Supporting information

for

Profiling of Aldehyde-containing Compounds by Stable Isotope Labelling- Assisted Mass Spectrometry Analysis

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Synthesis of 4-APC and 4-APC-d_4

N-(4-(2-Bromoethoxy)phenyl)acetamide p-Hydroxyacetanilide (2.0 g) and 1,2-dibromoethane(24.7 g) were added to saturated sodium carbonate solution (20 mL). Tetrabutylammonium perchlorate (0.1 g) was added as a catalyst. The mixture was vigorously stirred for 28 h at 80 °C after which water and the excess of 1,2-dibromoethane were evaporated under high vacuum. The residue was extracted with chloroform (250 mL) to remove inorganic salts and amounts of disubstituted products. The chloroform extract was washed with a saturated sodium carbonate solution (2×). The chloroform layer was dried over $MgSO_4$, filtered, and concentrated. Recrystallization from ethanol/water yielded a white solid. ^{1}HNMR (CD_3Cl/drop DMSO-d_6, 200 MHz) δ 7.5 (d, 2H, CHCNHAc), 6.8 (d, 2H, CHCOCH2), 4.2 (t, 2H, OCH2), 3.6 (t, 2H, CH2Br), 2.0 (s, 3H, COCH3).

(2-(4-Acetamidophenoxy)ethyl)trimethylammonium Bromide N-(4-(2-Bromoethoxy)phenyl)acetamide (0.5 g) was mixed with a solution of Me3N in EtOH (5 mL). Cosolvent methylethylketone (5 mL) was added, and themixture was stirred at room temperature for 4 days, during which time the product precipitated. It was filtered and recrystallized from hot EtOH/water to afford C as pinkish/beige transparent crystals. ^{1}H NMR (D2O, 200 MHz) δ7.3 (d, 2H, CHCNHAc), 7.0 (d, 2H, CHCOCH2), 4.4 (m, 2H, OCH2), 3.7 (m, 2H, CH2NMe3), 3.2 (s, 9H, NMe3), 2.1 (s, 3H, COCH3).

(4-(2-(trimethylammonio)ethoxy)benzenaminium halide (2-(4-Acetamidophenoxy)ethyl)trimethylammonium (0.2 g) was heated at reflux with hydrochloric acid (5 N, 3 mL) for 30 min. After cooling, the solvent was evaporated from the solution leaving a solid residue, which was recrystallized from hot EtOH/water. Filtering, washing, and drying afforded shiny beige crystals with excellent purity. ^{1}HNMR (D_2O, 200 MHz) δ 7.4 (d, 2H, CHCNH3), 7.1 (d,

2H, CHCOCH2), 4.5 (m, 2H, OCH2), 3.8 (m, 2H, CH2NMe3), 3.2 (s, 9H, NMe3).

4-APC-d_4 was prepared in a similar procedure with 1,2-dibromoethane-d_4 instead of 1,2-dibromoethane. ^{1}HNMR (D_2O, 200 MHz) δ 7.2 (d, 2H, CHCNH3), 7.0 (d, 2H, CHCOCH2), 3.1 (s, 9H, NMe3).

Table S1. The slopes of regression lines of the measured peak intensity ratios versus the mean concentration ratios (1:20, 1:10, 1:5, 1:1, 5:1, 10:1and 20:1) of 4-APC/4-APC-d_4 labelled standards of hexanal, benzaldehyde and heptanal using QTRAP mass spectrometer.

Analytes	Linear range (light / heavy)	Regression line		
		Slope	Intercept	R^2
Hexanal	0.05-20	1.064	0.0229	0.9914
Benzaldehyde	0.05-20	0.951	0.0122	0.9973
Heptanal	0.05-20	0.985	-0.0009	0.9972

Table S2. The measured concentration of creatinine in pooled urine sample of healthy controls and lung cancer patients using LC-UV spectrometer.

Analytes	Concentration (mmol/L)
Healthy controls	7.1 ± 0.4
Lung cancer patients	4.8 ± 0.3

Table S3. List of the measured peak intensity ratios (pooled urine sample of lung cancer patients / pooled urine sample of healthy controls) of 16 aldehyde-containing compounds using QTRAP mass spectrometer.

Number	Retention Time (min)	4-APC Labelled (*m/z*)	4-APC-d_4 Labelled (*m/z*)	Forward Labelling	Reverse Labelling
1	17.46/17.42	279.2496	283.1369	1.6±0.1	1.7±0.1
2	14.96/14.89	285.1660	289.1653	2.6±0.1	2.8±0.1
3	20.38/20.31	293.2646	297.1595	1.8±0.4	1.9±0.2
4	23.19/23.10	307.2795	311.2139	2.1±0.3	1.9±0.2
5	25.67/25.68	321.2946	325.3206	1.5±0.2	1.7±0.3
6	14.19/14.08	265.2419	269.1554	2.1±0.3	2.3±0.2
7	14.00/13.97	281.1802	285.2436	0.3±0.0	0.3±0.1
8	13.25/13.14	267.1811	271.1567	0.5±0.1	0.3±0.0
9	16.80/16.69	295.1655	299.1372	1.6±0.2	1.8±0.3
10	26.63/26.60	335.3114	339.3357	1.6±0.2	1.8±0.3
11	18.16/18.13	351.2148	355.2672	1.3±0.2	1.3±0.0
12	16.05/16.02	369.1517	373.2130	1.6±0.2	1.8±0.0
13	20.79/20.81	217.1268	221.1210	1.3±0.0	1.4±0.1
14	6.48/6.38	273.1210	277.1547	1.5±0.2	1.6±0.0
15	25.53/25.24	337.0839	341.1340	1.4±0.1	1.7±0.2
16	21.99/21.43	395.9088	400.0807	1.8±0.3	1.9±0.2

Table S4. The measured *m/z* of 4-APC and 4-APC-d_4 labelled aldehyde-containing compounds from white wine and their prospective molecular formulas using QTOF-MS spectrometer.

No.	RT (min) (4-APC/4-APC-d_4 Labelled)	4-APC Labelled (*m/z*)	4-APC-d_4 Labelled (*m/z*)	Prospective Formulas	Compound Name
1	17.35/17.28	279.2496	283.2743	$C_6H_{12}O$	Hexanal
2	14.77/14.72	285.1384	289.0646	C_7H_6O	Benzaldehyde
3	20.33/20.29	293.2646	297.2895	$C_7H_{14}O$	Heptanal
4	23.22/23.17	307.2795	311.3049	$C_8H_{16}O$	Octanal
5	2.76/2.74	223.1709	227.1902	C_2H_4O	Acetaldehyde
6	6.42/6.26	251.2132	255.2390	C_4H_8O	Butanal
7	17.07/17.02	265.2419	269.1560	$C_5H_{10}O$	Pentanal
8	17.10/17.03	261.2134	265.2422	C_2H_4NO	Aminoacetaldehyde
9	8.80/8.74	275.1780	279.2035	$C_5H_4O_2$	Furfural
10	16.37/16.34	299.2190	303.2082	C_8H_8O	Phenylacetaldehyde
11	27.80/27.76	335.3114	339.3357	$C_{10}H_{20}O$	Decanal
12	16.76/16.63	342.1907	346.2894	$C_9H_9NO_2$	p-Acetaminobenzaldehyde
13	16.73/16.76	343.2091	347.2346	$C_9H_8O_3$	Caffeic aldehyde
14	24.32/24.36	349.3255	353.3519	$C_{11}H_{22}O$	Undecanal
15	29.04/28.92	383.3114	387.2120	$C_{14}H_{20}O$	9Z-13-Tetradecadien-11-ynal
16	19.54/19.56	393.3190	397.3447	$C_{12}H_{22}O_3$	12-Oxo-dodecanoic acid
17	2.77/2.73	224.2809	228.3021		
18	18.39/18.32	237.2011	241.2217		
19	17.07/17.04	262.2011	266.2270		

No., number; RT, retention time.

Figure S1. The pathway for the synthesis of 4-APC and 4-APC-d_4

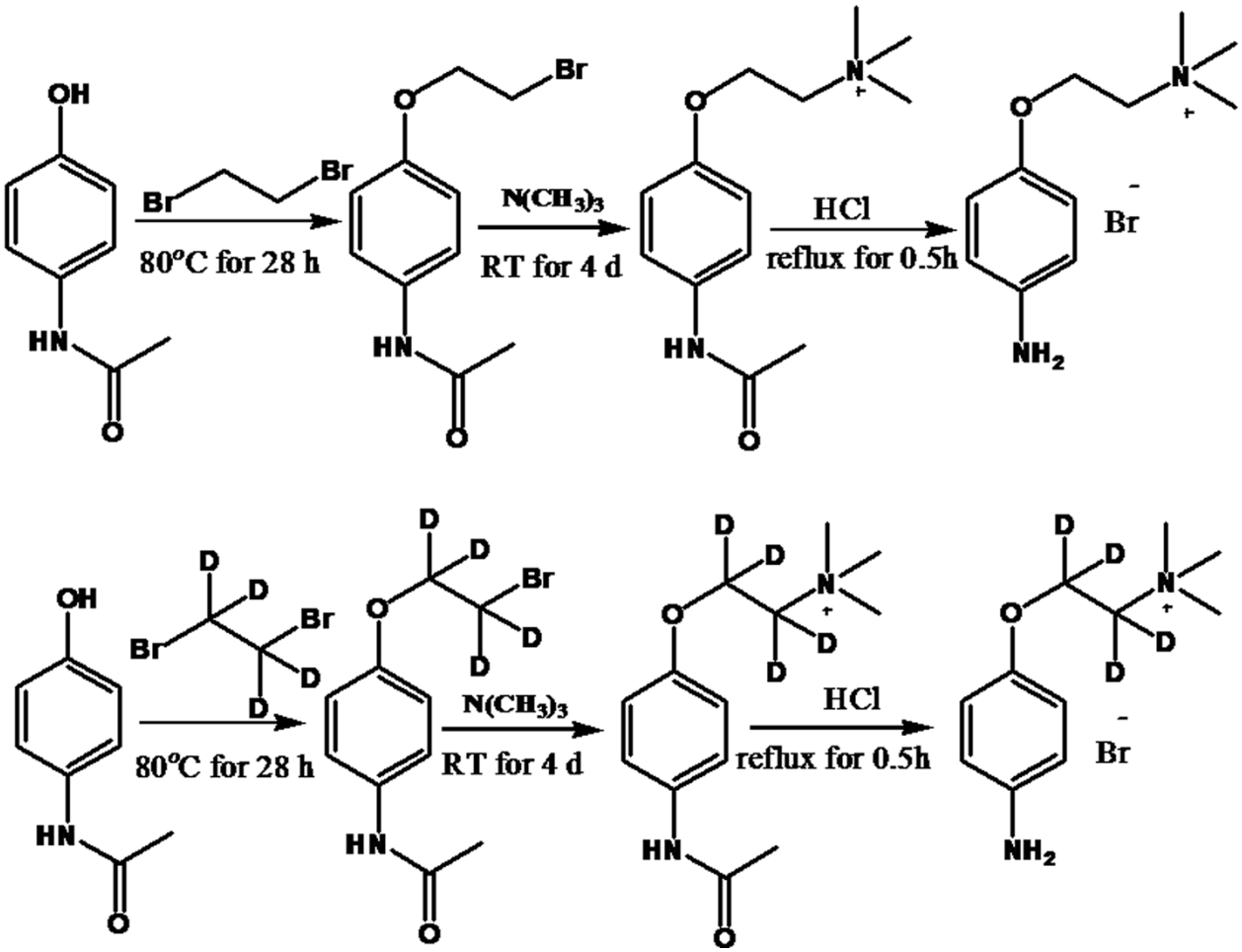


Figure S2. The proposed fragmentation pathway of 4-APC labelled aldehydes.

