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Determination of Δ⁹-tetrahydrocannabinol and two of its metabolites in whole blood, plasma and urine by UHPLC-MS/MS using QuEChERS sample preparation

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Introduction

In France, as in other countries, cannabis is the most widely used illicit drug. In forensic as well as in clinical contexts, Δ⁹-tetrahydrocannabinol (THC), the main active compound of cannabis, and two of its metabolites [11-hydroxy-Δ⁹-tetrahydrocannabinol (11-OH-THC) and 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)] are regularly investigated in biological fluids for example in Driving Under the Influence of Drug context (DUID) (figure 1).
Historically, the concentrations of these compounds were determined using a time-consuming extraction procedure and GC-MS. The use of LC-MS/MS for this application is relatively recent, due to the low response of these compounds in LC-MS/MS while low limits of quantification need to be reached. Recently, on-line Solid-Phase-Extraction coupled with UHPLC-MS/MS was described, but in our hands it gave rise to significant carry-over after highly concentrated samples. We propose here a highly sensitive UHPLC-MS/MS method with straightforward QuEChERS sample preparation (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe).

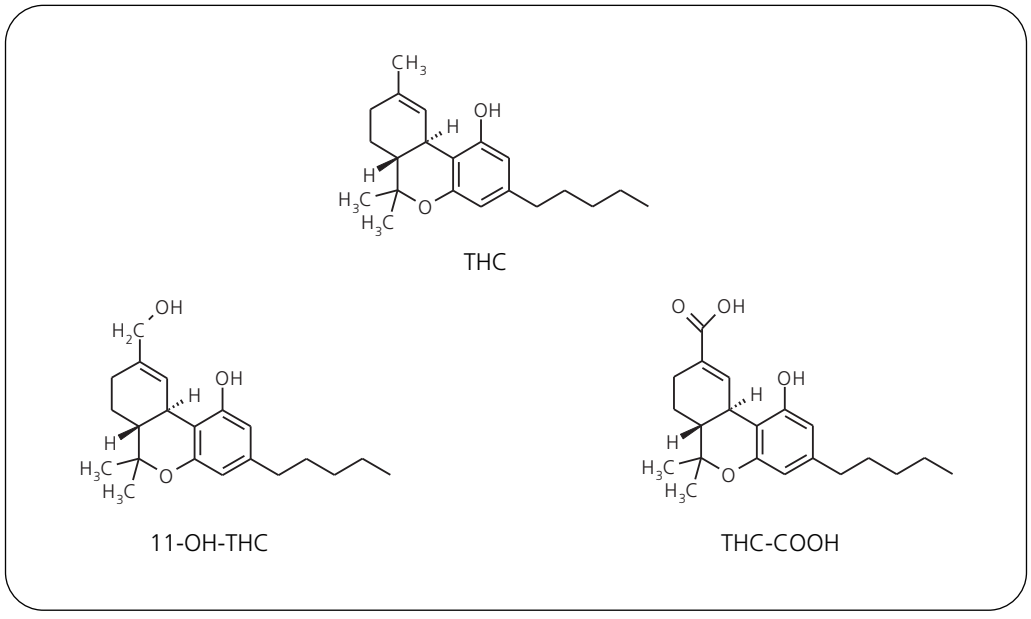

Figure 1: Structures of THC and two of its metabolites

Methods and Materials

Isotopically labeled internal standards (one for each target compound in order to improve method precision and accuracy) at 10 ng/mL in acetonitrile, were added to 100 µL of sample (urine, whole blood or plasma) together with 50 mg of QuEChERS salts ($MgSO_4$/NaCl/Sodium citrate dehydrate/Sodium citrate sesquihydrate) and 200 µL of acetonitrile. Then the mixture was shaken and centrifuged for 10 min at 12,300 g. Finally, 15 µL of the upper layer were injected in the UHPLC-MS-MS system. The whole acquisition method lasted 3.4 min.

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UHPLC conditions (Nexera MP system)

Column	: Kinetex C18 50x2.1 mm 2.6 µm (Phenomenex)
Mobile phase A	: 5mM ammonium acetate in water
B	: CH_3CN
Flow rate	: 0.6 mL/min
Time program	: B conc. 20% (0-0.25 min) - 90% (1.75-2.40 min) - 20% (2.40-3.40 min)
Column temperature	: 50 °C

MS conditions (LCMS-8040)

Ionization	: ESI, negative MRM mode
Ion source temperatures	: Desolvation line: 300°C Heater Block: 500°C
Gases	: Nebulization: 2.5 L/min Drying: 10 L/min

MRM Transitions:

