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Dowling, G, Kavanagh, PV, Eckhardt, HG, Twamley, B, Hessman, G, McLaughlin, G, O' Brien, J and Brandt, SD

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 clonazolam, for use as reference materials, utilizing polymer supported reagents

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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 clonazolam, 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-^{1}H and ^{1}H-^{1}H 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) ^{1}H NMR spectrum for extract from clonazolam tablets.

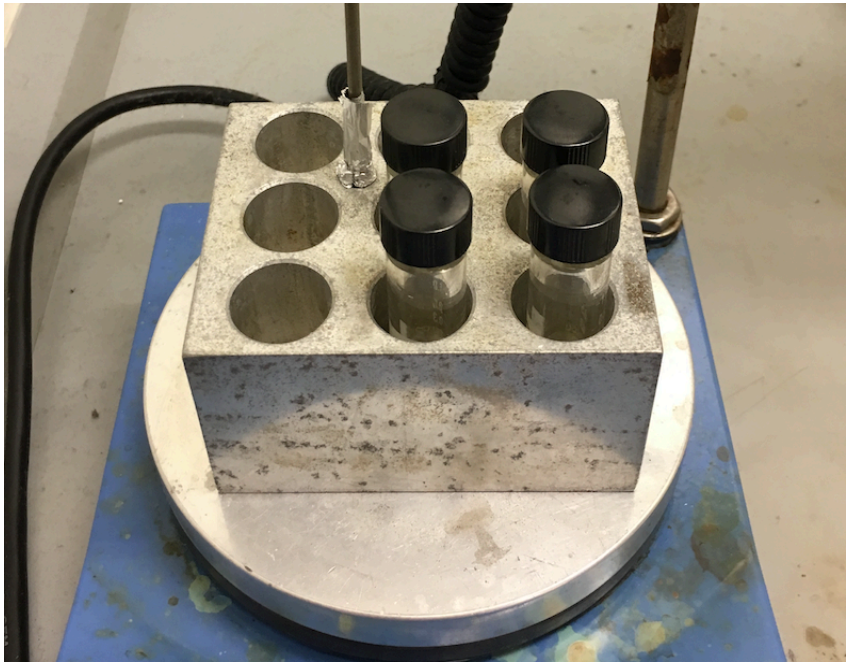
Supporting information 4: Single crystal x-ray diffraction (x-ray crystallography data) for clonazolam.

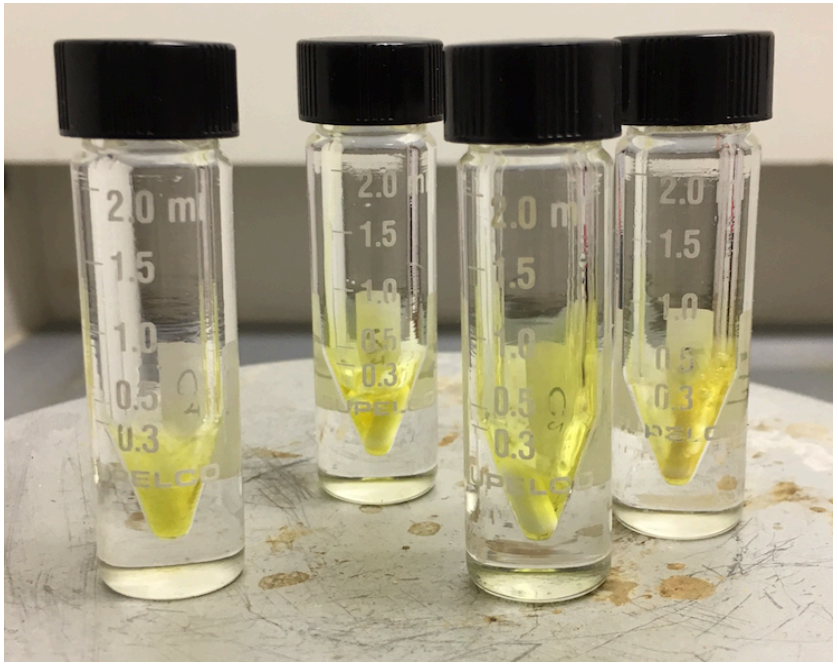
Supporting information 5: Quantification of clonazolam in tablets.

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





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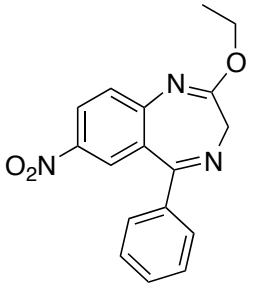
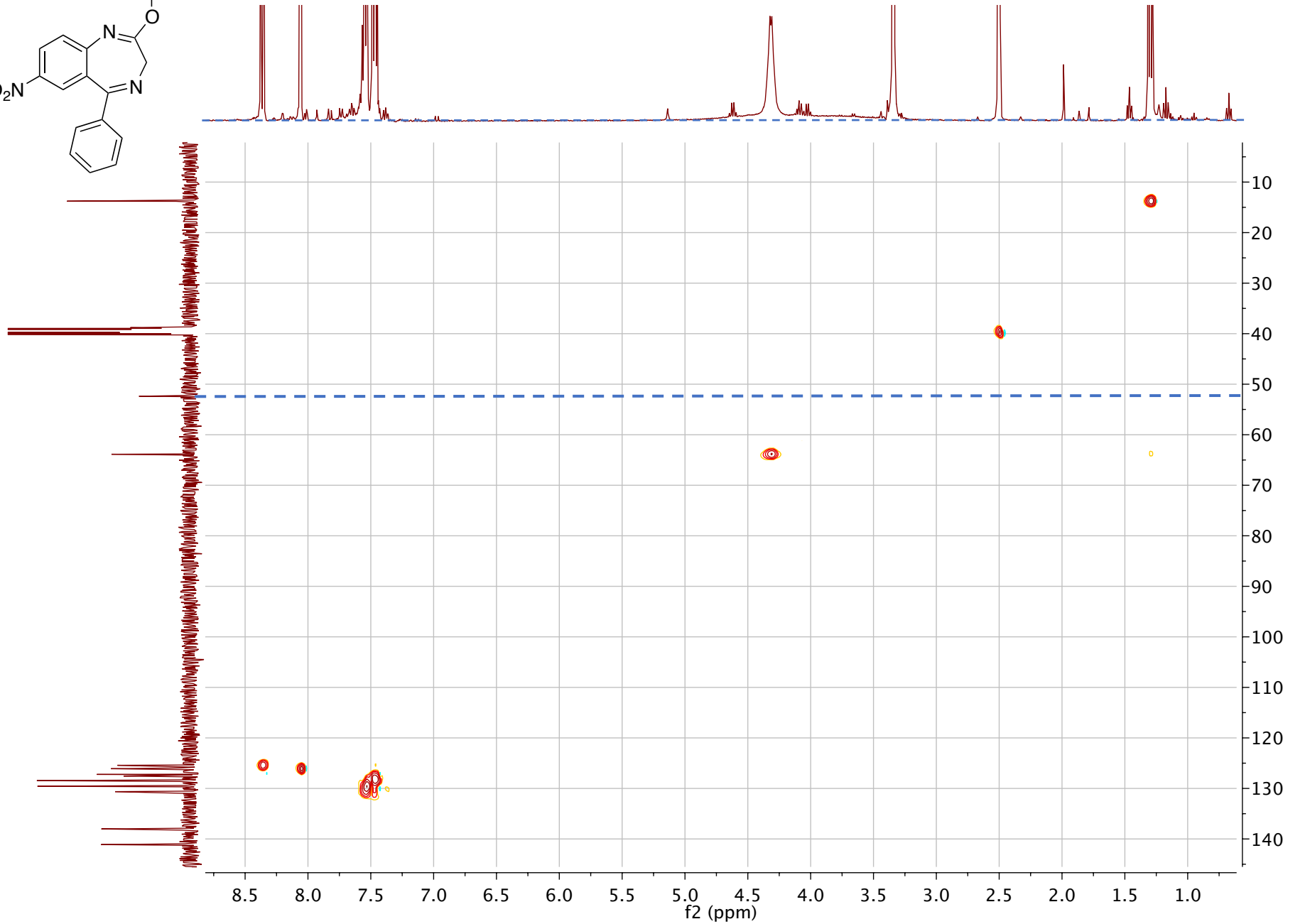
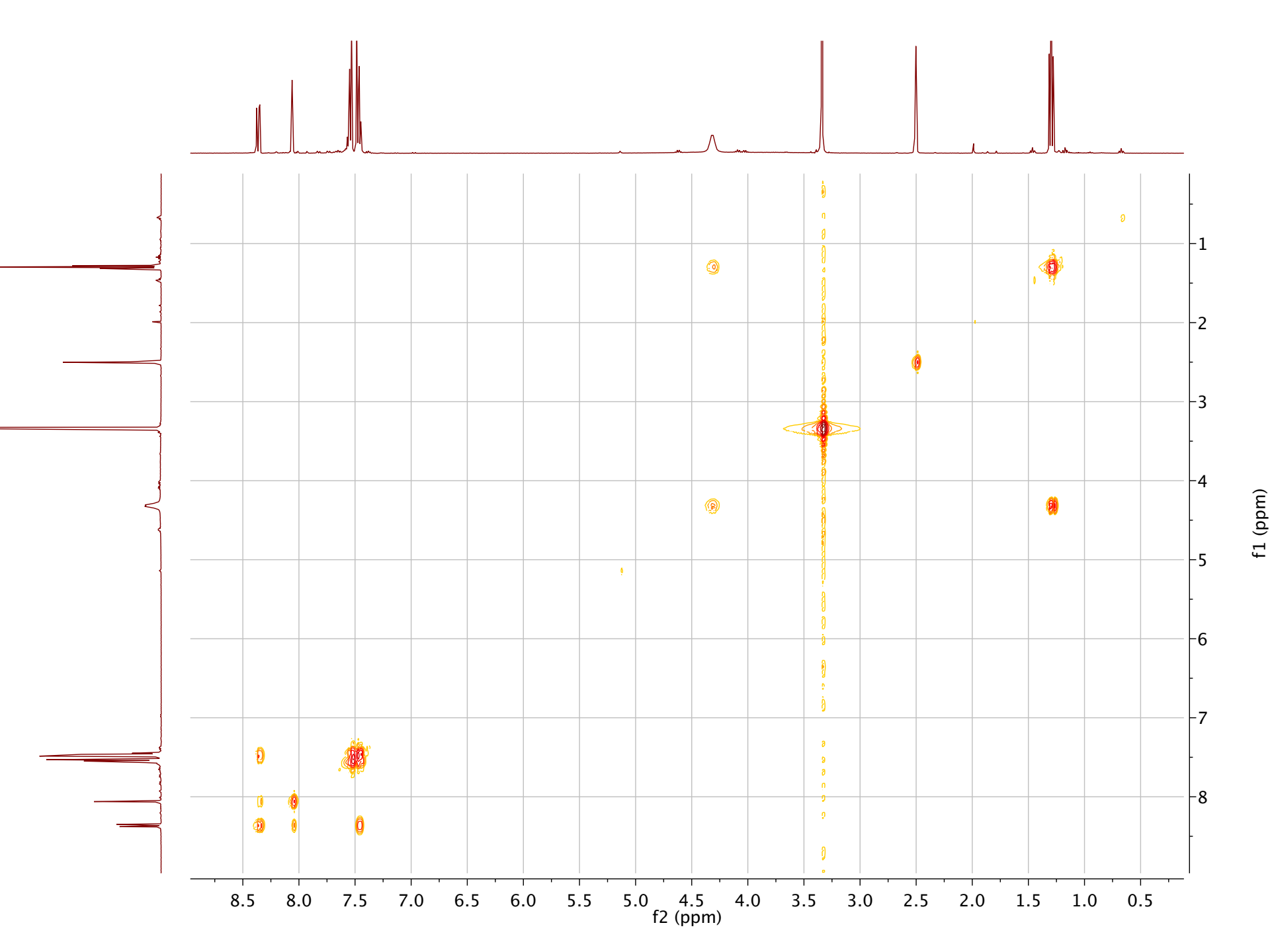
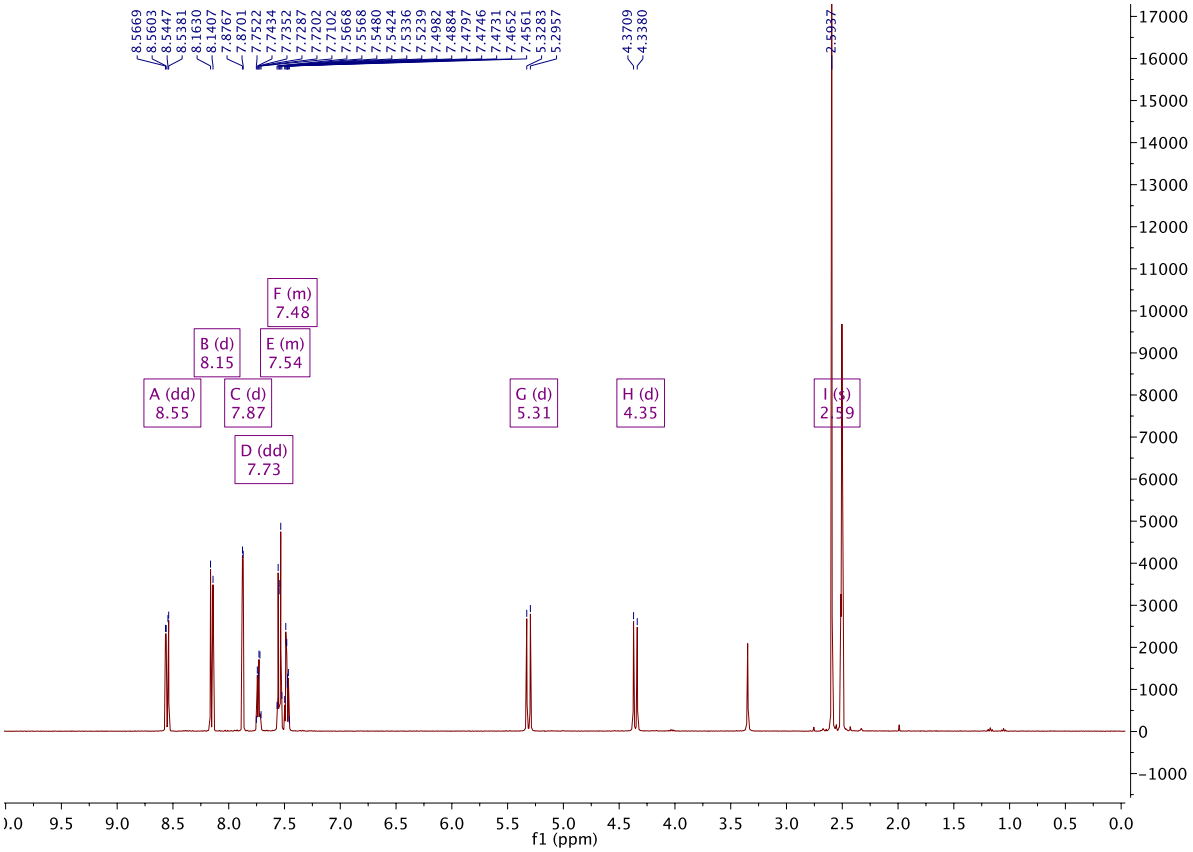


