Analysis of Cannabinoid in Urine by LC/MS

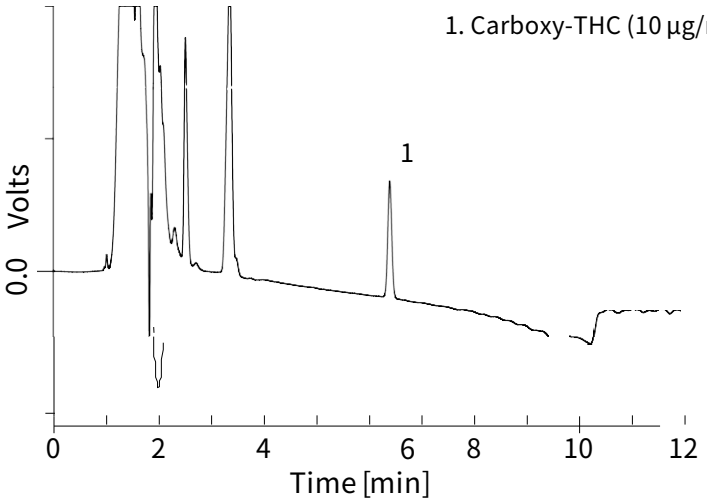
Detection and determination of 11-nor-9-carboxy-Delta 9-carboxylic acid (THCA), which is a metabolite of principal psychoactive constituent of cannabis plant, is necessary to confirm cannabis use from urine. Sample pretreatment method suitable for handling low volume and low concentration sample has been desired for the determination because THCA concentration in urine is in ppb level.

In this note, MonoSpin, which provides high recovery even from sample of less than 100 µL, was used for the sample pretreatment, and the purified solution was injected into LC/MS/MS system. The results showed good linearity, recovery, and reproducibility.

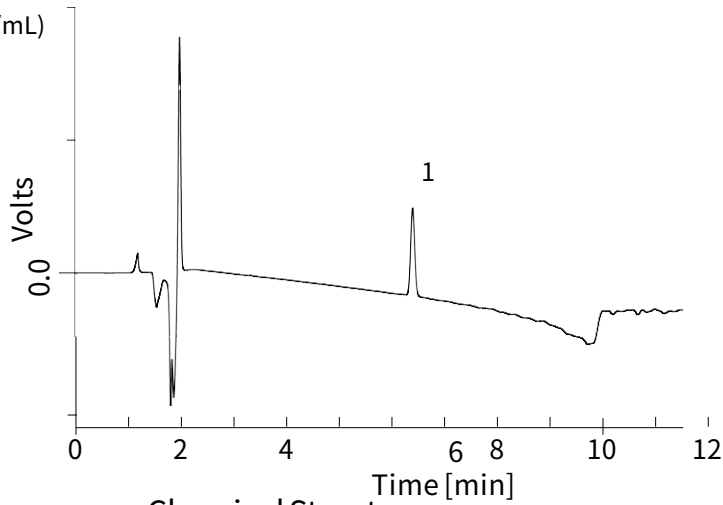
(Y. Yui and S. Ota)

Evaluation of purification by MonoSpin C18-CX (analyzed by HPLC-UV system)

＜Before purification＞



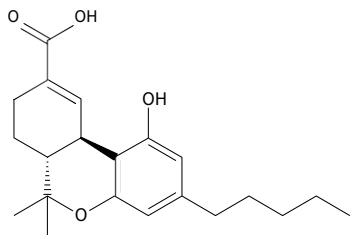
＜After purification＞



Conditions

Column	: Inertsil ODS-3 (5 µm, 150 × 4.6 mm I.D.)
Eluent:	: A) 0.1 % HCOOH in H_2O B) 0.1 % HCOOH in CH_3CN A/B = 60/40 - 10 min - 0/100, v/v
Flow rate:	: 1.0 mL/min
Col. Temp.:	: 40 °C
Detection:	: UV 214 nm
Injection Vol.:	: 10 µL
Sample:	: (±)-Carboxy-THC 10 µg/mL

