Application Note : 1214

Tetrahydrocannabinol(THC) Confirmation in Oral Fluids by Laser Diode Thermal Desorption (LDTD) – MS/MS

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Keywords: THC Analysis, Oral Fluids, Ultra High-Throughput, LDTD

Introduction

Drug testing in Oral Fluids is a constantly evolving analysis procedure which benefits from 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. In a clinical environment, oral fluids can be used in patient screening for rapid confirmation of the presence or absence of orally administered drugs.

The LDTD ion source uses an infrared laser diode to desorb samples that have been dried onto a 96-well LazWell™ plate. The rapid desorption produces neutral species which are carried into a corona discharge region to undergo an efficient protonation and are subsequently transferred directly into the mass spectrometer for detection.

Oral Fluid Collection

- The Intercept® device by OraSure is used for saliva collection. Standard curves and QC's are prepared in the Oral Fluid Calibration Buffer.

Figure 1: Intercept® Oral Fluid Drug Test

LDTD-MS/MS System

Figure 2: LDTD system on Thermo Vantage Mass Spectrometer.

Sample Method

Extraction Procedure

200 µL Oral Fluid Calibration Buffer
20 µL IS (THC-d9 at 1 µg/mL in MeOH:H_2O (75:25))
- Mix

400 µL Ethyl Acetate
- Mix and centrifuge (2 min. / 14000 rpm)

Spot 8 µL of organic phase in LazWell plate
- Let sit on bench until dry. Do not evaporate with air flow.

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	2	0
	5	65
	5.1	0
	7.1	0

MS/MS Method

	Transition	CE	S-Lens
THC	313->245	30	100
THC-d9	316->182	25	90
Mode:	Negative		

Results and Discussion

Linearity Results

As shown in **Figure 3,** excellent linearity (r^2 > 0.99) with no signs of carryover effect is achieved in the quantification range (1 to 1,000 ng/mL).

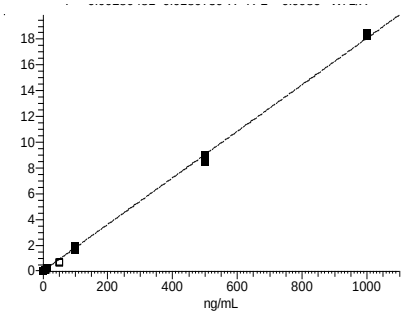

Figure 3: THC standard curve

	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

Accuracy and Precision

As shown on **Table 2 and 3,** the inter-run and intra-run accuracy and precision are between 91.7 to 102.2% and 0.6 to 10.9%, respectively.

	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 2: Inter-run precision and accuracy for THC

	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 3: Intra-run precision and accuracy for THC

Stability Verification

Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of the drug. After 8h, all samples were re-spotted and analyzed. Linearity, precision and accuracy were evaluated to determine the stability. **Table 4** shows that a wet stability of 8h is obtained with good precision and accuracy of LOQ standard.

The stability of dry samples in LazWell plate was also determined. All standards and QCs are spotted, dried and kept at room temperature for 48h. Then, standards and QCs were analyzed and the linearity, precision and accuracy are verified. **Table 4** shows the dry stability results and the storage conditions of the LazWell.

	Wet Stability (4°C)	Dry in LazWell (RT)
Time (h)	8	48
Temp. (°C)	4°C	RT
Conc. (ng/mL)	1	1
N	4	3
Mean (ng/mL)	1.00	1.11
%RSD	6.16	6.4
%Nom	99.5	111.1

Table 4: Stability Results for THC

Correction Factor

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

Conclusions

The ease of use of the Intercept® oral fluid sampling device from OraSure provides an accurate and fast sampling method for many drugs of abuse. The combination of the oral fluid extraction procedure with the analysis speed of the LDTD-MS/MS is an ideal solution in high-throughput drug analysis.

A fast, sensitive and reproducible method for the analysis of THC in oral fluid matrix is achieved using a simple buffer extraction method combined to the speed of analysis of the LDTD-MS/MS with a total sample-to-sample analysis time of **8 seconds**.

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