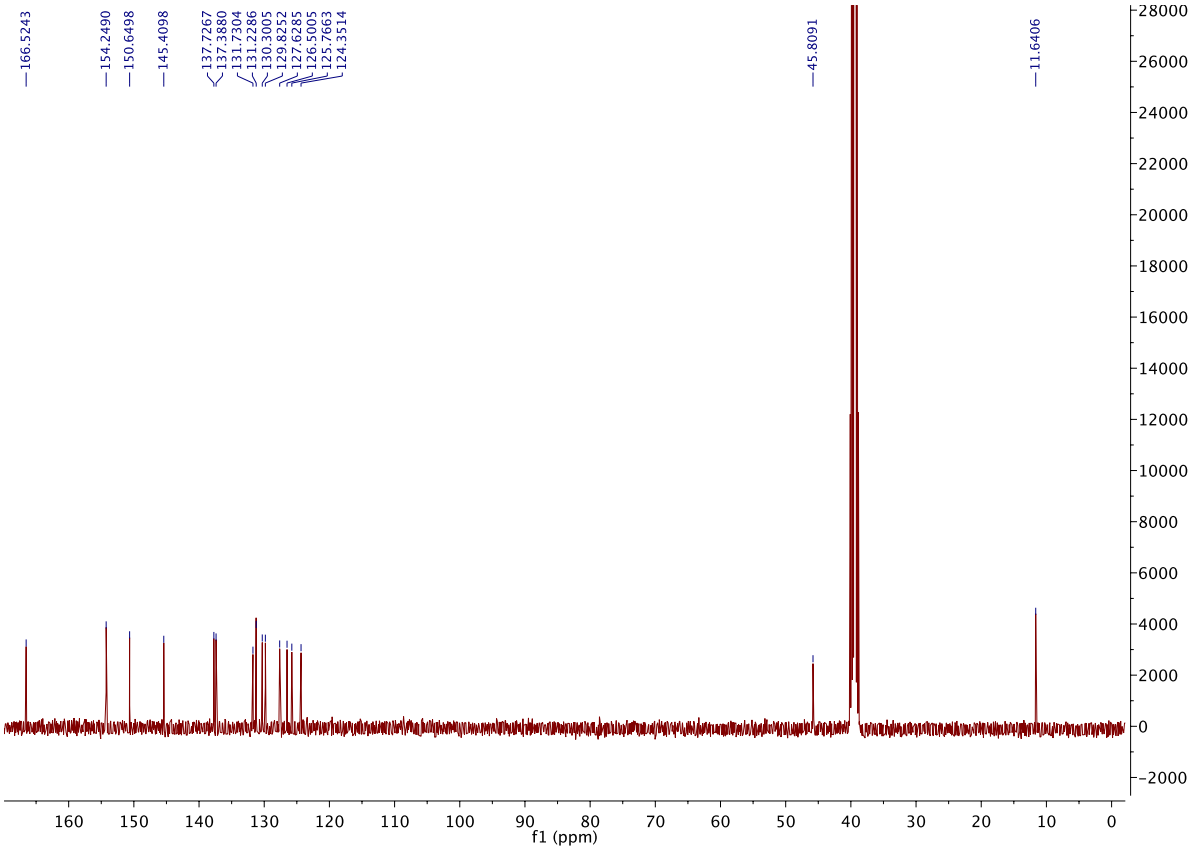


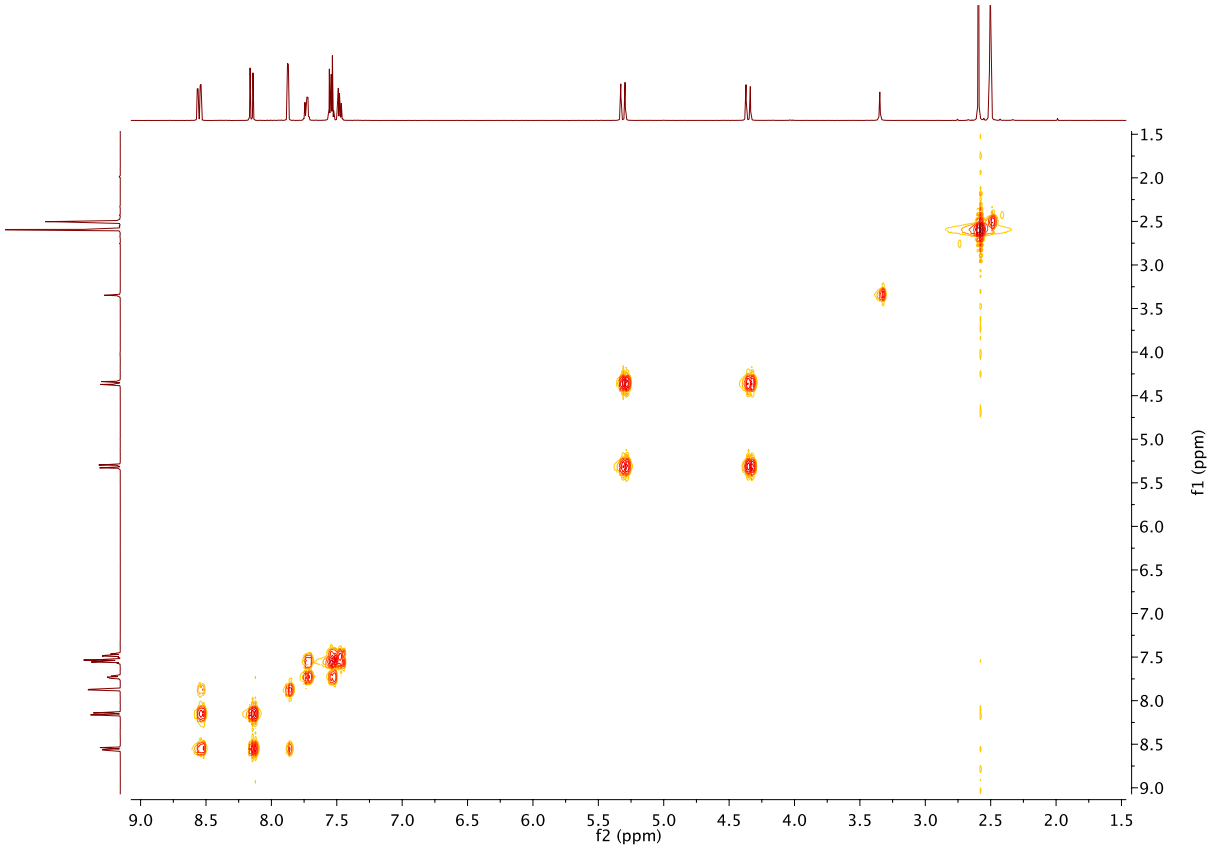

8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
1
2
3
4
5
6
7
8
f1 (ppm)

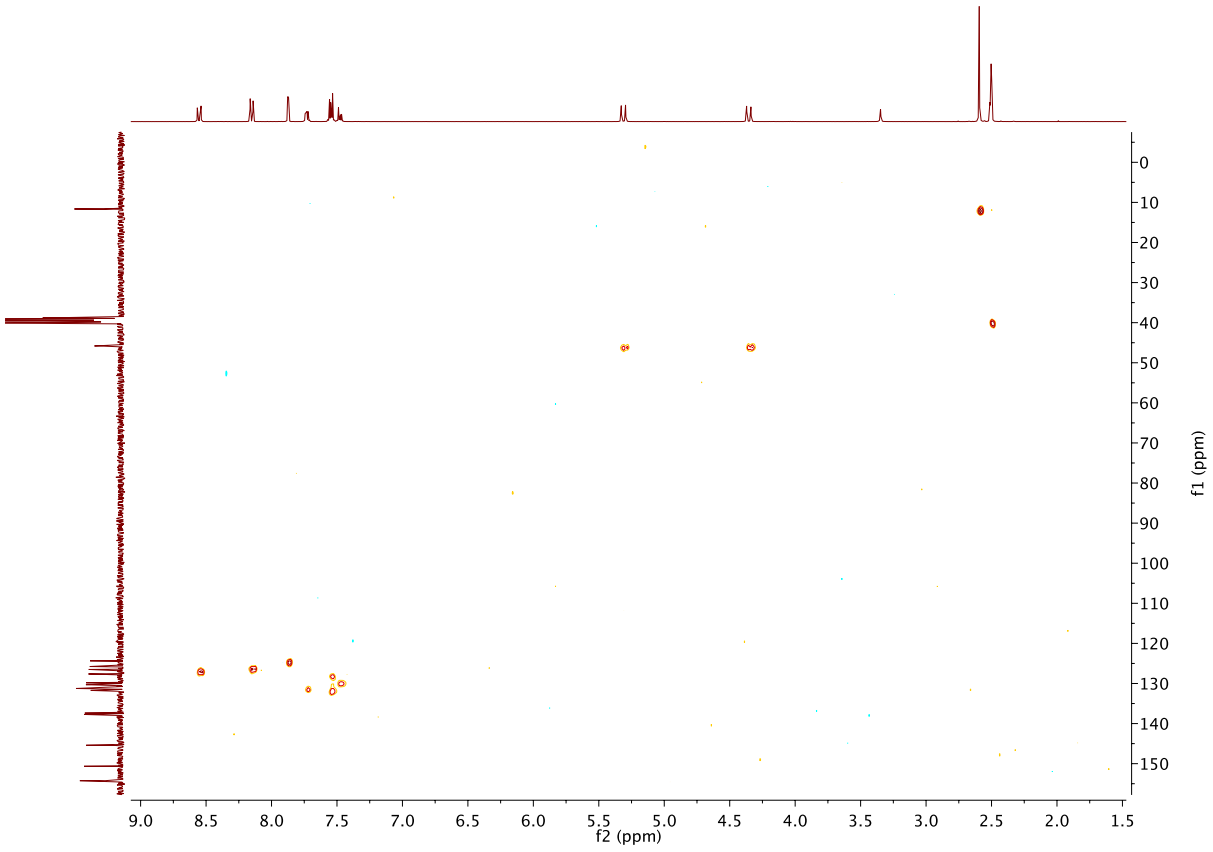
Supporting information

Clonazolam (powder, vendor sample) NMR data



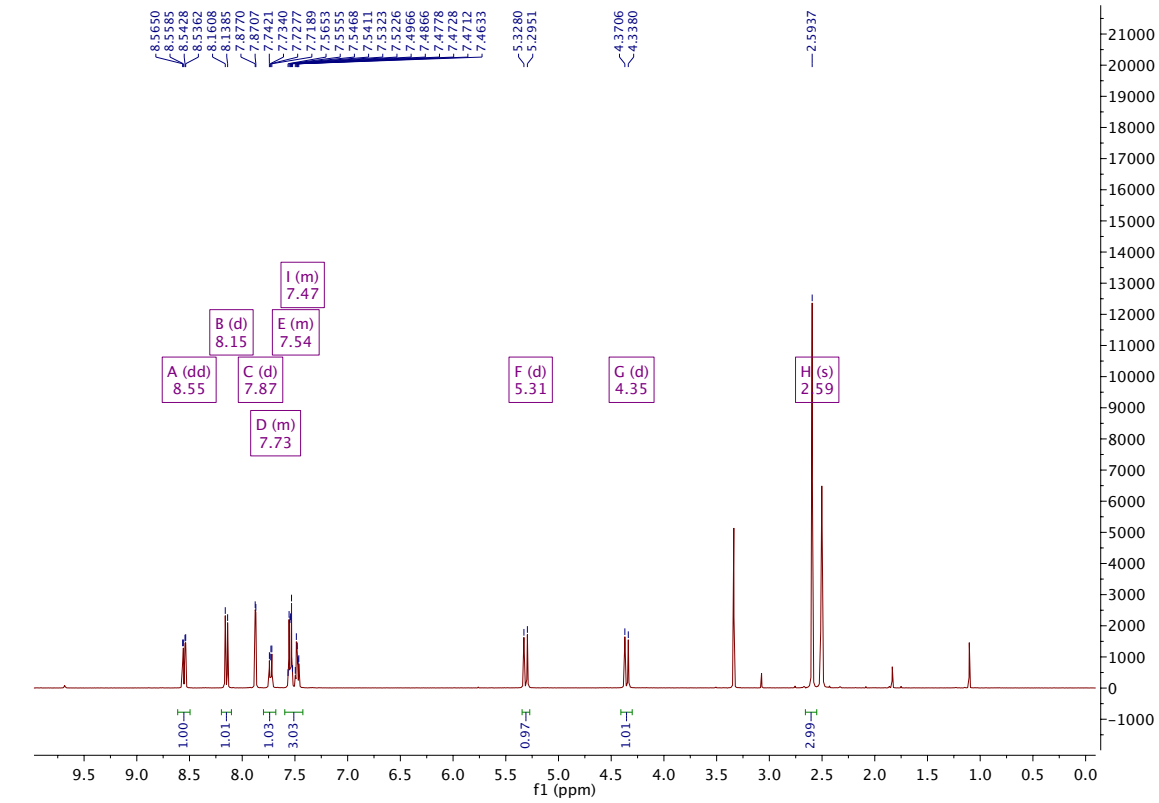



f2 (ppm)
f1 (ppm)

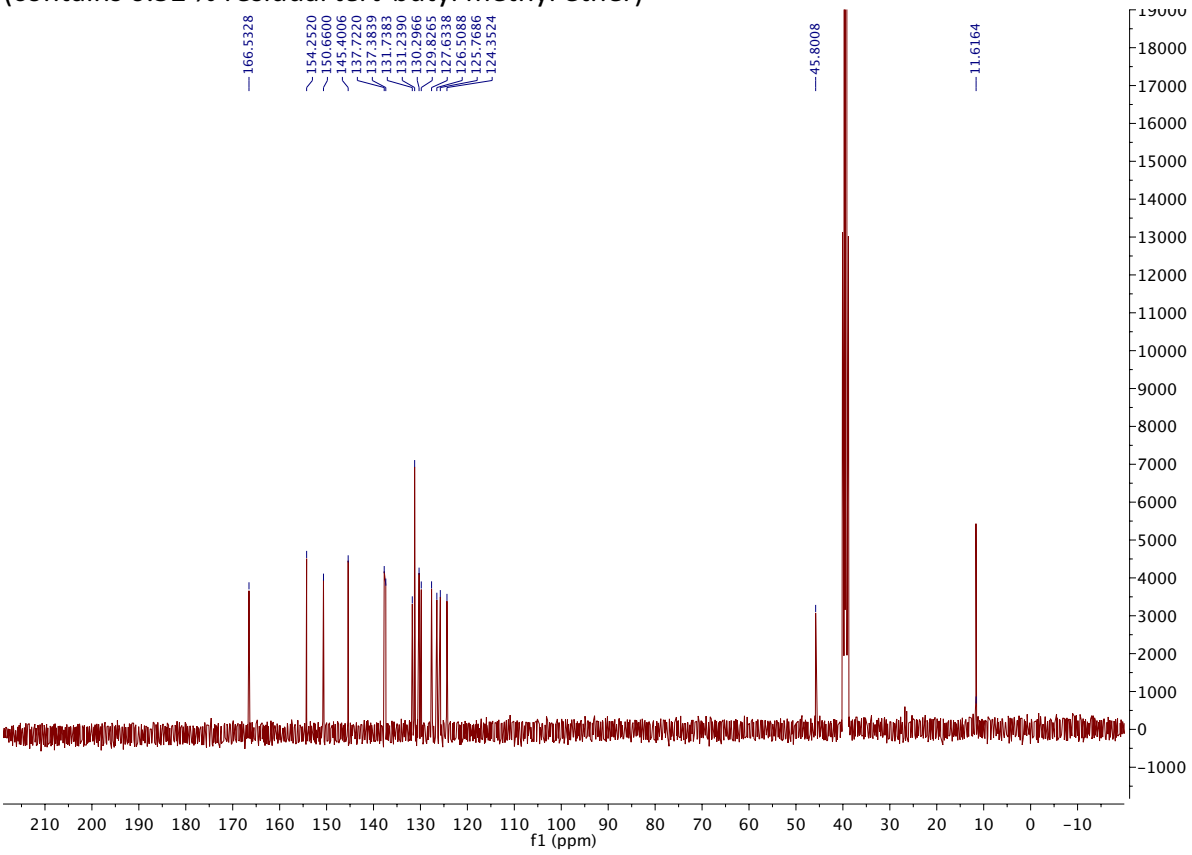

f2 (ppm)
f1 (ppm)

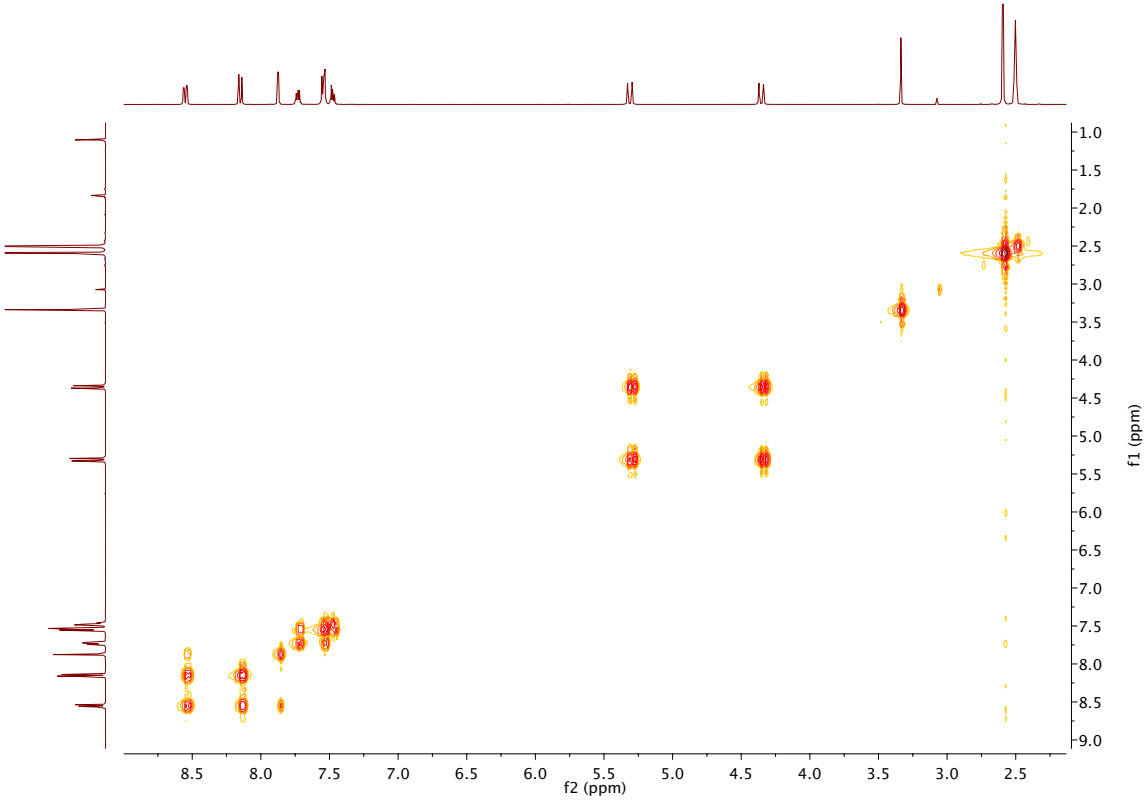
Clonazolam (powder, vendor sample): a light yellow powder: m.pt 222-224 °C; ^{1}H NMR (400 MHz, d_6 DMSO) δ 8.55 (dd; *J* = 8.9, 2.6 Hz; 1 H; Ar H-5), 8.15 (d; *J* = 8.9 Hz; 1 H; Ar H-6), 7.87 (d; *J* = 2.6 Hz; 1 H; Ar H-3), 7.71 - 7.75 (m; 1 H Ar H-3'), 7.59 – 7.52 (m; 2 H; Ar H-4' and 5'), 7.51 – 7.45 (m; 1 H; Ar H-6'), 5.31 (d; *J* = 13.1 Hz; 1 H; one H from CH_2), 4.35 (d; *J* = 13.2 Hz; 1 H; one H from CH_2), 2.59 (s; 3 H; CH_3). ppm; ^{13}C NMR (101 MHz, d_6 DMSO) δ 166.52 (Ar-C=N), 154.25 (CH_2-C=N), 150.65 (CH_3-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 (CH_2), 11.64 (CH_3) ppm; HRMS found, 354.0753 $[M+H]^+$, $C_{17}H_{13}ClN_5O_2$, requires 354.0752 (Δ = 0.3 ppm).

