Validation of High-Throughput Analysis of THC in Oral Fluid using Laser Diode Thermal Desorption (LDTD) combined with Mass Spectra

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OVERVIEW

Purpose
• Ultra-Fast THC Quantitation in Saliva sample using LDTD-MS/MS

Method
• Extraction of THC in Saliva with the Intercept® device
• Quick Liquid-Liquid extraction
• Deposit of a small volume of the organic phase in LazWell plate
• Analysis using LDTD-MS/MS system

Results
• Excellent linearity over the calibration range ($R^2 > 0.99$)
• Accuracy ranging from 93.8 and 109.7%
• Precision ranging from 0.7 and 10.4 %
• Good sample stability (Wet and Dry in LazWell)
• **Samples are analyzed with a run time of 10 seconds using LDTD-MS/MS system**

INTRODUCTION

Drug testing in oral fluids is constantly evolving due to increasingly sensitive methods of detection. Testing for drugs of abuse in oral fluids can strongly benefit the criminal justice field as a less invasive and cost-effective approach for drug detection when compared to blood or urine sampling.

Oral fluid analysis is an increasingly useful and non-invasive method that has facilitated laboratory analysis for many drugs of abuse. Using the Intercept device in combination with Laser Diode Thermal Desorption (LDTD) technology, we propose to validate an ultra-fast and accurate method for THC analysis in oral fluid.

LDTD™ Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96 well plate**.** The energy is then transferred through the sample holder. The sample is dried and vaporized prior being carried by a gas to a corona discharge region for ionization. This type of ionization is characterized by a strong resistance to ionic suppression because of the absence of solvent. LDTD ionization reduces sample-to-sample analysis time to 10 seconds and allows high throughput capabilities without carry over.

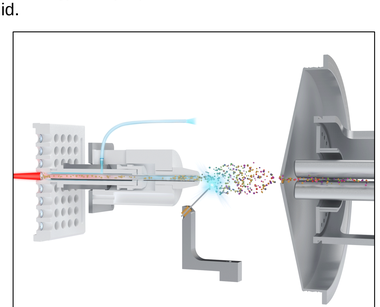

Figure 1 Schematic of the LDTD ionization source.

METHOD

Swabbing procedure

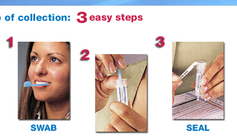

Figure 2 Oral Fluid swabbing procedure

Figure 3 Intercept® Oral Fluid Drug device

Buffer Extraction procedure
• 200 µL Oral fluid Calibrator Buffer (Glass tube)
• 20 µL Internal Standard (THC-d3, 1µg/ml in MeOH:Water (75:25))
• 400 µL EDTA Buffer (0.5M, pH8)
  • Vortex 30 seconds
• 400 µL 1-Chlorobutane
  • Vortex 30 seconds
• Centrifuge 6000 rpm / 2 minutes
• Spot 6 µL of upper phase in LazWell plate.
• Evaporate at room temperature

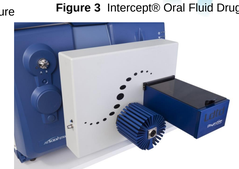

Figure 4 LDTD system on AB Sciex Qtrap 5500

Instrumentation
• Phytronix Technologies LDTD ion source (model T-960);
• AB Sciex Qtrap 5500

LDTD Parameters
• Laser power pattern :
  ➢ Increase laser power to 65 % in 3.0 s
  ➢ Decrease laser power to 0 %
• Carrier gas flow: 3 L/min (Air)

MS/MS Parameters
• MRM mode:
  ➢Negative
  ➢CE: 35
  ➢DP: 100
    ➢THC: 313 -> 245
    ➢THC-d3: 316 -> 248

RESULTS:

Linearity
As shown in **Figure 5,** excellent linearity ($r^2 > 0.99$) with no signs of carryover effect is achieved within the quantification range (1 to 1000 ng/mL).

	r^2	Slope (ratio area / concentration)	y-Intercept
Run 1	0.9986	0.0181	-0.0028
Run 2	0.9981	0.0170	-0.0076
Run 3	0.9986	0.0166	-0.0052

Table 1 Calibration Curve Parameters

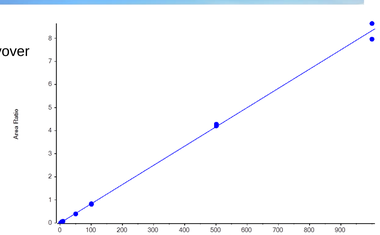

Figure 5 Typical Standard curve

Precision and Accuracy
As shown in the following **Table 2 and 3,** the intra-run and inter-run precision/accuracy, respectively:

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/mL)	1	5	100	500	1000
N	4	4	4	4	4
Mean (ng/mL)	1.02	4.58	95.78	484.29	1019.18
%RSD	10.9	1.5	9.0	3.4	0.6
%Nom	102.2	91.7	95.8	96.9	101.9

Table 2 Intra-run precision and accuracy

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	5	100	500
N	12	12	12
Mean (ng/mL)	4.77	94.48	498.02
%RSD	8.4	2.9	3.2
%Nom	95.3	94.5	99.6

Table 3 Inter-run precision and accuracy

Wet stability
Following the extraction process, all samples were stored at 4°C to evaluate wet stability. After a given time, all samples were re-spotted and analyzed. Linearity, precision and accuracy are verified for the stability run. **Table 4** shows that a wet stability of drug is obtained with good precision and accuracy at LOQ level.

