Electronic Supplementary Information for

Development of a High-Resolution Paper-Spray Mass Spectrometry Method using Street Drugs for the Early Detection of Emerging Drugs in the Unregulated Supply

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Table S1: TSQ Fortis™ triple quadrupole mass spectrometer instrument parameters

Parameter	**Value**
Global Parameters	
Method Duration (min)	2.0
Spray Voltage (V)	Time Dependent: Start (min) Voltage (V) 0 0 0.1 3800 1.1 0 1.2 4000
Ion Transfer Tube Temp (°C)	300
Sample Rewetting (A)	2 dispenses of 10 µL
Spray Solvent (B)	11 dispenses of 10 µL
Experiment 1	
Experiment Type	SRM
Start Time (min)	0
End Time (min)	1.2
Data Mode	centroid
Collision Gas Pressure (mTorr)	2.0
Q1 Resolution (FWHM)	0.7
Q3 Resolution (FWHM)	1.2
Polarity	positive

Experiment 2	
Experiment Type	full scan
Start Time (min)	1.2
End Time (min)	2.0
Data Mode	centroided
Collision Gas Pressure (mTorr)	2.0
Polarity	positive
Q3 Resolution (FWHM)	0.7
Source Fragmentation (V)	5.0

Table S2: Orbitrap Exploris ™ 120 parameters used for high resolution measurements.

Parameter	**Value**
Global Settings	
Positive Spray Voltage	3800
Ion Transfer Tube Temperature	300
Mild Trapping	False
Scan MasterScan	
Resolution	120,000
Scan Range (*m/z*)	50–600
RF Lens(%)	70
Use Easy-IC™	Off
AGC Target	Standard
Data Type	Profile
Ionization Polarity	Positive
Filter Dynamic Excluion	
Exclude After n Times	1
Exclusion Duration	20 s
Mass tolerance low	5 ppm
Mass tolerance high	5 ppm
Filter Targeted Exclusion Mass List	
Mass Tolerance Low	10 ppm
Mass Tolerance High	10 ppm
Scan ddMS^n Scan	
Isolation Window (*m/z*)	2
Collision Energy Type	Normalized
HCD Collision Energies (%)	30, 50, 70
Resolution	30,000
RF Lens (%)	70
AGC Target	Standard
Easy-IC™	Off
Data Type	Centroid

Table S3: List of compounds used for matching by the peak search algorithm, and the corresponding confirming ions. Entries without confirming ions can be manually identified using *in silico* fragmentation software.

Name	Monoisotopic Mass	Ion 1	Ion 2	Ion 3
Propionyl chloride	92.0029			
Niacin	123.032	80.0493	78.0338	96.0443
N-phenylpropanamide	149.0841			
Methamphetamine	149.1204	91.0542	119.0855	
Acetaminophen	151.0633	110.0593	134.0587	135.0626
4-Anilinopiperidine	176.1313			
Phenacetin	179.0946	110.06	138.0913	152.0706
(2-Bromoethyl)benzene	183.9888			
4-Bromo-o-phenylenediamine	185.9793			
Citric acid	192.027			
MDMA	193.1103	163.0754	133.0643	105.0699
Caffeine	194.0804	138.0662	110.0713	
1-Boc-4-piperidone	199.1208			
Medetomidine	200.1313	95.0604	133.1011	
N-phenethyl-4-piperidone	203.131			
Phenylethylmalonamide	206.1055			
Prolintane	217.183	91.0542	72.0808	105.0699
Primidone	218.1055	91.0542	119.0855	162.0913
Xylazine	220.1034	90.0372	164.0528	147.0917
Acetamiprid	222.0672	126.0105	56.0495	
Phenobarbital	232.0848			
Norfentanyl	232.1576	84.0808	150.0913	177.1386
MD-MAPA	236.0685			
Ketamine	237.092	125.0153	220.0888	179.0622
BTMPS_2	240.1963	58.0648	81.0694	123.1162
Etomidate	244.1212	95.024	105.0699	113.0346
PMK ethyl glycidate	250.0841	147.0432	177.054	119.0487
N-phenethyl-N-phenylpropionamide	253.1467			
AP-237	272.1961	117.0676	91.0521	149.0205
UF-17	274.2045	150.091	174.1275	230.1544
N-Boc-4-AP	276.1838			
ANPP	280.1939	105.0699	188.1434	134.0964
Diazepam	284.0716	154.0418	193.0886	222.1151
Hydromorphone	285.1365	185.0597	227.0703	157.0648
Morphine	285.1365	201.091	229.0859	183.0804
Oxazepam	286.0509	241.0527	231.0684	269.0476
Para-methyl AP-237	286.2045	131.0833	91.0523	116.0596
AP-238	286.2045	117.0683	169.1315	91.0525

