Determination of 32 Cathinone Derivatives and other Designer Drugs in Serum by Comprehensive LC/Triple Quadrupole/MS/MS Analysis

Application Note

Authors

Madeleine J. Swortwood and
Anthony P. DeCaprio
Department of Chemistry and
Biochemistry and International
Forensic Research Institute
Florida International University
Miami, FL 33199

Diane M. Boland
Miami-Dade Medical Examiner
Department Toxicology Laboratory,
Miami, FL 33136

Abstract

There are few comprehensive screening techniques for the detection and quantification of designer drugs in biological specimens. A liquid chromatography triple quadrupole tandem mass spectrometry (LC/Triple Quadrupole/MS/MS) method that encompasses over thirty important compounds within the phenethylamine, tryptamine, and piperazine designer drug classes was developed and validated. The assay was selective for all analytes showing acceptable accuracy and precision and limits of quantification (LOQ) were in the range of 1–10 ng/mL for each compound with limits of detection (LOD) near 10 pg/mL. The validated method was used to analyze post-mortem specimens from two cases that were suspected of containing designer drugs. The method was able to identify and quantify seven of these compounds at concentrations as low as 11 ng/mL.

Agilent Technologies

Introduction

The illicit drug market has seen a large influx of designer drugs such as Cathinone derivatives a.k.a. bath salts and structural analogs of DEA schedule I and II substances, which is serious, as the safety profiles are unknown and potentially dangerous. This LC/MS line of equipment provides broad based screening capabilities to laboratories for these new and emerging drugs.

Materials and Methods

Chemicals and materials

The drugs were purchased from LipoMed (Cambridge, MA), from Cerilliant (Round Rock, TX) and from Grace Davison Discovery Sciences (Deerfield, IL). Table 1 details the structures and drug classes (that is, phenethylamines, tryptamines, and piperazines), compound abbreviations, and chemical names.

Table 1. Classification of Targeted Analytes with Structures and Abbreviations

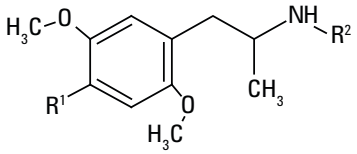
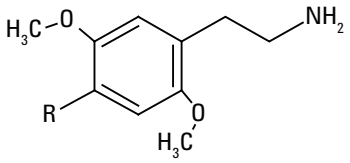
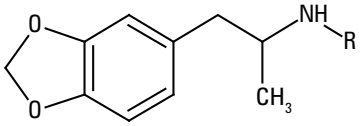
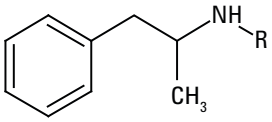
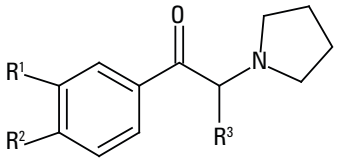
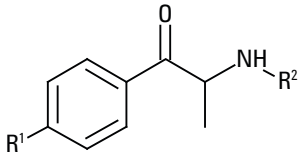
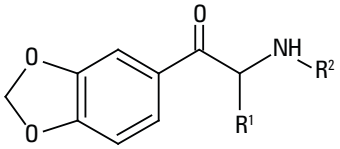
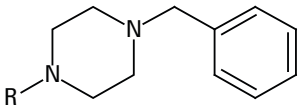
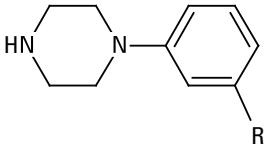
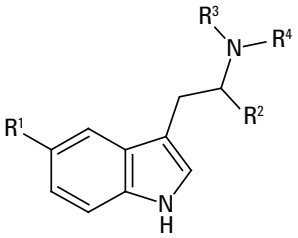
Class	Basic structure	Substituents	Name/abbreviation
Phenethylamines 2,5-dimethoxy amphetamines		R^1 = Br R^2 = H R^1 = C_2H_5 R^2 = H R^1 = CH_3 R^2 = H R^1 = O-CH_3 R^2 = H	DOB DOET DOM TMA
Phenethylamines 2Cs		R = Br R = C_2H_5 R = I R = S-$CH(CH_3)_2$ R = S-C_3H_7	2C-B 2C-E 2C-I 2C-T-4 2C-T-7
Phenethylamimes 3,4-methylene- dioxyamphetamines		R = H R = C_2H_5 R = CH_3	MDA MDEA MDMA
Phenethylamines Amphetamines		R = H R = CH_3 R = C_2H_5	Amphetamine Methamphetamine Ethylamphetamine
Phenethylamines Pyrrolidinophenones		R^1 = R^2 = O-CH_2-O R^3 = C_3H_7	MDPV
Phenethylamines β-keto-amphetamines	 	R^1 = R^2 = CH_3 R^1 = R^2 = H R^1 = H R^2 = CH_3 R^1 = O-CH_3 R^2 = CH_3 R^1 = CH_3, C_2H_5 R^1 = F R^2 = CH_3 R^1 = R^2 = CH_3 R^1 = C_2H_5 R^2 = CH_3	Mephedrone Cathinone Methcathinone Methedrone 4-Methylethcathinone Flephedrone Methylone Butylone

