Dilute-and-shoot Determination of THC-COOH/THC-COOH-glucuronide in Human Serum by LC-MS/MS

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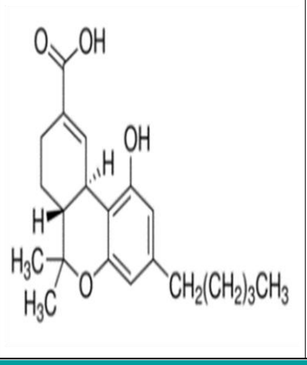
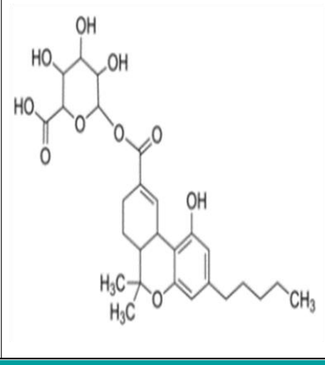
SUMMARY

Cannabis is widely used as a psychoactive drug for medical and recreational purposes. It is not uncommon for women to use it during pregnancy. Research has shown that exposure to first and second-hand cannabis smoke during pregnancy can result in negative health impacts on both baby and mother. 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) and THC-COOH-glucuronide are the major metabolites that have long half-life and are detected in higher concentration after cannabis use. Therefore, they serve as biomarkers for monitoring. In this study, we developed a dilute-and-shoot method for rapid analysis of THC-COOH and THC-COOH-glucuronide in serum using LC-MS/MS.

Analyte Structure/Property

Figure 1: Structures and properties of Analytes

Compounds	THCCOOH	THCCOO-Glu
Name	11-nor-9-Carboxy-Δ^9-THC	11-Nor-Δ9-THC-9-carboxylic acid glucuronide
Molecular Weight	344.44	520.57
Molecular Formula	C21H28O4	C27H36O10
$T_{1/2}$	5.2 to 6.2 days	2.3-10.3 days
Structure		

Method Details

100 µL of internal standard in DI was added to 100 µL of serum., followed by protein precipitation using 1.8 mL of acetonitrile containing 0.1% formic acid. The supernatant was centrifuged and filtered using 0.2 µm PTFE. 10 µL of filtrate was loaded onto a Eclipse Plus Phenyl-Hexyl column (4.6mm × 100 mm, 5µm) with guard column ZORBAX Eclipse Plus Phenyl-Hexyl, 95Å, (4.6 x 12.5 mm, 5 µm) using an Agilent 1200 LC. Detection was performed with an AB Sciex 5500 in negative MRM mode. Identification and quantitation was performed using two MRM transitions in conjunction with the retention time.

Table 1: Gradient for HPLC Method

Time(min)	B (%)	Flow Rate (µl/min)
0	40	1000
1	40	1000
2.5	75	1000
5	82.5	1000
5.01	100	1000
9	100	1000
9.01	0	1000
12	0	1000
12.01	40	1000
15.5	40	1000

Mobile Phase: A: 0.1% Formic acid B: CAN

Column Temperature: 40° C

Injection Volume: 10 mL

Table 2: Injector Program

Line	Function
1	DRAW def. amount from sample
2	WASH NEEDLE in flush port for 20 sec.
3	INJECT
4	WAIT 7.00 min.
5	VALVE bypass
6	WAIT 0.50 min.
7	VALVE mainpass
8	WAIT 0.50 min.
9	VALVE bypass
10	WAIT 0.50 min.
11	VALVE mainpass
12	WAIT 0.50 min.
13	VALVE bypass
14	WAIT 0.50 min.
15	VALVE mainpass

Method Performance

Table 3: Analyte MS Parameters and RT

Compounds	Retention time	DP*	Quant Ion			Qual Ion		
	(min)		MRM 1	CE**	CXP***	MRM 2	CE	CXP
THCCOOH	5.00	-52	343/245.2	-45	-20.0	343/190.9	-52	-14.2
THCCOO-d9	4.94	-46	352.1/308.4	-30	-8.6	352.2/254.2	-40	-20.0
THC-COO-Glu	4.30	-50	519/343	-33	-9.0	519/299	-48	-21.0
THC-COO-Glu-d3	4.28	-45	522.3/193	-24	-11.0	522.3/302	-50	-24.0

DP*: Declustering Potential CE**: Collision Energies CXP***: Collision Cell Exit Potential