Chemical Structure



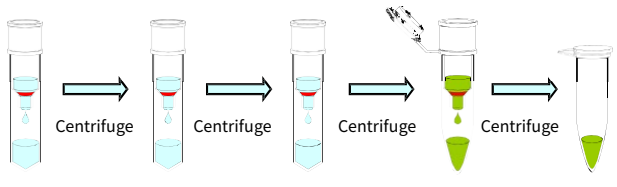
(±)-11-nor-9-Carboxy-Δ^9-THC
(THC-COOH)

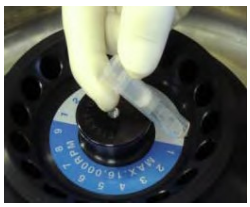
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

What is MonoSpin ?

MonoSpin is a series of spin columns for solid phase extraction (SPE). Owing to the high permeability of monolithic silica disk packed into the spin column, the procedures, such as conditioning, sample loading, washing, and elution can be carried out only by centrifuging the column. It is also the advantage that the elution volume is only 200 µL.

An Example of Procedures





GL Sciences

Sample Pretreatment using MonoSpin C18 - CX

MonoSpin C18 - CX has octadecyl group and cation - exchange group on the surface of its silica monolithic support. In this case, acidic buffer was used for sample loading. THC - COOH, which hardly dissociates under acidic condition, could be retained with hydrophobic interaction.

Sample solution		
0.1 % formic acid in water		490 µL
Sample matrix		490 µL
Standard solution	*	10 µL
+) Solution of internal standard*		10 µL
		1000 µL

* Their concentration is described in the results.

1. Conditioning
(Equilibrating)

Wash with 300 µL of mthenol and300 µL of 0.1 % formic acid in water

Centrifuge** → 2 min each

2. Sample loading

Load 200 µL of samplesolution

Centrifuge → 2 min

3. Wash

Wash with 300 µL of 0.1 % formic acid in water

Centrifuge → 2 min

4. Elution

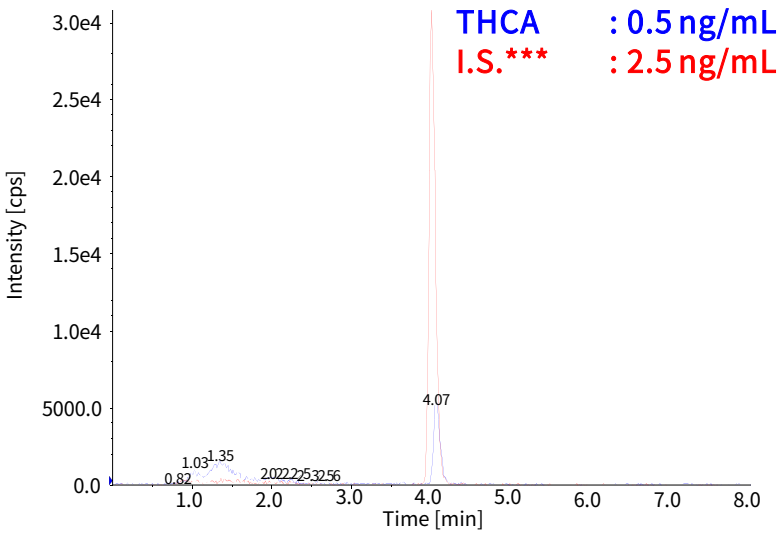
Elute with100 µL of 5% ammonia in methanol

Centrifuge → 1 min

5. HPLC Analysis

** All the centrifugation was operated at 3,000 x *g*.

Evaluation of Recovery by Standard Addition



Conditions;

System	: LC800 (GL Sciences) API3000 (AB SCIEX)
Column	: InertSustain C18 (2 µm, 100 × 2.1 mm I.D.)
Eluent	: A) 0.1% HCOOH in H_2O B) 0.1% HCOOH in CH_3CN A/B = 35/65 - 6 min - 5/95 - 4 min - 5/95 - 0.1 min - 35/65 - 5 min – 35/65, v/v
Flow Rate	: 200 µL/min
Col. Temp.	: 40 °C
Detection	: MS/MS (ESI, Nega, MRM)
Injection Vol.	: 5 µL