Table 1. Classification of Targeted Analytes with Structures and Abbreviations (continued)

Class	Basic structure	Substituents			Name/abbreviation
Piperazines Benzylpiperazines		R = H R = CH_2-C_6H_5			BZP DBZP
Piperazines Phenylpiperazines		R = Cl R = CF_3			mCPP TFMPP
Tryptamines		$R^1 = R^3 = R^4 = H$ $R^1 = R^2 = H$ R^1 = O-CH_3 R^1 = O-CH_3	$R^2 = CH_3$ $R^3 = R^4 = CH_3$ $R^2 = H$ $R^2 = H$	 $R^3 = R^4 = CH_3$ $R^3 = R^4 = CH(CH_3)_2$	AMT DMT 5-MeO-DMT 5-MeO-DiPT

Methanolic solutions of the following deuterated internal standards were purchased from LipoMed as 0.1 mg/mL standards: d6-amphetamine, d5-MDMA, and d3-mephedrone. Methanolic solutions of the following deuterated internal standards were purchased from Cerilliant as 0.1 mg/mL standards: d7-BZP, d3-methylone, and d4-TFMPP. DBZP was purchased as a bulk powder from Sigma-Aldrich (St. Louis, MO) as it was not available as a calibrated reference standard.

Resprep Drug Prep I cartridges (200 mg; 10 mL) for solid-phase extraction were purchased from Restek (Bellefonte, PA) for manual extraction performed on a Supelco Visiprep-DL Disposable Liner SPE vacuum manifold.

Serum samples

Bioreclamation (Westbury, NY) was the source for obtaining the pooled blank human serum recovered from whole blood donations used for method development and validation. Quantitative analysis was performed on authentic post-mortem blood specimens and were stored at –20 °C.

Sample preparation

Serum samples (1 mL) were diluted with 2 mL of sodium phosphate buffer (100 mM, pH 6.0). 20 µL of internal standards (IS) containing 1 µg/mL each of d6-amphetamine, d7-BZP, d5-MDMA, d3-mephedrone, d3-methylone, and d4-TFMPP, was added to the samples, vortexed and loaded onto a mixed-mode (Drug Prep I) SPE cartridge that was previously conditioned with 3 mL of methanol, 3 mL of water, and 1 mL of phosphate buffer. After extraction, cartridges were sequentially washed with 1 mL of water, 1 mL of 0.1 M acetic acid, and then 1 mL of methanol. Analytes were then eluted slowly using two rounds of 1.5 mL of elution buffer, which consisted of dichloromethane (DCM), isopropanol (IPA), and ammonium hydroxide (80:20:2 v/v/v) [1, 2]. The combined eluates were acidified with 100 µL of HCl–IPA (1:3 v/v) before evaporation in an Eppendorf Vacufuge at 30 °C. When dry, the residue was reconstituted in 50 µL of mobile phase and 5 µL of extract was injected into the LC/MS/MS system.

LC/Triple Quadrupole/MS/MS analysis

Instrumentation

The samples were analyzed using an Agilent 1290 Infinity Binary Pump LC coupled to an Agilent 6460 Triple Quadrupole MS/MS with Jet Stream technology and electrospray ionization (ESI) using Agilent MassHunter software. An Agilent ZORBAX Rapid Resolution HD Eclipse Plus C18 LC column (50 × 2.1 mm, 1.8 µm particle size) was used for separation. Data acquisition was performed in Dynamic MRM mode with positive ESI using one principal MRM transition for quantitation and one additional transition to serve as a qualifier for each analyte.

LC conditions

Chromatographic separation occurred with gradient elution at a flow rate of 0.5 mL/min using 2 mM ammonium formate/0.1% formic acid in water as mobile phase A and acetonitrile/water (90:10 v/v) with 0.1% formic acid as mobile phase B. The gradient was as follows: 5% B up to 35% B in 6 minutes as the analytical run, followed by a 30-second ramp up to 95% B and then a 1-minute hold at 95% B for cleanup before a 3.5-minute re-equilibration at 5% B. The analytical column was kept at 40 °C in a temperature controlled column compartment during separation.

