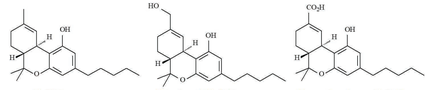
SIMULTANEOUS QUANTITATION OF Δ⁹-TETRAHYDROCANNABINOL, CANNABIDIOL AND THEIR METABOLITES IN HUMAN PLASMA COUPLED TO A HIGHLY SENSITIVE METHOD FOR QUANTITATION OF TRACE AMOUNT OF Δ⁹-TETRAHYDROCANNABINOL

Guillaume Malik, Alan Dzerk, Kirk Newland, Curtis Sheldon and Timothy Sangster

celerion
Translating Science to Medicine

INTRODUCTION AND OBJECTIVES

The rise of cannabis legalization in several countries increased the development of new therapeutics to treat conditions such as epilepsy, anxiety disorders or chronic pain. Therefore, it is highly important to develop reliable methods for the quantitation of cannabidiol (CBD), Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and their phase 1 metabolites.



A key goal for the development was to include trace level quantitation for Δ⁹-THC as many products will claim removal of the psychoactive cannabinoid. In order to achieve improved sensitivity over the published approaches, a novel derivatization was employed allowing quantitation as low of 10.0 pg/mL.

SAMPLE PROCESSING OPTIMIZATION

- Protein precipitation and Liquid-Liquid extraction gave good recovery but selectivity was poor and low concentration samples had too many interferences
- Direct purification with SPE gave good results for metabolites but poor recovery was seen for THC and CBD
- Combination of protein precipitation with SPE C18 gave the best results for all analytes tested and was optimized as described below
- Derivatization of Δ⁹-THC was performed post SPE extraction, enabling the extraction to be highly convergent

GENERAL ANALYTICAL CONDITIONS

Detector: AB SCIEX Triple Quad™ 6500

Analyte	Ions monitored (m/z)			Dwell (mSec)
CBD/THC	315.3	→	193.3	70
d_3-CBD/ d_3-THC	318.3	→	196.3	35
7-OH-CBD/11-OH-THC	331.2	→	193.1	70
d_3-7-OH-CBD/d_3-11-OH-THC	334.2	→	196.1	35
11-nor-9-OH-D9-THC	345.2	→	299.2	40
d_3-11-nor-9-COOH-THC	348.2	→	302.1	20

Column: Restek Raptor C18 2.1 x 150 mm 2.7 u
Mobile Phase (MPH) A: 100:0.25 H2O:HCOOH
Mobile Phase (MPH) B: 50:50 ACN:MeOH
Temperature: 50 °C
Flow Rate:0.5 mL/minute
Diversion is used

Time (minutes)	%A	%B
0.00	38	62
0.20	38	62
4.00	14	86
4.01	2	98
4.50	2	98
5.11	38	62
5.60	38	62

OPTIMIZED SAMPLE PROCESSING

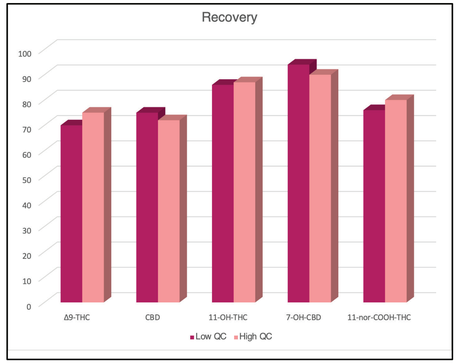
0.200 mL of plasma sample volume + 0.025 mL of IS
↓
Add 0.6 mL of ACN
↓
Vortex Plate for 5 min and centrifuge at 4500 rpm (ambient, 5min)
↓
Transfer 0.7 mL of supernatant to a new plate
↓
Dry down samples, reconstitute and load on a C18 SPE
↓
Wash and Elute with 0.7 mL ACN

Dry down and reconstitute with injection solvent

Transfer 0.150 mL of eluent
↓
Dry down and add derivatizing reagent
↓
Dry down and add injection/quenching solvent

EXTRACTION/DERIVATIZATION EFFICIENCY

- Recovery was consistent between low and high concentration samples for all analytes
- All analytes were efficiently extracted (above 70%)
- Limited matrix enhancement/suppression was observed
- The small sample volume required allowed for automation use during the extraction procedure



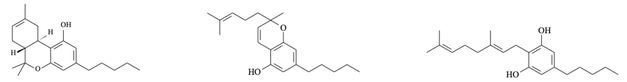
QUANTITATION RESULTS

- Quantitation at 4 QC concentrations is both accurate and precise for all analytes (only data from 1 AP batch are shown, 6 replicates for each level)
- LLOQ QCs also quantitate well except for 7-OH-CBD due to low sensitivity for this specific metabolite

