

Supporting Information

An approach to shortening the timeframe between the emergence of new compounds on the drugs market and the availability of reference standards: the microscale syntheses of nitrazolam and clonazepam, for use as reference materials, utilizing polymer supported reagents

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Supporting information 1: Photographs of microscale reaction set-up.

Supporting information 2: ^{13}C - ^1H and ^1H - ^1H 2D NMR for 2-ethoxy-7-nitro-5-phenyl-3*H*-benzo[*e*][1,4]diazepine (5a).

Supporting information 3: NMR data for nitrazolam (synthesized) and clonazolam (vendor powder sample and synthesized), and low-field (benchtop, 60 MHz) ^1H NMR spectrum for extract from clonazolam tablets.

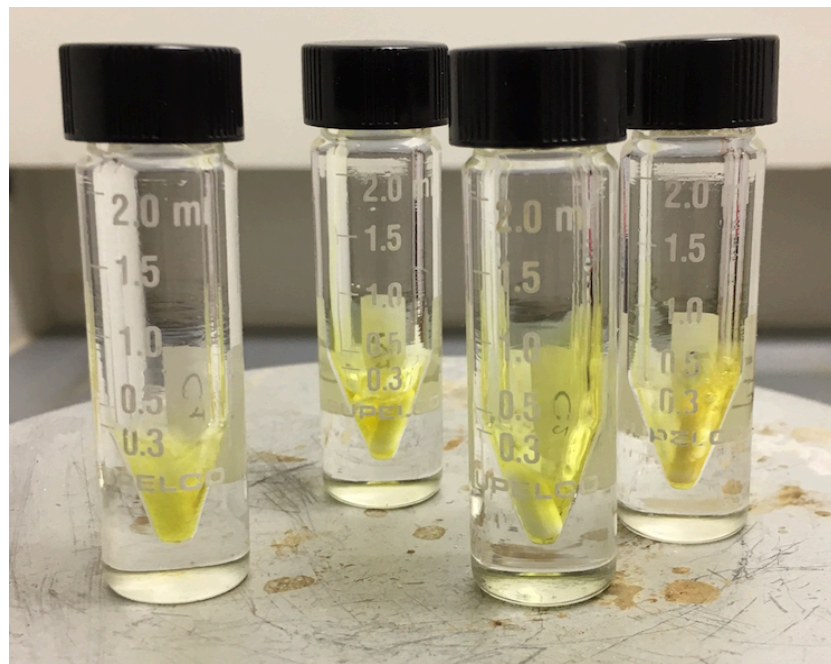
Supporting information 4: Single crystal x-ray diffraction (x-ray crystallography data) for clonazolam.

Supporting information 5: Quantification of clonazolam in tablets.

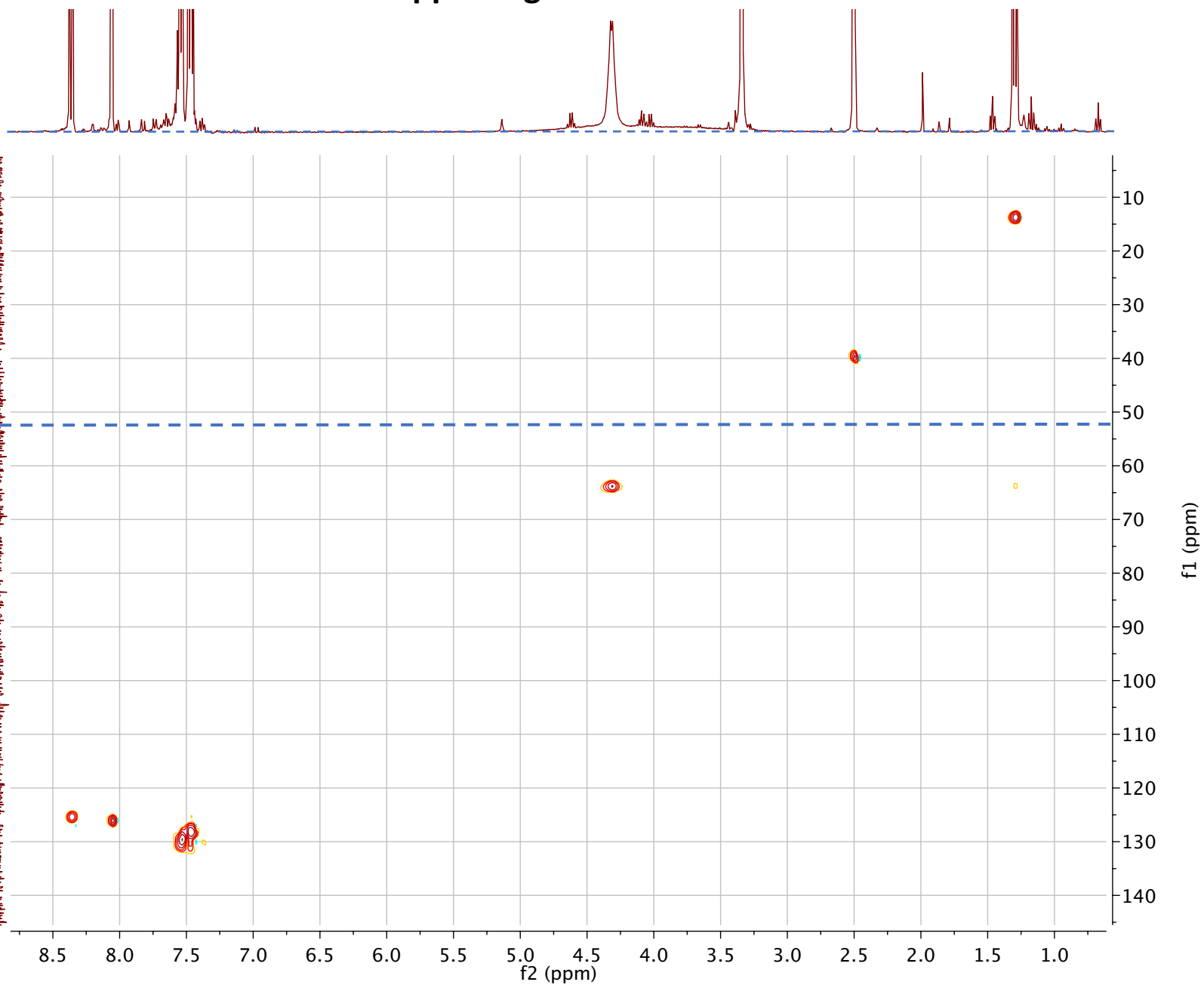
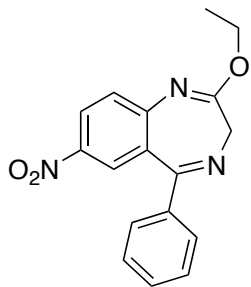
Supporting information 6: Microscale reaction by-product profiling – (a-d). GC-EIMS data for nitrazolam, (e-h). GC-EIMS data for clonazolam and (i). EI mass spectral fragmentation pathways for nitrazolam and clonazolam microscale by-products.

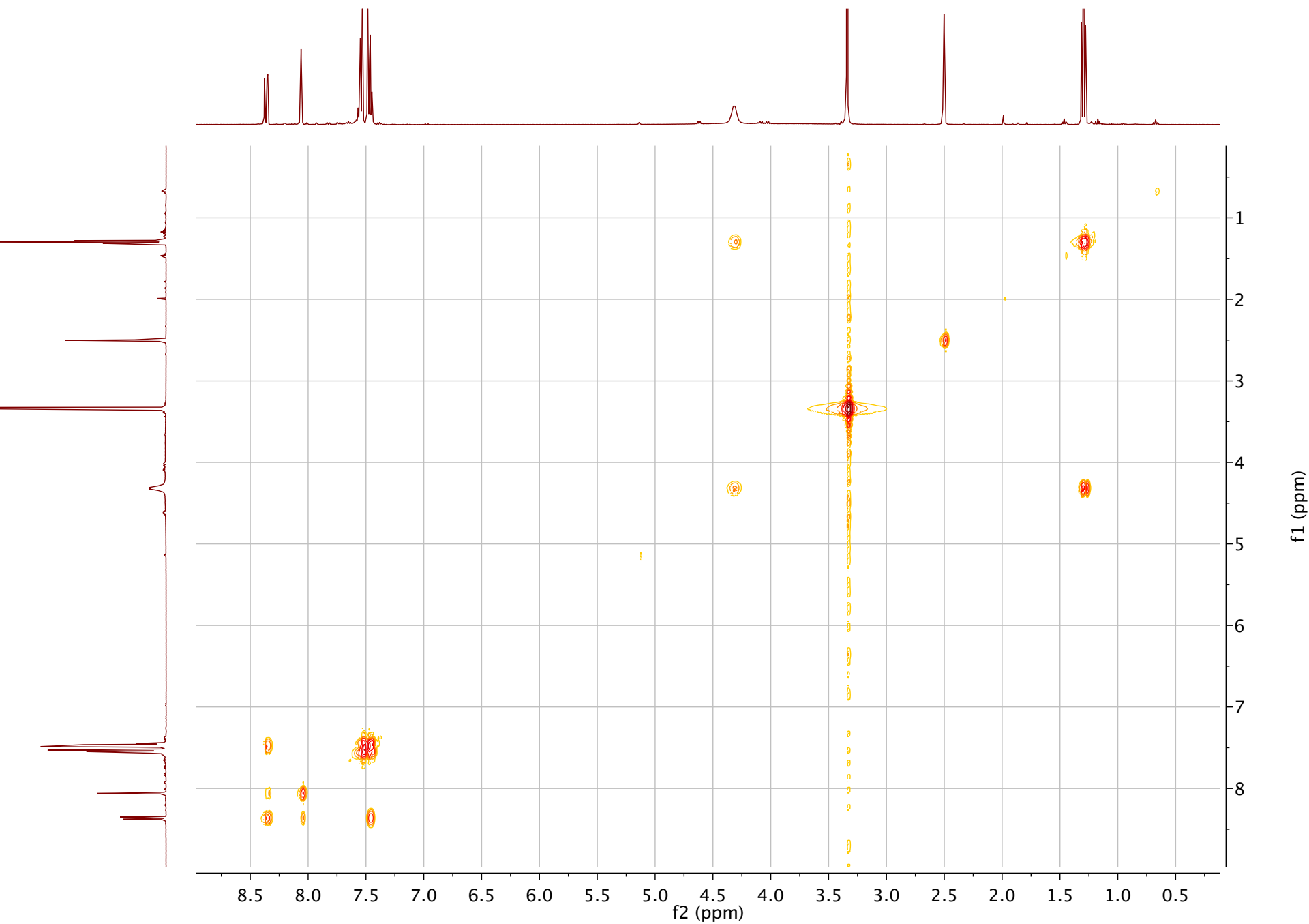
Supporting information 7: Preparative scale crude products - GCMS and LCMS data.

Supporting information 1



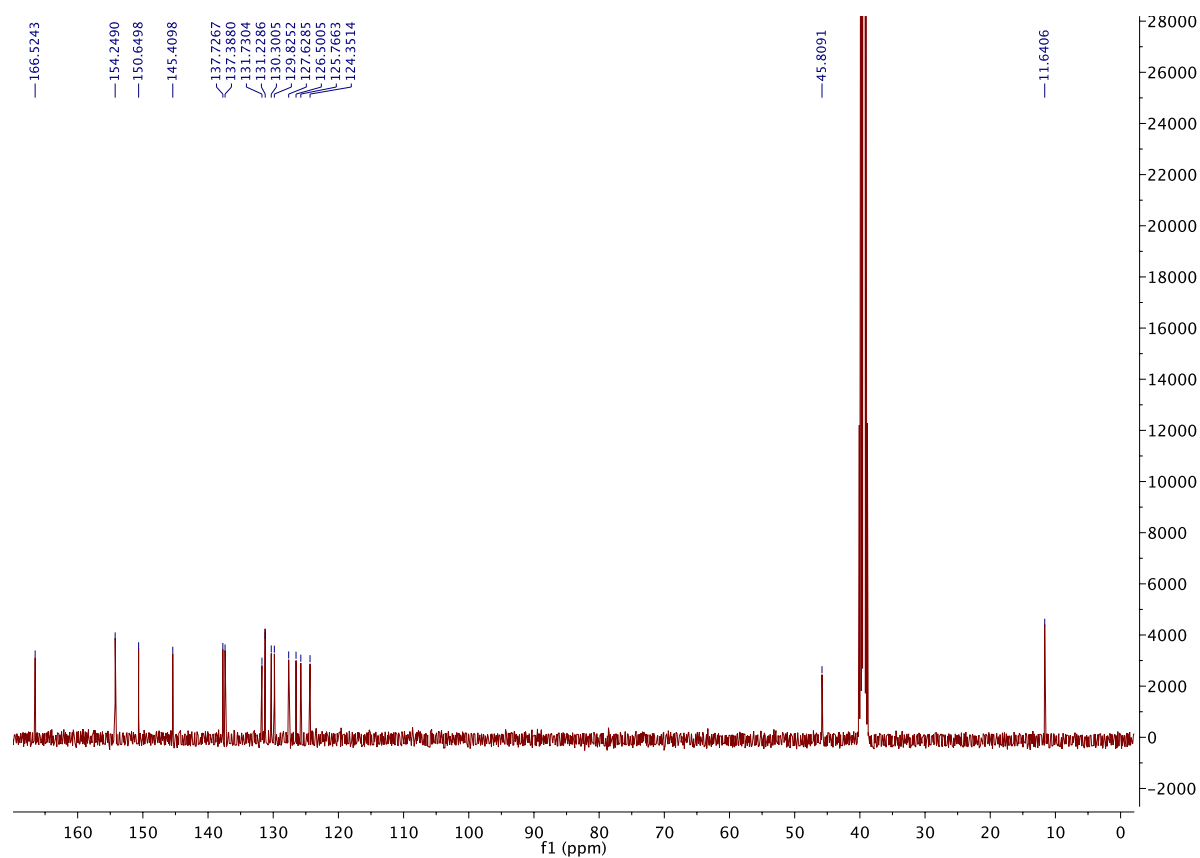
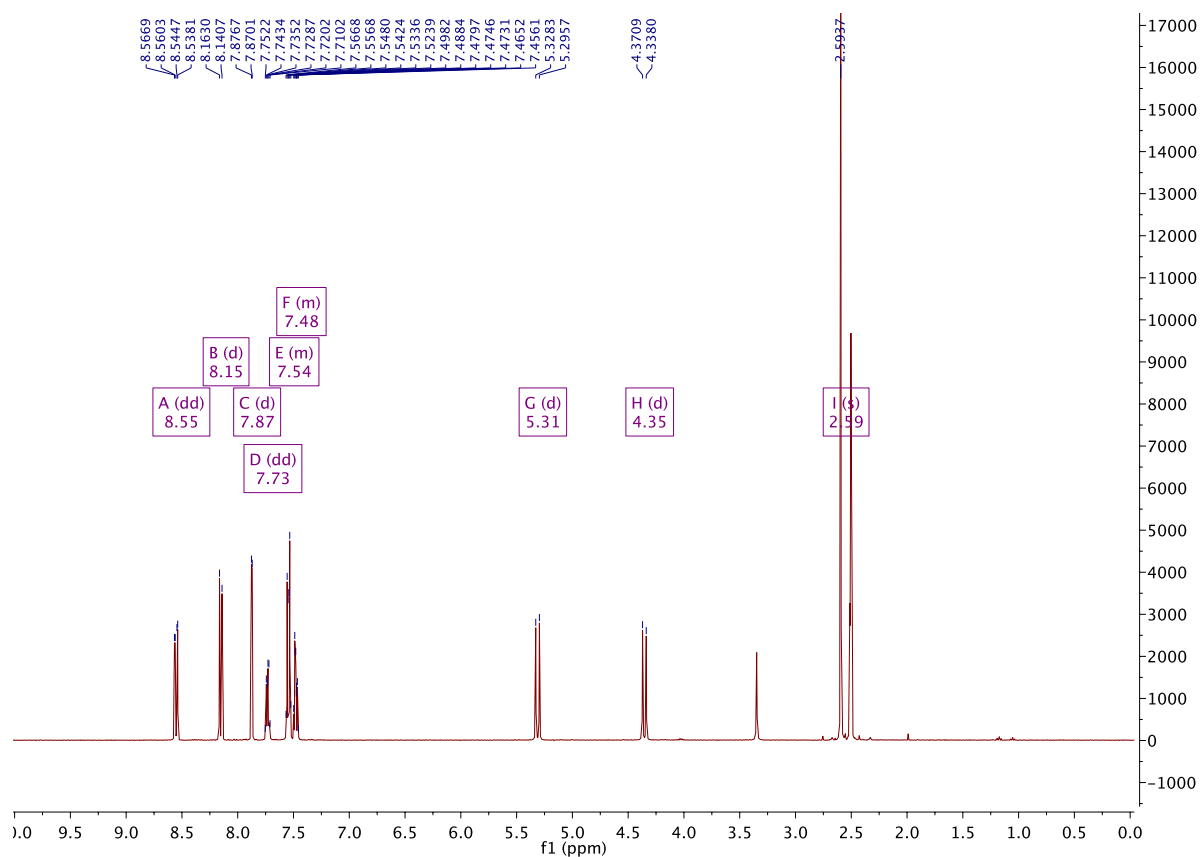
Supporting information 2