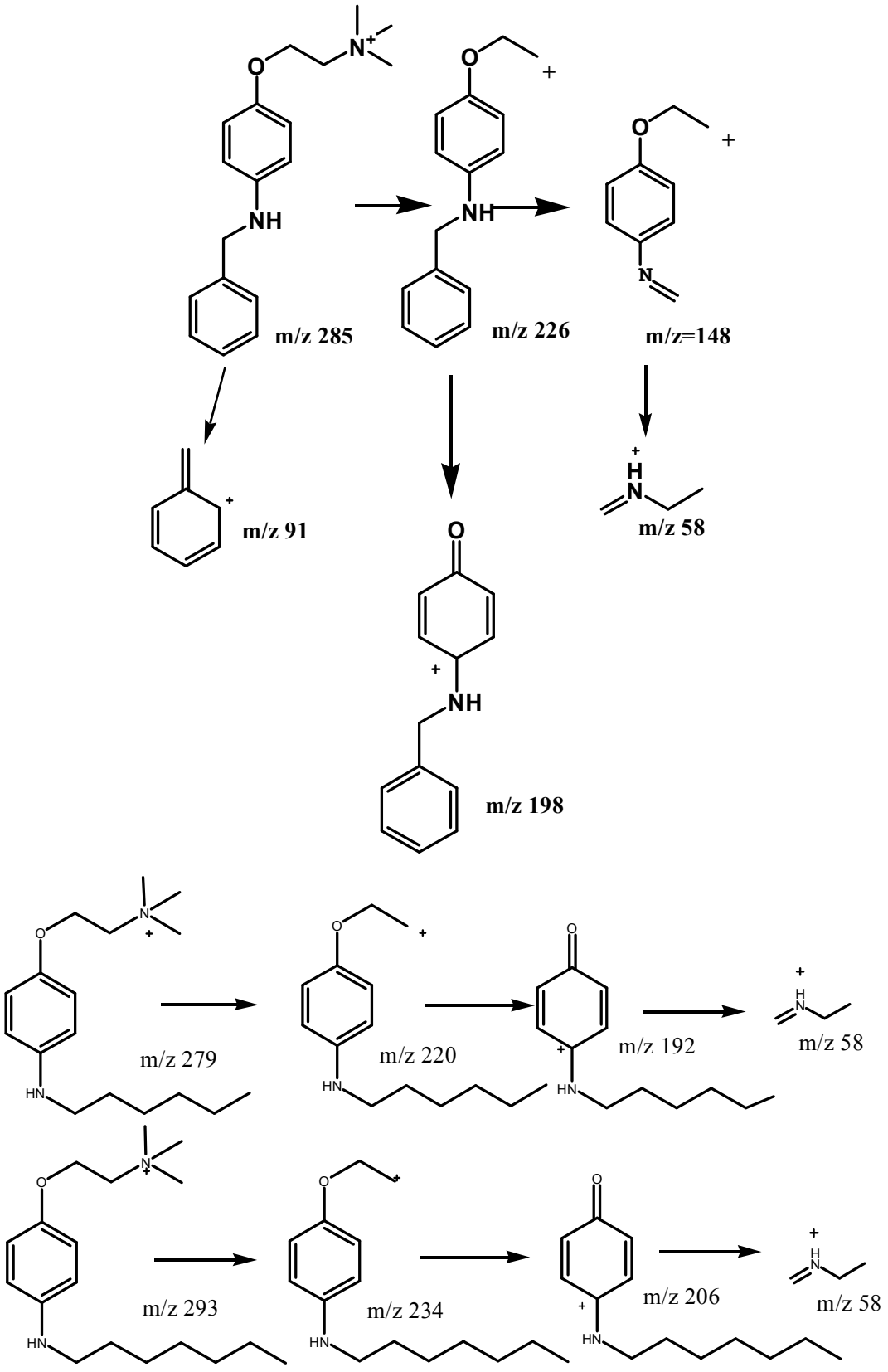
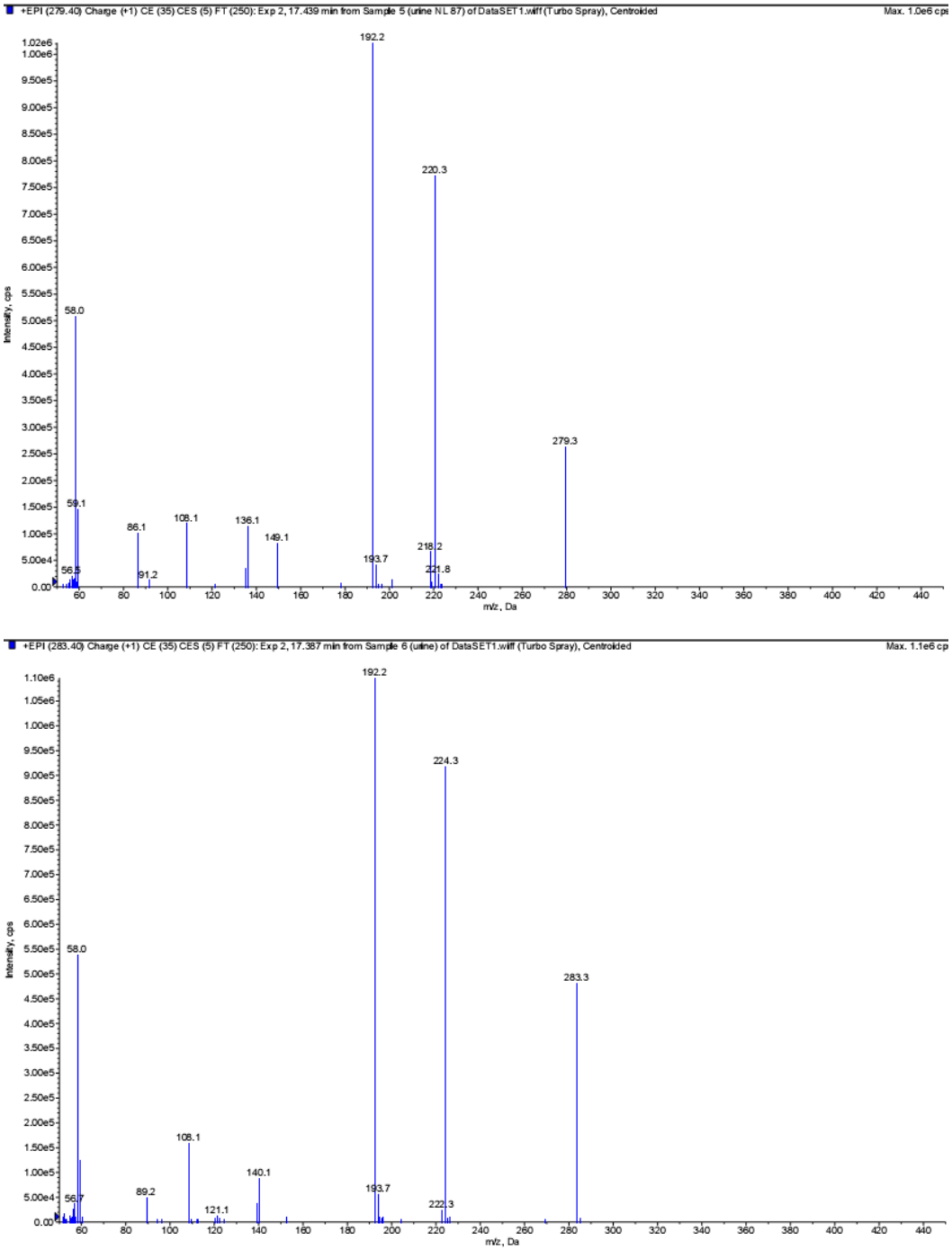
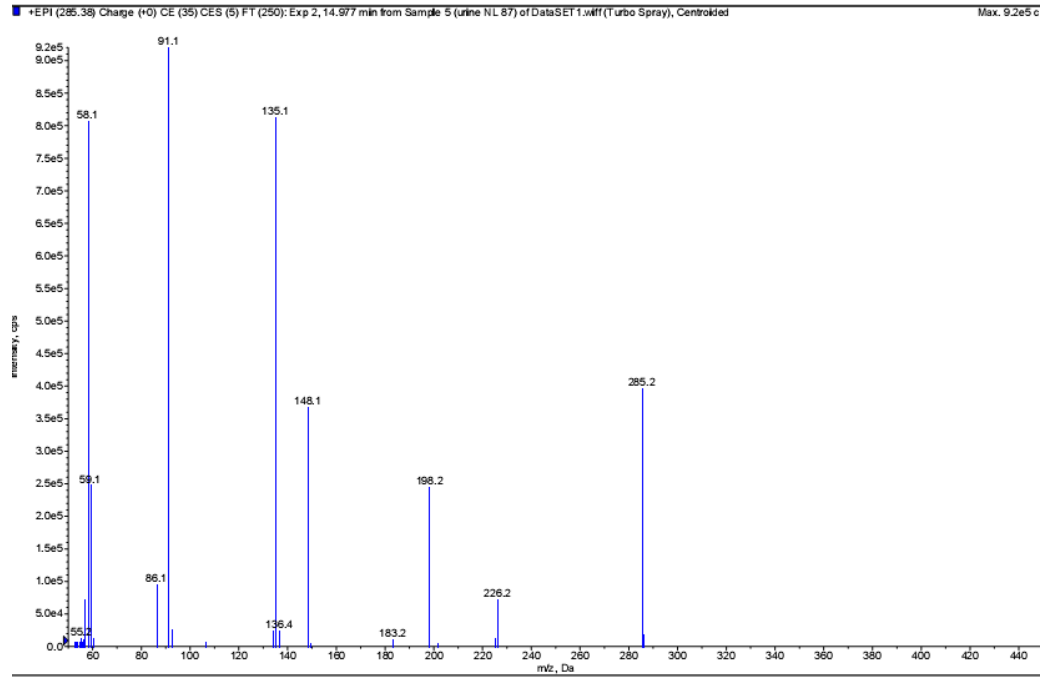


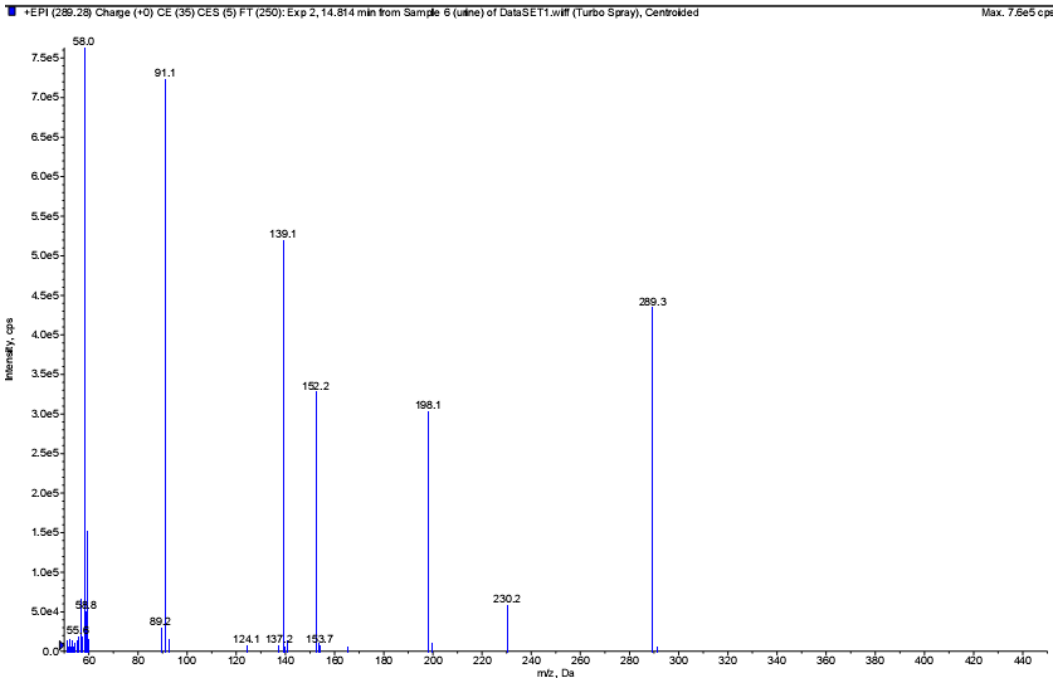
Figure S3. The product ion mass spectra of 4-APC and 4-APC-d_4 labelled compounds in human urine using QTRAP mass spectrometer.

