



# Ultra High Throughput Analysis Method of Synthetic Phenethylamines (25I-NBOMe and 25B-NBOMe) in urine, saliva and serum by LDSTD-MS/MS

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## OVERVIEW

### Purpose

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

### Method

- Standards, QC and sample preparation using 3 different matrices
- Liquid-Liquid extraction
- Deposit of small volume of the organic phase in a LazWell™ plate
- Ultra-Fast quantitative analysis using LDSTD® -MS/MS system

### Results

- Excellent linearity over the calibration range ( $R^2 > 0.998$ )
- Accuracy ranging from 93.6 to 111.0%
- Precision ranging from 0.3 to 4.45 %
- **All samples are analyzed with a run time of 9 seconds using LDSTD® -MS/MS system**

## INTRODUCTION

Emerging synthetic drugs of abuse have been introduced on the illegal drug market and pose a challenge for analytical forensic toxicologist. These drugs continually increase in numbers and slightly vary across different chemical classes, making them difficult to detect by current analytical methods. In November 2013, the United States Drug Enforcement Administration (DEA) issued a final order to place these two synthetic phenethylamines into the Controlled Substances Act. A comprehensive detection and quantification method for the analysis of these 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 LDSTD® coupled to a tandem mass spectrometry system. Method development was performed in urine, saliva (OraSure® buffer) and serum matrices and the analysis time needed was 9 seconds per sample.

### LDSTD® Ionization Source:

The LDSTD® 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. LDSTD® ionization reduces sample-to-sample analysis time to 9 seconds and allows high throughput capabilities without carry over.

