SUPPLEMENTARY MATERIAL

FLEXIBLE HIGH RESOLUTION-MASS SPECTROMETRY APPROACH FOR SCREENING NEW PSYCHOACTIVE SUBSTANCES IN URBAN WASTEWATER

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MATERIALS AND METHODS

Chemicals and materials

The deuterated analogues of illicit drugs including in the mixed solution were: cocaine-d3, benzoylecgonine-d3, cocaethylene-d8, amphetamine-d6, methamphetamine-d9, 3,4 methylenedioxyamphetamine-d5, 3,4-methylenedioxymethamphetamine-d5, 3,4 methylenedioxyethamphetamine-d5, 1,3-benzodioxolyl-N-methylbutanamine-d5, ketamine-d4, norketamine-d4, codeine-d6, oxycodone-d6, hydrocodone-d6, morphine-d3, morphine-3β-D-glucuronide-d3, 6-acetylmorphine-d6, methadone-d3, 2-ethylidene-1,5- dimethyl-3,3-diphenylpyrrolidine-d3. All analytical standards were purchased from Cerilliant Corporation (Round Rock, Texas, USA). Individual standards were available as solutions in methanol (MeOH) or acetonitrile (ACN) at concentrations of 1, 0.4 or 0.1 mg mL^{-1}. Each stock solution of 10 μg mL^{-1} and the working solutions (1 and 0.1 μg mL^{-1}) were prepared with MeOH and stored at -20° C in the dark. Mixed solutions were prepared daily at a concentration of 2 ng (reconstitution in 200 μL, 10% methanol (aq)) and injected before and after each sequence for assessing the sensitivity and selectivity of the instrument. The MS^2 spectra database is available on request.

Instrumental analysis

The specific parameters of each experiment are reported in the following lines:

1) Full MS experiment:

MS parameter	Value
Microscan	3
Resolution	70 000
AGC Target	1 e^6
Maximum IT	200 ms
Scan spectra	100 to 600 m/z

2) Data-dependant analysis (Full MS-$ddMS^2$ TOP5)

Full MS parameter	Value
Microscan	1
Resolution	70 000
AGC Target	1 e^6
Maximum IT	100 ms
Scan spectra	100 to 500 m/z

ddMS^2 parameter	Value
Microscan	1
Resolution	17 500
AGC Target	1 e^5
Maximum IT	50 ms
Loop count	5
MSX count	1
Top N	5
Isolation window	3.0 m/z
Normalized Collision Energy	35, 50 eV

As we selected the "preliminary suspects" with the first Full MS experiment, an inclusion list was created for the data dependant analysis, in which a retention time (RT) window of ±1 min was established for each suspect compound.

3) Data-independent analysis (DIA)

When no MS^2 scans were collected with Full MS-ddMS^2, the Full MS scan was followed by a DIA experiment. Again, an inclusion list was created establishing a retention time (RT) window of ±1 min for each suspect compound.

Full MS parameter	Value
Microscan	1
Resolution	70 000
AGC Target	1 e^6
Maximum IT	100 ms
Scan spectra	100 to 500 m/z

DIA parameter	Value
Microscan	1
Resolution	35 000
AGC Target	2 e^5
Maximum IT	auto
Loop count	2
MSX count	1
Isolation window	3.0 m/z
Normalized Collision Energy	35, 50 eV

RESULTS AND DISCUSSION

NPS spectral database

Table S1. Retention time (RT), parent and product ions (theoretical accurate m/z values and chemical formulae) obtained for NPS fragmentation with Q-Exactive mass analyser.