MS parameters and screening procedure

MS source parameters were as follows: gas temperature, 320 °C; gas flow 8 L/min; nebulizer 27 psi; sheath gas heater 380 °C; sheath gas flow 12 L/min; capillary voltage 3,750 V; and charging voltage 500 V. Agilent MassHunter Optimizer software was used to optimize the data acquisition parameters for MRM mode by automatically selecting the best precursor ions and associated fragmentor voltages in addition to selecting the best fragment ions and collision energies for each transition. Enhanced sensitivity was achieved with the Dynamic MRM acquisition capabilities of the Agilent system, which utilizes analyte retention times, detection windows (ΔtR), and a constant scan cycle time for precise detection of multiple analytes in a small ΔtR . All detection windows were set at 0.4 minutes (±0.2 minutes around tR). Table 2 summarizes the Dynamic MRM parameters.

Table 2. Dynamic MRM MS Method Parameters

No.	Drug	Transitions*	CE (V)	Fragmentor (V)	t_R (min)	Internal standard
1	DOB	**274.01 → 256.9**	14	100	3.846	d6-Amphetamine
		274.01 → 228.9	10			
2	DOET	**224.3 → 207**	5	85	4.547	d6-Amphetamine
		224.3 → 91	49			
3	DOM	**210.3 → 193.1**	5	75	3.538	d6-Amphetamine
		210.3 → 165	13			
4	TMA	**226.3 → 209**	5	80	2.075	d6-Amphetamine
		226.3 → 91	45			
5	2C-B	**260.01 → 242.9**	4	90	3.403	d5-MDMA
		260.01 → 227.9	6			
6	2C-E	**210.3 → 193**	5	80	4.119	d5-MDMA
		210.3 → 163	25			
7	2C-I	**308.1 → 290.9**	9	90	3.906	d5-MDMA
		308.1 → 91	49			
8	2C-T-4	**256.4 → 239**	5	90	4.675	d5-MDMA
		256.4 → 197	17			
9	2C-T-7	**256.4 → 239**	9	85	4.959	d5-MDMA
		256.4 → 166.9	29			
10	MDA	**180.1 → 163**	4	70	1.658	d6-Amphetamine
		180.1 → 105	20			

*Quantifying transition in bold, qualifying transition in normal text.

Table 2. Dynamic MRM MS Method Parameters (continued)

No.	Drug	Transitions*	CE (V)	Fragmentor (V)	t_R (min)	Internal standard
11	MDEA	**208.14 → 163**	8	90	2.220	d5-MDMA
		208.14 → 105	24			
12	MDMA	**194.1 → 163**	8	85	1.849	d5-MDMA
		194.1 → 105	24			
13	Amphetamine	**136.11 → 91**	16	75	1.490	d6-Amphetamine
		136.11 → 119	4			
14	Methamphetamine	**150.13 → 91**	16	80	1.715	d5-MDMA
		150.13 → 119	4			
15	Ethylamphetamine	**164.11 → 91**	20	85	2.093	d5-MDMA
		164.11 → 119	8			
16	MDPV	**276.3 → 126**	25	130	3.383	d3-Methylone
		276.3 → 135	25			
17	Mephedrone	**178.25 → 160**	10	85	2.123	d3-Mephedrone
		178.25 → 144	30			
18	Cathinone	**150.2 → 132**	10	80	1.031	d3-Mephedrone
		150.2 → 117	22			
19	Methcathinone	**164.23 → 146**	10	85	1.196	d3-Mephedrone
		164.23 → 130	34			
20	Methedrone	**194.25 → 176**	10	80	1.745	d3-Mephedrone
		194.25 → 161	18			
21	4-MEC	**192.28 → 174.1**	10	95	2.482	d3-Mephedrone
		192.28 → 145	18			
22	Flephedrone	**182.21 → 164**	10	85	1.422	d3-Mephedrone
		182.21 → 148	34			
23	Methylone	**208.24 → 160**	14	80	1.397	d3-Methylone
		208.24 → 132	26			
24	Butylone	**222.26 → 174**	14	95	2.035	d3-Methylone
		222.26 → 204	10			
25	BZP	**177.11 → 91**	20	100	0.589	d7-BZP
		177.11 → 65	50			
26	DBZP	**267.21 → 91**	32	125	3.520	d7-BZP
		267.21 → 175	12			
27	mCPP	**197.11 → 153.9**	20	120	2.878	d4-TFMPP
		197.11 → 118	36			
28	TFMPP	**231.11 → 188**	20	125	3.826	d4-TFMPP
		231.11 → 118	44			

*Quantifying transition in bold, qualifying transition in normal text.