Table 4: Method performance

Method performance			
Parameters	**Unit**	**THC-COOH**	**THC-COO-Glu**
Linearity	ng/mL	1 - 2000	1 - 1000
LoD & LoQ	ng/mL	1	1
Intra-day precision	%	2.5 - 10	1.6 - 7.9
Inter-day precision	%	4.2 - 7.3	4.4 - 8.3
Accuracy	%	90.8 - 118.4	86.7 - 117.8
Interference	--	No observed	No observed
Matrix effect	%	-8.6 - 2.4	-4.5 - 8.7
Recovery	%	95.1 - 106.6	97.8 - 106.2
Extract Stability	Days	> 8 days	> 8 days
No carryover observed after		2000	2000

Figure 2: Chromatography of THC-COOH and THC-COO-glucuronide

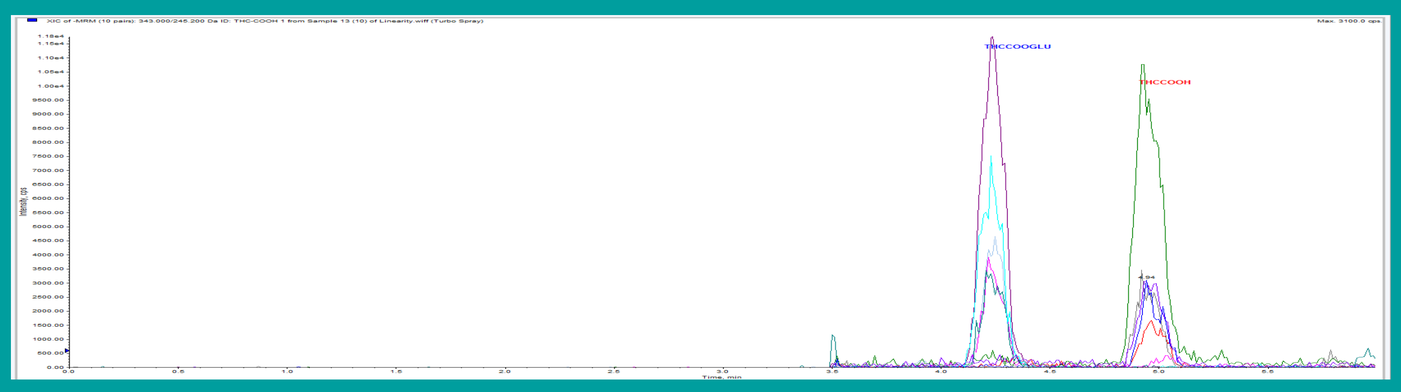
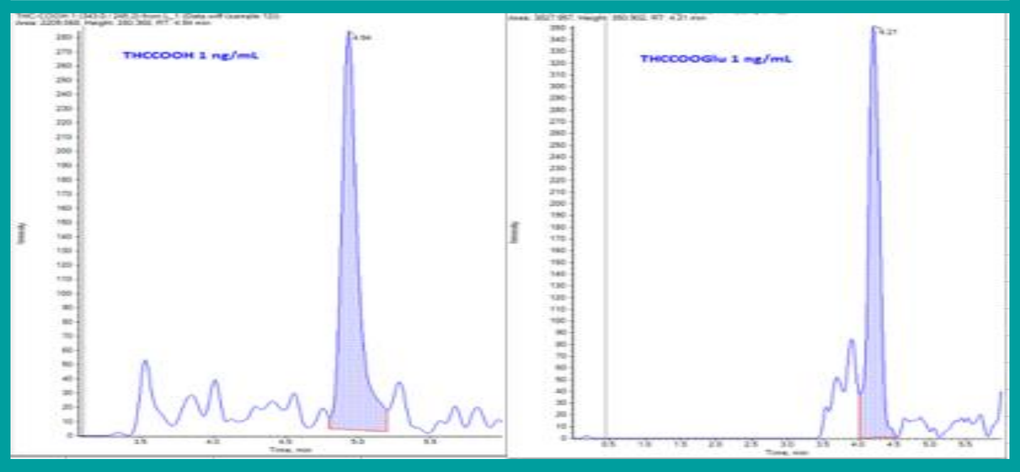


Figure 3: Extracted ion chromatography for both analytes at 1 ng/mL



CONCLUSIONS

A fast, simple, sensitive, robust and high throughput method for the determination of THC-COOH and THC-COOH-glucuronide in serum using LC-MS/MS was developed and validated. The method achieved a low LoQ for THC-COOH-glucuronide compared with other published methods. It meets the high sensitivity requirement for biomonitoring applications

ACKNOWLEDGEMENT

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