POSTER NOTE

No. 64893

Screening, Confirmation and Quantitation of Synthetic Cathinones and Cannabinoids in Urine by High-Resolution Accurate-Mass Mass Spectrometry

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INTRODUCTION

Forensic laboratories need reliable and flexible methods for detecting novel psychoactive compounds such as synthetic cathinones and cannabinoids. The methods need to be easily modifiable to include new compounds. LC-MS is ideally suited for this type of application since it can easily detect different classes of compounds in a single analytical run.

OBJECTIVE

To demonstrate the performance of high-resolution mass spectrometry for identification, confirmatio and quantitation of synthetic cathinones and cannabinoids in urine.

MATERIALS AND METHODS

Sample Processing

- A single point calibrator at cut-off concentration, and two QCs (one each at 50% and 150% of the calibrator concentration) were prepared by fortifying blank urine with 32 synthetic cathinones and cannabinoids (**Table 1).**
- Calibrator, QCs and an unknown donor sample were processed by a collaborating laboratory using protein precipitation followed by dilution.

Liquid Chromatography

Two chromatographic gradients were used. The first was a "fast and dirty" two-minute screening method that provided limited chromatographic separation of isobaric compounds. The second was a nine-minute gradient used for confirmation.

- Thermo Scientific™ Dionex™ UltiMate™ 3000 HPG-3400RS pump with OAS-3300TXRS autosampler.
- Mobile Phase A: 10 mM ammonium formate in water
- Mobile Phase B: 10 mM ammonium formate in methanol
- Method 1 (Screening)
  - Column: Thermo Scientific HyPURITY™ C18 Javelin guard column, 20x2.1 mm
  - Gradient: see **Figure 1a**
- Method 2 (Confirmation)
  - Column: Thermo Scientific Accucore™ Phenyl-Hexyl, 2.6 µm, 50 x 2.1 mm
  - Gradient: see **Figure 1b**

Mass Spectrometry

- Thermo Scientific Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer
- HESI ionization source
- Full scan (FS) MS spectra at a resolution of 70,000 (FWHM at *m/z* 200)
- Data-dependent MS-MS fragmentation (ddMS^2) spectra at a resolution of 17,500 (FWHM at *m/z* 200) triggered on compound *m/z* from inclusion list

Method Evaluation

Compounds were identified using retention time and accurate *m/z* (5 ppm mass accuracy) from the full-scan data. Semi-quantitation was performed on the FS extracted ion chromatographic peak using the single-point calibrator and linear-through-zero calibration curves. Confirmation was accomplished by spectral library matching with the MS2 spectra in both methods. Isotopic pattern matching was added to the longer method.

To assess method performance, the calibrator and each QC sample were injected ten times with each method to determine mass accuracy, peak area precision, and quantitative performance. The unknown sample previously analyzed by collaborating laboratory was injected three times with each method to determine identification accuracy.

Data was acquired and processed with Thermo Scientific TraceFinder™ software version 4.1

Figure 1. Short (a) and Long (b) Chromatographic Gradients

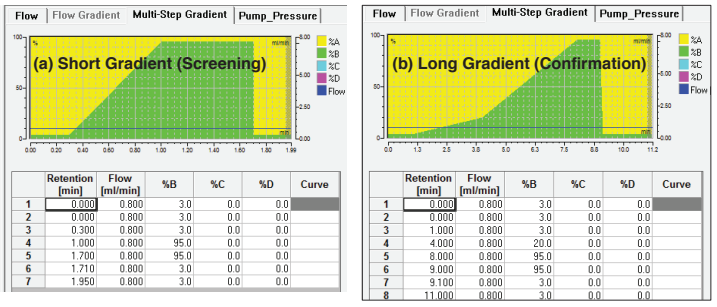

Table 1. Synthetic Cathinones and Cannabinoids with Calibrator Concentration

Analyte	ng/mL	Analyte	ng/mL
3-Fluoromethcathinone	500	JWH 018 N-pentanoic acid metabolite	50
3-FMC Ephedrine metabolite	100	MAM2201 N-pentanoic acid metabolite	25
4-Methylethcathinone	50	MDPV	50
5-Fluoro PB-22 3-carboxyindole metabolite	100	MDPV-D8	75
AB-FUBINACA	50	Mephedrone	100
AB-PINACA Pentanoic acid metabolite	250	Methcathinone	100
ADBICA N-pentanoic acid metabolite	250	Methedrone	100
ADB-PINACA pentanoic acid metabolite	100	Methylone	100
AKB48 N-pentanoic acid metabolite	25	Naphyrone	25
alpha-PVP	25	N-Ethylcathinone	50
AM2201 4-hydroxypentyl metabolite	25	N-Ethylcathinone Ephedrine Metabolite	100
AM2201 4-Hydroxypentyl metabolite D-5	37.5	N-Ethylcathinone Ephedrine metabolite-D5	150
BB-22 3-carboxyindole metabolite	100	PB-22 3-carboxyindole metabolite	250
Buphedrone	100	Pentylone	50
Buphedrone Ephedrine metabolite	100	p-methoxy-methamphetamine	100
Butylone	50	UR-144 N-pentanoic acid metabolite	50
Cathinone	500	XLR-11 4-Hydroxypentyl metabolite-D5	37.5
Ethylone	50	XLR11 N-(4-hydroxypentyl) metabolite	25