Ortho-methyl boc-4-AP	290.1994	108.0802	84.0805	128.0702
Despropionyl ortho-methylfentanyl	294.2096	188.1441	134.0968	105.0707
3,4-Difluoro-U-47700	296.17	252.1166	141.0127	172.0548
F-ANPP	298.1845	105.0699	188.1434	134.0964
Clonidine	299.0174	212.9981	161.9872	
Chlorodiazepoxide	299.0825			
Hydrocodone	299.1521	199.0754	241.0859	183.0804
Temazepam	300.0666	255.0684	193.0886	228.0575
Cocaine	303.1471	182.1176	82.0651	105.0335
Desalkylquazepam	304.0237	271.0402	236.073	109.044
Bromantane	305.0779	135.1174	93.0697	195.1018
Alprazolam	308.0829	281.0714	273.1213	241.0527
4'Cl-deschloroalprazolam	308.0829	281.0716	274.1227	131.0601
Deschloroetizolam	308.1095	280.075	255.0917	226.0647
Ethyl-4-ANPP	308.2252			
Naphthyl-U-47700	310.2045	266.1532	155.0483	91.0539
Metamizole	311.094			
Furanyl UF-17	312.1837	268.1206	188.0688	95.0118
Flunitrazepam	313.0863	268.1006	300.0905	239.0979
Desalkylgidazepam	314.0055	208.0993	183.9769	158.0483
7-Bromo-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one	314.1155			
Bromazepam	315.0007	182.0838	209.0947	261.0022
Clonazepam	315.0411	270.0554	302.0453	214.0418
Oxycodone	315.1471	241.1097	256.1332	298.1438
4'-Chlorodiazepam	318.0326	98.9844	256.0733	154.0418
Nitrazolam	319.1069	292.095	274.121	246.1024
Lorazepam	320.0119	275.0137	229.0527	303.0086
Acetylfentanyl	322.2045			
Quinine	324.1838	81.0699	160.0757	184.0757
Midazolam	325.0782	291.1166	244.0324	209.0635
Flualprazolam	326.0734	293.1114	223.0691	165.0218
Fluetizolam	326.1001	298.0682	98.9834	243.0508
Acetylmorphine	327.1471	211.0754	165.0698	193.0648
Metizolam	328.0549	300.0176	275.0349	192.0546
Trifluoromethyl-U-47700	328.1762	284.1265	173.0207	204.0657
Meclonazepam	329.0567			
Flubromazepam	331.996	226.0901	183.9756	208.9709
Fluclotizolam	332.0299	298.0675	228.0268	279.0161
N-boc norfentanyl	332.21			
Dipyanone	335.2249	265.1531	105.0295	223.1066
Fentanyl	336.2202	188.1434	105.0699	146.0964
Metodesnitazene	337.2154	100.1113	121.064	72.0806