Compound 1, *m/z* 279/283, t=17.46 min

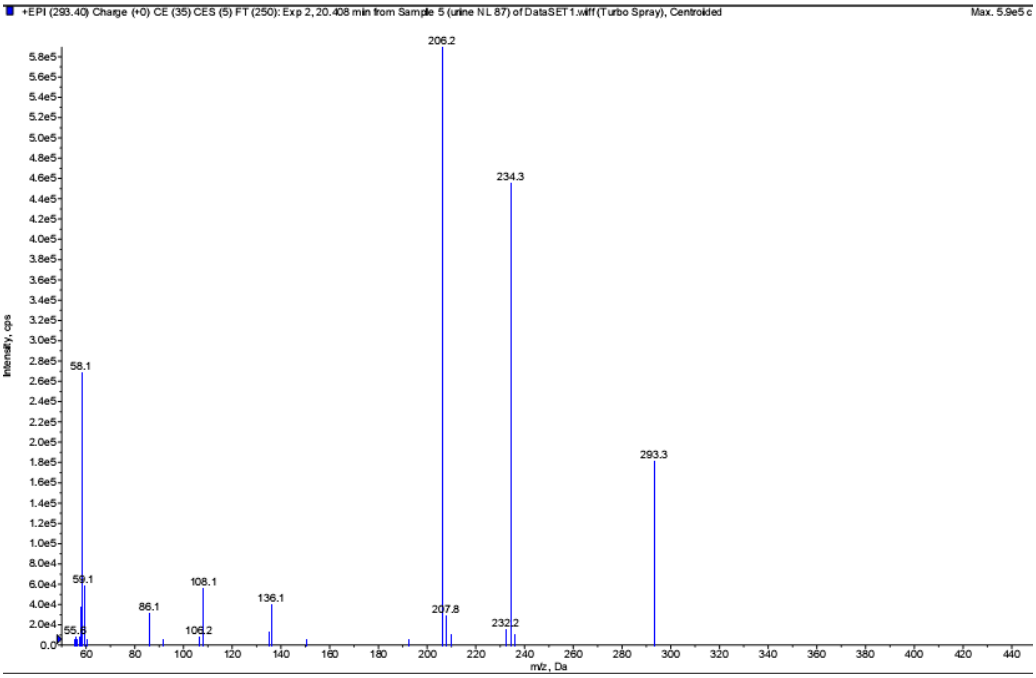


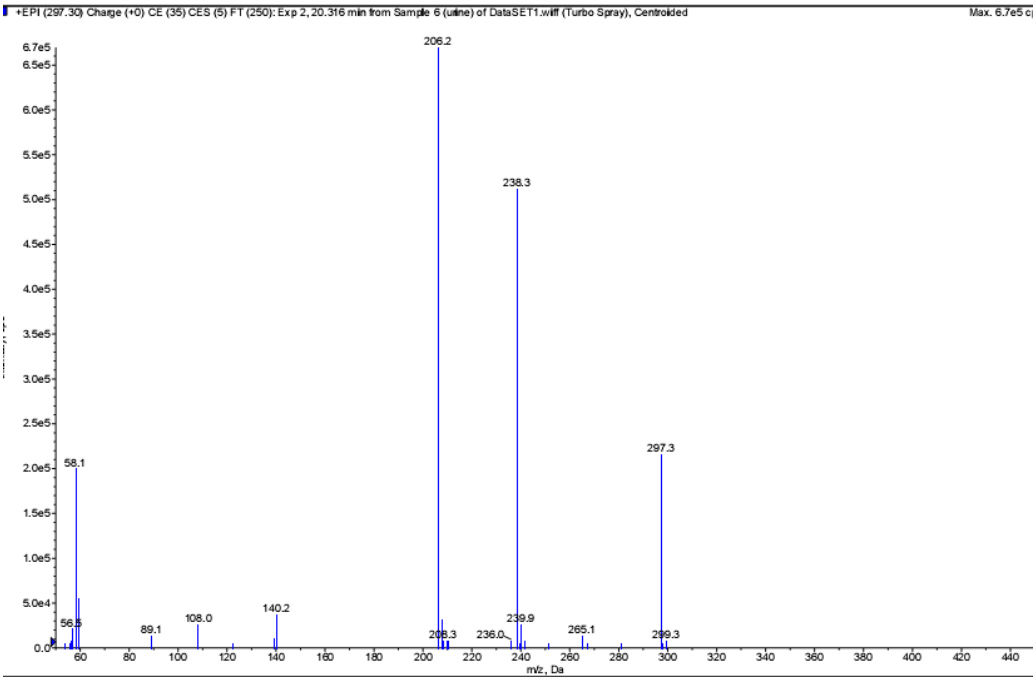
Compound 2, *m/z* 285/289, t=14.96 min



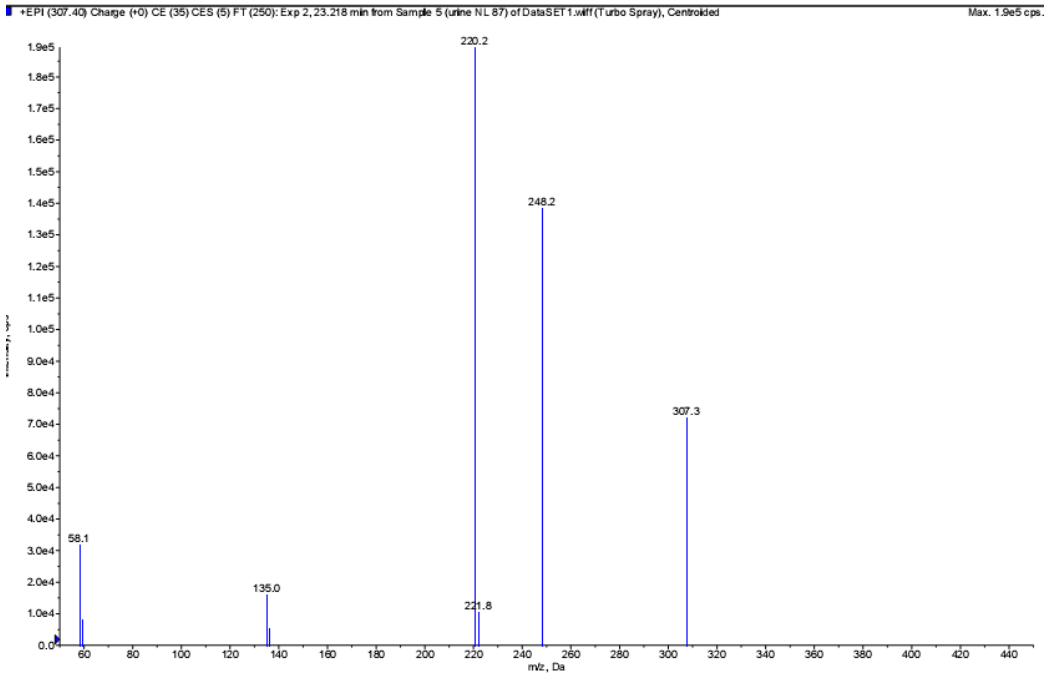


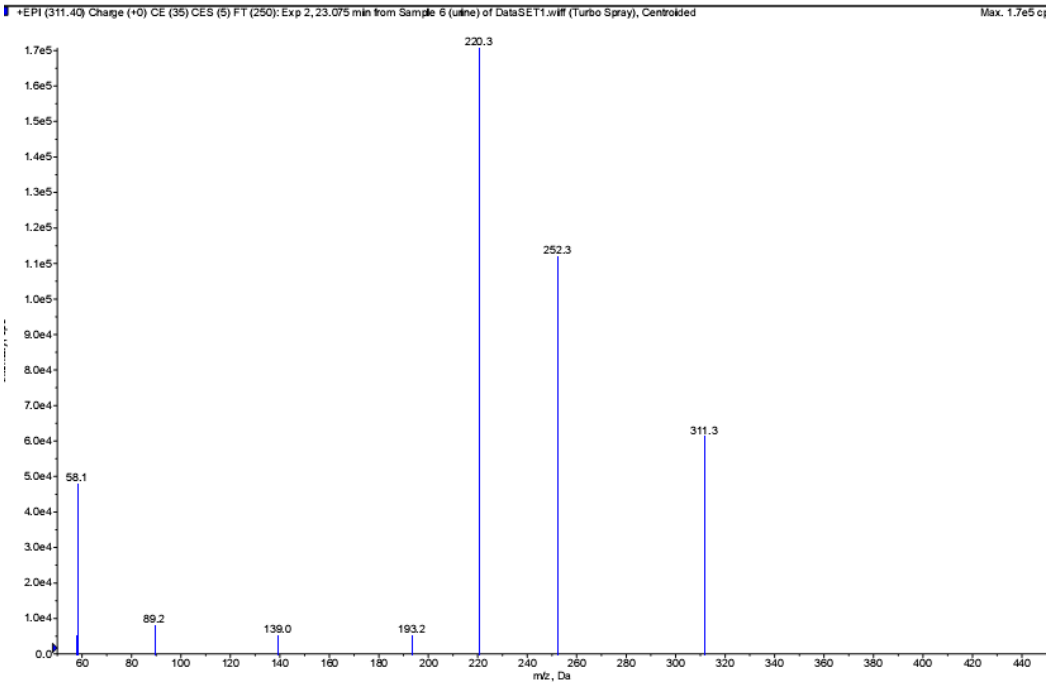
Compound 3, *m/z* 293/297, t=20.38 min



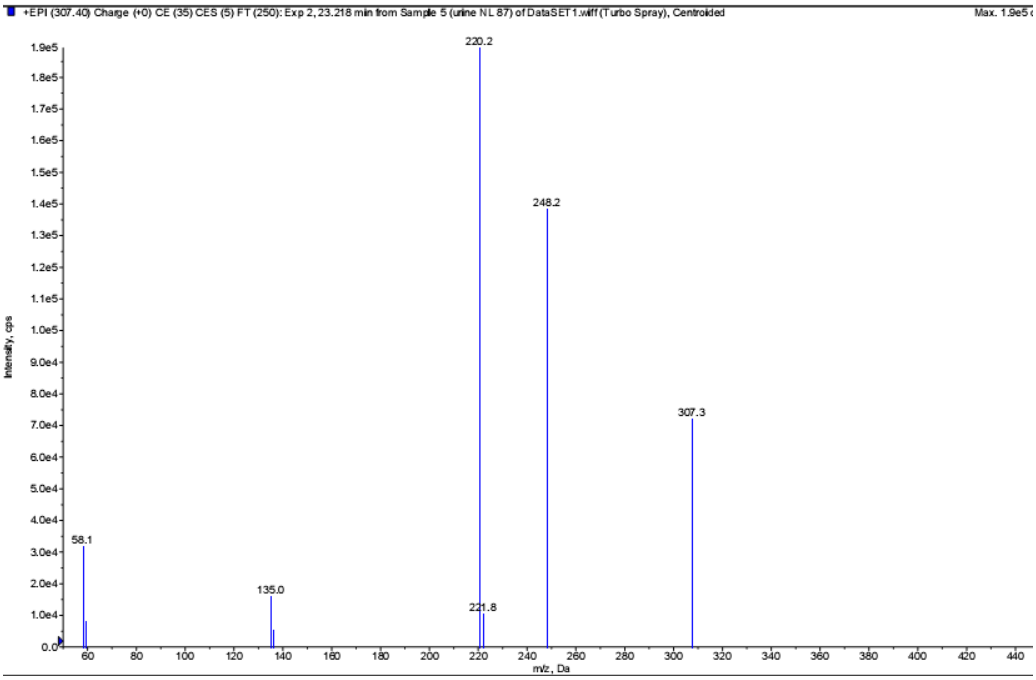


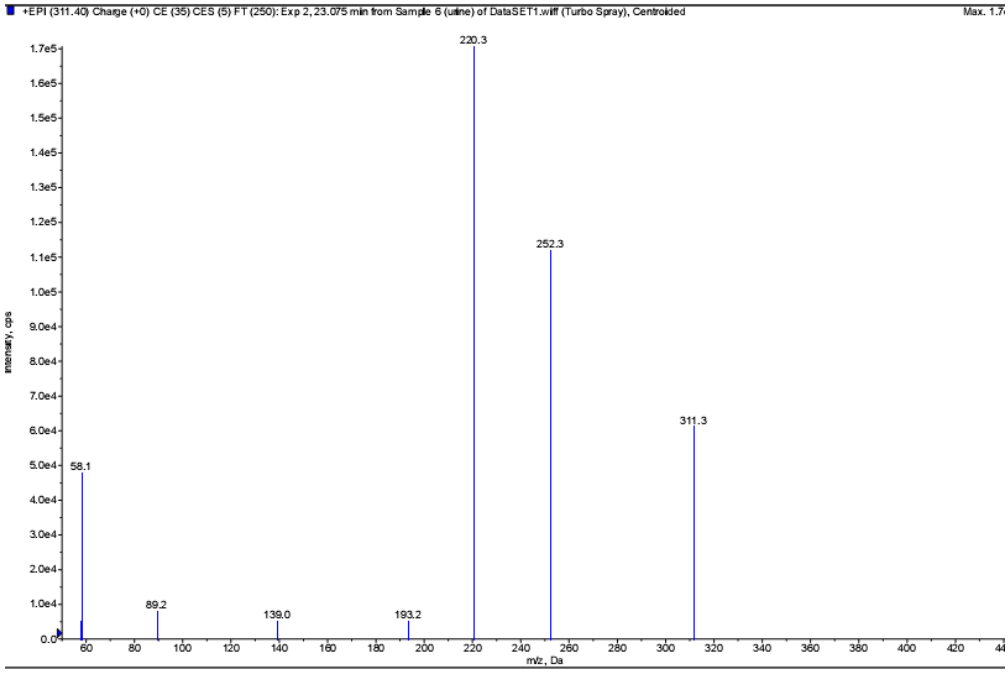
Compound 4, *m/z* 307/311, t=23.19 min



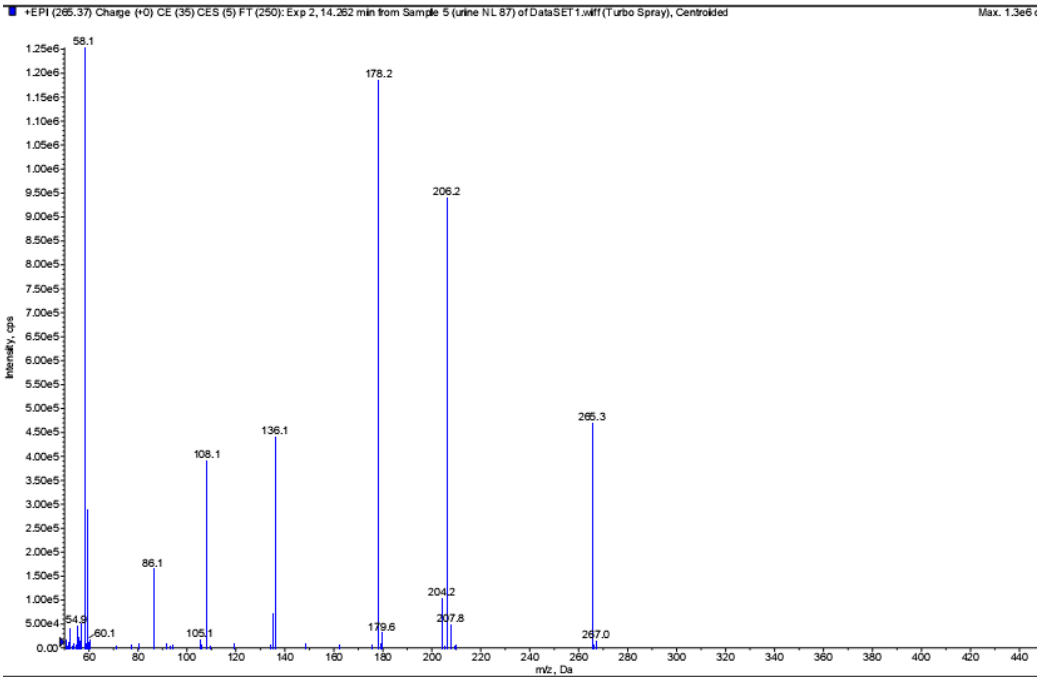


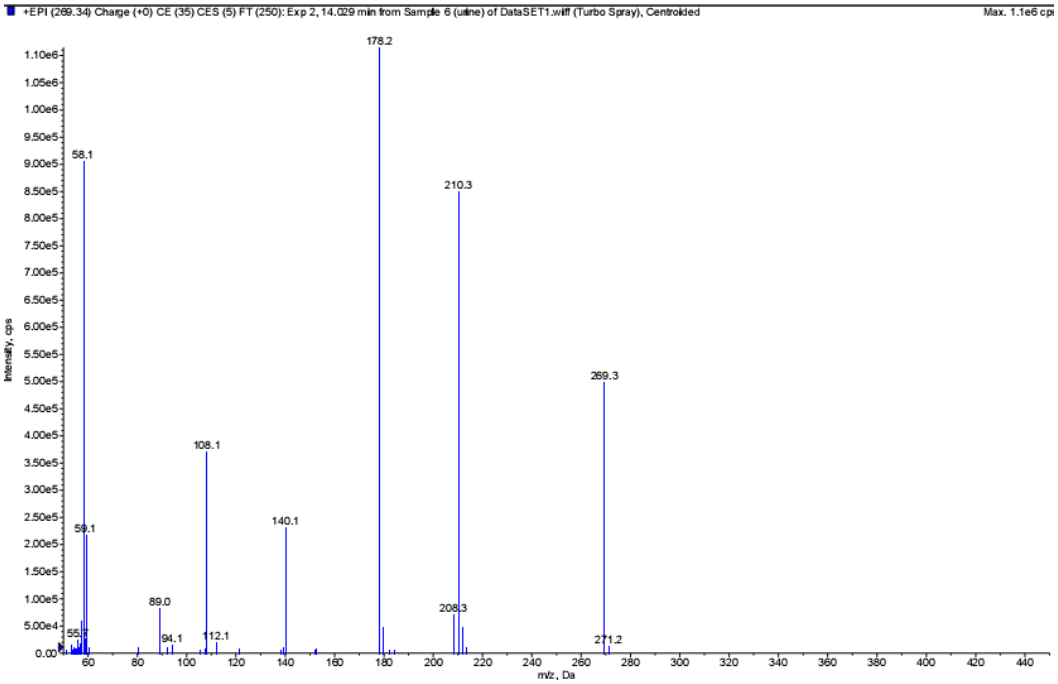
Compound 5, *m/z* 321/325, t=25.67 min



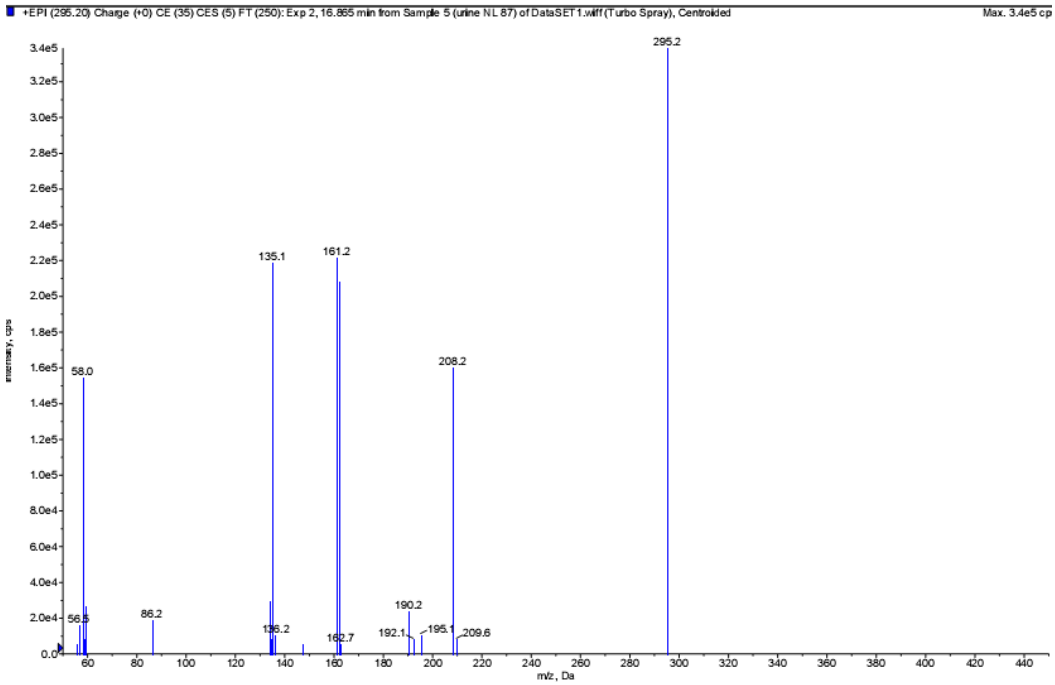