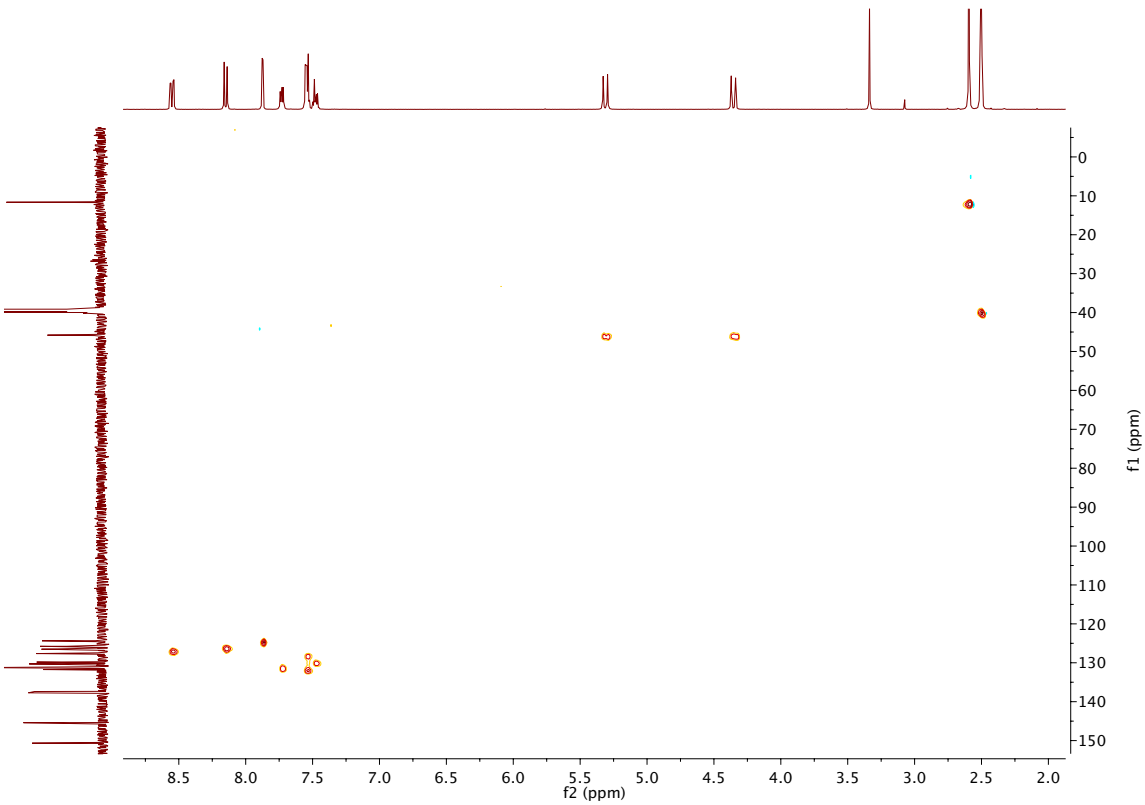
Clonazolam (synthesized) – NMR data



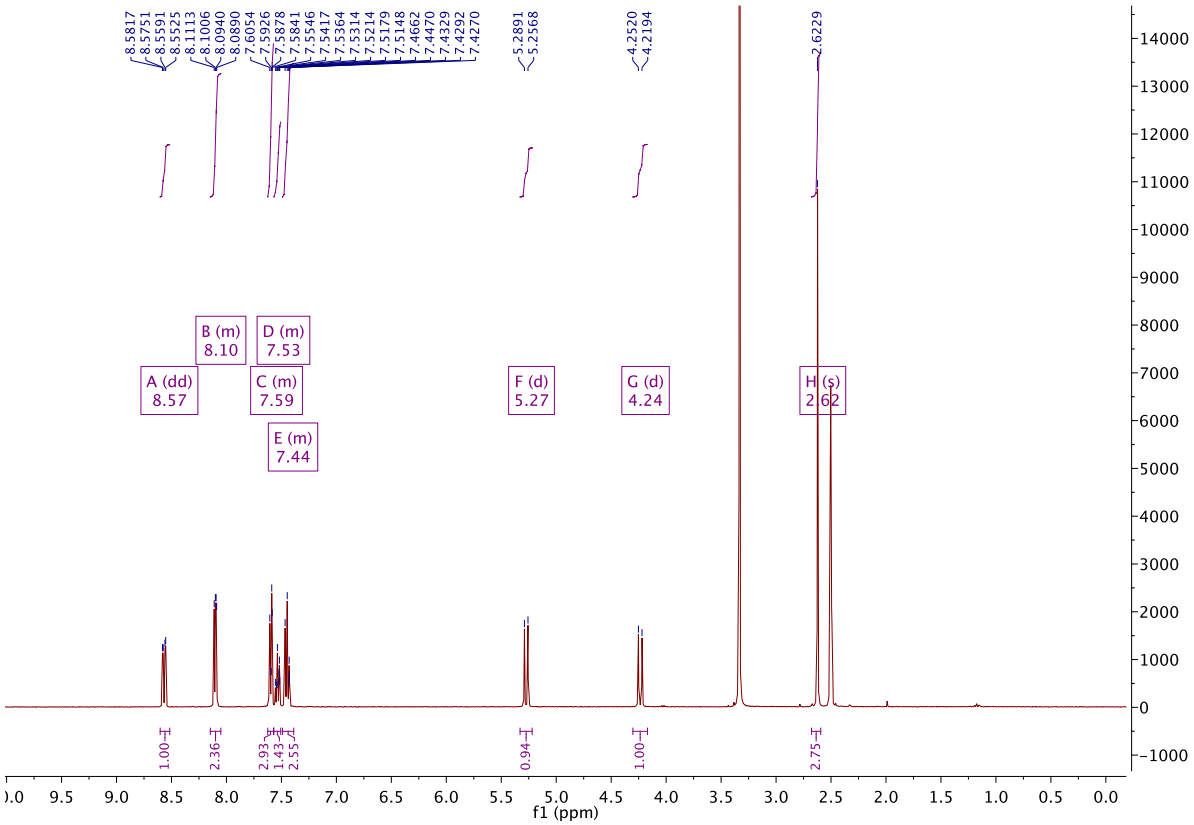
(contains 0.52 % residual *tert*-butyl methyl ether)

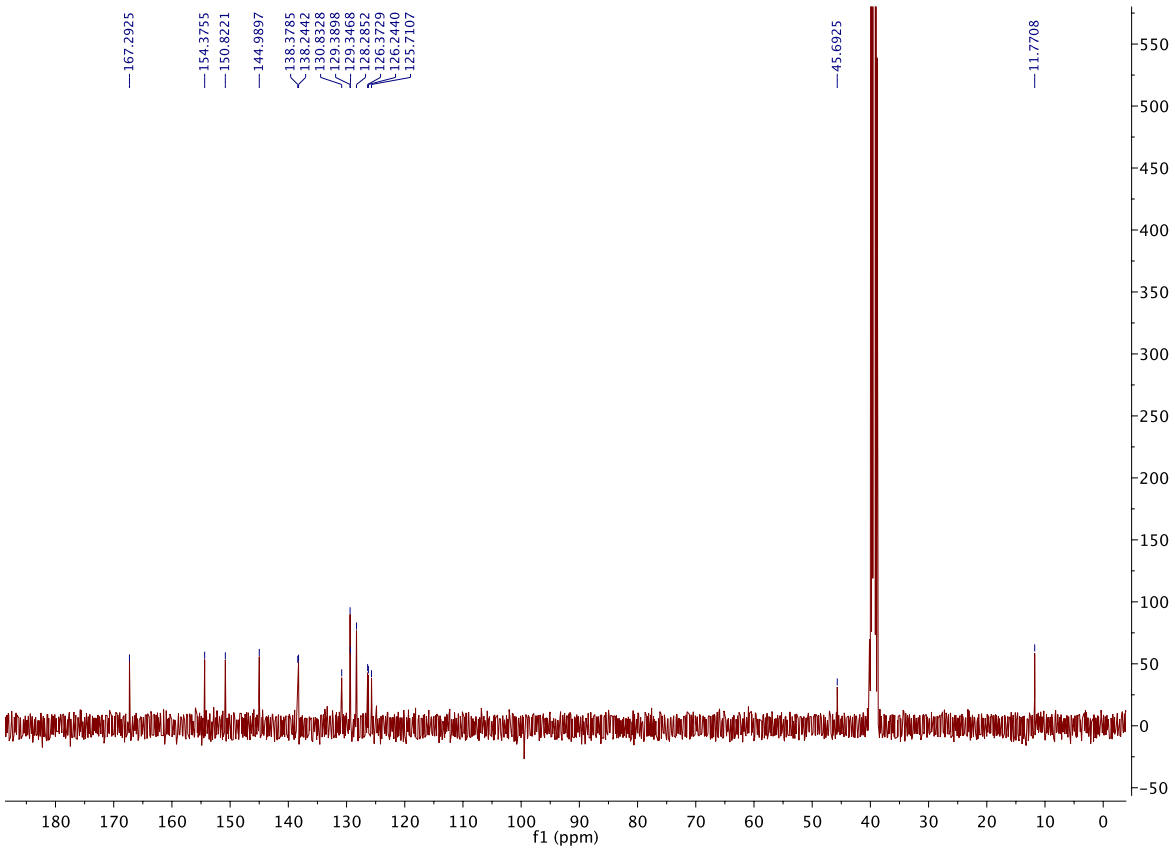


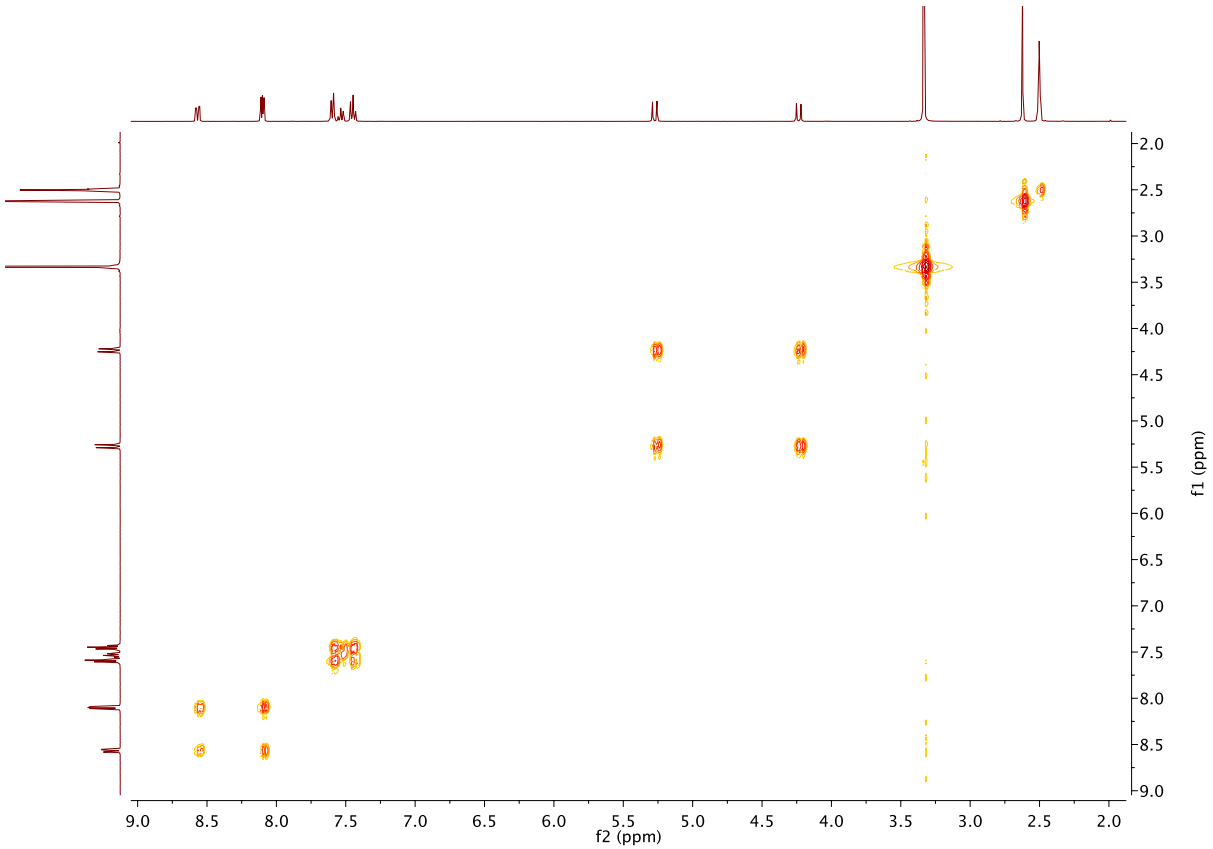

f2 (ppm)
f1 (ppm)

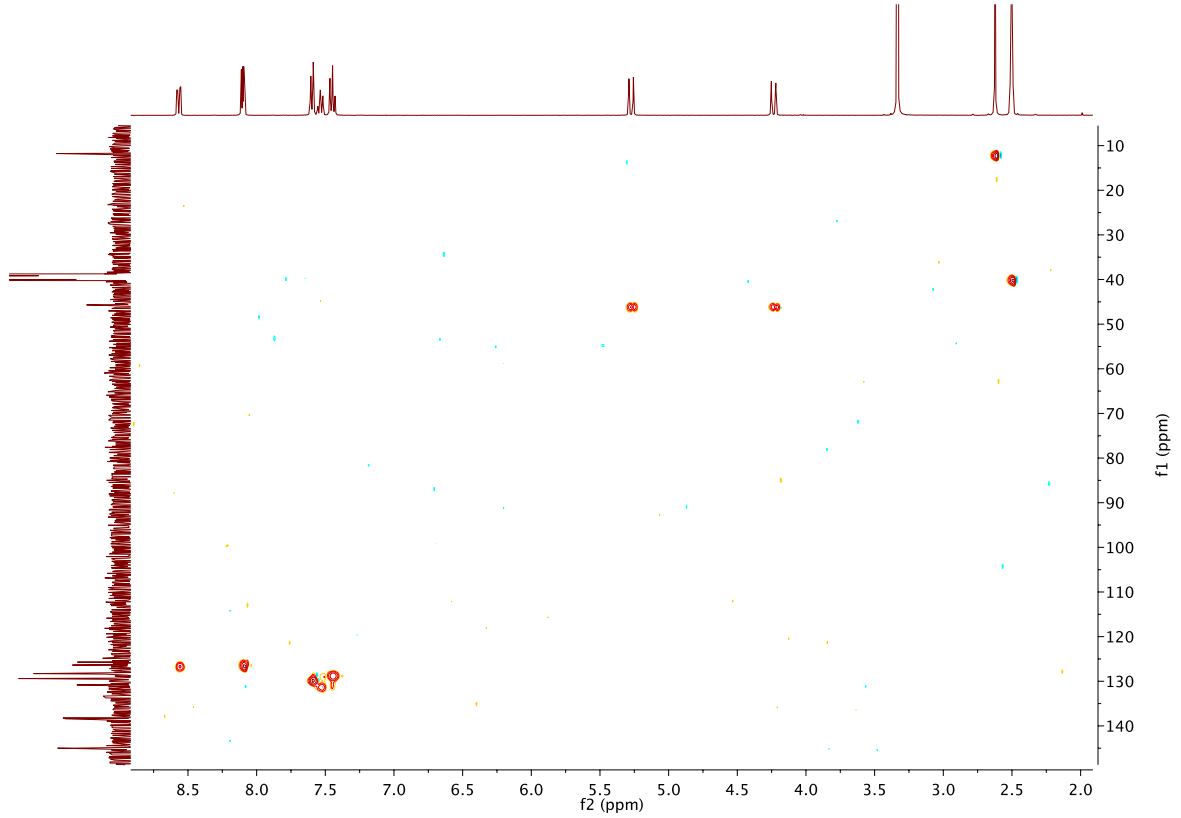

f2 (ppm)
f1 (ppm)

Nitrazolam (synthesized) NMR data



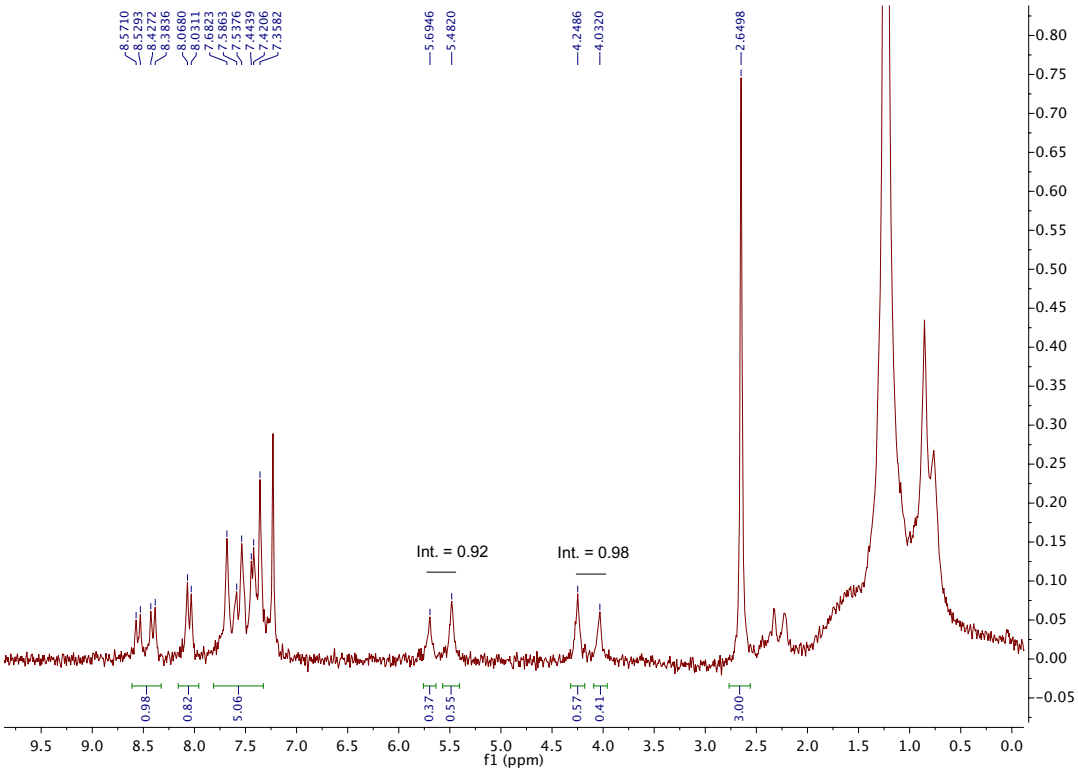



f1 (ppm)
f2 (ppm)

