Electronic Supplementary Material (ESI) for Analyst.

Detection of mephedrone and its metabolites in fingerprints from a controlled human administration study by liquid chromatography-tandem mass spectrometry and paper spray-mass spectrometry

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Supplementary Information

1. Validation results: LC-MS/MS

2.1 Selectivity

No interferences were observed in the extracted blank matrix.

2.2 Linearity

Mean linearity of $r^2 > 0.996$ was achieved for all analytes in all three validation runs.

2.3 LOD and LLOQ

Presented in the paper.

2.4 Precision and accuracy

Intra-day and inter-day precision and accuracy results, summarised in Table S1, were found to be within the acceptable limits. The intra-day inaccuracy was within ± 15% of the target concentration and ranged from 96.6-115% for MEPH, 101-105% for DHM, 90.9-106% for NOR, 85.6-111% for HYDROXY, 85.5-108% for 4-CARBOXY and 88.3-107% for DHNM. The intra-day imprecision was < 13.8% and ranged from 0.523-5.10% for MEPH, 1.58-4.48% for DHM, 2.048.95% for NOR, 2.64-9.09% for HYDROXY, 2.51-13.8% for 4-CARBOXY and 0.906-5.06% for DHNM. Inter-day precision and accuracy results were acceptable over the validated range with % CV < 14.5% and accuracy within ± 7.50% of the target concentration.

*Table S1. Precision and accuracy at QC Low, QC Medium and QC High; * average value of 18 measurements over 3 days*

Analyte	True value (ng/fingerprint)	Mean (ng/fingerprint), (% CV), % accuracy			
		Day 1 (n=6)	Day 2 (n=6)	Day 3 (n=6)	Inter-day (n=3)*
MEPH	0.08	0.080 1.31% 100%	0.085 3.44% 107%	0.081 5.10% 101%	0.082 4.58% 102%
	1	1.04 2.76% 104%	1.05 3.89% 105%	0.992 2.28% 99.2%	1.02 3.78% 102%
	4	4.06 0.523% 101%	4.58 1.06% 115%	3.86 3.11% 96.6%	4.25 9.61% 106%
DHM	0.04	0.041 3.07% 103%	0.042 2.62% 104%	0.041 2.23% 103%	0.042 2.63% 103%
	0.5	0.503 1.79% 101%	0.510 2.20% 102%	0.505 2.43% 101%	0.506 2.10% 101%
	4	4.10 4.48% 103%	4.10 1.58% 102%	4.19 3.41% 105%	4.19 7.37% 105%
NOR	0.08	0.077 5.12% 96.7%	0.081 3.68% 101%	0.075 8.95% 93.1%	0.078 6.66% 96.8%
	1	0.913 4.71% 91.3%	0.951 5.28% 95.1%	0.909 2.87% 90.9%	0.925 4.74% 92.5%
	4	3.66 2.04% 91.6%	3.87 2.66% 96.6%	4.26 5.25% 106%	4.03 14.5% 101%
HYDROXY	0.05	0.049 5.91% 98.1%	0.052 2.64% 104%	0.049 5.53% 98.2%	0.050 3.21% 100%
	1	1.01 3.14% 101%	0.892 8.43% 89.2%	1.11 4.15% 111%	1.00 10.7% 100%
	4	4.16 8.28% 104%	4.29 3.64% 107%	3.42 9.09% 85.6%	3.96 11.8% 98.9%
4-CARBOXY	0.05	0.045 10.7% 90.1%	0.050 5.41% 101%	0.048 6.91% 95.2%	0.048 5.49% 95.3%
	1	0.958	0.863	10.6	0.960

		2.51%	8.05%	2.91%	10.1%
		95.8%	86.3%	106%	96.0%
		3.98	4.31	3.42	3.90
	4	13.4%	7.58%	13.8%	11.5%
		99.4%	108%	85.5%	97.6%
		0.074	0.083	0.080	0.079
	0.08	5.06%	1.99%	4.96%	1.12%
		92.7%	104%	99.6%	98.7%
		0.883	1.01	0.980	0.957
DHNM	1	3.67%	2.47%	1.83%	0.484%
		88.3%	101%	98.0%	95.7%
		3.83	4.27	4.23	4.17
	4	3.62%	0.906%	2.53%	1.44%
		95.8%	107%	106%	104%

2.5 Recovery and matrix effect

As shown in Table S2, recovery was found to be greater than 60.6 ± 6.16% for all analytes, with NOR showing the lowest recovery of 60.6 ± 6.16% and 63.5 ± 4.32% at QC Low and QC High, respectively. The highest recovery of 91.3 ± 5.53% and 91.1 ± 7.57% was achieved for DHM at QC Low and QC High, respectively. The IS-corrected matrix effect values were within ± 7.3% for all analytes, showing that significant matrix effects do not affect the assay.