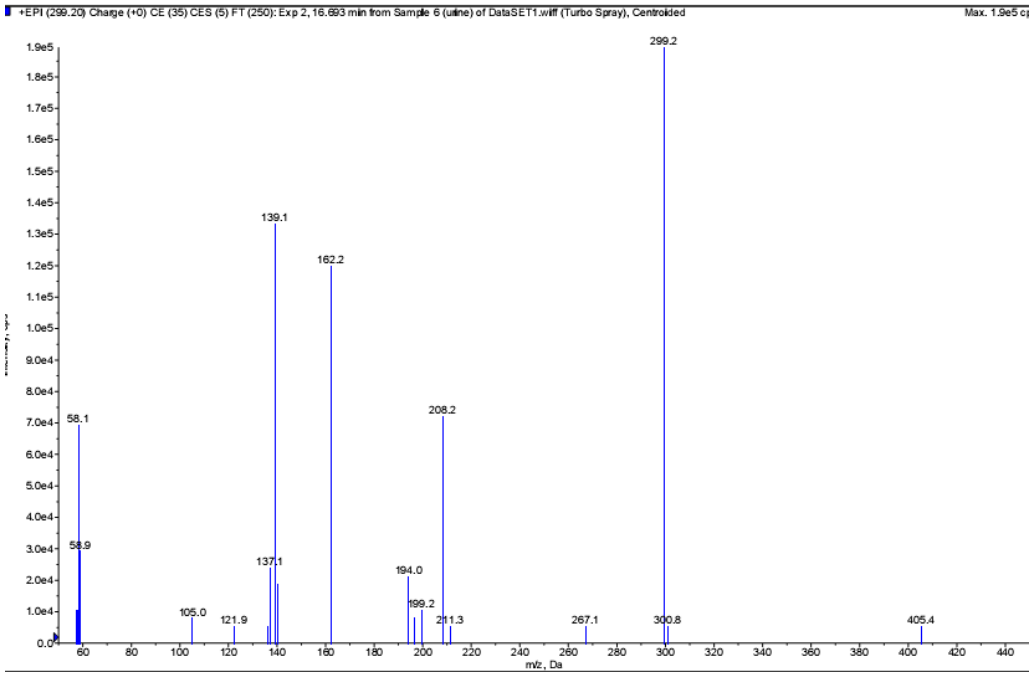
Compound 6, *m/z* 265/269, t=14.19 min



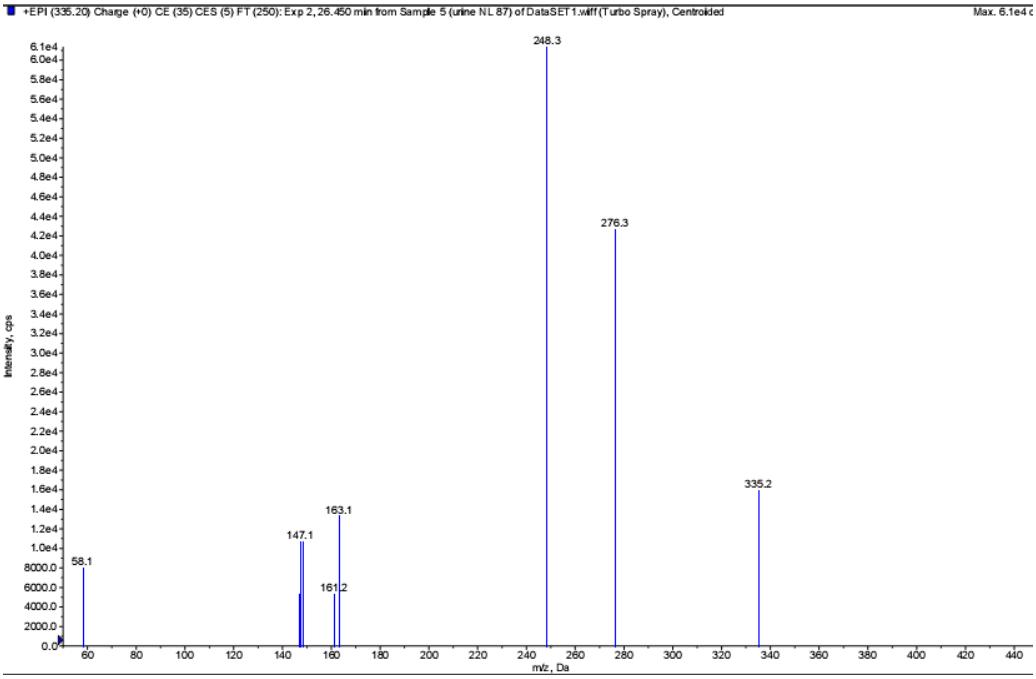


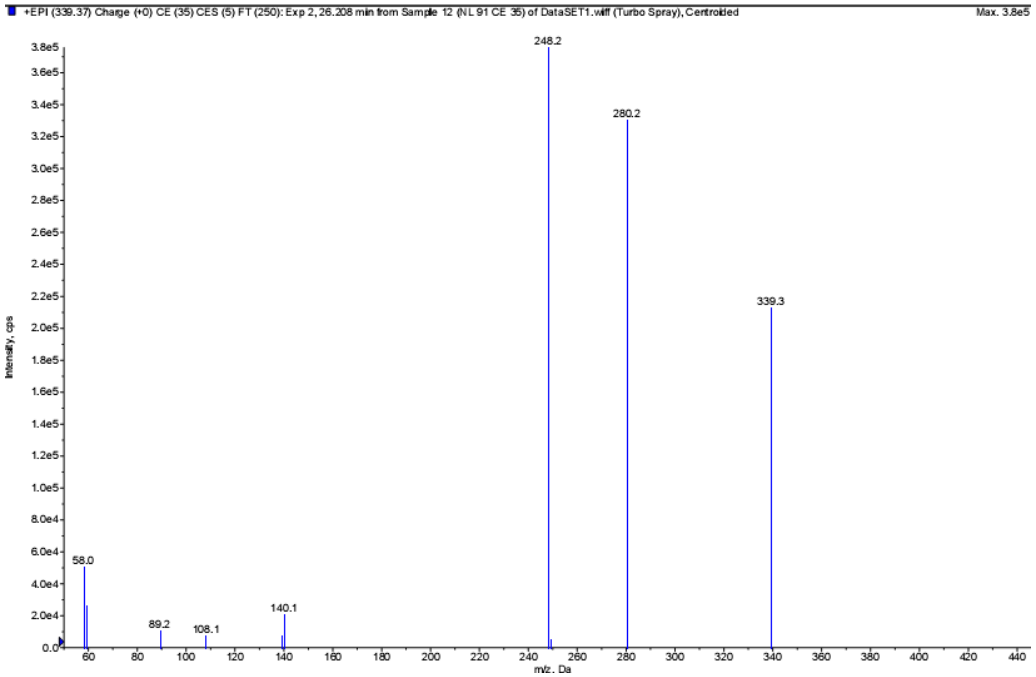
Compound 9, *m/z* 295/299, t=16.80 min



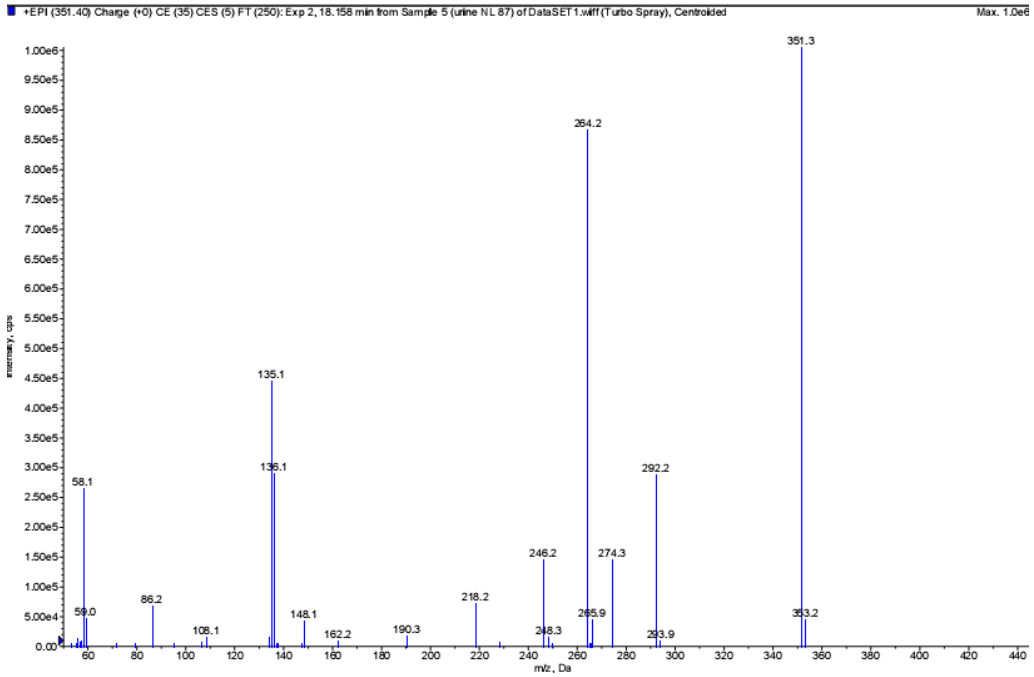


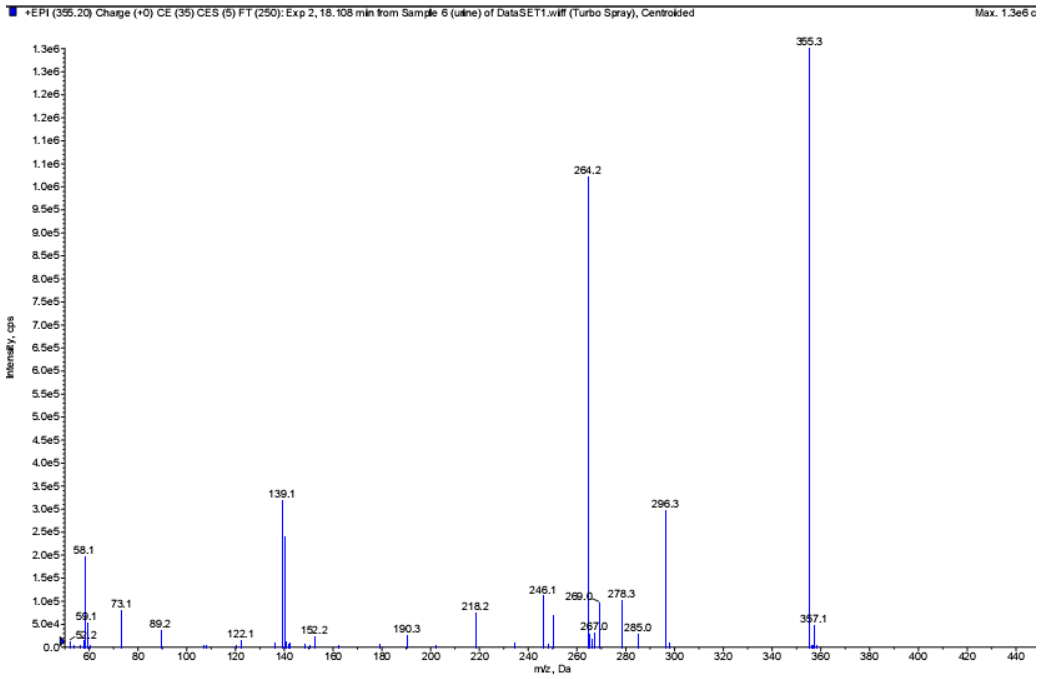
Compound 10, *m/z* 335/339, t=26.63 min



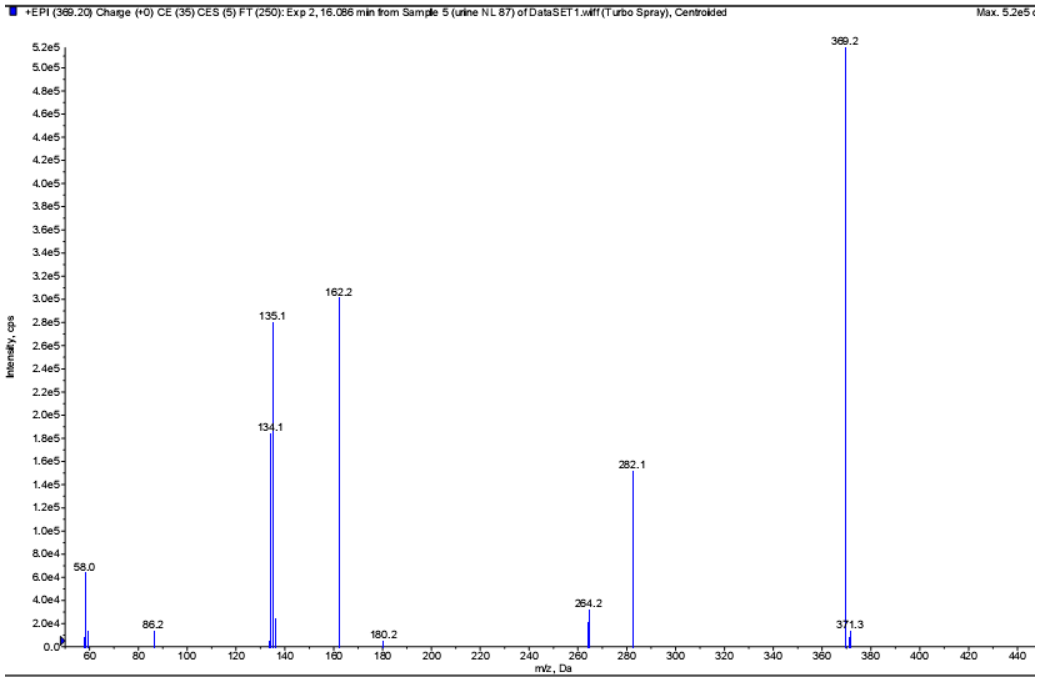


Compound 11, m/z 351/355, t=18.16 min





Compound 12, m/z 369/373, t=16.05 min



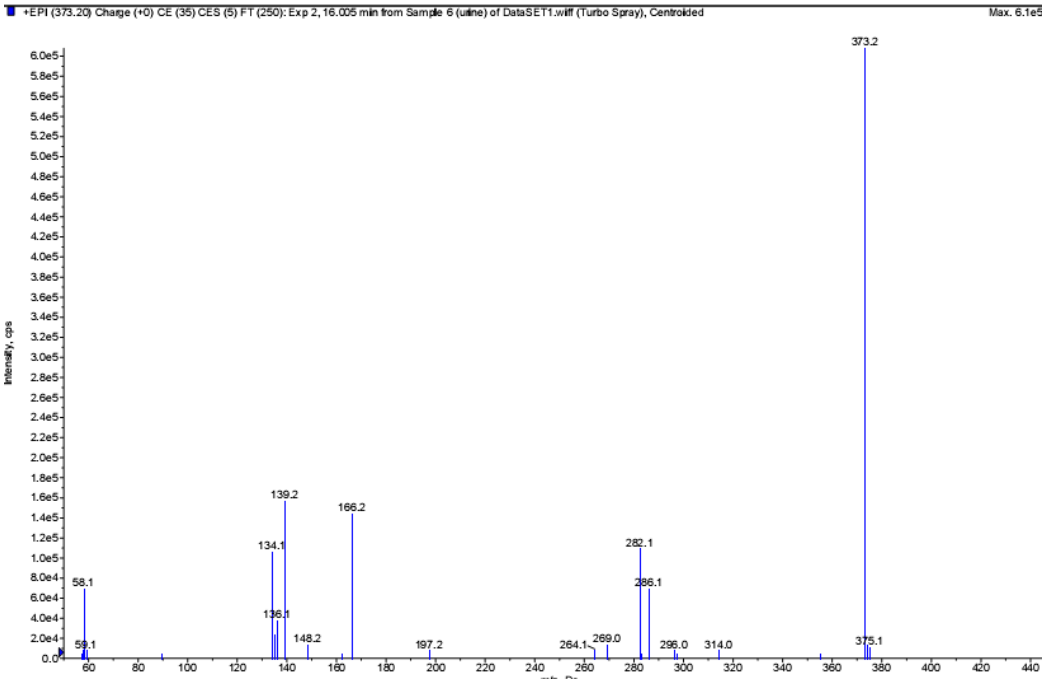


Figure S4. The flow chart illustrating the structure and individual steps of the approach.