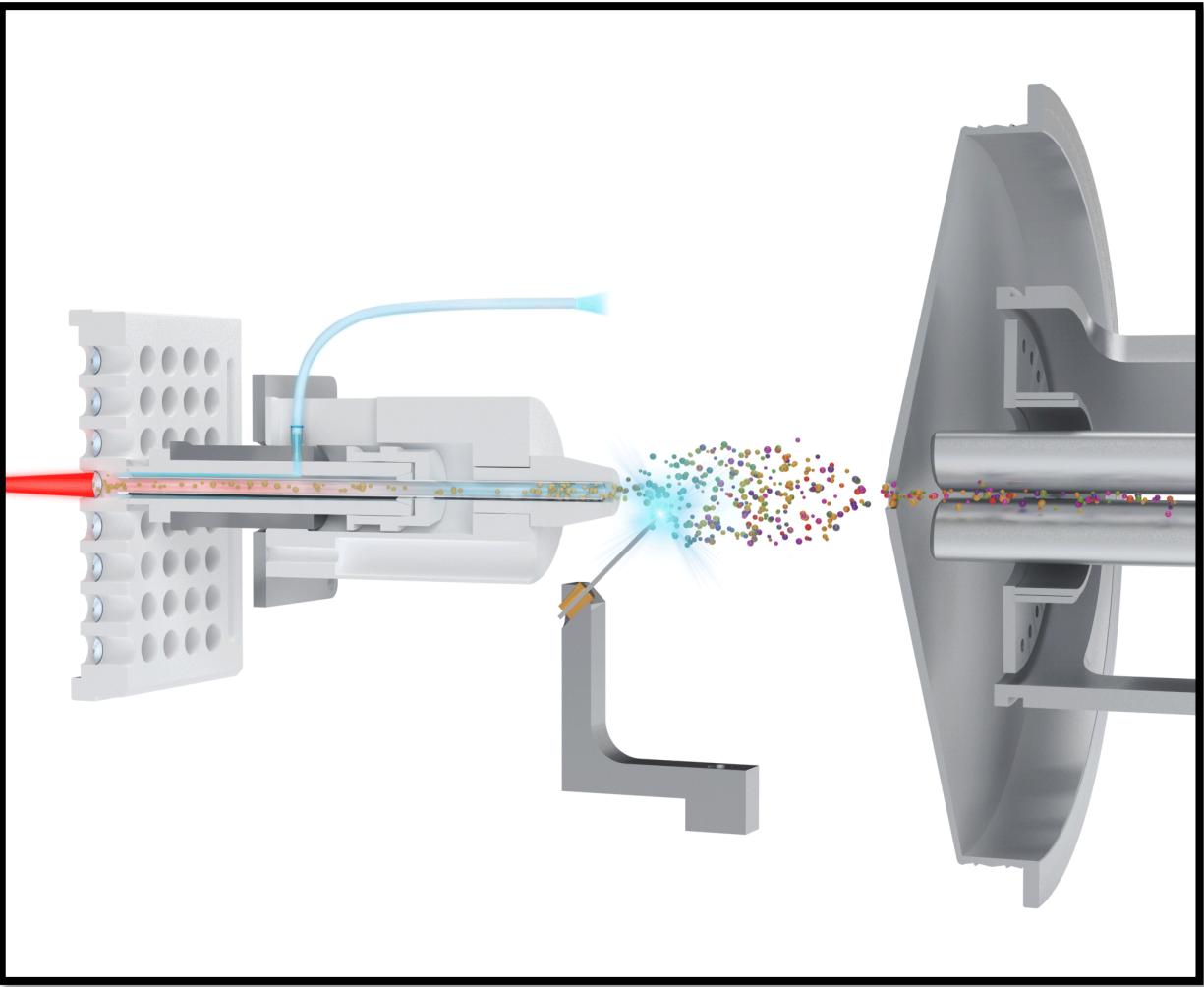


Figure 1 Schematic of the LDSTD® ionization source.

