MACHEREY-NAGEL
Chromatography application note

MN application note 07/2018

Determination of cannabinoids (THC) in plasma and serum samples with GC-MS

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Abstract

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

Introduction

There is an increasing interest in the determination of cannabinoids from different matrices like plasma or serum for pharmacokinetic studies, 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, 2]. The ratio of THC-OH to the parent compound can be used to interpret the approximate time of use. Cannabis consumption can be proofed by gas chromatography–mass spectrometry (GC-MS) methods by analyzing THC and its metabolized hydroxylated and carboxylated forms (THC-OH, THC-COOH). After a liquid-liquid extraction procedure of biological sample fluid 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.

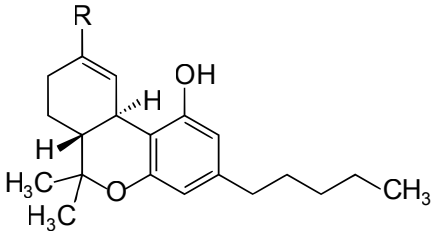

Figure 1: THC and its metabolites.

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 biological fluid sample by stirring
- Fill 0.5 mL sample into a safe-lock tube
- Add standard solution, internal standard solution as described in table 2
- Add 25 µL acetic acid (25 %) and 1000 µL of a mixture of 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 500 µL of the organic phase for derivatization in a vial
- 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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Determination of cannabinoids (THC) in plasma and serum

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

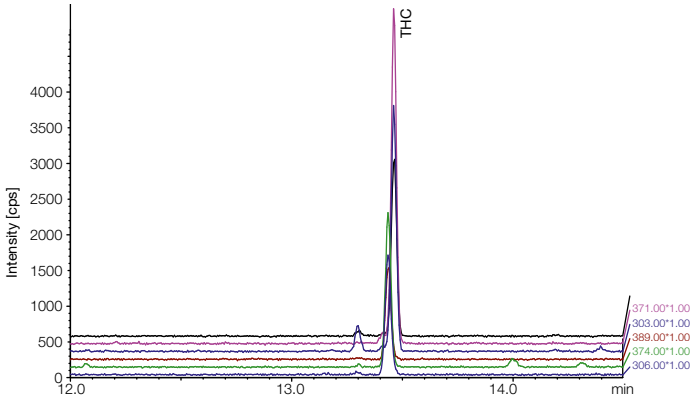

Figure 2: Chromatograms of THC and THC-D_3 from standard solution (β = 100 ng/mL).

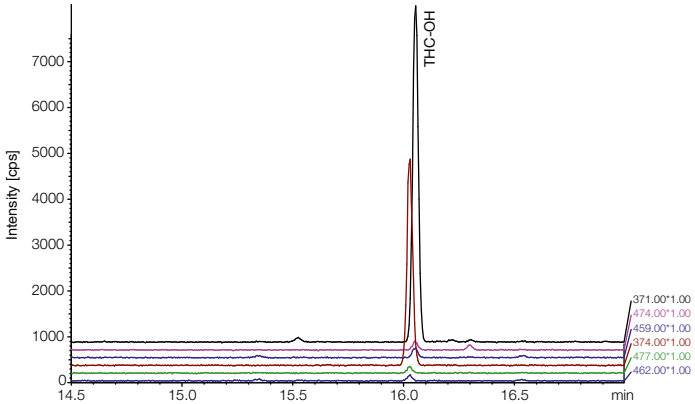

Figure 3: Chromatograms of THC-OH and THC-OH-D_3 from standard solution (β = 100 ng/mL).

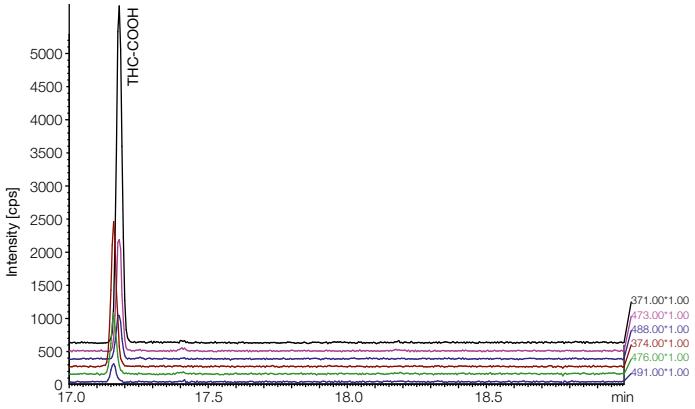

Figure 4: Chromatograms of THC-COOH and THC-COOH-D_3 from standard solution (β = 100 ng/mL).

Determination of cannabinoids (THC) in plasma and serum

Chromatograms (cont.)

