DoCoLab

UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

Fast quantification of THCA using microwave-accelerated derivatisation and GC-MS/MS analysis

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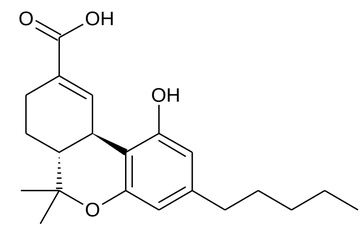


INTRODUCTION AND AIM

The psychoactive substances from *Cannabis sativa* are amongst the most widely used illicit drugs in the world. Considering the various effects of cannabis, there is a **need for rapid and sensitive detection methods in many fields**: workplace drug testing, clinical and forensic toxicology, and the fight against doping.

Although they have limited potential to improve athletic performance, cannabinoids allow the athlete to relax and escape from pressure. Additionally, the use of cannabis reduces alertness and quick reflexes, making it dangerous in automobile and team sports. Therefore natural (e.g. cannabis, hashish, marijuana) or synthetic ∆9-tetrahydrocannabinol (THC) are also **prohibited in competition** by the World Anti-Doping Agency (WADA).

To differentiate active use from passive inhalation, a **threshold concentration of 15 ng/mL** of 11-nor-∆9-tetrahydrocannabinol-9-carbolxylic acid (THCA) in urine is set by WADA.

Both the **limited reporting period and the limited amount of urine** available are important factors in the development of a confirmation procedure.

EXPERIMENTAL

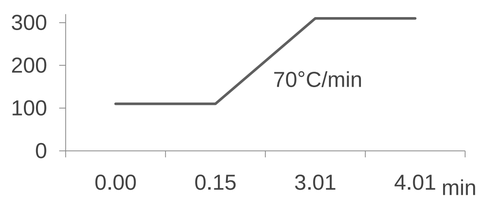
Chromatography

Agilent GC 7890 gas chromatograph
Gerstel MPS2 autosampler

Injection: PTV Solvent Vent

Volume	10 µL		
Speed	0,5 µL/sec		
Temperature		110°C	0,15 min
	12°C/min	310°C	2 min
	12°C/min	380°C	1 min
Vent flow	60 mL/min		
Vent pressure	5 psi		0,1 min

Column: HP-1MS
12 m x 250 µm and 0.25 µm film thickness



Carrier gas: He @ 3 mL/min
Transfer line: 310°C

Mass spectrometry

Agilent 7000B triple quadrupole mass spectrometer

Compound	Transition	Collision energy (eV)	Transition ratio (%)
THCA	371 -> 305	10	
THCA	371 -> 289	10	98.8
THCA	371 -> 265	10	86.8
THCA	371 -> 95	10	78.4
THCA-d9	380 -> 67	25	
THCA-d9	380 -> 101	25	35.2
THCA-d9	380 -> 84	25	50.5
THCA-d9	380 -> 292	25	43.2

QQQ collision cell:
- Quench gas: He at 2.25 mL/min
- Collision gas: N2 at 1.5 mL/min.

Microwave calibration

Domestic microwave oven (Samsung M643 – 750W)

Calorimetric methodology: [1]
-1L distilled water
- measure ΔT
- Calculation absorbed power P

$$\Delta T = \frac{P.t}{V.C_P.\rho}$$

P = microwave power (W),
t = time of heating (s),
V = volume of heated water (m³),
C_P = heat capacity (J/kg.K)
ρ = density (kg/m³).

Sample preparation

1 mL urine
+ 50 µL THCA-d9 (0.5 µg/mL)

Alkaline Hydrolysis
@ 56°C
+ 100 µL 6M NaOH
7 min

Extraction
vortex
+ 1.5 mL Acetic Acid
+ 3 mL Hexane/Ethyl acetate (9/1)
1 min

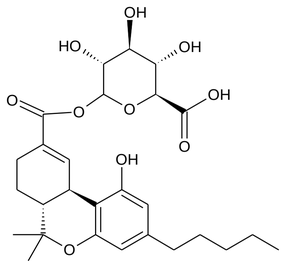
Microwave assisted derivatisation
@ 750 W
+ 20 µL ACN
+ 50 µL MSTFA
+ 50 µL MSTFA/ethanethiol/NH4I (500:4:2)
1.5 min

RESULTS AND DISCUSSION

Sample preparation procedure

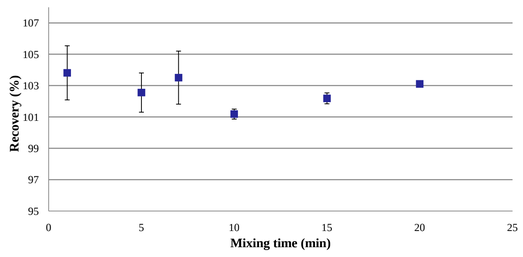
- Urine volume: The combination of large volume injection using a PTV injector and tandem mass spectrometry for higher selectivity and sensitivity allowed for using only **1 mL of urine**.

- Alkaline hydrolysis: using NaOH for complete **hydrolysis in 7 min**.



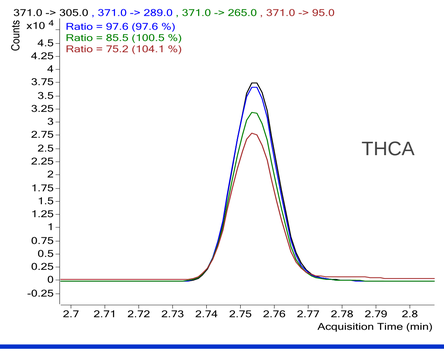
- Sample clean-up: While in the past, when using single MS technology, a preliminary extraction was needed for sample clean-up in order to obtain sufficient sensitivity, this is not longer necessary when using MS/MS.