Table 2. Dynamic MRM MS Method Parameters (continued)

No.	Drug	Transitions*	CE (V)	Fragmentor (V)	t_R (min)	Internal standard
29	AMT	**175.2 → 158**	9	75	2.037	d6-Amphetamine
		175.2 → 143	25			
30	DMT	**189.11 → 58.1**	8	85	1.775	d5-MDMA
		189.11 → 144	16			
31	5-MeO-DMT	**219.3 → 58.1**	9	85	1.955	d5-MDMA
		219.3 → 174	9			
32	5-MeO-DiPT	**275.4 → 174**	17	100	3.627	d5-MDMA
		275.4 → 114.1	13			
33	d6-Amphetamine (IS)	**142.25 → 93**	13	75	1.470	-
		142.25 → 125.1	5			
34	d5-MDMA (IS)	**199.29 → 165**	9	90	1.839	-
		199.29 → 107	25			
35	d3-Mephedrone (IS)	**181.27 → 163**	9	90	2.115	-
		181.27 → 148	21			
36	d3-Methylone (IS)	**211.21 → 163**	13	85	1.390	-
		211.21 → 135	29			
37	d7-BZP (IS)	**184.11 → 98.1**	21	105	0.562	-
		184.11 → 70.1	57			
38	d4-TFMPP (IS)	**235.11 → 190**	21	125	3.815	-

*Quantifying transition in bold, qualifying transition in normal text.

Quantification

Quantification of the analytes was done using Agilent MassHunter Quantitative Analysis software version B.04.00. Peak area ratios (that is, drug versus IS) were calculated and plotted against concentrations within the software.

Assay validation

The LC/MS/MS assay was fully validated according to generally accepted guidelines. The experimental design for the validation experiments was based on those proposed by Peters *et al*. [3]. The parameters evaluated included selectivity, matrix effects, recovery, process efficiency, linearity, processed sample stability, freeze-thaw stability, precision, and accuracy.

Preparation of stock and spiking solutions

Separate aqueous stock solutions were prepared during method development and optimization for each analyte at a concentration of 1 µg/mL from the commercially available calibrated reference standards (1 mg/mL for targeted compounds, 0.1 mg/mL for internal standards). An aqueous spiking solution of the 32 analytes was prepared at a concentration of 10 µg/mL each. This stock solution was used for the preparation of diluted aqueous spiking solutions at a concentration of 1 µg/mL each.

Preparation of Matrix samples

Samples for linearity of calibration, QC samples, samples for the evaluation of selectivity, matrix effects, process stability, freeze thaw and precision and accuracy were made by the appropriate addition of spiking solutions into the indicated matrix.

Blood samples from two authentic post-mortem cases were submitted for analysis and assayed with the described validated method.

Results and Discussion

LC-MS/MS analysis

The Agilent MassHunter Optimizer software was able to identify the two most common fragments, which were used for the quantifying and qualifying transitions, the collision energy, and the fragmentor voltage (summarized in Table 2). The gradient method allowed for separation of the 32 analytes in less than a 6-minute run time (Figure 1).

Assay validation

Selectivity

Using Dynamic MRM, no interfering peaks were observed when the analytes or internal standards were analyzed individually. Compounds with similar transitions, such as DOM and 2C-E, could still be differentiated due to the difference in retention times. Upon analysis of blank pooled serum, interfering peaks were minor and did not elute at the same time as any of the targeted analytes or internal standards. Only deuterated compounds were chosen as internal standards, to avoid possible overestimation of the internal standard signal that can occur when using therapeutic drugs as IS [2]. The method proved to be highly selective.