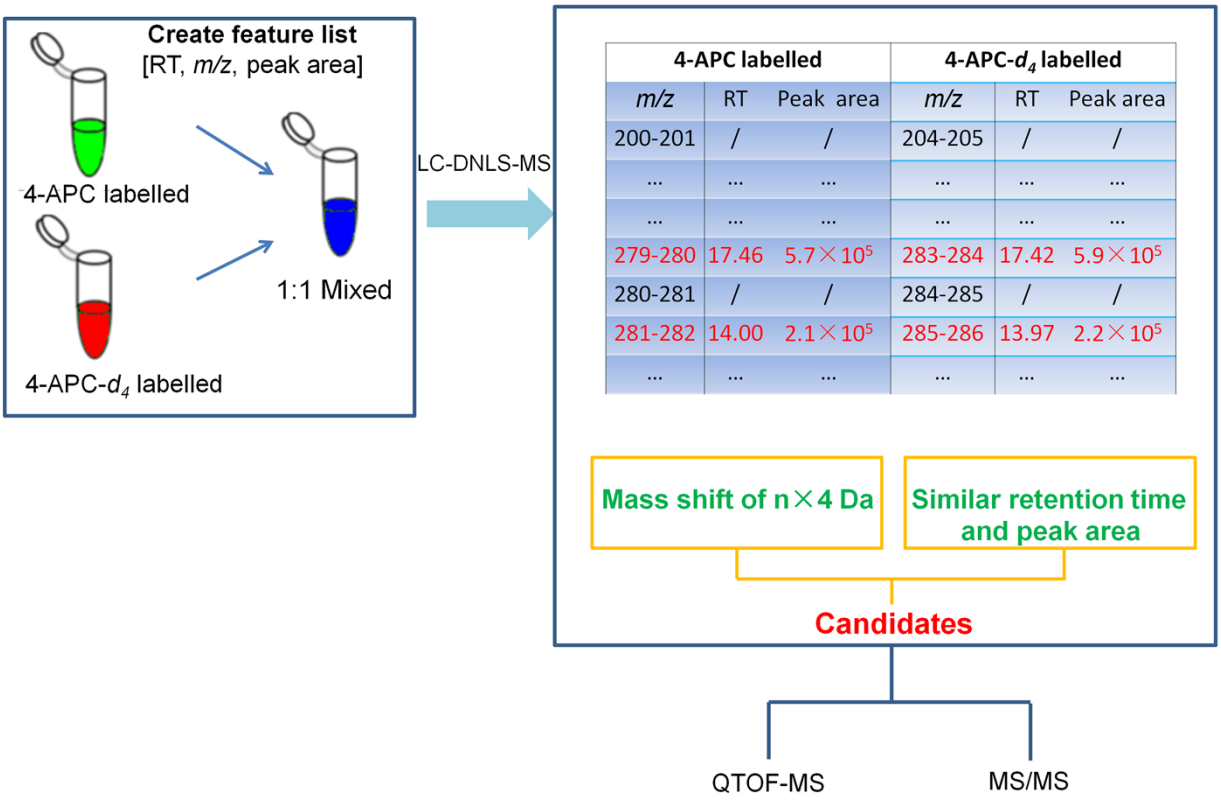
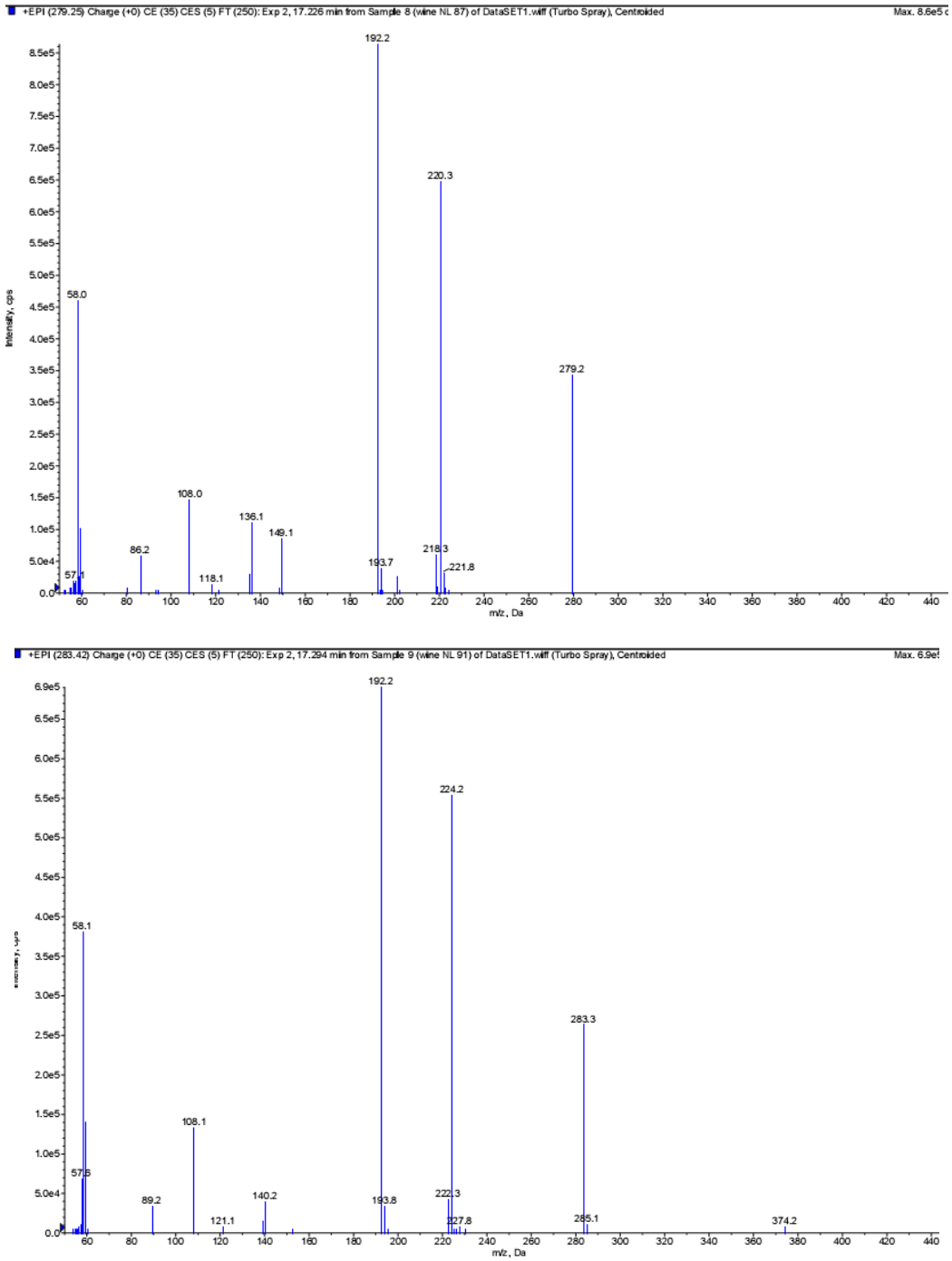
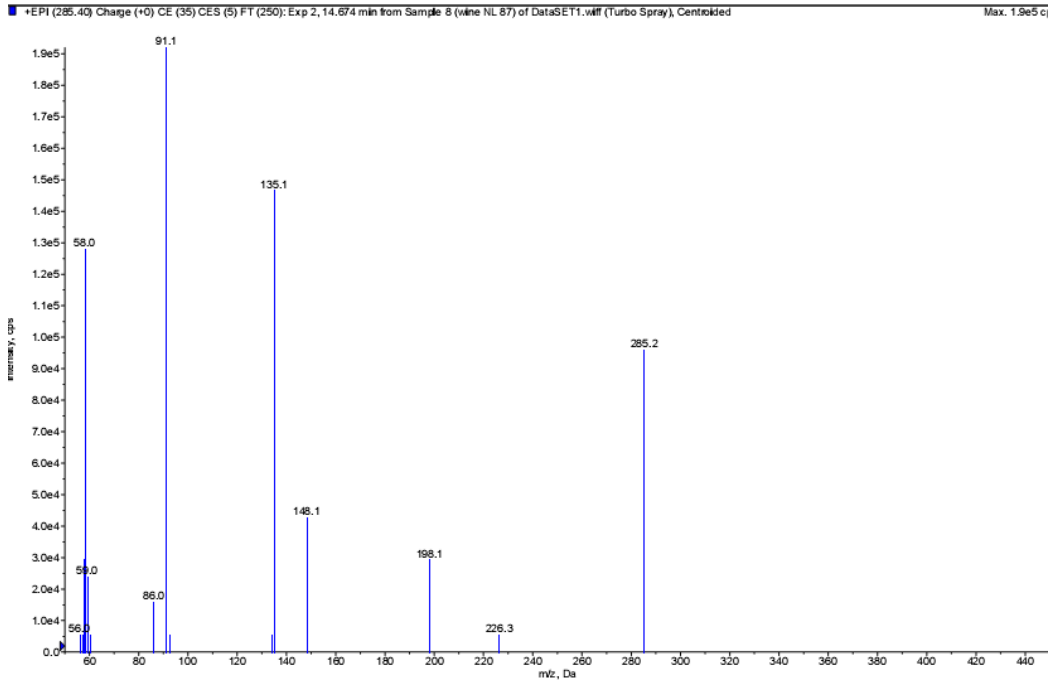


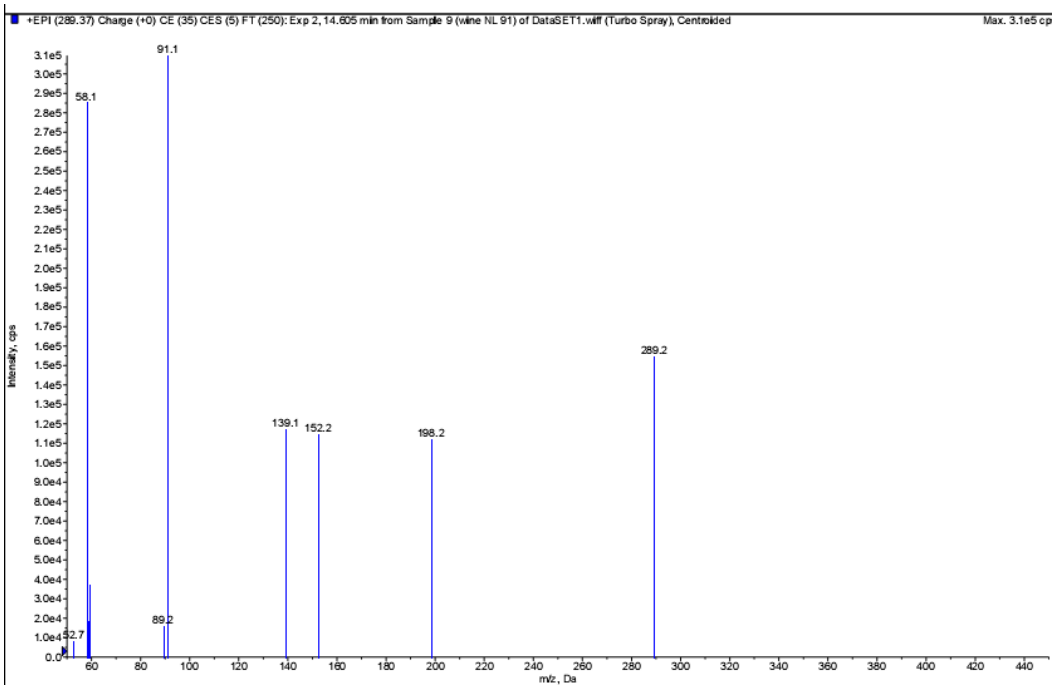
Figure S5. The product ion mass spectra of 4-APC and 4-APC-d_4 labelled compounds in white wine using QTRAP mass spectrometer.