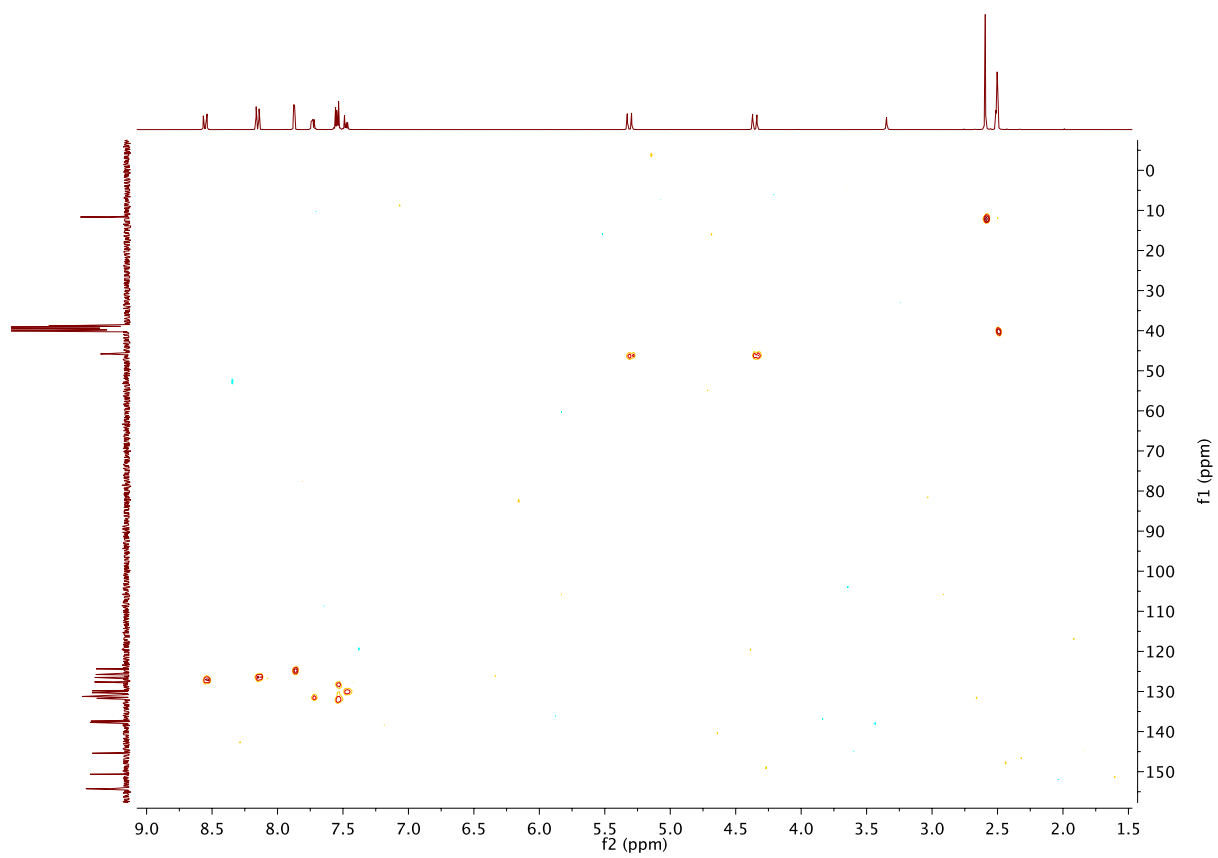
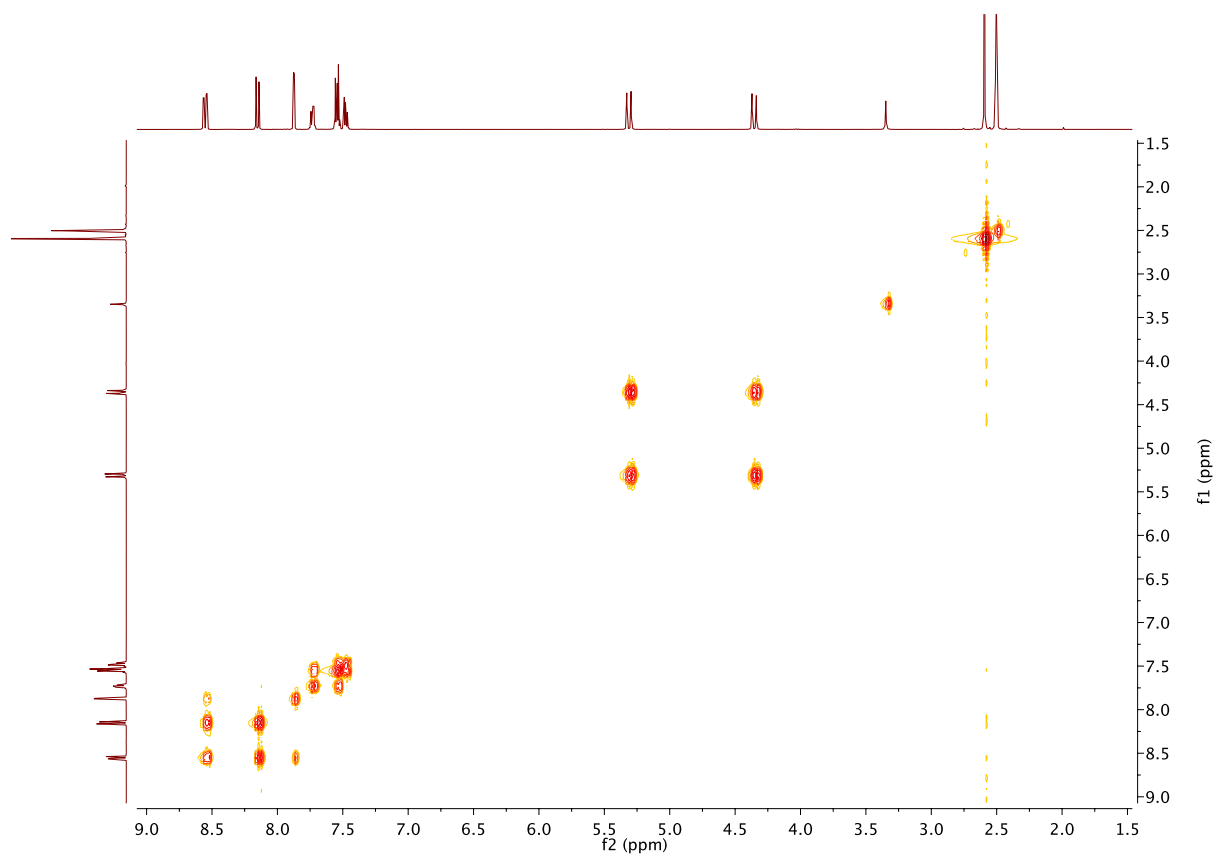




Supporting information

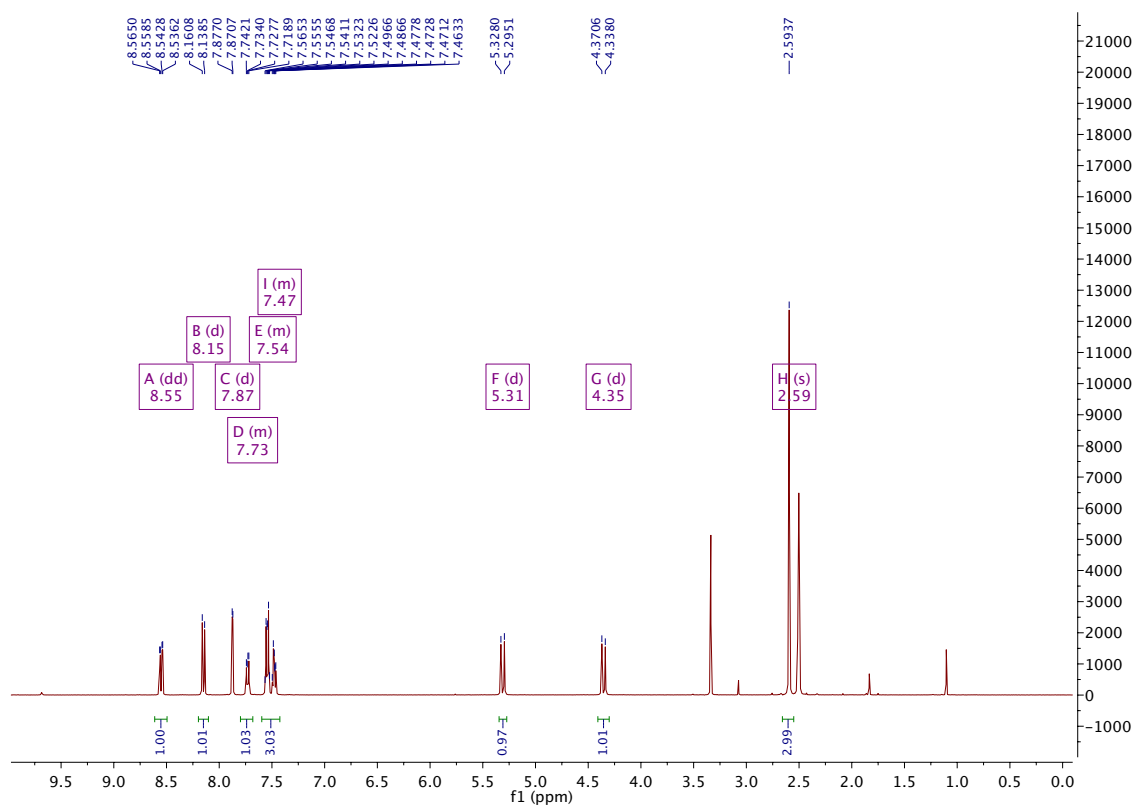
Clonazepam (powder, vendor sample) NMR data



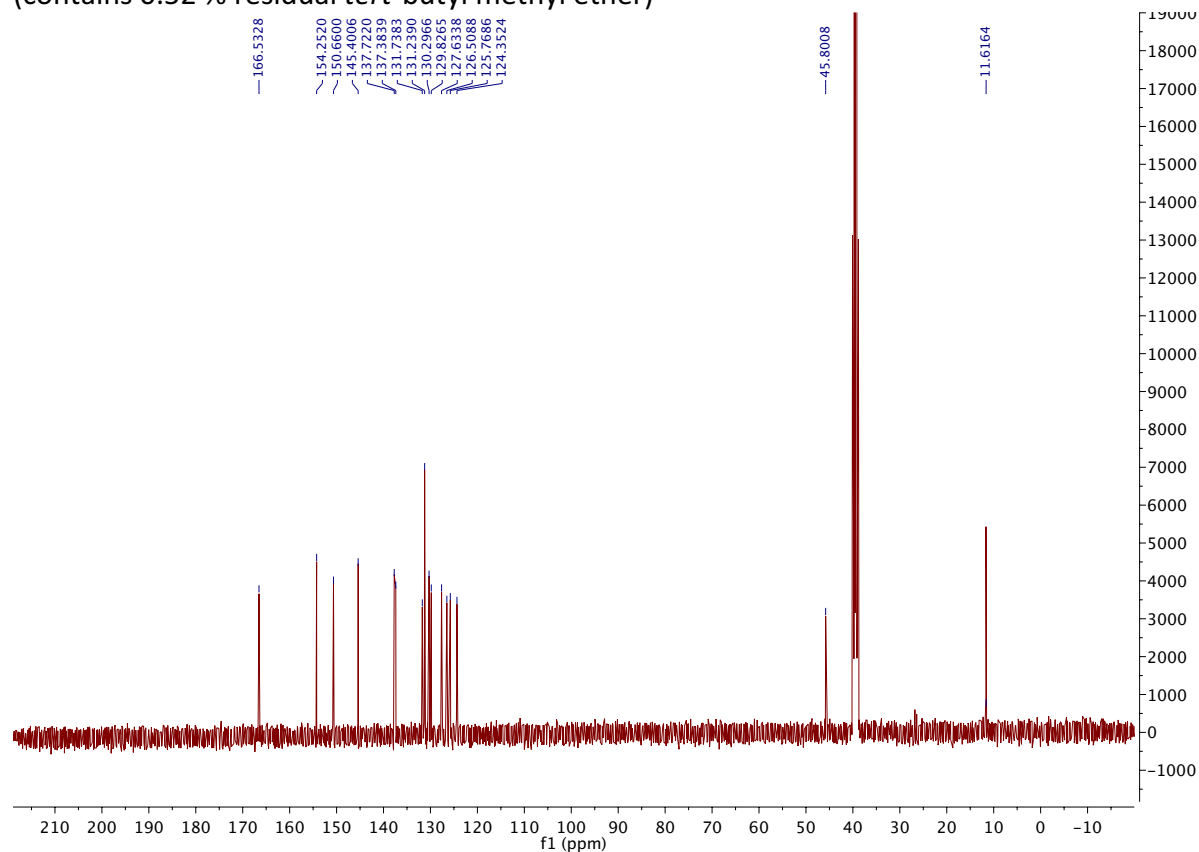


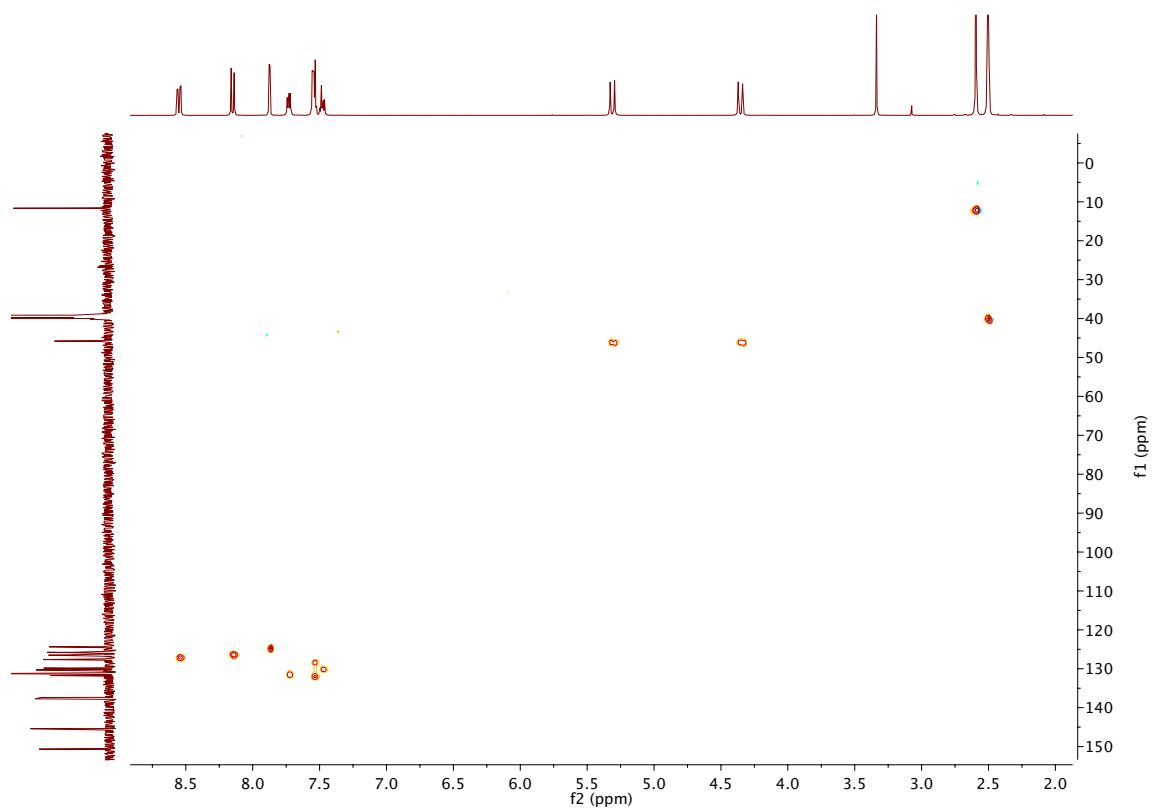
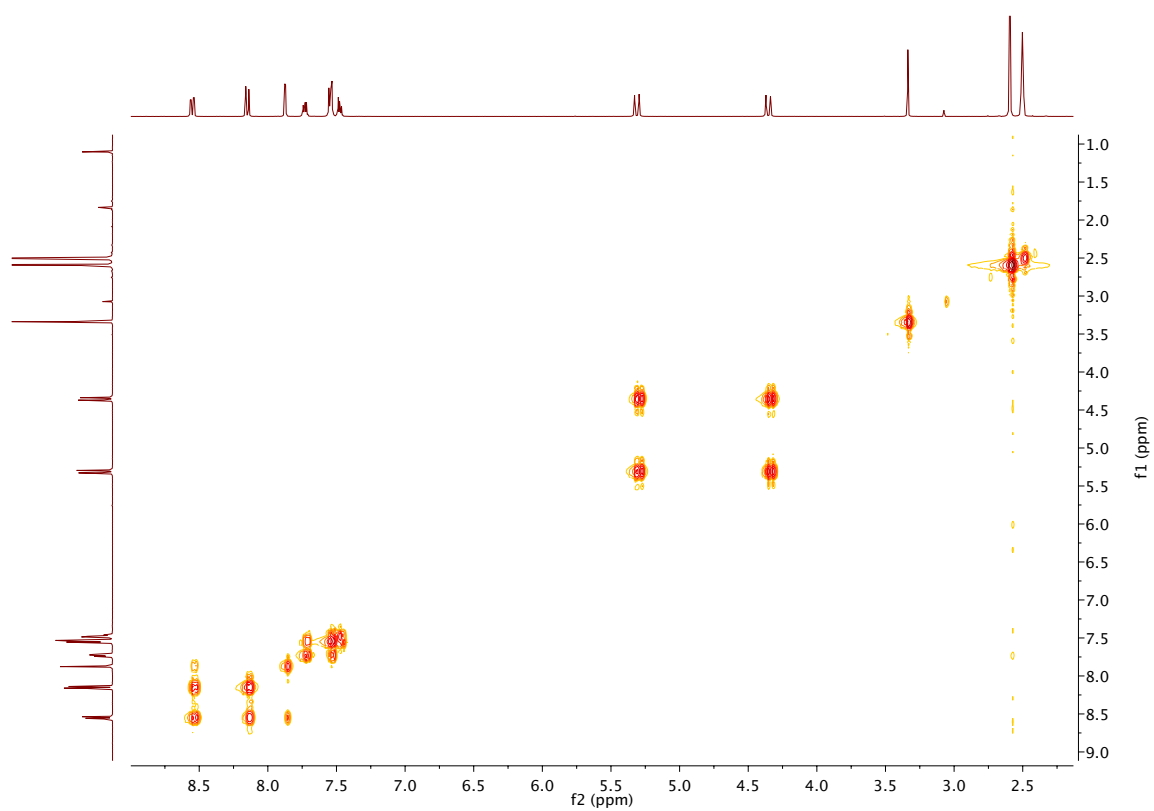
Clonazepam (powder, vendor sample): a light yellow powder: m.pt 222-224 °C; ¹H NMR (400 MHz, d₆ DMSO) δ 8.55 (dd; *J* = 8.9, 2.6 Hz; 1 H; Ar H-5), 8.15 (d; *J* = 8.9 Hz; 1 H; Ar H-6), 7.87 (d; *J* = 2.6 Hz; 1 H; Ar H-3), 7.71 - 7.75 (m; 1 H Ar H-3'), 7.59 – 7.52 (m; 2 H; Ar H-4' and 5'), 7.51 – 7.45 (m; 1 H; Ar H-6'), 5.31 (d; *J* = 13.1 Hz; 1 H; one H from CH₂), 4.35 (d; *J* = 13.2 Hz; 1 H; one H from CH₂), 2.59 (s; 3 H; CH₃). ppm; ¹³C NMR (101 MHz, d₆ DMSO) δ 166.52 (Ar-C=N), 154.25 (CH₂-C=N), 150.65 (CH₃-C=N), 145.41 (Ar C-4), 137.73 (Ar C-1 or 2), 137.39 (Ar C-1 or 2), 131.73 (Ar C-4' or 5'), 131.24 (Ar C-1' or 2'), 131.23 (Ar C-3'), 130.30 (Ar C-1' or 2'), 129.83 (Ar C-6'), 127.63 (Ar C-4' or 5'), 126.50 (Ar C-5), 125.77 (Ar C-6), 124.35 (Ar C-3), 45.81 (CH₂), 11.64 (CH₃) ppm; HRMS found, 354.0753 [M+H]⁺, C₁₇H₁₃ClN₅O₂, requires 354.0752 (Δ = 0.3 ppm).