Compound	MRM	Dwell time (msec)
THC	313.10>245.25 (Quan)	60
	313.10>191.20 (Qual)	60
	313.10>203.20 (Qual)	60
$THC-D_3$	316.10>248.30 (Quan)	5
	316.10>194.20 (Qual)	5
11-OH-THC	329.20>311.30 (Quan)	45
	329.20>268.25 (Qual)	45
	329.20>173.20 (Qual)	45
$11-OH-THC-D_3$	332.30>314.40 (Quan)	5
	332.30>271.25 (Qual)	5
THC-COOH	343.20>245.30 (Quan)	50
	343.20>325.15 (Qual)	50
	343.20>191.15 (Qual)	50
	343.20>299.20 (Qual)	50
$THC-COOH-D_3$	346.20>302.25 (Quan)	5
	346.20>248.30 (Qual)	5

Pause time	: 3 msec
Loop time	: 0.4 sec (minimum 20 points per peak for each MRM transition)

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Results

Chromatographic conditions

A typical chromatogram of the 6 compounds is presented in figure 1.

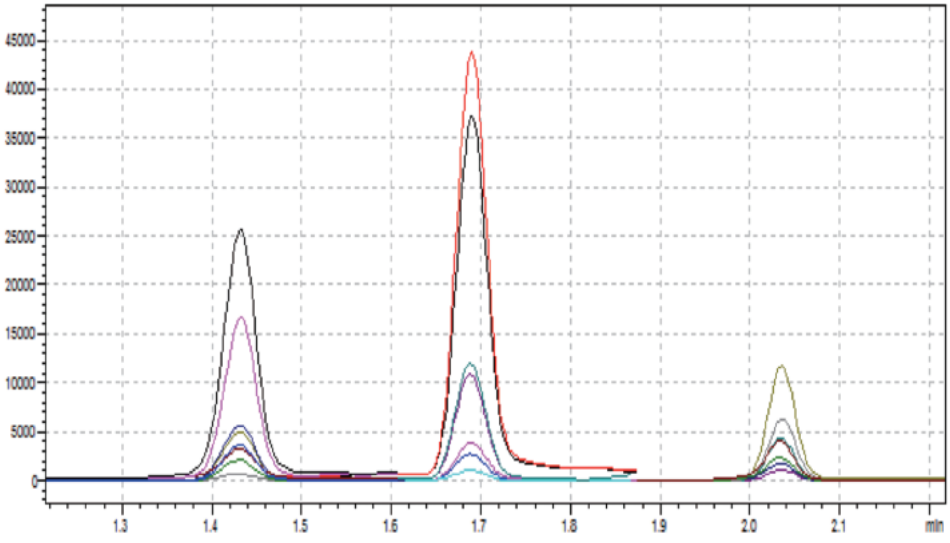

Figure 1: Chromatogram obtained after an injection of a 15 µL whole blood extract spiked at 50 µg/L

Extraction conditions

As described by Anastassiades et al. J. AOAC Int 86 (2003) 412-31, the combination of acetonitrile and QuEChERS salts allowed the extraction/partitioning of compounds of interest from matrix. This extraction/partitioning process is not only obtained with whole blood and plasma-serum where deproteinization occurred and allowed phase separation, but also with urine as presented in figure 2.

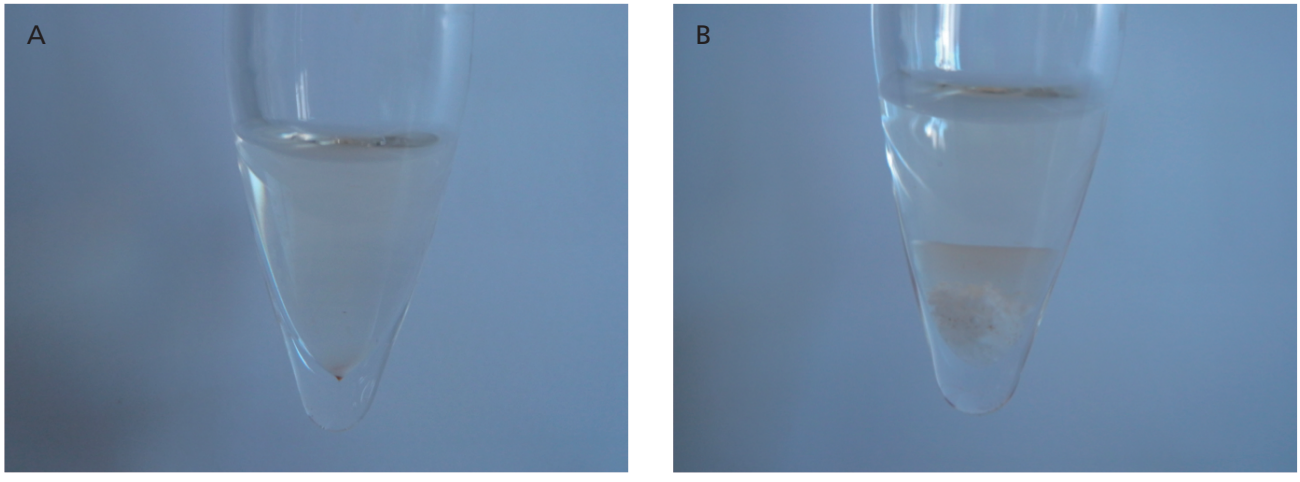

Figure 2: influence of QuEChERS salts on urine extraction/partitioning: A: acetonitrile with urine sample lead to one phase / B: acetonitrile, QuEChERS salts and urine lead to 2 phases.

