Method Development of NBOMes in Oral Fluid by Solid Supported Liquid Extraction and Gas Chromatography Mass Spectrometry

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Introduction

NBOMes ((2,5-dimethoxyphenyl)-N-[(2- methoxyphenyl)methyl]ethanamine) are a class of novel psychoactive substances (NPS) that are derived from a specific group of substituted phenethylamines – the 2C series. The 2C series contains methoxy groups on the 2 and 5 positions of a benzene ring of the phenethylamine backbone structure. NBOMes have an additional 2-methoxybenzyl on the nitrogen backbone of the 2C series, which is believe to result in increased potency. Insufficient data exists about NBOMes, however, it is thought that NBOMes interact with the serotonin neurotransmitter in the brain. General effects of NBOMes include hallucinations, tachycardia, seizures and death can result at low levels. NBOMes are usually administered sublingually on blotter paper and often mistaken for Ecstasy or LSD.

Oral fluid is an emerging biological specimen that is readily collected using non-invasive procedures. Currently, there is not any literature on the detection of NBOMes in oral fluid, however, detecting NBOMes and other novel psychoactive substances in oral fluid is of value. Since NBOMes are primarily administered sublingually, it is probable that the drugs may contaminate the oral mucosa as well as accumulate in oral fluid due to ion trapping after passively diffusing from the blood into oral fluid.

Supported liquid extraction (SLE) is an extraction technique that requires minimal preparation, solvent, time, and produces minimal waste. SLE is based on pKa and polarity. An initial treatment of samples is required to leave the analytes of interest un-ionized, load the sample, allow the samples to adsorb onto the cartridge and elute with a solvent to collect un-ionized analytes and leave ionized compounds behind. Eight NBOMes (figure 1) were analyzed in oral fluid using SLE and GC/MS.

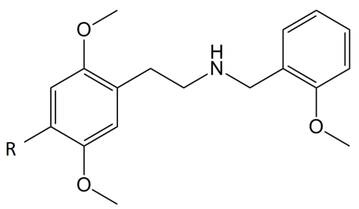

Figure 1. General Structure of NBOMe Analytes

R Group Substituent	Resulting NBOMe
Bromine	25B-NBOMe
Chlorine	25C-NBOMe
Methyl Group	25D-NBOMe
Ethyl Group	25E-NBOMe
Hydrogen	25H-NBOMe
Iodine	25I-NBOMe
N_2O	25N-NBOMe
SC_2H_5	25T2-NBOMe

Methods

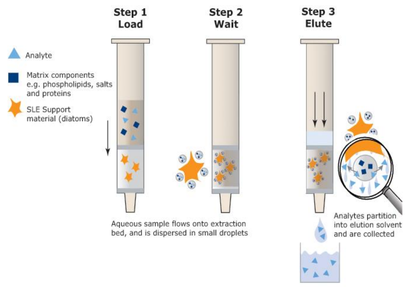

Figure 2. Schematic of ISOLUTE SLE+ extraction procedure. The SLE method encompasses a "Load, Wait, Elute" method with no preconditioning, equilibrating nor washing of the cartridges like SPE requires.

Extraction Method

- 500 µL of oral fluid spiked with analytes of interest
- 400 µL of sample applied to SLE cartridge
- Samples were pretreated with 250 µL of 5% ammonium hydroxide
- Samples adsorbed for 5 minutes and eluted with 1 mL of DCM/EtOAc/IPA (85/10/5) twice
- Samples were dried to completion at 33˚C and derivatized with PFPA:EtOAc (2:1) at 80˚C for 30 minutes
- Samples were reconstituted in 50 µL of ethyl acetate
- Samples were analyzed on an Agilent 7890A gas chromatograph coupled with a 5975C mass spectrometry.

Optimization Results

Several parameters of the extraction were evaluated in an effort to optimize the extraction procedure in terms of time and volume of solvents. The results were evaluated in terms of percent recovery.

Sample Pretreatment Experiments

Percent NH_4OH	Percent Recovery Average (n=8)	Volume NH_4OH	Percent Recovery Average (n=8)
1%	91%	100 µL	85%
2%	120%	250 µL	99%
5%	101%	500 µL	109%

- The concentration of ammonium hydroxide and volume added to samples were varied
- Based on percent recovery, it was determined 250 uL of 5% ammonium hydroxide was optimal

Extraction Solvent Experiments

Extraction Solvent	Percent Recovery	Extraction Volume	Percent Recovery Average (n=8)
DCM/EtOAc/IPA (85/10/5)	Highest: 97% Lowest: 57%	2x 500 µL	81%
MTBE	Highest: 133% Lowest: 24%	2x 1 mL	84%
DCM/IPA (95/5)	Highest: 100% Lowest: 42%	2x 1.5 mL	80%

- The extraction solvent and composition of solvents and volume of extraction solvent used were varied.
- It was determined that extracting with 1 mL twice with DCM/EtOAc/IPA (85/10/5) was optimal.