Clonazepam (synthesized) – NMR data

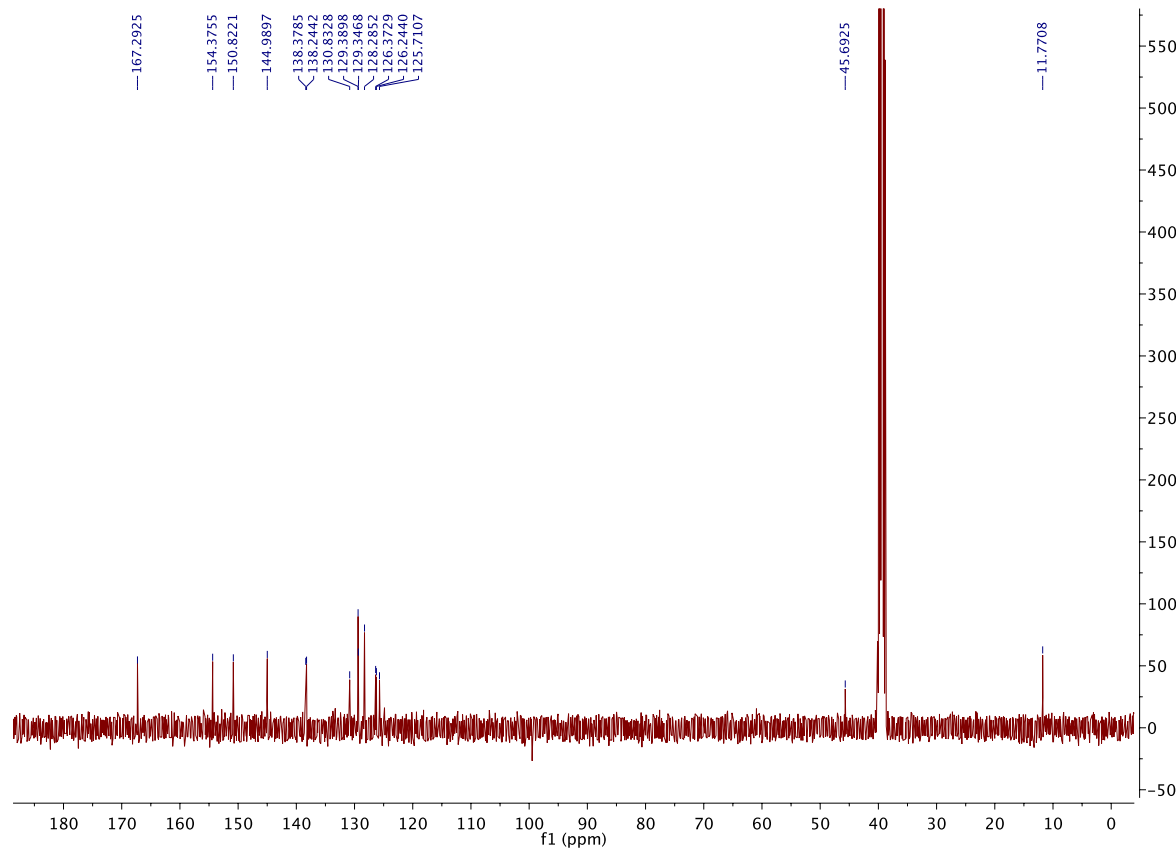
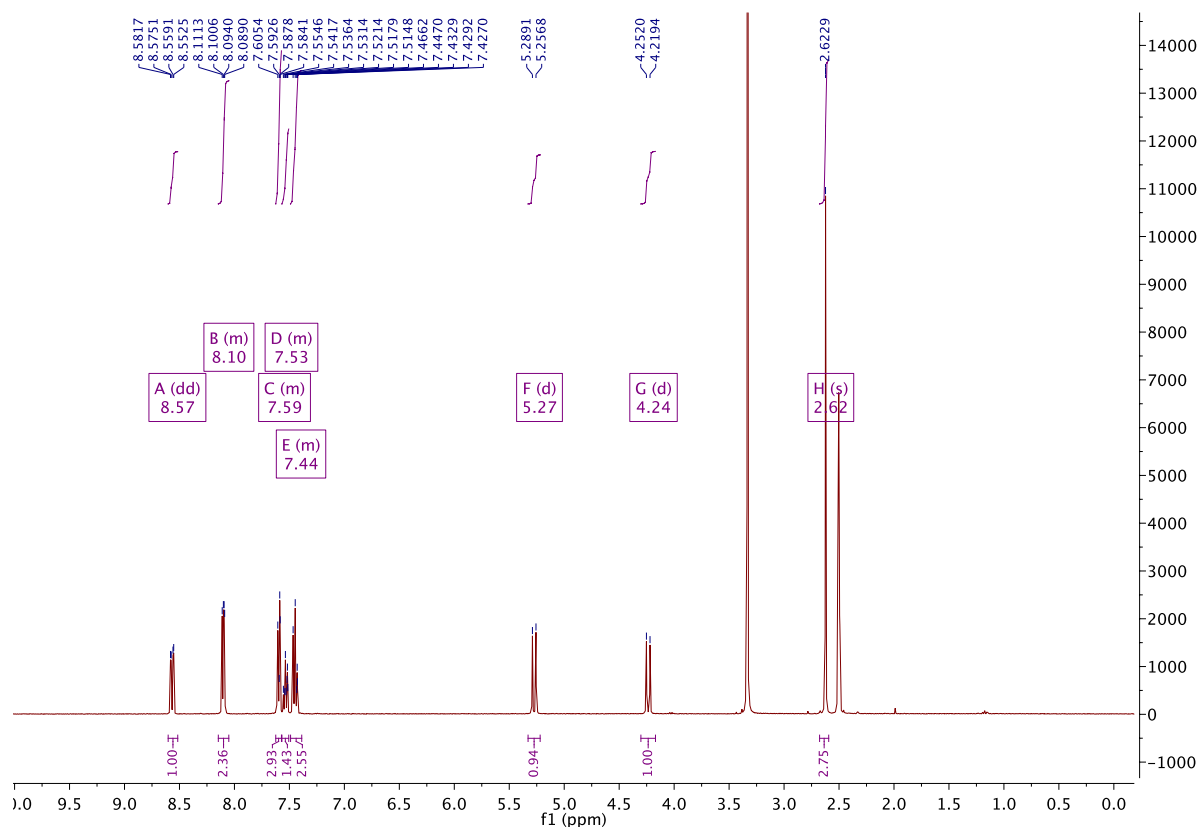


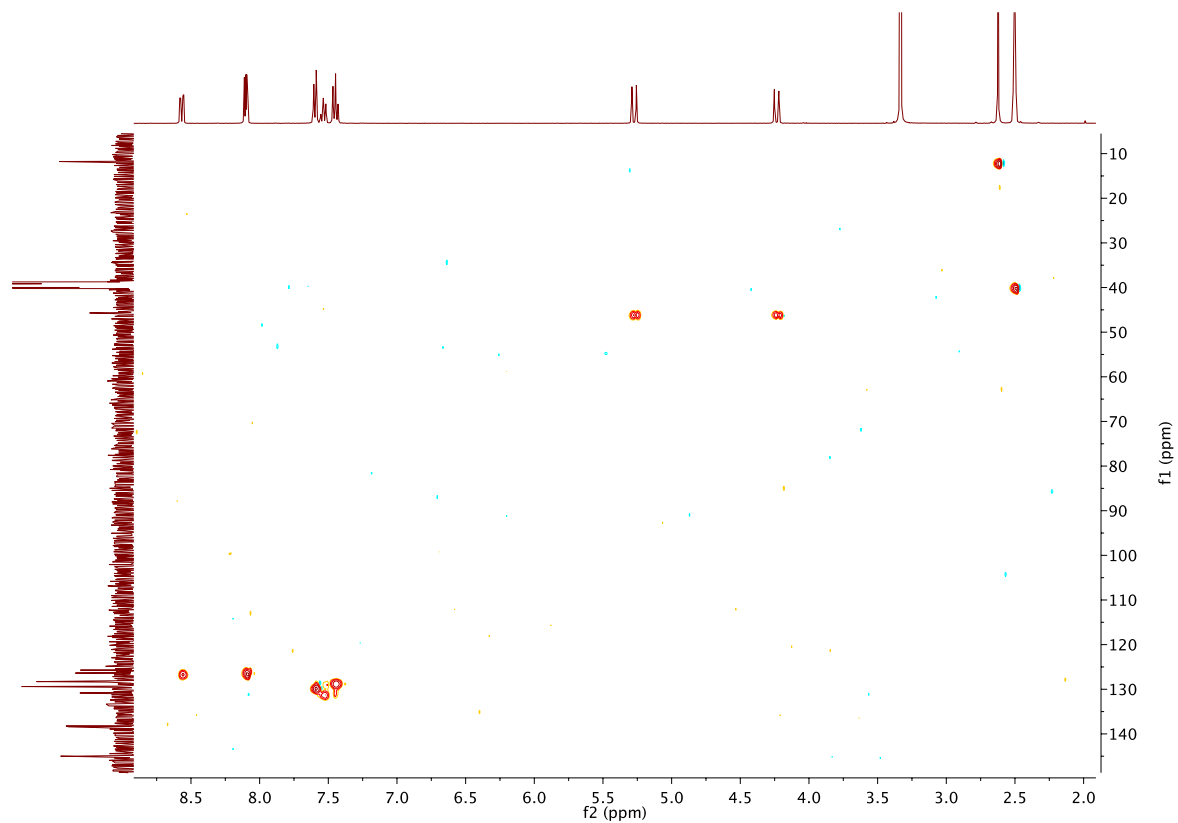
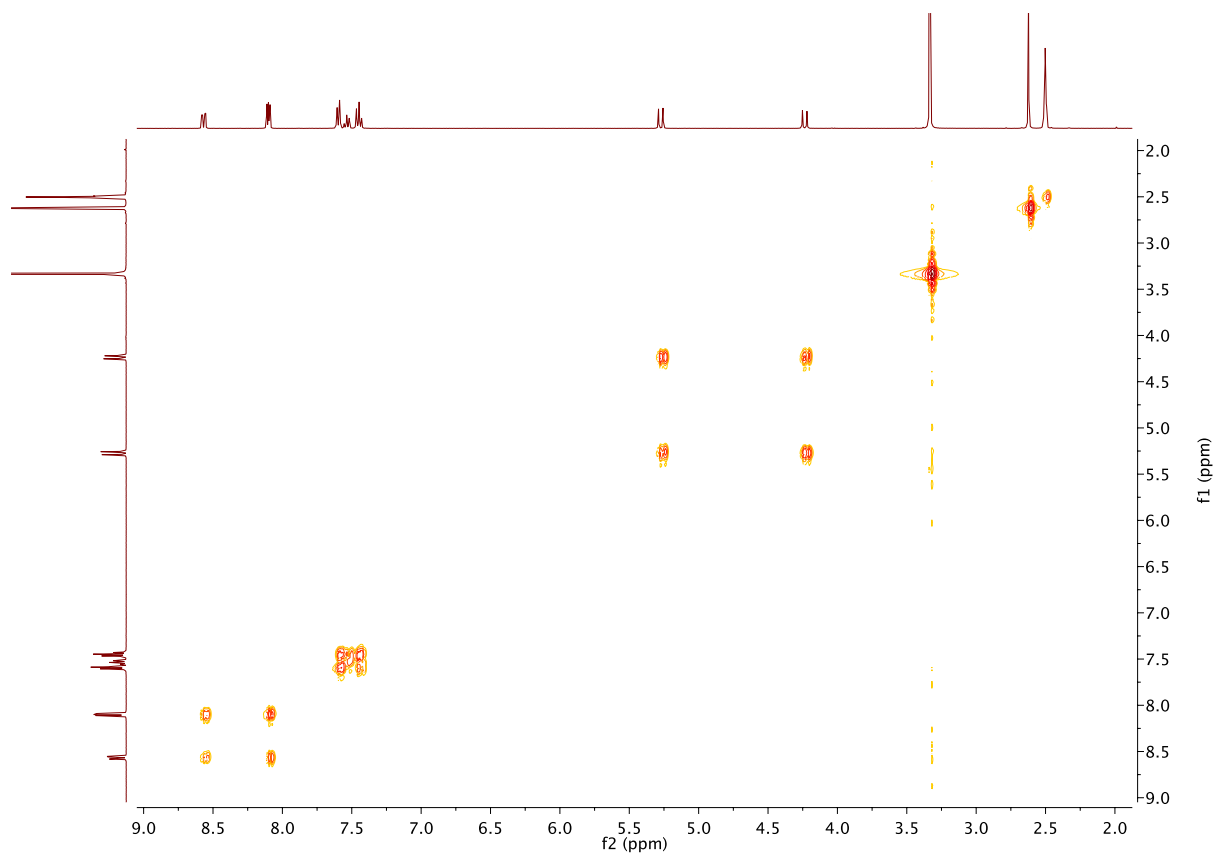
(contains 0.52 % residual *tert*-butyl methyl ether)





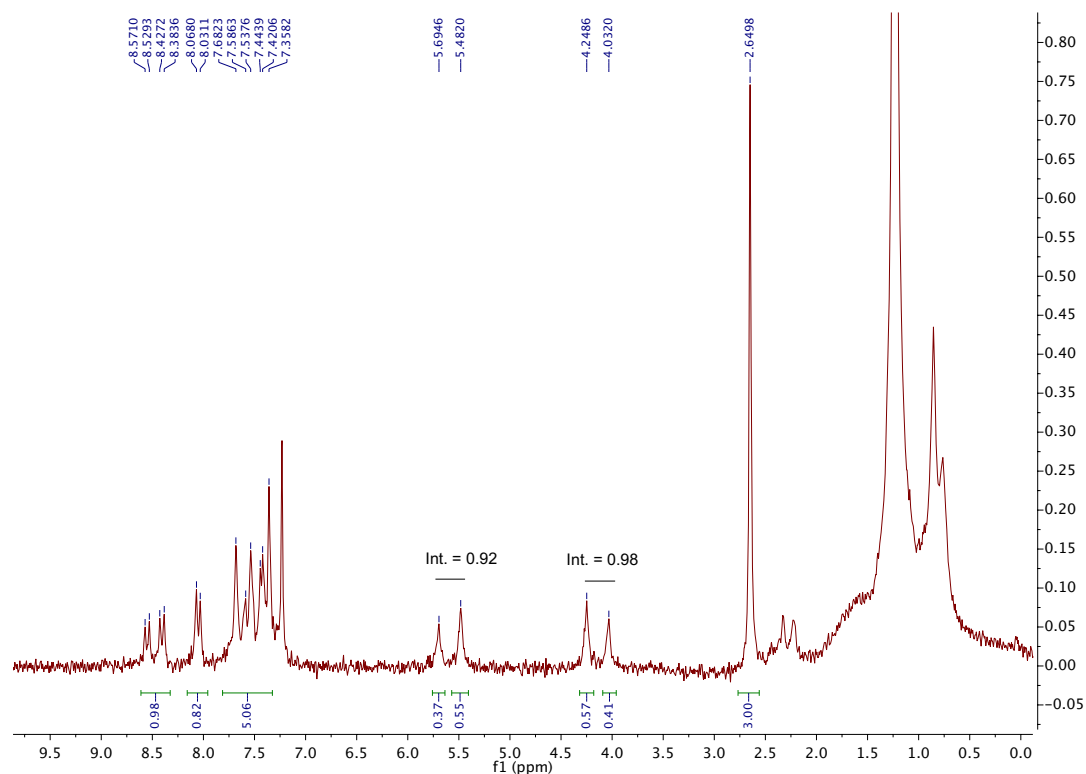
Nitrazolam (synthesized) NMR data



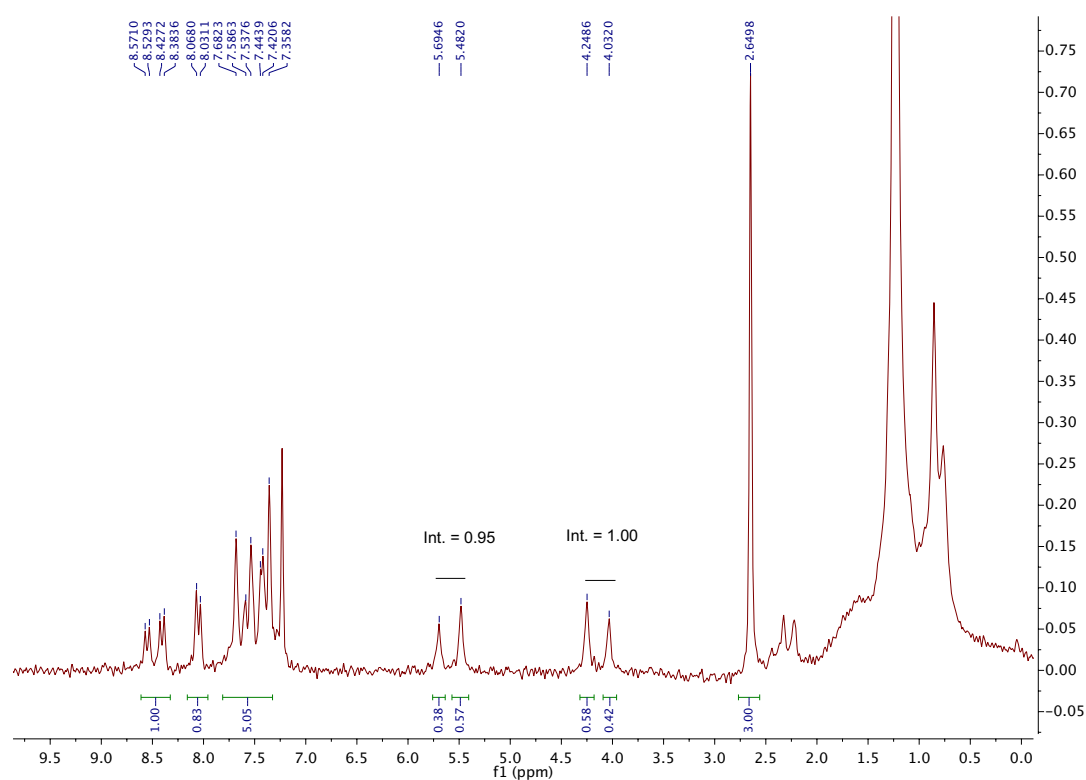


¹H spectrum for clonazepam (tablets, analysed by bench-top NMR, 60 MHz)

δ (CDCl₃, 60 MHz) 8.48 (dd; *J* = 9.0, 2.5 Hz; 1 H; Ar-H), 8.05 (d, *J* = 2.5 Hz; 1 H; Ar-H), 7.74 – 7.30 (m; 5 H; Ar H), 5.59 (d; *J* = 13.1 Hz; 1 H; CH₂), 4.14 (d; *J* = 13.1 Hz; 1 H; CH₂) and 2.65 (s; 3 H; CH₃) ppm.



With smoothing (apodization in Mnova, Exponential = -0.4 Hz, Gaussian = 1.0 Hz).



Supporting Information 4:

1. Crystallography

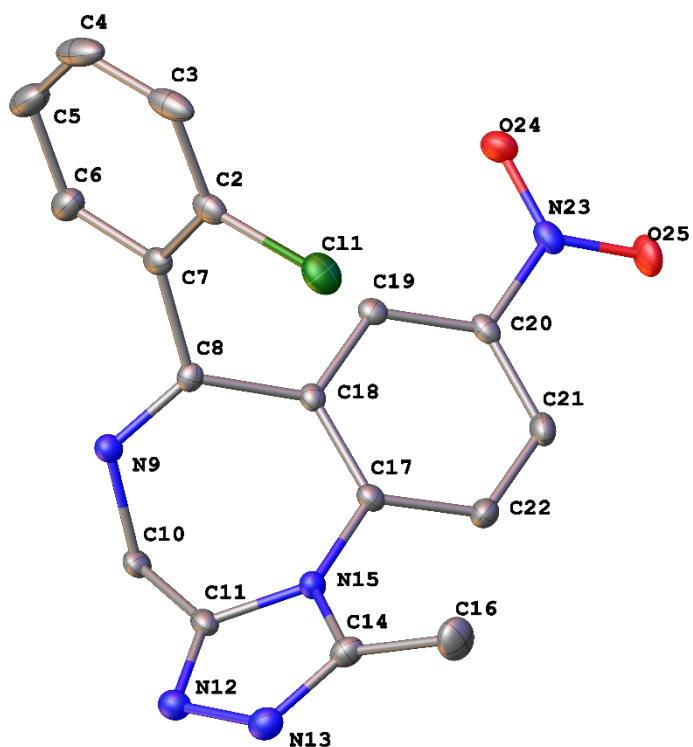


Fig. S1a Labelled molecular structure of 1 with atomic displacement shown at 50% probability.