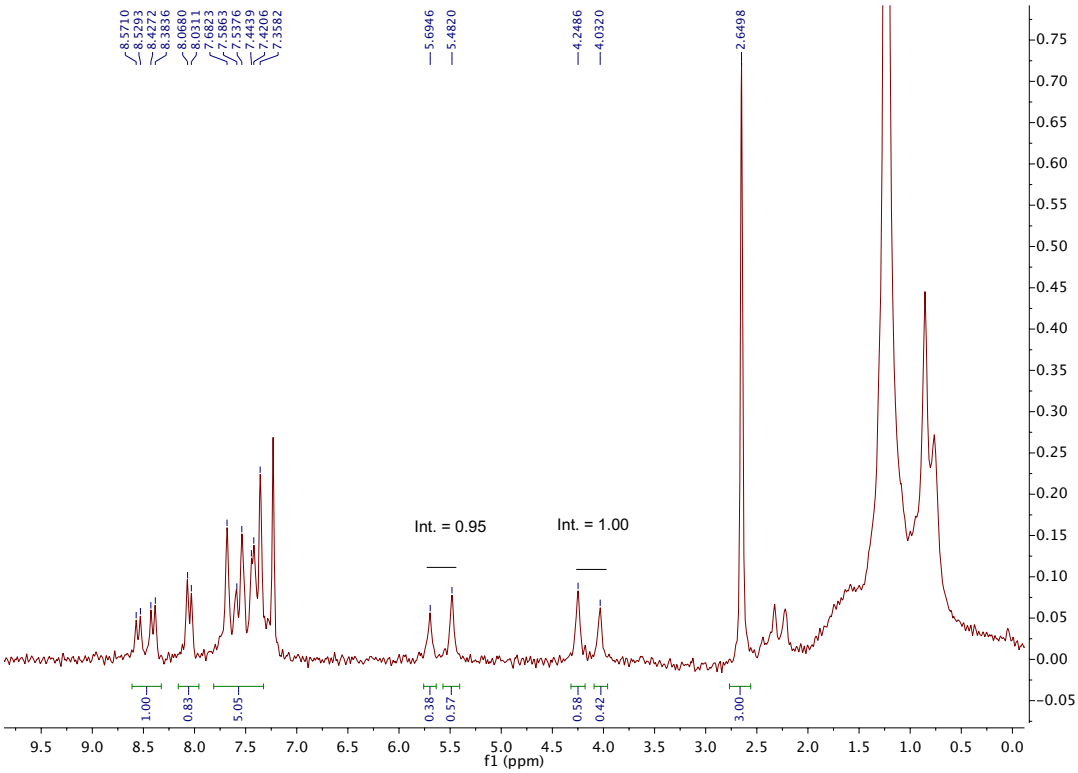

f1 (ppm)
f2 (ppm)

^{1}H spectrum for clonazolam (tablets, analysed by bench-top NMR, 60 MHz)

δ ($CDCl_3$, 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; CH_2), 4.14 (d; *J* = 13.1 Hz; 1 H; CH_2) and 2.65 (s; 3 H; CH_3) ppm.



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



Supporting Information 4:

1. Crystallography

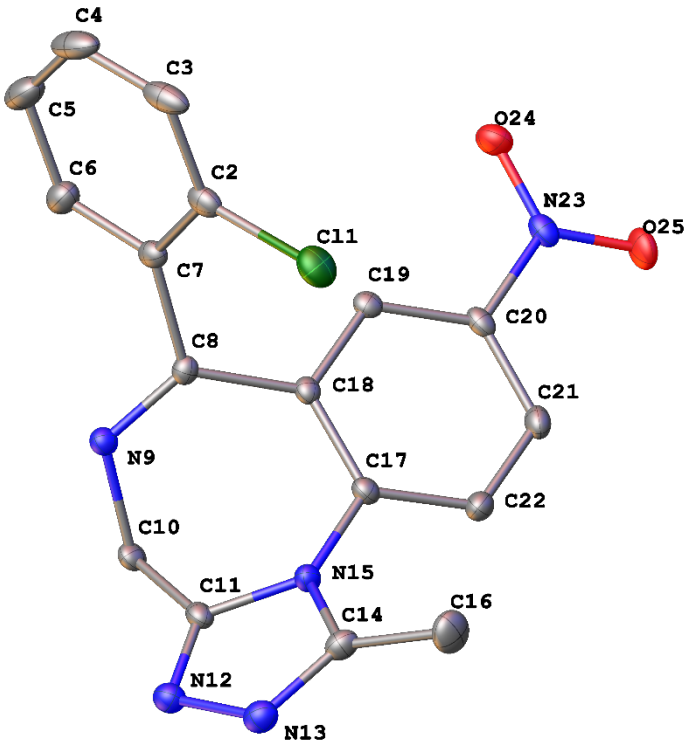


Fig. S1a Labelled molecular structure of 1 with atomic displacement shown at 50% probability.

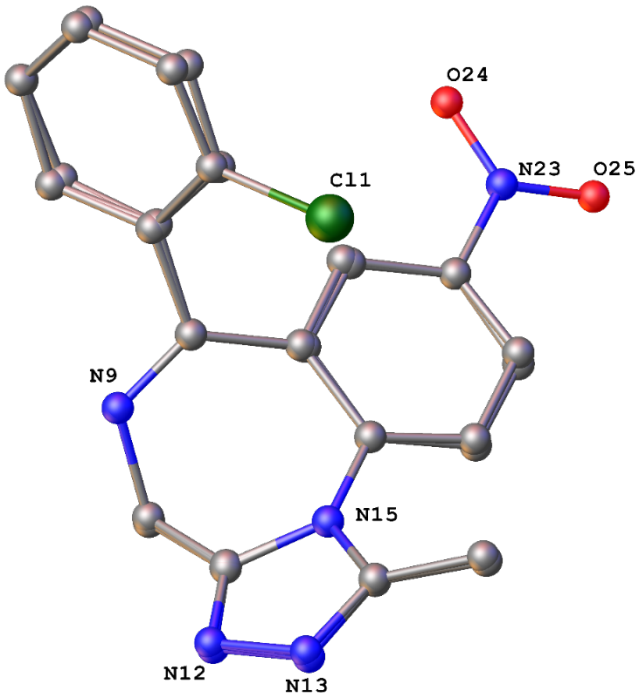


Fig. S1b. Overlay plot of 1 and Alprazolam. Data taken from CCDC 106079. Only heteroatom labelling from 1 used in the plot.

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 ATOM	DEVIATION
CL1	3	-0.28402	-0.49645	-0.37528	N23	0.315
N1	4	-0.36948	-0.75561	-0.71038	N15	0.034
N2	4	-0.68766	-0.80944	-0.65401	N9	0.078
N3	4	-0.33270	-0.84720	-0.82733	N13	0.091
N4	4	-0.50343	-0.83544	-0.83509	N12	0.089
C1	1	-0.20502	-0.55776	-0.53476	C21	0.196
C2	1	-0.30562	-0.57335	-0.47354	C20	0.140
C3	1	-0.43022	-0.64689	-0.48665	C19	0.107
C4	1	-0.45245	-0.70863	-0.56520	C18	0.072
C5	1	-0.35044	-0.69497	-0.62909	C17	0.081
C6	1	-0.23044	-0.61953	-0.61507	C22	0.130
C7	1	-0.58635	-0.78510	-0.57763	C8	0.070
C8	1	-0.67782	-0.74970	-0.73866	C10	0.068
C9	1	-0.52254	-0.78336	-0.76528	C11	0.032
C10	1	-0.25965	-0.79777	-0.75226	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

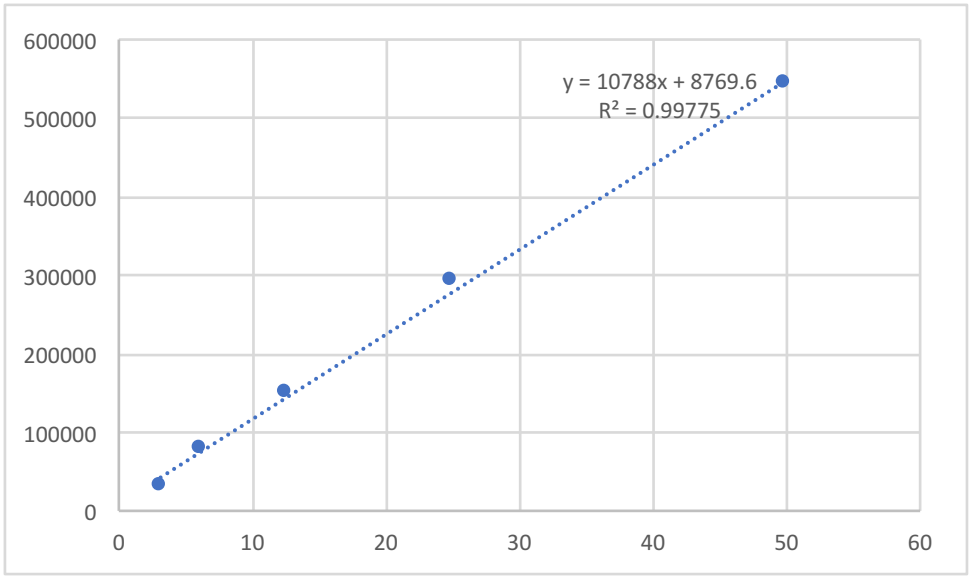
Supporting information 5

Clonazolam tabs (0.5 mg/tab)

Dil. 4

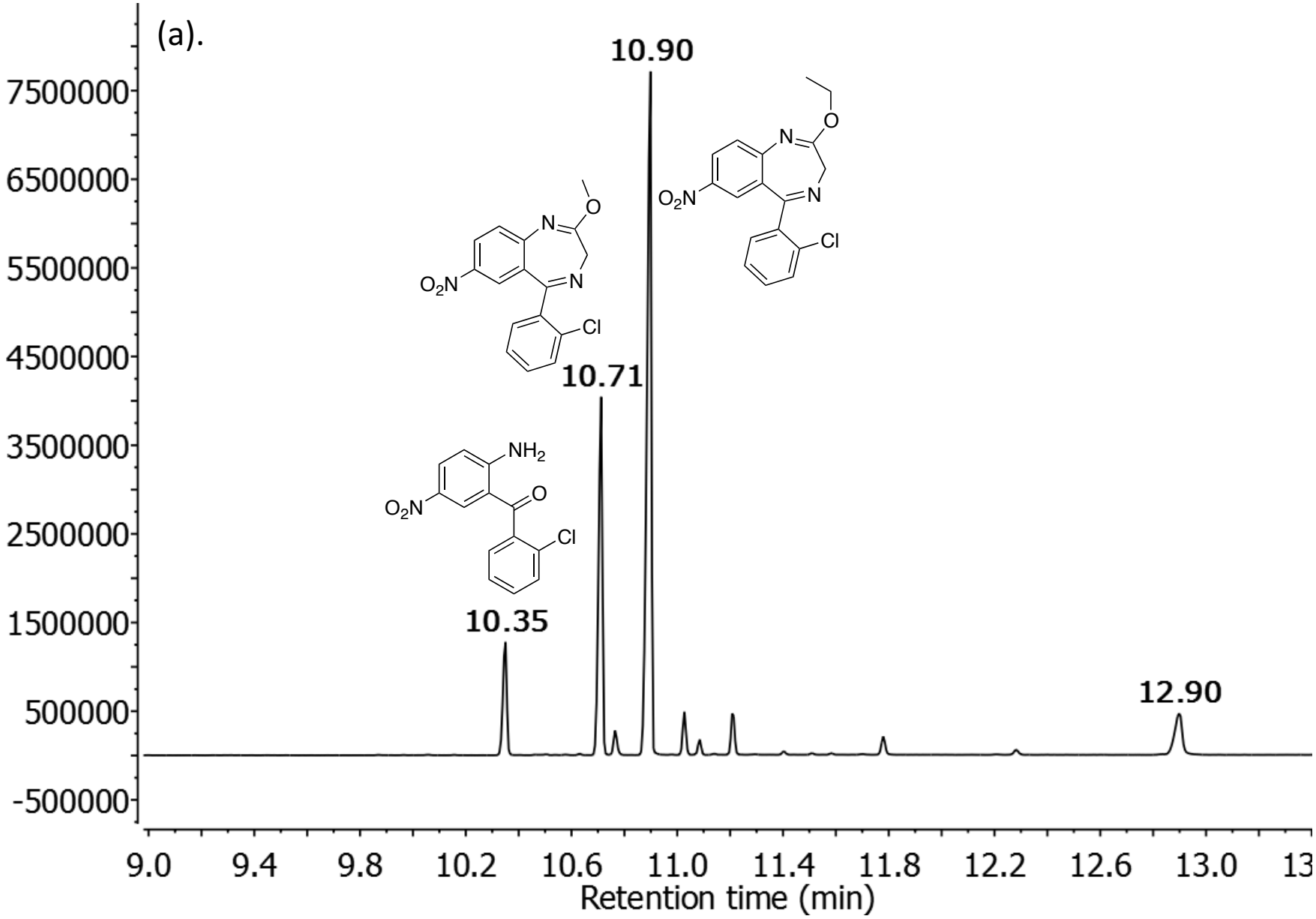
Std. (mcg/mL)	Peak area
3.125	30010
6.25	78074
12.5	148923
25	290623
50	541325

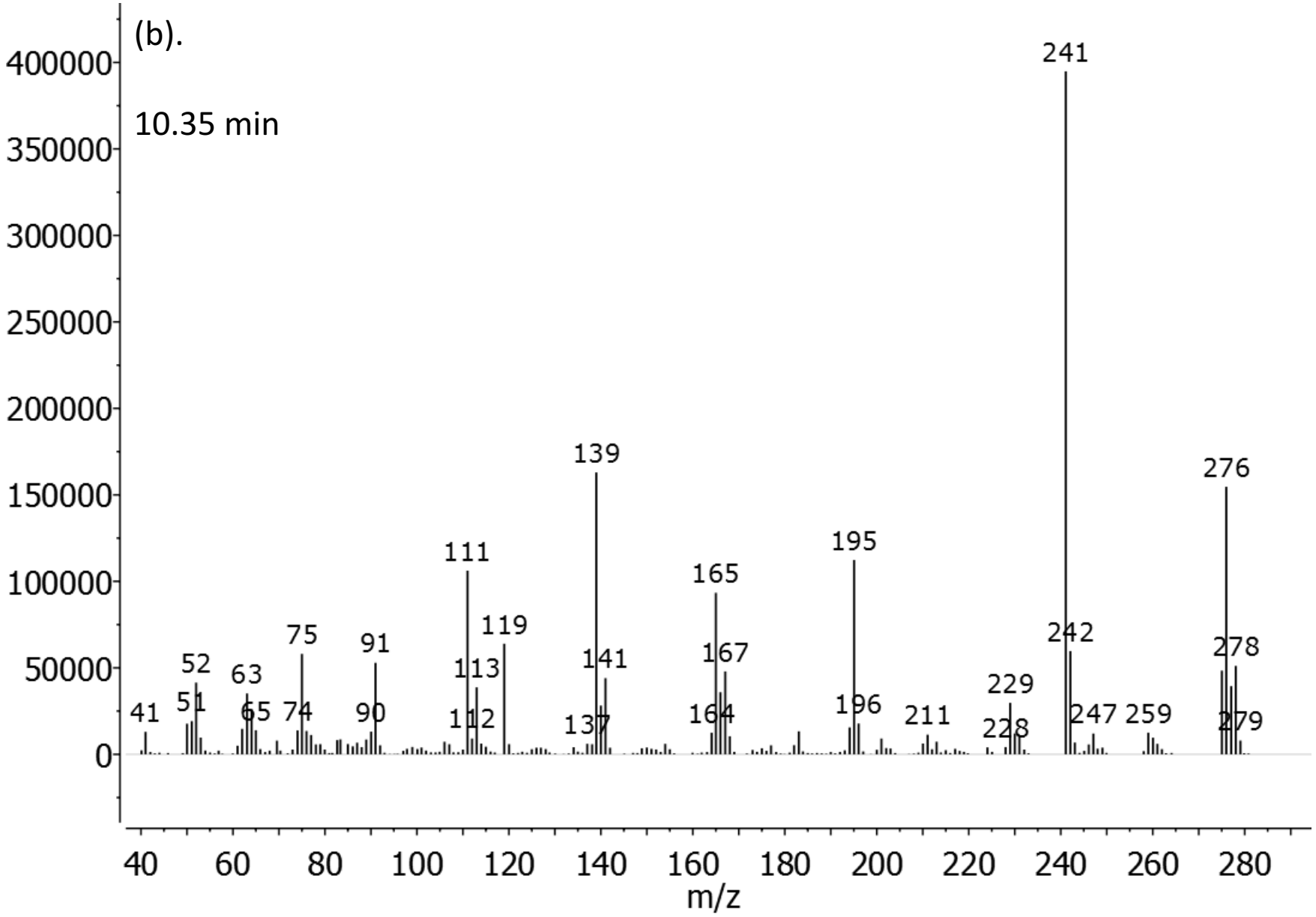
			x4x2x5
		mcg/mL	mg/tablet
Tab A (97 mg)	147175	12.83	0.51
	148077	12.91	0.52
Tab B (99 mg)	142173	12.37	0.49
	153852	13.45	0.54
		Av.	**0.52** mg per tab

