MACHEREY-NAGEL
Chromatography application note

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Determination of cannabinoids (THC) in urine samples with GC-MS

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Abstract

This application describes the determination of cannabinoids from urine matrix, prior to GC-MS analysis.

Introduction

There is an increasing interest in the determination of cannabinoids from different matrices like urine, hair and blood for pharmacokinetic studies, drug treatment, workplace drug testing, drug impaired driving investigations, and for evaluating the time of cannabis use. Fast and sensitive procedures capable of quantifying THC and its metabolites are necessary for all this application fields.

Delta-9-tetrahydrocannabinol (THC) is the major psychoactive component of marijuana and it will be quickly metabolized to hydroxylated and carboxylated forms (THC-OH, THC-COOH) after consumption [1]. THC, THC-OH and THC-COOH are conjugated to glucuronide conjugates in human body to enhance water solubility facilitating urinary excretion [2]. Total urine cannabinoid conjugates can only be quantified by gas chromatography–mass spectrometry (GC-MS) methods after hydrolysis prior to extraction of urine sample. After a liquid-liquid extraction procedure of hydrolyzed urine sample the extract was concentrated and derivatized with MSTFA [3].

Using deuterated internal standards by GC-MS facilitates the identification and quantitation of the focused analytes in sample extracts.

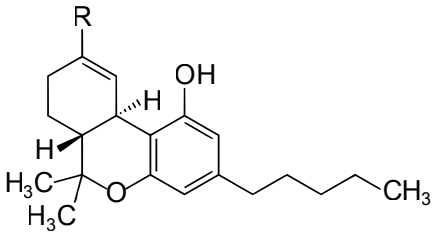

Figure 1: THC and its metabolites.

Analyte	R	Formula	M [g/mol]
THC	CH_3	$C_{21}H_{30}O_2$	314.5
THC-OH	CH_2OH	$C_{21}H_{30}O_3$	330.5
THC-COOH	COOH	$C_{21}H_{28}O_4$	344.4

Table 1: Compounds of interest.

Sample pretreatment

- Homogenize urine sample by stirring
- Fill 1 mL sample into a safe-lock tube (2 mL)
- Add standard solution, internal standard solution like described in table 2
- Add 50 µL of β-glucuronidase solution (β = 2500 units/mL) and 125 µL phosphate buffer with pH 6.8
- Shake mixture and incubate in a shaking bath at 37 °C over night (shaking speed 2000)
- Cool down samples at room temperature
- Add 125 µL sodium hydroxide solution (0.5 N) and 500 µL of a mixture (ethyl acetate – *n*-hexane (9+1, v+v))
- Shake vigorously for 30 sec
- Centrifuge at room temperature at 13400 rpm for 10 min
- Take up organic phase for derivatization in a vial
- Add 125 µL acetic acid (99 %) and 500 µL of a mixture (ethyl acetate – *n*-hexane (9+1, v+v)) to aqueous phase
- Shake vigorously for 30 min
- Centrifuge at room temperature at 13400 rpm for 10 min
- Add organic phase to the organic phase of first extraction procedure
- Concentrate organic phase at 40 °C under nitrogen stream to dryness
- Add 50 µL ethyl acetate and 50 µL MSTFA (REF 701270.201)
- Shake vigorously for 30 sec and incubate mixture at 80 °C for 30 min
- Take sample solution for GC-MS analysis

Spike level [ng/mL]	Volume internal standard mixture [µL]	Volume standard mixture [µL]
0	50	0 (β = 1 µg/mL)
25	50	50 (β = 1 µg/mL)
50	50	100 (β = 1 µg/mL)
100	50	40 (β = 5 µg/mL)
150	50	60 (β = 5 µg/mL)
200	50	80 (β = 5 µg/mL)
250	50	100 (β = 5 µg/mL)

Table 2: Pipette scheme for sample pretreatment.

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Subsequent analysis: GC-MS

Chromatographic conditions

Column:
Optima® 5 HT, 0.25 µm, 30 m, 0.25 mm ID (REF 726106.30)

Injection volume:
1 µL

Injection Mode:
Splitless

Injection Temperature:
250 °C

Carrier Gas:
Helium

Column Flow:
1.31 mL/min

Oven Programm:
70 °C [2 min] → [20 °C/min] → 250 °C [4 min] → [20 °C/min] → 300 °C [17 min]

MS conditions:
GCMS-QP2010plus, Shimadzu, ion source EI, scan type SIM

Tune:
Autotune

Ion Source temperature:
200 °C

Interface temperature:
250 °C

Solvent delay:
4 min

Analyt	Retention time [min]	M/Z Registered in SIM mode
Tetrahydrocannabinol (THC)	13.465	303, 371, 386
Hydroxy Tetrahydrocannabinol (THC-OH)	16.055	371, 459, 474
Carboxy Tetrahydrocannabinol (THC-COOH)	17.180	371, 473, 488
D_3-Tetrahydrocannabinol (THC-D_3)	13.440	306, 374, 389
D_3-Hydroxy Tetrahydrocannabinol (THC-OH-D_3)	16.030	374, 462, 477
D_3-Carboxy Tetrahydrocannabinol (THC-COOH-D_3)	17.160	374, 476, 491

Table 3: M/Z Registered in SIM mode for cannabinoids.