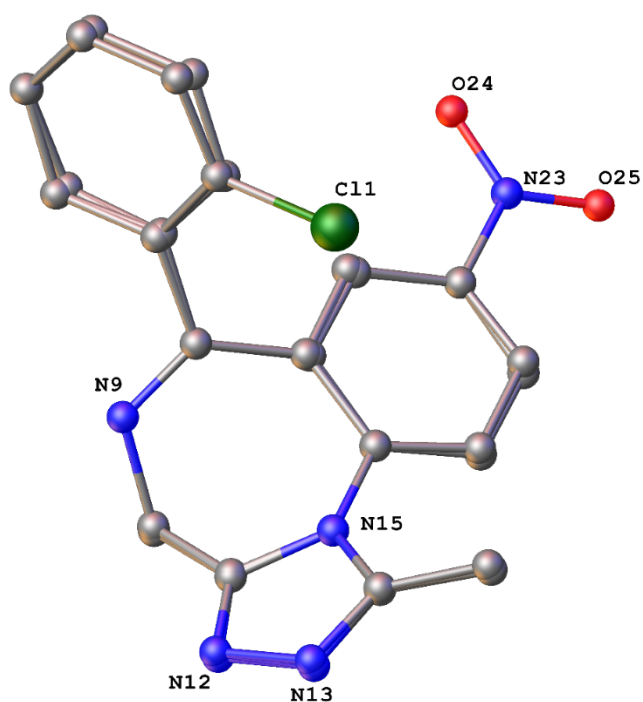


Fig. S1b. Overlay plot of 1 and Alprazolam. Data taken from CCDC 106079. Only heteroatom labelling from 1 used in the plot.

Overlay MODEL FITTING FOR 1 in P2(1)/n

ATOM MODEL DEVIATION

1	Other	Deviation (Å)
N9	N2	0.078
N12	N4	0.089
N13	N3	0.091
N15	N1	0.034
C2	C12	0.168
C3	C13	0.281
C4	C14	0.182
C5	C15	0.070
C6	C16	0.164
C7	C11	0.078
C8	C7	0.070
C10	C8	0.068
C11	C9	0.032
C14	C10	0.044
C16	C17	0.082
C17	C5	0.081
C18	C4	0.072
C19	C3	0.107
C20	C2	0.140
C21	C1	0.196
C22	C6	0.130

WEIGHTED R.M.S. DEVIATION =0.1232 Å

IDEALIZED CRYSTAL COORDINATES FOR MODEL ATOMS

NAME	SFAC	X	Y	Z	NEAREST ATOMDEVIATION	
CL1	3	-0.28402		-0.49645	N23	0.315
N1	4	-0.36948		-0.75561	N15	0.034
N2	4	-0.68766		-0.80944	N9	0.078
N3	4	-0.33270		-0.84720	N13	0.091
N4	4	-0.50343		-0.83544	N12	0.089
C1	1	-0.20502		-0.55776	C21	0.196
C2	1	-0.30562		-0.57335	C20	0.140
C3	1	-0.43022		-0.64689	C19	0.107
C4	1	-0.45245		-0.70863	C18	0.072
C5	1	-0.35044		-0.69497	C17	0.081
C6	1	-0.23044		-0.61953	C22	0.130
C7	1	-0.58635		-0.78510	C8	0.070
C8	1	-0.67782		-0.74970	C10	0.068
C9	1	-0.52254		-0.78336	C11	0.032
C10	1	-0.25965		-0.79777	C14	0.044

C11	1	-0.59693	-0.84424	-0.49235	C7	0.078
C12	1	-0.45514	-0.87849	-0.43049	C2	0.168
C13	1	-0.46742	-0.93051	-0.35085	C3	0.281
C14	1	-0.62408	-0.94601	-0.33090	C4	0.182
C15	1	-0.76333	-0.91141	-0.38994	C5	0.070
C16	1	-0.75531	-0.85731	-0.47219	C6	0.164
C17	1	-0.07576	-0.79274	-0.71727	C16	0.082

Supporting information 5

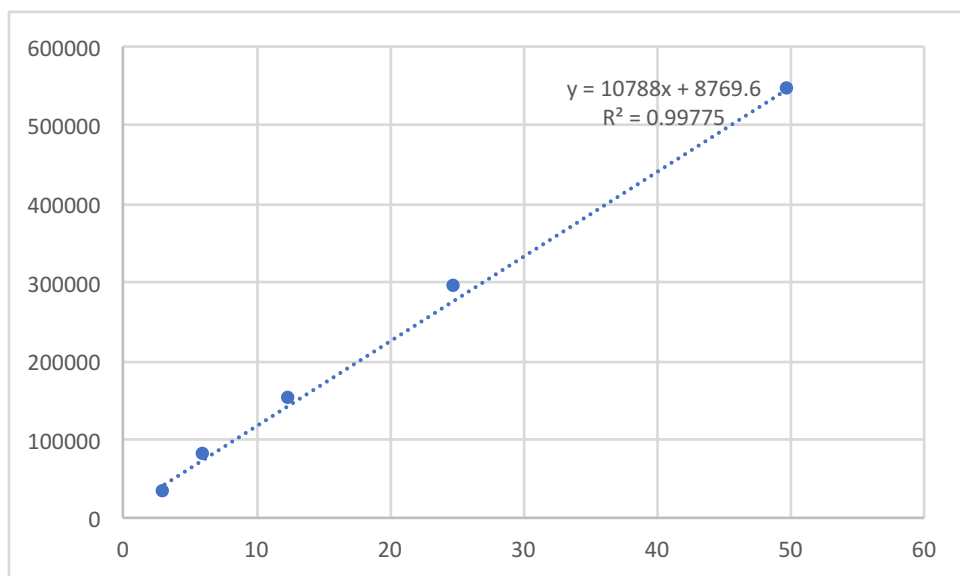
Clonazepam tabs (0.5 mg/tab)

Dil. 4

Std. (mcg/mL)	Peak area
3.125	30010
6.25	78074
12.5	148923
25	290623
50	541325

		x4x2x5	
		mcg/mL	mg/tablet
Tab A (97 mg)	147175	12.83	0.51
	148077	12.91	0.52
Tab B (99 mg)	142173	12.37	0.49
	153852	13.45	0.54

Av. 0.52 mg per tab



Tabs. weights

Tablet (mg)