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Table 2. Performance Evaluation Results for Short Chromatographic Method %CVs are for n=11 replicates at each concentration. Delta ppm is the average across all concentrations and all replicates (n=33).

Compound	Delta ppm	Peak Area %CV			Calc Conc. %CV		
		Cal	QC-Lo	QC-Hi	Cal	QC-Lo	QC-Hi
3-Fluoromethcathinone	1.03	3.97	6.87	2.36	4.25	1.78	2.15
3-FMC ephedrine met.	0.47	2.74	5.65	1.71	1.78	3.01	1.47
4-Methylethcathinone	0.49	3.51	7.30	3.89	3.26	2.21	4.23
5-fluoro PB-22 3-carboxyindole met.	0.17	5.41	10.50	6.44	5.94	5.47	7.85
AB-FUBINACA	-0.05	3.44	10.90	1.70	2.89	6.50	1.80
AB-PINACA pentanoic acid met.	-0.16	2.72	10.50	2.03	3.30	5.14	2.99
ADBICA N-pentanoic acid met.	-0.13	5.31	12.60	3.45	6.05	8.34	3.68
ADB-PINACA pentanoic acid met.	-2.22	3.34	7.52	3.51	6.46	5.01	4.90
AKB48 N-pentanoic acid met.	0.13	2.58	13.90	2.37	4.19	5.21	2.48
alpha-PVP	0.19	2.90	8.12	2.50	3.45	1.71	2.51
AM2201 4-hydroxypentyl met.	-0.08	3.11	8.69	3.23	1.98	1.92	2.13
AM2201 4-Hydroxypentyl met. D-5	-0.27	4.44	NC	NC	3.10	NC	NC
BB-22 3-carboxyindole met.	0.28	5.53	6.96	4.28	7.15	3.55	3.98
Buphedrone	0.66	2.10	6.26	1.40	2.12	3.73	2.46
Buphedrone Ephedrine met.	0.39	2.21	7.04	1.73	2.18	0.61	2.85
Butylone	0.35	2.06	7.34	2.29	2.37	2.79	2.77
Cathinone	0.78	4.97	8.44	2.36	4.15	6.93	3.43
Ethylone	0.27	2.06	7.34	2.29	2.37	2.79	2.77
JWH 018 N-pentanoic acid met.	-0.03	3.73	7.83	2.63	5.35	3.64	3.19
MAM2201 N-pentanoic acid met.	0.03	1.97	7.05	2.85	1.69	3.96	2.64
MDPV	0.60	2.76	7.50	2.52	2.05	1.34	2.07
MDPV-D8	0.44	4.62	NC	NC	1.92	NC	NC
Mephedrone	0.59	2.10	6.26	1.40	2.12	3.74	2.46
Methcathinone	0.85	10.70	6.91	5.61	9.77	8.33	5.28
Methedrone	0.74	1.91	9.09	2.57	2.27	1.80	3.41
Methylone	0.53	3.68	11.70	1.50	3.58	4.81	1.51
Naphyrone	0.27	1.92	8.65	2.88	1.34	2.15	4.23
N-Ethylcathinone	0.82	2.10	6.26	1.40	2.12	3.73	2.46
N-Ethylcathinone Ephedrine met.	0.67	2.21	7.04	1.73	1.42	0.86	2.33
N-Ethylcathinone Ephedrine Met-D5	0.64	3.59	NC	NC	1.82	NC	NC
PB-22 3-carboxyindole met.	0.20	5.13	13.60	7.19	5.87	3.85	7.69
Pentylone	0.34	2.85	5.79	2.99	2.71	0.98	3.53
p-methoxymethamphetamine	0.39	2.21	7.04	1.73	1.42	0.86	2.33
UR-144 N-pentanoic acid met.	-0.14	1.83	10.70	2.10	2.11	0.39	1.76
XLR-11 4-Hydroxypentyl met.-D5	-0.24	5.95	NC	NC	2.32	NC	NC
XLR11 N-(4-hydroxypentyl) met.	0.74	2.58	12.90	2.03	1.41	1.16	1.39

Table 3. Performance Evaluation Results for Long Chromatographic Method %CVs are for n=11 replicates at each concentration. Delta ppm is the average across all concentrations and all replicates (n=33).