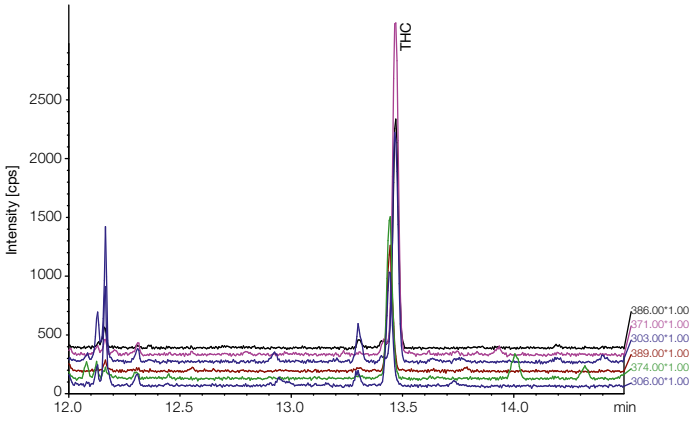

Figure 5: Chromatograms of THC and THC-D_3 from spiked plasma sample (β = 100 ng/mL).

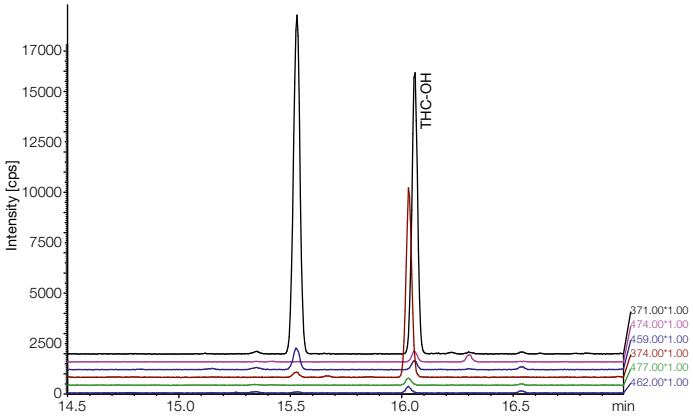

Figure 6: Chromatograms of THC-OH and THC-OH-D_3 from spiked plasma sample (β = 100 ng/mL).

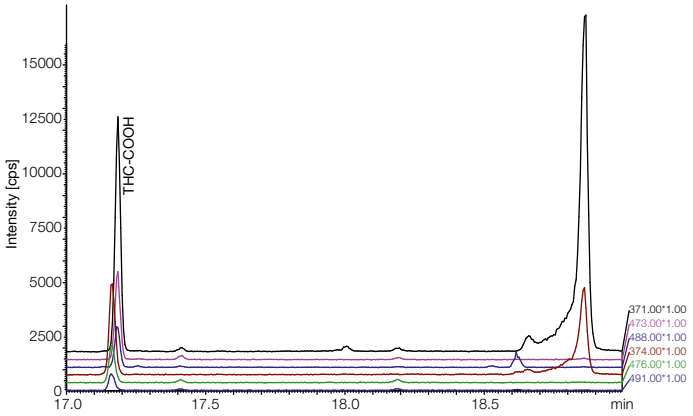

Figure 7: Chromatograms of THC-COOH and THC-COOH-D_3 from spiked plasma sample (β = 100 ng/mL).

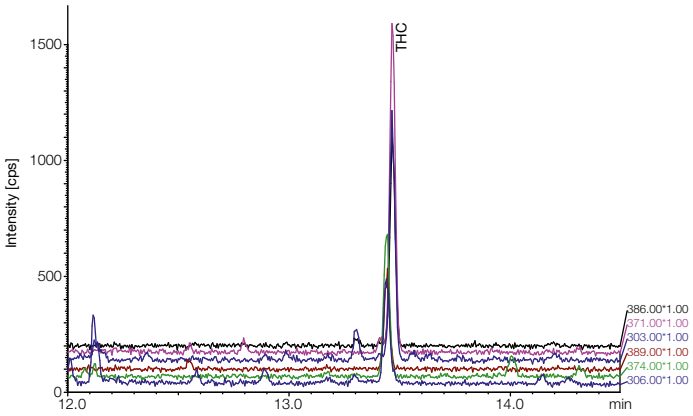

Figure 8: Chromatograms of THC and THC-D_3 from serum (β = 100 ng/mL).