Compound 1, *m/z* 279/283, t=17.35 min

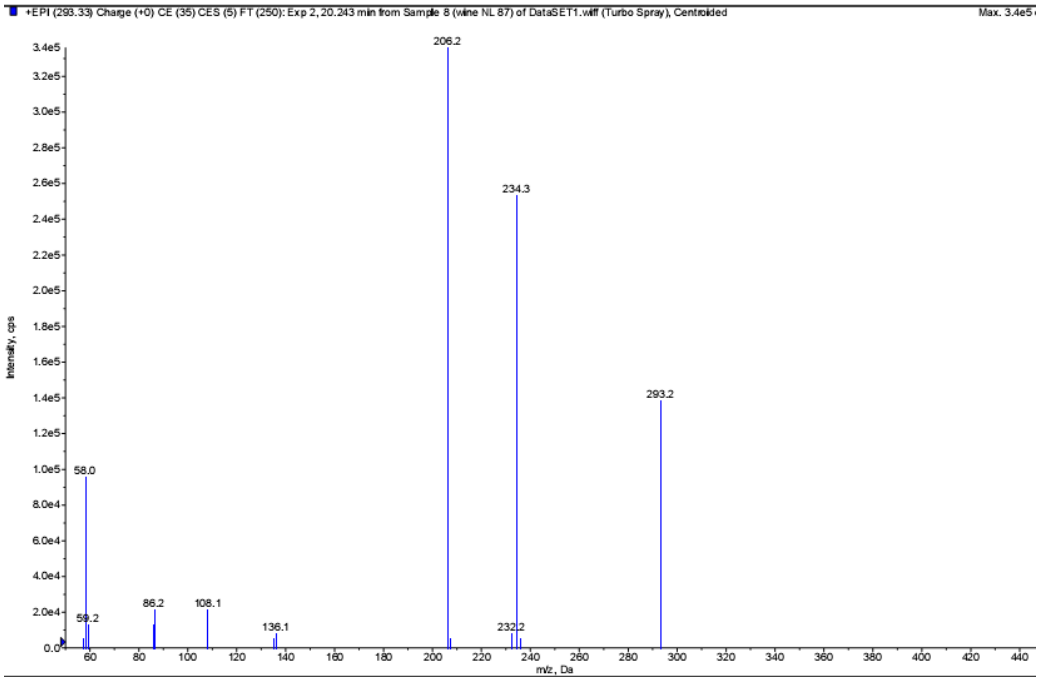


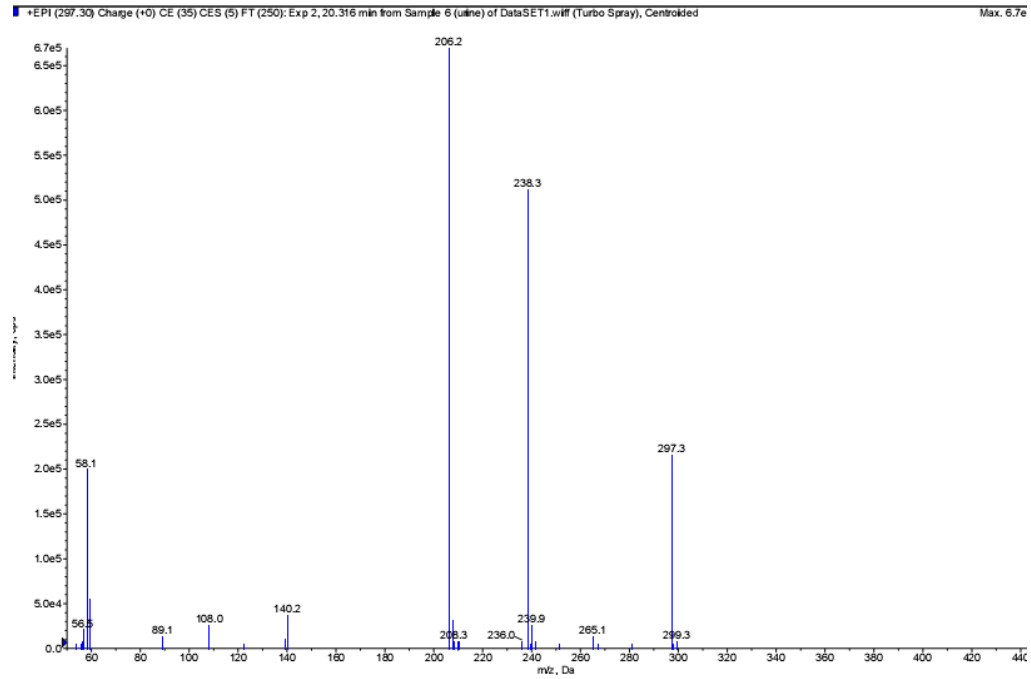
Compound 2, *m/z* 285/289, t=14.77 min



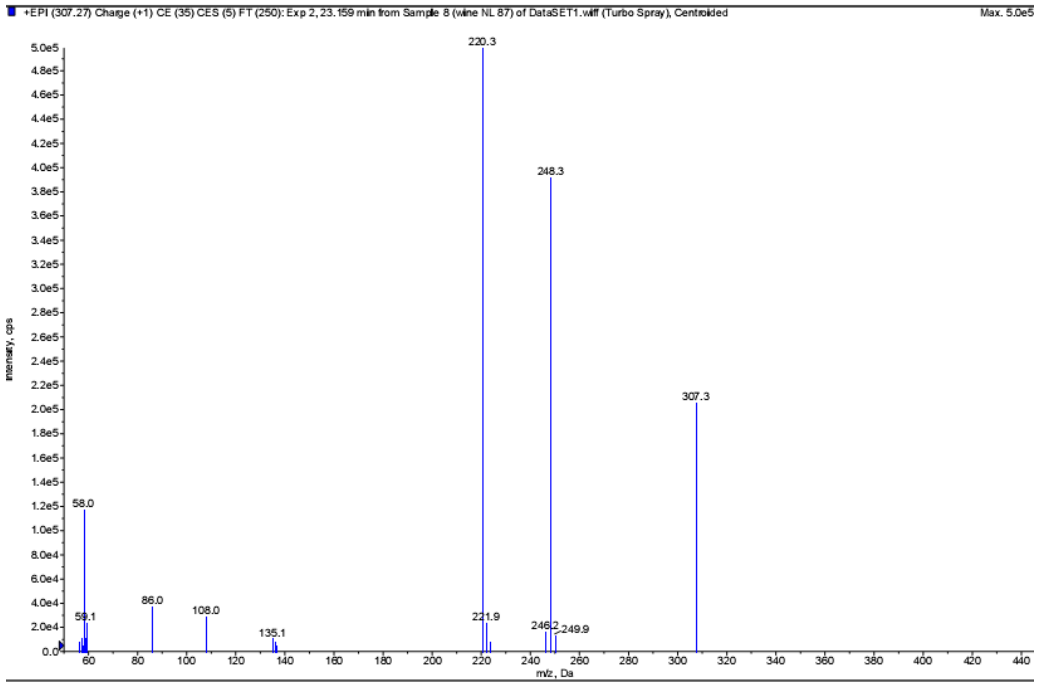


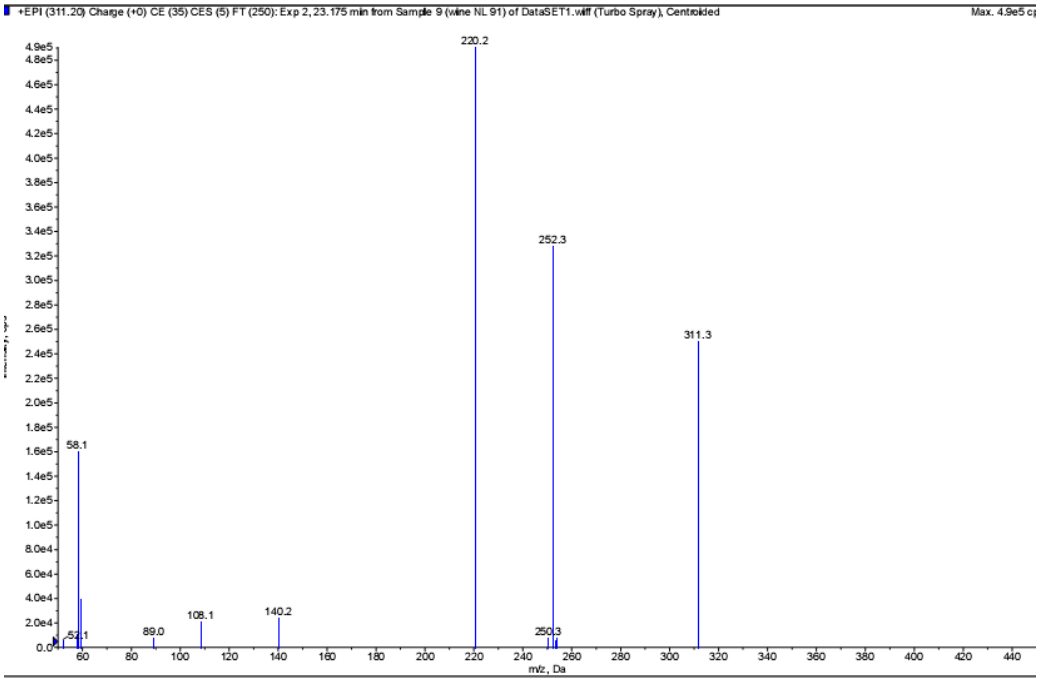
Compound 3, *m/z* 293/297, t=20.33 min



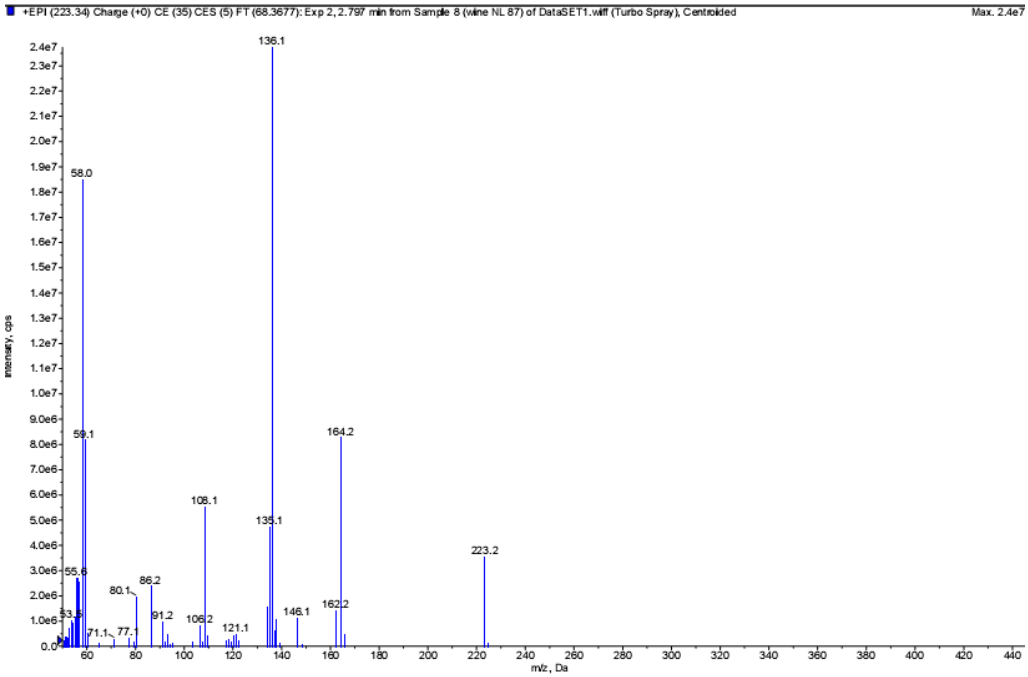


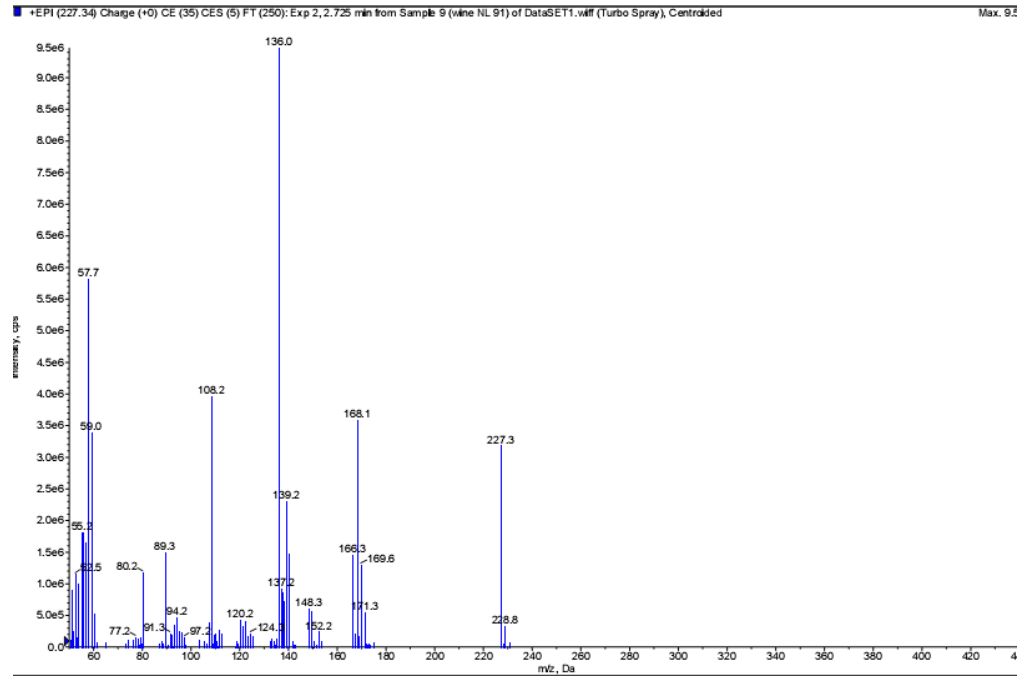
Compound 4, *m/z* 307/311, t=23.22 min



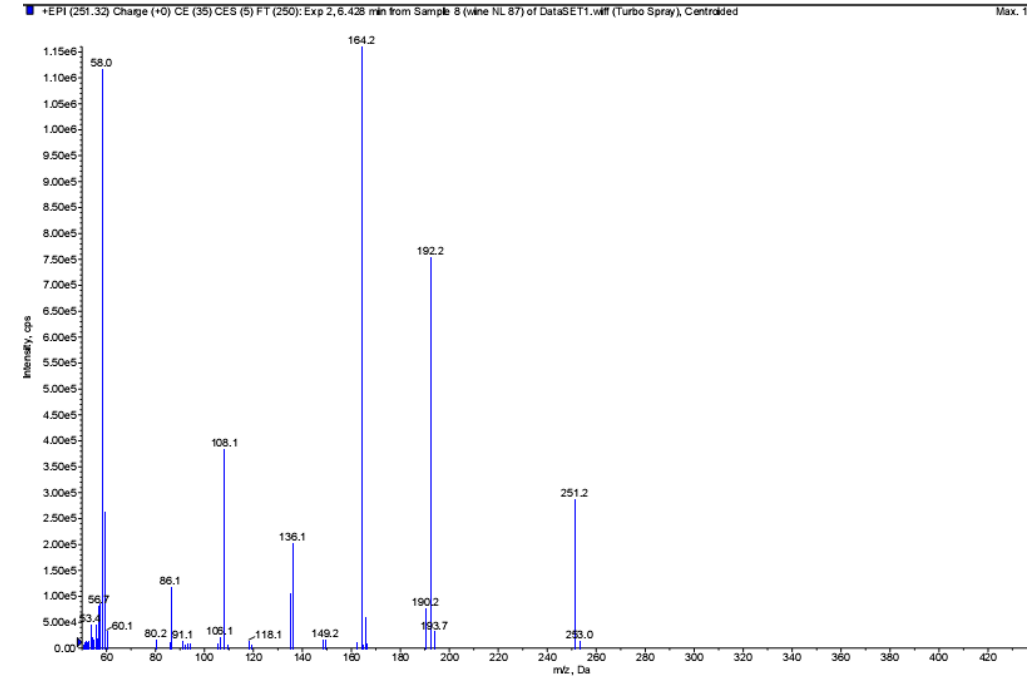


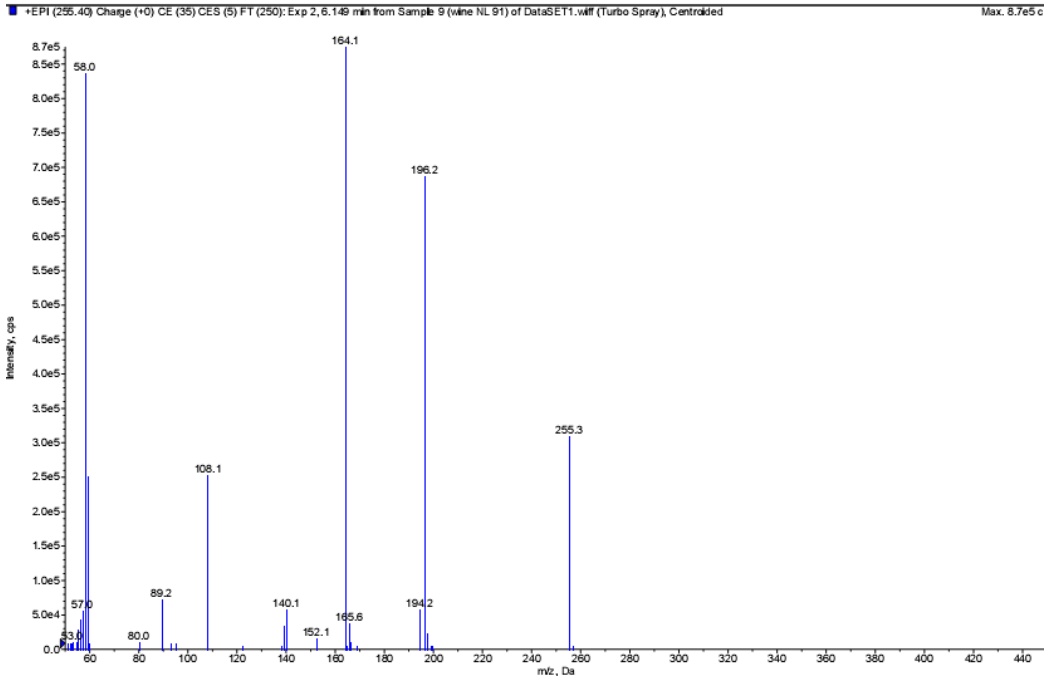
Compound 5, m/z 223/227, t=2.76 min



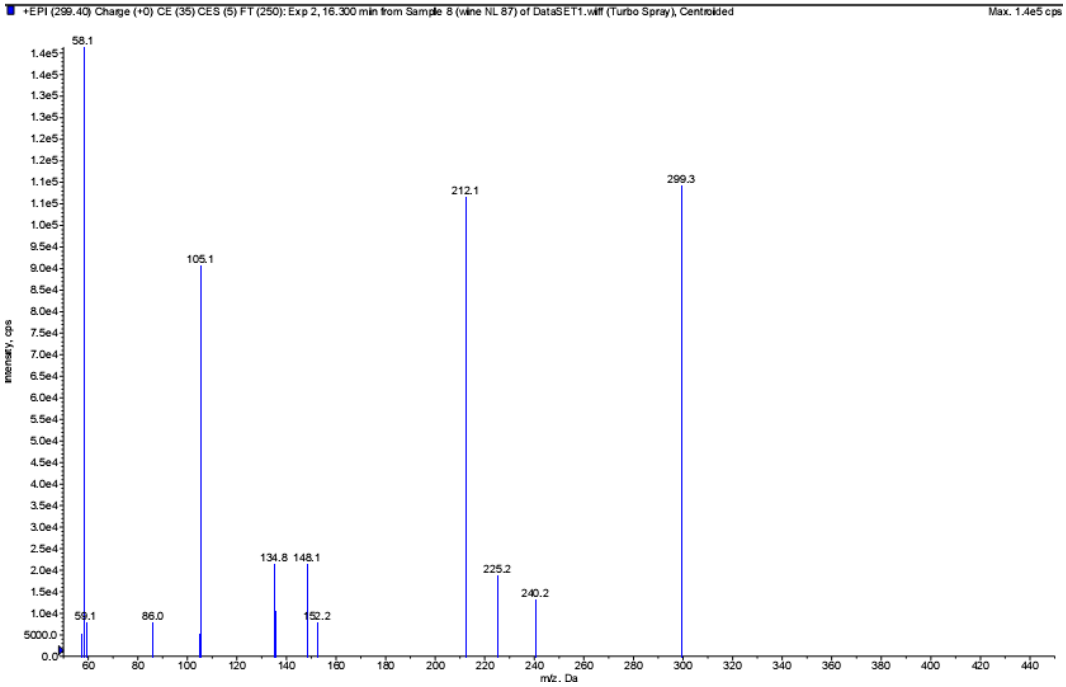


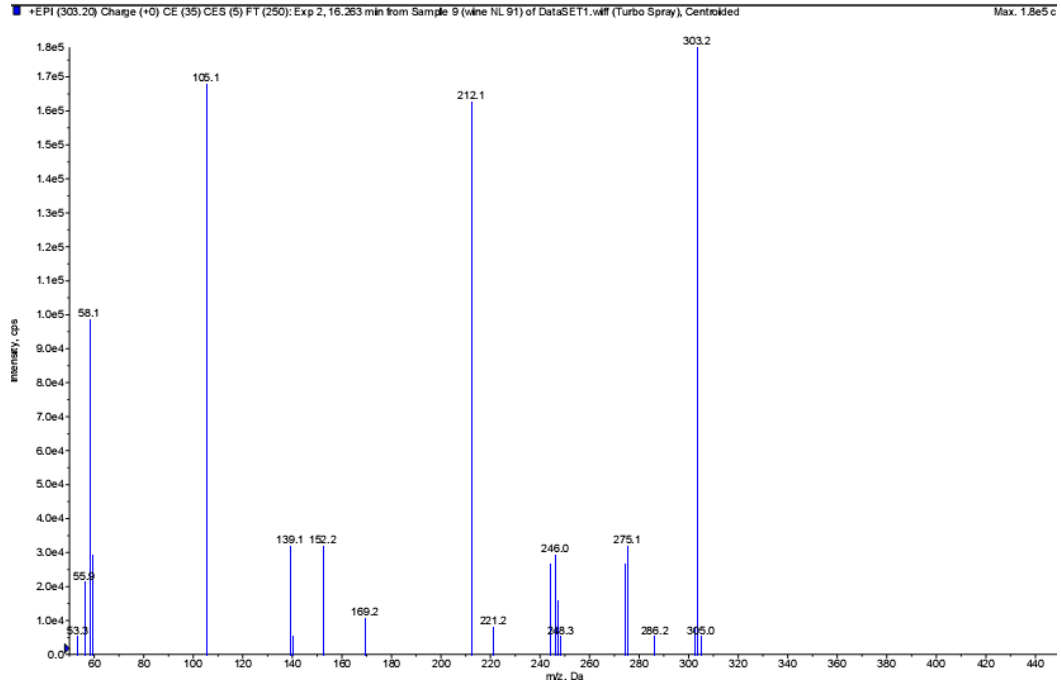
Compound 6, *m/z* 251/255, t=6.42 min



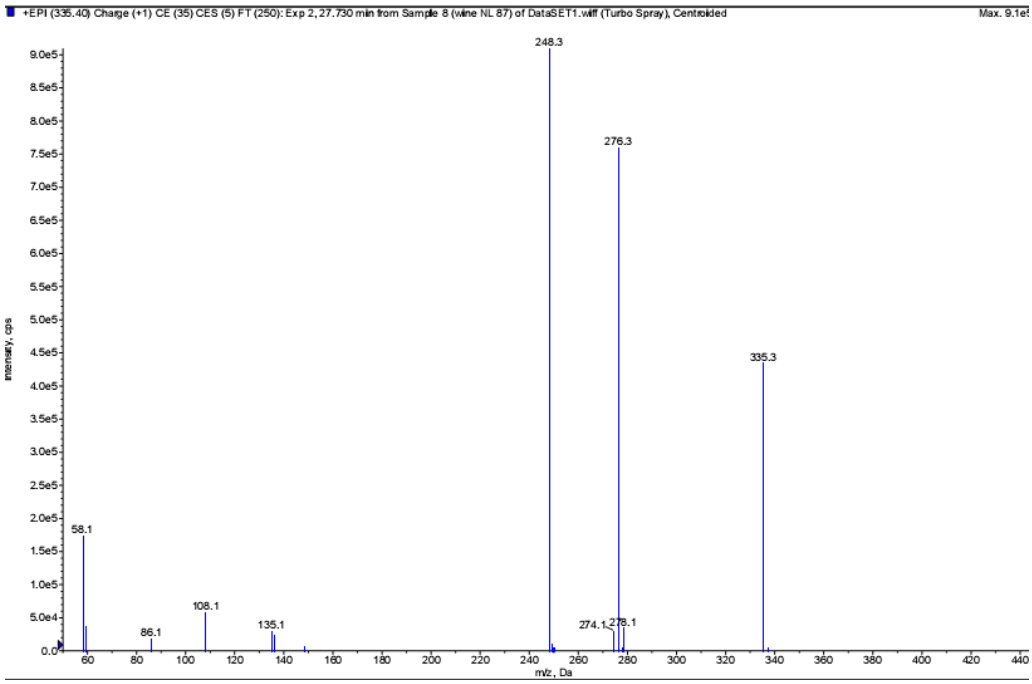


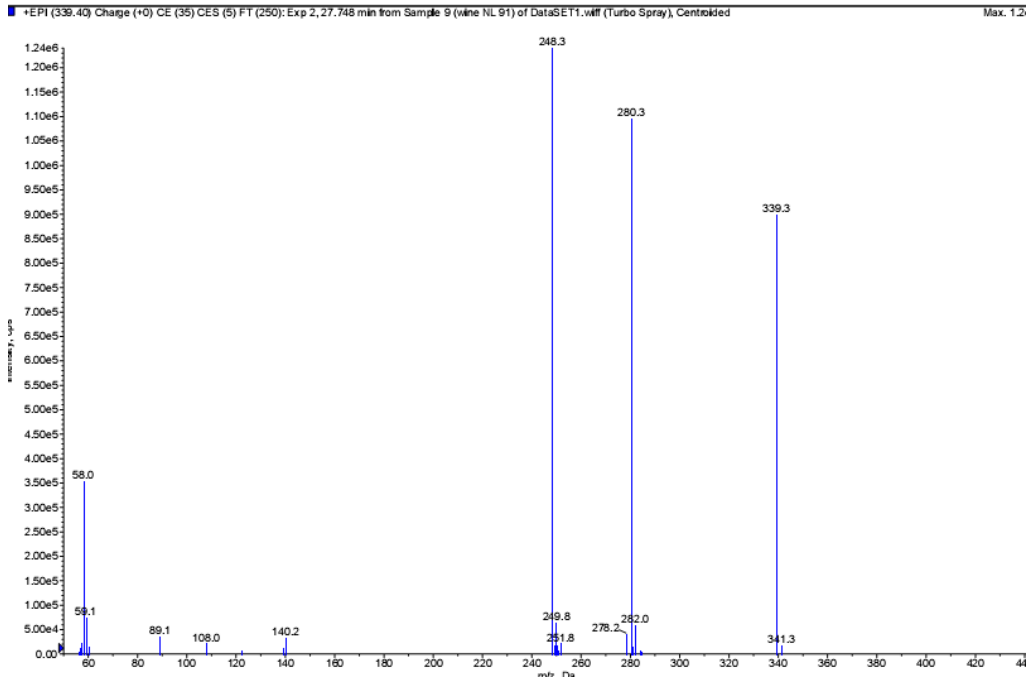
Compound 10, *m/z* 299/303, t=16.37 min