N-methyl U-47931E	338.0994	294.0438	215.9798	183.9407
Para-fluoro acetyl fentanyl	340.1951			
Acetylcodeine	341.1627	225.091	197.0961	58.0651
Etizolam	342.0706	314.0387	259.0217	138.0372
N-ethyl-U-47700	342.1265	298.073	172.9533	218.0115
2F-viminol	346.242	273.1717	202.0994	142.1569
Cyclopropyl fentanyl	348.2202	188.1434	105.0699	132.0808
Ortho-methylfentanyl	350.2358	105.0699	188.1434	146.0964
Adinazolam	351.125	58.063		
Etodesnitazene	351.231	100.1129	72.0831	
Bromazolam	352.0324	325.0169	274.1178	205.075
Methoxyacetylfentanyl	352.2151	105.0699	188.1434	146.0964
Pyrazolam	353.0276	167.073	206.0838	275.1165
Clonazolam	353.068	308.0823	273.1135	204.0808
Fluorofentanyl	354.2107	188.1434	105.0699	146.0964
Chlorphine	355.1451	218.1282	139.03	84.0792
Para-methylcyclopropylfentanyl	362.2358	188.1413	242.1505	105.0682
5-Methyl etodesnitazene	365.2467	100.1116	72.0808	
N-desethyl etonitazene	368.1848	72.0807	298.1195	129.0545
Heroin	369.1576	58.0651	211.0754	268.1332
Flubromazolam	370.0229	392.1119	343.0115	237.0948
Flunitazene	370.1805	100.111	109.0435	72.08
Chlorofentanyl	370.1812	188.1406	105.0679	250.0935
N-pyrrolidino metonitazene	380.1848	98.0966	121.0649	
Metonitazene	382.2005	100.1105	121.0624	72.0797
N-desethyl isotonitazene	382.2005	312.135	72.0808	270.0884
Quetiapine	383.1667	221.1072	253.0794	210.0372
Phenethyl-ANPP	384.2565			
Phenazolam	385.9934	308.0825	360.9808	
N-phenethyl noroxymorphone	391.1783	374.176	105.0696	332.1659
Fluoro furanyl fentanyl	392.19	105.0699	188.1434	134.0964
N-pyrrolidino etonitazene	394.2005	98.0958	107.0508	135.08
Carfentanil	394.2256	113.0597	134.0964	146.0964
Methylenedioxynitazene	396.1797	100.1121	72.0809	120.0806
2',5'-Dimethoxyfentanyl	396.2413	248.1633	165.0899	206.1168
Brorphine	399.0946	218.1252	182.9766	104.0587
Fluoro phenethyl 4-ANPP	402.2471			
Para-methoxy furanyl fentanyl	404.21	188.1415	105.0686	134.0954
N-propionitrile chlorphine	408.1733	139.0302	271.155	84.0806
Protonitazepyne	408.2161	98.0956		
Etonitazepipne	408.2161	112.1112	135.0795	107.0484
T-butyl nitazene	408.2525			
5,6-Dichloro desmethylchlorphine	409.0515	125.014	208.0875	82.0643

Isotonitazene	410.2318	100.1109	72.0809	296.1022
Protonitazene	410.2318	100.112	72.081	
Bromofentanyl	414.1307	188.1418	105.0695	134.0975
Diltiazem	414.1613	178.0321	150.0372	137.0597
N-phenethyl-N-phenylpropionamide	416.1463			
Butonitazene	424.2474	100.1117	163.1104	107.047
Tianeptine	436.1224	228.0575	292.0194	193.0886
Rilmazafone	474.0974	418.0834	389.0568	341.0199
BTMPS	480.3927	140.1435	342.2641	58.0656

Spray Solvent Optimization

Methods

The ionization efficiency of two spray solvents consisting of a solution of acetonitrile, deionized water, and formic acid (90/9.9/0.1 v/v% acetonitrile/DI water/formic acid), and isopropanol were compared for opioid sample components. Four opioid samples that had previously been analyzed and quantified using the QqQ instrument were analyzed in triplicate for each of the spray solvents using full scan mode on an Orbitrap instrument. The intensity of the precursor ions for the drugs in each sample was compared and used to evaluate the optimal solvent.

Results

The results of the intensity comparison for six different components of an opioid samples are given in Figure S1. The acetonitrile-based spray solvent demonstrated higher intensity for all compounds analyzed, as well as a smaller standard deviation between replicate measurements (n=3), and was therefore selected to proceed with for future applications of the method.