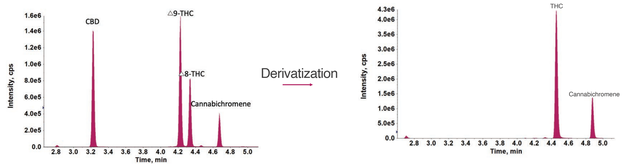
ANALYTES	VALUES	LLOQ QC	QC A	QC B	QC C	QC D
THC-Δ⁹ (0.010 - 10 ng/mL)	Mean	0.00971	0.0293	0.317	4.83	6.98
	%CV	8.5	8.1	1.7	1.8	0.5
	%bias	-2.9	-2.3	-0.9	-3.4	-5.7
CBD (0.200 - 200 ng/mL)	Mean	0.216	0.598	6.38	99.8	144
	%CV	7.1	2.7	1.9	1.4	1.8
	%bias	8.0	-0.3	-0.3	-0.2	-2.7
11-OH-THC (0.200 - 200 ng/mL)	Mean	0.194	0.578	6.34	98.9	143
	%CV	8.5	4.1	1.1	1.3	1.1
	%bias	-3.0	-3.7	-0.9	-1.1	-3.4
7-OH-CBD (0.750 - 750 ng/mL)	Mean	1.06	2.33	23.2	358	525
	%CV	17.7	8.8	1.2	2	1.3
	%bias	41.3	3.6	-3.3	-4.5	-5.4
11-nor-COOH-THC (0.250 - 250 ng/mL)	Mean	0.235	0.728	8.03	121	176
	%CV	2.3	1.1	1	1.6	1.3
	%bias	-6.0	-2.9	0.4	-3.2	-4.9

SELECTIVITY COMPOUNDS TESTED

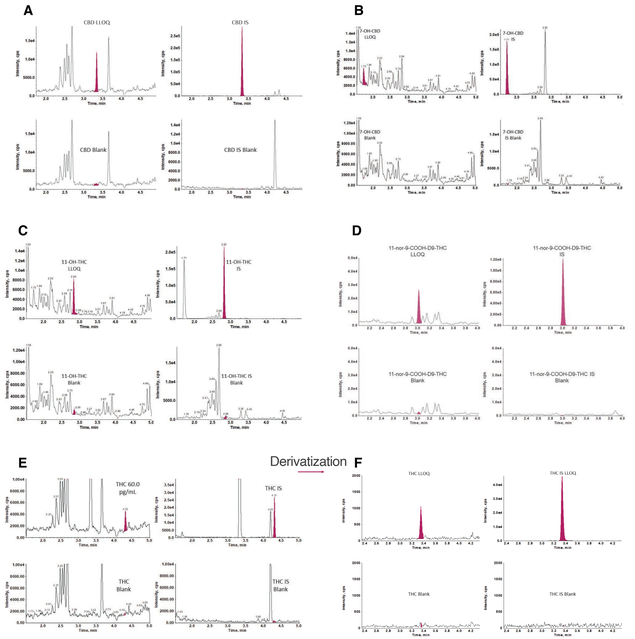
- Δ⁸-THC, Cannabichromene (isobaric metabolites) and Cannabigerol (THC,CBD mass+2) were evaluated for selectivity



- Δ⁸-THC and Cannabichromene are resolved from Δ⁹-THC and CBD (Cannabigerol does not interfere due to mass difference)
- Resolution is lost after derivatization (Δ⁸-THC and Δ⁹-THC are co-eluting, cannabichromene is still resolved after derivatization)



CHROMATOGRAPHY EXAMPLES



LLOQ chromatogram with blank overlay for A) CBD (0.2 ng/mL) ; B) 7-OH-CBD (0.750 ng/mL) ; C) 11-OH-THC (0.200 ng/mL) ; D) 11-nor-9-COOH-THC (0.250 ng/mL) E) Underivatized THC at 0.060 ng/mL (STD D) with blank overlay (S/N ratio = 3.9) ; F) derivatized LLOQ THC (0.0100 ng/mL; S/N ratio = 10.4) with blank overlay

SUMMARY AND LIMITATIONS

The method presented described the detection and accurate quantitation of Δ⁹-THC, CBD and their phase 1 metabolites. A partial derivatization post extraction (protein precipitation combined with a SPE clean up) allowed us to lower the LLOQ for Δ⁹-THC down to 10.0 pg/mL with great sensitivity. While the method is still in the development stage and requires full validation, we are in presence of a highly efficient method for a comprehensive metabolomics profile and of THC and CBD in human plasma.

One of the limitations is the non-selectivity toward Δ⁸-THC post-derivatization. The lack of resolution will however allow for the highly sensitive detection of total THC in products claiming containing only CBD as active ingredient.