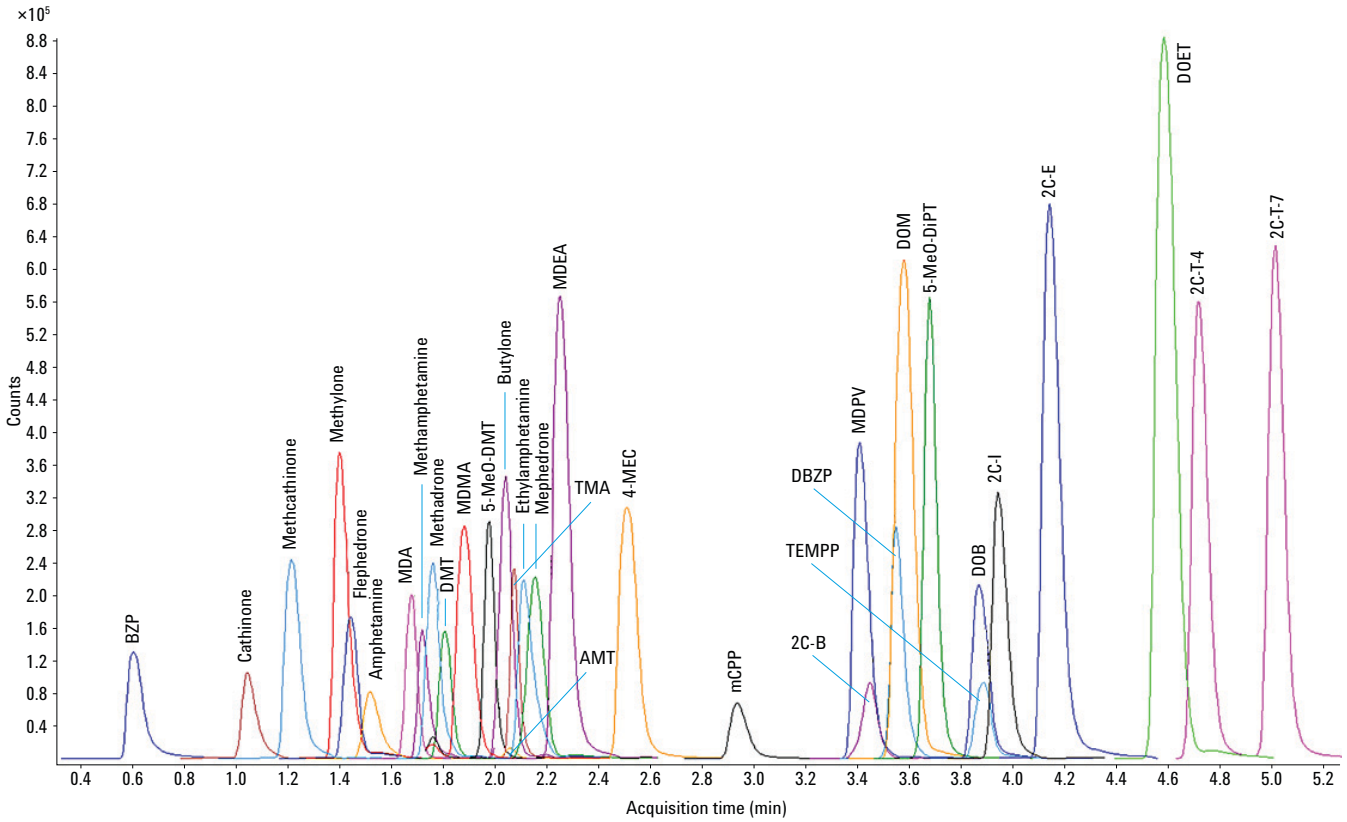


Figure 1. Chromatogram (counts versus retention time) of primary MRM transitions for 32 targeted analytes.

Matrix effects, recovery, and process efficiency

The Matrix Effects (ME), Recovery (R) and Process Efficiency (PE) were calculated for each analyte for both a LOW and HIGH analyte concentration (that is, 25 and 250 ng/mL nominal concentrations, respectively). Table 3 summarizes the means and RSDs, expressed as percentages.

The ion suppression or ion enhancement from matrix effects were generally acceptable (80–120%) at the lower analyte concentration. However, 5-MeO-DiPT, 5-MeO-DMT, DBZP, and BZP demonstrated significant ion suppression at 25 ng/mL but with fairly high %RSD, which might indicate some interferences or break-down at low concentrations. The matrix effects were slightly increased at the higher analyte concentration, an effect that might be a side effect of using a complex mixture of analytes and that may be diminished when examining single analytes. The recoveries were generally higher than 80%, demonstrating a sufficient extraction technique for most analytes. Lower recoveries were noted for 2C-T-4 and 2C-T-7, possibly due to different chemistries because of the presence of sulfur in the molecules. Recovery values higher than 100% may represent losses that could have occurred in the dry down stage when Set B included spiked elutions. The overall process efficiency was fairly reproducible and overall acceptable, taking into account both the matrix effects and recoveries.

Linearity of calibration

Agilent MassHunter Quantitative Analysis software was used to determine regression lines as well as to check precision, accuracy, ion response ratios, and retention times. A factor of 1/x was used to weight the linear regression models to account for heteroscedasticity. All R^2 values were a minimum of 0.990 in this experiment, bias within ±15% (±20% approximately the LOQ) and precision within ±15%RSD (±20% approximately the LOQ) were observed for all compounds from 10 ng/mL up to 250 ng/mL. For all further experiments, the following levels were used for calibration: 10, 25, 50, 100, 150, and 250 ng/mL. Data for all analytes were linear between 10 and 250 ng/mL.