98

97

98

99

100

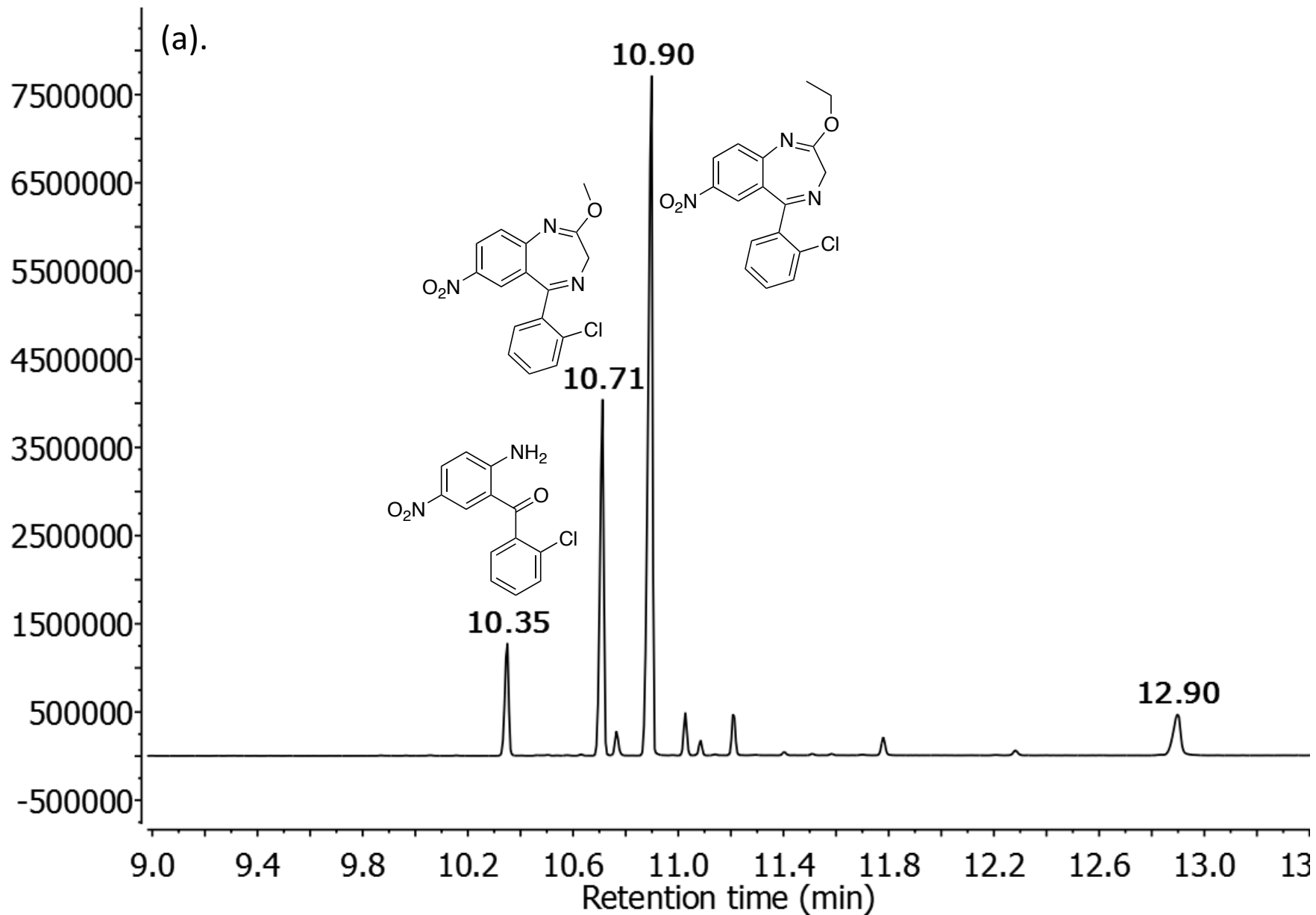
99

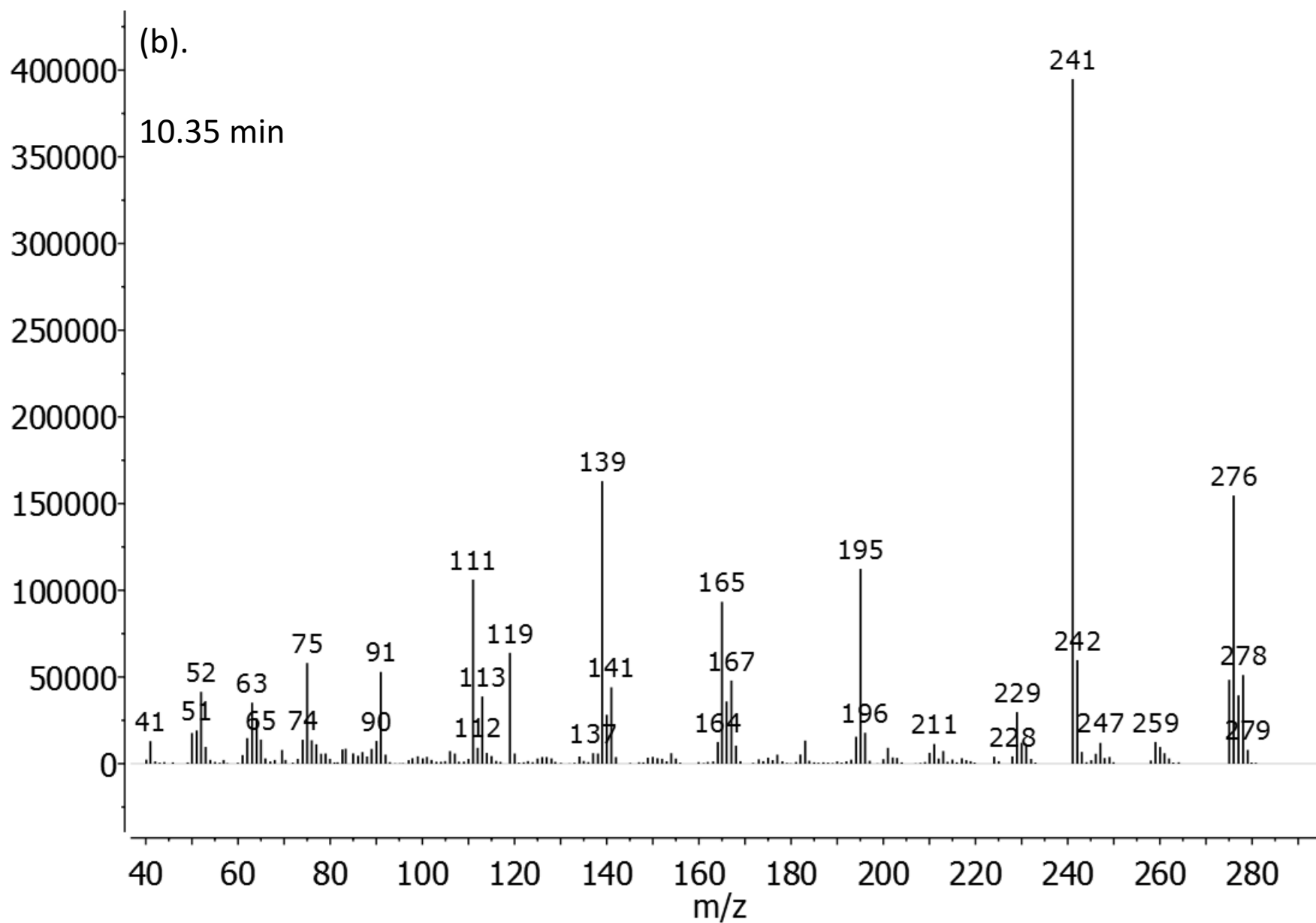
Av. 99

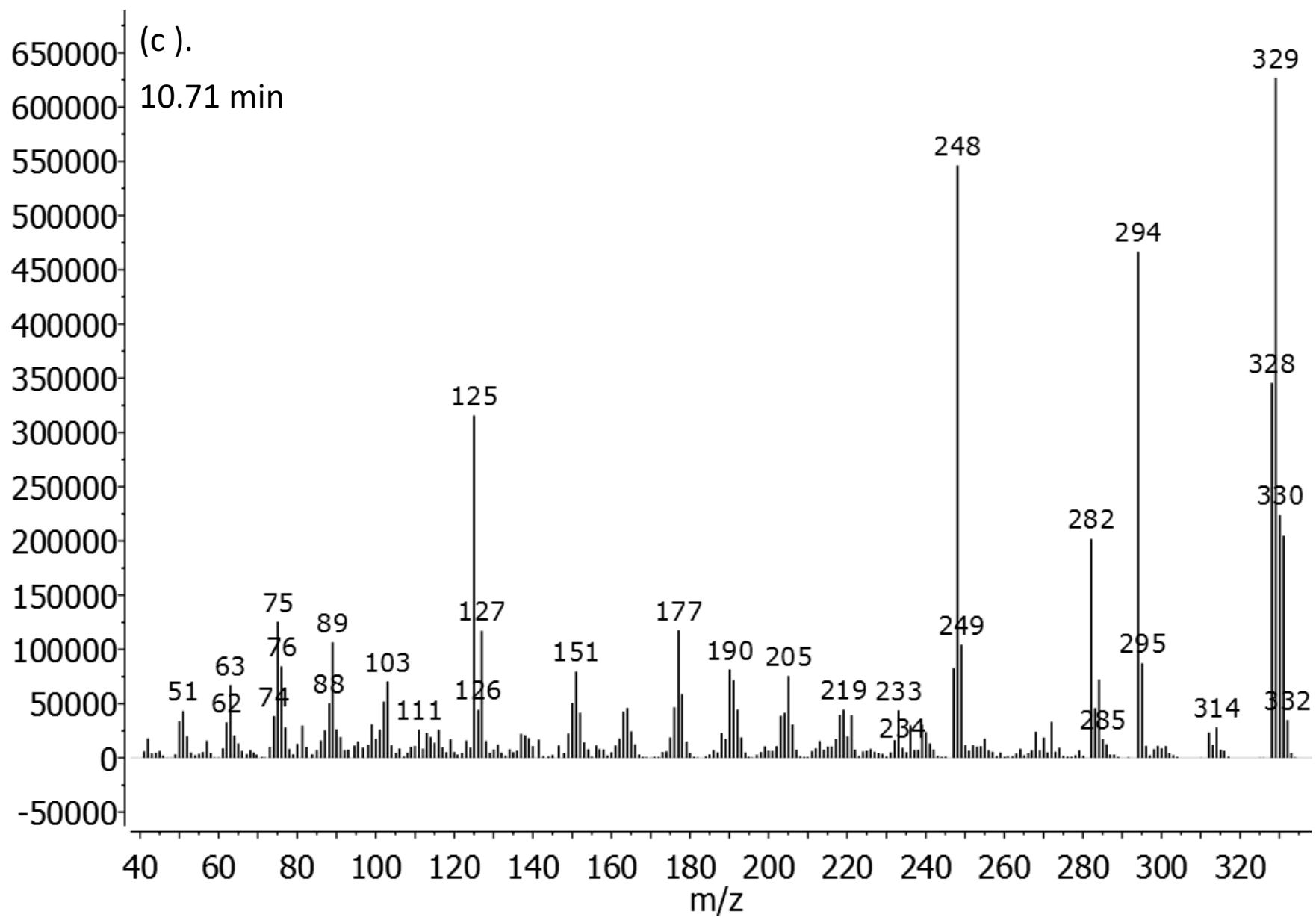
SD 1



Supporting information 6

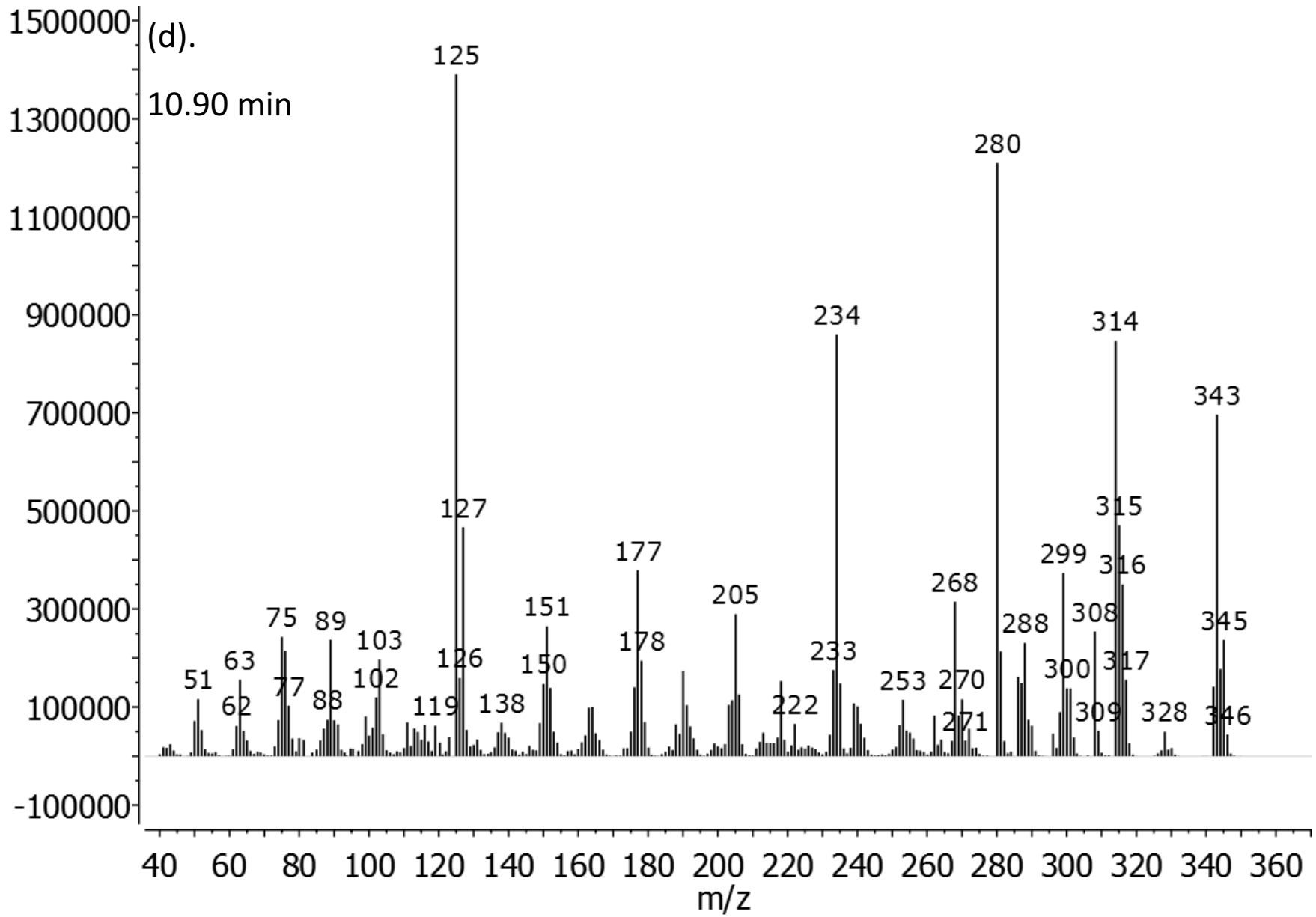


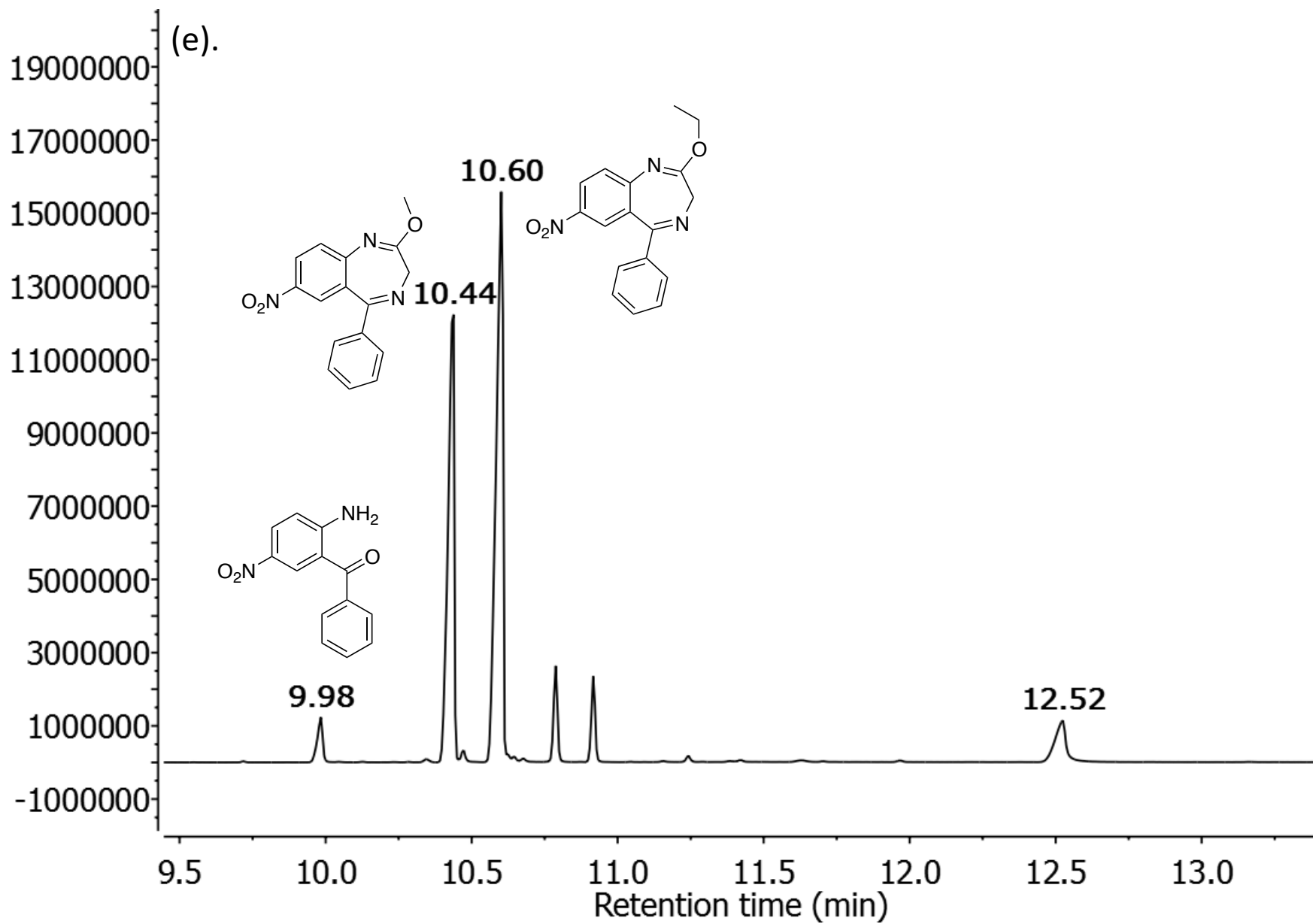


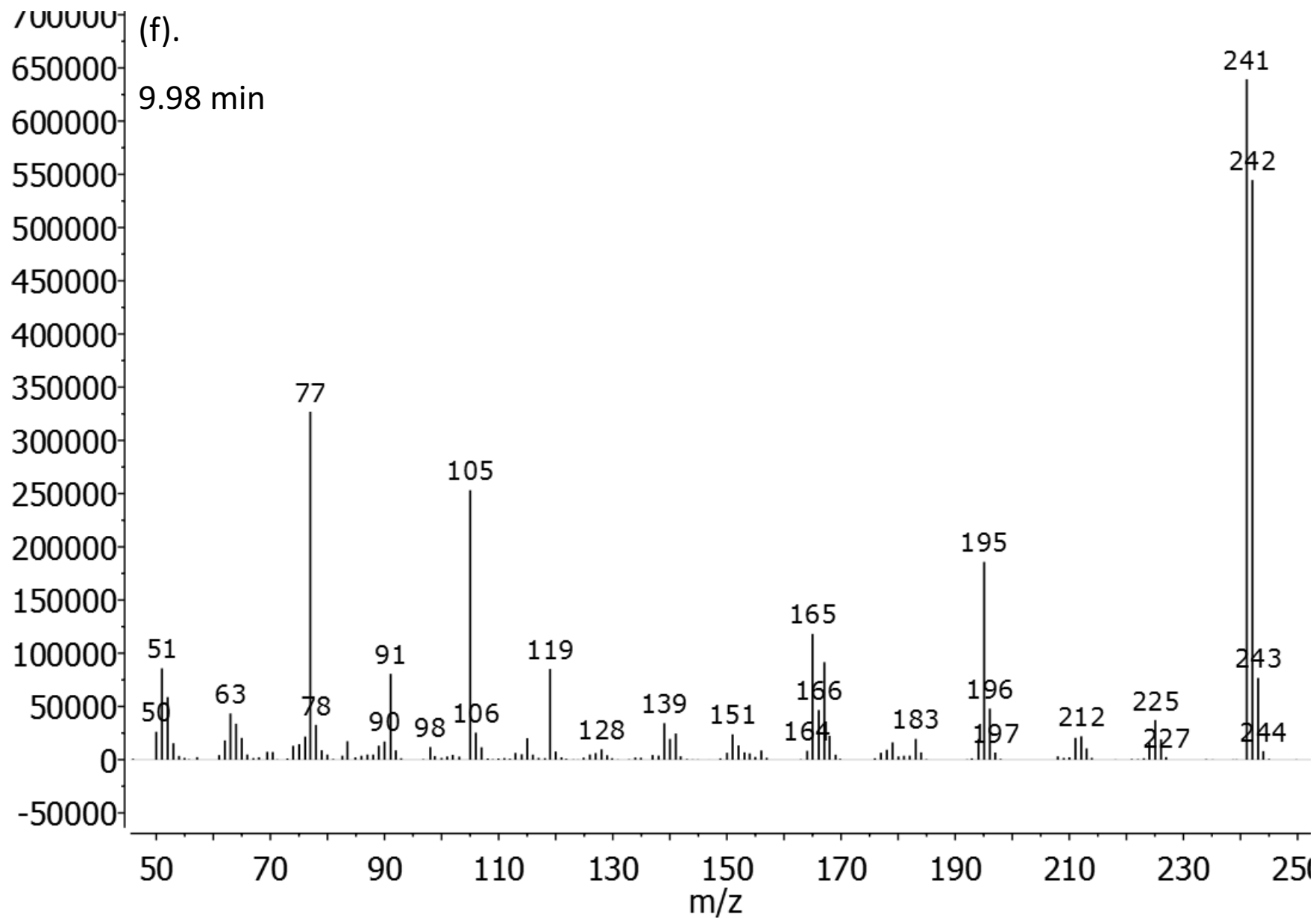


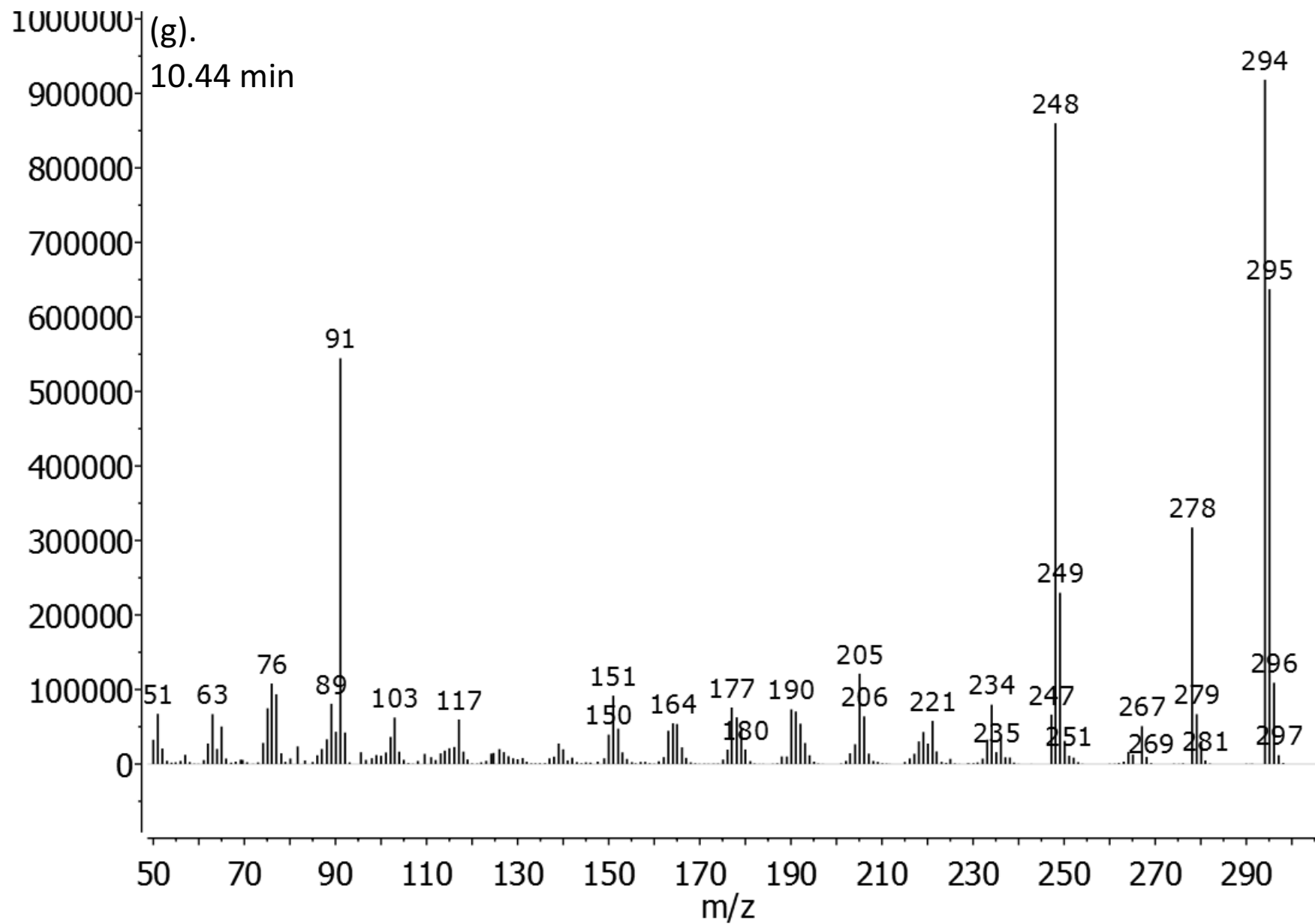
(d).

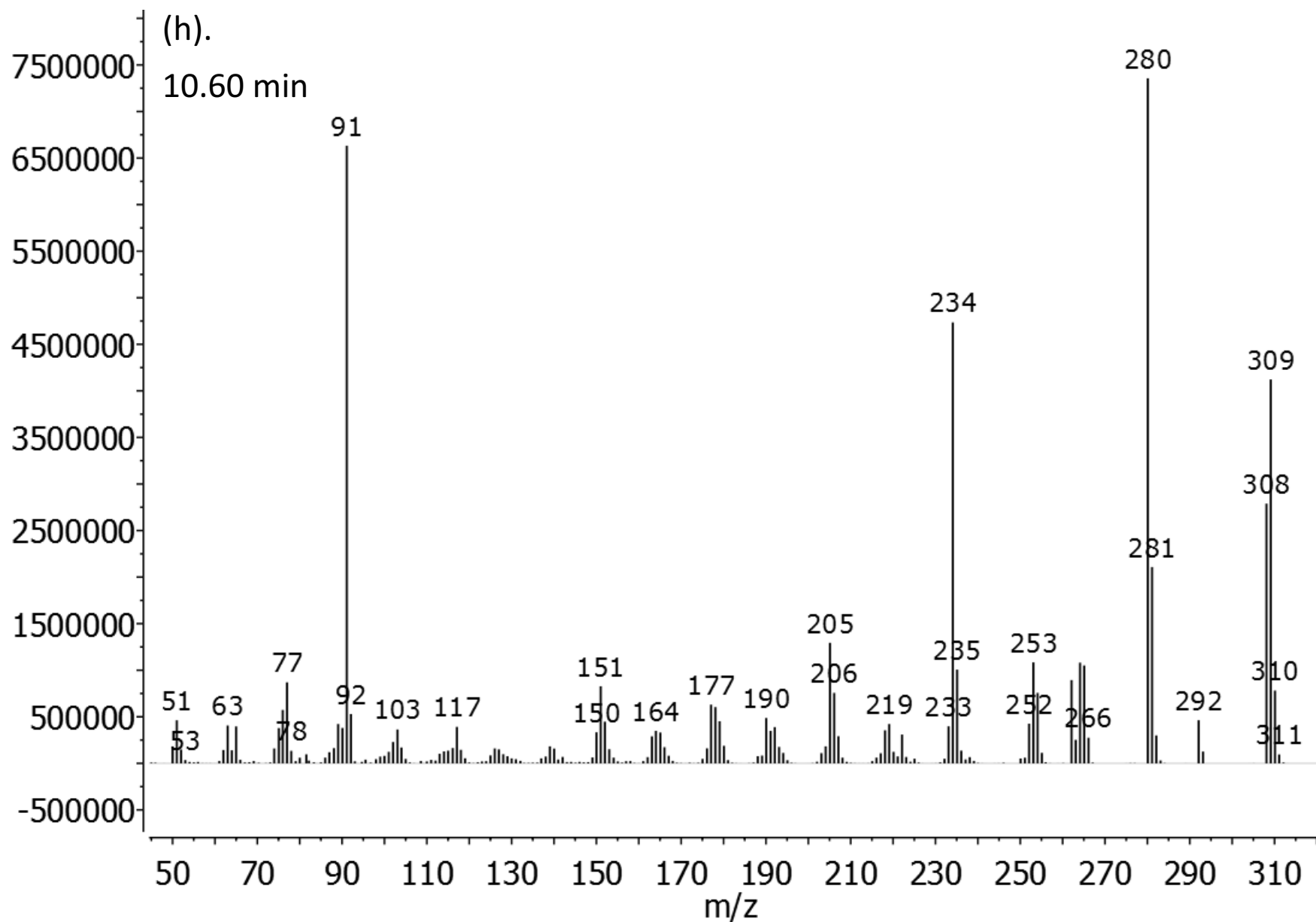
10.90 min



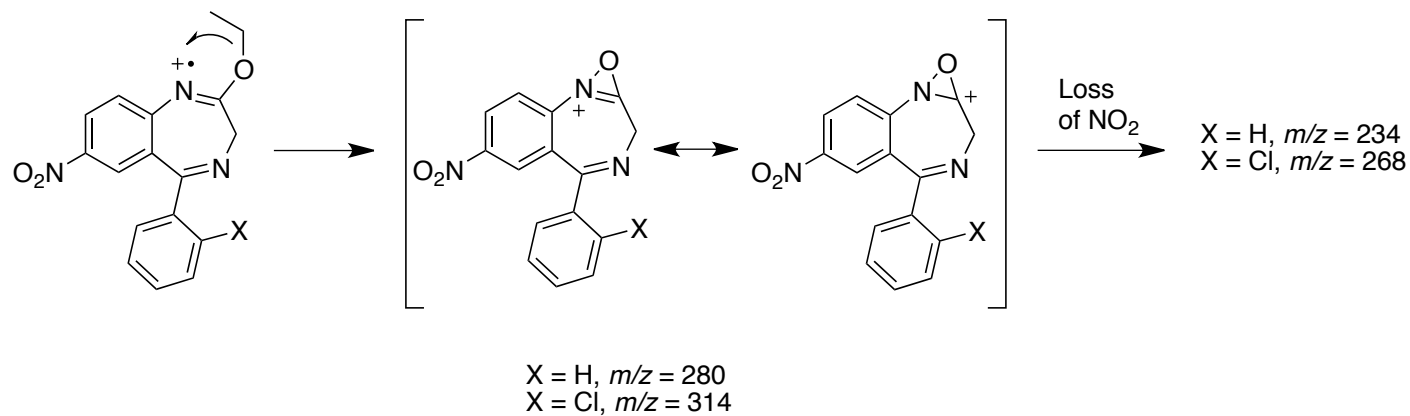
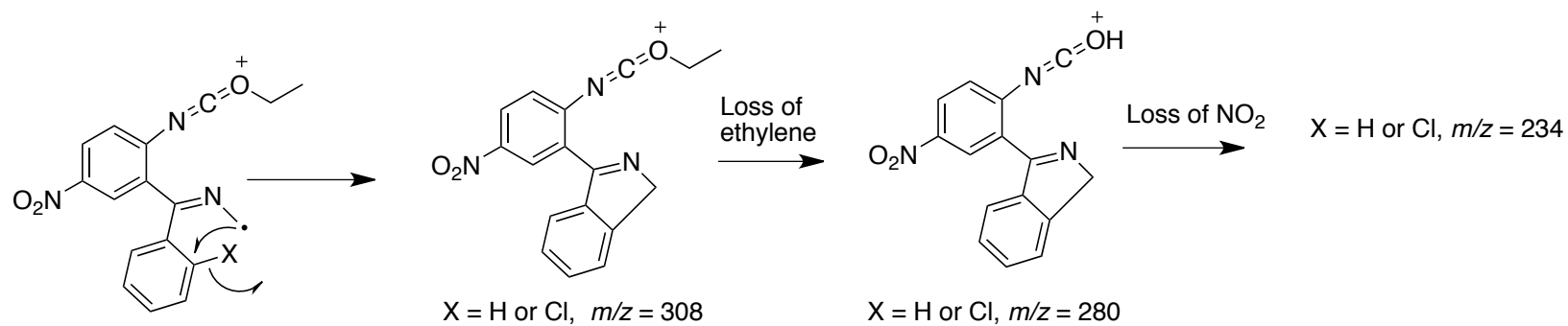
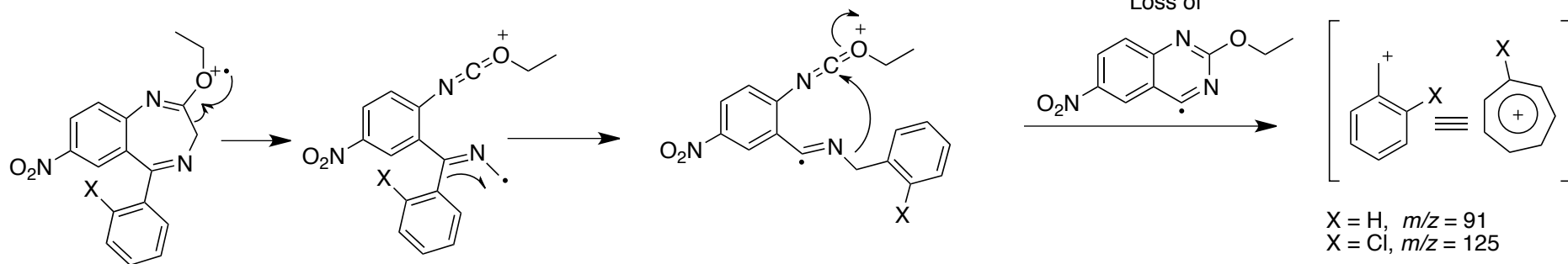


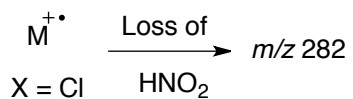
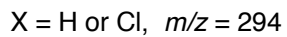
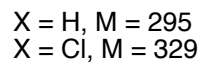






(i).