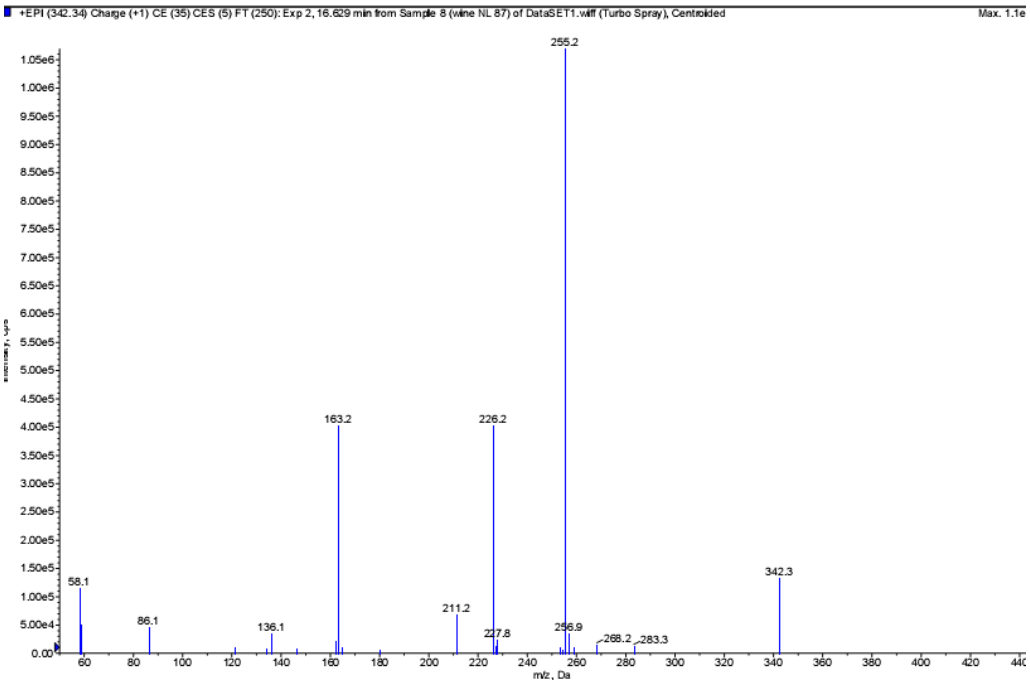


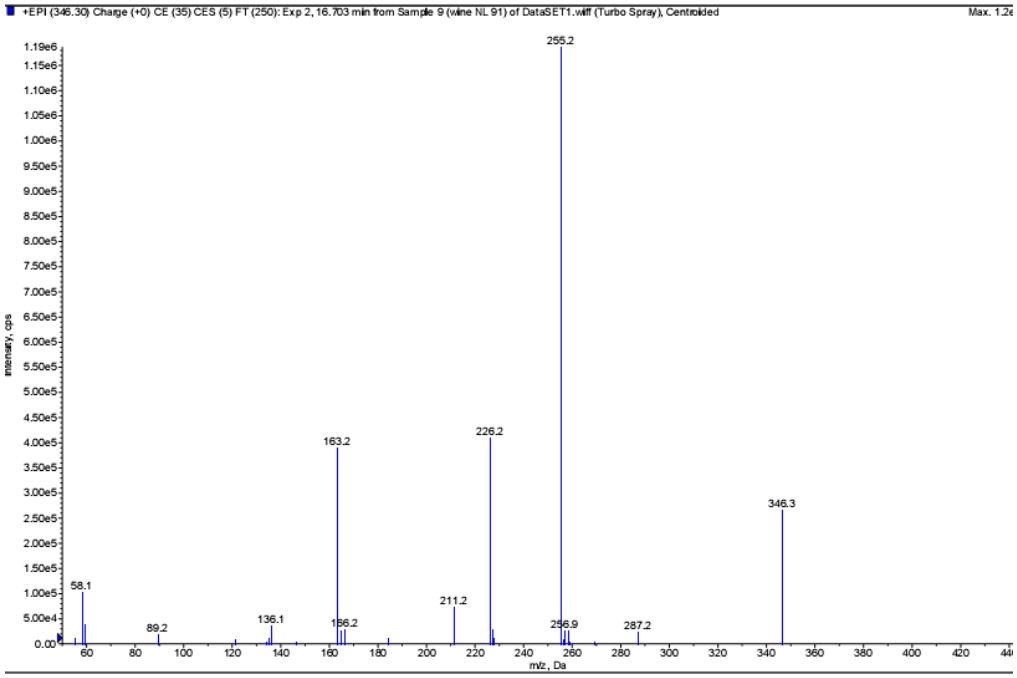
Compound 11, *m/z* 335/339, t=27.80 min



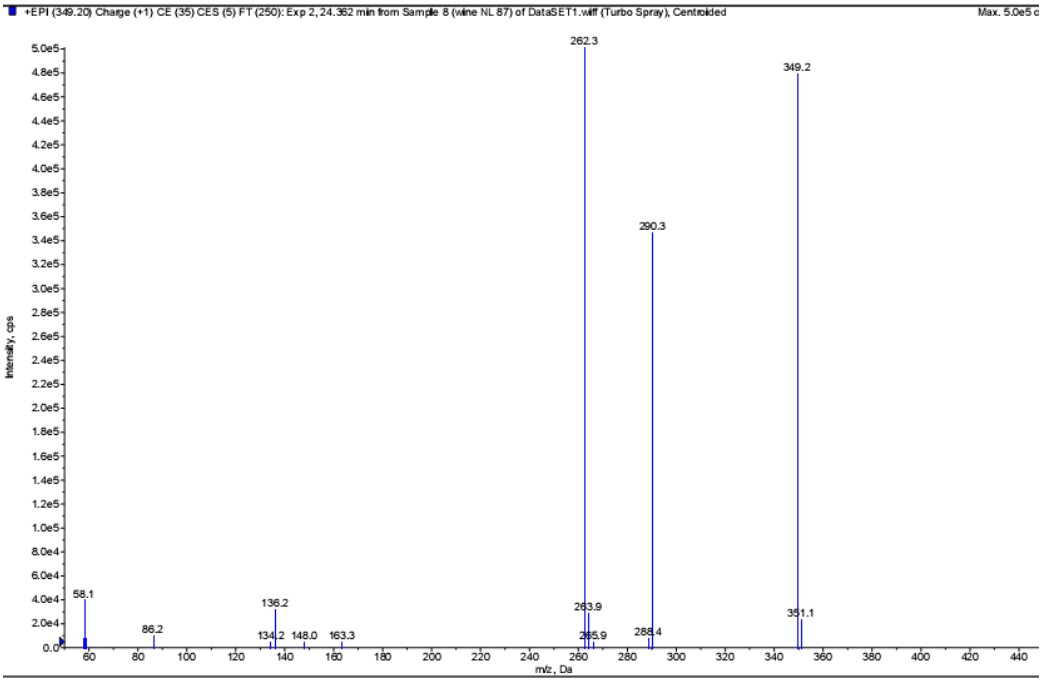


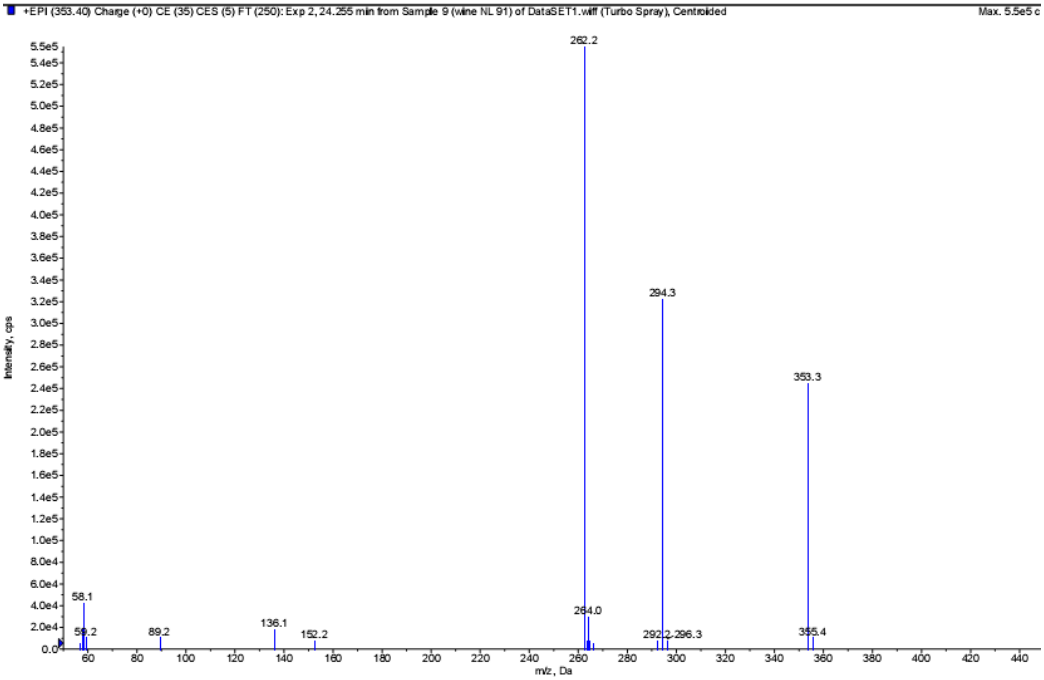
Compound 12, *m/z* 342/346, t=16.76 min



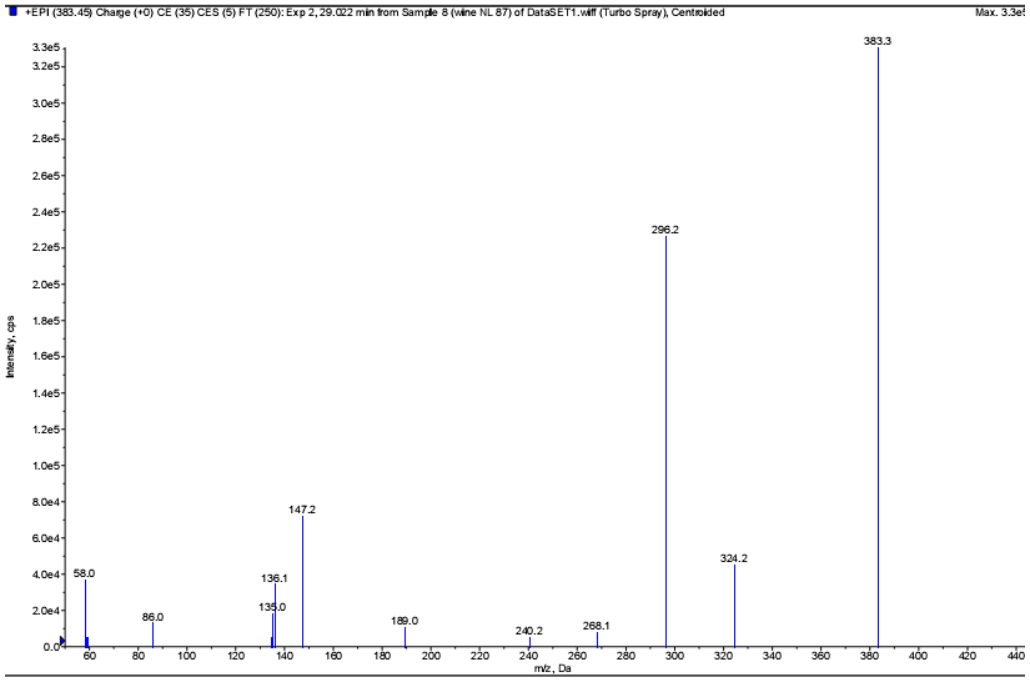


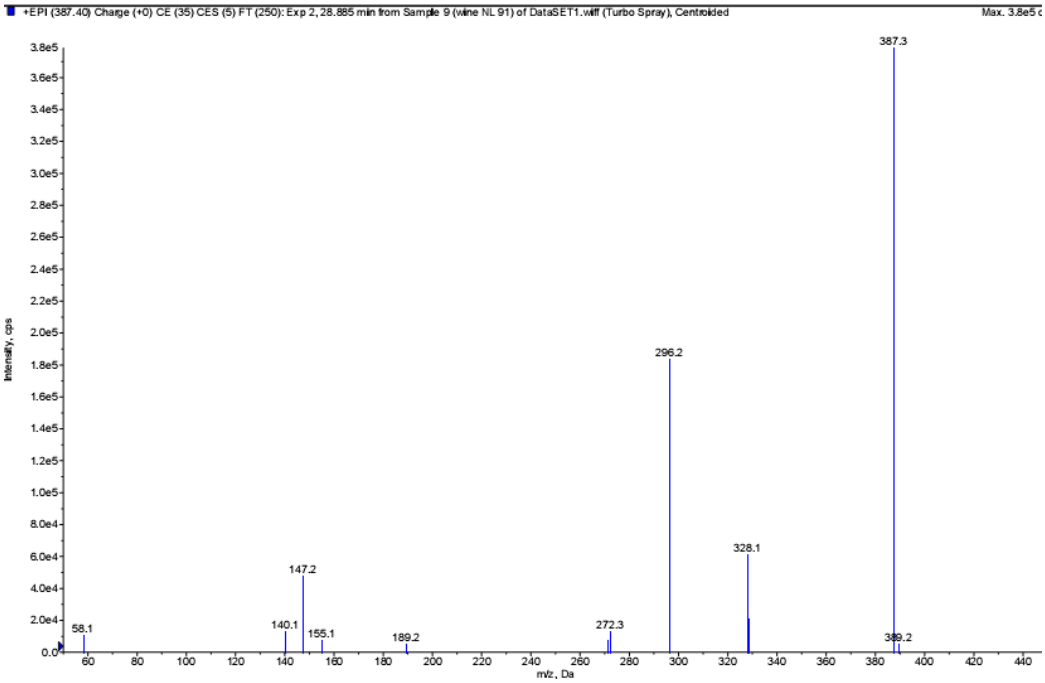
Compound 14, *m/z* 349/353, t=24.32 min



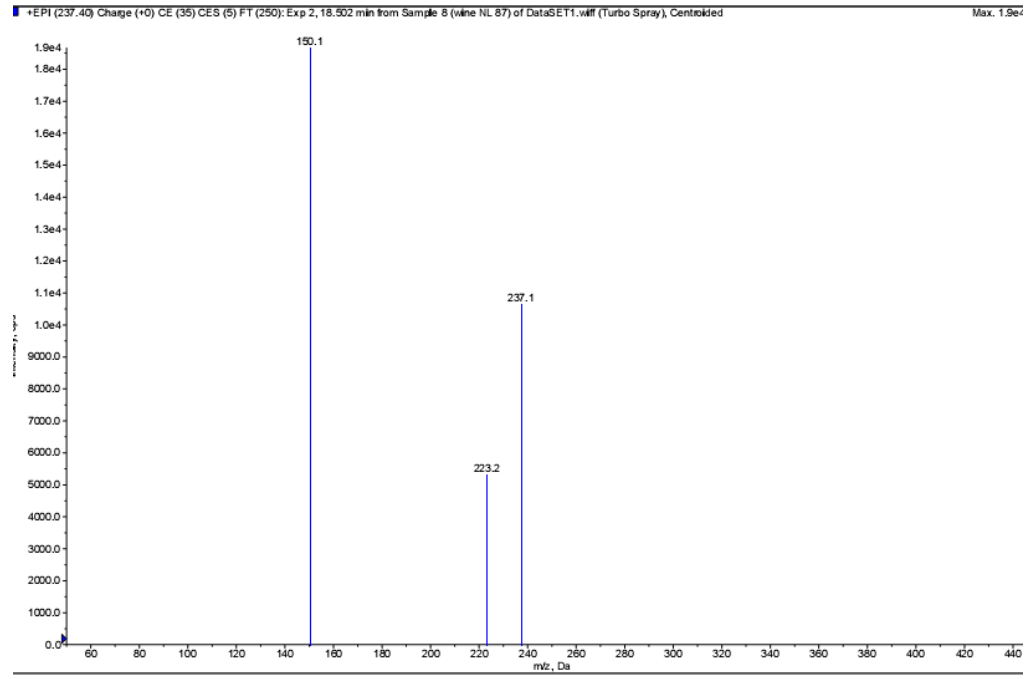


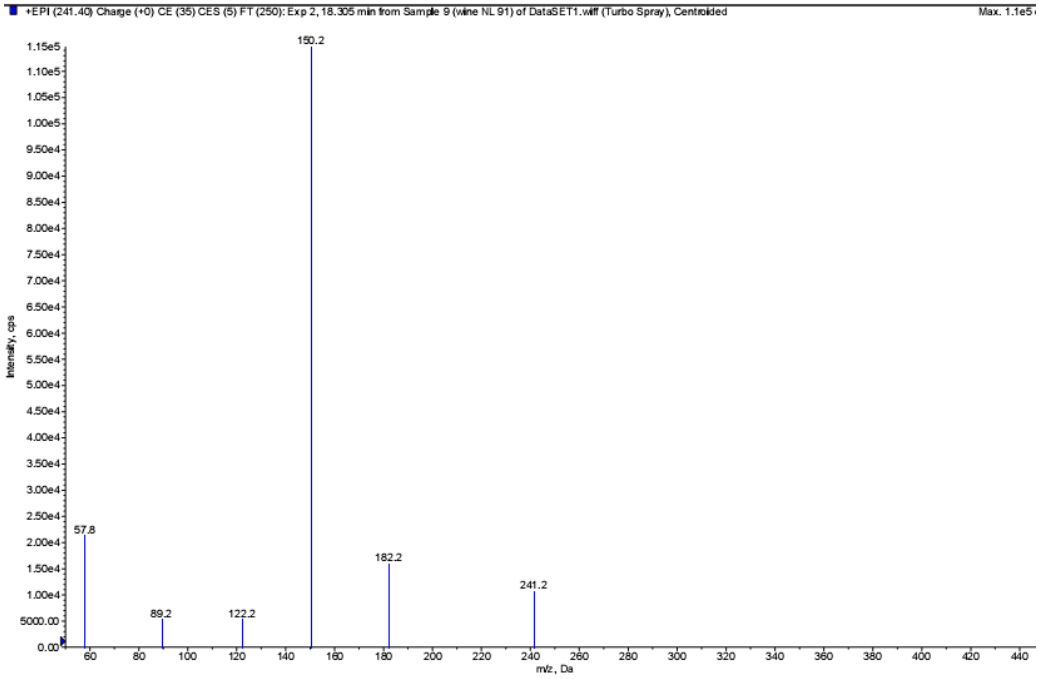
Compound 15, *m/z* 383/387, t=29.04 min



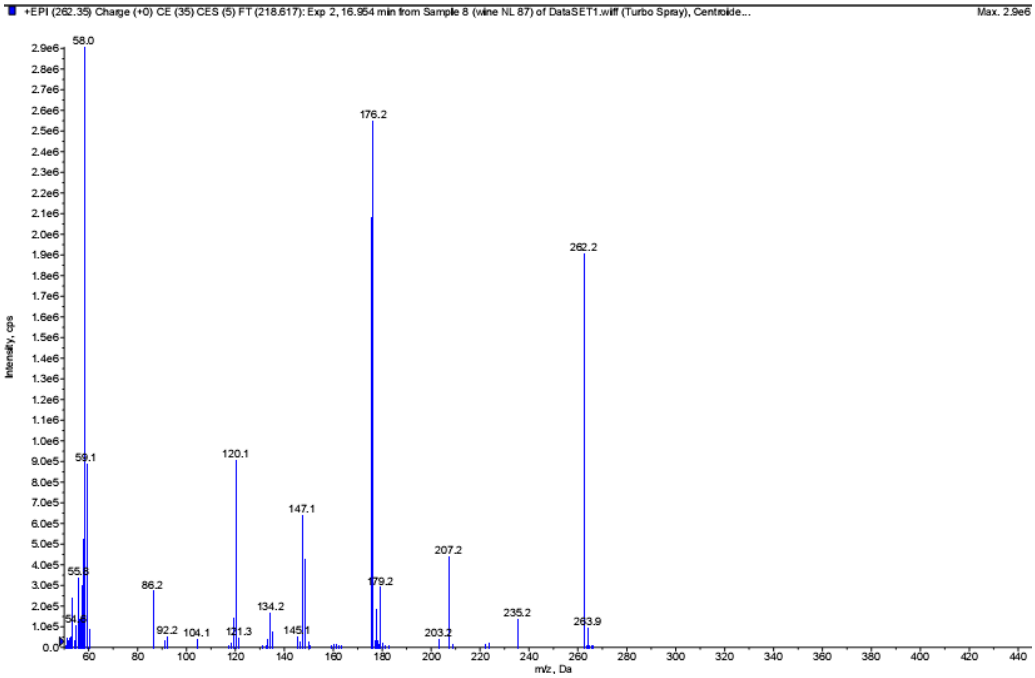


Compound 18, *m/z* 237/241, t=18.39 min





Compound 19, *m/z* 262/266, t=17.07 min



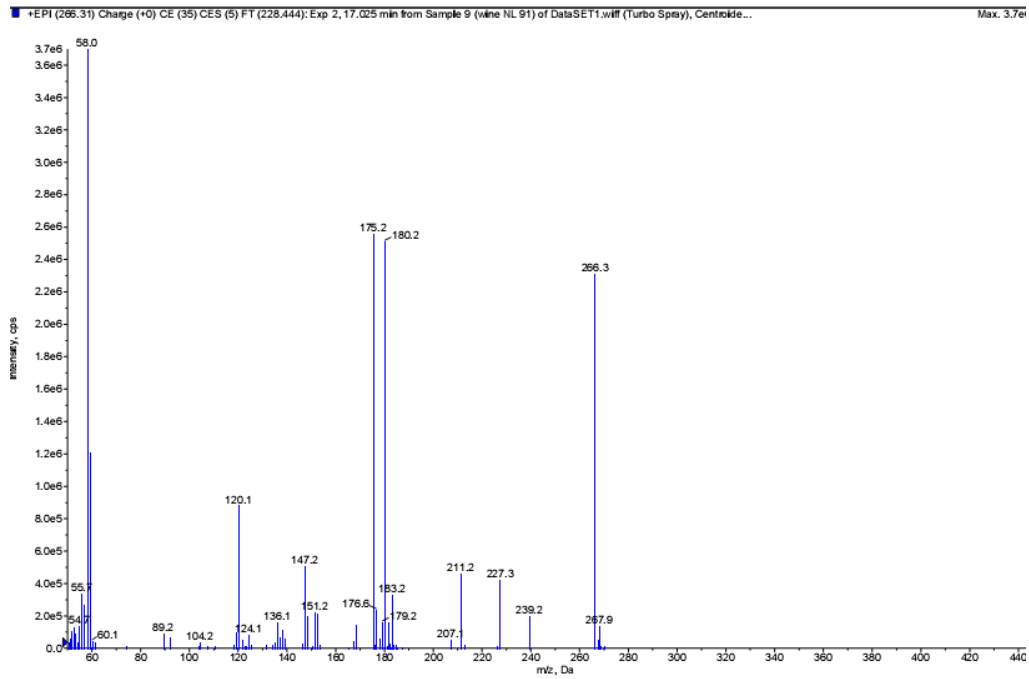


Figure S6. Comparison of the retention time of 4-APC and 4-APC-d_4 labelled hexanal, benzaldehyde, heptanal and octanal from the white wine