Final Optimized Method

In the final optimized method 500 µL of oral fluid was spiked to the desired concentration and pretreated with 250 µL of 5% NH_4OH. Samples were loaded onto the ISOLUTE SLE+ cartridges and eluted with two one milliliter washes of dichloromethane, ethyl acetate, and isopropanol (85/10/5). Show in the figure below is a total ion chromatogram for all 8 NBOMes extracted using this optimized method.

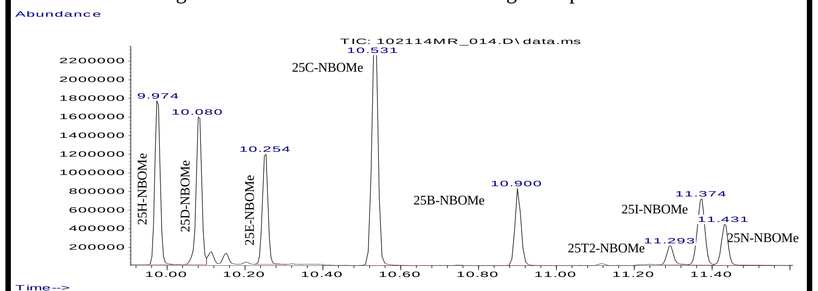

Figure 3. A representative chromatogram under optimized conditions, indicating all NBOMes are resolved from each other.

Acknowledgments

I would like to acknowledge Biotage for providing ISOLUTE+ SLE cartridges, The Center for Forensic Science Research and Education and Arcadia University for their support of this project.

Extraction Results

The optimized SLE method was compared to traditional techniques such as liquid liquid (LLE) and solid phase extraction (SPE) to determine if SLE was comparable in terms of recovery and which method was the most efficient in terms of time, amount of solvents used, and cost.

Comparison of Percent Recovery using SLE, LLE, and SPE

NBOMe	SLE Percent Recovery	LLE Percent Recovery	SPE Percent Recovery
25H-NBOMe	98%	97%	96%
25D-NBOMe	101%	100%	91%
25E-NBOMe	97%	101%	86%
25C-NBOMe	101%	101%	99%
25B-NBOMe	92%	101%	96%
25T2-NBOMe	120%	92%	29%
25I-NBOMe	91%	101%	100%
25N-NBOMe	73%	77%	80%

Comparison of Extraction Techniques

Extraction Technique	Sample Volume	Time of Extraction	Amount of Solvent Consumed	Cost of Cartridges
SLE	0.5 mL	Shortest	2.25 mL	$116 for 50
SPE	1 mL	Longest	14 mL	$113 for 50
LLE	0.5 mL	Intermediate	4 mL	N/A

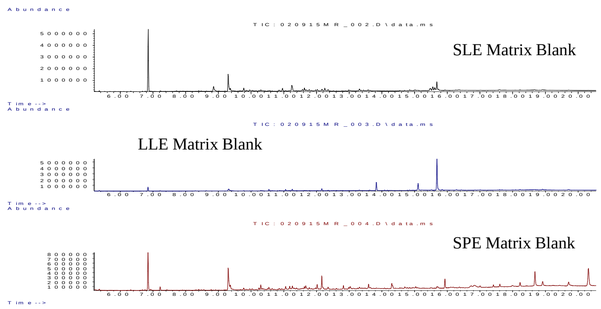

Figure 4. Matrix blanks for SLE, LLE, and SPE that demonstrate that SLE is a faster extraction technique with comparable chromatographic results as traditional LLE and SPE.

Conclusion

Oral fluid is a valuable specimen in DUI or drug intoxication cases because the collection of oral fluid can provide a more accurate depiction drugs eliciting the psychoactive effects. Using oral fluid, a successful method was developed for detecting NBOMes using SLE and GC/MS. SLE produces comparable, if not better recovery, uses less sample volume and is more efficient in terms of waste, time, and cost compared to SPE and LLE. Future work includes determining linear range, performing a method validation, determining comparison values for NBOMes in oral fluid and with other matrices such as blood and urine, and analyzing authentic samples.