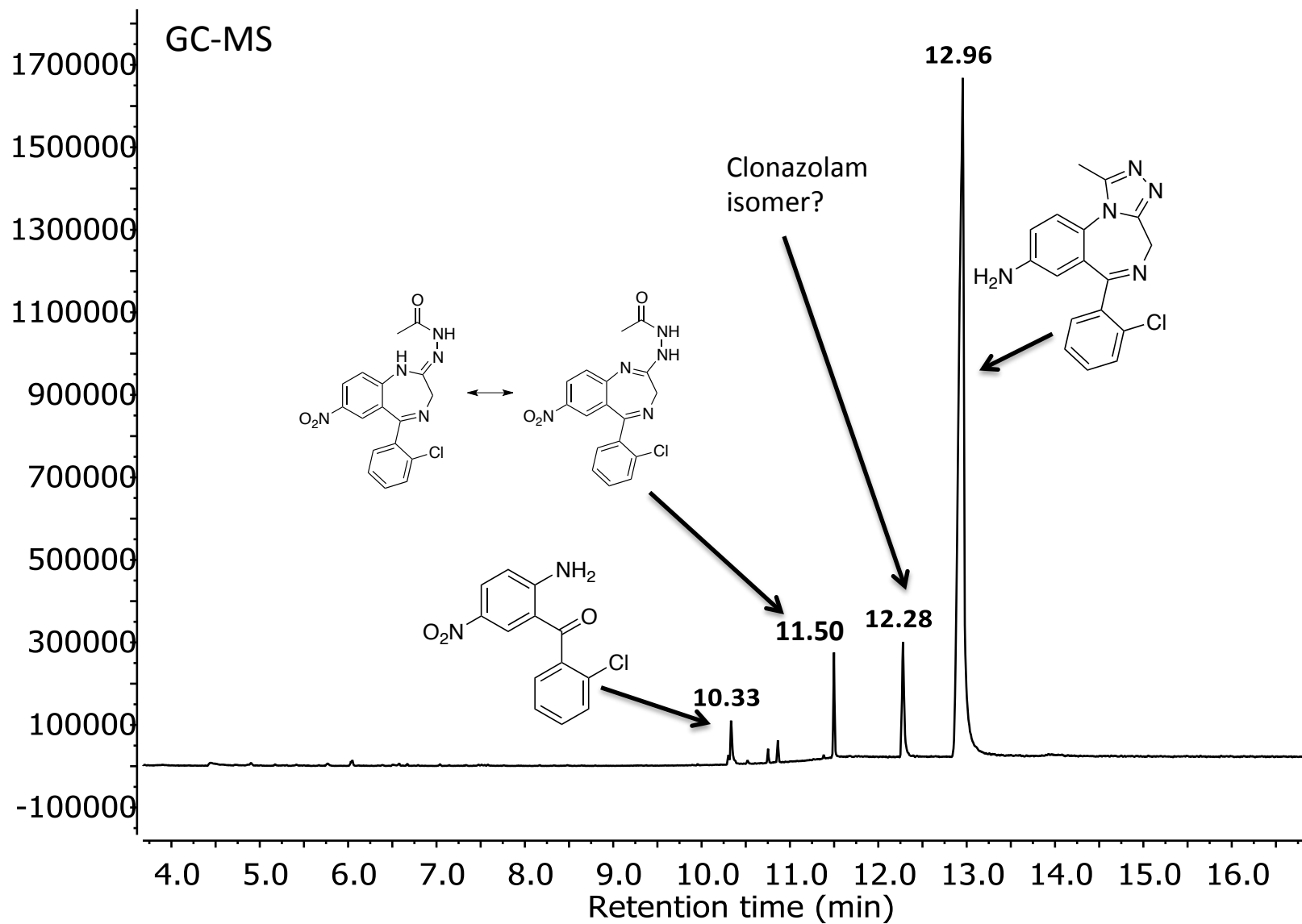


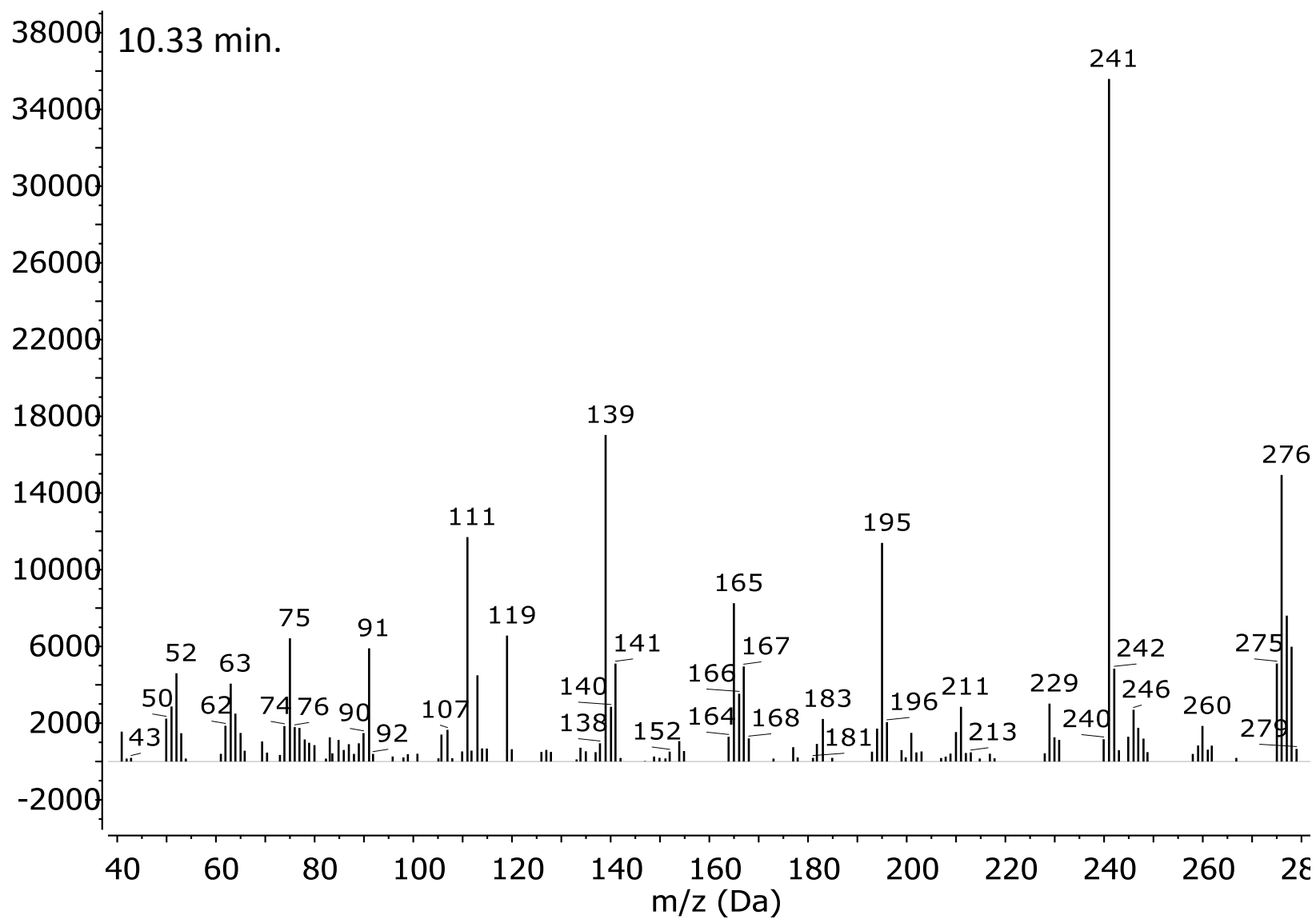


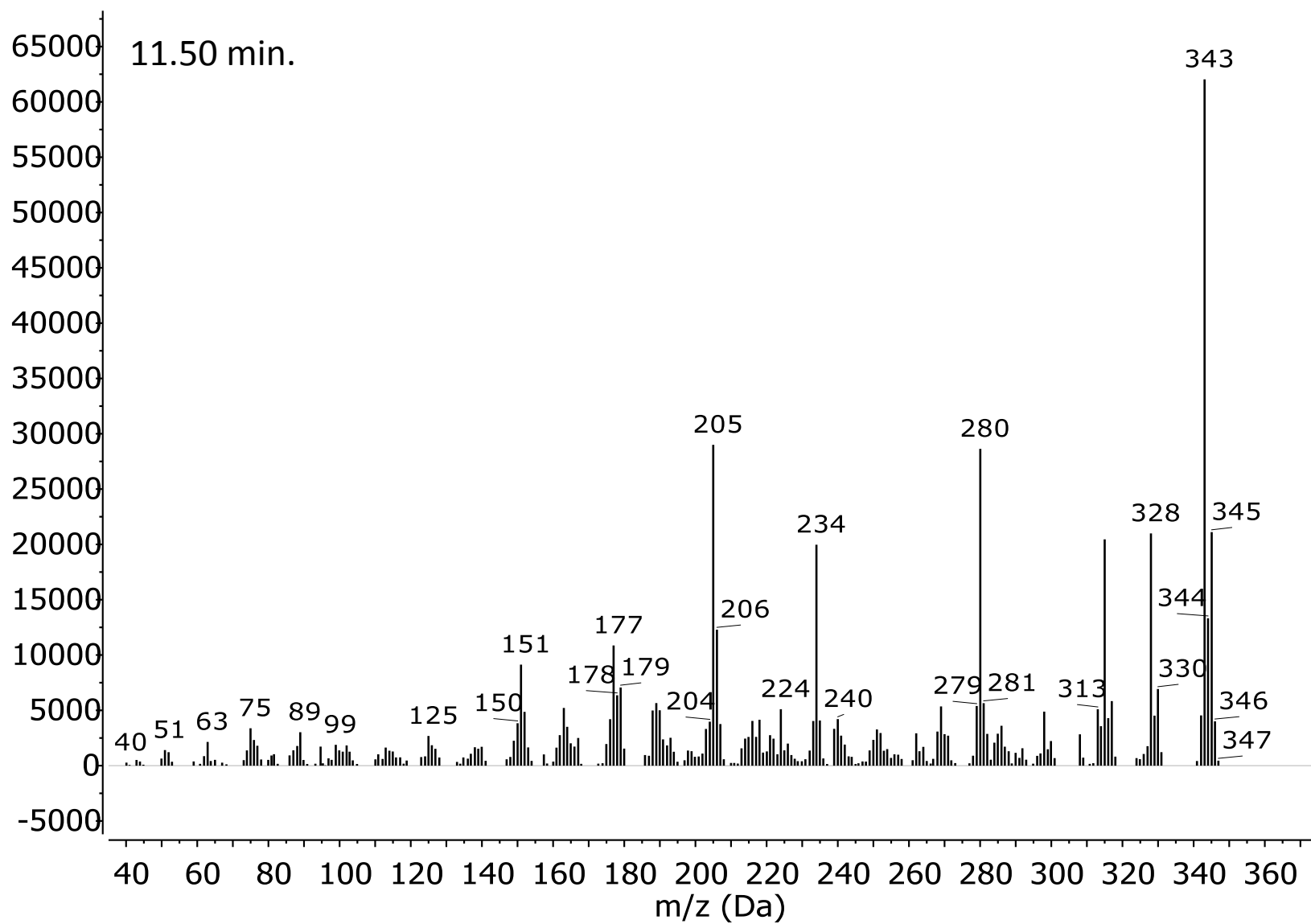
Supporting information 7

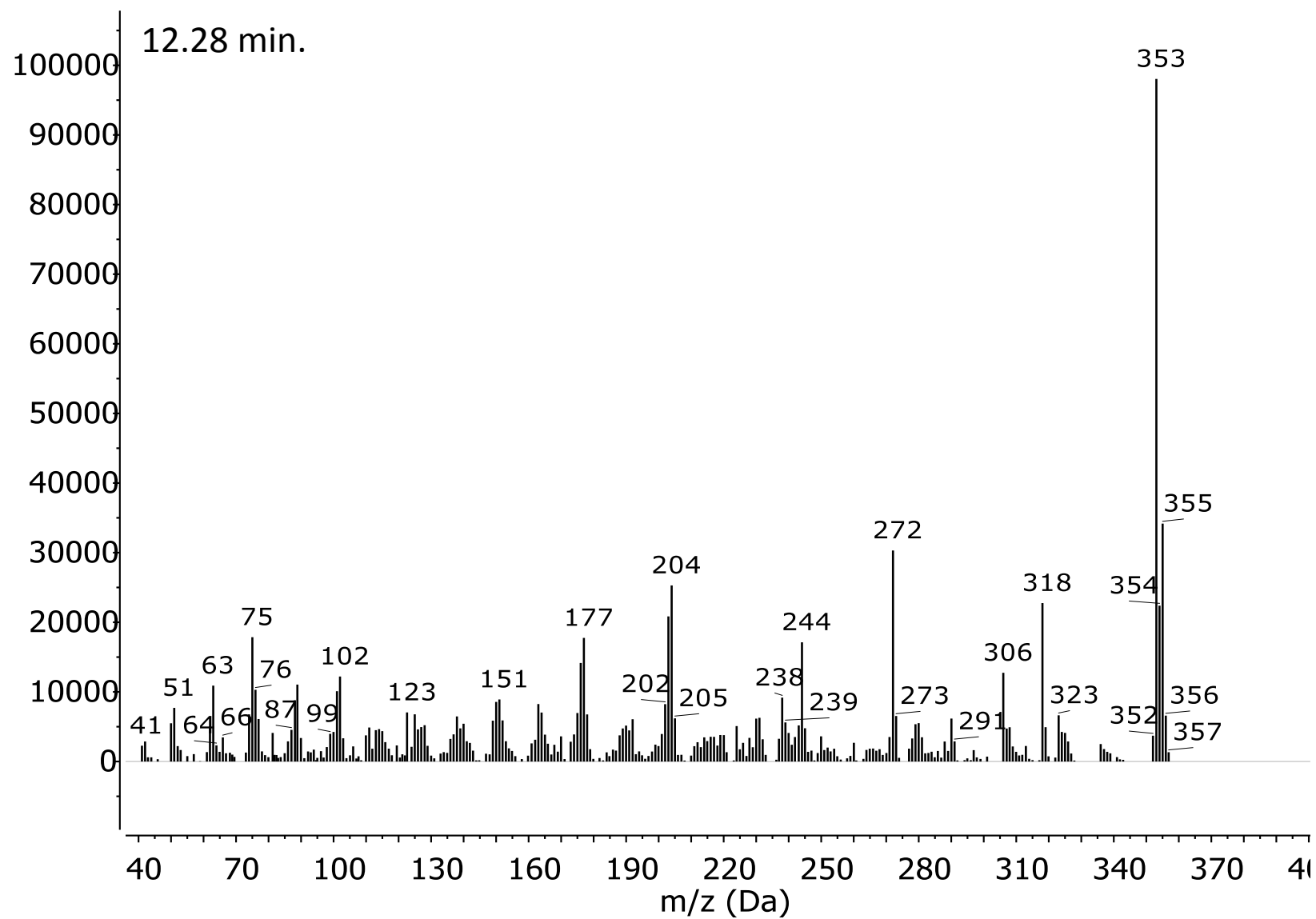
Clonazolam – crude product – preparative scale

