



## Methadone 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

- 10 µL Oral fluid Calibration Buffer
- 10 µL IS (Methadone-d9 at 50 ng/mL in MeOH:Water (1:1))
- 10 µL NaOH (0.1N in Water)
  - Mix
- 600 µL Ethyl Acetate\*
  - Mix and centrifuge (2 min. / 14000 rpm)
- 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
Methadone	310->265	15	80
Methadone-d9	319->268	15	80
Mode:	Positive		

## 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 100 ng/mL).

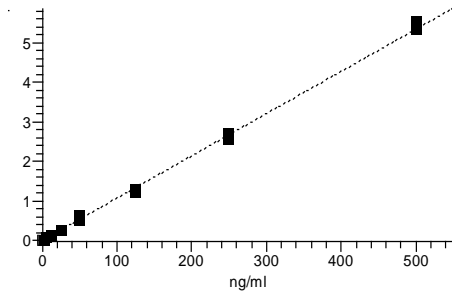


Figure 3: Methadone standard curve

	$r^2$	Slope (ratio area / concentration)	y- Intercept
Run 1	0.998	0.0107	0.0065
Run 2	0.9966	0.0135	0.0041
Run 3	0.9978	0.0107	0.0063
Run 4	0.9982	0.0131	0.0035

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 97.5 to 109.7% and 0.6 to 14.2%, respectively.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	12.5	50	250
N	12	12	12
Mean (ng/mL)	12.59	54.83	245.45
%RSD	6.2	6.1	3.1
%Nom	100.7	109.7	98.3

Table 2: Inter-run precision and accuracy for Methadone

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/mL)	5	12.5	50	250	500
N	3	3	3	3	3
Mean (ng/mL)	5.42	13.41	52.48	243.66	500.90
%RSD	2.5	1.3	14.2	2.2	0.6
%Nom	108.4	107.3	105.0	97.5	100.2

Table 3: Intra-run precision and accuracy for Methadone

### Detection limit (LOD)

A detection limit of 2.5 ng/mL can be reached with a blank interference of 6.6% at this concentration.

### Stability Verification

Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of the drug. After 41h, 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 41h 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 47h35. 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	Dry in LazWell (RT)
Time (h)	41	47h35
Temp. (°C)	4°C	RT
Conc. (ng/mL)	5	5
N	3	3
Mean (ng/mL)	5.03	4.79
%RSD	3.0	8.0
%Nom	100.6	95.7

Table 4: Stability Results for Methadone

### 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 Methadone 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**.