with standards spiked in white wine by DNLS analysis using QTRAP mass spectrometer. (A) 4-APC and 4-APC-d_4 labelled hexanal; (B) 4-APC and 4-APC-d_4 labelled benzaldehyde; (C) 4-APC and 4-APC-d_4 labelled heptanal; (D) 4-APC and 4-APC-d_4 labelled octanal.

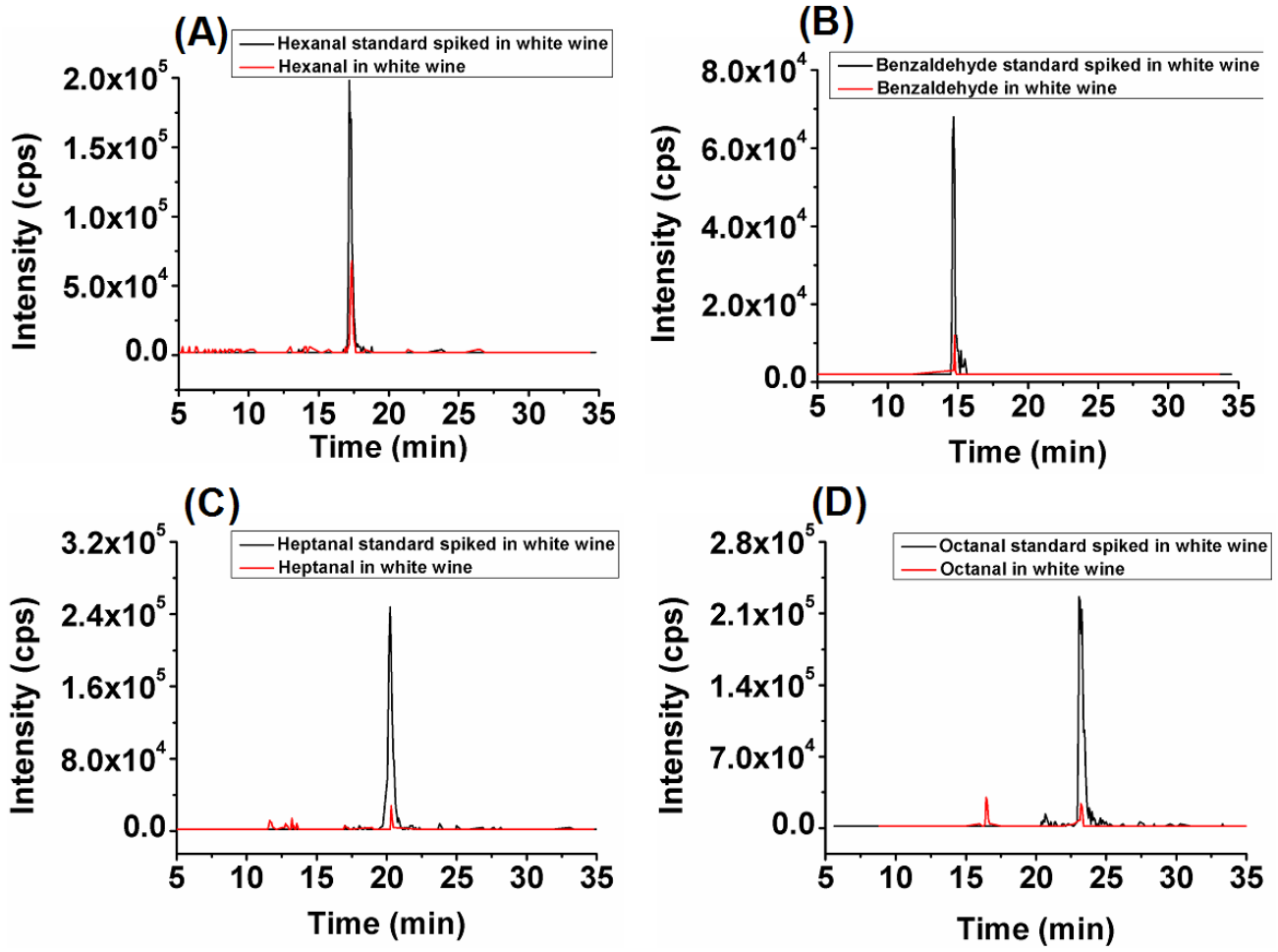


Figure S7. Comparison of the MS/MS spectrum of 4-APC labelled hexanal, benzaldehyde, heptanal, octanal and nonanal from the urine with the standards using QTRAP mass spectrometer. (A) 4-APC labelled hexanal standard; (B) 4-APC labelled hexanal in white wine; (C) 4-APC labelled benzaldehyde standard; (D) 4-APC labelled benzaldehyde in white wine; (E) 4-APC labelled heptanal standard; (F) 4-APC labelled heptanal in white wine; (G) 4-APC labelled octanal standard; (H) 4-APC labelled octanal in white wine.