Development of an ultra-rapid, high throughput analysis method of synthetic phenethylamines (25I-NBOMe and 25B-NBOMe) in urine, saliva and serum by laser diode thermal desorption-mass spectrometry

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OVERVIEW

Purpose

- Ultra-Fast analysis of synthetic phenethylamines (25I-NBOMe and 25B-NBOMe) in urine, saliva (OraSure® buffer) and serum samples using LDTD®-MS/MS

Method

- Standards, QC and samples preparation using different matrices
- Liquid-Liquid extraction
- Deposit of a small volume of the organic phase in LazWell™ plate
- Fast Analysis using LDTD®-MS/MS system

Results

- Excellent linearity over the calibration range ($R^2 > 0.998$)
- Accuracy ranging from 93.6 to111.0 %
- Precision ranging from 0.3 to 4,45 %
- Good sample stability (Wet and Dry in LazWell™)
- **All samples are analyzed with a run time of 9 seconds using LDTD®-MS/MS system.**

INTRODUCTION

Emerging drugs of abuse have been introduced on the illegal drug market and pose a challenge for analytical forensic toxicologists. These drugs have increased in numbers and vary across different chemical classes, making them difficult to detect by current analytical methods. In November 2013, the United States Drug Enforcement Administration issued a final order to temporarily schedule three synthetic phenethylamines into the Controlled Substances Act: 25I-NBOMe, 25C-NBOMe, and 25B-NBOMe. A comprehensive detection and quantification method for the analysis of emerging drugs of abuse in biological matrices is needed. We developed an ultra-rapid, high-throughput and cost effective analytical method for the detection of 25I-NBOMe and 25B-NBOMe using the LDTD® coupled to an API-5500 MS/MS system. Method development and validation were performed in urine, saliva (OraSure® buffer) and serum matrices and the analysis time needed was 9 seconds per sample.

LDTD® Ionization Source:

The LDTD® uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96-well plate**.** The energy is then transferred through the sample holder. The sample gets dried and vaporized prior being carried by a gas in a corona discharge region. This type of ionization is characterized by a strong resistance to ionic suppression because of the absence of solvent. LDTD® ionization reduces sample-to-sample analysis time to 9 seconds and allows high throughput capabilities without carry over.

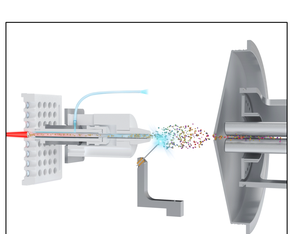

Figure 1 Schematic of the LDTD® ionization source.

METHOD

Liquid-liquid extraction

- 50 µL calibrators, QC or patient specimen
- 20 µL Internal standard (25I-NBOMe-D3, 50 ng/mL in MeOH)
- 200 µL buffer Na_2CO_3 0.5N pH 10
- 200 µL hexane/ethyl acetate : 75/25 v/v
  - Vortex and centrifuge at 14000 rpm for 2 minutes
- Transfer 6 µL of the organic layer in a Lazwell™ plate
- Analyze after complete solvent evaporation

Figure 2 LDTD® model S-960 coupled with AB Sciex QTRAP® 5500

Instrumentation

- LDTD® model S-960, Phytronix Technologies
- QTRAP® 5500 Systems, AB Sciex

LDTD Parameters

- Laser power pattern :
  - Increase laser power to 45 % in 6.0 s
  - Hold for 2 seconds
  - Decrease laser power to 0 %
- Carrier gas flow: 3 L/min (Air)
- Deposited sample volume: 6 µL

MS Parameters

- APCI (+) positive
- Scan time: 20 msec
- DP: 100
- MRM:

		CE (V)
25I-NBOMe (Quant):	428.0 → 121.0	25
25I-NBOMe (Conf):	428.0 → 91.0	60
25B-NBOMe (Quant):	380.0 → 121.0	25
25B-NBOMe (Conf):	380.0 → 91.0	60
25I-NBOMe-D3:	431.0 → 124.0	25

RESULTS:

Linearity

As shown in **Table 1,** an excellent linearity ($R^2 > 0.998$) with no signs of carryover effect is achieved within the quantification range **(0.1 to 100 ng/mL)**.

Table 1 Correlation for 25I-NBOMe and 25B-NBOMe in several matrices

Matrix	Drug	R^2
Urine	25I-NBOMe	0.998
	25B-NBOMe	0.998
Orasure® buffer (saliva)	25I-NBOMe	0.999
	25B-NBOMe	0.999
Serum	25I-NBOMe	0.999
	25B-NBOMe	0.999

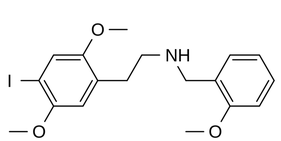

Figure 3 25I-NBOMe molecule

Figure 4 Typical Standard curve of 25I-NBOMe in saliva (OraSure® buffer)

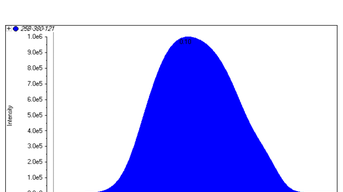

Figure 5 100 ng/mL standard desorption pic of 25B-NBOMe in urine

Precision and Accuracy

As shown in following **Table 2, 3, 4,** an intra-run precision for 25I-NBOMe and 25B-NBOMe in different matrices between 0.30 % and 4.45 % and an accuracy between 93.6 % and 111.0 % were obtained.

