MACHEREY-NAGEL
Chromatography application note

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Determination of THC in human urine

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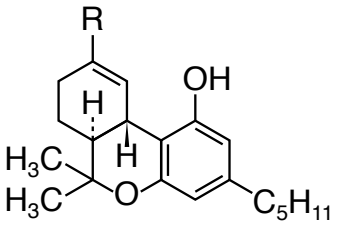
Abstract

This application note describes the determination of THC and its metabolites (THC–OH and THC–COOH) from urine in the lower ng/mL range using manual solid phase extraction or the LCTech FreeStyle™ liquid handling system. Since the target analytes are secreted as glucuronide conjugates in human urine, it is necessary to cleave the glucuronides prior to solid phase extraction. This is done by basic hydrolysis. The pretreated sample solution is then subjected to a solid phase extraction (SPE) step and finally analyzed by HPLC.

Introduction

Cannabis, also known as Marihuana or Hashish, is the most widely consumed drug in the world. Its consumption leads to mood-altering behavior like euphoria, relaxation and altered-time perception. A routinely consumption can lead to dependence and tolerance [1,2]. In recent years there is also an increasing interest in therapeutic effects of cannabinoids and the development of potential cannabinoid medications. Therefore it is investigated for treatment of chronic pain, muscle spasticity, nausea and AIDS wasting disease, for instance [1,3,4]. This also leads to an increasing demand for the development of accurate and sensitive analytical methods for the quantification of cannabinoids in biological fluids [1]. The measurement of urine cannabinoids is necessary for pharmacokinetic studies, drug treatment, workplace drug testing and for drug impaired driving investigations. Detailed urine collection procedures with comprehensive chain-of-custody documentation have been developed for forensic applications [1].

Compounds of interest



Analyte	R	Formula	Mass [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: Overview of the analytes.

Sample preparation

Sample pretreatment – alkaline hydrolysis

5 mL urine are spiked with a) 50 µL standard solution and b) 100 µL standard solution resulting in samples with a) 50 ng each of THC, THC–OH and THC–COOH and b) 100 ng each of the respective standards. The glucuronide is hydrolyzed by treating 5 mL of the spiked urine (a) c = 10 ng/mL b) c = 20 ng/mL) with 300 µL NaOH solution (10 mol/L) at 60 °C for 15 min in a heating block. The hydrolyzed solution is cooled and mixed with 200 µL glacial acetic acid. Then 2 mL ammonium acetate solution (50 mmol/L) are added and the mixture is adjusted to pH 6–7 with either acetic acid or diluted NaOH solution. The sample is transferred into a vial N 18. The hydrolysis tube is rinsed with 3 mL methanol which are combined with the sample solution.

Solid phase extraction (manual procedure)

(All steps with a flow of 1–2 mL / min)

Column type:

CHROMABOND® HR-X polypropylene columns (85 µm), 3 mL, 200 mg, (REF 730931)

Conditioning:

2 mL methanol, 2 mL water, 2 mL ammonium acetate buffer (50 mmol/L)

Sample application:

with a flow of 1–2 mL/min

Washing:

5 mL water – methanol (7:3, v/v), then drying for 10 min

Elution:

3 mL hexane – ethyl acetate – glacial acetic acid (75:25:1, v/v/v)

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Automated solid phase extraction (LCTech FreeStyle™)

Step		Volume	Dispensing speed	Waiting time after dosage	Dispense into
Conditioning	with methanol	2 mL	2 mL/min	10 s	waste
	with water	2 mL	2 mL/min	10 s	waste
	with ammonium acetate buffer (50 mmol/L)	2 mL	2 mL/min	10 s	waste
Load	quantitative transfer over sample loop from vial type 1@16 mL	12 mL	1 mL/min	25 s	
	rinse vial 3 times with water – methanol (7:3, v/v) tube rinse volume: 1 mL	1 mL	0.3 mL/min		waste
Washing	water – methanol (7:3, v/v)	5 mL	2 mL/min	35 s	waste
Drying	with nitrogen (120 s)				waste
Elution	hexane – ethyl acetate – glacial acetic acid (75:25:1, v/v/v)	3 mL	5 mL/min	15 s	vial type 1@4 mL
Drying	with air	20 mL	100 mL/min		

Table 2: Conditions of the SPE method (automated).

Eluent exchange

Eluent exchange is performed manually. Eluates from the SPE are evaporated to dryness at 40 °C under a stream of nitrogen and then redissolved in an organic solvent suited for the subsequent analysis. For HPLC-MS/MS 1 mL methanol is used.

Subsequent analysis: HPLC-MS/MS

Chromatographic conditions:

Column:

EC 50/2 NUCLEOSHELL® RP 18, 2.7 µm, (REF 763132.20)

Eluent:

A) H_2O (ultrapure) + 0.1 % formic acid; B) acetonitrile + 0.1 % formic acid; 50–100 % B in 2.5 min, 100 % B for 2.5 min, 100–50 % B in 0.1 min, 50 % B for 2.4 min

Flow rate: 0.3 mL/min

Temperature: 40 °C

Injection volume: 5 µL

MS conditions:

API 3200, ion source ESI, positive ionization mode

Curtain gas 20 psig, ion spray voltage 5500 V, temperature 550 °C, nebulizer gas 20 psig, turbo gas 20 psig, CAD 6.0 psig

MRM transitions

Analyte	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
THC	315.2	193.2	123.1
THC–OH	331.2	313.3	43.1
THC–COOH	345.2	327.3	299.4

Table 3: MRM transitions for the analysis of THC and THC derivates.

