Application note

Automated determination of THC contents in human urine

Category	Sample preparation, solid phase extraction
Matrix	Human urine
Method	automated SPE, HPLC-MS
Keywords	Drugs, THC, cannabis, Freestyle, Automation, solid phase extraction
Application ID	

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Abstract

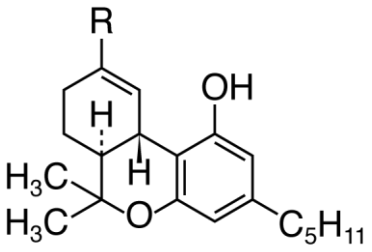
This application note describes the automated determination of THC metabolites and its derivatives from human urine in the lower ng scale. It is shown, how the analysis can be automated using the automated sample preparation system FREESTYLE™ SPE from LCTech, Dorfen, Germany in combination with CHROMABOND® HR-X SPE cartridges. The subsequent analysis was done via HPLC-MS using a NUCLEOSHELL® RP 18 column.

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 example[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].

In this application note the analysis of Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of cannabis, and its derivatives 11-Hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-Nor-Δ9-tetrahydrocannabinol COOH (THC-COOH) from human urine is shown. The time limiting factor for the lab staff, especially for high-troughput laboratories is the solid-phase extraction prior to the analysis, as all steps have to be done manually. In order to automate the whole process, an automated sample-preparation system was used, the FREESTYLE™ SPE system from LCTech, Dorfen, Germany. This system is a modular and completely automated sample preparation system that can work completely unsupervised. Every manual SPE method can be fast and easily transferred to the FREESTYLE™ system. In combination with CHROMABOND® HR-X columns a completely automated sample preparation with excellent recovery rates was established for the analysis of THC and its derivatives from human urine. The subsequent analysis was done using HPLC-MS with a MACHEREY-NAGEL NUCLEOSHELL® RP 18 column for fast and excellent results.

Figure 1 shows the molecular structures of THC and its derivatives THC-OH and THC-COOH.



Analyte	-R	Formula	Mass (g/mol]
THC	$-CH_3$	$C_{21}H_{30}O_2$	314,5
THC-OH	$-CH_2-OH$	$C_{21}H_{28}O_4$	330,5
THC-COOH	-COOH	$C_{21}H_{30}O_3$	344,4

Figure 1:
Molecular structures of THC and its derivatives THC-OH and THC-COOH

Materials

All used solvents and chemicals are of p.A. grade (≥99.0%), all solvents used for the HPLC-MS analysis are of ULC/MS grade.

Experimental sample preparation

Samples of human urine are spiked with a standard solution to get defined concentrations of THC, THC-OH and THC-COOH. Therefore the standard solutions of THC, THC-OH and THC-COOH are combined to get a standard solution that contains all of the three analytes in defined concentration.
5 mL urine are spiked with 50 µL of that combined standard solution.
All measurements were conducted in triple determination.

Table 1: Overview of the standard solutions and spiked urine sample

Solution	Concentration
Δ^9-Tetrahydrocannabinol from Lipomed THC-135-1LE (THC)	**(10 µg/mL in methanol)**
11-Hydroxy-Δ^9-tetrahydrocannabinol from Lipomed THC-318-1LM (THC-OH)	**(10 µg/mL in methanol)**
11-Nor-Δ9-tetrahydrocannabinol COOH from Lipomed THC-316-1LM (THC-COOH)	**(10 µg/mL in methanol)**
Combined standard solution	**(1x10^3 mg/mL (each))**
Spiked urine sample	**10 ng/mL (5 mL urine spiked with 50 µL standard solution)**

As THC and its metabolites are secreted as glucuronide conjugates in urine, it is necessary to cleave the glucuronide moiety before the solid phase extraction is conducted. In this case the hydrolysis was done via base to avoid the formation of glucuronides in the spiked urine samples.
300 µL NaOH solution (10 M) were given to 5 mL of the urine samples and shaken for 15 min at 60°C in a heating block. The solution is cooled to room temperature and 200 µL acetic acid are added. At last, 2 mL ammonium acetate solution (50 mM) is given to the solution and the pH is adjusted to pH 6-7 with acetic acid or NaOH solution.

Solid phase extraction (manual)

Before adapting the solid phase extraction to the automated FREESTYLE ™ SPE system, a manual method for the extraction was developed. The used conditions are given in Table 2.