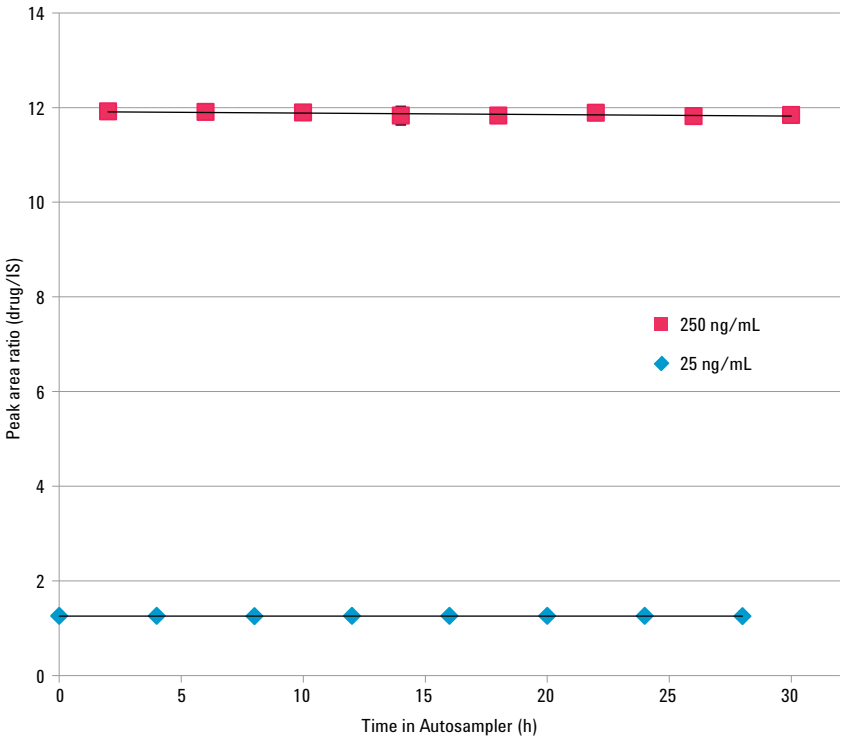


Figure 2. Example of processed sample stability for methylone.

Table 3. Matrix Effects, Recovery, and Process Efficiency

	Matrix Effects				Recovery				Process efficiency			
	LOW		HIGH		LOW		HIGH		LOW		HIGH	
Compound	**mean***	**%RSD**	**mean**	**%RSD**	**mean**	**%RSD**	**mean**	**%RSD**	**mean**	**%RSD**	**mean**	**%RSD**
BZP	71.2	15.8	66.2	51.6	125.7	15.3	135.5	56.0	89.4	7.4	89.7	22.9
Cathinone	92.0	9.4	146.7	9.6	133.9	14.3	126.9	24.7	123.1	11.7	186.2	25.4
Methcathinone	109.9	4.7	124.0	7.4	80.6	11.9	99.1	13.9	88.6	11.5	122.9	14.3
Methylone	95.8	3.9	96.4	4.0	106.0	11.1	104.8	4.9	101.5	10.7	101.0	3.7
Flephedrone	100.5	2.8	85.9	24.1	100.6	11.8	131.0	25.7	101.1	12.0	112.5	9.3
Amphetamine	102.5	2.8	115.9	19.0	100.2	16.1	87.6	19.2	102.8	16.2	101.6	3.7
MDA	88.7	8.8	92.3	12.4	101.4	9.0	108.0	16.6	90.0	3.3	99.6	12.4
Methedrone	108.2	3.0	104.4	10.8	101.9	13.3	111.4	11.2	110.3	13.1	116.3	5.7
Methamphetamine	99.9	2.9	77.7	8.1	106.3	19.6	114.4	10.8	106.2	19.7	88.8	8.7
DMT	118.0	25.2	116.7	27.4	91.0	28.1	107.1	27.1	107.4	22.5	125.0	37.7
MDMA	97.8	3.8	101.3	8.8	97.1	6.5	95.1	9.6	94.9	7.2	96.3	4.3
5-MeO-DMT	69.8	23.4	82.1	36.3	107.5	54.6	107.7	40.8	75.0	54.3	88.5	54.3
Butylone	102.5	3.7	84.2	7.3	102.0	12.8	112.8	9.8	104.6	12.6	94.9	7.1
AMT	113.5	11.6	49.7	52.7	99.7	21.9	128.7	79.3	113.2	21.9	63.9	63.0
TMA	103.0	6.2	123.0	11.3	96.6	14.0	81.4	20.7	99.5	14.7	100.1	18.1
Ethylamphetamine	102.6	3.1	82.5	10.4	101.1	11.6	110.5	12.0	103.7	11.7	91.2	6.2
Mephedrone	99.6	3.0	106.1	3.5	105.9	14.5	102.0	3.0	105.4	14.6	108.2	3.2
MDEA	106.3	4.9	121.5	14.7	92.1	10.5	85.8	22.3	97.9	9.7	104.3	17.2
4-MEC	115.2	5.7	148.0	9.1	102.2	16.0	94.1	13.3	117.8	15.1	139.2	15.4
mCPP	114.0	6.4	138.7	12.4	89.3	18.5	99.0	22.4	101.8	17.5	137.4	20.6
MDPV	115.1	8.9	153.3	16.3	93.0	15.1	84.1	17.6	107.1	13.0	128.9	11.9
2C-B	86.2	11.1	81.7	13.8	106.5	24.0	124.3	21.1	91.8	21.8	101.5	18.8
DBZP	50.6	11.0	90.0	48.5	141.4	37.9	76.9	62.3	71.5	37.9	69.2	41.1
DOM	118.8	6.8	126.2	15.9	84.1	7.7	79.1	16.3	99.9	4.5	99.9	5.3
5-MeO-DiPT	74.7	31.3	118.4	43.9	97.2	35.4	85.4	41.3	72.6	28.1	101.0	52.4
DOB	101.1	3.1	145.9	20.3	89.1	9.2	76.1	20.5	90.1	9.0	110.9	5.8
TFMPP	99.3	3.1	100.6	4.6	111.2	11.7	109.9	4.7	110.4	11.9	110.6	3.4
2C-I	85.6	13.2	115.5	25.0	92.0	23.6	91.3	24.7	78.8	20.0	105.4	6.9
2C-E	95.1	6.5	128.0	26.1	103.0	11.4	82.7	26.6	98.0	9.8	105.8	6.1
DOET	119.2	7.4	138.4	16.1	83.7	8.9	75.3	18.2	99.8	6.0	104.2	9.5
2C-T-4	101.0	7.4	146.4	30.1	66.0	23.0	79.9	29.2	66.7	22.1	117.0	16.3
2C-T-7	110.0	5.3	163.4	33.2	61.6	23.1	74.9	31.9	67.8	23.0	122.4	20.4

