Simultaneous Identification of Twenty-Two Synthetic Cathinones in Urine using LC/Q-TOF-MS

Lindsay Glicksberg, BS*; Sarah Kerrigan, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

ABSTRACT

Solid phase extraction (CEREX Polycrom Clin II) and LC/Q-TOF-MS (Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS) equipped with a Poroshell 120 EC-C18 column were used to identify twenty-two synthetic cathinones in urine: methcathinone, ethcathinone, pentedrone, buphedrone, 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (flephedrone, 4-FMC), 4-methylethcathinone (4-MEC), 4-ethylmethcathinone (4-EMC), mephedrone, methedrone, 3,4-dimethylmethcathinone (3,4-DMMC), ethylone, butylone, pentylone, eutylone, methylone, methylenedioxypyrovalerone (MDPV), 4-methylpyrrolidinobutiophenone (MPBP), 3,4-methylenedioxypyrrolidinobutiophenone (MDPBP), α-pyrrolidinopentiphenone (α-PVP), pyrovalerone, and naphyrone. A total of nine deuterated internal standards were employed (methylone-d3, eutylone-d5, pentylone-d3, butylone-d3, MDPV-d8, naphyrone-d5, mephedrone-d3, α-PVP-d8, and ethylone-d3). A targeted analysis was performed using a minimum of two transitions from each precursor ion. Unlike other published methods, water losses were not permitted and regioisomers of fluoromethcathinone were separated. Fragments were structurally identified and transitions were selected to enhance overall specificity. The procedure was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation. The parameters assessed included analytical recovery, calibration model, carryover, bias, limit of detection, limit of quantitation, matrix effect, interferences and dilution integrity.

INTRODUCTION

The ongoing proliferation of designer drugs present a variety of public health and public safety concerns. Synthetic cathinones are capable of producing a variety of psychostimulant effects and according to the National Forensic Laboratory Information System (NFLIS), their use has escalated considerably. There have been numerous published reports involving synthetic cathinones in antemortem and postmortem toxicology investigations. Due to limitations in immunoassay-based screening technologies, many forensic toxicology laboratories must rely on more labor intensive chromatographic-based screening approaches in order to detect these drugs in biological evidence.

MATERIALS AND METHODS

Synthetic cathinone reference standards and deuterated internal standards were purchased from Cerilliant (Round Rock, TX). Drug-free urine (1 mL) was fortified with target compounds at the appropriate concentration and internal standard (25 ng/mL). Extraction of synthetic cathinones was achieved using CEREX Polycrom Clin II solid phase extraction columns (SPEWare, Baldwin Park, CA).

Analysis was performed using an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS equipped with a 2.7 µm Poroshell 120 EC-C18 column (2.1 x 100 mm) and a 2.7 µm Poroshell 120 EC-C18 guard column (2.1 x 5 mm). The mobile phase consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile (B). Compounds were separated using the following gradient elution profile at 0.4 mL/min: 96% A and 4% B (0-0.5 mins), increased to 10% B over 5 minutes and held until 11 minutes. A 60% A and 40% B composition was held for one minute before rinsing with 100% B followed column equilibration. Targeted analysis was performed using a minimum of two transition ions from each precursor ion and a mass tolerance of 5 ppm **(Table 1)**. All qualitative and quantitative analysis was performed using Agilent MassHunter Qualitative and Quantitative Analysis software. Acceptance criteria included a retention time (RT) within 2% and all ion ratios within ±20% of the established value.

RESULTS & DISCUSSION

Analytical recovery was assessed by comparing relative peak areas of extracted (n=4) and non-extracted samples (n=4) at 25 ng/mL. Solid phase extraction yielded extraction efficiencies in the range 84-104% for all drugs. Quantitative analysis was achieved using weighted quadratic calibration models from 0 to 1000 ng/mL. Limits of detection (LOD) and quantitation (LOQ) were determined in drug-free urine fortified with reference materials. Three independent sources of matrix were analyzed in duplicate over three days. LODs ranged from 0.25-5 ng/mL and LOQs ranged from 0.25-10 ng/mL. Bias and precision were assessed using pooled fortified matrix at 10, 100, and 800 ng/mL in triplicate over five runs. Bias for all twenty-two analytes ranged from -1-12%, -3-4%, and 1-8% at 10, 100 and 800 ng/mL, respectively. Inter-assay and intra-assay precision were assessed. Inter-assay precision ranged from 4-12%, 2-12%, and 3-9% at 10, 100, and 800 ng/mL, respectively. Intra-assay precision ranged from 1-11%, 0-7%, and 0-8% for 10, 100, and 800 ng/mL, respectively. Ion suppression and enhancement was evaluated quantitatively using the post-extraction addition approach. Matrix effect for all analytes and internal standards were evaluated at 20 ng/mL (-22% to -1%) and 200 ng/mL (-21% to -2%) using ten drug-free urine samples from independent sources.