Compound	$[M+H]^+$	RT (min)	Theo m/z $[M+H]^+$	Fragment 1	Fragment 2	Fragment 3
				Theo m/z (Formula)	Theo m/z (Formula)	Theo m/z (Formula)
Synthetic cannabinoids						
MDMB-CHMICA	$C_{23}H_{33}N_2O_3$	25.1	385.2486	240.1381 ($C_{16}H_{18}NO$)	144.0443 (C_9H_6NO)	-
5Fpentyl-3-pyridinolindole	$C_{19}H_{20}FN_2O$	16.4	311.1555	232.1133 ($C_{14}H_{15}NOF$)	291.1493 ($C_{19}H_{19}N_2O$)	144.0443 (C_9H_6NO)
Synthetic cathinones						
Methcathinone	$C_{10}H_{14}NO$	3.6	164.1071	131.0731 (C_9H_9N)	133.0650 (C_9H_9O)	-
Ethcathinone	$C_{11}H_{16}NO$	3.9	178.1226	105.0703 (C_8H_9)	133.0650 (C_9H_9O)	-
3,4-DMMC	$C_{12}H_{18}NO$	8.1	192.1384	159.1042 ($C_{11}H_{13}N$)	161.0960 ($C_{11}H_{13}O$)	-
3-MMC	$C_{11}H_{16}NO$	6.1	178.1226	160.1121 ($C_{11}H_{14}N$)	145.0887 ($C_{10}H_{11}N$)	119.0858 (C_9H_{11})
3-MeOMC	$C_{11}H_{16}NO_2$	5.2	194.1176	161.0835 ($C_{10}H_{11}NO$)	138.0683 ($C_8H_{10}O_2$)	145.0887 ($C_{10}H_{11}N$)
4-MEC	$C_{12}H_{18}NO$	6.8	192.1384	145.0887 ($C_{10}H_{11}N$)	147.0805 ($C_{10}H_{11}O$)	146.0965 ($C_{10}H_{12}N$)
4-FMC	$C_{10}H_{13}FNO$	4.4	182.0976	149.0636 (C_9H_8FN)	123.0607 (C_8H_8F)	-
Methylone	$C_{11}H_{14}NO_3$	4.0	208.0969	160.0757 ($C_{10}H_{10}NO$)	132.0809 ($C_9H_{10}N$)	-
Butylone	$C_{12}H_{16}NO_3$	5.7	222.1125	174.0914 ($C_{11}H_{12}NO$)	175.0628 ($C_{11}H_{11}O_2$)	146.0965 ($C_{10}H_{12}N$)
Pentylone	$C_{13}H_{18}NO_3$	7.6	236.1281	188.1071 ($C_{12}H_{14}NO$)	175.0630 ($C_{11}H_{11}O_2$)	135.0442 ($C_8H_7O_2$)
Dipentylone	$C_{14}H_{20}NO_3$	8.0	250.1438	100.1126 ($C_6H_{14}N$)	175.0754 ($C_{11}H_{11}O2$)	135.0442 ($C_8H_7O_2$)
Methedrone	$C_{11}H_{16}NO_2$	5.0	194.1176	161.0835 ($C_{10}H_{11}NO$)	138.0663 ($C_8H_{10}O_2$)	145.0887 ($C_{10}H_{11}N$)
Mephedrone	$C_{11}H_{16}NO$	6.1	178.1226	160.1121 ($C_{11}H_{14}N$)	145.0887 ($C_{10}H_{11}N$)	-
Buphedrone	$C_{11}H_{16}NO$	5.3	178.1226	131.0731 (C_9H_9N)	132.0809 ($C_9H_{10}N$)	147.0805 ($C_{10}H_{11}O$)
Pentedrone	$C_{12}H_{18}NO$	7.2	192.1384	132.0809 ($C_9H_{10}N$)	161.0961 ($C_{11}H_{13}O$)	-
MDPV	$C_{16}H_{22}NO_3$	8.7	276.1594	126.1279 ($C_8H_{16}N$)	135.0442 ($C_8H_7O_2$)	175.0754 ($C_{11}H_{11}O_2$)
α-PVP	$C_{15}H_{22}NO$	8.2	232.1696	91.0548 (C_7H_7)	126.1279 ($C_8H_{16}N$)	161.0691 ($C_{11}H_{13}O$)
α-PVT	$C_{13}H_{20}NOS$	6.8	238.1258	126.1278 ($C_6H_{16}N$)	97.0111 (C_5H_5S)	167.0524 ($C_9H_{11}OS$)

3,4-DMeO- α-PVP	$C_{17}H_{26}NO_3$	8.4	292.1907	151.0754 ($C_9H_{11}O_2$)	126.1279 ($C_8H_{16}N$)	221.1173 ($C_{13}H_{17}O_3$)
4-Cl-α-PPP	$C_{13}H_{17}ClNO$	8.0	238.0993	98.0969 ($C_6H_{12}N$)	139.0310 (C_8H_8Cl)	167.0258 (C_9H_8OCl)
Phenethylamines						
25B-NBoMe	$C_{18}H_{23}BrNO_3$	14.7	380.0860	121.0650 (C_8H_9O)	91.0548 (C_7H_7)	-
25C-NBoMe	$C_{18}H_{23}ClNO_3$	14.2	336.1361	121.0650 (C_8H_9O)	91.0548 (C_7H_7)	-
25I-NBoMe	$C_{18}H_{23}INO_3$	15.5	428.0717	121.0650 (C_8H_9O)	91.0548 (C_7H_7)	-
25iP-NBoMe	$C_{21}H_{30}NO_3$	17.6	344.2219	121.0650 (C_8H_9O)	91.0548 (C_7H_7))	-
2-Cl-4,5-MDMA	$C_{11}H_{15}ClNO_2$	9.0	230.0942	185.0363 ($C_9H_{10}O_2Cl$)	198.0441 ($C_{10}H_{11}O_2Cl$)	178.0987 ($C_{11}H_{14}O_2$)
PMA	$C_{10}H_{16}NO$	5.2	166.1226	121.0650 (C_8H_9O)	149.0962 ($C_{10}H_{13}O$)	-
PMMA	$C_{11}H_{18}NO$	5.8	180.1383	121.0650 (C_8H_9O)	149.0962 ($C_{10}H_{13}O$)	-
NEDPA	$C_{16}H_{20}N$	10.9	226.1590	181.1010 ($C_{14}H_{13}$)	166.0776 ($C_{13}H_{10}$)	103.0546 (C_8H_7)
2-PEA	$C_8H_{12}N$	3.2	122.0966	105.0703 (C_8H_9)	79.0549 (C_6H_7)	-
5-IT	$C_{11}H_{15}N_2$	4.6	175.1229	158.0963 ($C_{11}H_{12}N$)	130.0652 (C_9H_8N)	117.0575 (C_8H_7N)
6-APDB	$C_{11}H_{16}NO$	5.5	178.1226	133.0648 (C_9H_9O)	161.0959 ($C_{11}H_{13}O$)	-
Opioids						
MT-45	$C_{24}H_{33}N_2$	15.2	349.2633	181.1010 ($C_{14}H_{13}$)	166.0776 ($C_{13}H_{10}$)	-
Tryptamines						
AMT	$C_{11}H_{15}N_2$	5.9	175.1229	158.0963 ($C_{11}H_{12}N$)	130.0652 (C_9H_8N)	-
5-MeO-MIPT	$C_{15}H_{22}N_2O$	7.2	247.1805	174.0914 ($C_{11}H_{12}NO$)	121.0650 (C_8H_9O)	203.1067 ($C_{12}H_{15}N_2O$)
5-MeO-DALT	$C_{17}H_{22}N_2O$	9.3	271.1805	110.0966 ($C_7H_{12}N$)	174.0912 ($C_{11}H_{12}NO$)	-
Aminorex derivatives						
4-methylaminorex	$C_{10}H_{13}N_2O$	6.0	177.1022	160.119 ($C_{10}H_{12}N_2$)	145.0885 ($C_{10}H_{11}N$)	134.0964 ($C_9H_{12}N$)
4,4’ – DMAR	$C_{11}H_{15}N_2O$	8.2	191.1179	148.1121 ($C_{10}H_{14}N$)	131.0857 ($C_{10}H_{11}$)	-
Ketamine analogues						
Methoxetamine	$C_{15}H_{22}NO_2$	7.3	248.1640	121.0650 (C_8H_9O)	175.1117 ($C_{12}H_{15}O$)	203.1067 ($C_{13}H_{15}O_2$)