*Data in %, see under Methods for calculation details.

Precision and accuracy

The QC samples were analyzed at LOQ (10 ng/mL), LOW (25 ng/mL), MED (100 ng/mL), and HIGH (250 ng/mL) in duplicate on each of eight days. The results showed acceptable accuracy and precision within 15%. The results for DMT, 5-MeO-AMT, AMT, TMA, DBZP, 5-MeO-DiPT, and DOET failed some of the parameters (data not shown) and did not meet complete validation criteria, likely due to the lack of proper internal standards. Table 4 summarizes this data.

Limits

The LOQs and LODs were determined by spiking samples with decreasing concentrations of drug and analyzing along with a calibration curve. LOQs were in the range of 1–10 ng/mL, whereas LODs were in the range of 10–100 pg/mL. The LOQs accommodate very low level concentrations with the ability to accurately and precisely quantify the drugs that are present. As demonstrated below, LOQs for the method were sufficiently sensitive to allow confirmation of MDPV in a case sample that was undetected by previous screens.

	Repeatability (%RSD)				Intermediate precision (%RSD)				Accuracy, bias (%)			
Analyte	**LLOQ**	**LOW**	**MED**	**HIGH**	**LLOQ**	**LOW**	**MED**	**HIGH**	**LLOQ**	**LOW**	**MED**	**HIGH**
BZP	3.4	4.2	4.3	6.6	4.0	5.4	5.0	6.6	-7.7	-1.0	2.0	0.2
Cathinone	13.3	9.3	11.9	10.8	15.3	9.9	11.9	12.3	-9.9	-9.9	-2.9	0.6
Methcathinone	7.0	5.5	11.0	12.5	8.6	6.8	11.0	13.9	-5.7	-10.0	-4.9	0.6
Methylone	6.5	6.0	5.4	6.1	6.9	6.8	5.9	6.1	-10.2	-2.2	0.2	-4.6
Flephedrone	6.8	7.3	2.9	8.2	7.4	8.0	3.1	8.2	-13.2	-8.0	-4.4	-6.7
Amphetamine	4.9	4.8	3.8	11.8	6.1	5.7	3.9	11.9	-6.5	-0.6	4.3	2.6
MDA	5.2	5.4	12.7	11.9	6.5	6.3	12.8	11.7	-6.7	2.3	0.8	-6.2
Methedrone	4.1	3.4	4.8	12.5	6.5	4.8	5.1	12.2	-2.1	-4.2	-0.7	-2.7
Methamphetamine	5.4	5.5	7.7	11.4	7.9	6.4	7.7	11.5	-4.8	-1.5	2.0	-6.6
MDMA	5.4	5.8	4.0	9.7	5.9	6.3	4.4	9.7	-6.0	-0.4	3.0	1.9
Butylone	4.2	4.3	5.2	13.6	6.2	5.3	5.4	13.6	-6.6	0.3	-0.2	-5.3
Ethylamphetamine	9.2	7.0	6.8	6.1	10.6	7.8	7.1	6.2	-0.2	0.1	0.7	5.0
Mephedrone	4.7	5.6	3.2	8.5	5.3	6.3	3.6	8.5	-9.5	-5.0	-2.0	-3.7
MDEA	9.3	7.1	13.1	5.4	11.9	7.9	13.1	6.4	0.6	-3.9	-1.8	0.6
4-MEC	4.3	6.6	13.8	11.3	6.5	7.5	13.8	12.0	3.1	-6.9	-2.9	2.8
mCPP	3.6	8.8	13.6	12.1	6.7	10.6	13.6	12.1	9.6	0.9	0.2	8.8
MDPV	8.7	8.7	12.4	7.8	10.4	9.1	12.4	8.8	8.3	-5.1	-4.8	5.8
2C-B	14.6	5.7	7.2	7.6	15.5	6.7	7.3	7.7	-9.9	-3.5	-5.3	-2.1
DOM	11.3	6.3	12.5	13.7	13.8	7.1	13.0	13.7	-3.7	-0.2	-3.1	-10.8
DOB	13.6	7.7	12.8	11.1	14.7	8.1	13.0	11.1	-10.7	1.2	-1.8	-11.9
TFMPP	2.1	3.8	4.4	7.2	4.4	4.6	4.6	7.4	-8.5	-2.9	0.8	-0.5
2C-I	19.1	9.9	12.3	11.9	19.5	10.4	12.6	11.9	-13.3	2.8	-3.0	-9.5
2C-E	12.5	7.0	5.7	15.0	12.7	7.3	6.0	14.9	-6.3	-3.6	-2.1	-0.4
2C-T-4	17.1	10.9	11.8	14.2	17.3	11.4	13.6	14.2	1.9	-2.9	-12.9	-4.1
2C-T-7	18.0	10.1	11.2	9.2	18.4	10.5	12.5	9.5	1.2	-5.0	-11.1	-3.2