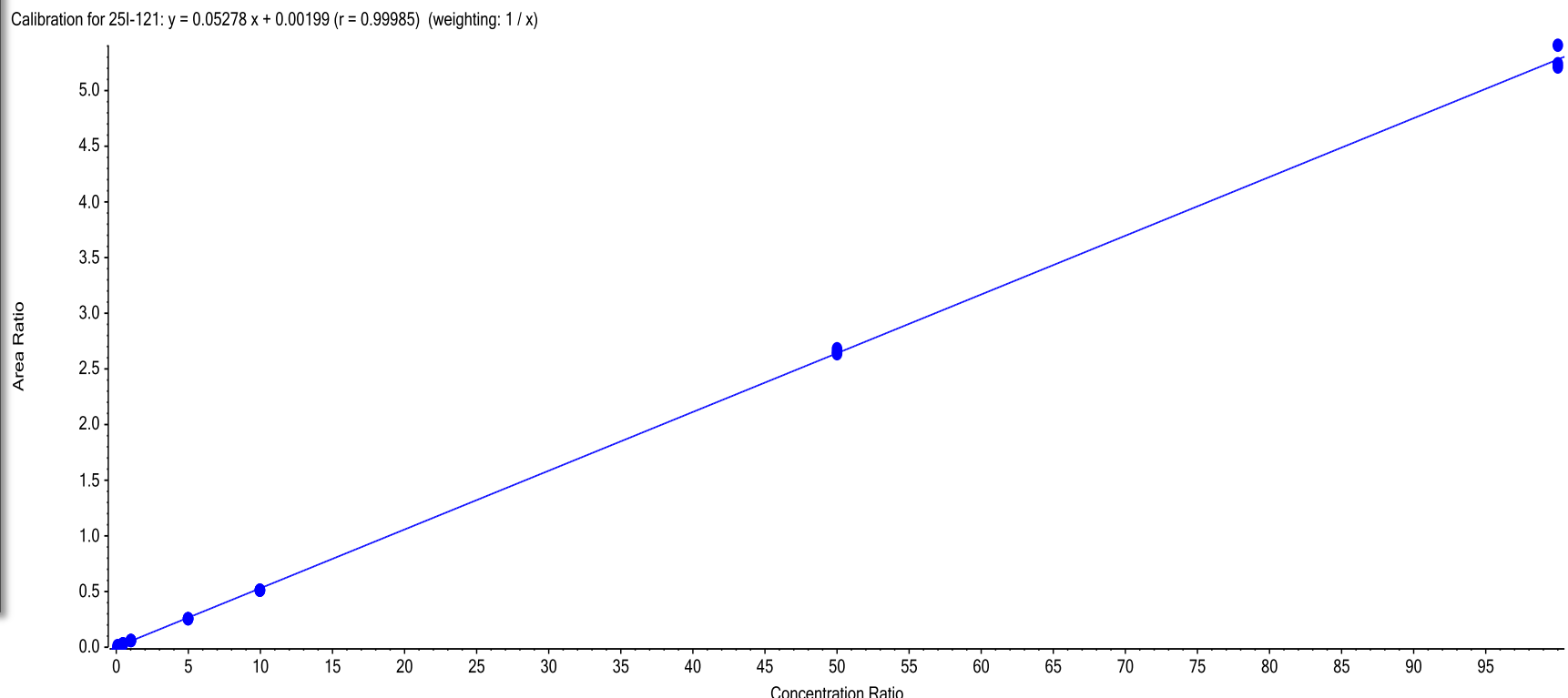


Figure 4 Typical Standard curve of 25I-NBOMe in saliva (OraSure® buffer)

## METHOD

### Liquid-liquid extraction

- 50 µL Standard, QC or patient specimen
- 20 µL Internal standard (25I-NBOMe-D3, 50 ng/mL in MeOH)
- 200 µL buffer Na<sub>2</sub>CO<sub>3</sub> 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

### Instrumentation

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

### LDSTD 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)



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

### MS Parameters

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

	Q1	Q3	CE (V)
➢ 25I-NBOMe :	428.0 → 121.0	25	
➢ 25B-NBOMe :	380.0 → 121.0	25	
➢ 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 <sup>2</sup>
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

