Acyl Glucuronides separation method for plasma analysis by LDTD-MS/MS in less than 9 seconds per sample

WP 634

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INTRODUCTION

Acyl glucuronidation is the major metabolic conjugation reaction of most carboxylic acid drugs in mammals. Quantitation of such drugs in pharmacokinetic study (PK) is known to require attention to the inherent reactivity of acyl glucuronides. Many reactions lead to the release of the parent drug causing overestimation. Using the Laser Diode Thermal Desorption ion source for analysis of those compounds may induce thermal conversion of glucuronides to the parent drugs. Systematic sample preparation strategy has been developed to separate acyl glucuronides from compounds of interest prior to analysis.

LDTD™ Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support which is a 96 well plate (Figure 1). The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization, and is the result of the absence of solvent and mobile phase. This allows for very high throughput capabilities of 10 seconds sample-to-sample analysis time, without carry over.

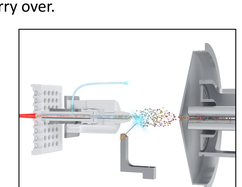


Figure 1 Schematic of the LDTD ionization source

METHOD

Human plasma was spiked with 11-*nor*-9-Carboxy-THC (THC-COOH) to generate a standard curve.

To monitor conversion effect, three QCs were prepared with the same concentration of THC-COOH (90.9 ng/mL) ;

- One with THC-COOH only
- One with THC-COOH + Low range THC-COOH glucuronide (45.5 ng/mL)
- One with THC-COOH + High range THC-COOH glucuronide (454.5 ng/mL)

Salt Assisted Liquid-Liquid Extraction (SALLE)
50 µL human plasma
100 µL satured NaCl solution
400 µL acetonitrile including internal standard (THC-COOH-d9) 25 ng/mL
Mix
Spot 4 µL on a Lazwell precoated with 8 µL EDTA solution 200 µg/mL

Table 1a SALLE extraction

Protein precipitation
50 µL human plasma
400 µL acetonitrile including internal standard (THC-COOH-d9) 25 ng/mL
Mix
Spot 4 µL on a Lazwell precoated with 8 µL EDTA solution 200 µg/mL

Table 1b Protein precipitation

Liquid- liquid extraction (LLE)	
10 µL internal standard (THC-COOH-d9) 500 ng/mL	
100 µL buffer	Acetate buffer pH 4 **OR** Phosphate buffer pH 7
50 µL human plasma	
Mix	
400 µL Hexane-Ethyl acetate mix	50: 50 **OR** 75:25 **OR** 90:10
Mix	
Spot 4 µL on a Lazwell precoated with 8 µL EDTA solution 200 µg/mL	

Table 1c Liquid-liquid extraction

LDTD parameters	
Laser power pattern	Increase laser power to 45% in 6.0 sec
	Stay at 45% 2.0 sec
	Decrease laser power to 0 % in 0.1 sec
Carrier gas flow	3 L/ min (air)

Table 2 LDTD parameters used

MS/MS parameters			
Transitions	343-245	CE:30	S-Lens:123
	343-191	CE:30	S-Lens:117
	352-254	CE:30	S-Lens:120
Mode	SRM APCI Negative		
Scan time	0.05 sec		
Discharge current	3 µA		
Instrument	Thermo Vantage MS/MS		

Table 3 MS/MS parameters used

RESULTS

Reference test uses protein precipitation. Other types of separation were tested like SALLE extraction and Liquid-liquid extraction. A method using Log D computational determination as a function of pH to find the optimal conditions was used. A series of organic solvent mixtures and buffers were tested for optimal separation changing polarity level and Log D of compounds for the liquid-liquid extraction.

Figure 4 shows software simulation of Log D as a function of pH with differences between the THC-COOH and the glucuronide form. Based on this simulation, we tested a LLE at pH 4 & 7 because the Log D difference allows extraction of the drug but not the glucuronide upon the solvent polarity.

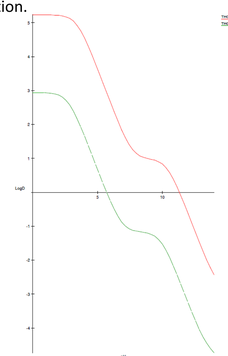
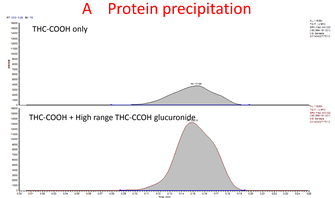


Figure 4 Log D in function of pH graph for THC-COOH and its glucuronide

Extraction type	AVERAGE THC-COOH only	Nominal %	AVERAGE THC-COOH + LOW glucoronide	Nominal %	AVERAGE THC-COOH + HIGH glucoronide	Nominal %
PROTEIN EXTRACTION	78.956	86.861	110.403	121.456	234.300	257.756
SALLE EXTRACTION	98.215	108.048	111.243	122.379	180.293	198.342
LLE 50 % HEXANE PH7	83.533	91.896	82.508	90.768	84.235	92.668
LLE 75% HEXANE PH4	96.666	106.343	93.041	102.355	93.684	103.062
LLE 75% HEXANE PH7	92.102	101.323	92.549	101.814	91.661	100.837
LLE 90% HEXANE PH4	84.975	93.482	97.373	107.122	88.525	97.387
LLE 90% HEXANE PH7	93.442	102.796	91.200	100.330	95.298	104.839

Table 4 Comparison of THC-COOH concentration obtained with different types of extraction

A Protein precipitation



B LLE pH3 90% hexane 10% ethyl acetate

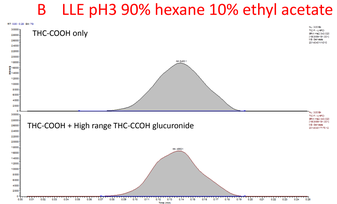


Figure 2 a-b Comparison between 2 types of extraction for the same QC

Adding to the THC-COOH sample the same amount of THC-COOH glucuronide, in pure solution, presents an in-source conversion of 34.6% at the THC-COOH transition.

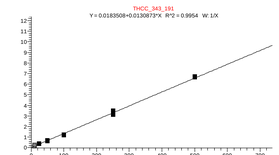


Figure 3 Typical standard curve

For Extraction procedures:

- Protein precipitation
  QC containing a high amount of glucuronide showed overestimation of **158%** of the parent drug (Figure 2A).
- SALLE extraction
  THC-COOH overestimation up to **98%** at high glucuronide concentration.
- LLE with a mixture of hexane and ethyl acetate
  QC with glucuronide showed same level of QC without glucuronide: **Efficient separation** (Figure 2B).

Further Log D simulations for carboxylic acid compounds show similar behavior between the drug and acyl glucuronide form allowing separation conditions using liquid-liquid extraction. Desorption of Glu-THC-COOH does not show the molecular mass in the spectra. This functional group does not vaporize while in its original shape. Only the parent drug is present in the mass spectra.

Tests with other glucuronides containing a phenolic bound do not show a molecular mass or a parent ion in the spectra. Thermal degradation occurs at a much higher temperature than typical laser level in most analysis. **With a regular desorption profile, no interfering conversion is observed for glucuronides on phenolic bound.**

CONCLUSIONS

• Log D software simulation of THC-COOH and its glucuronide has confirmed that the 2 analytes could be effectively separated in a liquid-liquid extraction.

• Presence of glucuronides in sample does not interfere with quantitation of parent drug using LLE instead of protein precipitation in 9 seconds LDTD-MS/MS analysis.