Proof of applicability

Post-mortem heart blood specimens from two forensic cases were submitted for analysis, as designer drugs were suspected to be present in these cases. The first case was a 31-year-old black male. The decedent died as a result of a suicidal gunshot wound to the head. During routine urinalysis, MDMA was found by GC/MS in the drug screen and was later confirmed in urine by GC/MS. Methylone was suspected in the GC/MS full scan confirmatory method but since a quantitative method was not in place the specimen was submitted for confirmation and quantification by the present validated LC/MS/MS method. The second case was a 26-year-old white male. The decedent had been huffing computer aerosol and was ruled an accidental death with cause of death attributed to acute polydrug toxicity, specifically citing 1,1-difluoroethane, MDMA, and 5-MeO-DiPT. In the initial GC/MS urine drug screen, MDA, MDMA, and 5-MeO-DiPT ("Foxy") were found. In addition, BZP, MDMA, 5-MeO-DiPT, and TFMPP were found in blood during a basic drug screen by GC/MS. In both cases, the amphetamine immunoassays were negative for both of the urines. Duplicate 1-mL portions of blood from each case were spiked with internal standard solution and extracted as described above. The concentrations of the analytes were calculated using a calibration curve and the QC samples in the same run were checked for acceptable accuracy and precision. Table 5 shows a summary of the quantitative results.

The concentrations of BZP and 5-MeO-DiPT were greater than the upper limit of quantification and would need to be reanalyzed after performing a sample dilution in order to ensure that they would be within the linear range. It is important to note that the calibration curve for Foxy and the daily QC samples were acceptable on the day that these samples were analyzed. The concentrations of the other compounds detected were comparable to those that were initially quantified by the submitting laboratory. Significantly, MDPV was found in Case 2 by the present method but had been missed in the initial GC-MS screens. The presence of bath salts was confirmed for both cases (methylone and MDPV, respectively) and establishes that these compounds are present in the local community. As the validated serum extraction procedure was adapted to whole blood in these cases, additional validation studies are currently underway for this type of biological specimen, particularly with regard to matrix effects and recovery.

Table 5. Summary of Quantitative Results for Case Samples

Compound	Case 1[a]	Case 2[a]
BZP	nd[b]	>250[c]
Methylone	63	nd
MDA	nd	36
MDMA	58	115
MDPV	nd	11
5-MeO-DiPT	nd	>250[c]
TFMPP	nd	93

[a] Data in ng/mL
[b] Not detected
[c] Present above highest calibration level.

Conclusions

An LC/Triple Quadrupole/MS/MS assay developed for the determination of 32 designer drug entities in serum was fully validated as per international guidelines. This was done only for 25 analytes because the remaining analytes from the tryptamine group did not meet the criteria for precision and accuracy. Using this method for extraction with serum specimens resulted in high recovery with minimal matrix effects, and was also used for the analysis of two whole blood specimens suspected of involving bath salts. This validated assay can be applied to forensic toxicological casework and future studies will continue to include many additional designer drugs that continue to be introduced to the market. Additional information regarding the full validation of this method can be found in Swortwood, *et al* [4]. Work is currently underway to develop an expanded screen of nearly 300 designer drugs and their metabolites.

References

1. A. Wohlfarth, W. Weinmann, and S. Dresen (2010) *Anal. Bioanal. Chem*. 396:2403-2414.
2. S.P. Vorce and J.H. Sklerov (2004) *J. Anal. Toxicol*. 28:407-410.
3. F.T. Peter, O.H. Drummer, and F. Musshoff (2007) *Forensic Sci. Int.* 165:216-224.
4. M.J. Swortwood, D.B. Boland, and A.P. DeCaprio (2012) *Anal. Bioanal. Chem.* 10.1007/s00216-012-6548-8

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
February 27, 2013
5991-1956EN

Agilent Technologies