Table S2. Analyte recovery and matrix effect at QC Low and QC High

Analyte	Recovery (% CV), n=6		Matrix Effect (% CV), n=6	
	QC LOW	QC HIGH	QC LOW	QC HIGH
MEPH	72.2% (4.27%)	61.9% (5.67%)	101% (1.02%)	96.6% (1.77%)
DHM	91.3% (5.53%)	91.1% (7.57%)	101% (1.34%)	99.1% (0.375%)
NOR	60.6% (6.16%)	63.5% (4.32%)	99.8% (2.99%)	92.7% (1.43%)
HYDROXY	82.5% (5.60%)	62.7% (4.32%)	97.9% (5.02%)	96.1% (6.63%)
4-CARBOXY	83.4% (5.33%)	75.2% (4.24%)	98.6% (8.44%)	99.4% (6.24%)
DHNM	84.9% (8.11%)	70.6% (5.30%)	102% (3.63%)	93.6% (2.39%)

2.6 Carryover

Carryover was not observed.

2.7 Dilution integrity

Good precision (9.91%) and accuracy (92.9%) were achieved for mephedrone following 1 in 100 dilution.

2. Validation results: PS-MS

2.1 Selectivity

Due to the lack of sample preparation and chromatography prior to the ionisation step, isobaric interferences (peak intensity of around 10^3-10^5) for all analytes were present in all tested solvents.

2.2 Linearity

Mean linearity of $r^2 > 0.990$ was achieved for MEPH and DHM in all three validation runs (Figure S1).

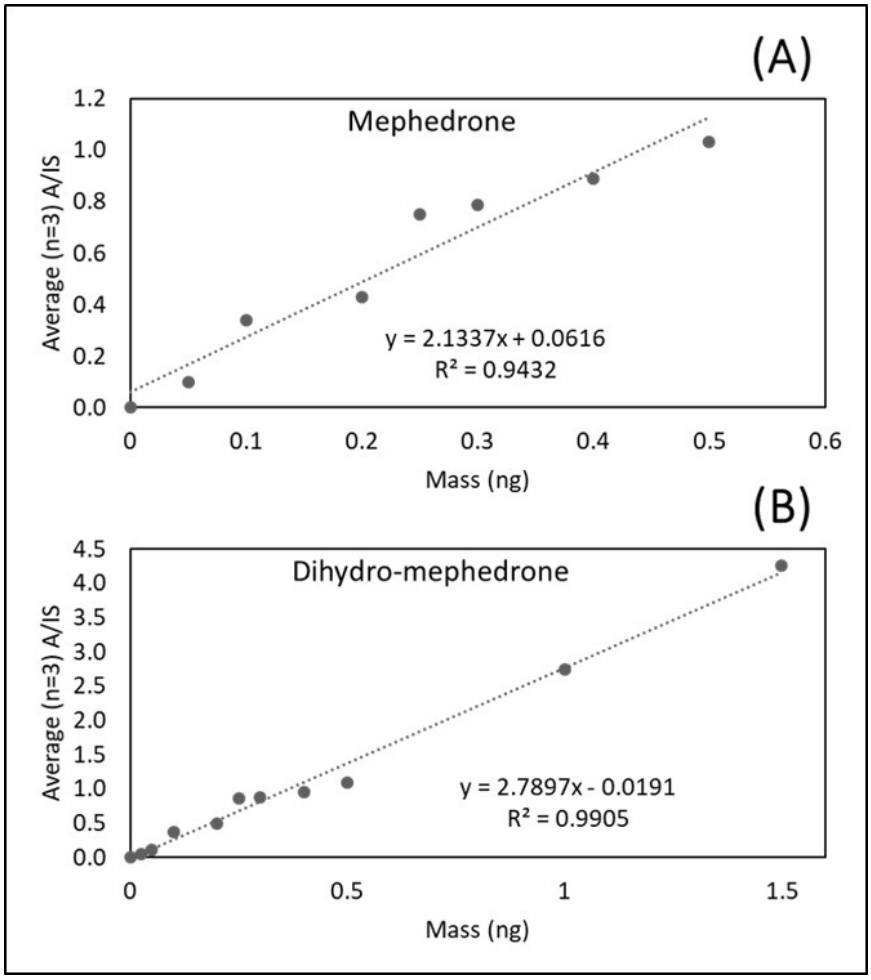

Figure S1. Calibration curves and the corresponding errors expressed as %CV for MEPH (A) and DHM (B); data expressed as ratio of analyte to internal standard (A/IS)

2.3 LOD and LLOQ

Presented in the paper.

