Confirmation of Methadone and 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) in Urine and analysis by LDTD–MS/MS

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Introduction

Confirmation of Methadone and 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) consumption in patients is often conducted in urine since it is a non invasive procedure. A liquid-liquid extraction procedure prior to the Laser Diode Thermal Desorption (LDTD) analysis can be done as an effective way to speed up and reduce the cost of the evaluation.

The LDTD ion source uses an infrared laser diode to desorb samples that have been previously dried onto a 96-well LazWell™ plate after sample preparation extraction. 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.

LDTD-MS/MS System

Figure 1: LDTD system on Thermo Vantage Mass Spectrometer.

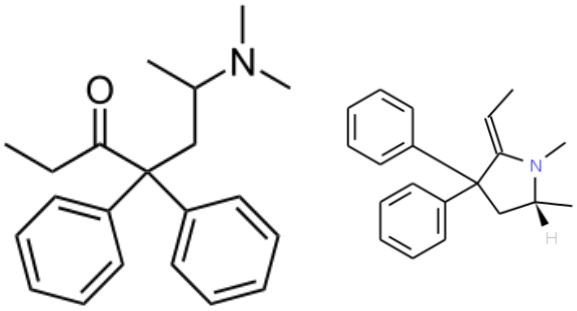

Figure 2: Molecular structures of Methadone (right) and EDDP Perchlorate (left)

Sample Method

Extraction procedure

10 µL urine sample
10 µL IS (50 ng/mL Methadone-d9 in MeOH:H_2O (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
EDDP	278->234	30	110
Mode:	Positive		

Results and Discussion

Linearity Results

As shown in **Figure 3** and **Figure 4,** excellent linearity (r^2 > 0.99) with no signs of carryover effect is achieved for Methadone and EDDP in the quantification range (30 to 7,500 ng/mL).

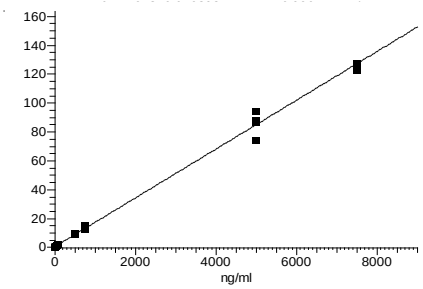

Figure 3: Methadone standard curve

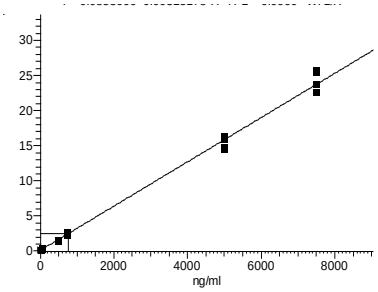

Figure 4: EDDP standard curve

Accuracy and Precision

As shown on **Table 2 and 3,** the inter-run accuracy and precision are between 96.6 to 103.8% and 4.5 to 7.6%, respectively, for both drugs.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	50	750	5000
N	12	12	12
Mean (ng/mL)	50.35	757.81	4956.02
%RSD	6.4	7.6	6.2
%Nom	100.7	101.0	99.1

Table 1: Inter-run precision and accuracy for Methadone

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	50	750	5000
N	12	12	12
Mean (ng/mL)	51.88	736.51	4829.55
%RSD	7.3	5.7	4.5
%Nom	103.8	98.2	96.6

Table 2: Inter-run precision and accuracy for EDDP

Stability Verification

Following the SPE extraction process, all samples were stored at 4°C to evaluate the wet stability of the drugs. After 48h, all samples were re-spotted and analyzed. Linearity, precision and accuracy were evaluated to determine the stability. **Table 5** shows that a wet stability of 48h 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 5** shows that the dry stability results and the storage conditions of the LazWell.

	Wet Stability		Dry in LazWell	
Time (h)	48		48	
Temp. (°C)	4°C		RT	
Conc. (ng/mL)	30		30	
N	4		4	
Drug	Methadone	EDDP	Methadone	EDDP
Mean (ng/mL)	30.34	29.34	31.27	24.78
%RSD	9.9	6.4	5.6	7.1
%Nom	101.1	97.8	104.2	82.6

Table 3: Stability Results for Methadone and EDDP

Conclusions

The liquid-liquid extraction ensures accurate and precise results with a linear standard curve (r^2 > 0.99) for both drugs.

A fast analysis can be achieved using LDTD-MS/MS system. This system allows a total sample-to-sample analysis time of **8 seconds**.