Application Note : 1209

Solid-Phase Extraction 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

Analysis of Methadone and 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) in urine can require a sample clean-up step to reduce the interference effect from the matrix. To obtain an optimal sample clean-up, the Silia*PrepX*™ HLB SPE cartridges are used in the extraction procedure prior to the Laser Diode Thermal Desorption (LDTD) analysis.

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.

Solid Phase Cartridge

The Silia*PrepX* HLB cartridge is used for the sample extraction procedure.

Figure 1: Silia*PrepX* HLB SPE cartridge

Formats	Qty / Pk	Product number
1 mL / 30 mg	100	SPE-P0002-01AA
3 mL / 60 mg	50	SPE-P0002-03BB
6 mL / 100 mg	30	SPE-P0002-06C
6 mL / 200 mg	30	SPE-P0002-06G
6 mL / 500 mg	30	SPE-P0002-06P
2 mL / 10 mg	1	96W-P0002-1A
2 mL / 30 mg	1	96W-P0002-AA

Table 1: Silia*PrepX* HLB Formats

LDTD-MS/MS System

Figure 2: LDTD system on Thermo Vantage Mass Spectrometer.

Sample Method

Extraction procedure

Cartridge: Silia*PrepX* HLB (1 mL / 30 mg)
Activation: 1 mL MeOH
1 mL NH_4OH (2% v/v) in Water
Load: 200 µL sample
40 µL IS (Methadone-d9 at 200 ng/mL in MeOH)
200 µL NH_4OH (4% v/v) in Water
Wash 1: 1 mL Water/MeOH (50/50) + NH_4OH (2% v/v)
Wash 2: 1 mL MeOH /Water (80/20) + NH_4OH (2% v/v)
Elution: 1 mL MeOH/Water (80/20)
Spot: 2 µL in LazWell plate

*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 (10 to 500 ng/mL and 10 to 1,000 ng/mL respectively).

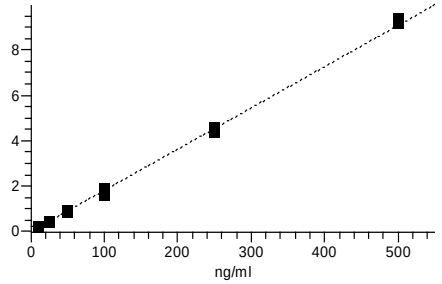

Figure 3: Methadone standard curve

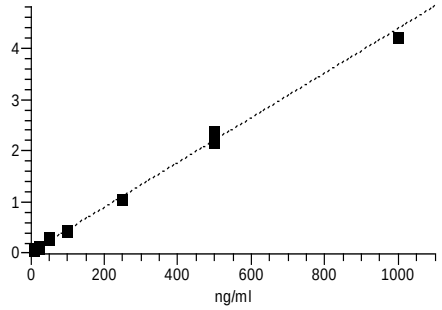

Figure 4: EDDP standard curve

Accuracy and Precision

As shown on **Table 2 and 3,** the inter-run accuracy and precision are between 92.2 to 104.5% and 3.4 to 10.8%, respectively, for both drugs.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	25	100	250
N	12	12	12
Mean (ng/mL)	23.12	93.88	251.80
%RSD	5.6	8.5	3.4
%Nom	92.2	93.9	100.7

Table 2: Inter-run precision and accuracy for Methadone

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	25	100	500
N	12	11	11
Mean (ng/mL)	24.52	94.40	522.74
%RSD	7.3	10.8	6.9
%Nom	98.1	94.4	104.5

Table 3: Inter-run precision and accuracy for EDDP

Recovery

Recovery at 1,000 ng/mL of concentration for each drug is reported in **Table 4** (N=4).

	Methadone	EDDP
Recovery (%)	91%	85%

Table 4: Recovery results for both drugs

Stability Verification

Following the SPE extraction process, all samples were stored at 4°C to evaluate the wet stability of the drugs. After 27h, 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 27h 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 25h. 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 (RT)	
Time (h)	27		25	
Temp. (°C)	4°C		RT	
Conc. (ng/mL)	10		10	
N	3		3	
Drug	Met	EDDP	Met	EDDP
Mean (ng/mL)	11.62	11.29	11.53	11.83
%RSD	3.3	9.0	9.5	5.9
%Nom	116.2	112.9	115.3	118.3

Table 5: Stability Results for Methadone and EDDP

Conclusions

The sample solid extraction using **Silia*PrepX* HLB** polymeric SPE cartridges 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**.

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