HRMS ions are ordered according to their relative abundance in the spectrum, from the most (Fragment 1) to the least abundant (Fragment 3).

Fig S1. Chromatograms and MS^2 spectra for 3,4-DMeO-alpha-PVP in A) wastewater and B) reference standard. Identification at confidence level 1.

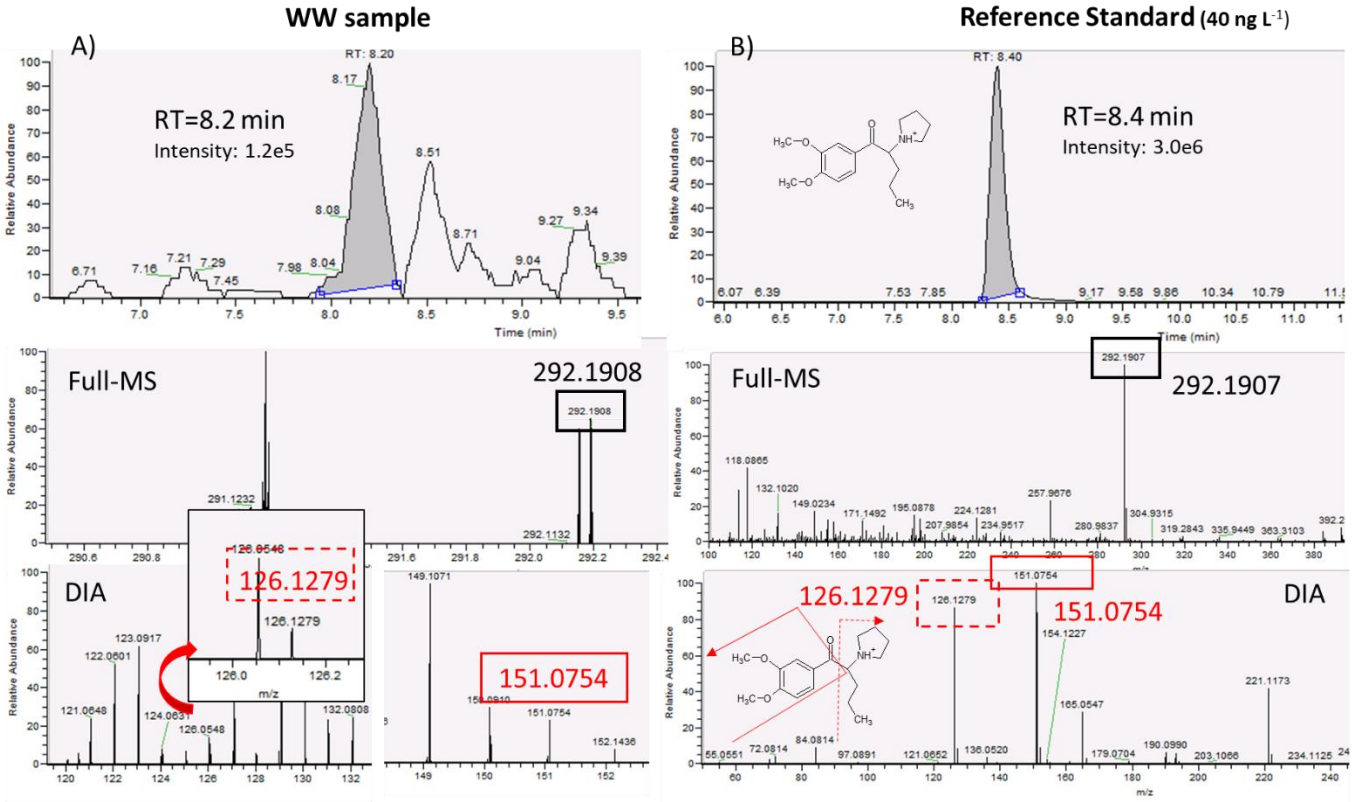

Fig S2. Chromatograms and MS^2 spectra for 2-phenetylamine (2-PEA) in A) wastewater and B) reference standard. Identification at confidence level 1.