Table 2: Conditions of the SPE method (manual)

Column	CHROMABOND® HR-X, 3 mL, 200 mg
Column conditioning	2 mL methanol (2 mL/min) 2 mL H_2O (Millipore) (2 mL/min) 2 mL ammonium acetate buffer (50 mM) (2 mL/min)
Loading	The sample is applied onto the column with a speed of 1-2 mL/min
Washing step	5 mL methanol - H_2O (Millipore) (V:V, 3:7) Drying for 10 min
Elution step	3 mL hexane - ethyl acetate - glacial acetic acid (V:V:V, 75:25:1)

Solid phase extraction (automated)

The manual solid phase extraction can be easily transferred to the FREESTYLE™ SPE system for an automated sample preparation. The software of the FREESTYLE™ SPE system contains pre-defined method wizards for every step making the method development very fast and easy. Up to fifteen solvents can be connected to the system.

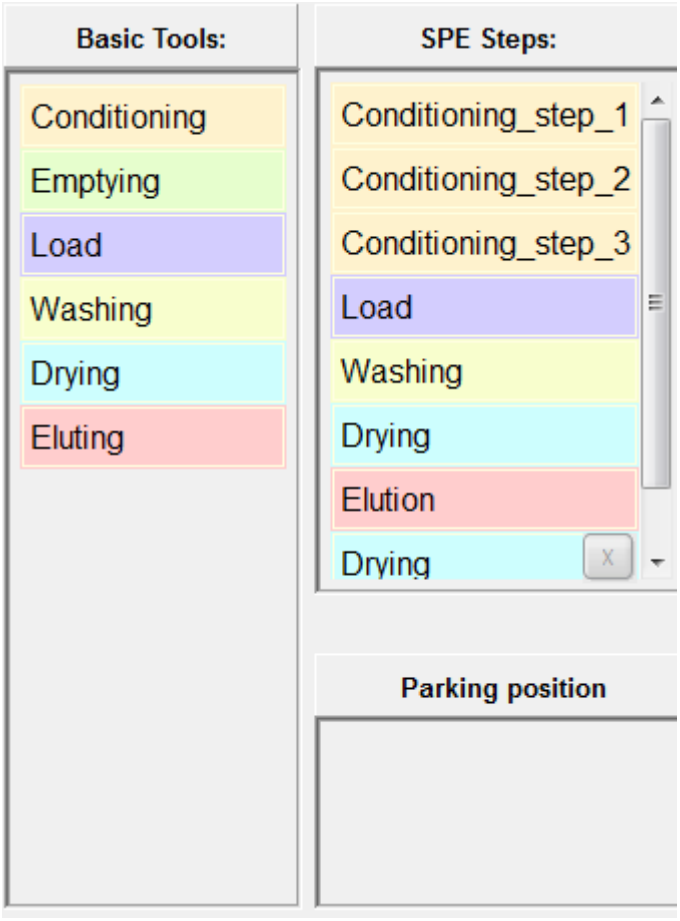


Figure 2: Predefined steps for the automated SPE method

Figure 3 shows the method wizard for the sample load step as example. For every step it can be defined which port of solvent is used, which volume of solvent is used and the speed the solvents is forced through the cartridge.