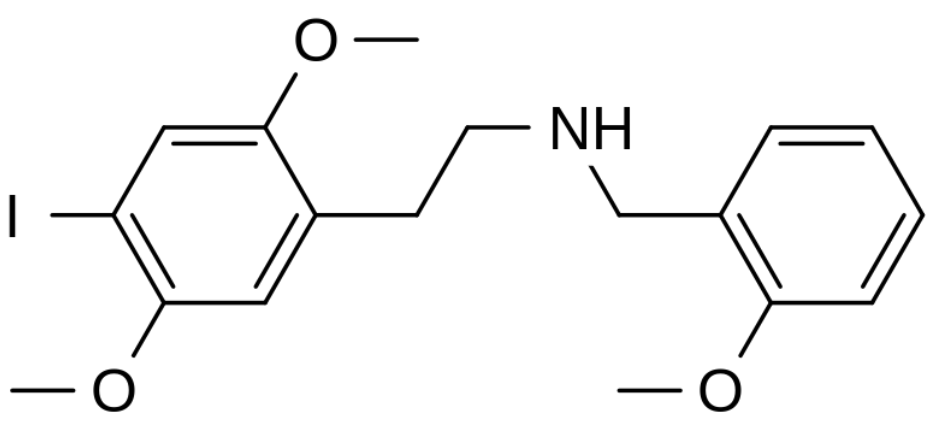


Figure 3 25I-NBOMe molecule

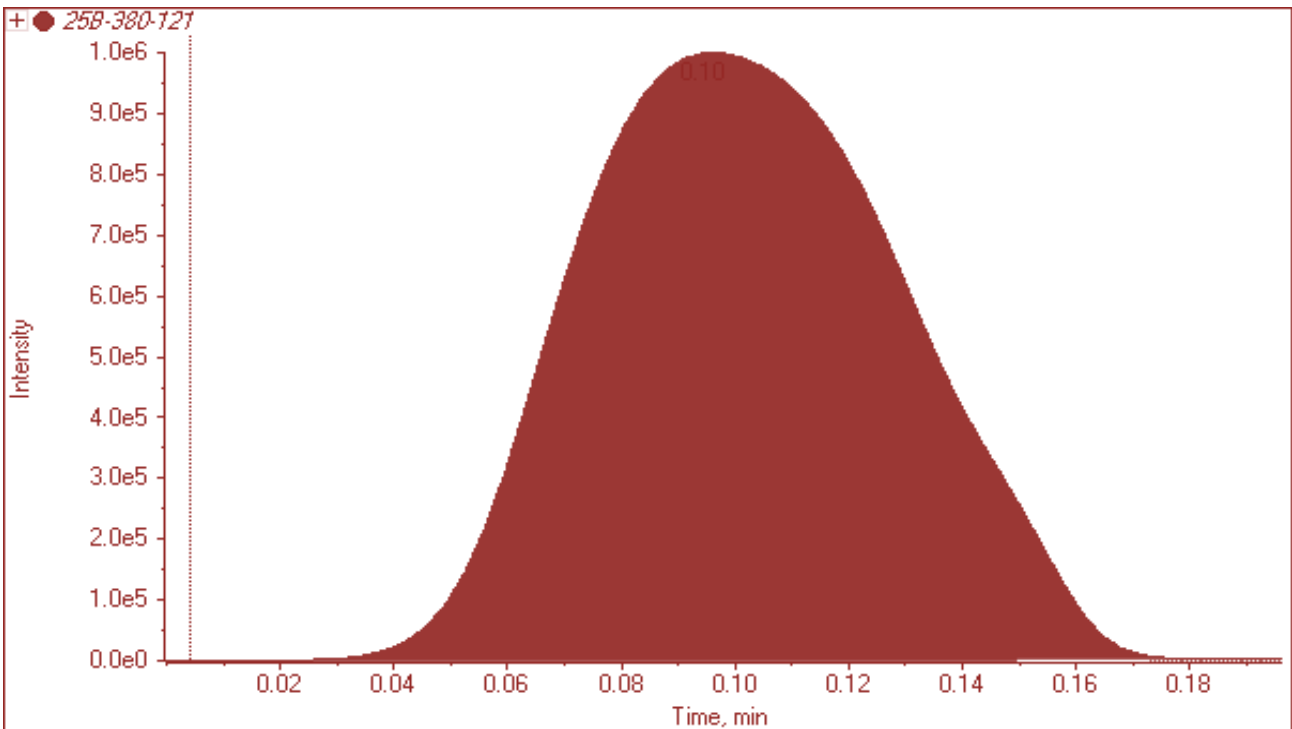


Figure 5 100 ng/mL standard desorption pic of 25B-NBOMe in urine

## RESULTS:

### 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%.

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

	Serum			
		LLOQ	QCM	ULOQ
	Conc. (ng/mL)	0.5	10	100
	n	3		
25I	Mean (ng/mL)	0.49	10.15	98.05
	%RSD	3.04	0.68	1.12
	%NOM	98.9	101.5	98.1
25B	Mean (ng/mL)	0.52	9.92	98.83
	%RSD	4.45	0.68	0.73
	%NOM	104.6	99.2	98.8

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

	Urine			
		LLOQ	QCM	ULOQ
	Conc. (ng/mL)	0.5	10	100
	n	3		
25I	Mean (ng/mL)	0.48	10.54	104.01
	%RSD	3.63	1.05	0.57
	%NOM	95.9	105.4	104.0
25B	Mean (ng/mL)	0.48	10.28	103.40
	%RSD	2.13	0.87	1.54
	%NOM	95.8	102.8	103.1

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

	Saliva (OraSure® buffer)			
		LLOQ	QCM	ULOQ
	Conc. (ng/mL)	0.5	10	100
	n	3		
25I	Mean (ng/mL)	0.51	9.70	100.17
	%RSD	2.38	1.37	1.97
	%NOM	101.5	97.0	100.2
25B	Mean (ng/mL)	0.51	9.81	100.21
	%RSD	1.89	1.18	2.40
	%NOM	101.2	98.1	100.2

### Drugs interferences

32 drugs, with a concentration of 1000 ng/mL, have been added to a 10 ng/mL quality control sample to analyze the interferences between these drugs and the 2 phenethylamines. **Table 5** shows the concentration results for both 25I-NBOMe and 25B-NBOMe drugs in the 3 different matrices while **Table 6** shows the drugs used in the analysis

Table 5 Results of Potential Drug Interference test

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 6 List of the 32 drugs used for the Drug interference test

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, and urine samples**
- **High Selectivity, Sensitivity and Specificity using Tandem Mass Spectrometry**
- **Versatility of LDSTD technology proven with human samples**
- **LDSTD provides the High-Throughput analysis of sample extract in 9 seconds sample-to-sample without any carry over**