Chromatograms

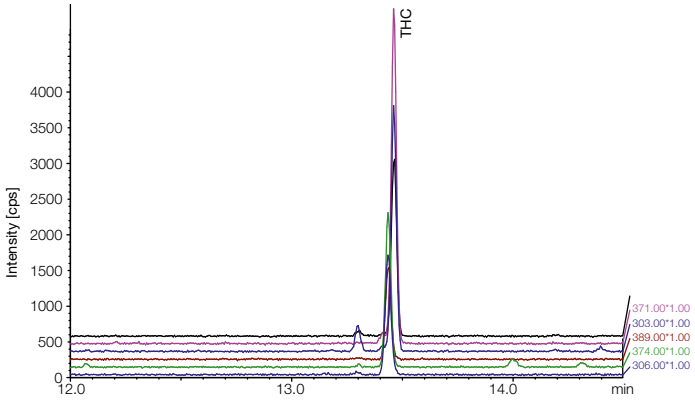

Figure 2: Chromatograms of THC and THC-D_3 from standard solution (β = 100 ng/mL).

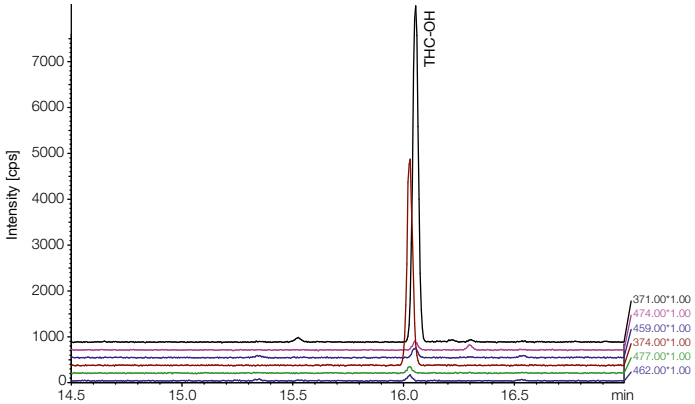

Figure 3: Chromatograms of THC-OH and THC-OH-D_3 from standard solution (β = 100 ng/mL).

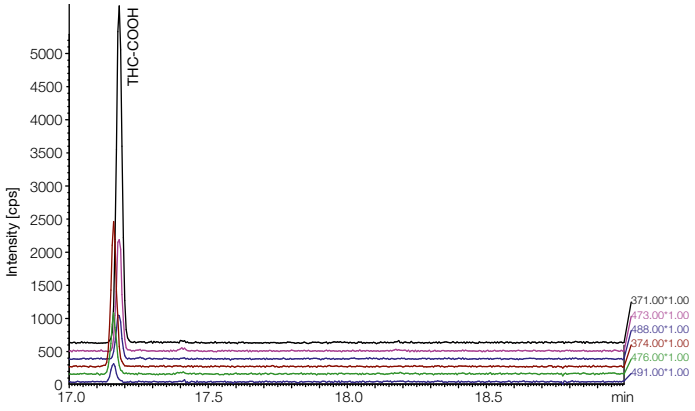

Figure 4: Chromatograms of THC-COOH and THC-COOH-D_3 from standard solution (β = 100 ng/mL).

Determination of cannabinoids (THC) in urine

Chromatograms (cont.)

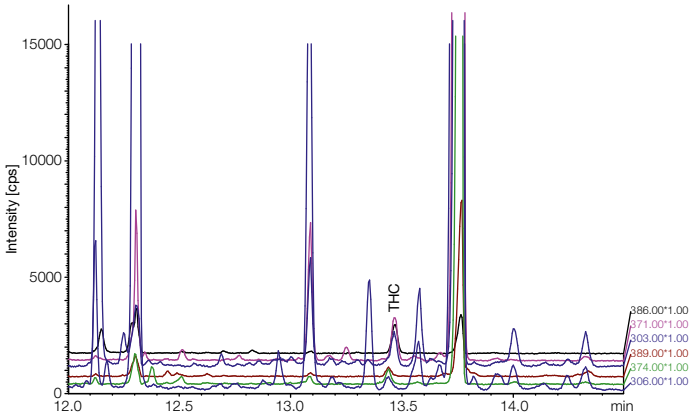

Figure 5: Chromatograms of THC and THC-D_3 from spiked urine sample (β = 150 ng/mL).