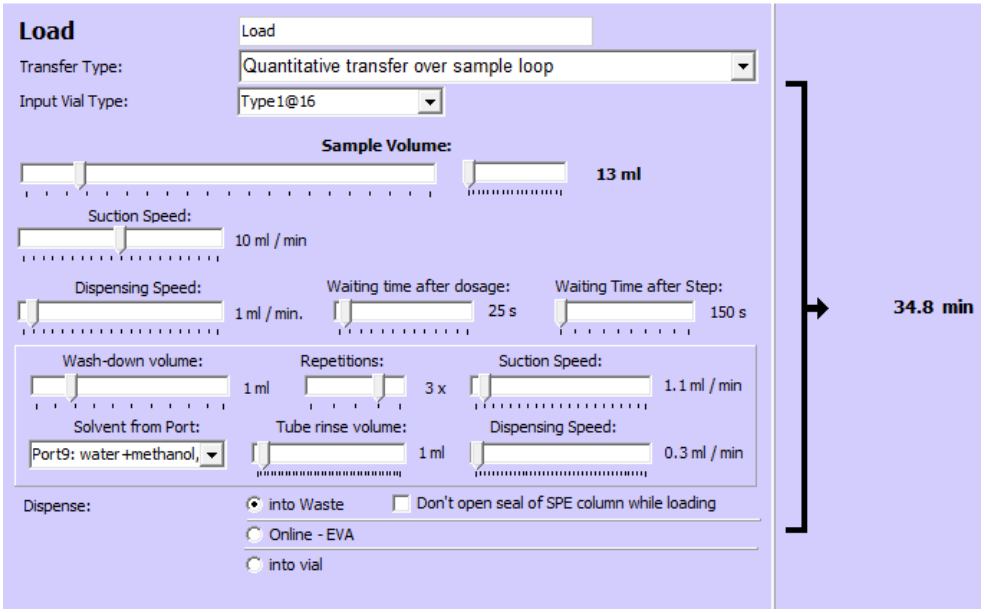


Figure 3: Software wizard for the loading step of the automated SPE method

Table 3 gives an overview over the conditions used for every step of the automated SPE method.

Table 2: Conditions of the SPE method (automated)

Column	**CHROMABOND® HR-X, 3 mL, 200 mg**
Column conditioning	**2 mL methanol (2 mL/min)** **2 mL H_2O (Millipore) (2 mL/min)** **2 mL ammonium acetate buffer (50 mM) (2 mL/min)**
Loading	**The sample is given onto the column with a speed of 1-2 mL/min**
Washing step	**5 mL methanol - H_2O (Millipore) (V:V, 3:7)** **Drying for 10 min**
Elution step	**3 mL hexane - ethyl acetate - glacial acetic acid (V:V:V, 75:25:1)**

Eluent exchange

In order to ensure that the analytes are dissolved in an HPLC/MS compatible solvent, an eluent exchange has to be performed prior to the analysis.
Therefore, the eluates from the solid phase extraction are evaporated to dryness at 40°C under nitrogen. The residue is dissolved in 1 mL methanol.

HPLC-MS/MS analysis

The subsequent HPLC-MS analysis was performed on a Dionex Ultimate 3200 HPLC system in HPG configuration with an ABSciex 3200 MS detector. The following conditions were used.

Table 4: Method for the HPLC-MS analysis

Column	**NUCLEOSHELL® RP 18, 2,7 µm, 50 x 2 mm**		
Eluent A	**H_2O (Millipore) + 0.1% formic acid**		
Eluent B	**acetonitrile + 0.1% formic acid**		
Gradient	**Time [min]**	**%A**	**%B**
	0.0	**50**	**50**
	2.5	**0**	**100**
	5.0	**0**	**100**
	5.1	**50**	**50**
	7.5	**50**	**50**
Flow rate	**0.3 mL/min**		
Injection Volume	**5 µL**		
Column Temperature	**40 °C**		
Detection	**API 3200** **Ion source: ESI** **Ionisation mode: positive** **Curtain gas: 20 psig** **Ionspray Voltage: 5500 V** **Temperature: 550 °C** **Gas 1: 20 psig** **Gas 2: 20 psig** **CAD: 6.0 psig** **MRM-Transitions**		
Run Time:	**7.5 min**		

Results

Two mass-transitions per analyte were detected as qualifier and quantifier. Figure 4 shows the two transitions for the THC-OH as example.