Interferences from endogenous compounds, isotopically labeled internal standards, common drugs, and structurally related compounds were also evaluated. In addition to the common drugs, more than twenty-five amphetamines and amphetamine-like designer drugs (including DO-, 2C- and 2CT-series drugs) were included in the interference study, totaling more than fifty compounds. Interferences were evaluated using negative and positive controls fortified with target analyte (10 ng/mL and 100 ng/mL) in the presence of the interferent at a 10- or 100-fold increased concentration (1000 ng/mL).

All twenty-two analytes eluted between three and eleven minutes **(Figure 1).** The weighted quadratic calibration model was established over five independent runs using six non-zero calibrators (510, 50, 100, 250, 500, 1000 ng/mL). Carryover was evaluated at 1000, 2500 and 5000 ng/mL. Negative controls were analyzed immediately following a high control and carryover was determined to be present if any reportable drug was present. No carryover was observed with the exception of naphyrone at 5000 ng/mL. Finally, dilution integrity was verified using two and four-fold dilutions, yielding accuracies within ±20% of the expected value. The LOD, LOQ, %CV for the precision studies, matrix effects, analytical recovery, bias, and dilution integrity for each synthetic cathinone are summarized in **Table 2**. Processed sample stability was evaluated by reanalyzing extracts at 25 and 350 ng/mL in triplicate over different time intervals. The processed samples were stored in the refrigerated autosampler and were stable over 48 hours.

Figure 1. Overlaid chromatograms of twenty-two synthetic cathinones (100 ng/mL) and nine internal standards (25 ng/mL).

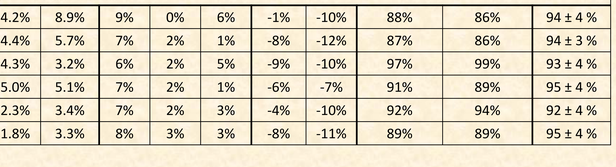

Table 2. LOD, LOQ, inter-assay precision, intra-assay precision, bias, matrix effects, dilution integrity and recovery for the twenty-two synthetic cathinones in urine.

Name	Internal Standard	LOD (ng/mL) (n=18)	LOQ (ng/mL) (n=18)	Intra-assay Precision (%CV) (n=3)			Inter-assay Precision (%CV) (n=15)			Bias (%Bias) (n=15)			Matrix Effects (%) (n=20)		Dilution Integrity Accuracy (%) (n=3)		Analytical Recovery (n=4)
				10 ng/mL	100 ng/mL	800 ng/mL	10 ng/mL	100 ng/mL	800 ng/mL	10 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	200 ng/mL	Two-Fold Dilution	Four-Fold Dilution	
Simple (Unsubstituted) Synthetic Cathinones																	
Methcathinone	Mephedrone-d3	0.25	0.25	3-6%	1-4%	0-5%	7.0%	3.0%	3.5%	8%	1%	5%	-13%	-14%	96%	99%	93 ± 10 %
Ethcathinone	Butylone-d3	1	2	1-9%	3-7%	1-4%	9.3%	6.3%	7.5%	12%	1%	8%	-5%	-9%	95%	92%	89 ± 4 %
Buphedrone	Mephedrone-d3	2	2	2-7%	1-4%	1-5%	8.3%	2.8%	4.7%	10%	2%	6%	-6%	-7%	96%	95%	95 ± 5 %
Pentedrone	Mephedrone-d3	5	5	0-7%	1-5%	2-5%	7.8%	3.6%	4.1%	8%	1%	5%	-5%	-9%	95%	97%	95 ± 5 %
Ring Substituted Synthetic Cathinones																	
3-FMC	Mephedrone-d3	1	10	1-11%	1-5%	1-5%	8.9%	4.7%	5.9%	9%	0%	2%	-17%	-18%	100%	99%	84 ± 12 %
4-FMC	Mephedrone-d3	1	1	1-4%	2-7%	3-6%	5.6%	4.5%	9.2%	7%	1%	4%	-2%	-16%	97%	102%	90 ± 9 %
4-MEC	Mephedrone-d3	1	1	2-7%	1-3%	0-5%	12.1%	11.5%	4.3%	11%	1%	4%	-10%	-16%	97%	101%	101 ± 4 %
4-EMC	Mephedrone-d3	2	5	2-4%	0-2%	0-5%	6.8%	2.2%	3.5%	8%	2%	3%	-14%	-5%	97%	97%	97 ± 4 %
Methedrone	Mephedrone-d3	1	1	1-6%	0-1%	1-5%	4.7%	1.7%	6.4%	8%	1%	2%	-12%	-9%	100%	101%	104 ± 6 %
Mephedrone	Mephedrone-d3	2	2	1-3%	1-2%	0-5%	4.8%	2.0%	3.3%	7%	2%	2%	-12%	-15%	91%	92%	97 ± 7 %
3,4-DMMC	Methylone-d3	5	5	4-7%	0-6%	2-9%	11.7%	8.6%	5.5%	-1%	-3%	3%	-15%	-21%	99%	97%	96 ± 7 %
Methylenedioxy-Type Synthetic Cathinones																	
Methylone	Methylone-d3	0.25	1	0-4%	1-2%	0-3%	4.4%	2.4%	2.5%	6%	1%	2%	-6%	-4%	93%	92%	99 ± 4 %
Ethylone	Ethylone-d3	1	5	2-4%	2-4%	2-5%	6.9%	3.0%	4.6%	7%	2%	1%	-5%	-13%	90%	90%	98 ± 3 %
Eutylone	Eutylone-d5	5	5	3-6%	1-3%	1-4%	6.7%	2.4%	5.8%	3%	2%	2%	-14%	-9%	92%	91%	98 ± 3 %
Butylone	Butylone-d3	1	2	1-5%	0-6%	1-4%	4.6%	4.1%	3.5%	6%	0%	4%	-10%	-18%	94%	89%	98 ± 3 %
Pentylone	Pentylone-d3	1	5	1-6%	1-4%	1-6%	11.6%	3.6%	5.8%	3%	4%	3%	-8%	-11%	92%	89%	100 ± 5 %
Pyrrolidine-Type Synthetic Cathinones																	
α-PVP	Alpha-PVP-d8	2	2	1-6%	0-2%	3-7%	6.7%	4.2%	8.9%	9%	0%	6%	-1%	-10%	88%	86%	94 ± 4 %
MDPBP*	Eutylone-d5	0.5	5	1-7%	1-3%	1-5%	7.1%	4.4%	5.7%	7%	2%	1%	-8%	-12%	87%	86%	94 ± 3 %
MPBP	Naphyrone-d5	1	5	4-7%	2-4%	1-4%	9.4%	4.3%	3.2%	6%	2%	5%	-9%	-10%	97%	99%	93 ± 4 %
MDPV*	MDPV-d8	1	2	1-3%	1-3%	2-7%	6.1%	5.0%	5.1%	7%	2%	1%	-6%	-7%	91%	89%	95 ± 4 %
Pyrovalerone	Naphyrone-d5	0.25	0.25	3-8%	1-2%	1-4%	8.7%	2.3%	3.4%	7%	2%	3%	-4%	-10%	92%	94%	92 ± 4 %
Naphyrone	Naphyrone-d5	0.5	0.5	2-4%	0-3%	1-3%	6.0%	1.8%	3.3%	8%	3%	3%	-8%	-11%	89%	89%	95 ± 4 %

