A Novel SLE-LDTD-MS/MS Method for the Screening of NBOMe Designer Drugs in Oral Fluid

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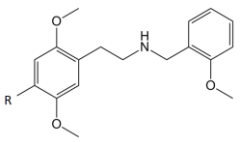
Introduction

NBOMes (Figure 1) are a class of novel psychoactive substances (NPSs) marketed as "legal highs." NBOMes are derived from substituted phenethylamines in the 2C series of analytes. The 2C series contain methoxy groups on the 2 and 5 positions of a benzene ring of the phenethylamine backbone structure. NBOMes also contain a 2-methoxybenzyl on the nitrogen backbone, which results in increased substitution and ultimately potency. NBOMes are typically administered sublingually via blotter paper and often confused or misrepresented as LSD. The general effects include hallucinations, tachycardia, agitation, and seizures. Oral fluid is an emerging biological matrix because of the ability to readily obtain a sample using a noninvasive collection procedure. Oral fluid contains primarily the parent compound, which is most commonly associated with the pharmacological effects, making it an ideal matrix for the detection of NBOMe analytes.

Analytes of interest



R = Br	25B-NBOMe
R = Cl	25C-NBOMe
R = CH_3	25D-NBOMe
R = C_2H_5	25E-NBOMe
R = H	25H-NBOMe
R = I	25I-NBOMe
R = N_2O	25N-NBOMe
R = SC_2H_5	25T2-NBOMe

Figure 1: *Chemical structures of NBOMes studied*

Reduced workflow Sample Preparation – "Load, Wait, Elute"

Fast effective sample cleanup was achieved using a "load, wait, elute" approach with supported liquid extraction (SLE).

The ISOLUTE SLE$^+$ format was employed in a 96 well format (400 µL). The principles of extraction were described in Figure 2. Relative recovery demonstrated 60-70% for both analytes yield LLOQs of 0.5 ng/mL. Optimized parameters are given in Table 1 for extending the linear dynamic range of the method. SLE-LDTD-MS was previously demonstrated for the screening of drugs of abuse in oral fluids[1].

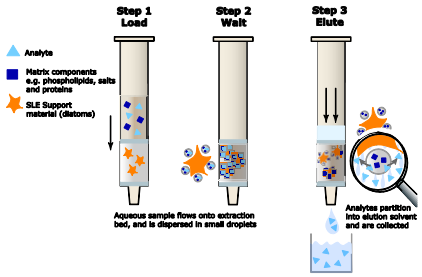


Figure 2. Schematic of ISOLUTE® SLE+ Supported Liquid Extraction procedure.

Table 1: *SLE+ Method*

Extraction Procedure	Range 1	Range 2
SLE	Biotage SLE+ 400µL	Biotage SLE+ 400µL
Loading	150µL sample 15µL ISTD 25I-NBOMe-D3 (1000ng/ml MeOH) 10µL NH_4OH (0.1%)	300µL sample 30uL ISTD 25I-NBOMe-D3 (100ng/ml MeOH) 20µL NH_4OH (0.1%)
Adsorption	Wait 5 minutes	Wait 5 minutes
Elution	4x 500µL MTBE	4x 500µL MTBE
LazWell Spotting	Deposit 3µL of elution phase on LazWell plate and let dry	Deposit 6µL of elution phase on LazWell plate and let dry

Mass Spectrometry

Samples were analyzed by laser diode thermal desorption (LDTD)-MS/MS (Figure 3).

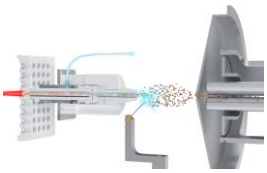


Figure 3: *LDTD source configuration*

Following sample preparation, a small volume is transferred and dried into a well cavity. The analytes of interest were vaporized indirectly by thermal action and ionized by APCI. The time required was **less than 9 seconds per sample**.

MS/MS compound selective parameters

The LDTD-MS was coupled to a SCIEX 5500 QTRAP (Figure 4). The MS/MS instrumentation was operated in multi-reaction monitoring mode (MRM). The optimized compound selective parameters were given in Table 2.

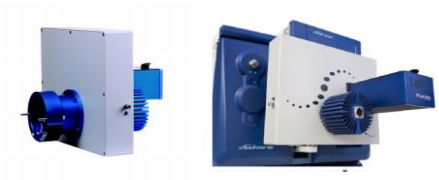


Figure 4: *LDTD source design for AB Sciex instrumentation*

Table 2: Compound selective MS/MS parameters

MRM	Q1	Q3	dwell	COMPOUND	Collision energy
1	m/z	m/z	msec	identification	eV
2	302.2	120.6	5	25H-121	22
3	302.2	165.1	5	25H-165	22
4	316.0	120.6	5	25D-121	22
5	316.0	179.0	5	25D-179	22
6	330.1	120.7	5	2.50E-120	22
7	330.1	192.9	5	2.50E-192	22
8	336.1	120.7	5	25C-121	22
9	336.1	90.9	5	25C-91	22
10	347.2	120.6	5	25N-121	22
11	347.2	90.8	5	25N-91	22
12	348.2	120.7	5	25T2-121	22
13	348.2	211.1	5	25T2-211	22
14	380.2	120.8	5	25B-121	22
15	380.2	91.0	5	25B-91	22
16	428.1	120.7	5	25I-121	22
17	428.1	272.1	5	25I-272	22
18	431.1	124.0	5	25I_d3-124-IS	22

