Application Note: 1203

Solid-Phase Extraction of Amphetamine and Methamphetamine in Urine and analysis by LDTD–MS/MS

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Introduction

Analysis of certain drugs of abuse 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*Prep*™ CleanDRUG SPE cartridges are used in the extraction procedure prior to ultra-fast analysis by Laser Diode Thermal Desorption (LDTD).

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: SilliaPrep 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 / 1 g	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)
Load:	200 µL sample
	40 µL IS (Amphetamine-d5 and Metamphetamine-d9 at 250 ng/mL in MeOH)
	600 µL Na Acetate (100 mM, pH 6)
Wash 1:	1 mL Water
Wash 2:	1 mL MeOH
Elution:	1 mL EtAc / IPA / NH_4OH (78/20/2)
	After elution, add 40 µL of formic acid
	Mix*
	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
Amphetamine	136->119	10	40
Amphetamine-d5	141->124	10	40
Metamphetamine	150->119	10	40
Metamphetamine-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 (20 to 2,000 ng/mL).

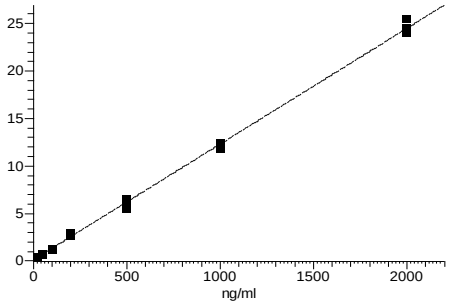

Figure 3: Amphetamine standard curve

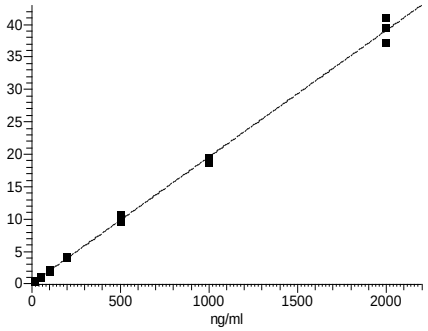

Figure 4: Methamphetamine standard curve

Accuracy and Precision

As shown in **Table 2 and 3,** the inter-run accuracy and the precision are situated between 92.8 to 105.4% and between 5.1 to 9.6%, respectively, for both drugs.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	50	200	1000
N	9	9	9
Mean (ng/mL)	50.47	208.87	990.69
%RSD	9.0	5.7	5.7
%Nom	100.9	104.4	99.1

Table 2: Inter-run precision and accuracy for Amphetamine

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	50	200	1000
N	9	9	8
Mean (ng/mL)	46	211	953
%RSD	9.6	5.1	8.7
%Nom	92.8	105.4	95.3

Table 3: Inter-run precision and accuracy for Methamphetamine

Recovery

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

	Amphetamine	Methamphetamine
Recovery (%)	101	74

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 74h, 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 74h 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 74h. Then, standards and QCs were analyzed and the linearity, precision and accuracy are verified. **Table 5** shows the dry stability results and the storage conditions of the LazWell.

	Wet Stability		Dry in LazWell (RT)	
Time (h)	74		74	
Temp. (°C)	4°C		RT	
Conc. (ng/mL)	20		20	
N	3		3	
Drug	**Amph.**	**Meth.**	**Amph.**	**Meth.**
Mean (ng/mL)	18.6	19.0	19.5	21.3
%RSD	9.53	8.50	13.71	2.00
%Nom	92.9	95.2	97.4	106.4

Table 5: Stability Results of Amphetamine and Methamphetamine

Conclusions

The solid phase extraction (SPE) using **Silia*Prep* CleanDRUG** 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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