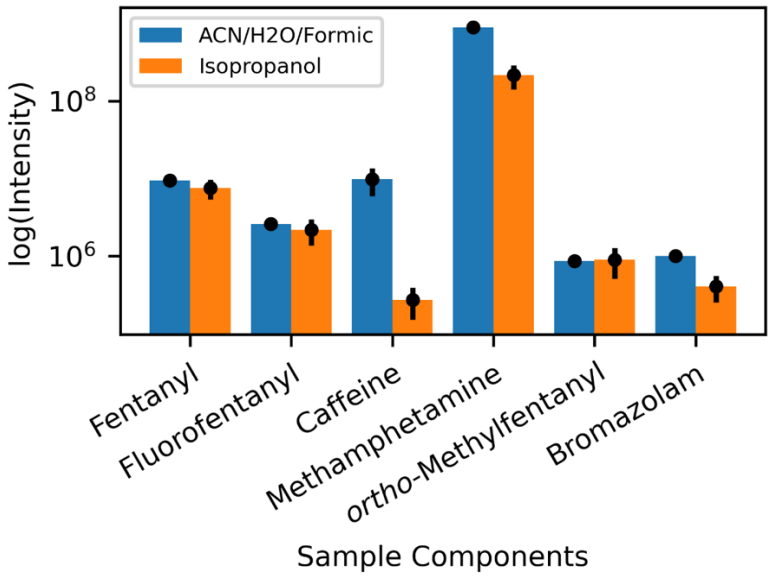

Figure S1: Comparison of signal intensity for the components in an opioid sample: bromazolam (1.7% w/w), caffeine, fentanyl (2.9% w/w), fluorofentanyl (0.8% w/w), methamphetamine, and *ortho*-methylfentanyl (0.5% w/w). Samples were analyzed in triplicate using full scan mode, with spray solvents composed of either acetonitrile:water:formic acid (90:10:0.1) or Isopropanol. Triplicate measurements were performed, with error bars representing standard deviation from the mean.

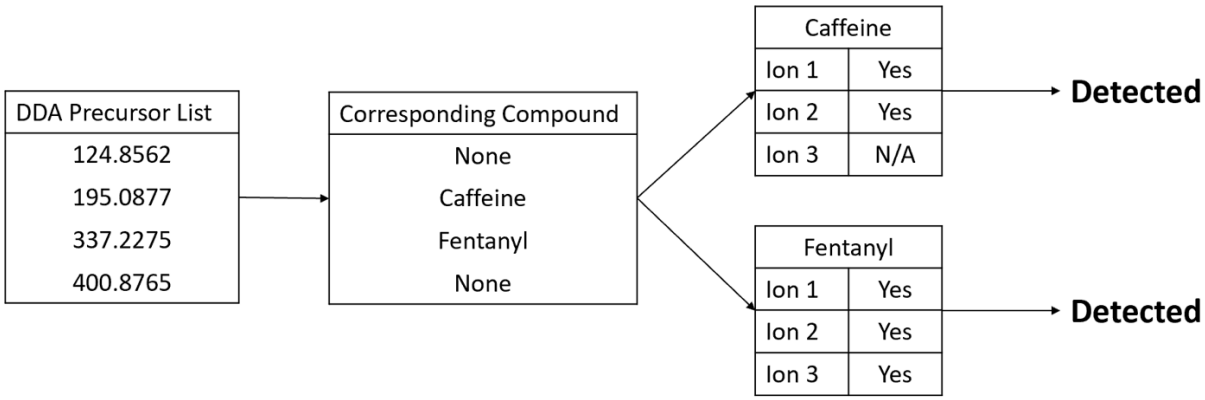

Figure S2: Schematic for the peak searching algorithm, with an example given for the detection of fentanyl and caffeine in a sample.

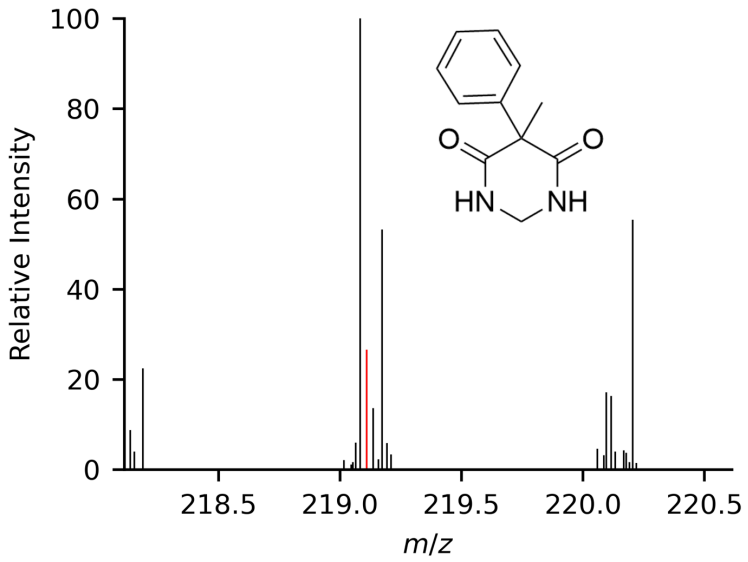

Figure S3: DDA precursor isolation window for primidone from the full scan spectrum. The primidone precursor ion at *m/z* 219.1128 is highlighted in red.

