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Fast quantification of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THCA) using microwave-accelerated derivatisation and gas chromatography-triple quadrupole mass spectrometry (GC-QqQ-MS).

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Abstract

A rapid and sensitive determination of cannabinoids in urine is important in many fields, from workplace drug testing over toxicology to the fight against doping. The detection of cannabis abuse is normally based on the quantification of the most important metabolite 11-nor-Δ9-tetrahydrocannabinol-9-carboxlylic acid (THCA) in urine. In most fields THCA needs to be present at a concentration of exceeding 15 ng/mL before a positive result can be reported.
In this paper a fast confirmation method is described to quantify THCA in 1 mL of urine. This method combines a 4 min GC-QqQ-MS method with a fast sample preparation procedure using microwave assisted derivatisation in order to complete the quantification of THCA in urine in 30 min.
The method is selective, linear over the range of 5 – 100 ng/mL and shows excellent precision, trueness, and hence. The estimated measurement uncertainty at the threshold level is small. The method also complies with applicable criteria for mass spectrometry and chromatography. Therefore the method can be used for rapid screening and confirmatory purposes.

Introduction

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 [1-3].
Although they have limited potential to improve athletic performance, cannabinoids allow the athlete to relax and escape from pressure. Since the use also reduces alertness and quick reflexes - making it dangerous in sports - natural or synthetic Δ9-tetrahydrocannabinol is prohibited in competition by WADA.
To differentiate active use from passive inhalation, a threshold concentration of 15 ng/mL of 11-nor-Δ9-tetrahydro-cannabinol-9-carbolxylic acid (THCA) in urine is set [4].
Both the limited reporting period and the limited amount of urine available are important factors in the development of a confirmation procedure.

Experimental

1 Instrumentation

An Agilent (Agilent Technologies, Palo Alto, USA) GC 7890 gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer and a MPS2 autosampler and Programmed Temperature Vaporizing injector (PTV) from Gerstel (Mülheim and der Ruhr, Germany) were used. The GC column – 12 m x 250 µm and 0.25 µm film thickness – was a HP-1MS from J&W Scientific (Agilent Technologies, USA). The used GC-QqQ conditions can be found in Table 1.
Quantification and confirmation were performed in selected reaction monitoring (SRM) mode (Table 2).
For derivatisation a domestic microwave oven (Samsung M643 -750W) was used. The effective capacity was verified with every batch.

 ISBN 978-3-86884-038-4

2 Sample preparation

1 mL of urine was spiked with 50 µL of the internal standard containing 0.5 µg/mL THCA-d9. Hydrolysis was performed by incubation for 7 min in an oven at 56°C ± 5°C after addition of 100 µL of 6M NaOH solution. After briefly cooling down 1.5 mL acetic acid and 3 mL n-hexane/ethyl acetate (9/1) were added and the mixture was extracted by vortexing for 1 min. The organic phase was evaporated under oxygen free nitrogen at 40°C ± 5°C. The dried residue was derivatised using 20 µL acetonitrile, 50 µL MSTFA and 50 µL of MSTFA/ethanethiol/NH_4I (500:4:2) in a microwave reactor at 750 W during 1.5 min.

3 Microwave calibration

The microwave calibration procedure was based upon a calorimetric methodology. In a glass beaker, 1L distilled water was heated in the microwave for 90 sec at 100 % power. The microwave power was calculated as follows [5]:

$\Delta T = (P \cdot t) / (V \cdot C_p \cdot \rho)$

In this formula P is the microwave power in Watt (W), t the time of heating in seconds, V the volume of heated water (m^3), c_p the heat capacity (J/kg.K) and ρ the density (kg/m^3).

Injection: PTV Solvent Vent				**Column**: HP-1MS			
Volume	10 µL			Parameters	12m x 250 µm x 0,25 µm		
Speed	0,5 µL/sec			Flow	He @ 3 mL/min		
Temperature		110°C	0,15 min	Temperature		110°C	0,15 min
	12°C/s	310°C	2 min		70°C/min	310°C	1 min
	12°C/s	380°C	1 min				
Vent flow	60 mL/min			Transfer line	310 °C		
Vent pressure	5 psi	until	0,1 min	Collision gas	He	2,25 mL/min	

Table 1. GC-QqQ conditions

Compound name	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

Table 2. Monitored transitions for THCA and THCA-d9 (internal standard), corresponding collision energies and transition ratios.