2.4 Precision and accuracy

Intra-day and inter-day precision and accuracy results, summarised in Table S3 and Table S4, were found to be within the acceptable limits. Intra and intra-day imprecision and accuracy were within ± 20% of the target value for both analytes.

*Table S3. Inter- and intra-day precision for MEPH and DHM measured using paper spray; * average value of 9 measurements over 3 days*

Analyte	pg/fingerprint	Mean (Analyte/IS ratio), (%CV)			
		Day 1 (n=3)	Day 2 (n=3)	Day 3 (n=3)	Inter-day (n=3)*
MEPH	500	0.74	0.67	0.65	0.69
		6%	2%	7%	7%
	1500	3.3	2.74	2.73	2.92
		2%	2%	7%	11%
DHM	500	0.76	0.85	0.68	0.76
		16%	1%	4%	11%
	1500	4.57	4.33	3.43	4.11
		2%	4%	8%	15%

Table S4. Intra-day accuracy and %CV of the paper spray method at QC Low and QC High (n=3)

	pg/fingerprint	Calculated mass of QC (ng)	%CV	Accuracy
MEPH	500	0.48	7%	96%
	1500	2.44	1%	97%
DHM	500	0.46	7%	91%
	1500	2.55	3%	102%

2.5 Matrix effects

Fingerprint samples were collected from the right thumb (RT) and right index (RI) and as 5 overlapping fingerprint samples (5F). As shown in Table S5, significant matrix effects were not observed for MEPH or DHM.

Table S5. Matrix effects for MEPH and DHM in the present of a single (right thumb, RT and right index, RI) or 5 overlapping fingerprints (5F) for a male and female donors

	Matrix effect, (%CV)					
	Male			Female		
Analyte	**RT**	**RI**	**5F**	**RT**	**RI**	**5F**
MEPH	-7 (11%)	-7 (7%)	-10 (6%)	-11 (7%)	-13 (7%)	-8 (5%)
DHM	-3 (6%)	-2 (3%)	-2 (7%)	-6 (11%)	-4 (7%)	-5 (4%)

2.6 Carryover

No carryover effect was observed.

2.7 Stability

Table S6. Week 1 and week 4 stability of MEPH and DHM prepared at 50 ng/mL in solution (Sol) and fingerprints (RT and RI) and stored at different temperatures (n=3); data is represented as +/-% change (%CV)

1 week @ +5°C			
	Fingerprint (RT)	**Fingerprint (RI)**	**Solution**
MEPH	-18% (12%)	-17% (15%)	-6% (18%)
DHM	-40% (15%)	-36% (15%)	-1% (5%)
4 weeks @ +5°C			
	Fingerprint (RT)	**Fingerprint (RI)**	**Solution**
MEPH	-37% (39%)	-36% (15%)	6% (6%)
DHM	-56% (40%)	-53% (22%)	-27% (16%)
1 week @ -20°C			
	Fingerprint (RT)	**Fingerprint (RI)**	**Solution**
MEPH	-24% (12%)	-23% (17%)	1% (12%)
DHM	-41% (16%)	-41% (16%)	16% (19%)
4 weeks @ -20°C			
	Fingerprint (RT)	**Fingerprint (RI)**	**Solution**
MEPH	-41% (33%)	-38% (27%)	12% (8%)
DHM	-63% (44%)	-67% (63%)	-21% (11%)

3. PS-MS parameters

Table S7 shows the MS parameters used for the PS-MS method.

Table S7. MS parameters used for the PS-MS method (Thermo Scientific Q-Exactive Plus Orbitrap)

Parameter	Operating Conditions
Electrospray source parameters	
Spray voltage	4 kV
Capillary temperature	250°C
S-Lens RF level	50
Full Scan	
Scan type	Full MS-SIM
Scan rage	*m/z* 66.7-500
Resolution	280,000 at *m/z* 200
Polarity	Positive
Automatic gain control target	10^6
Maximum inject time	500 ms
MS/MS	
Scan type	Parallel Reaction Monitoring
Resolution	17,500
Automatic gain control	200,000
Max inject time	30 ms
Isolation window	*m/z* 0.5
Normalised collision energy	Stepped: 30, 60, 90

4. PS-MS spectra

Figure S2 and Figure S3 show the MS and MS/MS spectra of all analytes, respectively.

