studied concentration. For THC reproducibility in the range 0.03-2 $ng.mg^{-1}$ is good and the limit of quantification of 0.05 ng.mg-1 is adequate since the expected concentrations are between 0.05 and 10 $ng.mg^{-1}$.

Conclusion

Up to now, the parameters studied showed that the method is valid for the quantification of THC. For the two other molecules the limit of quantification could be attained but with a more sophisticated technique. Furthermore, other parameters should be checked, namely upper limit of linearity and reproducibility as well.

Centre Hospitalier Universitaire Dijon

CHU de DIJON
Plateau Technique de Biologie
Laboratoire de Pharmacologie-Toxicologie
2 Rue Angélique Ducoudray
21070 Dijon