ISBN 978-3-86884-038-4

Results and Discussion

1 Sample preparation procedure

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. Since THCA is connected to the glucuronide using an ester bond, in its glucuronidated form, a faster alkaline hydrolysis can be used, which provides complete hydrolysis in 7 min [6]. Analysis of blank urine samples spiked at 15 ng/mL THCA using different extraction times, showed that the extraction can be shortened to 1 min of vortexing without compromising on efficiency, resulting in a drastic reduction of analysis time.
The derivatisation step required for GC-analysis is traditionally time-consuming. However, when supplying the energy transfer by microwave irradiation instead of thermal heating, this derivatisation time can be reduced. The derivatisation yield after 90 sec of irradiation at 750 W is comparable to the yield of the derivatisation using 30 min of conventional heating at 80°C. Hence, the optimized sample preparation procedure together with a GC run of only 4 min allows for a quantification of THCA in 1 mL of urine in less than 30 minutes.

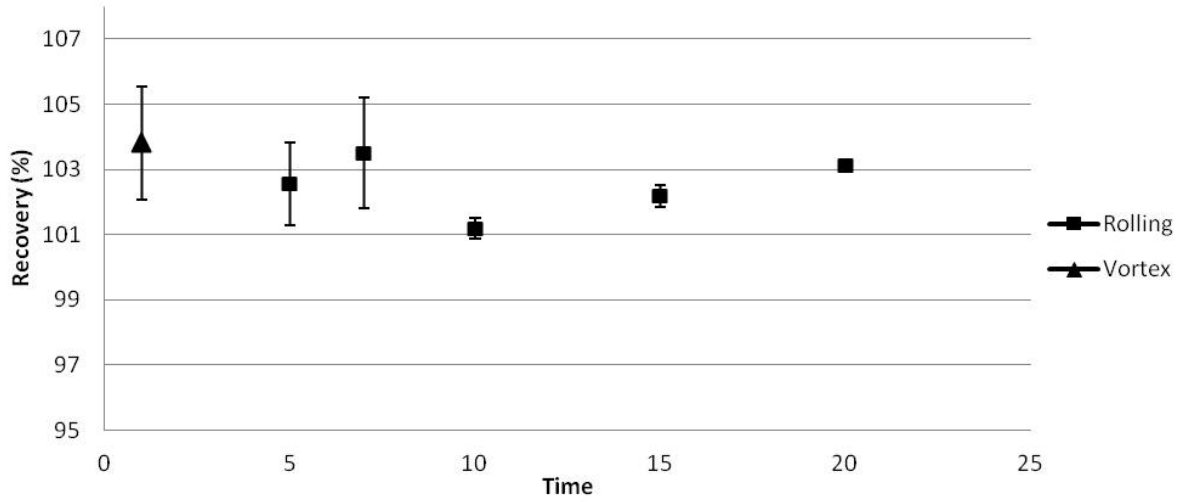

Fig. 1 Optimization extraction procedure: recoveries as a function of the mixing time for samples spiked at 15 ng/mL THCA (n=3).

2 Validation

The method is validated according to international standards [7,8]. For quantification calibration curves over 6 concentration levels (5, 10, 20, 50, 75 and 100 ng/mL) were constructed. Hereby the coefficient of determination r^2 was above 99 %. The results for precision and bias, calculated out of 3 replicates at every level, are shown in Table 3.
Over 40 quality control samples, spiked at 15 ng/mL THCA, the relative standard deviation (RSD) and bias were found to be 5.88 % and 2.50 % respectively. Using these values the calculation of 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 [4].

Conc.(ng/mL)	5	10	20	50	75	100
2/3RSDmax	23.68	21.33	19.22	16.75	15.75	15.09
RSD %	11.21	6.59	1.32	1.3	5.19	3.75
Bias %	6.87	-1.73	-6.83	-3.93	0.60	0.92

Table 3: Relative standard deviation and bias for every calibration level. The tolerances for precision were calculated following the Horwitz equation (RSDmax= 2(1-0,5logC)) and do not exceed 2/3 RSDmax.

ISBN 978-3-86884-038-4

Conclusions

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 in 1 mL of urine in less than 30 min.

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