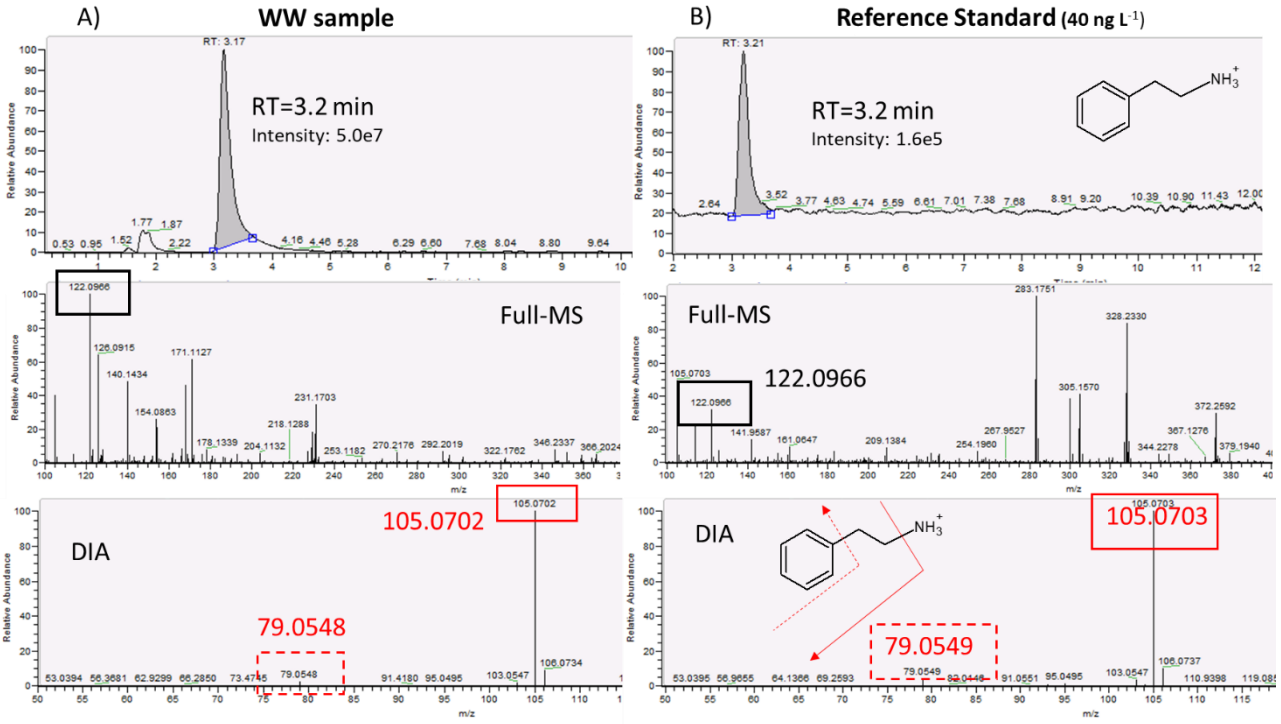

Fig S3. Chromatograms and MS^2 spectra for para-methoxyamphetamine (PMA) in A) wastewater and B) reference standard. Identification at confidence level 1.