*** Deuterium labeled compound ((±)-11-nor-9-Carboxy-Δ^9-THC-D_9, THC-COOH-d9) was used as internal standard.

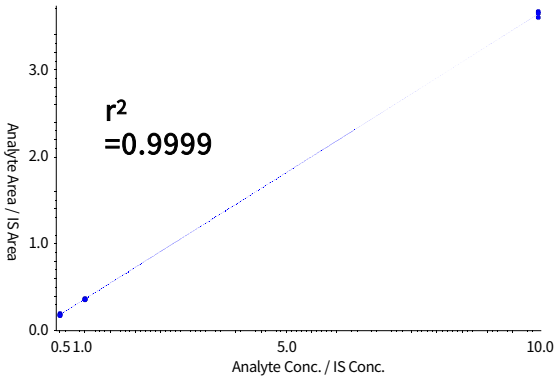
Scan Parameters

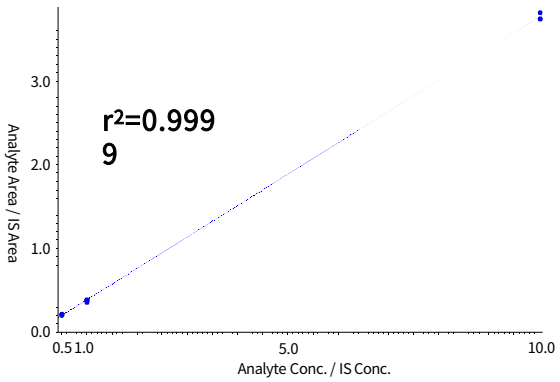
Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)
(±)-Carboxy-THC	343.0	299.0
(±)-Carboxy-THC-d9	352.1	308.1

Calibration Curves

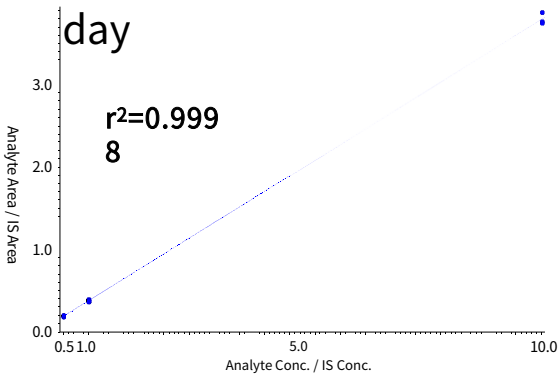
Calibration curve was plotted 4 times by conducting same experiment on different days. All the calibration curve showed good linear response (r^2 = 0.9998～1.000).

1st day 2nd day

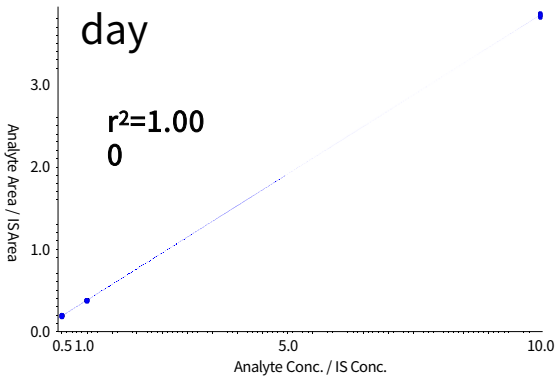




3rd day



4th day



Recovery, Reproducibility, and Accuracy

From the chromatograms, recovery, reproducibility, and accuracy were calculated for each day. Almost all results were satisfactory.

