Confirmation of Amphetamine, Methamphetamine, MDA, MDMA, and MDEA in Urine via analysis by LDTD–MS/MS

Serge Auger , Alex Birsan & Pierre Picard
Phytronix Technologies, Quebec, Canada

Keywords: Amphetamine, Methamphetamine, MDA, MDEA,MDMA, Urine, Liquid-Liquid Extraction, LDTD

Introduction

Confirmation of Amphetamine, Metamphetamine, MDA, MDMA and MDEA 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

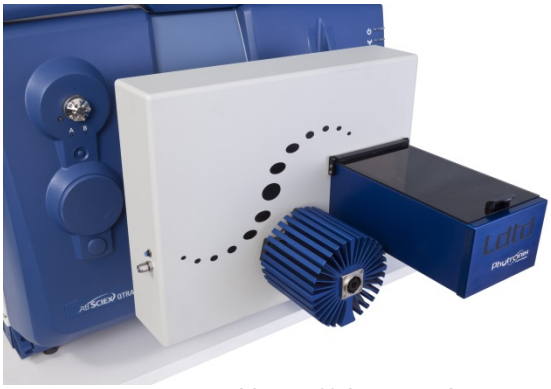

Figure 1: LDTD system on AB SCIEX 5500 Qtrap Mass Spectrometer

Sample Method

Extraction procedure

200 µL urine sample
40 µL IS (750 ng/mL MeOH:H_2O (1:1)
500 µL NaOH (1N in Water)
- Mix

500 µL 1-Chlorobutane
- Mix and centrifuge (2 min. / 14000 rpm)

Transfer 100 µL upper layer
Add 10 µL HCl (0.01N in MeOH)*
Spot 6 µL of organic phase in Lazwell plate
- Evaporate to dryness

*HCl salt formation is used to reduce the amine volatility.

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	5	0
	10	25
	10.1	0
	11	0

MS/MS Method

	Transition	CE	S-Lens
Amphetamine	136->119	10	100
Amphetamine-d5	141->124	10	100
Metamphetamine	150->119	15	100
Metamphetamine-d11	161->127	15	100
MDA	180->133	12	100
MDEA	208->163	20	100
MDEA-d5	213->163	20	100
MDMA	194->163	20	100
MDMA-d5	199->165	20	100
Mode:	Positive		

Results and Discussion

Linearity Results

As shown in **Figure 2** excellent linearity (r^2 > 0.99) with no signs of carryover effect is achieved in the quantitative range (50 to 5000 ng/mL).

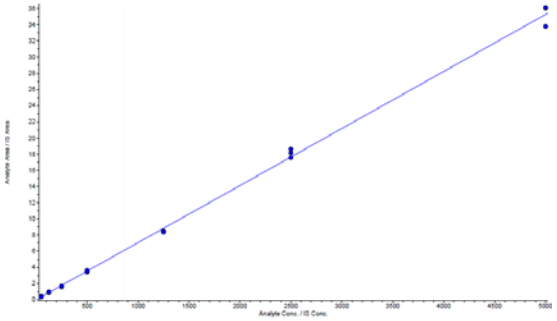

Figure 2: Amphetamine standard curve

Accuracy and Precision

As shown on **Table 1 to 5,** the inter-run accuracy and precision are between 98.4 to 107.7% and 2.6 to 9.3%, respectively, for both drugs.

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	50	200	1000
N	12	12	12
Mean (ng/mL)	50.05	206.99	985.42
%RSD	4.6	4.7	3.0
%Nom	100.1	103.5	98.5

Table 1: Inter-run precision and accuracy for Amphetamine

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	50	200	1000
N	12	12	12
Mean (ng/mL)	50.07	205.21	1004.93
%RSD	3.4	2.6	3.4
%Nom	100.1	102.6	100.5

Table 2: Inter-run precision and accuracy for Metamphetamine

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	125	500	2500
N	9	9	9
Mean (ng/mL)	126.0	491.9	2502.2
%RSD	9.3	7.1	5.1
%Nom	100.8	98.4	100.1

Table 3: Inter-run precision and accuracy for MDMA

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	125	500	2500
N	9	9	9
Mean (ng/mL)	131.7	538.4	2550.0
%RSD	6.8	5.5	7.7
%Nom	105.3	107.7	102.0

Table 4: Inter-run precision and accuracy for MDA

	QC-Low	QC-Med	QC-High
Conc. (ng/mL)	125	500	2500
N	9	9	9
Mean (ng/mL)	128.6	531.0	2565.6
%RSD	8.9	8.0	4.9
%Nom	102.8	106.2	102.6

Table 5: Inter-run precision and accuracy for MDEA

Cross validation

72 real patient urine samples have been tested with this method to correlate with results obtained by LC-MS/MS method. **Figure 3 and 4** shows a correlation (>0.99) between results for Amphetamine and Metamphetamine. All negative samples are detected as negative in both methods. Similar results are obtained for MDA, MDMA and MDEA.

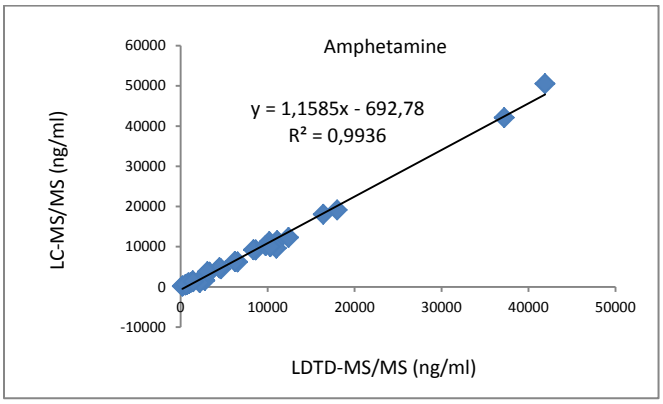

Figure 3: Cross validation LC-MS/MS vs LDTD-MS/MS for Amphetamine

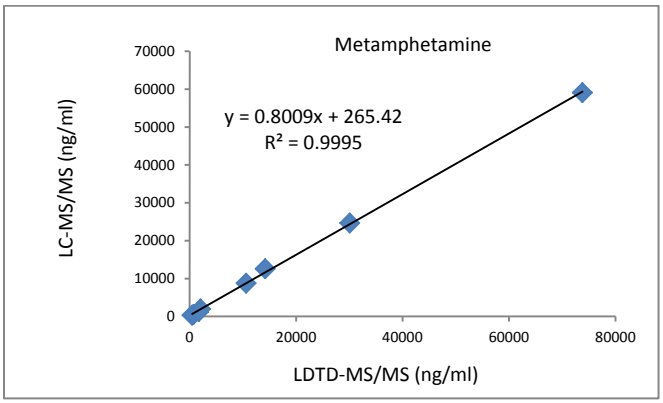

Figure 4: Cross validation LC-MS/MS vs LDTD-MS/MS for Metamphetamine

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 that allows a total sample-to-sample analysis time of **11 seconds** with no carryover.