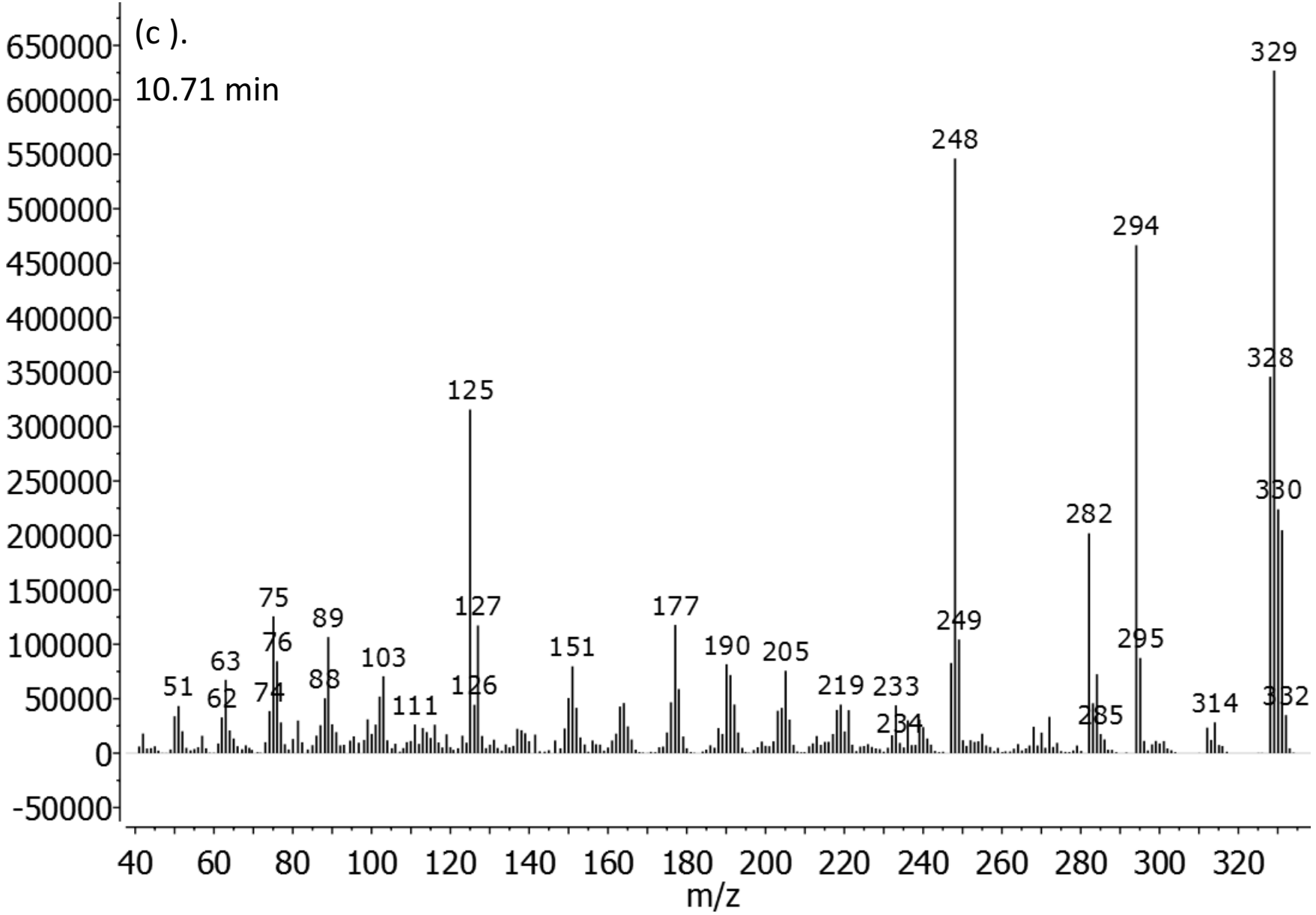


	Tabs. weights
	Tablet (mg)
	98
	97
	98
	99
	100
	99
Av.	99
SD	1

Supporting information 6




(b).
10.35 min
400000
350000
300000
250000
200000
150000
100000
50000
0
241
139
276
195
111
165
119
75
91
242
278
52
63
167
113
141
229
41
51
65
74
90
112
137
164
196
211
228
247
259
279
40
60
80
100
120
140
160
180
200
220
240
260
280
m/z



(c).
10.71 min
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
-50000
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
m/z
51
62
63
74
75
76
88
89
103
111
125
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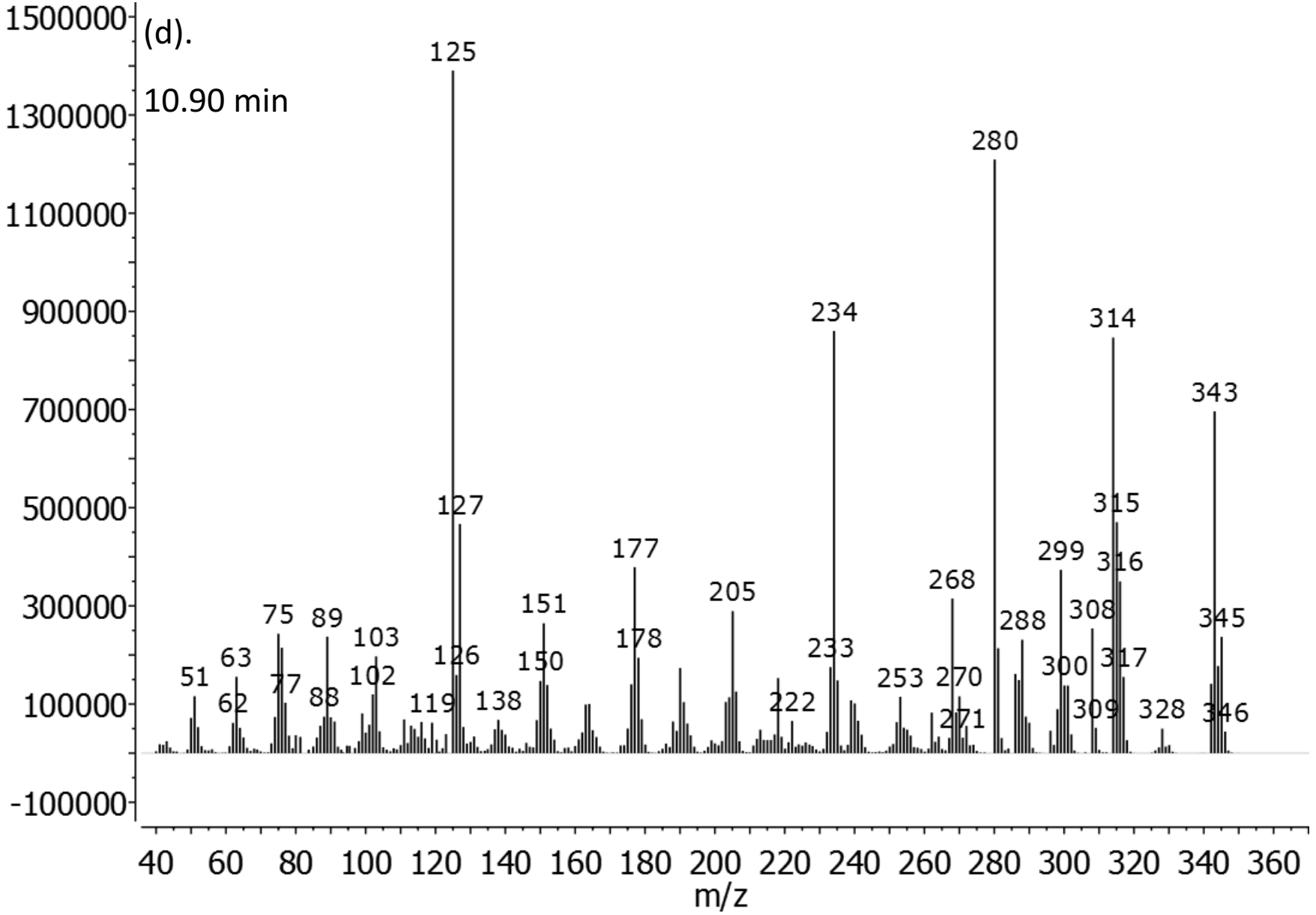
(d).
10.90 min
1500000
1300000
1100000
900000
700000
500000
300000
100000
-100000
125
280
234
314
343
127
315
177
299
316
268
205
151
75
89
103
288
308
345
178
233
63
126
150
300
317
51
77
102
253
270
62
88
119
138
222
271
309
328
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60
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100
120
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200
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240
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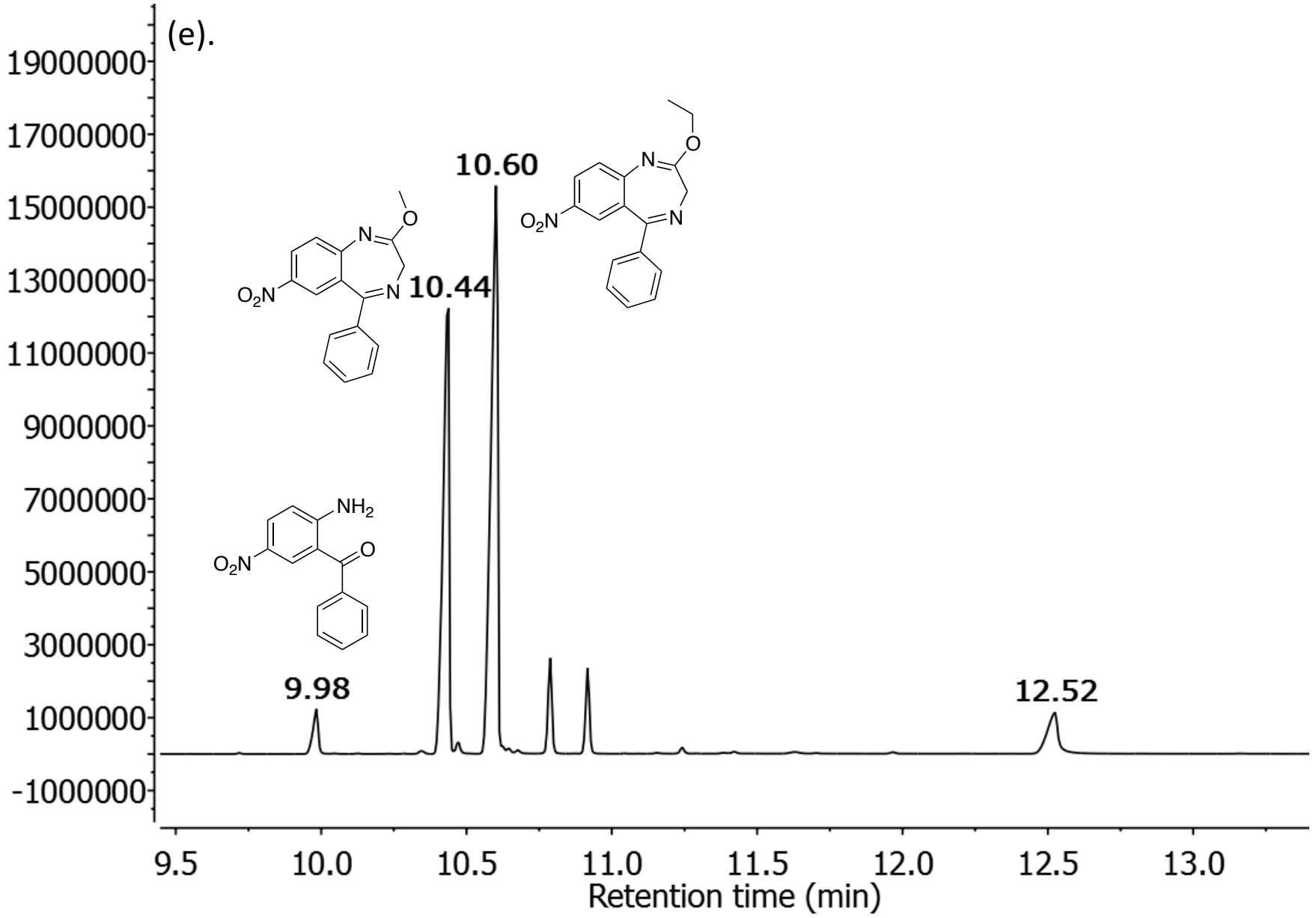
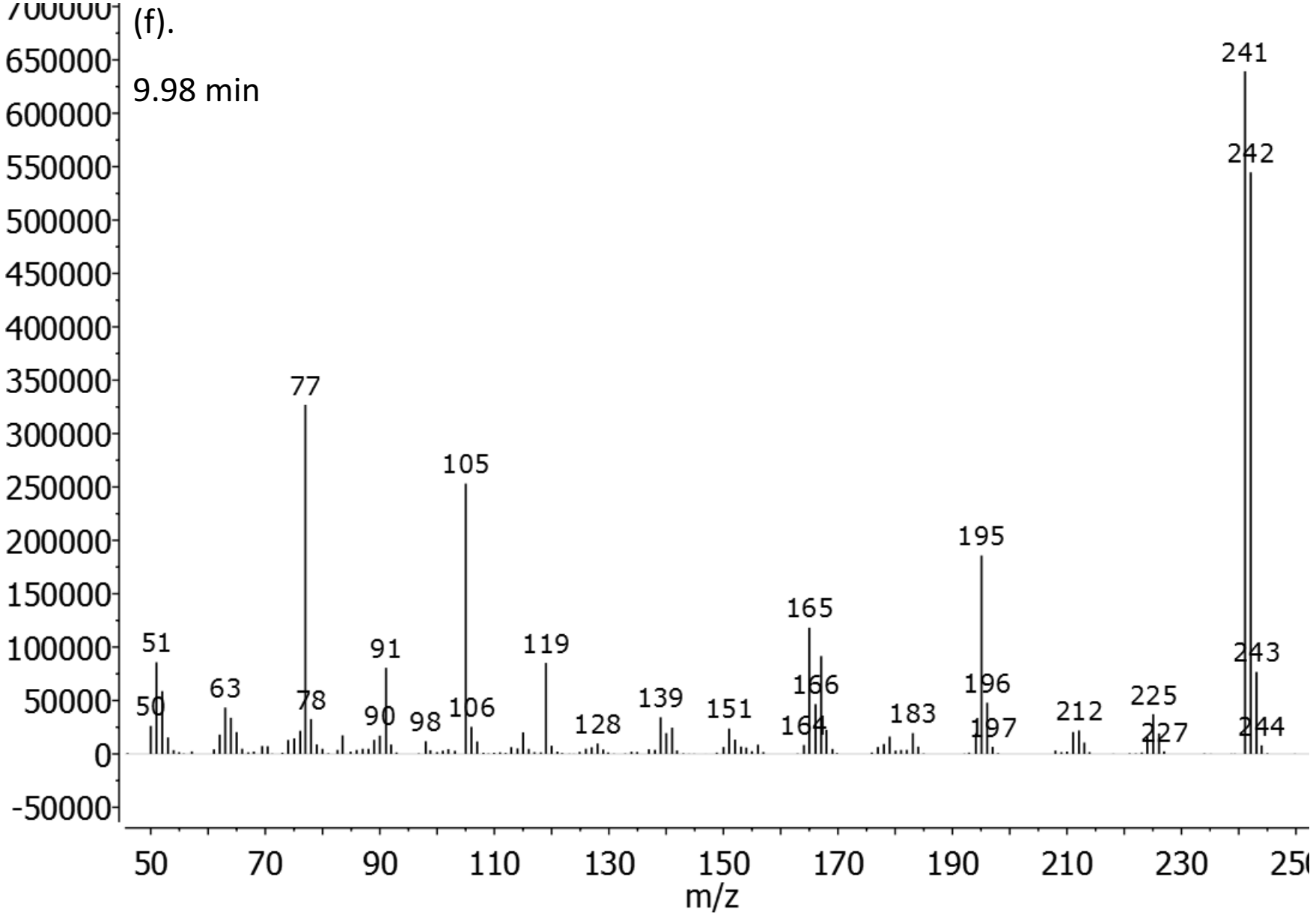
280
300
320
340
360
m/z


(e).
19000000
17000000
15000000
13000000
11000000
9000000
7000000
5000000
3000000
1000000
-1000000
9.98
10.44
10.60
12.52
9.5
10.0
10.5
11.0
11.5
12.0
12.5
13.0
Retention time (min)