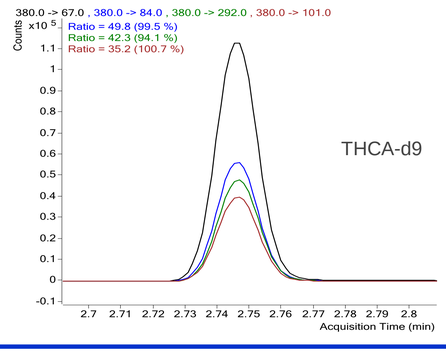
- Liquid-liquid extraction: Compared with the traditional 20min of rolling, only **1 min of vortexing** showed equivalent recoveries and hence resulted in a drastic reduction of analysis time.



- Microwave assisted derivatisation (MAD): Supplying the energy transfer by microwave irradiation instead of thermal heating can reduce the derivatisation time. The derivatisation yield after **90 sec of irradiation at 750W** is comparable to the yield of the derivatisation using 30 min of conventional heating at 80°C.

- GC-run: The GC-MS method resulted in a perfect separation of the target compound from any matrix interferences in less than **4 min**.

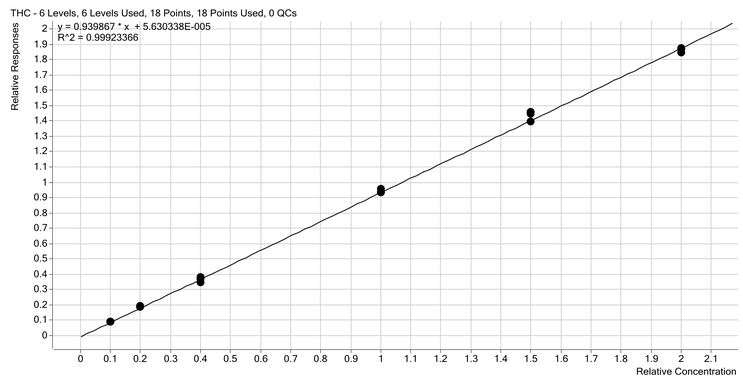




Method validation

- Calibration curves: For the quantification of THCA, calibration curves over 6 concentration levels (5, 10, 20, 50, 75 and 100 ng/mL) were constructed, using the method of least squares. Hereby the coefficient of determination R^2 was above 99%

- Accuracy & precision: At each level, precision and bias were determined out of 3 replicates. For every calibration level the obtained RSD was lower than 2/3rd of the calculated RSDmax (Horwitz). The bias was always below 15%



- WADA IDCR: Retention times did not differ more than 2% between all samples. All calculated transition ratios are in the confidence intervals imposed by WADA. Additionally the S/N ratio of the least intense diagnostic transition is calculated. On every concentration level of the calibration curve, this ratio was higher than 3 to 1 [2].

- Selectivity: Analysis of ten blank urines and urines spiked with mixtures of other WADA prohibited drugs and/or metabolites showed that there are no interferences of the urine matrix and spiked compounds with the target analytes.

- Detection limits: LOD = 0.0567 ng/mL ; LOQ = 0.1889 ng/mL
Although this is a low detection limit, it is of little importance in doping control since THCA is a threshold substance, but it does show the possibility to detect trace amounts of this compound in a biological matrix with limited sample preparation [3].

- Combined uncertainties (uc): Over 40 quality control samples, spiked at 15 ng/mL THCA, the RSD and bias were found to be 5.88% and 2.50% respectively. Using these values the combined standard uncertainty u_c resulted in 0.632 ng/mL. This is well below the maximal combined standard uncertainty $u_{c\ Max}$ of 1.5 ng/mL imposed by WADA.

Green chromatography

Sample preparation is probably the most polluting part of an analytical method because of the high quantities of organic solvents used. Special attention was paid to the impact on the environment and the associated overall cost per analysis.

Alkaline hydrolysis:
No need to adjust the pH to the optimum for the β-glucuronidases. In this way the otherwise necessary buffer solutions are avoided and the cost is reduced.

Liquid-Liquid extraction:
Only 3 mL of organic solvents are consumed and there is no need for SPE cartridges

CONCLUSION

A selective method for the quantification of THCA in urine was developed and validated. Special attention was paid to the limited volume of urine available and the total analysis time.
The use of microwave assisted derivatisation allows for a fast and complete silylation of THCA in 90 sec, whereas this step would take at least 30 min using conventional heating in an oven. In order to monitor the performance of the domestic microwave a calorimetric methodology was used.
This method can be applied as a confirmation procedure after a positive finding of THCA in a screening method and allows for the quantification is less than 30 min.

REFERENCES:
[1] F. Botre, L. Amendola, C. Colamonici, M. Mazzarino, Anal Chim Acta, 475 (2003) 125-136
[2] WADA, 2010. WADA Technical Document – TD2010IDCR, Identification criteria for qualitative assays incorporation column chromatography and mass spectometry.
[3] J. Mocak, A.M. Bond, S. Mitchell, G. Scollary, Pure Appl Chem, 69 (1997) 297-328.

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