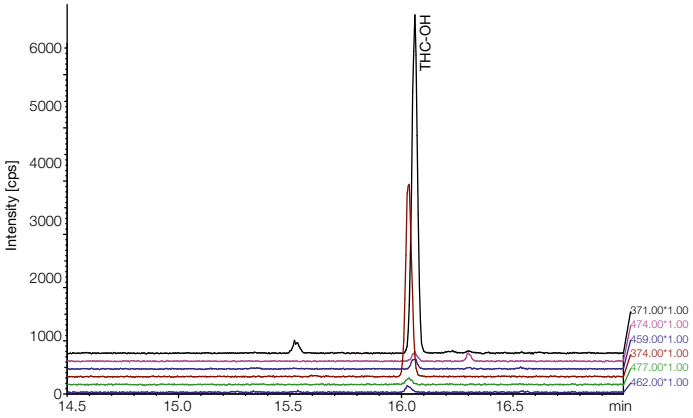

Figure 9: Chromatograms of THC-OH and THC-OH-D_3 from serum (β = 100 ng/mL).

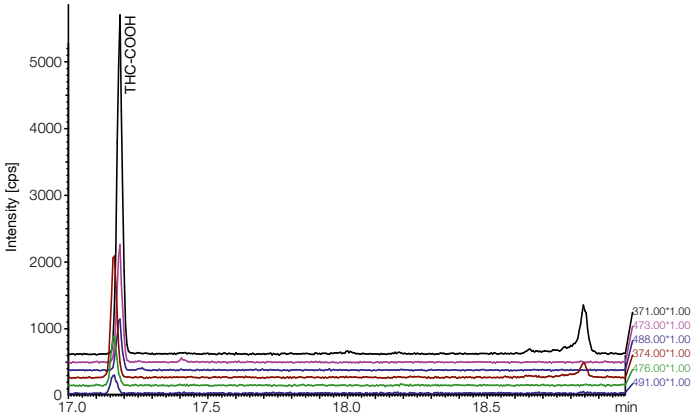

Figure 10: Chromatograms of THC-COOH and THC-COOH-D_3 from serum (β = 100 ng/mL).

Determination of cannabinoids (THC) in plasma and serum

Calibration curves

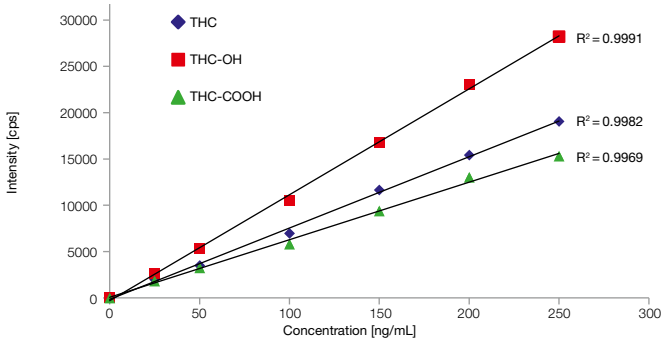

Figure 11: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from standard solutions.

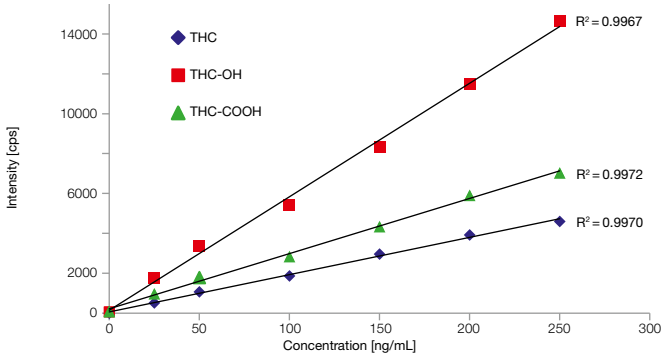

Figure 12: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from plasma samples.

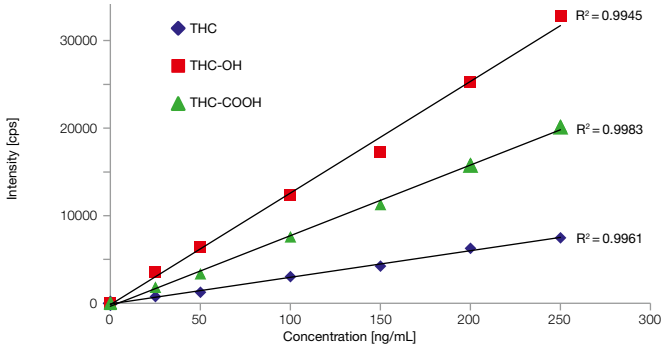

Figure 13: Calibration curves for cannabinoids in concentration range between 25 ng/mL and 250 µg/mL with an excellent coefficient of determination from serum samples.

Conclusion

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

In summary the presented application describes a quick and convenient method for the determination of cannabinoids from plasma and serum samples with 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.l Stadelmann, P. Roser, T. Schefter, F. Pragst, Journal of Analytical Toxicology, Volume 29, Issue 8, 1 November 2005, Pages 782–789.

Additional information

The following application regarding “Determination of cannabinoids (THC) in plasma and serum 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. 215350

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