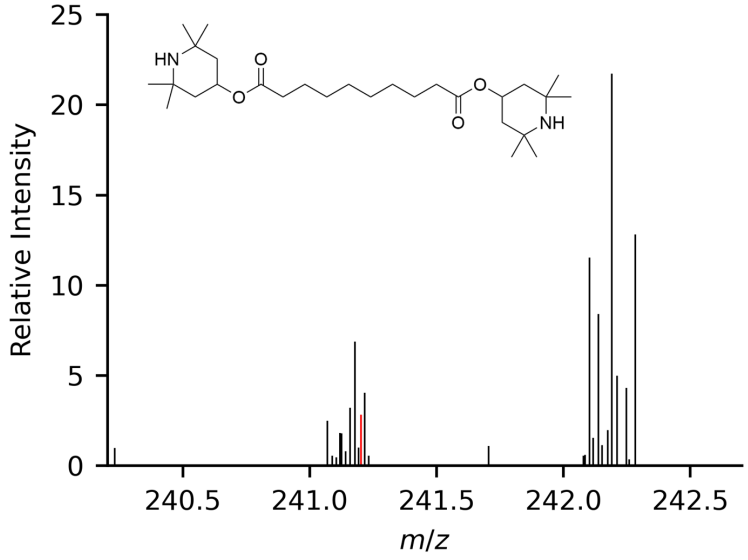

Figure S4: DDA precursor isolation window for BTMPS from the full scan spectrum. The BTMPS $[M + 2H]^{2+}$ precursor ion at *m/z* 241.2039 is highlighted in red.

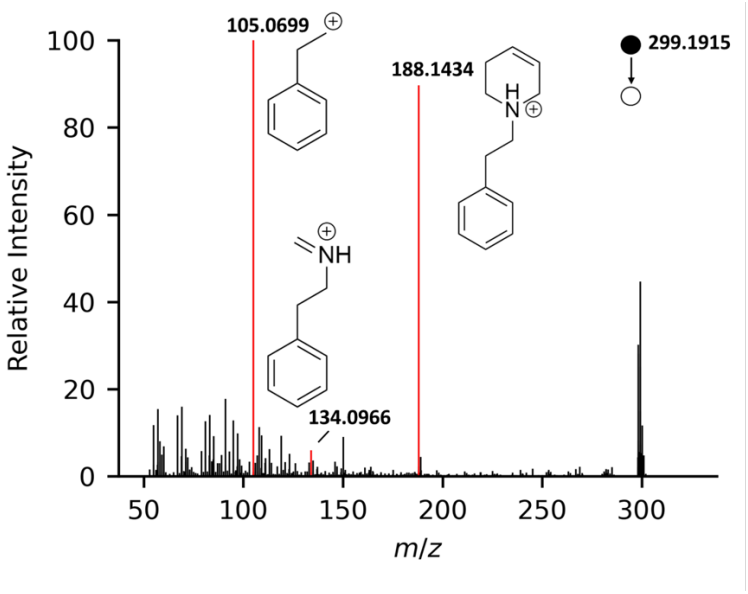

Figure S5: HRMS/MS $[M+H]^+$ spectrum of F-ANPP, with the three selected confirming ions highlighted.

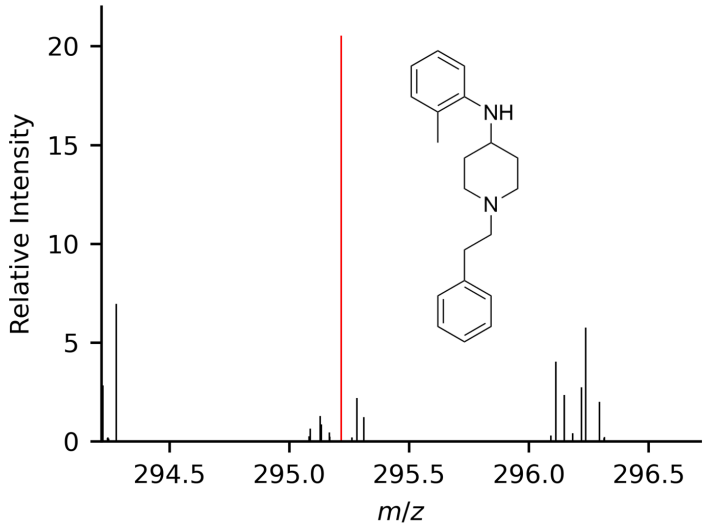

Figure S6: DDA precursor window for ortho-methyl ANPP from the full scan spectrum. The ortho-Methyl ANPP precursor ion at *m/z* 295.2169 is highlighted in red.