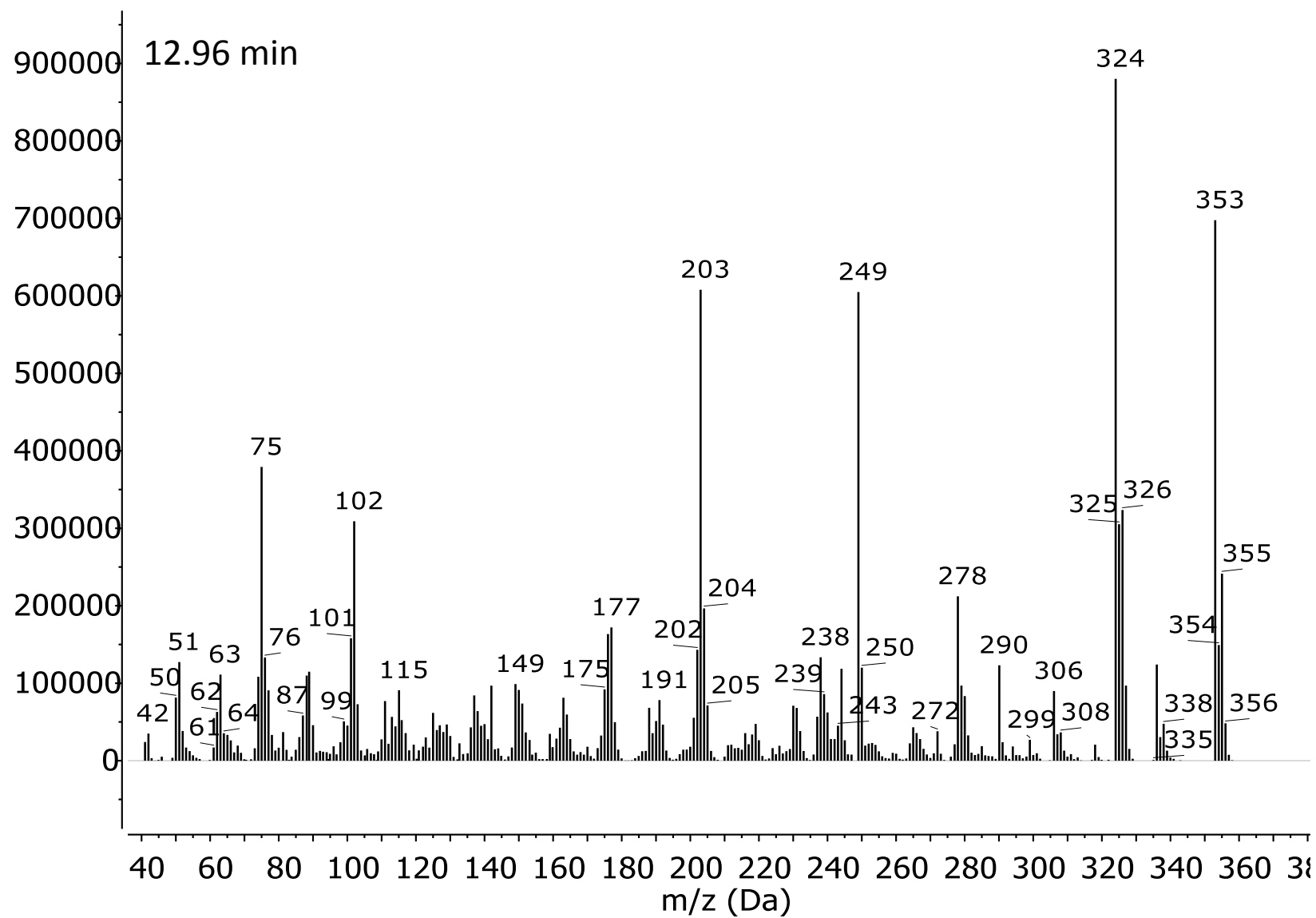
GC-MS



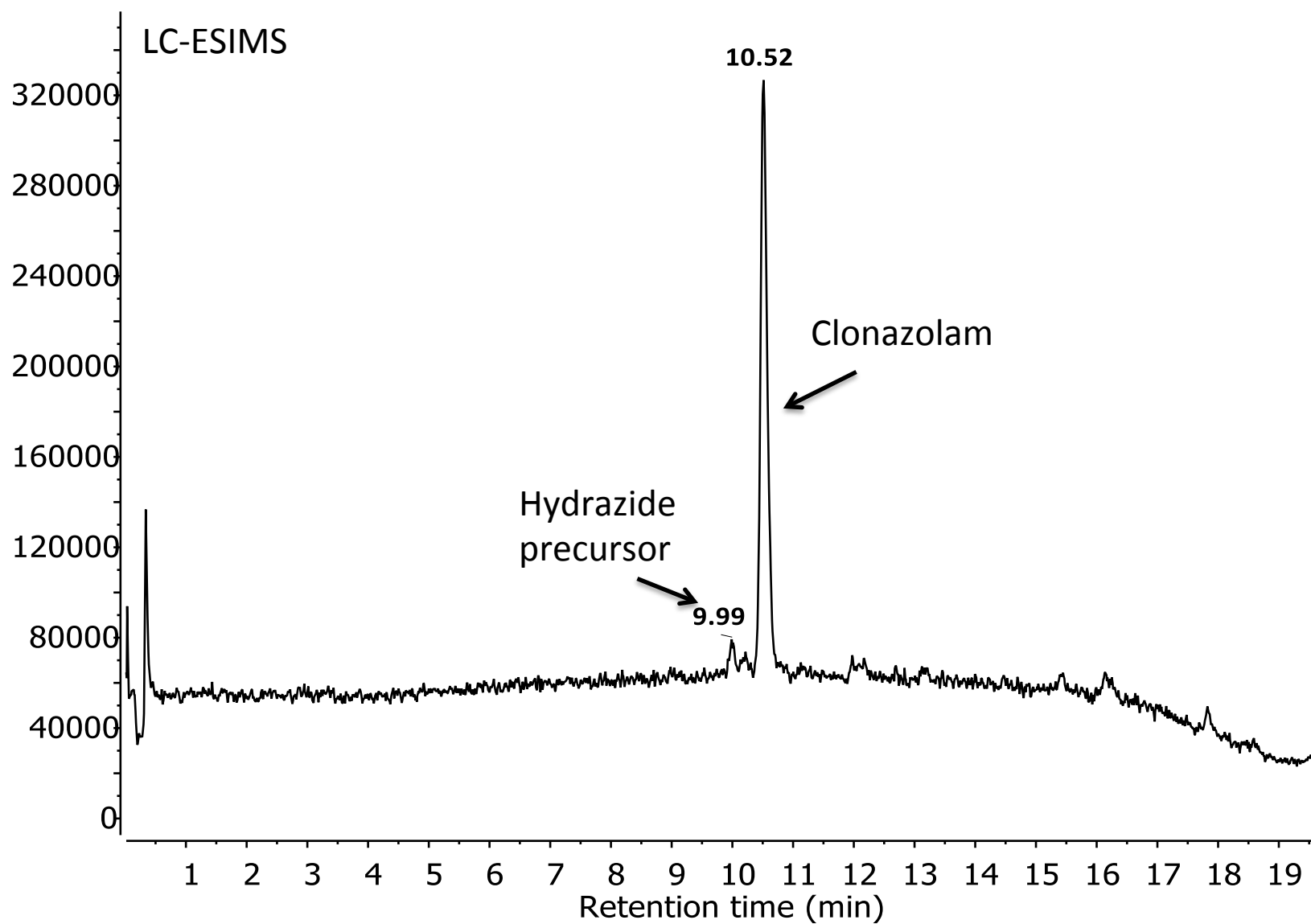


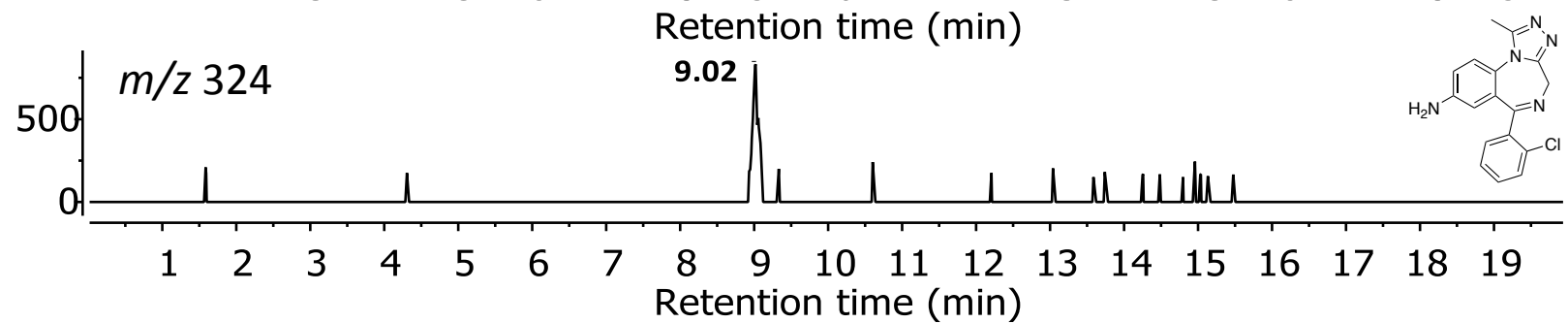
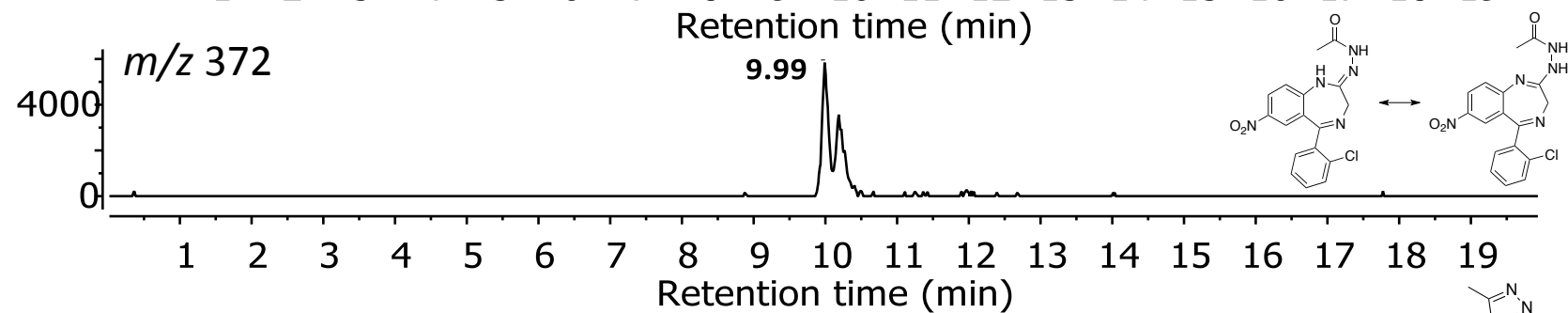
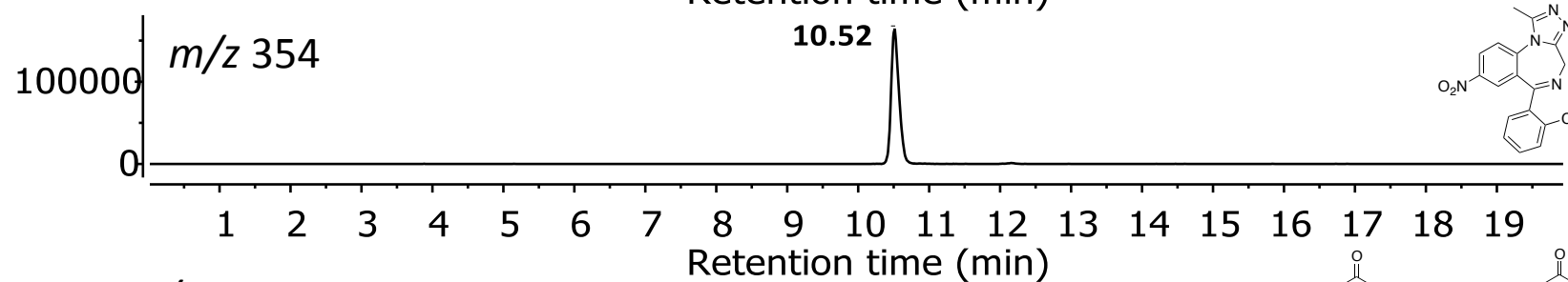
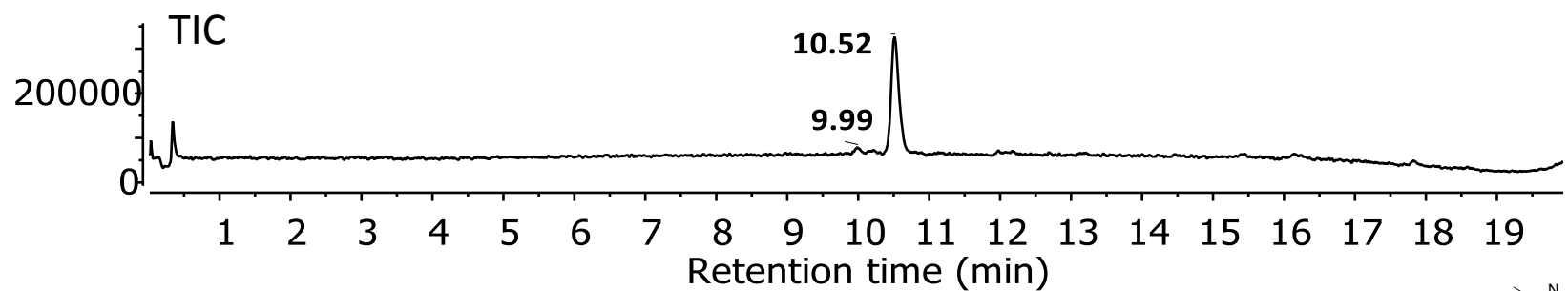