(f).
9.98 min
700000
650000
600000
550000
500000
450000
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350000
300000
250000
200000
150000
100000
50000
0
-50000
50
70
90
110
130
150
170
190
210
230
m/z
241
242
243
244
77
105
195
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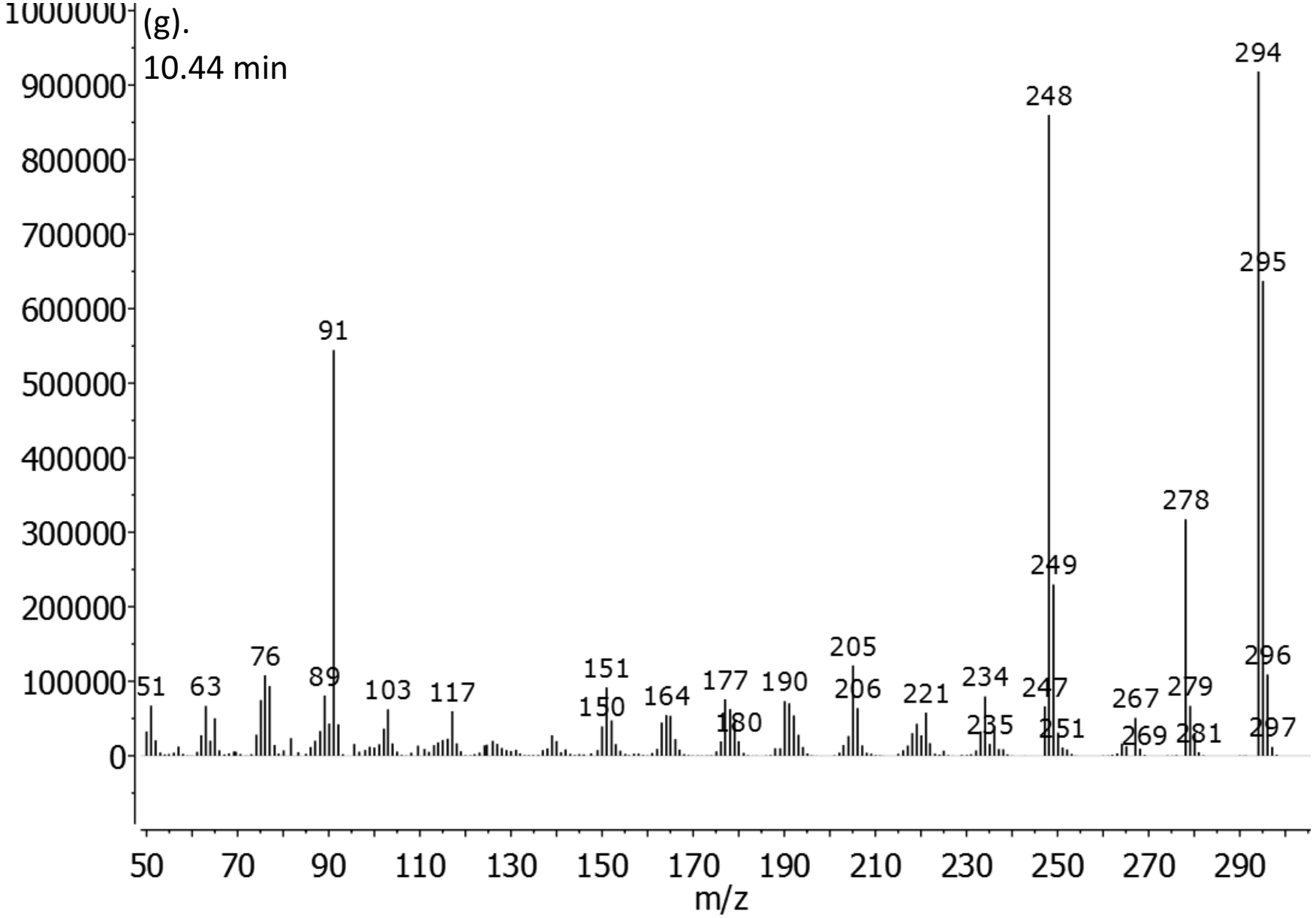
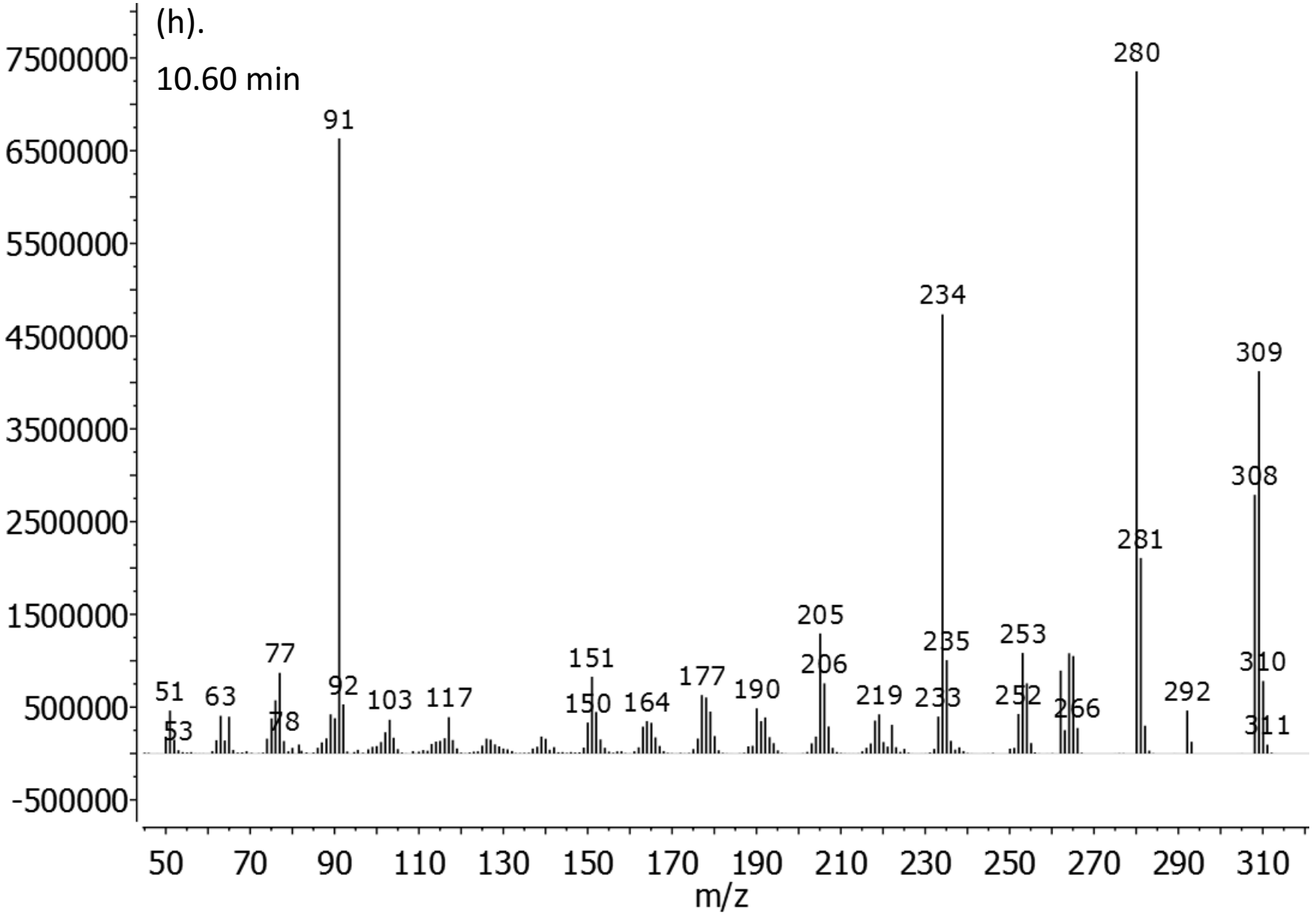
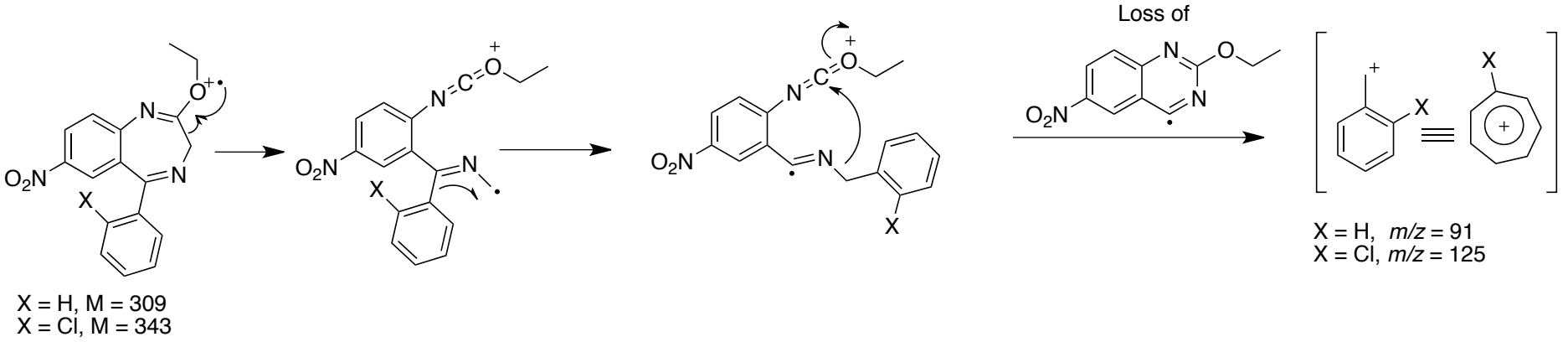
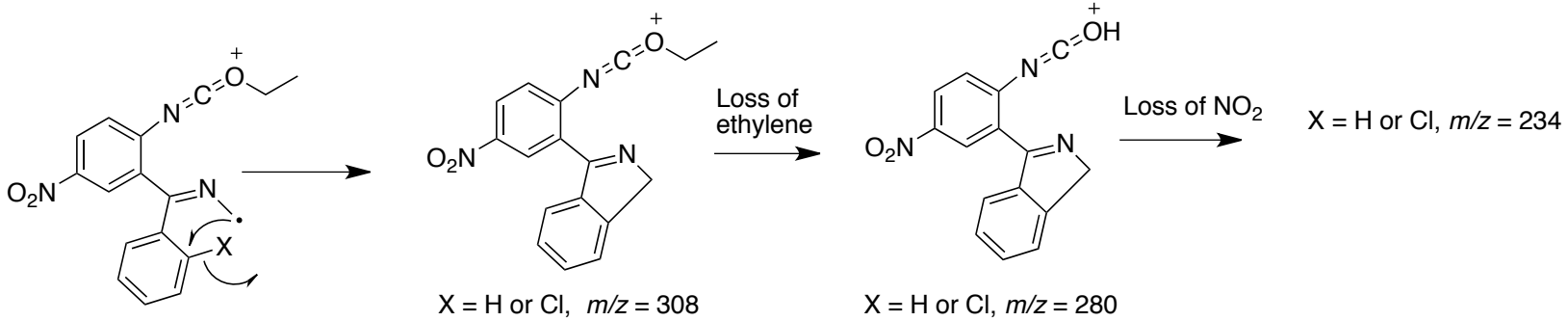
212
225
227
50

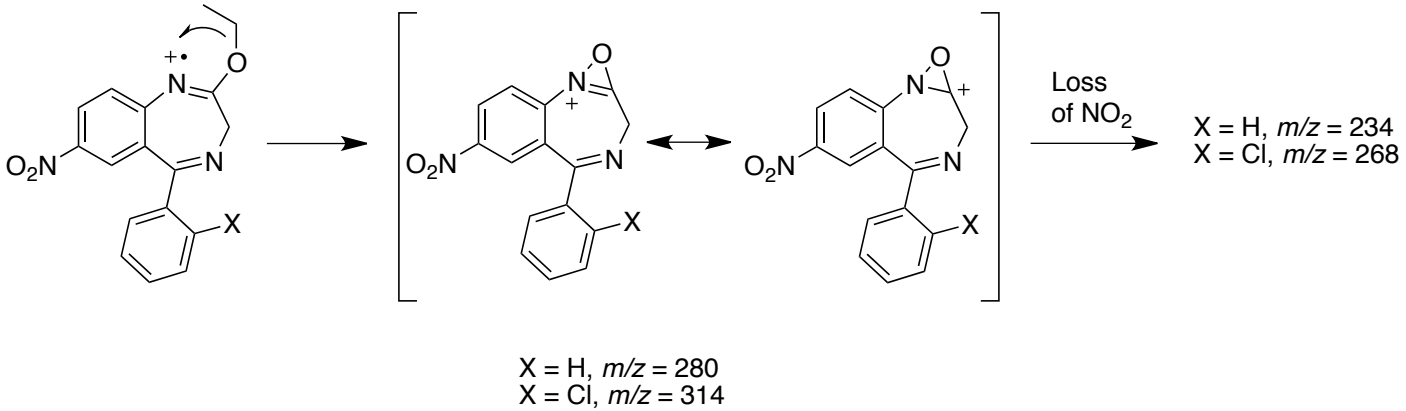

(g).
10.44 min
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
51
63
76
89
91
103
117
151
150
164
177
180
190
205
206
221
234
235
247
248
249
251
267
269
278
279
281
294
295
296
297
50
70
90
110
130
150
170
190
210
230
250
270
290
m/z

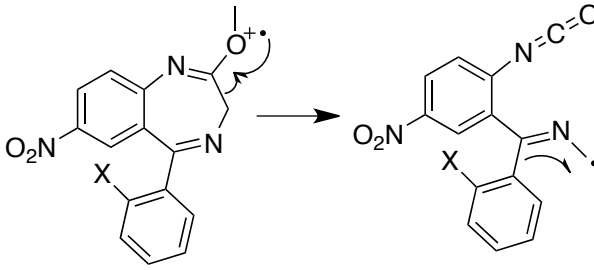

(h).
10.60 min
7500000
6500000
5500000
4500000
3500000
2500000
1500000
500000
-500000
91
280
234
309
308
281
205
235
253
310
77
151
177
190
206
51
63
92
103
117
150
164
219
233
252
266
292
53
78
311
50
70
90
110
130
150
170
190
210
230
250
270
290
310
m/z

(i).

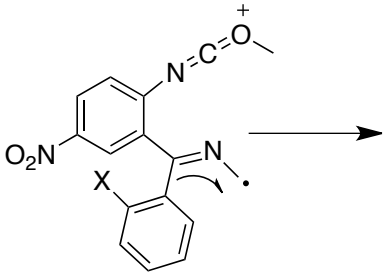

Loss of
X = H, M = 309
X = Cl, M = 343
X = H, m/z = 91
X = Cl, m/z = 125

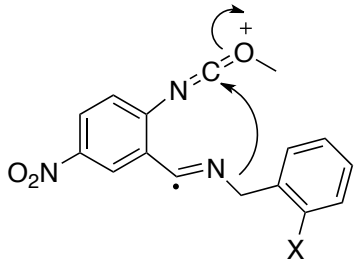

Loss of ethylene
Loss of NO2
X = H or Cl, m/z = 308
X = H or Cl, m/z = 280
X = H or Cl, m/z = 234


Loss of NO2
X = H, m/z = 280
X = Cl, m/z = 314
X = H, m/z = 234
X = Cl, m/z = 268

