Extraction of cannabinoids from whole blood and quantitation by GC-MS

Cindy UGO

Introduction

A reliable and precise testing method for quantification of illegal drugs, such as cannabinoids, is necessary to aid traffic security and reduce car accidents.
We have undertaken a Cannabinoid testing method with the use of classic GC-MS. There are advantages to this technique including ease of use; however, blood is a complex matrix that contains interferences that prevent quantification. Therefore, several methods were tested to ascertain the best method, which was further optimized and finally validated.
Cannabinoids include: cannabis, Δ9–transtetrahydro-cannabinol or Δ 9-THC, and its metabolites, 11-OH-Δ9-THC and Δ9-THC-COOH. Each compound was quantified by a different internal dosage, in order to elucidate information about the consumption of the drug.

Experimental

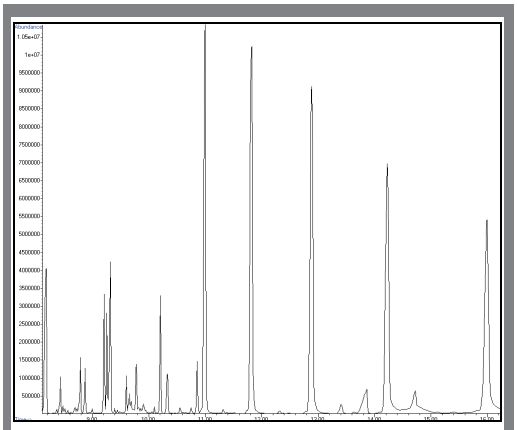
2 mixtures (1mg/L) of the 2 molecules were prepared:
- solution A: normal molecules
- solution B: counter parts, deutered and used as internal standards

Firstly, reference blood was doped with 100µL of diluted solution A, making the final concentration 100 µg/L.
Molecules were extracted from whole blood under the following conditions: 1 mL of blood + 200 µL of acetic acid + 9 mL of Hexan/Ethyl acetate (90/10) + 100 µL diluted solution A were mixed in a 12mL glass tube. The tube was agitated for 10 minutes then centrifuged for 5 minutes. The organic phase was recovered, then, 20 µL of solution B was added and evaporated under gas flow.
Molecules were derived with the addition of a derivation agent to the mixture: 40 µL of BSTFA-1%TMCS [N,O-bis(trimethylsilyl) trifluoroacetamid]-[trimethylchlorosilan]. The mixture was then decanted into a vial, and heated at 80 °C for 20 minutes in a heating chamber.
Lastly, the sample was analyzed by GC-MS with the following steps: heated at 60°C for 1 minute, temperature increase of 30°C/min until 295°C was reached, then held at 10 minutes at 295°C. The total analyzation time was 19 minutes

Results and discussion

The chromatogram (figure1) result of the injection displays the complexity of blood, as seen by the number of peaks. However, there was no observed interference to the target molecules. The retention times were in the same order. For the non-deutered/deutered couples, the difference was restricted, to only 2 or 3 seconds. This gap was significant: it is characteristic of the cannabinoid molecules.
The 3 mass spectra showed numerous peaks of different m/z ratio (mass to charge ratio for the detected ions), some of which were common to the 3 cannabinoid molecules. About 10 peaks were chosen and included into the chromatographic method, which required a preliminary analysis. For each compound, the 2 most abundant peaks were chosen for quantification. The table (figure1) only presents the ions of non-deutered molecules; the ions of the deutered molecules have 3 units added to the previous ratio.

The concentration calculations were made from the known quantity of introduced deutered molecules in the blood, following extraction. The yields were determined according to a standard, which was subjected to the same method, without an extraction phase. We used a ratio between the slope of the extracted scale, and the slope of the non-extracted scale, in order to minimize the errors for slight concentrations.



Compound	tr (min)	Ions
Δ9–THC	9.3	371,386
OH–THC	10.2	371,459
THC-COOH	10.8	371,473

Figure 1: Chromatogram, retention times tr and ions chosen for a cannabinoids extraction sample (100 µg/mL)

Conclusion

This method was simple and fast, but some parameters could be improved.
Given the complexity of the spectrum, a purification phase would be necessary to limit interference, particularly in judicial cases in which blood is often dirty.
When many samples were analyzed at the same time, another parameter has to be checked; pollution of the column was possible, and would engender new peaks on the following spectrum and generate interferences.

Toxicology Laboratory of Police Prefecture
2 place Mazas
75012 PARIS