Solid-Phase Extraction of Fentanyl and Norfentanyl in Urine and analysis by LDTD–MS/MS

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Introduction

Analysis of narcotic drugs, such as Fentanyl and Norfentanyl, in urine needs a good sample clean-up to reduce interference and matrix suppression effect. To obtain a good sample clean-up, the Silia*Prep*™ CleanDrug 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*Prep* CleanDrug cartridge is used for the sample extraction procedure.

Figure 1: Silia*Prep* CleanDrug SPE cartridge

Silia*Prep* CleanDRUG Formats		
Formats	**Qty / Pk**	**Product number**
1 mL / 50 mg	100	SPEC-R651230B-01B
1 mL / 100 mg	100	SPEC-R651230B-01C
3 mL / 200 mg	50	SPEC-R651230B-03G
3 mL / 500 mg	50	SPEC-R651230B-03P
6 mL / 500 mg	50	SPEC-R651230B-06P
6 mL / 1g	50	SPEC-R651230B-06S
2 mL / 50 mg	1	96W-R651230B-B
2 mL / 100 mg	1	96W-R651230B-C

Table 1: Silia*Prep* CleanDRUG product number

LDTD-MS/MS System

Figure 2: LDTD system on Thermo Vantage Mass Spectrometer.

Sample Method

Extraction Procedure

Cartridge:	Silia*Prep* CleanDrug (1 mL / 100 mg)
Activation:	1 mL MeOH
	1 mL Water
	1 mL Na Acetate (100 mM, pH 6) in Water
Load:	200 µL sample
	40 µL IS (Fentanyl-d5 and Norfentanyl-D5 at 200 ng/mL in MeOH)
	600 µL Na Acetate (100 mM, pH 6) in Water
Wash 1:	1 mL Water
Wash 2:	1 mL MeOH
Elution:	1 mL EtAc/IPA/NH_4OH (78/20/2)
	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
Fentanyl	337->188	22	120
Fentanyl-d5	342->188	22	120
Norfentanyl	233->150	15	85
Norfentanyl-d5	238->155	15	85
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 in the quantification range (10 to 1,000 ng/mL).

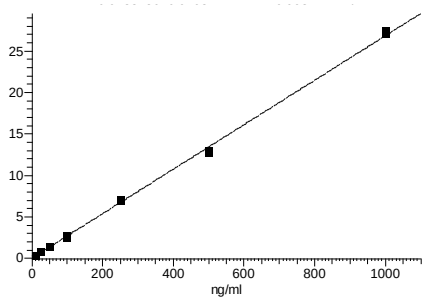

Figure 3: Fentanyl standard curve

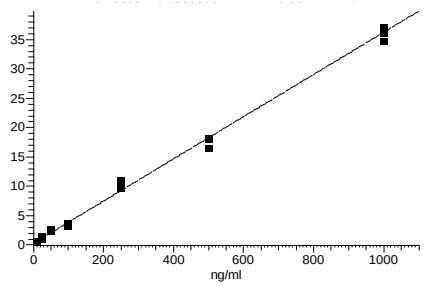

Figure 4: Norfentanyl standard curve

Accuracy and Precision

As shown on **Table 2 and 3,** the inter-run accuracy and the precision are between 93.2 to 110.3% and 1.4 to 15.6%, respectively, for both drugs.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	25	100	500
N	12	12	12
Mean (ng/mL)	26.38	95.25	481.44
%RSD	2.0	3.9	1.4
%Nom	105.5	95.2	96.3

Table 2: Inter-run precision and accuracy for Fentanyl

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	25	100	500
N	12	12	12
Mean (ng/mL)	27.58	93.17	489.69
%RSD	15.6	8.1	6.2
%Nom	110.3	93.2	97.9

Table 3: Inter-run precision and accuracy for Norfentanyl

Recovery

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

	Fentanyl	Norfentanyl
Recovery (%)	95	79

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 drug. 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 25. Then, standards and QCs were analyzed and the linearity, precision and accuracy are verified. **Table 5** shows the dry stability and the storage conditions of the LazWell.

	Wet Stability		Dry in LazWell (RT)	
Time (h)	48		25	
Temp. (°C)	4°C		RT	
Conc. (ng/ml)	10		10	
N	4		4	
Drug	Fent.	Norf.	Fent.	Norf.
Mean (ng/ml)	8.39	9.48	9.60	8.78
%RSD	0.9	12.9	2.2	8.3
%Nom	83.9	94.8	96.0	87.8

Table 5: Stability Results for Fentanyl and Norfentanyl

Conclusions

The sample solid extraction using **Silia*Prep* CleanDrug** SPE cartridges ensures accurate and precise results with a linear standard curve (r^2 > 0.99) for both drugs.

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

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