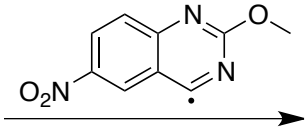


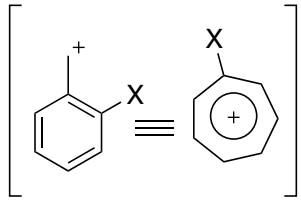
X = H, M = 295
X = Cl, M = 329



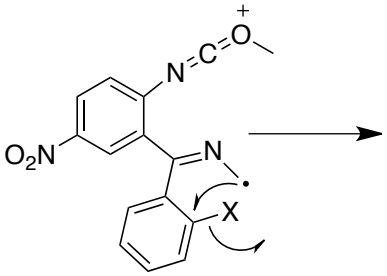


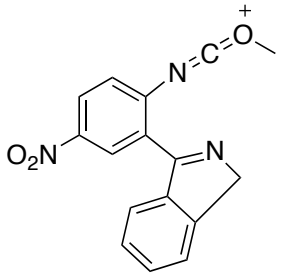
Loss of





X = H, *m/z* = 91
X = Cl, *m/z* = 125





X = H or Cl, *m/z* = 294

Loss of NO_2

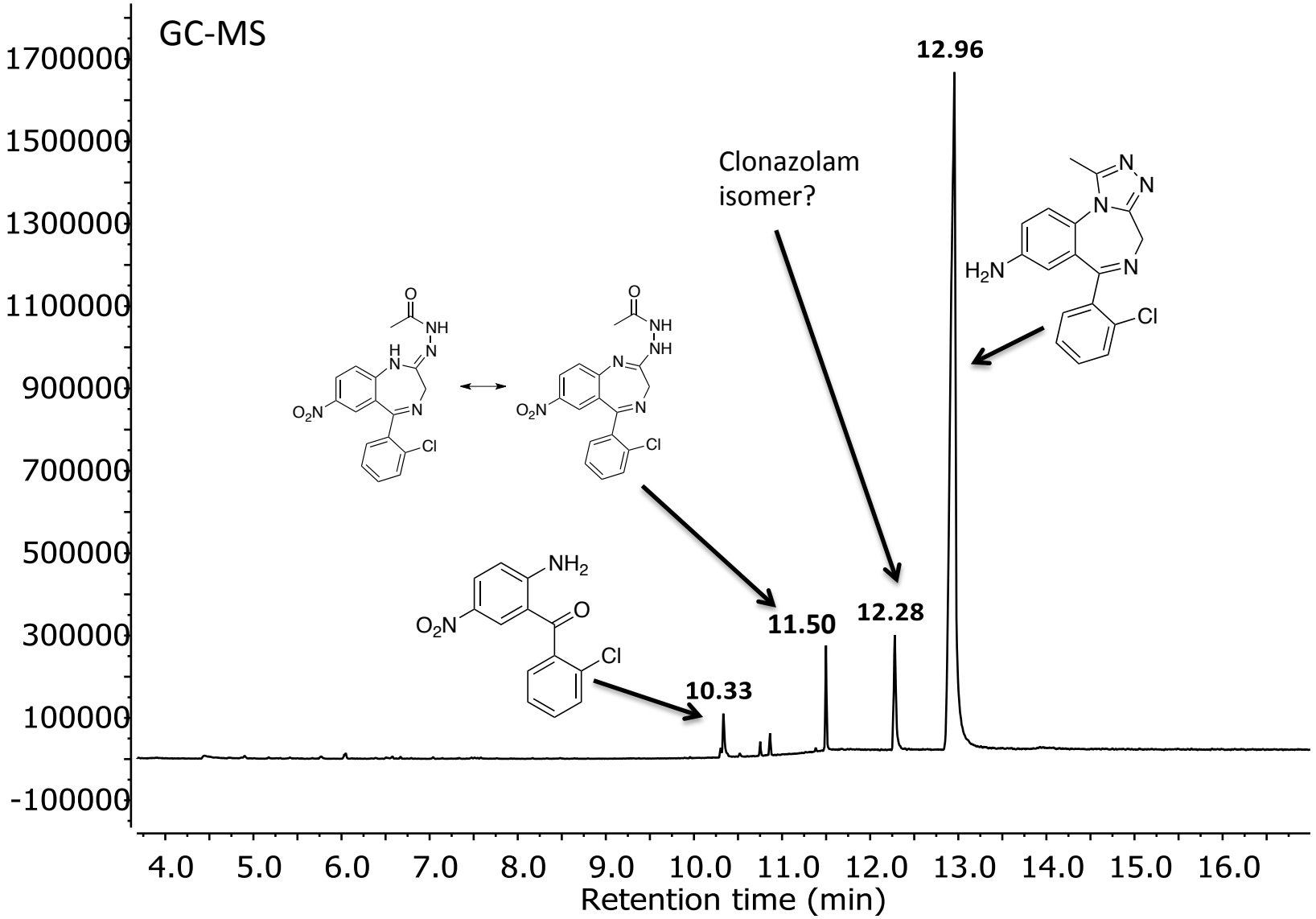
X = H or Cl, *m/z* = 248

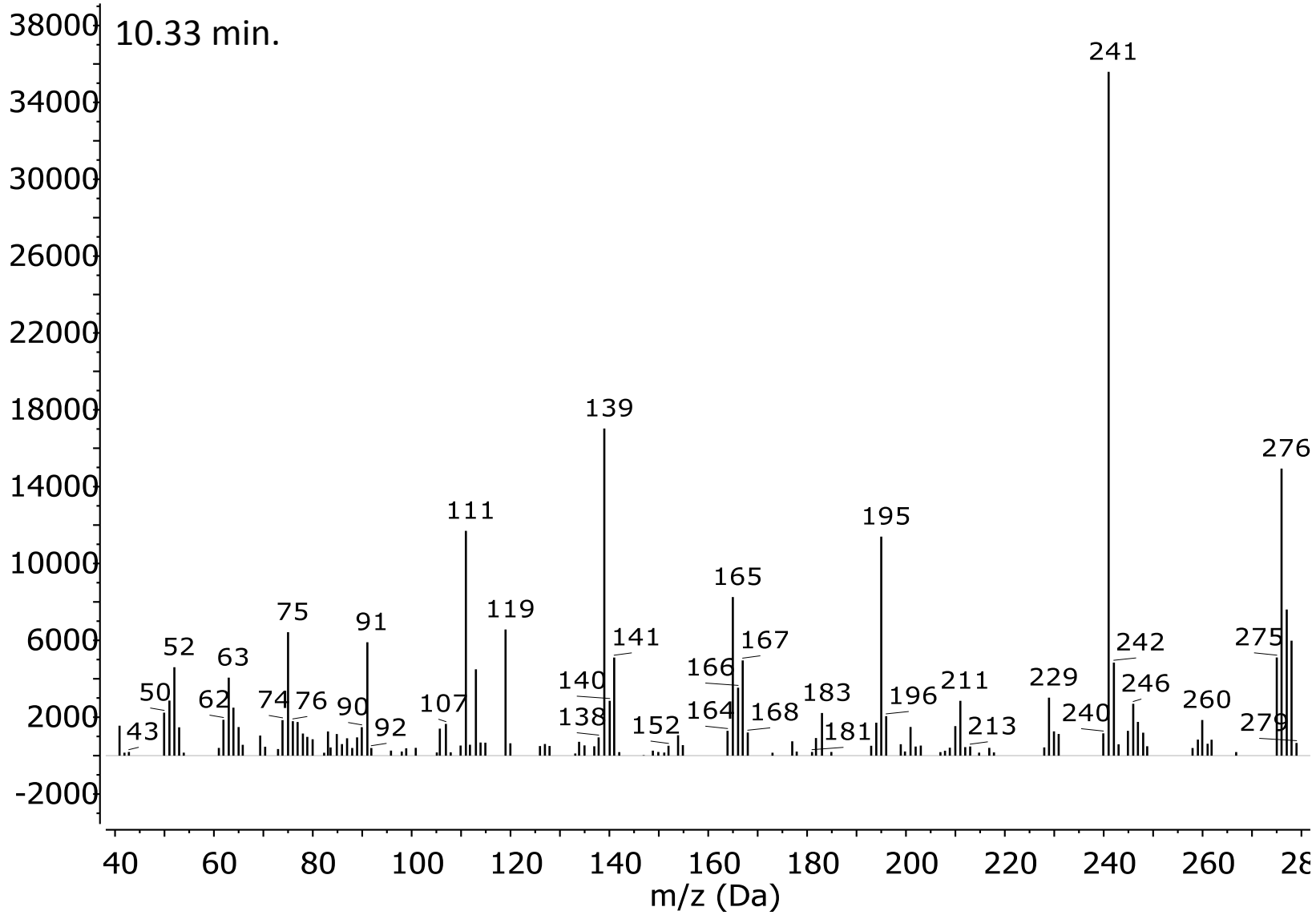
$M^{+\bullet}$ —Loss of $HO^{\bullet}$→ *m/z* 278 (X = H)

$M^{+\bullet}$ —Loss of HNO_2→ *m/z* 282 (X = Cl)

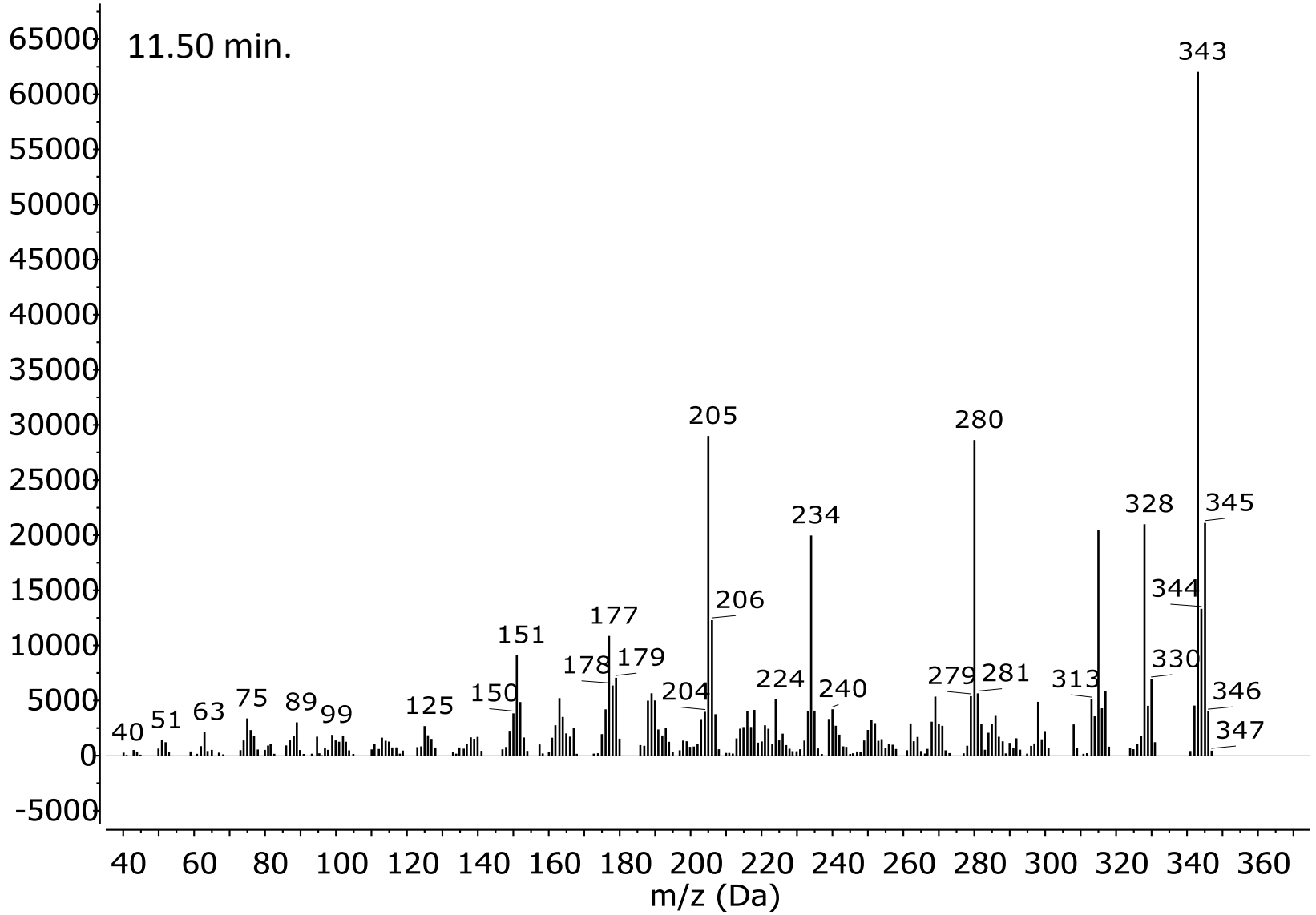
Supporting information 7

Clonazolam − crude product − preparative scale

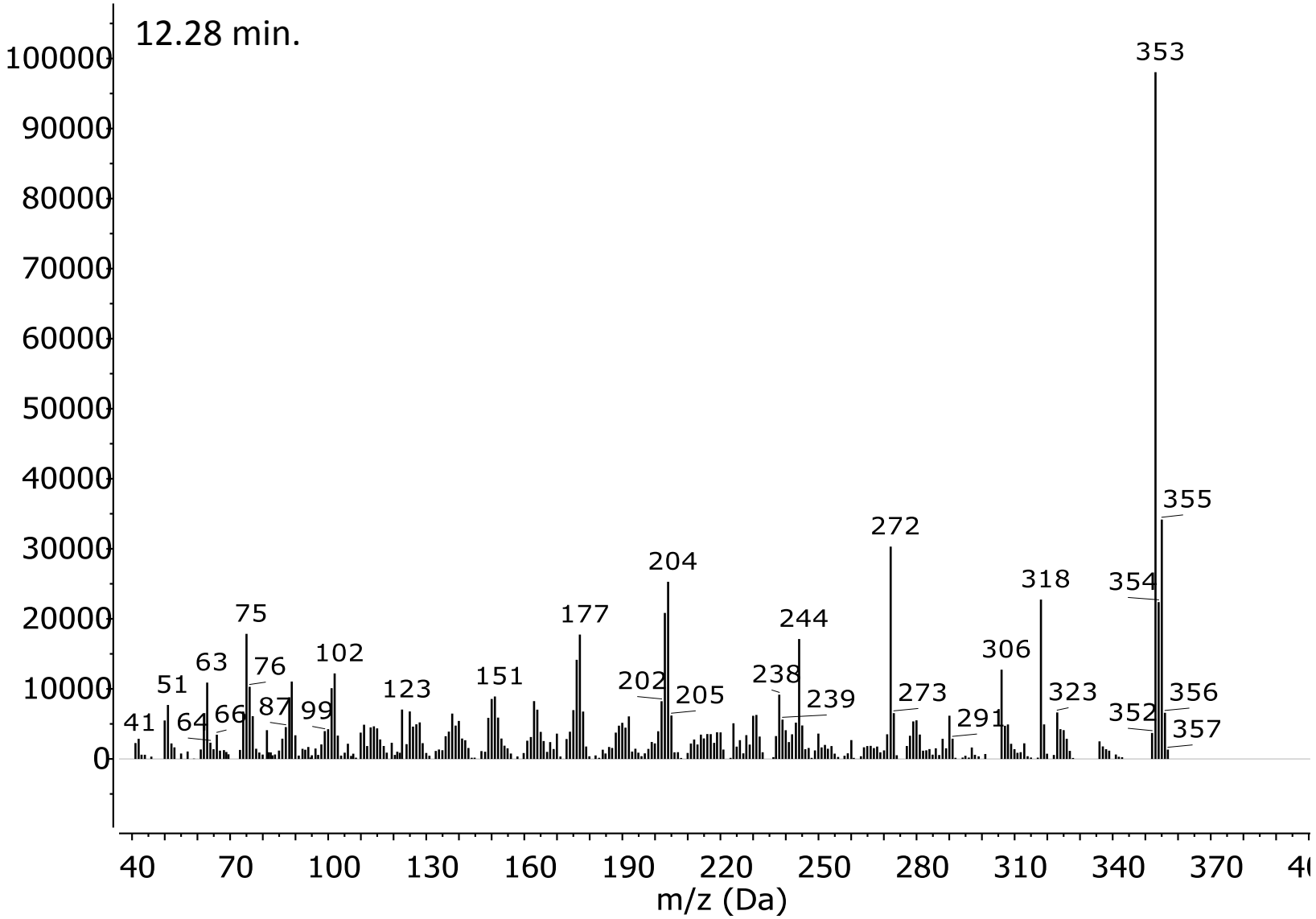


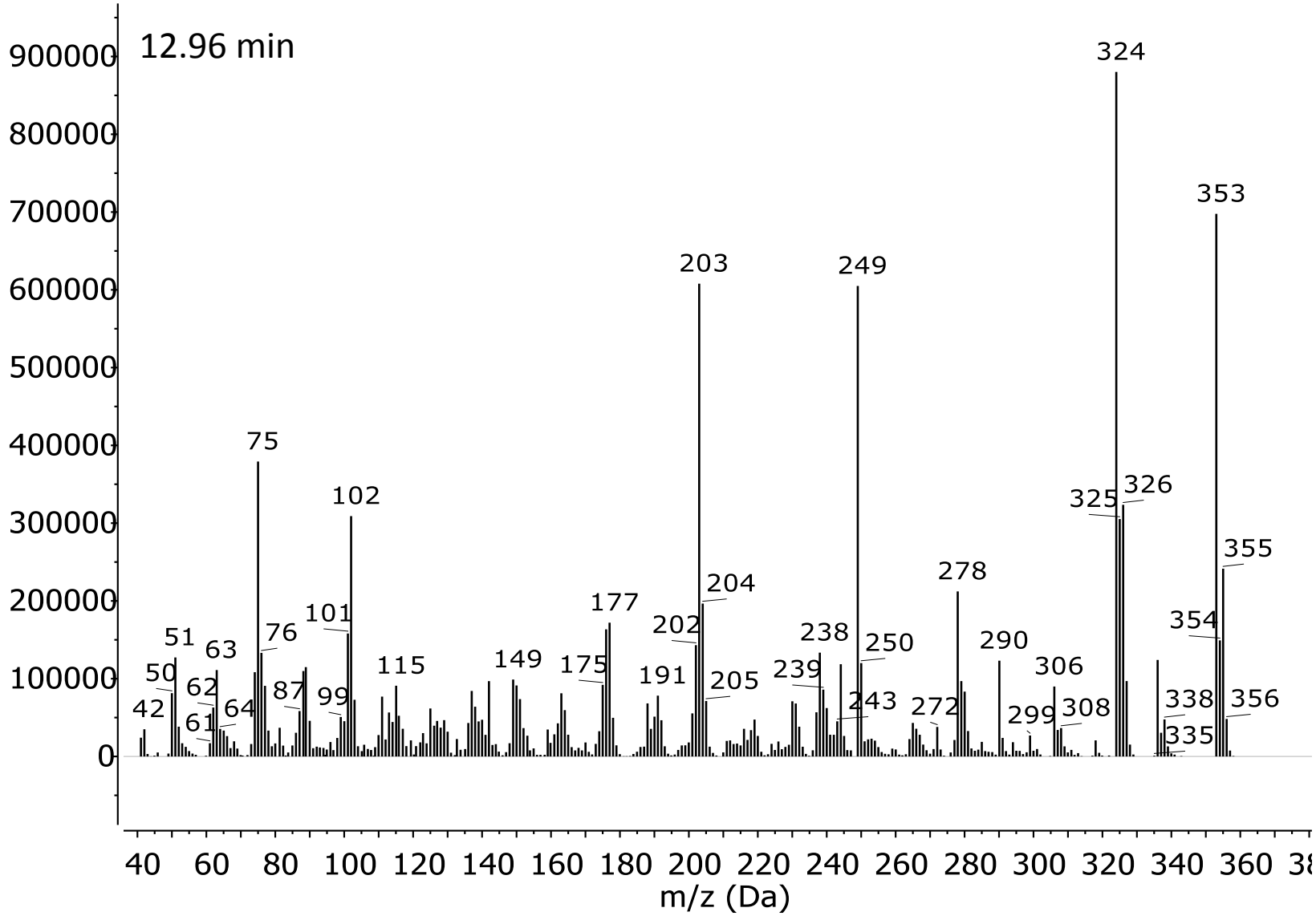


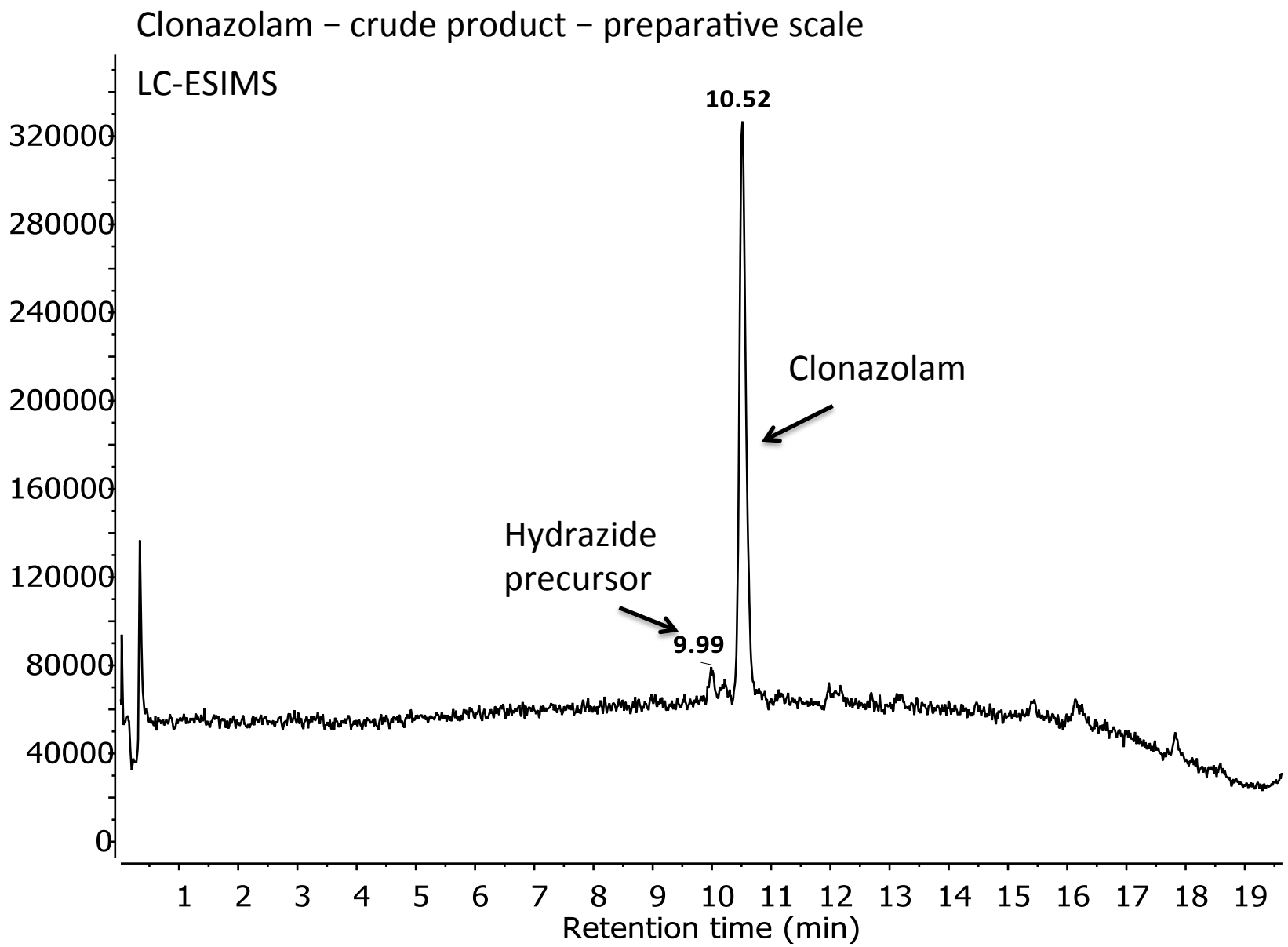
10.33 min.
38000
34000
30000
26000
22000
18000
14000
10000
6000
2000
-2000
241
139
276
111
195
165
119
75
91
141
167
242
275
52
63
166
140
183
196
211
229
246
260
50
62
74
76
90
107
138
152
164
168
181
213
240
279
43
92
40
60
80
100
120
140
160
180
200
220
240
260
m/z (Da)