Chromatograms

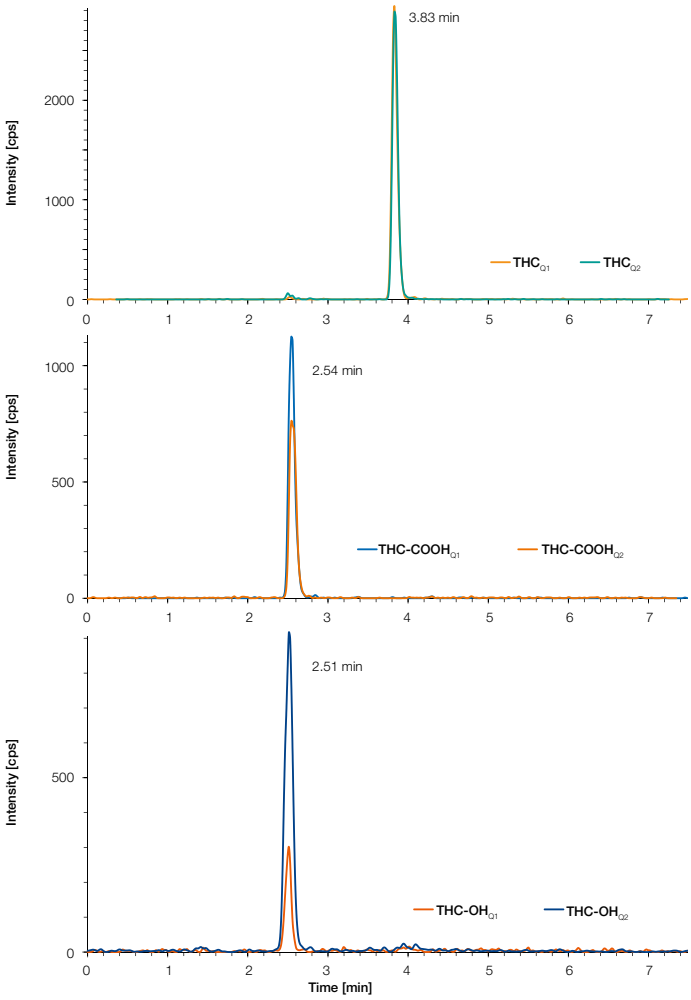

Figure 1: Chromatograms of HPLC-MS/MS analysis.

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Calibration curves

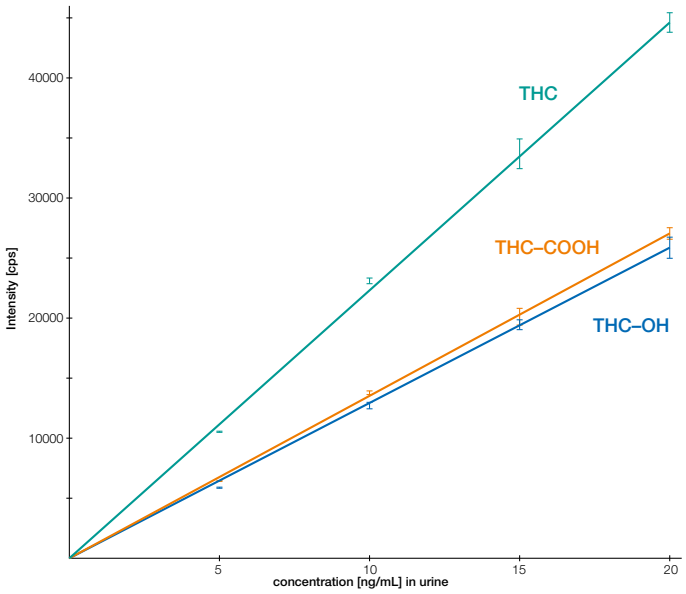

Figure 2: Calibration curves of THC and THC-derivates in urine matrix.

Recovery rates

Analyte	Recovery in % (n = 3)	Standard deviation in %
Manual SPE		
THC	80	3
THC–OH	77	10
THC–COOH	98	8
Automated SPE (LCTech FreeStyle™)		
THC	90	9
THC–OH	80	6
THC–COOH	93	8

Table 4: Recovery rates for the manual and automated SPE methods (average from triple determination).

LOD/LOQ

	THC	THC–OH	THC–COOH
LOD (ng/mL urine)	0.12	0.75	0.33
LOQ (ng/mL urine)	0.17	1.29	0.75

Table 5: Limit of quantification (LOQ) and limit of detection (LOD), the concentration that provides a signal-to-noise ratio (S/N) of > 3 was considered as LOD and S/N > 10 was considered as LOQ.

Conclusion

Using the automated FreeStyle™ SPE sample preparation system in combination with CHROMABOND® HR-X columns and a subsequent LC-MS analysis using NUCLEOSHELL® RP 18 showed reliable results for the analysis of THC and its metabolites from human urine. Using the automated system even better recovery rates were found than using a manual SPE method.

References

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Additional information

The following applications regarding “The Determination of THC in human urine” and further applications can be found on our online application database at *www.mn-net.com/apps*:

SPE (manual procedure): MN Appl. No. 305990
SPE (automated procedure): MN Appl. No. 306000
HPLC: MN Appl. No. 127380

Product information

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

REF 730931, CHROMABOND® HR-X, 3 mL, 200 mg
REF 702098, Screw neck vials N 18, 16.0 mL
REF 702284, Screw neck vials N 9, 1.5 mL
REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red
REF 702973, Screw neck vials N 13, 4.0 mL
REF 702052, N 13 PP Screw cap, black, colored top, silicone white / PTFE red
REF 763132.20, EC 50/2 NUCLEOSHELL® RP 18, 2.7 µm

Acknowledgment

We thank the company LCTech GmbH for the cooperation and for providing the robotic system, called FreeStyle™ SPE Module.