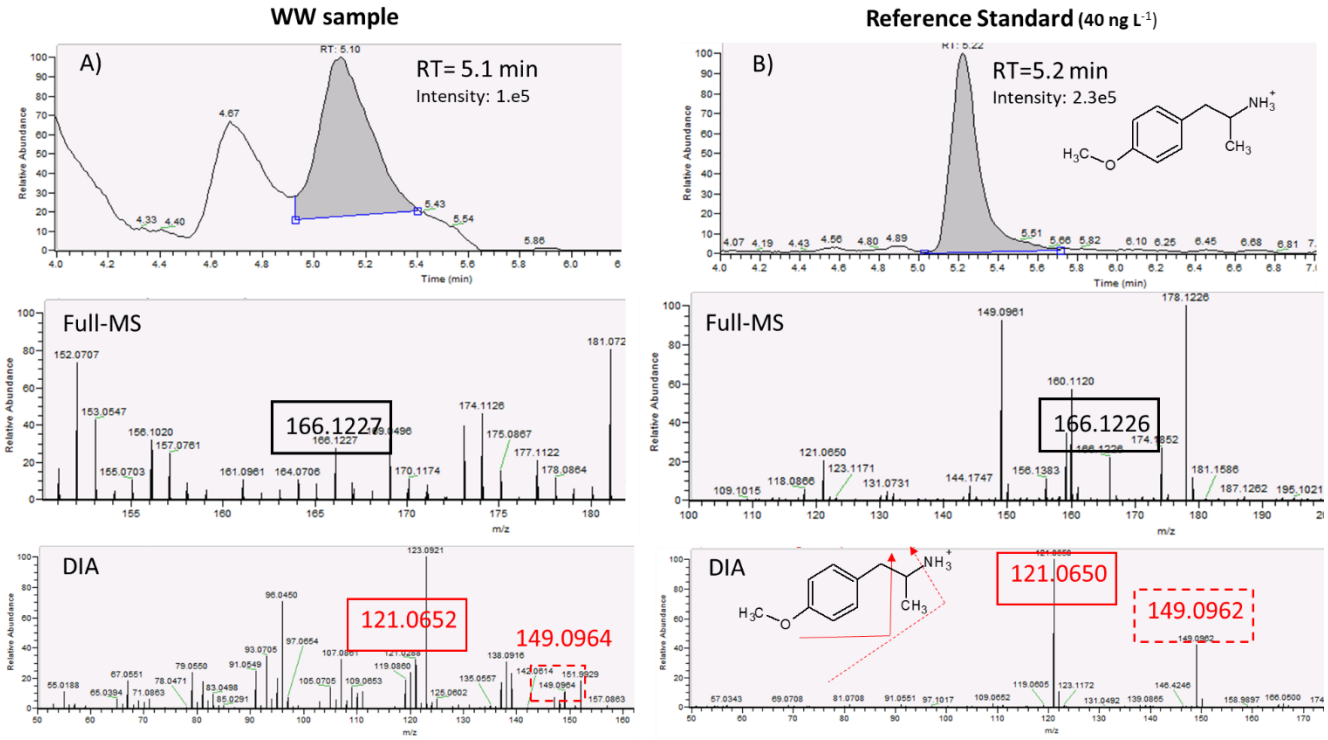

Fig S4. Chromatograms and MS^2 spectra for (A) 2-methoxyamphetamine (2-MA) in wastewater and B) analytical standard of *para*-methoxyamphetamine (PMA). Identification at confidence level 2.

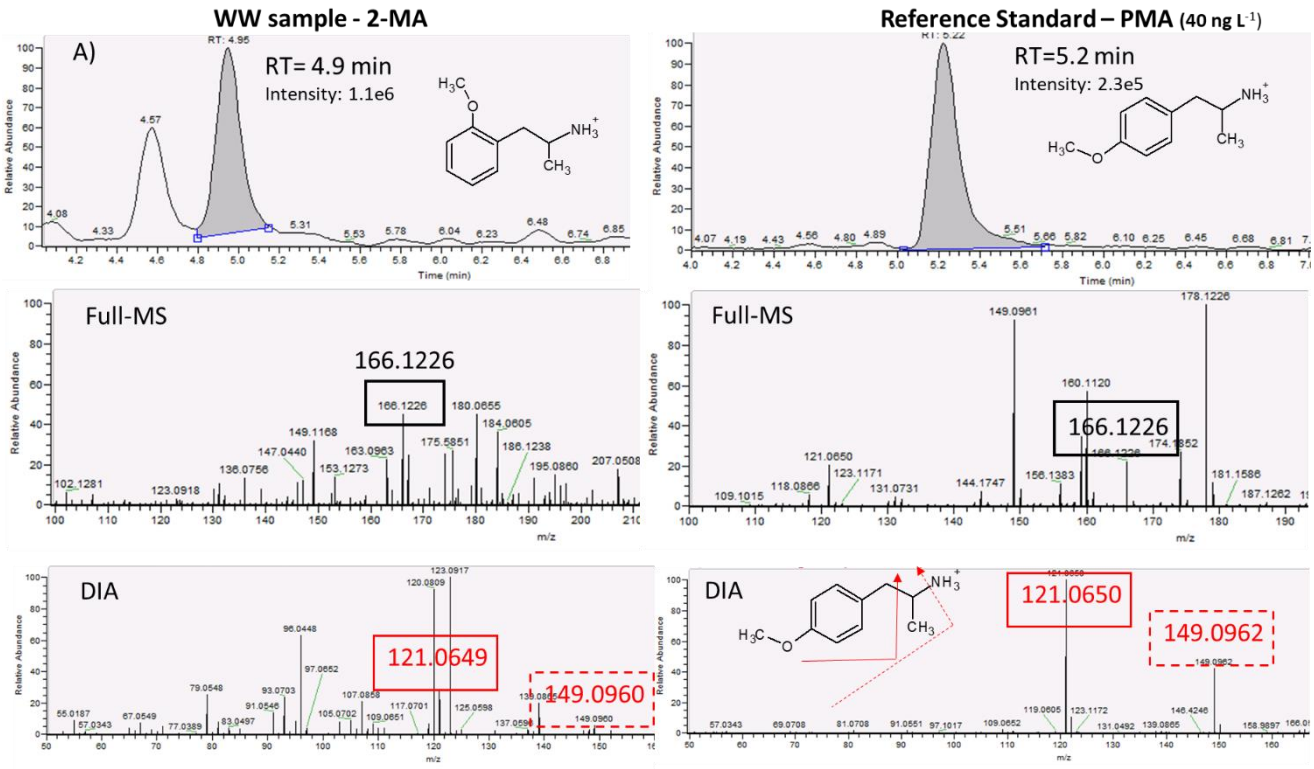

Fig. S5. Chromatogram and MS^2 spectra for the DOiP in wastewater. Identification at confidence level 3.