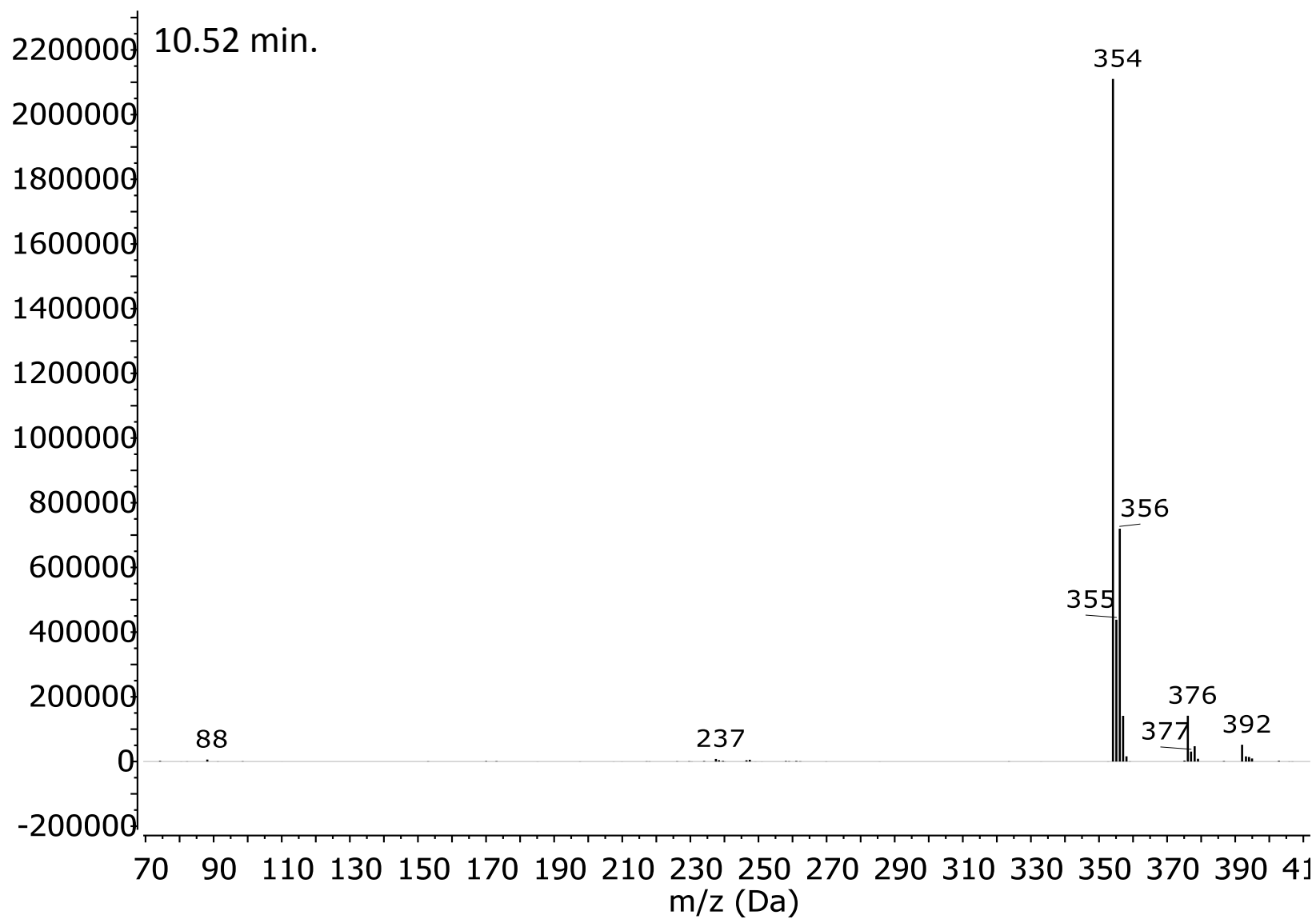


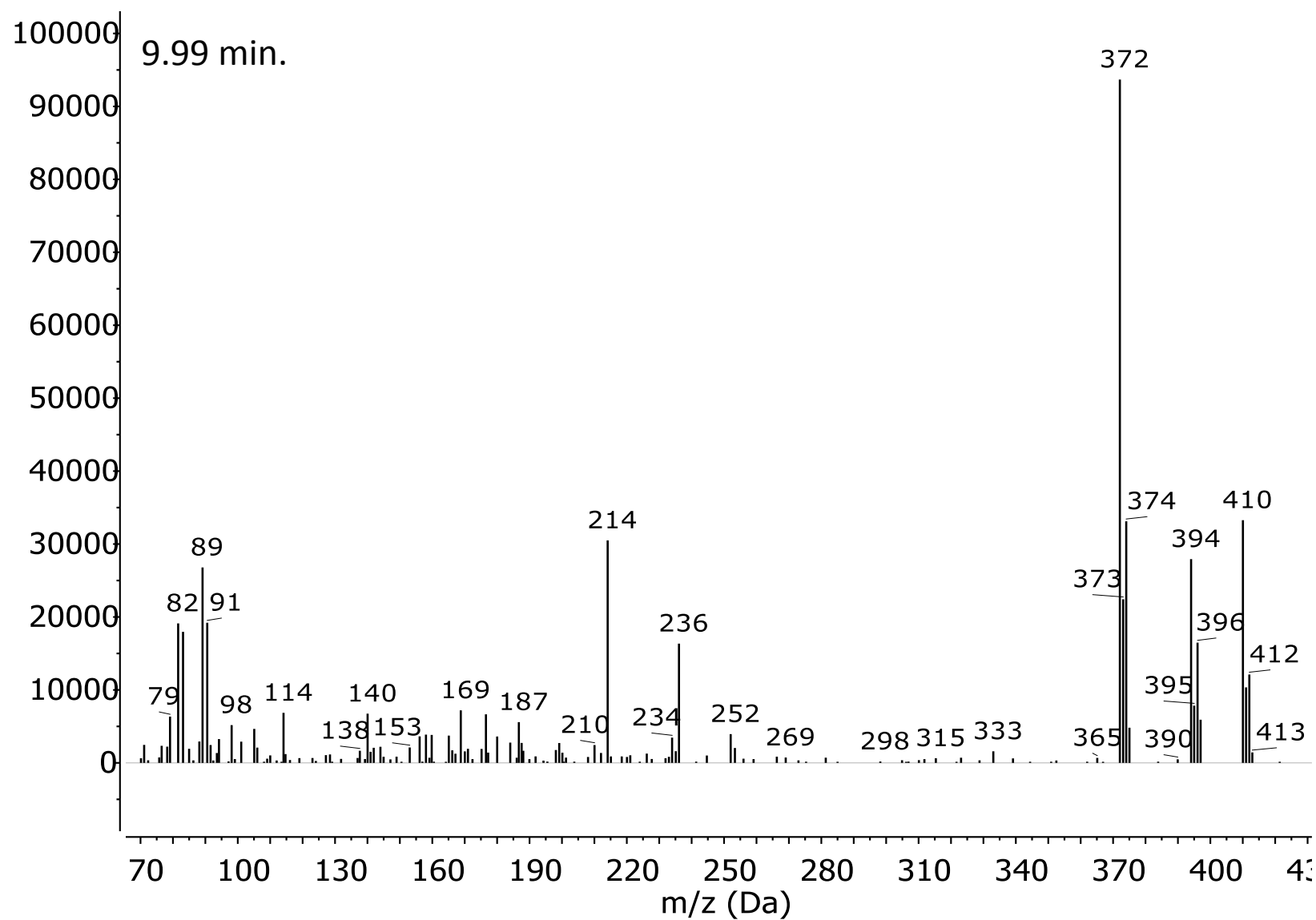


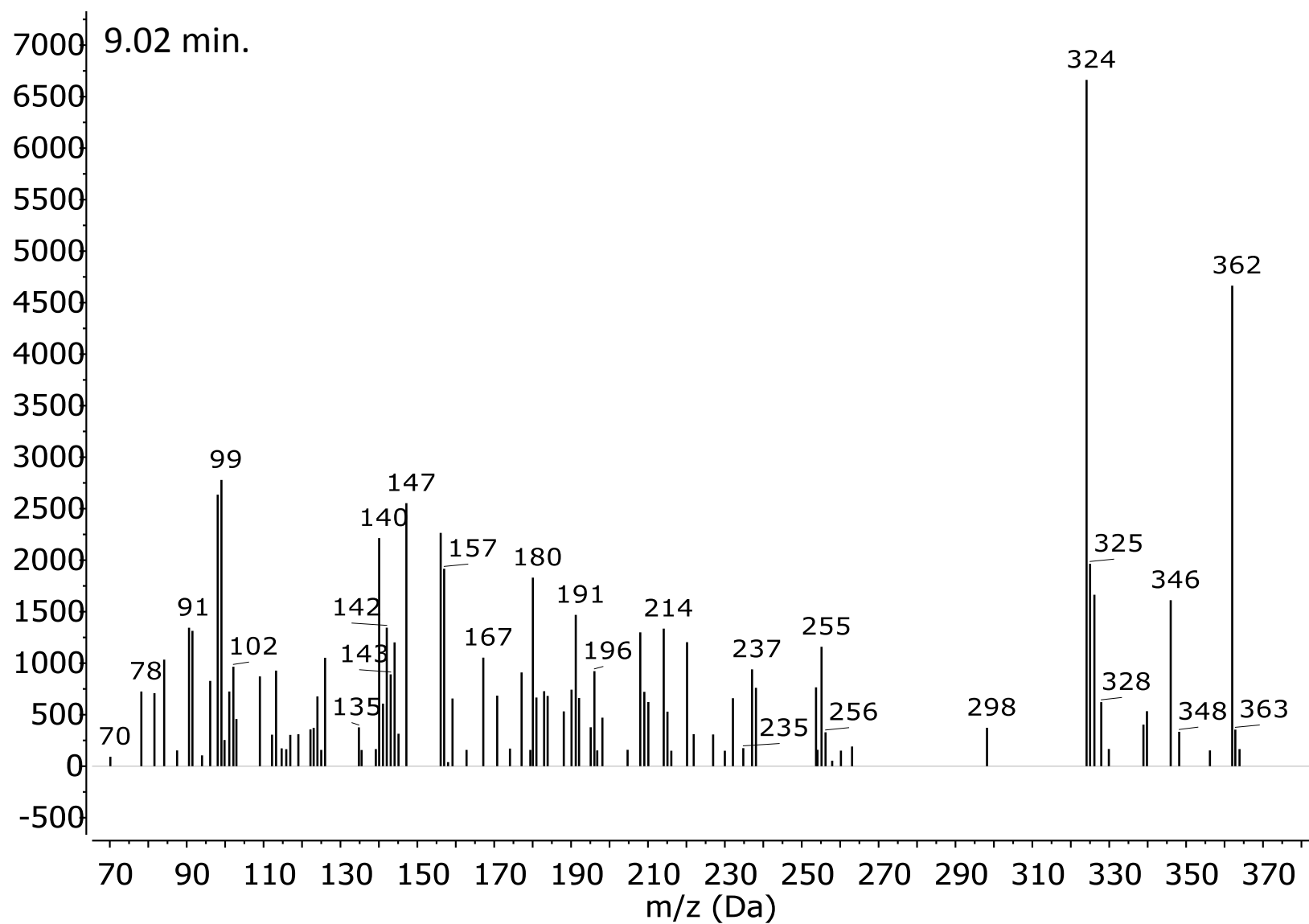
Clonazepam – crude product – preparative scale



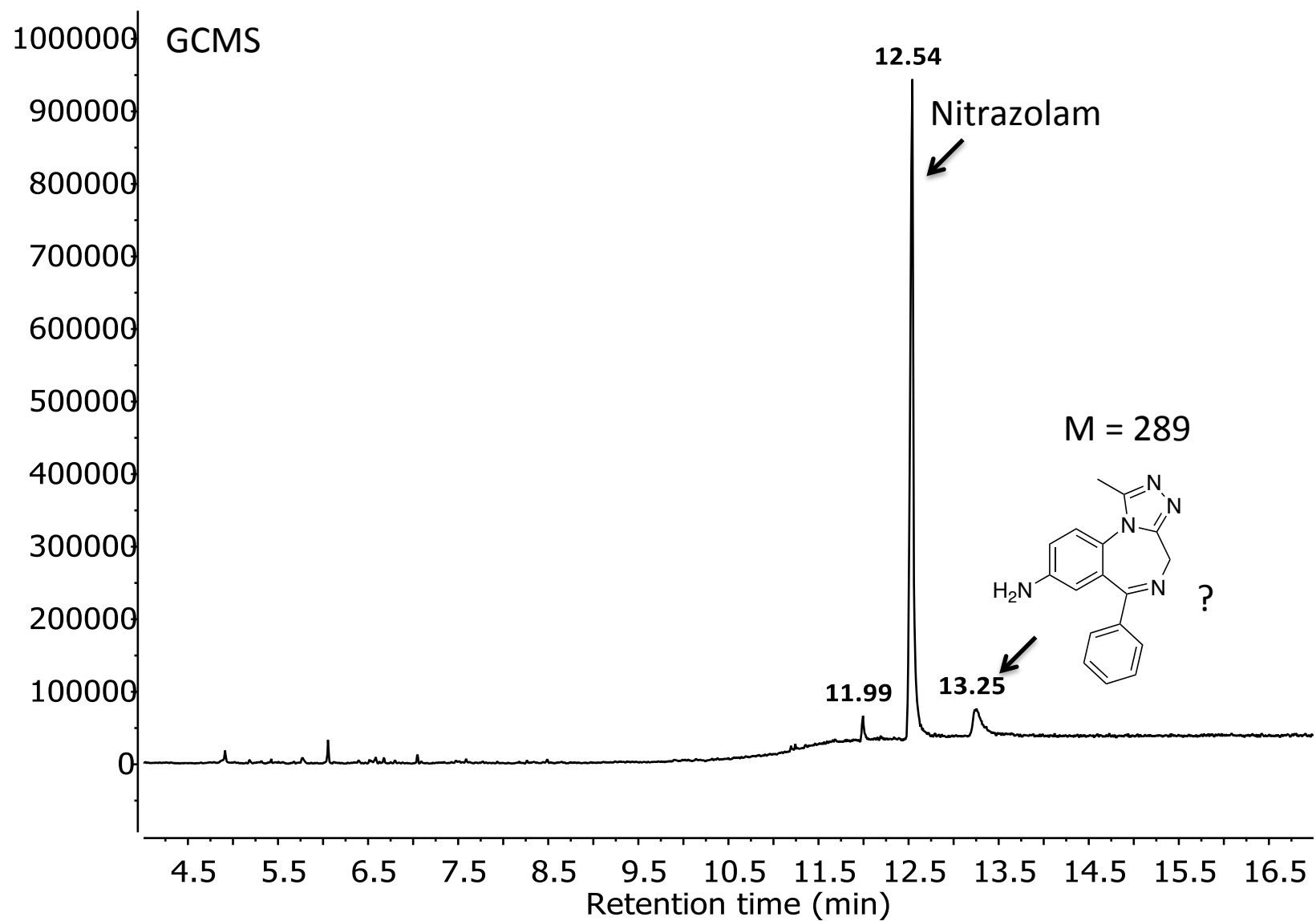


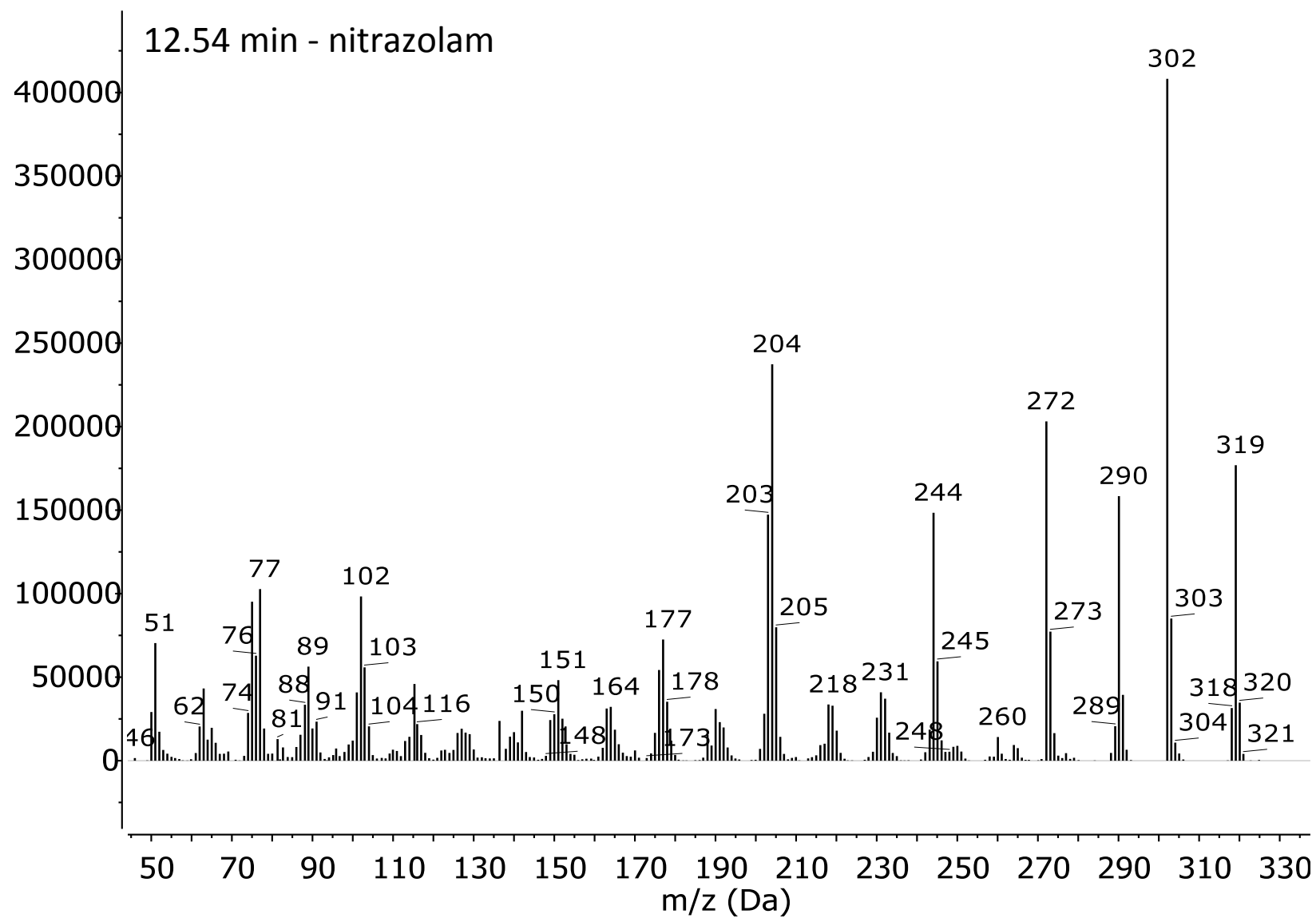


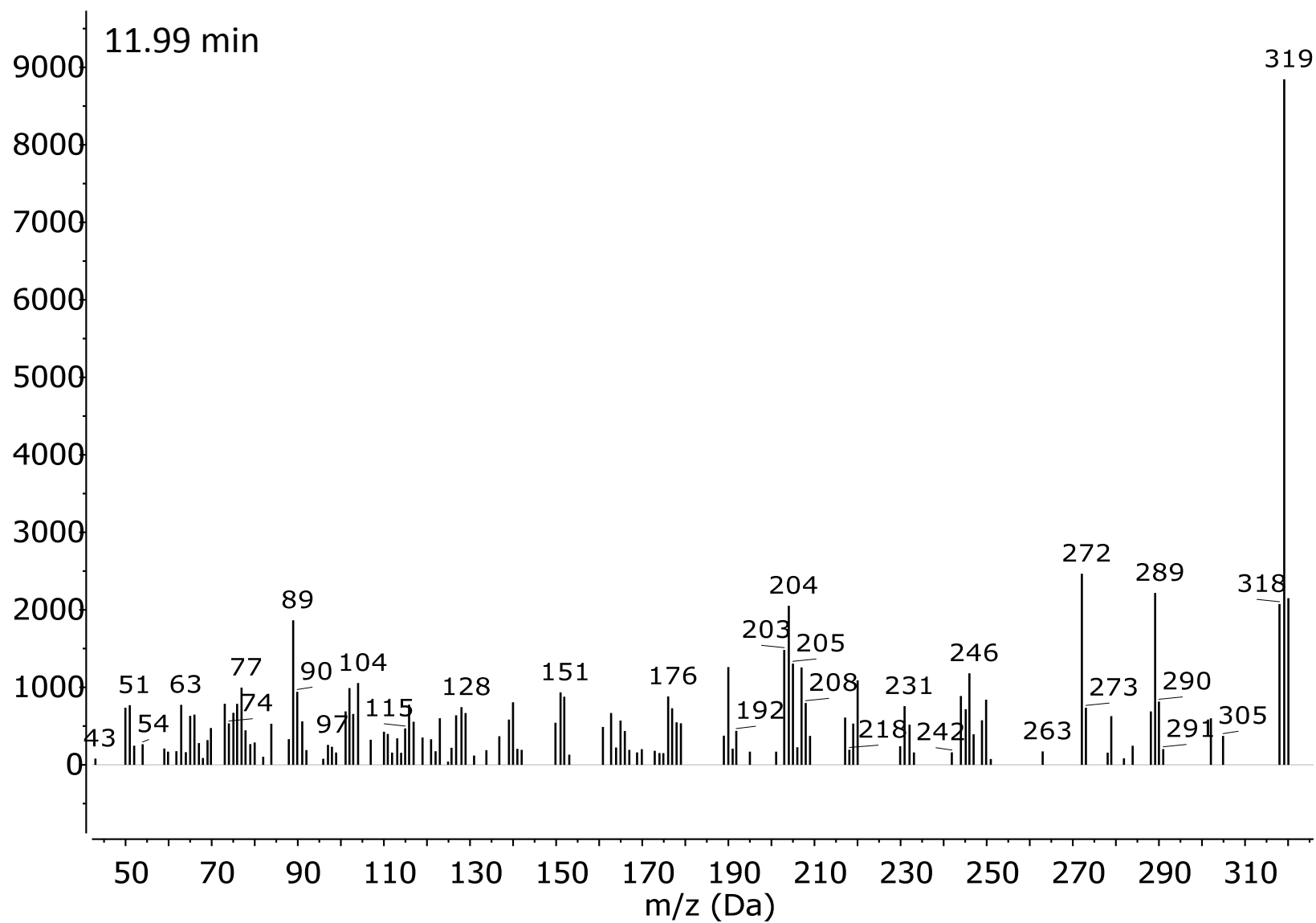


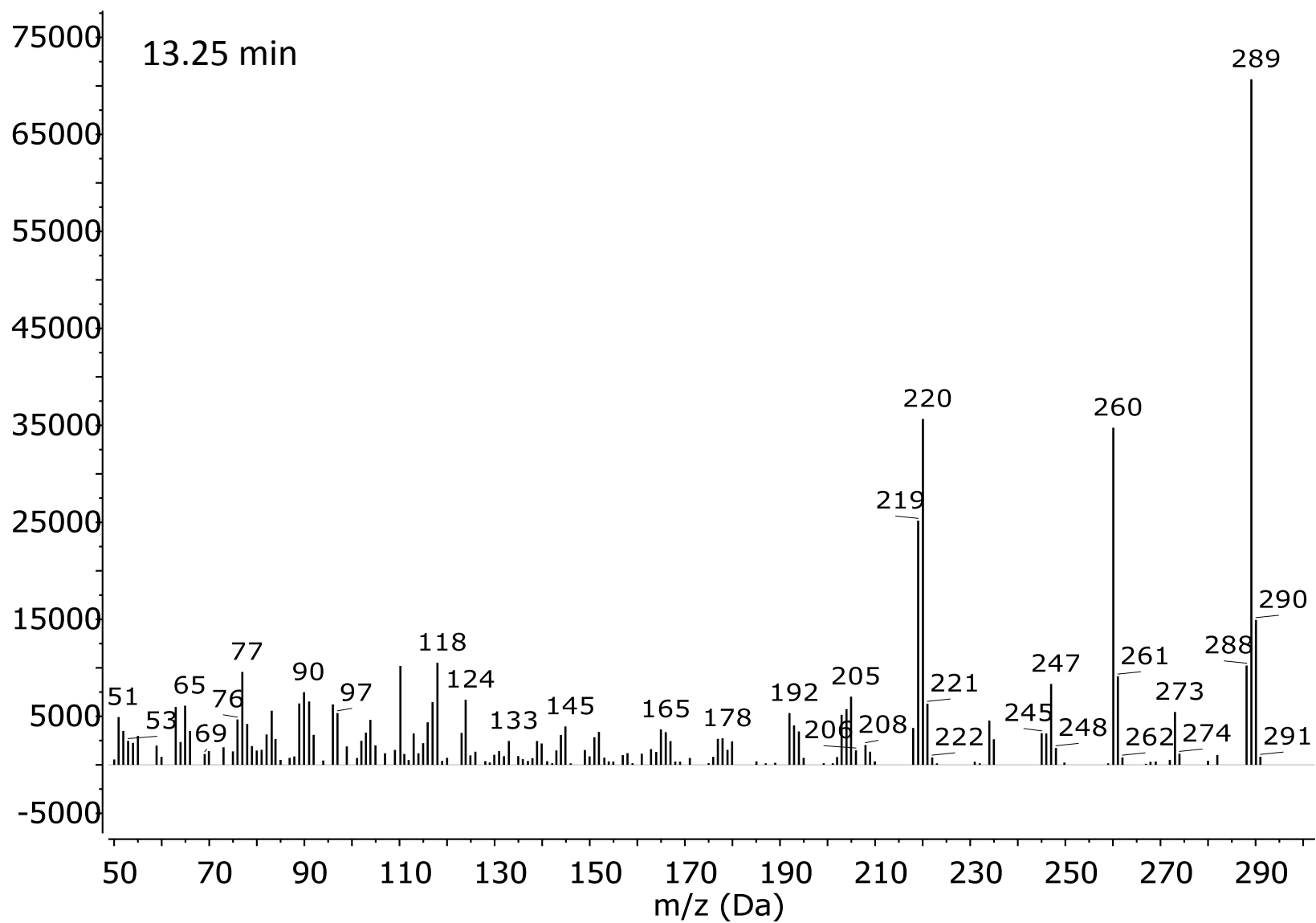


Nitrazolam preparative scale - crude









Nitrazolam preparative scale - crude

