Amphetamine and Methamphetamine Confirmation in Oral Fluids by Laser Diode Thermal Desorption (LDTD) – MS/MS

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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

100 µL Oral fluid Calibration Buffer
20 µL IS (Amphetamine-d5 and Methamphetamine-d9 at 250 ng/mL in MeOH)
100 µL NaOH (0.1N in Water)
- Mix

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

Transfer 400 µL organic phase
Add 20 µL HCl (0.02N) in MeOH
Spot 2 µL of organic phase in LazWell plate
- Evaporate to dryness

*Organic phase can be evaporated and reconstituted to further concentrate the sample

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	2	0
	5	45
	7	45
	7.1	0
	8	0

MS/MS Method

	Transition	CE	S-Lens
Amphetamine	136->119	10	40
Amphetamine-d5	141->124	10	40
Methamphetamine	150->119	10	40
Methamphetamine-d9	159->125	10	40
Mode:	Positive		

Results and Discussion

Linearity Results

As shown in **Figure 3 and 4,** excellent linearity (r^2 > 0.99) with no signs of carryover effect is achieved in the quantification range (10 to 1,000 ng/mL).

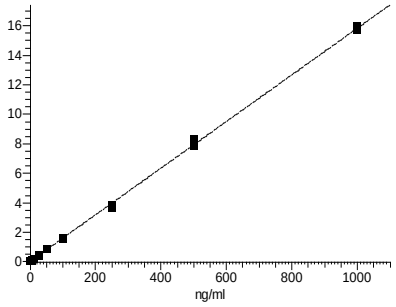

Figure 3: Amphetamine standard curve

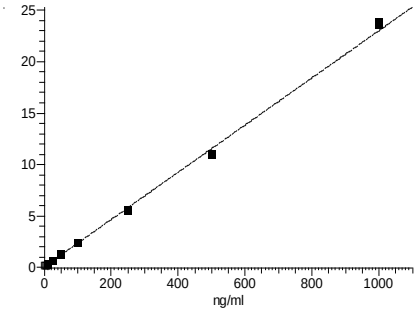

Figure 4: Methamphetamine standard curve

Accuracy and Precision

As shown on **Table 1 and 2,** the inter-run accuracy and the precision are between 95.9 to 102.0% and 2.7 to 9.6% respectively.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	25	100	500
N	8	8	9
Mean (ng/mL)	25.50	99.00	501.45
%RSD	9.6	3.9	3.7
%Nom	102.0	99.0	100.3

Table 1: Inter-run precision and accuracy for Amphetamine

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	25	100	500
N	8	8	9
Mean (ng/mL)	23.97	97.78	493.67
%RSD	7.3	5.4	2.7
%Nom	95.9	97.8	98.7

Table 2: Inter-run precision and accuracy for Methamphetamine

Detection limit (LOD)

A detection limit of 0.5 ng/mL can be reached with a blank interference less than 1% at this concentration.

Stability Verification

Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of the drugs. After 92h, all samples were re-spotted and analyzed. Linearity, precision and accuracy were evaluated to determine the stability. **Table 3** shows that a wet stability of 92h 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 in two specific stability conditions. After the stability time, standards and QCs were analyzed and the linearity, precision and accuracy are verified. **Table 3** shows the dry stability given the storage conditions of the LazWell at 66h with a combination of RT/4°C.

	Wet Stability		Dry in LazWell (RT+4°C)	
Drug	Amph.	Methamph..	Amph.	Methamph..
Time (h)	92	92	66	66
Temp. (°C)	4°C	4°C	RT (5h20) + 4°C (60h40)	RT (5h20) + 4°C (60h40)
Conc. (ng/mL)	10	10	10	10
N	3	3	3	3
Mean (ng/mL)	11.10	11.40	10.04	10.55
%RSD	9.8	2.7	3.5	3.6
%Nom	111.0	114.0	100.4	105.5

Table 3: Stability Results for Amphetamine and Methamphetamine

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 Amphetamine and Methamphetamine 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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