*MDPV and MDPBP can be classified as pyrrolidine-type and methylenedioxy-type.

RESULTS (CONT.)

Table 1. The collision energies, retention time, and ion transitions selected for the twenty-two synthetic cathinones. The quantitation ion is in bold.

Cathinone	Transitions	CE (V)	Retention Time (min)
Methcathinone	164.1070-->**131.0731** 164.1070-->105.0703	20	3.295
3-FMC	182.0975-->**149.0634** 182.0975-->123.060	20	3.821
4-FMC	182.0976-->**149.0636** 182.0976-->123.0605	20	3.978
Methylone	208.0968-->**160.0757** 208.0968-->132.0807	20	4.036
Ethcathinone	178.1226-->**131.0721** 178.1226-->117.0586 178.1226-->105.0700	20	4.171
Ethylone	222.1125-->**174.1222** 222.1125-->146.0958	30	5.038
Methedrone	194.1178-->**161.0833** 194.1178-->146.0598 194.1178-->135.0803	20	5.171
Buphedrone	178.1226-->**131.0731** 178.1226-->91.0549 178.1226-->145.0880	20	5.289
Butylone	222.1125-->**174.0914** 222.1125-->146.0964	30	6.141
Mephedrone	178.1226-->**145.0889** 178.1226-->119.0853	20	6.325
Eutylone	236.1281-->**188.1069** 236.1281-->174.0547 236.1281-->161.0598	30	6.829
4-MEC	192.1383-->**145.0886** 192.1383-->159.1041 192.1383-->131.0738	20	7.071
MDPBP	262.1438-->**161.0597** 262.1438-->191.0704 262.1438-->112.1125	20	7.142
Pentedrone	192.1383-->**132.0810** 192.1383-->161.0958 192.1383-->91.0546	20	7.402
Pentylone	236.1281-->**188.1070** 236.1281-->175.0682	30	7.773
3,4-DMMC	192.1387-->**159.1043** 192.1287-->144.0802	20	7.995
alpha-PVP	232.1696-->**161.0954** 232.1696-->91.0549	20	8.031
4-EMC	192.1383-->**145.0889** 192.1383-->105.0701	20	8.167
MPBP	232.1696-->**161.0960** 232.1696-->133.1010 232.1696-->112.1120	20	8.308
MDPV	276.1594-->**205.0857** 276.1594-->126.1277 276.1594-->175.0756	20	8.371
Pyrovalerone	246.1852-->**175.1110** 246.1852-->126.1280 246.1852-->105.0701	20	9.329
Naphyrone	282.1852-->**211.1122** 282.1852-->126.1280 282.1852-->141.0701	20	10.626

CONCLUSIONS

LC/Q-TOF-MS was used to identify twenty-two synthetic cathinones in urine following solid phase extraction. This procedure was developed as part of a larger study to systematically evaluate the stability of synthetic cathinones in biological evidence. The method was validated in accordance with SWGTOX Standard Practice for Method Validation recommendations.

ACKNOWLEDGEMENTS

This project was supported by Award No. 2013-R2-CX-K006 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.