Compound	Delta ppm	Peak Area %CV			Calc Conc. %CV		
		Cal	QC-Lo	QC-Hi	Cal	QC-Lo	QC-Hi
3-Fluoromethcathinone	-0.48	1.93	0.99	1.55	1.08	1.22	0.86
3-FMC ephedrine met.	-0.61	1.92	0.77	1.12	1.36	0.52	1.39
4-Methylethcathinone	-1.47	1.36	1.31	1.23	1.70	1.26	1.33
5-FluoroPB-22 3-carboxyindole met.	-2.28	2.30	2.04	1.98	3.07	2.15	2.48
AB-FUBINACA	-2.22	2.21	1.59	1.69	2.44	1.57	1.62
AB-PINACA pentanoic acid met.	-2.85	2.59	1.24	1.07	2.91	1.69	1.15
ADBICA N-pentanoic acid met.	-2.62	2.18	1.00	1.71	2.44	1.22	1.67
ADB-PINACA pentanoic acid met.	-4.20	6.92	5.67	8.15	6.61	5.52	8.56
AKB48 N-pentanoic acid met.	-2.16	1.27	2.84	1.66	1.60	2.51	1.52
alpha-PVP	-2.53	1.98	1.16	1.06	0.70	0.60	0.67
AM2201 4-hydroxypentyl met.	-1.90	1.03	2.06	1.26	0.62	0.90	1.78
AM2201 4-Hydroxypentyl met. D-5	-2.52	1.08	NC	NC	1.08	NC	NC
BB-22 3-carboxyindole met.	-1.81	2.27	3.05	3.35	2.60	2.48	3.04
Buphedrone	-1.26	1.26	0.91	1.58	1.17	0.83	0.89
Buphedrone Ephedrine met.	-1.54	1.08	1.00	1.66	0.90	1.28	0.75
Butylone	-1.40	1.09	1.33	1.23	1.69	1.18	1.15
Cathinone	-1.47	1.43	1.99	1.09	1.34	1.57	1.25
Ethylone	-2.36	1.14	1.07	1.53	1.50	0.95	1.37
JWH 018 N-pentanoic acid met.	-2.36	1.88	0.79	2.31	1.85	1.51	2.53
MAM2201 N-pentanoic acid met.	-1.89	0.95	0.97	2.08	0.96	1.45	2.06
MDPV	-2.30	1.23	0.84	0.98	1.53	0.70	1.15
MDPV-D8	-2.66	4.09	NC	NC	1.69	NC	NC
Mephedrone	-1.39	1.39	1.13	1.54	1.39	1.13	1.54
Methcathinone	-0.97	2.06	0.93	1.55	1.60	0.79	0.89
Methedrone	-1.02	1.17	0.77	1.34	1.05	1.20	0.90
Methylone	-1.42	1.29	1.41	1.50	1.95	1.52	1.21
Naphyrone	-0.90	1.74	0.66	1.50	0.81	0.69	1.08
N-Ethylcathinone	-1.25	4.40	0.27	1.45	1.85	0.72	1.08
N-Ethylcathinone Ephedrine met.	-1.12	1.27	0.58	1.26	0.55	0.24	0.51
N-Ethylcathinone Ephedrine Met-D5	-1.76	2.26	NC	NC	1.39	NC	NC
PB-22 3-carboxyindole met.	-1.73	3.12	1.41	3.85	3.18	1.26	3.64
Pentylone	-1.69	1.54	2.01	1.27	1.21	1.46	1.22
p-methoxymethamphetamine	-2.28	1.06	0.92	1.55	1.08	0.85	0.76
UR-144 N-pentanoic acid met.	-2.02	1.03	2.28	2.61	1.57	2.05	2.70
XLR-11 4-Hydroxypentyl met.-D5	-2.45	4.16	NC	NC	1.31	NC	NC
XLR11 N-(4-hydroxypentyl) met.	-1.57	1.59	0.75	1.54	0.68	1.00	0.46

RESULTS

Data from the short screening method showed mass accuracies within 1 ppm for all except one compound, which was within 2.2 ppm. The long method, which was run several days after the short method and near the end of the recommended instrument calibration stability, showed mass accuracies within 3 ppm except for the same single compound, which was within 4.2 ppm.

Peak area precision was better than 13.9% and 8.1% for all compounds and all concentrations for the short and long methods, respectively.

Calculated concentration precision was better than 9.8 % and 8.5% across all compounds and all concentrations for the short and long methods, respectively.

Compound specific details for the above results are shown in **Table 1** and **Table 2**.