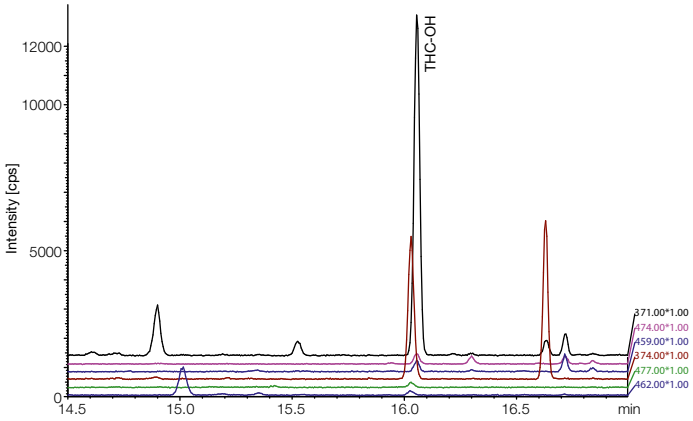

Figure 6: Chromatograms of THC-OH and THC-OH-D_3 from spiked urine sample (β = 150 ng/mL).

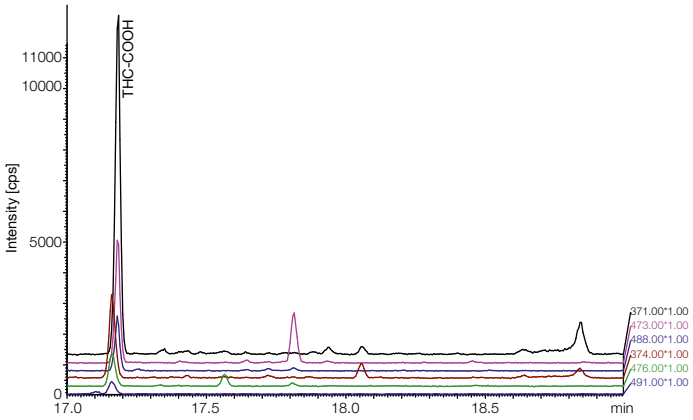

Figure 7: Chromatograms of THC-COOH and THC-COOH-D_3 from spiked urine sample (β = 150 ng/mL).

Calibration curves

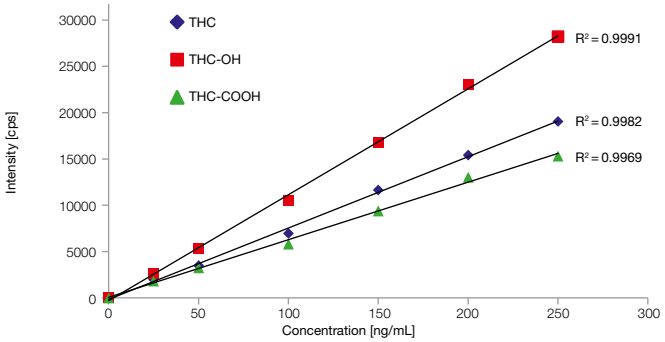

Figure 8: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from standard solutions.

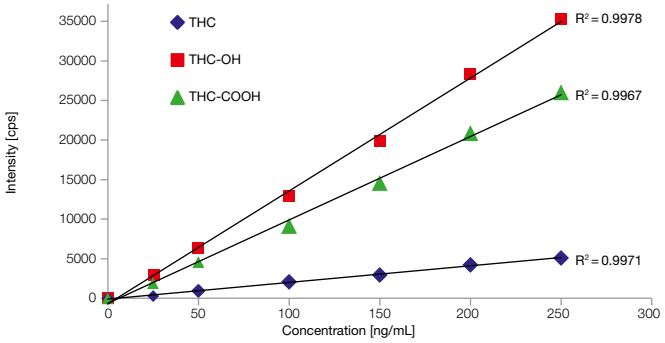

Figure 9: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from urine samples.

Conclusion

This application note shows that the determination of cannabinoids from urine samples could be carried out successfully with all the tested products. The calibration curve from standard solution and urine samples indicate good linearity with excellent correlation coefficients. Sample preparation and derivatization procedure were simple and efficient for the determination of THC and its metabolites from urine sample matrix. By using a MS detector with higher sensitivity or a solid phase extraction method, it would be possible to determine cannabinoids from urine in lower concentration levels. In summary the presented application describes a quick and convenient method for the determination of cannabinoids from urine with a simple and efficient sample preparation procedure.

References

1. Constituents of Cannabis sativa. Georg Thieme Verlag Stuttgart · New York.
2. F. Grotenhermen, Journal of Cannabis Therapeutics, Vol. 3(1) 2003, Clinical Pharmacokinetics of Cannabinoids.
3. T. Nadulski, F. Sporkert, M. Schnelle, A.M.I Stadelmann, P. Roser, T. Schefter, F. Pragst, Journal of Analytical Toxicology, Volume 29, Issue 8, 1 November 2005, Pages 782–789.

Determination of cannabinoids (THC) in urine

Additional information

The following application regarding “Determination of cannabinoids (THC) in urine samples with GC-MS” and further applications can be found on our online application database at *www.mn-net.com/apps*

GC: MN Appl. No. 215340

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 726106.30, Optima® 5 HT, 0.25 µm, 30 m, 0.25 mm ID

REF 701270.201, *N*-Methyl-*N*-trimethylsilyl-trifluoracetamid, 20 x 1 mL

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white/PTFE red

Image credits