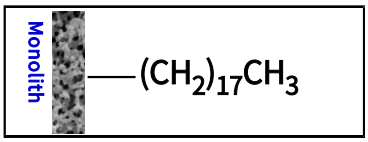
Recovery (%)	1st day	2nd day	3rd day	4th day	Inter-day average
0.5 ng/mL	86.8	94.4	87.0	88.5	89.2
1.0 ng/mL	99.0	100.8	101.4	101.7	100.7
10 ng/mL	88.1	91.1	91.6	92.8	90.9

Reproducibility (%)	1st day	2nd day	3rd day	4th day	Inter-day average
0.5 ng/mL	2.67	0.74	2.17	3.20	2.2
1.0 ng/mL	1.00	2.85	2.79	0.57	1.8
10 ng/mL	1.14	1.05	1.79	0.80	1.2

Accuracy (%)	1st day	2nd day	3rd day	4th day	Inter-day average
0.5 ng/mL	101.0	105.3	101.0	101.7	102.2
1.0 ng/mL	99.2	97.1	99.5	98.9	98.7
10 ng/mL	99.9	99.9	99.9	100.1	100.1

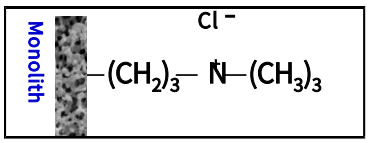
The series of MonoSpin

MonoSpin C18



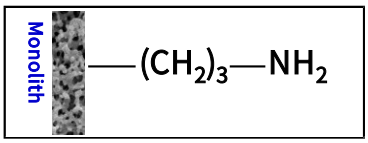
Octadecyl group is chemically bonded, and non-polar compounds can be retained because of its hydrophobic interaction. It can be used for extraction or desalting.

MonoSpin SAX



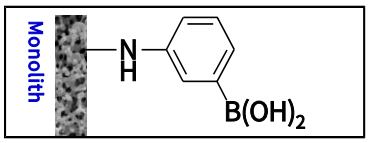
Trimethylaminopropyl group is bonded. It offers strong anion-exchange and weak hydrophobic interaction. It is suitable for extarction of acidic drugs.

MonoSpin NH_2



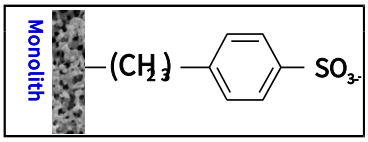
Aminopropyl group is bonded. It is suitable for extarction of hydrophilic compounds, such as sugar chain.

MonoSpin PBA



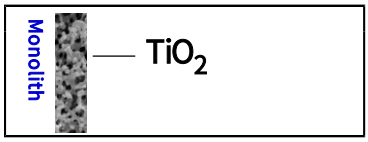
Phenylboronic acid is chemiaclly bonded. Componds containing *cis*-diol group can be retained with high selectivity.

MonoSpin SCX



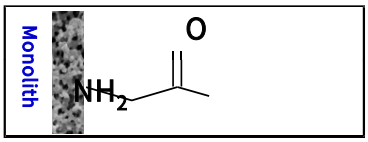
Propylbenzenesulfonic acid is bonded. It offers strong cation-exchange and hydrophobic interaction. It is suitable for extarction of basic drugs.

MonoSpin TiO



Monolithic silica is coated with titanium dioxide. It is suitable for extraction of phoshate-containing compounds.

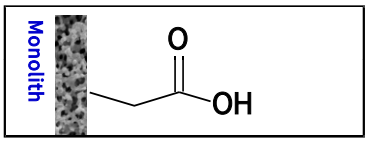
MonoSpin Amide



Amide group is bonded. Wide variety of hydrophilic compounds, such as sugar chain, can be retained and extracted in HILIC mode.

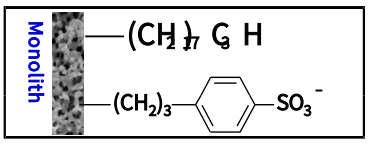
(used in this note)

MonoSpin CBA



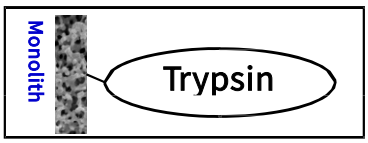
Carboxyl group is bonded to silica monolith. It is suitable for extraction of basic compounds through weak cation-exchange interaction.

MonoSpin C18-CX



Functional groups of both C18 and SCX are bonded. C18-CX is often superior to C18 or SCX in purification and clean-up of basic drugs in serum and urine.

MonoSpin Trypsin



Trypsin is immobilized on the surface of silica monolithic support. It enables rapid protein- digesting.

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