Wet stability	
Time (h)	8
Temp. (°C)	4°C
Conc. (ng/mL)	1
N	4
Mean (ng/mL)	1.00
%RSD	6.16
%Nom	99.5

Table 4 Wet stability results

Dry sample in LazWell plate Stability
The stability of dry samples in LazWell plate was also verified. All standards and QCs are spotted, dried and kept in specific stability conditions. After the stability time, standards and QCs were analyzed and the linearity, precision and accuracy are verified. **Table 5** shows the dry stability storage conditions of the LazWell at which we still maintained good precision and accuracy at LOQ level.

Dry stability	
Time (h)	48
Temp. (°C)	RT
Conc. (ng/mL)	1
N	3
Mean (ng/mL)	1.11
%RSD	6.4
%Nom	111.1

Table 5 Dry stability results

Real patient sample comparison
13 real patient saliva samples have been tested with this method to correlate with LC-MS/MS results. **Figure 6** shows a correlation >95% between results using both methods.

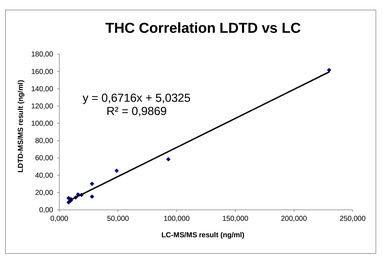

Figure 6 Correlation between THC concentrations in real saliva samples obtained with LDTD-MS/MS and LC-MS/MS

Potential drug interference
Potentially interfering drugs were spiked in a QC sample (90.9 ng/ml). Final concentration of interference compounds of group 1,3,4,6 and 7 were 10 µg/ml. For group 2 and 5, the interference compound concentration were 2 µg/ml and THCC interference concentration was evaluated at 0.1 µg/ml (**Table 6 and 7**).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Conc. (ng/mL)	90,9	90,9	90,9	90,9	90,9	90,9	90,9	90,9
N	3	3	3	3	3	3	3	3
Mean (ng/mL)	88,8	90,9	95,0	86,4	88,9	86,0	84,7	84,9
%RSD	1	0,1	6,5	5,2	5,1	1,8	6,1	5,6
%Nom	97,7	100,0	104,5	95,1	97,8	94,6	93,1	93,4

Table 6 Precision and accuracy result of potential drug interference experiment

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Methadone	EDDP	MDMA	Propanolol	OH-Midazolam	Caffein	Albumine	THCC
6-Acetylmorphine	Buprenorphine	Benzoylecgonine	Nordiazepam	7-aminoflunitrazepam	Acetaminophen		
Codein	Norbuprenorphine	Cocain	Lonazepam	OH-Ethylflurazepam	Ibuprofen		
Fentanyl	Triclabendazole	MDEA	Clonazepam	OH-Alprazolam	Ascorbic acid		
Hydrocodone	Ketamine	MDA	Diazepam	OH-Triazolam	Naloxone		
Hydromorphone	Imipramine	PCP	Estazolam	Ramitidine	Desipramine		
Morphine	Carisoprodol	Cotinine	Oxazepam	Nicotine	Glipizide		
Norfentanyl	Zolpidem	Amobarbital	Chlordiazepoxide		Dextromethorphan		
Norpropoxyphene	LSD	Butabarbital	Flunitrazepam				
Oxycodone		Secobarbital	Alprazolam				
Oxymorphone		Butalbital	Amphetamine				
		Pentobarbital	Metamphetamine				
		Phenobarbital					

Table 7 List of spiked drugs for potential signal interference check

Matrix Effect
Saliva from ten volunteers (non THC smokers) was collected using the Intercept device. These samples were then spiked with THC for a final concentration of 110 ng/ml (**Table 8**).

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Conc. (ng/mL)	110	110	110	110	110	110	110	110	110	110
N	3	3	3	3	3	3	3	3	3	3
Mean (ng/mL)	107,0	108,7	110,8	108,2	112,4	119,2	114,2	116,6	114,0	115,9
%RSD	3,6	0,3	4,7	1,8	0,3	6,2	4,7	5,7	2,0	2,6
%Nom	97,3	98,8	100,7	98,4	102,1	108,4	103,8	106,0	103,6	105,3

Table 8 Precision and accuracy result of Matrix effect experiment

Correction factor
Values reported represent diluted oral fluid. To convert to whole saliva, you must multiply by a factor of 3X.

CONCLUSIONS

- Fast Saliva sampling using Intercept® devices
- Fast LLE extraction of Extraction Buffer
- High Selectivity, Sensitivity and Specificity using Tandem Mass Spectrometry
- Good validation and real sample comparison result.
- LDTD provides Ultra High-Throughput analysis of THC in **10 seconds sample-to-sample.**