Three compounds, MDPV, mephedrone and methylone were identified and confirmed in the unknown sample. The compounds were identified by retention time and accurate *m/z* from the FS data. They were confirmed with isotopic pattern matching and fragmentation spectra matching to a spectral library (**Figure 2**). A fourth compound was identified by *m/z*, retention time, and isotopic pattern matching as methedrone. However, it failed the spectral matching (**Figure 3 and Figure 4**). It was suspected that this compound might be a metabolite of one of the confirmed compounds. A literature search revealed a possible match in hydroxytolyl-mephedrone which was confirmed with a theoretical fragmentation spectra match performed in Thermo Scientific Mass Frontier™ software (**Figure 5**).

Table 4. Summary of Screening Results for Unknown sample. Results shown include Identified Compound Name, Confirmation Status, Peak Area, Calculated Concentration, Library Search Status, Library Score, Name of Match in Library, Isotopic Pattern Matching Score, # of Isotopes Matched, Delta *m/z* in ppm for detected peak, and Retention Time. Note that Methedrone failed confirmation due to a low Library Score.

Compound	Confirm	Area	ng/mL	LS	LS (%)	Lib Match Name	IP Score (%)	# Isotopes	m/z (Delta in ppm)	Actual RT
MDPV	C	1.45E+07	9.64	Pass	100	MDPV	100	3 of 4	-1.98	5.64
Mephedrone	C	6.99E+06	11.3	Pass	100	Mephedrone	100	4 of 5	-1.46	4.00
Methedrone	I	2.18E+07	24.9	Fail	63	Methedrone	100	4 of 6	-1.21	3.49
Methylone	C	1.23E+07	15.5	Pass	100	Methylone	100	3 of 5	-1.46	2.97

Figure 2. Data results showing positive identification and confirmation of Mephedrone in the unknown sample. The compound peak is identified by retention time and accurate mass chromatogram (5 ppm window). Confirmation is based on spectral library and isotopic pattern matching.

Identification/Quantitation Library Spectral Matching – and – Isotopic Pattern Matching

Figure 3. Data results showing positive identification and negative confirmation of Methedrone in the unknown sample. The compound is identified by accurate mass and retention time. Isotopic pattern matching also passed, but the spectral library match did not meet the required limit.

Identification/Quantitation Library Spectral Matching (fail) – and – Isotopic Pattern Matching (pass)

Figure 4. Comparison of fragmentation spectra of (a) known methedrone in calibrator, (b) methedrone Library spectra, and (c) unknown sample.

(a) Methedrone in Calibrator

(b) Methedrone Library Spectra

(c) Peak in Unknown Sample

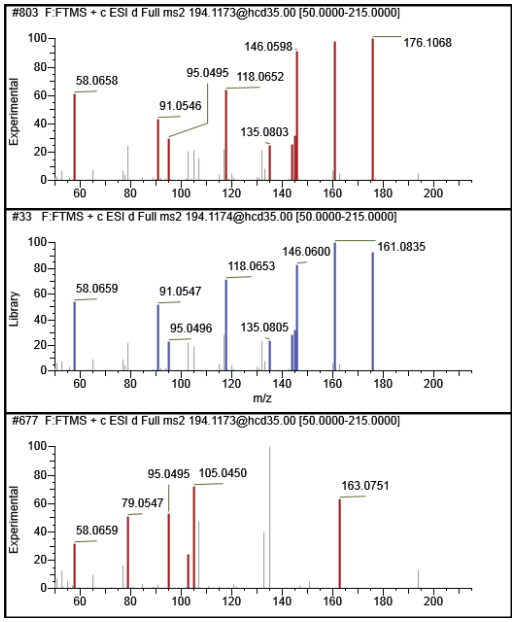
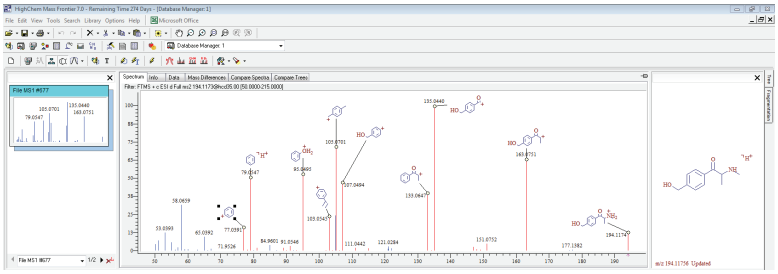


Figure 5. Identification of fragments in Hydroxytolyl-Mephedrone spectrum using theoretical fragmentation in Mass Frontier software. Red highlighted fragments are matches to the experimental spectra (in blue).



CONCLUSIONS

- The developed methods accomplished their goals of identifying, confirming and quantifying 32 synthetic cathinones and cannabinoids in urine.
- The short method was intended as a screening-only method, not requiring definitive confirmation. It surpassed that goal by also providing confirming fragmentation spectral matches.
- The longer confirmatory method provided better confirmation with higher quantitative precision and library matching scores.
- Theoretical fragmentation can provide confidence in identification of unknown peaks.

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PN64893-EN 11/16S