Results

Experiment 1: Summary of method development study

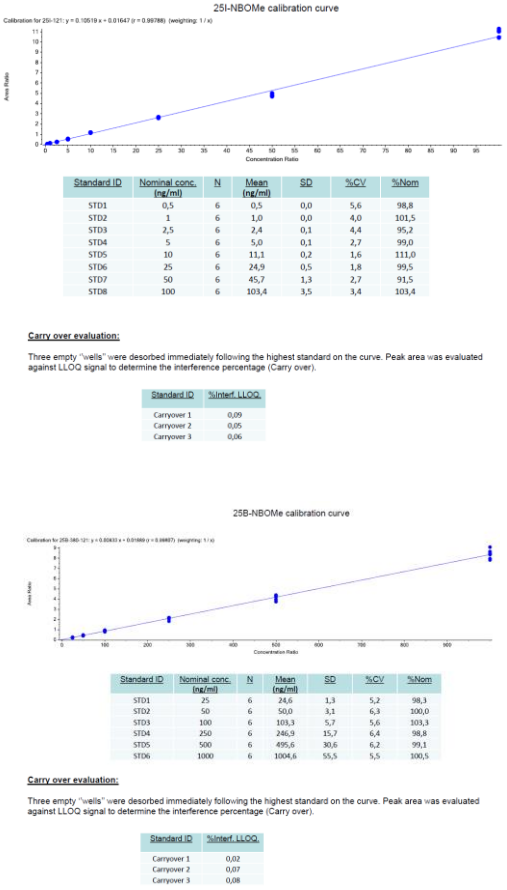
Linearity and specificity were determined for 2 of the analytes of interest (R=Br, I) to verify method performance. The results are reported in Figure 5.

Experiment 2: Fortified Oral Fluid Specimen Set (n=8) submitted blind to the analyst

A set of oral fluid specimens was prepared at the Center for Forensic Science Research and Education. The samples were collected with Salivettes and fortified to different concentration levels. The samples were submitted blind to the Phytronix applications lab.

Additional analytes were added to extend the panel capturing current trends in this emerging threat to public health: 25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25E-NBOMe, 25I-NBOMe, 25N-NBOMe, and 25T2-NBOMe. The compound selective transitions were optimized prior to analysis. Results are detailed in Figure 6.

Experiment 1:



Standard ID	Nominal conc. (ng/ml)	N	Mean (ng/ml)	SD	%CV	%Nom
STD1	0,5	6	0,5	0,0	5,6	98,8
STD2	1	6	1,0	0,0	4,0	101,5
STD3	2,5	6	2,4	0,1	4,4	95,2
STD4	5	6	5,0	0,1	2,7	99,0
STD5	10	6	11,1	0,2	1,6	111,0
STD6	25	6	24,9	0,5	1,8	99,5
STD7	50	6	45,7	1,3	2,7	91,5
STD8	100	6	103,4	3,5	3,4	103,4

Standard ID	%Interf. LLOQ
Carryover 1	0,09
Carryover 2	0,05
Carryover 3	0,06

Standard ID	Nominal conc. (ng/ml)	N	Mean (ng/ml)	SD	%CV	%Nom
STD1	25	6	24,6	1,3	5,2	98,3
STD2	50	6	50,0	3,1	6,3	100,0
STD3	100	6	103,3	5,7	5,6	103,3
STD4	250	6	246,9	15,7	6,4	98,8
STD5	500	6	495,6	30,6	6,2	99,1
STD6	1000	6	1004,6	55,5	5,5	100,5

Standard ID	%Interf. LLOQ
Carryover 1	0,02
Carryover 2	0,07
Carryover 3	0,08

Figure 5: *Examples of linearity and specificity*

Experiment 2:

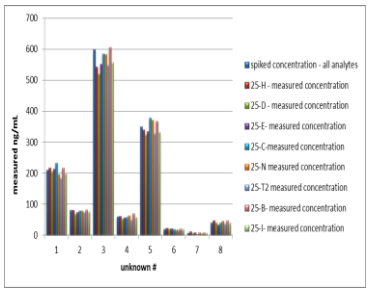


Figure 6: *Unknown sample set fortified to 8 concentration levels for a panel of NBOMe designer drugs*

Conclusions

- SLE-LDTD-MS demonstrated a viable workflow solution for the screening of NBOMe designer drugs in oral fluids.
- SLE proved effective in minimizing matrix effects by producing clean extracts.
- This method demonstrated good sensitivity with LLOQ values determined at 0.5 ng/mL for both analytes.
- This method demonstrated acceptable precision and accuracy across the calibration range of interest.
- Linearity of r2> 0.99 for all analytes
- Reproducibility with CV < 10% with n=4 (<15% at LLOQ)
- Reference QC high and low within 15% accuracy

Reference

Birsan, A "Generic SLE extraction method for ultra-fast drug screening in saliva using LDTD-MS/MS analysis" *Mass Spectrometry Applications to the Clinical Laboratory (MSACL)*, San Diego, CA, March 2014

(This poster was first presented at TAMS, RTP, 2014)