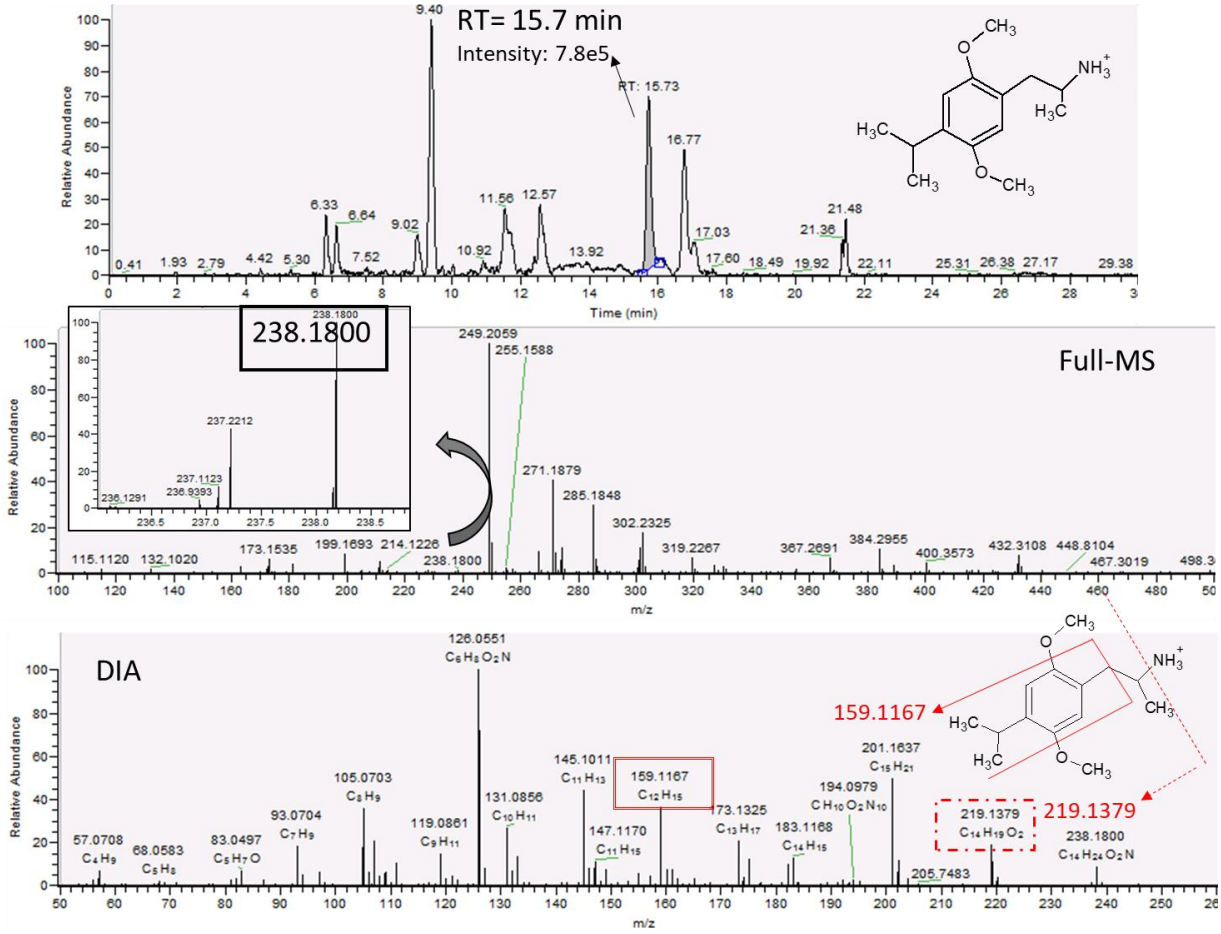

Fig. S6. Chromatogram and MS^2 spectra for the HDMP-28 in wastewater. Identification at confidence level 3.