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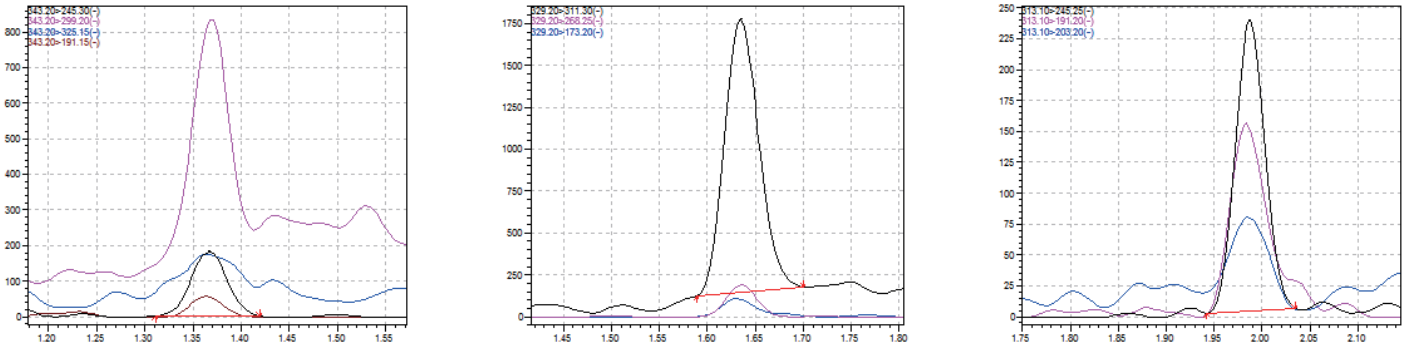
Validation data

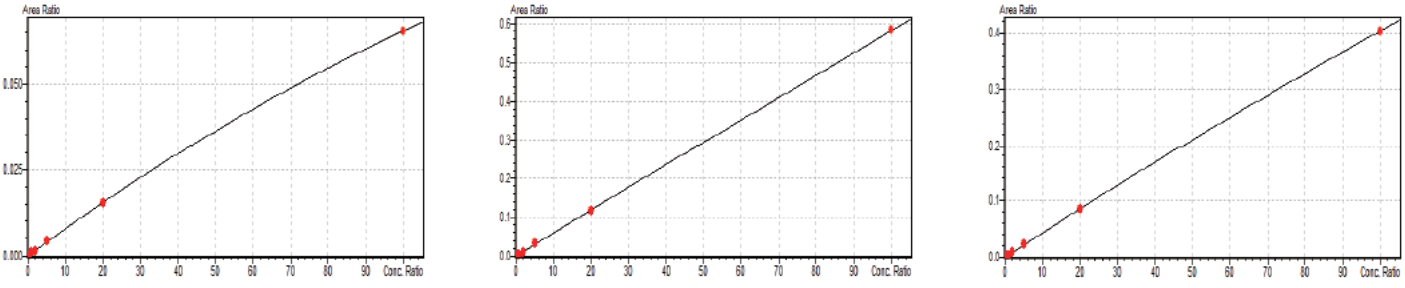
One challenge for the determination of cannabinoids in blood using LC-MS/MS is the low quantification limits that need to be reached. The French Society of Analytical Toxicology proposed 0.5 µg/L for THC et 11-OH-THC and 2.0 µg/L for THC-COOH. With the current application, the lower limit of quantification was fixed at 0.5 µg/L for the three compounds (3.75 pg on column). The corresponding extract ion chromatograms at this concentration are presented in figure 3.



THC-COOH 11-OH-THC THC

Figure 3: Chromatogram obtained after an injection of a 15 µL whole blood extract spiked at 0.5 µg/L (lower limit of quantification).

The upper limit of quantification was set at 100 µg/L. Calibration graphs of the cannabinoids-to-internal standard peak-area ratios of the quantification transition versus expected cannabinoids concentration were constructed using a quadratic with 1/x weighting regression analysis (figure 4).



THC-COOH 11-OH-THC THC

Figure 4: Calibration curves of the three cannabinoids

Contrary to what was already observed with on-line Solid-Phase-Extraction no carry-over effect was noted using the present method, even when blank samples were injected after patient urine samples with concentrations exceeding 2000 µg/L for THC-COOH.

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Conclusions

- Quick sample preparation based on QuEChERS salts extraction/partitioning, almost as short as on-line Solid Phase Extraction.
- Low limit of quantification compatible with determination of DUID.
- No carry over effect noticed.

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