Table 2 Intra-run for 25I and 25B in urine matrix

Urine		LLOQ	QCL	QCM	QCH	ULOQ
	Conc. (ng/mL)	0.5	1	10	50	100
	n			3		
25I	Mean (ng/mL)	0.48	1.12	10.54	45.79	104.01
	%RSD	3.63	3.11	1.05	0.75	0.57
	%NOM	95.9	111.8	105.4	91.6	104.0
25B	Mean (ng/mL)	0.48	1.11	10.28	46.81	103.40
	%RSD	2.13	3.92	0.87	0.30	1.54
	%NOM	95.8	111.0	102.8	93.6	103.1

Table 3 Intra-run for 25I and 25B in serum matrix

Serum		LLOQ	QCL	QCM	QCH	ULOQ
	Conc. (ng/mL)	0.5	1	10	50	100
	n			3		
25I	Mean (ng/mL)	0.49	1.01	10.15	51.96	98.05
	%RSD	3.04	1.87	0.68	1.11	1.12
	%NOM	98.9	100.8	101.5	103.9	98.1
25B	Mean (ng/mL)	0.52	0.98	9.92	51.41	98.83
	%RSD	4.45	2.61	0.68	1.61	0.73
	%NOM	104.6	97.8	99.2	102.8	98.8

Table 4 Intra-run for 25I and 25B in saliva matrix

Saliva (OraSure® buffer)		LLOQ	QCL	QCM	QCH	ULOQ
	Conc. (ng/mL)	0.5	1	10	50	100
	n			3		
25I	Mean (ng/mL)	0.51	0.98	9.70	50.32	100.17
	%RSD	2.38	0.53	1.37	0.60	1.97
	%NOM	101.5	98.2	97.0	100.6	100.2
25B	Mean (ng/mL)	0.51	0.98	9.81	50.06	100.21
	%RSD	1.89	2.35	1.18	1.35	2.40
	%NOM	101.2	98.4	98.1	100.1	100.2

Wet stability

Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of the drugs. After a given time, all samples were re-spotted and analyzed. Linearity, precision and accuracy are verified for the stability run. **Table 5** shows that good precision and accuracy are obtained with the LLOQ level for both drugs in saliva matrix.

*Wet stability and Dry stability were also done with urine and serum matrices with results within the quantification range.

Table 5 Wet stability results in saliva

Wet stability in Orasure® buffer*		
Drug	**25I**	**25B**
Time (h)	12	12
Temp. (°C)	4°C	4°C
Conc. (ng/mL)	0.10	0.10
N	3	3
Mean (ng/mL)	0.10	0.11
%RSD	0.60	1.66
%Nom	102.7	108.9

Dry stability

The stability of dry samples in LazWell™ plate was also verified. All standards and QCs are spotted, dried and kept in specific stability conditions. After the stability time, standards and QCs were re-analyzed and the linearity, precision and accuracy are verified. **Table 6** shows that good precision and accuracy are obtained with the LOQ level for both drugs in saliva matrix.

Table 6 Dry stability results in saliva

Dry stability in Orasure® buffer*		
Drug	**25I**	**25B**
Time (h)	12	12
Temp. (°C)	RT	RT
Conc. (ng/mL)	0.5	0.5
N	2	2
Mean (ng/mL)	10.0	10.4
%RSD	6.18	16.50
%Nom	109.6	103.8

Drug interferences

32 drugs, with a concentration of 1000 ng/mL, have been added to a 10 ng/mL quality control sample to verify potential interferences between these drugs and the 2 phenethylamines. **Table 7** shows the concentration results for both 25I-NBOMe and 25B-NBOMe drugs in the 3 different matrices while **Table 8** shows the drugs used in the analysis.

Table 7 Potentially drug Interference results

Matrix	Drug	n	QC Conc. (ng/mL)	Mean Conc. (ng/mL)	% difference
Urine	25I-NBOMe	6	10	9.01	9.9
	25B-NBOMe	6	10	8.49	15.1
Saliva	25I-NBOMe	6	10	9.26	7.4
	25B-NBOMe	6	10	9.11	8.9
Serum	25I-NBOMe	6	10	7.99	20.1
	25B-NBOMe	6	10	9.19	8.1

Table 8 List of the 32 drugs used for the interferences analysis

Phentermine	Eslicarbazepin	Amphetamine
THC	EDDP perchlorate	Methamphetamine
THCC	Nordiazepam	Cocaine
Piroxicam	Diazepam	MDEA
Norpropoxyphene	Estazolam	MDA
Norfentanyl oxalate	Temazepam	MDMA
Hydrocodone	Alprazolam	Benzoylecgonine
Morphine	Lorazepam	Fentanyl
Norcodeine	Triazolam	Phenobarbital
Oxycodone	Codeine	PCP
Oxymorphone	Norhydrocodone	

CONCLUSIONS

- Fast extraction of 25I-NBOMe and 25B-NBOMe for serum, saliva (OraSure® buffer) and urine samples.
- High Selectivity, Sensitivity and Specificity using Tandem Mass Spectrometry.
- Versatility of LDTD® Technology proven with human samples.
- LDTD® provides ultra-fast High-Throughput analysis of sample extract in **9 seconds sample-to-sample without any carry over.**

1) Salary/Consultant Fees: Phytronix