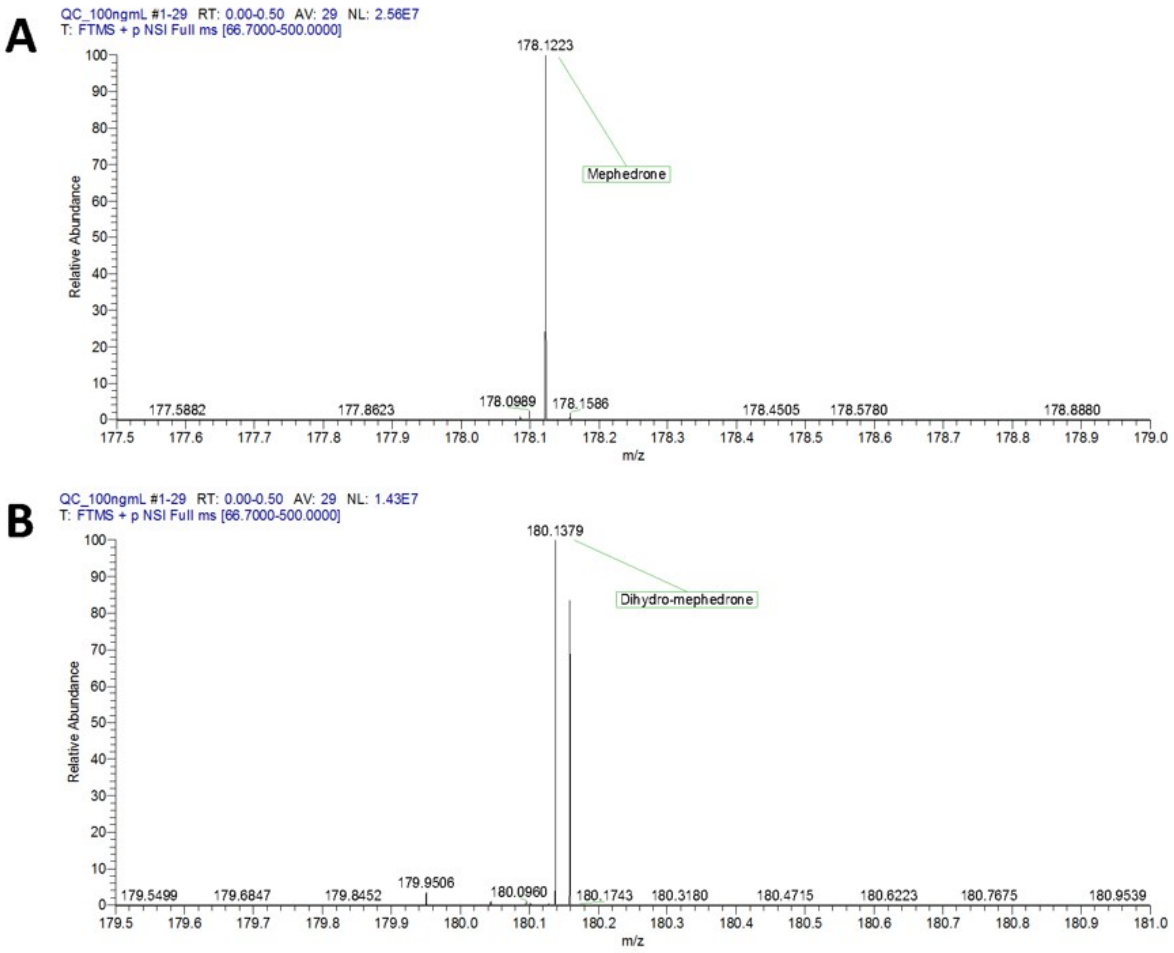

Figure S2. Example mass spectra for (A) MEPH and (B) DHM

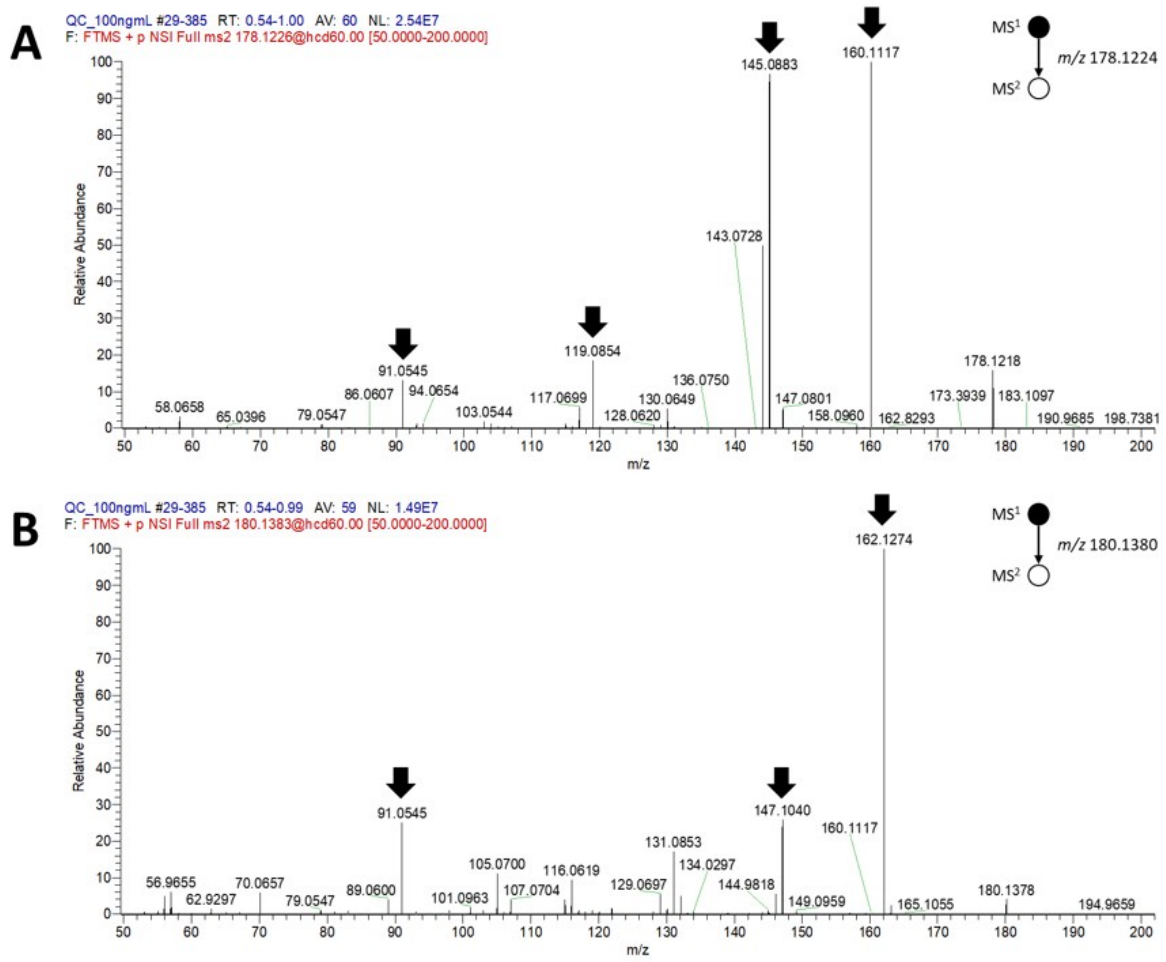

Figure S3. Example MS/MS spectra for (A) MEPH (m/z 178.1 > 160.11) and (B) DHM (m/z 180.1 > 162.13) from a standard (250 pg/fingerprint). Peak assignment was confirmed by agreement with the standard to within 5 ppm.

5. Urine screen

The urine samples were analysed using a standard stimulant (including mephedrone) immunoassay screen at Abbott. The immunoassay was run on a Beckman Coulter 5800 and reagents were purchased from ThermoFisher (see Table S8 for further details). Any positive samples were confirmed by a validated LC-MS method using an Orbitrap MS system.

Table S8. Analytes and their corresponding cut-off values

Analyte	Cut-off
Amphetamines	1000 ng/mL
Barbiturates	200 ng/mL
Benzodiazepines	200 ng/mL
Cannabis	50 ng/mL
Cocaine	300 ng/mL
Methadone	300 ng/mL
Opiates	300 ng/mL
Buprenorphine	10 ng/mL
Ketamine	500 ng/mL
Tramadol	200 ng/mL
Propoxyphene	300 ng/mL
PCP	25 ng/mL
Methaqualone	300 ng/mL
6AM	10 ng/mL
LSD	0.5 ng/mL

LC-MS details:

Mass Spectrometer: Thermo Exactive Orbitrap HCD System with Ion Max Source and H-ESI II Probe

Pump: Accela UHPLC

Autosampler: Accela

Column: Hypersil GOLD 5-x3.1 1.9uM Column, C18