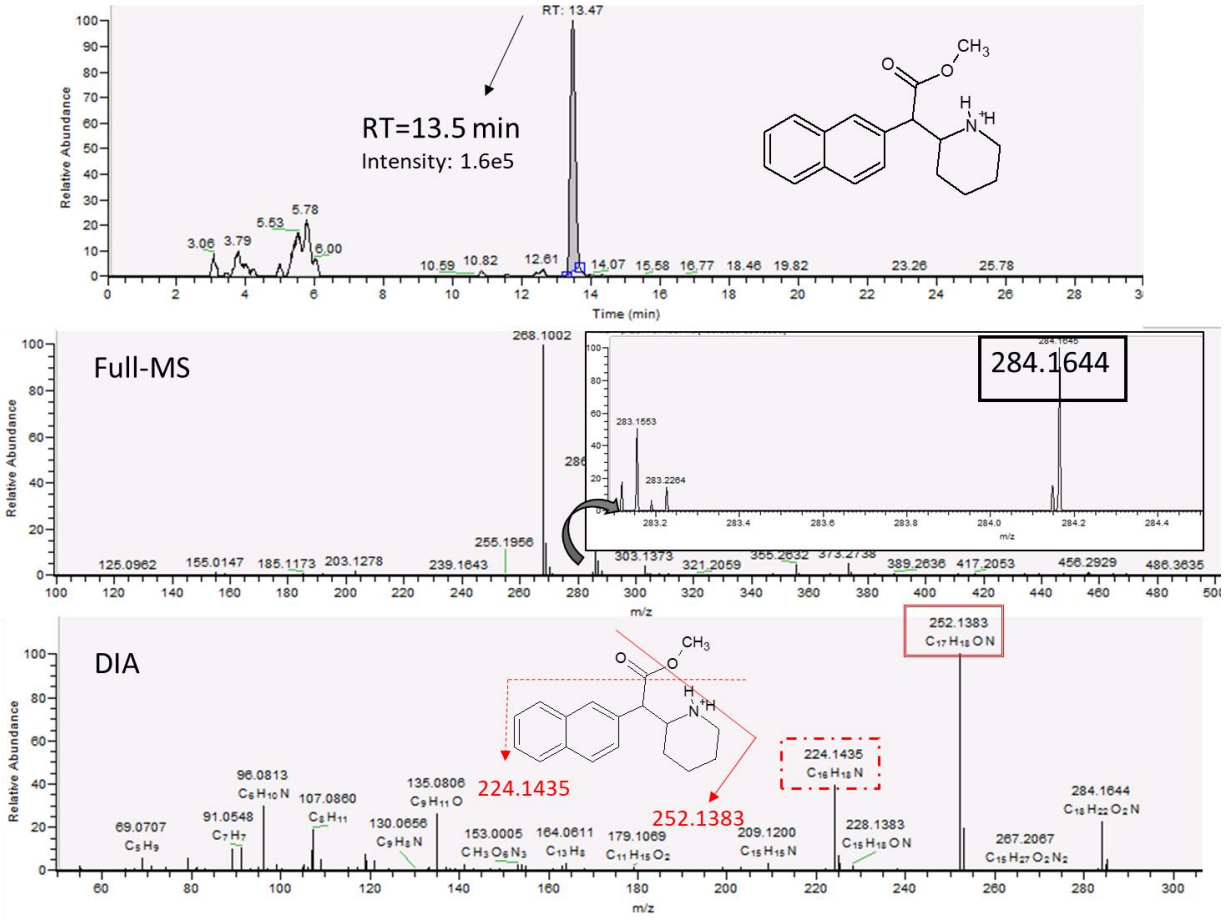

Fig. S7. Chromatogram and MS^2 spectra for the Isopropylphenidate in wastewater. Identification at confidence level 3.

