Serviço de Química e Toxicologia Forenses

DETERMINATION OF BIOMARKERS OF CANNABIS CONSUMPTION IN HAIR SAMPLES: PRELIMINARY RESULTS

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Introduction

Over 96 million European adults are estimated to have consumed illicit drugs at some point in their lives, and cannabis is still the most consumed drug [1].

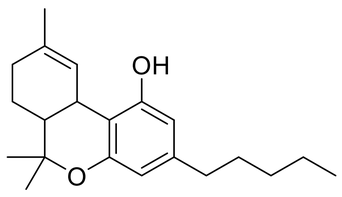
The Portuguese data is obtained using surveys, but this type of study has several disadvantages, such as under or overestimation of consumption rates, which may lead to biased conclusions.

Consequently, it is desirable that these studies are accompanied by drug monitoring in biological samples to circumvent the associated drawbacks.

Objectives

- ❑ Develop and validate an LC-MS/MS analytical methodology for representative cannabis drugs in hair samples.
- ❑ Apply the developed method to hair samples collected from students attending Portuguese universities.
- ❑ Compare the results with data obtained from self-completion questionnaires and with the reported values in official statistical bulletins.
- ❑ Identify risky groups and possible targets for preventive measures and public policies to manage drug abuse.

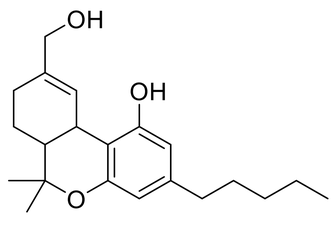
Analytes



THC

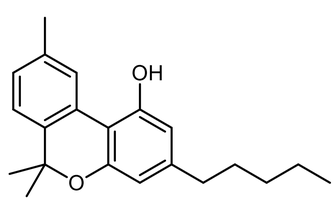
Main psychoactive substance of cannabis.

Cutoff value: 50 pg/mg [2].

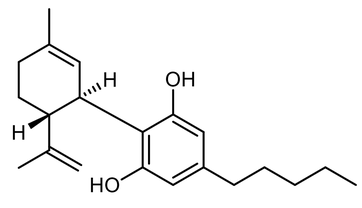


THC-OH

Main metabolite of THC.

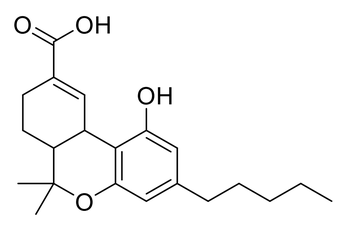


CBN



CBD

Main component of medicinal cannabis.



THC-COOH

Main secondary metabolite of THC.

Cutoff value: 0.2 pg/mg [2].

Mandatory to identify active consumption, since it is formed exclusively by metabolism [3].

This poses an analytical challenge due to the acidic nature of the compound (which causes low incorporation rates).

Highly sensitive analytical techniques are required to detect it, since this cutoff value is not reachable with a single mass spectrometer [4].

Optimization of the LC-MS/MS Parameters

- ❑ Scan type: MRM
- ❑ Polarity: Positive
- ❑ Temperature: 250 ºC
- ❑ Oven temperature: 40 ºC
- ❑ Injection volume: 20 µL

Table 1: MRM transitions and retention times for THC-OH, CBD, CBN, THC, THC-d_3 and THC-OH-d_3.

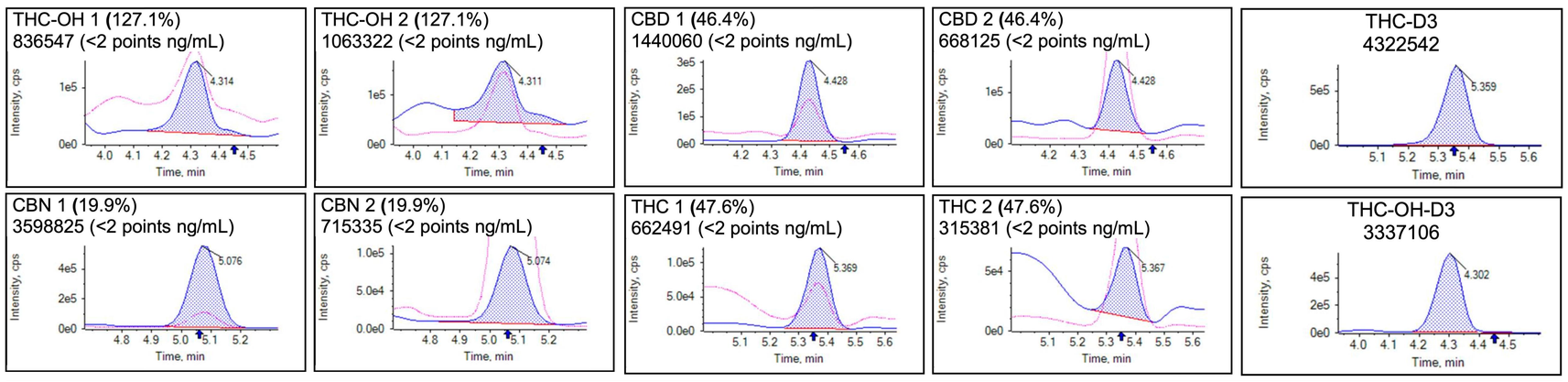
Compound	Transitions (Da)	Retention Time (min)
THC-OH	331.123 → 193.100 → 201.100	4.27
CBD	315.100 → 193.100 → 123.000	4.39
CBN	311.200 → 223.000 → 241.000	5.00
THC	315.123 → 193.000 → 123.100	5.30
THC-d_3	334.123 → 196.100	4.27
THC-OH-d_3	348.106 → 302.100	4.36

- ❑ Scan type: MS/MS/MS (MS^3)
- ❑ Polarity: Negative
- ❑ Temperature: 600 ºC [4]
- ❑ Oven temperature: 40 ºC
- ❑ Injection volume: 20 µL

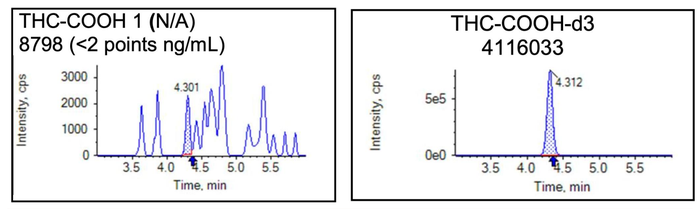
Table 2: First and second precursors and MS^3 range scanned (m/z) for THC-COOH and THC-COOH-d_3.

Compound	1st Precursor	2nd Precursor	Start (Da)	Stop (Da)
THC-COOH	343.100	299.200	244.700	245.700
THC-COOH-d_3	346.100	302.200	247.700	248.700

LC-MS/MS Results



LC-MS/MS in MRM positive mode after analyte extraction (50 pg/mg).



LC-MS/MS in MS/MS/MS (MS^3) negative mode after analyte extraction (0.2 pg/mg).

Optimization of the extraction method

20 mg of hair

Digestion: 500 µL of MeOH + 500 µL of NaOH for 25 min at 50 °C

Extraction: LLE with 4 mL of hexane:ethyl acetate (9:1, v/v)

The cutoff value of 0.2 pg/mg for THC-COOH was reached using these solvents [4].

Dissolution of the dried residues: 40 µL of pentanol:methanol with 0.1% formic acid:pure water with 0.1% formic acid (50:30:20, v/v/v).

Acknowledgements

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References

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