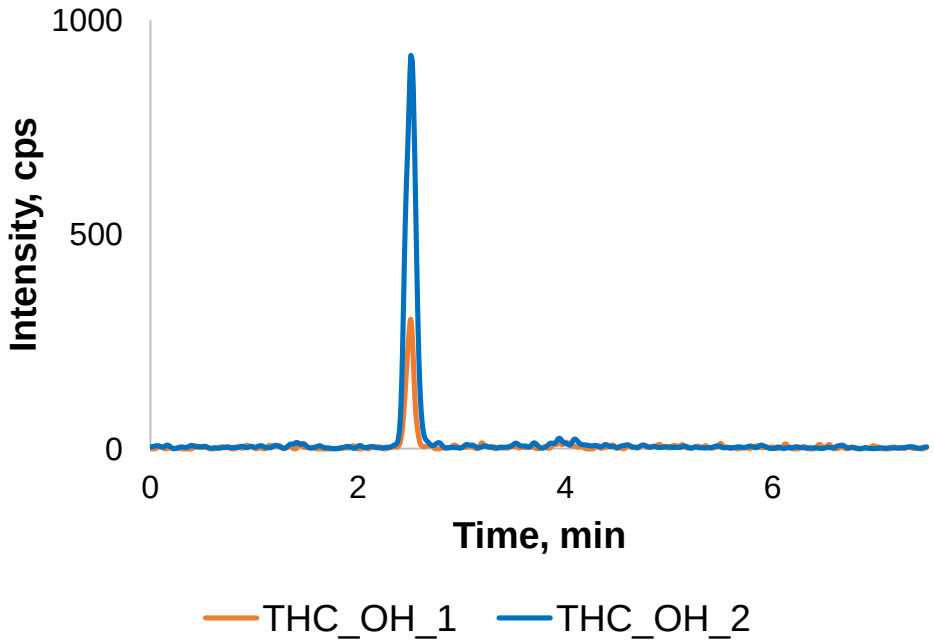


Figure 4: Chromatogram of the HPLC-MS analysis

Table 5 shows the results of the MS analysis.

Table 5: Results of the MS analysis

Analyte	$[M+H]^+$	Q1 (Quantifier)	Q2 (Qualifier)
THC	315.2	193.2	123.1
THC-OH	331.2	313.3	43.1
THC-COOH	345.2	327.3	299.4

Based on a calibration with different standard solutions, the peak areas were integrated and the recovery rates for both, the manual and the automated SPE method were calculated.
In Table 6 the recovery rates as results of a triple determination of both methods are compared to each other. It can be seen, that for the automated method even higher recovery rates than for the manual method are found.

Table 6: Recovery rates for the manual and automated SPE methods (average from triple determination)

Analyte	Recovery rate [%]	
	manual	FREESTYLE™ SPE
THC	80 ± 3	90 ± 9
THC-OH	77 ± 10	80 ± 6
THC-COOH	98 ± 8	93± 8

Conclusion

Using the automated FREESTYLE™ SPE sample preparation system in combination with CHROMABOND® HR-X cartridges 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.

Literature

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Physical properties of recommended columns

For the solid phase extraction, CHROMABOND® HR-X SPE cartridges from MACHEREY-NAGEL are used. Table 7 gives an overview over the physical properties of those cartridges.

Table 7: Properties of the recommended SPE cartridges

Stationary Phase	CHROMABOND® HR-X
Carrier material	Polymeric
Surface modification	none
Pore size	55-60 Å
Surface area (BET)	1000 m^2/g
Particle size	85 µm
Shape	Spherical
Cartridge size, amount of sorbens	3 mL, 100 mg
REF number (MN)	730931 (30 pc./pg.)

For the HPLC-MS analysis a NUCLEOSHELL® RP 18 column is recommended. In Table 8 the properties of the used column are summarized.

Table 8: Properties of the recommended HPLC column

Stationary phase	NUCLEOSHELL® RP 18
USP Code	L1
Pore size	90 Å
Surface area	130 m^2/g
%C	7.8
Endcapping	Multi-endcapping
Particle size	2.7 µm
Shape	Spherical
Dimensions	50 x 2 mm
REF Number (MN)	763132.20

Recommended Instrumentation

The automated solid-phase extraction is performed on a FREESTYLE SPE system from LCTech, Dorfen, Germany. Its modular configuration allows to set up the system tailor-made to special desires. Table 9 summarizes the modules used for this application note.

Table 9: Used modules of the FREESTYLE™ system

FREESTYLE™ Module	REF number (LCTech)
FREESTYLE™ Basic	12663
FREESTYLE™ SPE	12668
Upgrade to use 6 instead of 3 solvents	12952
Discharge function for nitrogen	12905
Rack for 60 x 4 mL sample flasks	11926
Rack for 30 x 16 mL sample flasks	11933
Rack for up to 18 SPE columns	13416
Carrier for racks, 100 mm broad	11915
Carrier for racks, 120 mm broad	12103
Lock ring for 3 mL standard SPE columns (10 pc./pg.)	12334
Plug for 3 mL SPE columns (100 pc./pg.)	13491
Screw vial, about 16 mL (100 pc./pg.)	V0016
Plug with hole (100 pc./pg.)	V0016-SL
Septa for 16 mL vials (100 pc./pg.)	V0016-D

For more information about the system click here or contact LCTech:

http://www.lctech.de/en/automated-preparation/freestyle-spe

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