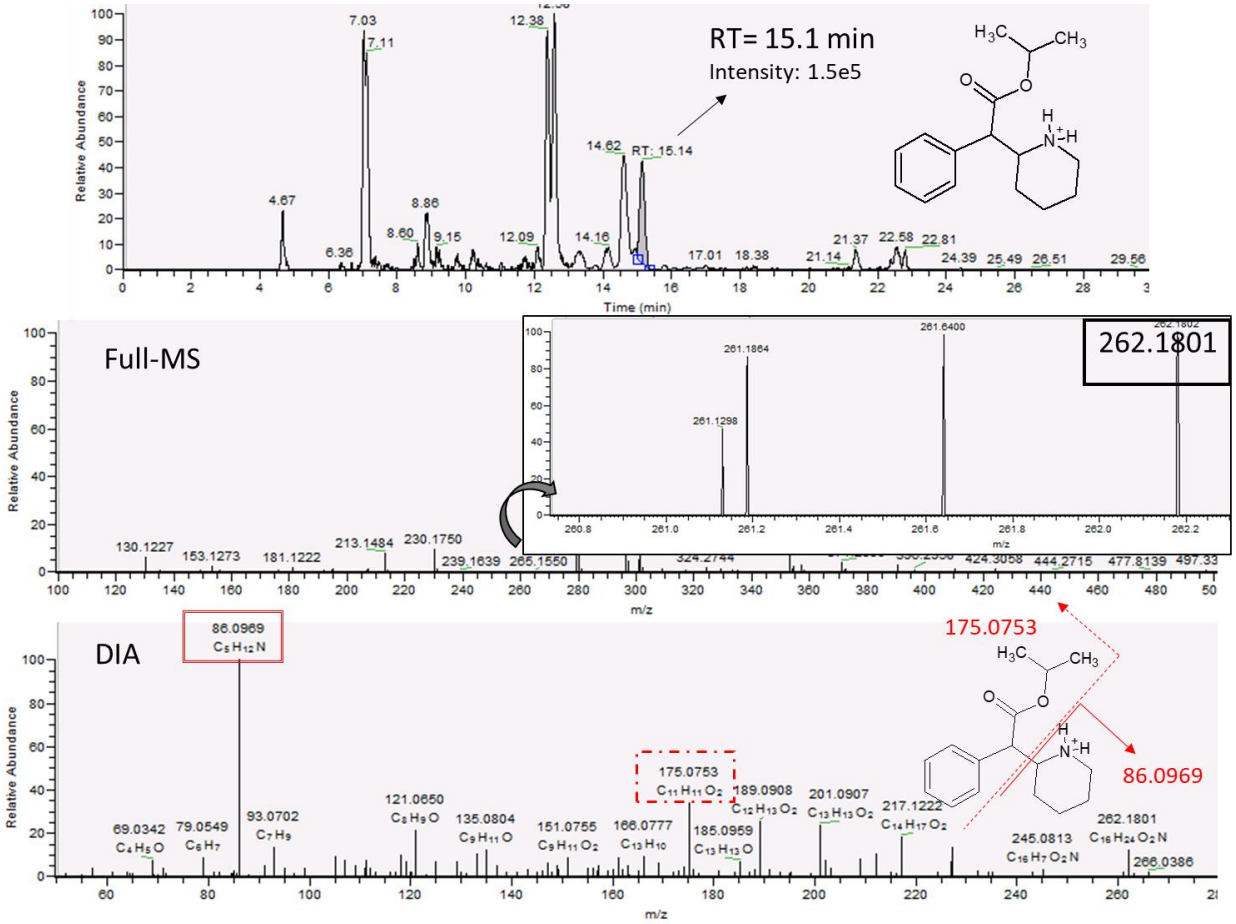

Fig. S8. Chromatogram and MS^2 spectra for the diphenidine in wastewater. Identification at confidence level 3.

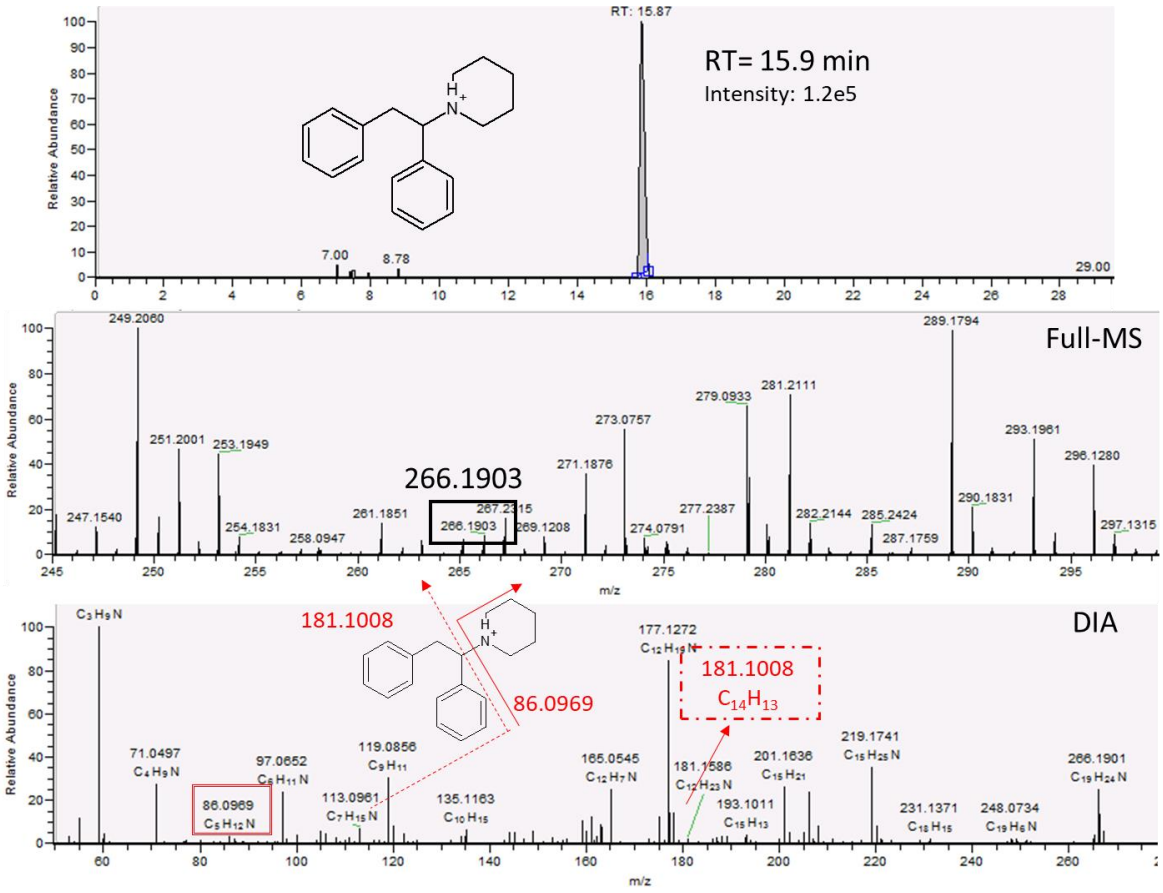

Fig. S9. Chromatogram and MS^2 spectra for the AMB-FUBINACA in wastewater. Identification at confidence level 3.