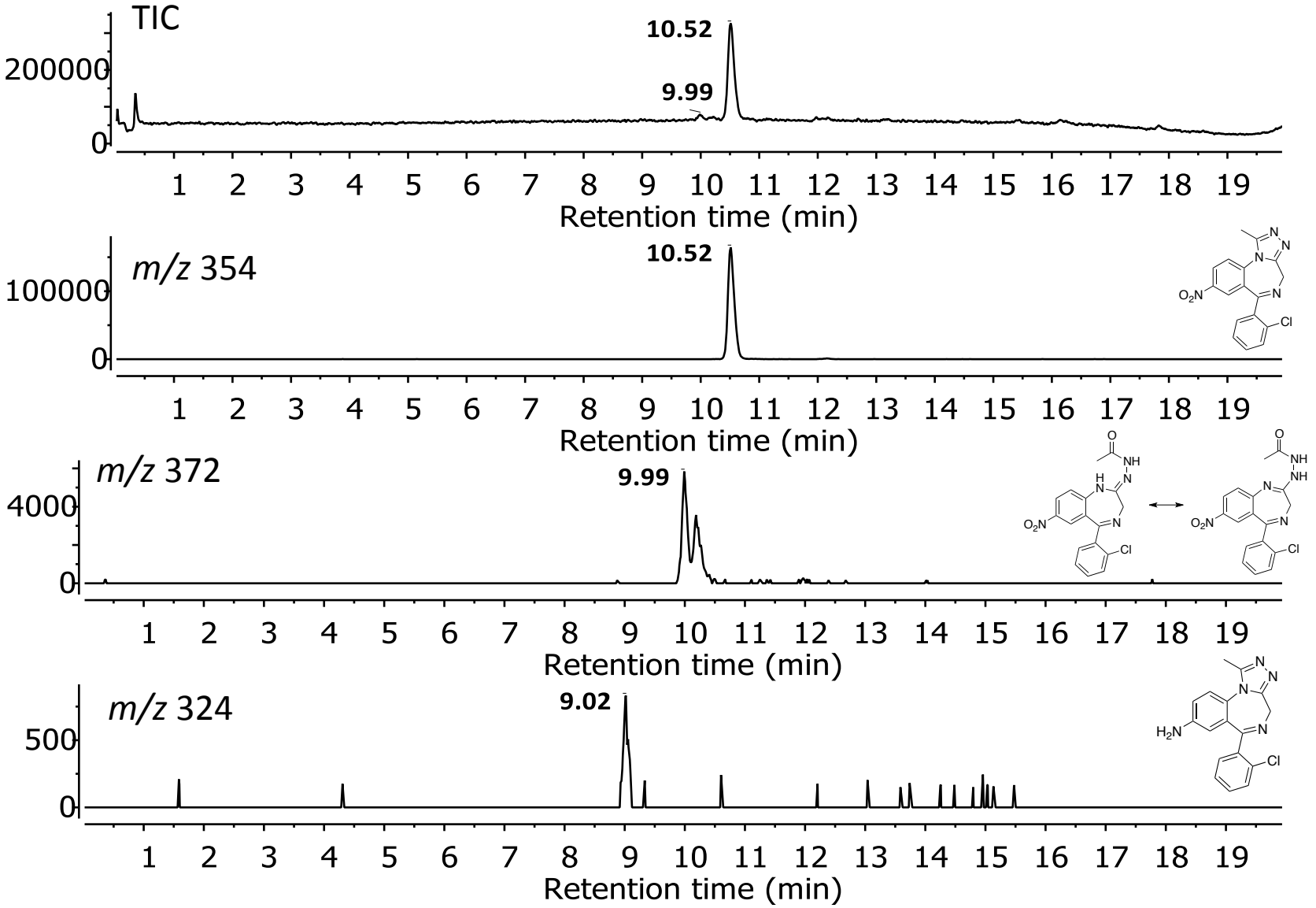


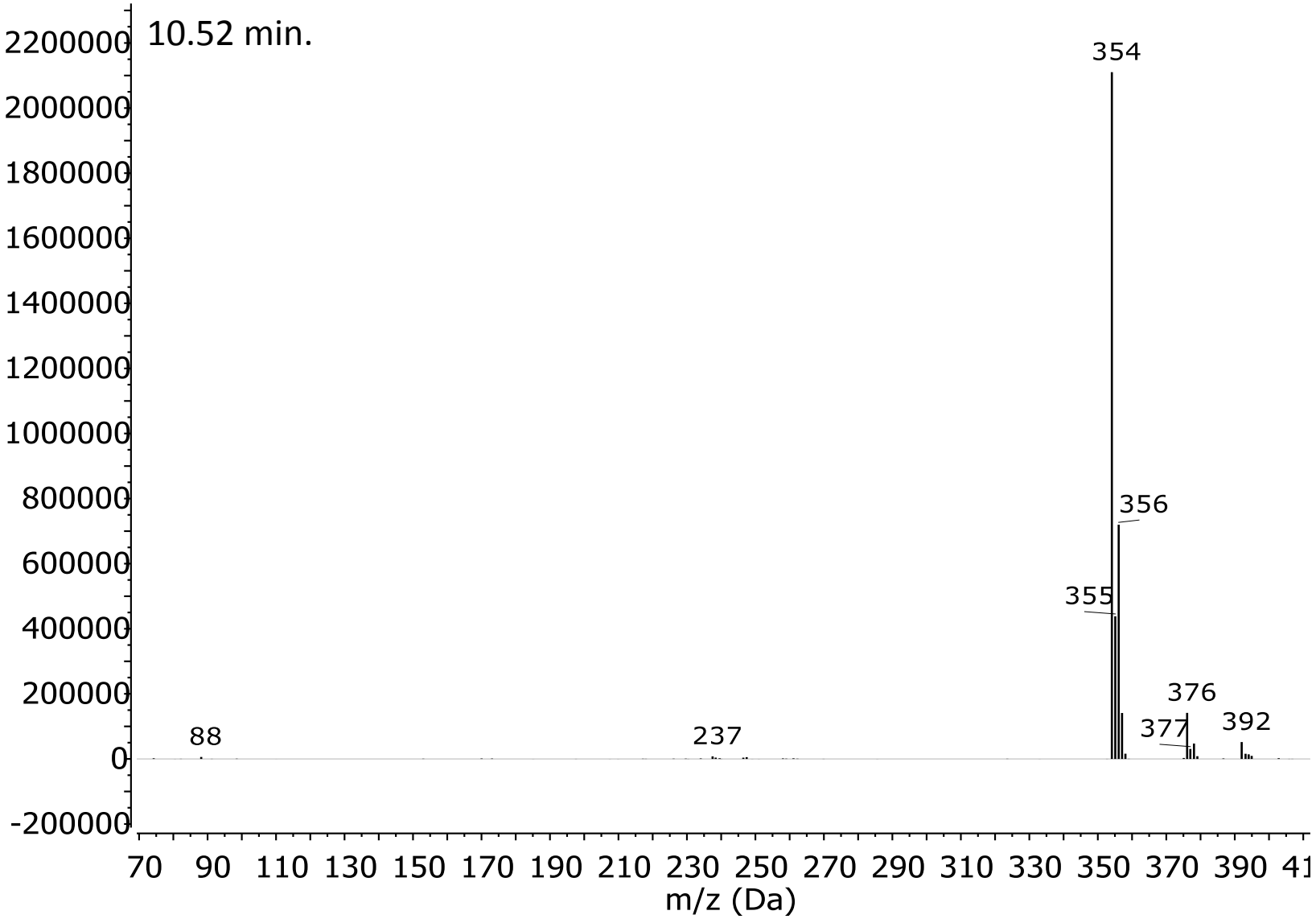
11.50 min.
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
40
51
63
75
89
99
125
150
151
178
177
179
204
205
206
224
234
240
279
280
281
313
328
330
343
344
345
346
347
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
m/z (Da)

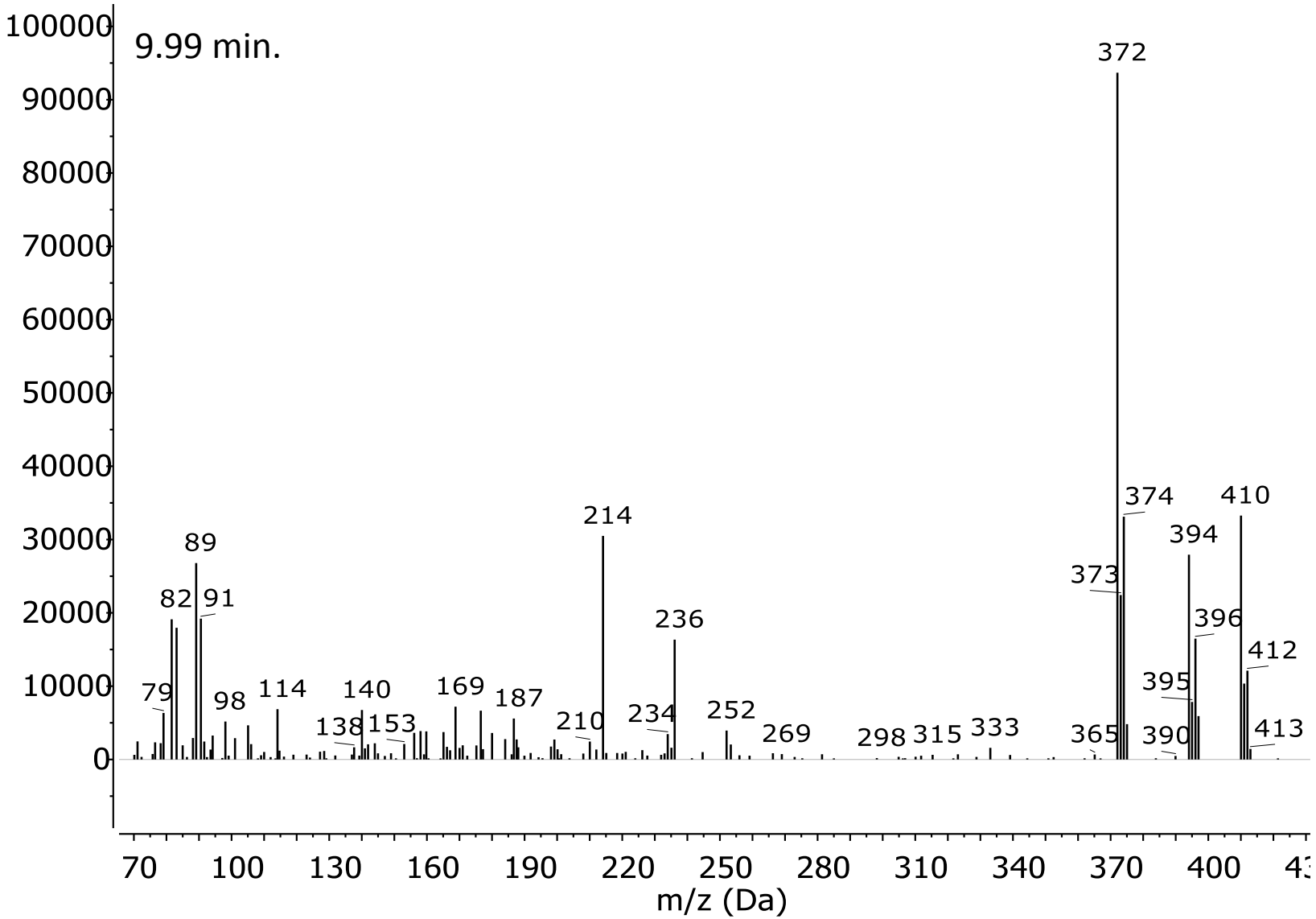

12.28 min.
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
353
355
272
204
318
354
75
177
244
306
102
63
76
51
123
151
202
238
205
239
273
323
356
41
64
66
87
99
291
352
357
40
70
100
130
160
190
220
250
280
310
340
370
m/z (Da)

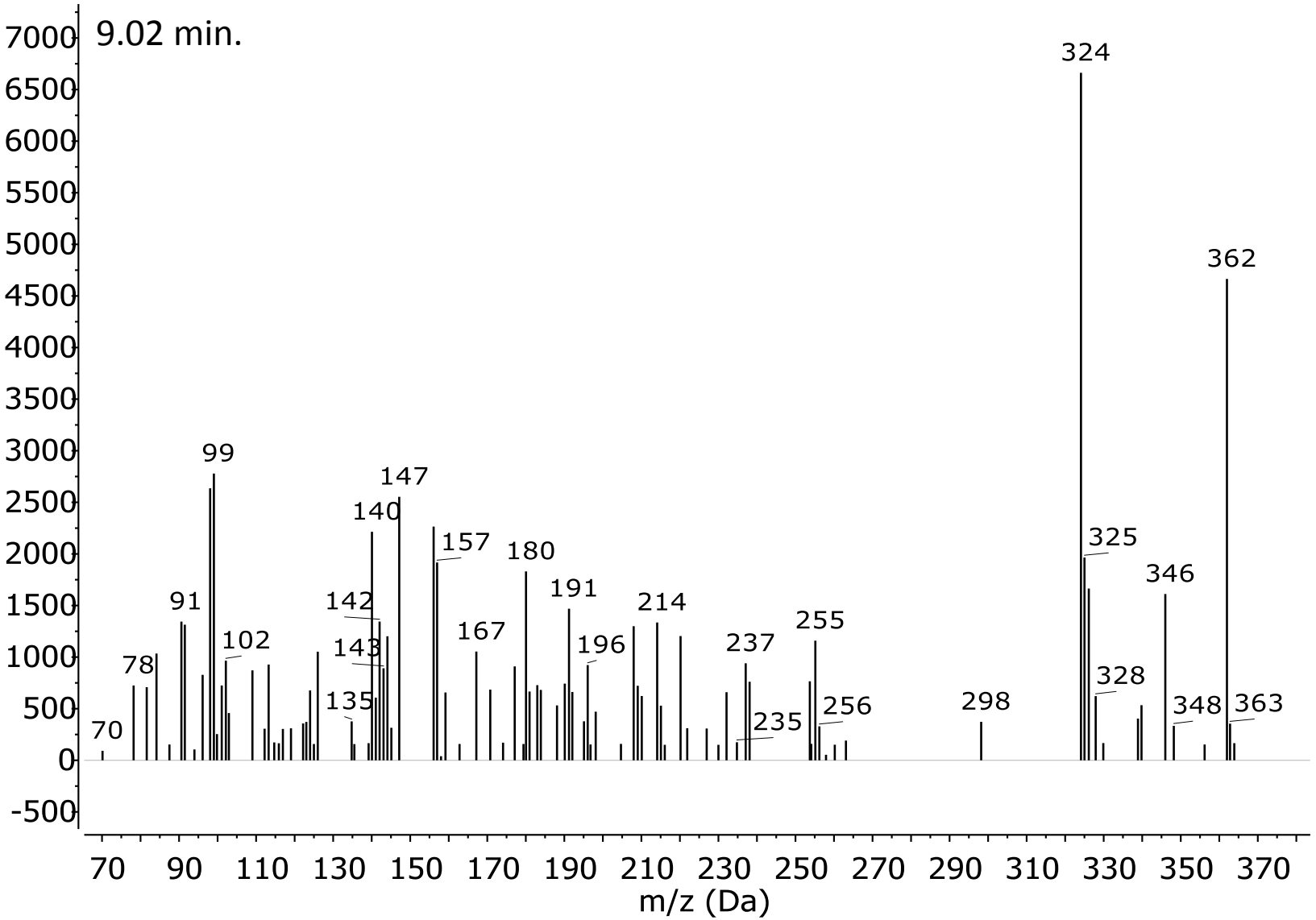

12.96 min
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
324
353
203
249
75
102
325
326
355
278
204
177
101
202
238
250
290
354
51
63
76
115
149
175
191
205
239
306
50
62
87
99
243
272
299
308
338
356
42
61
64
335
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
m/z (Da)

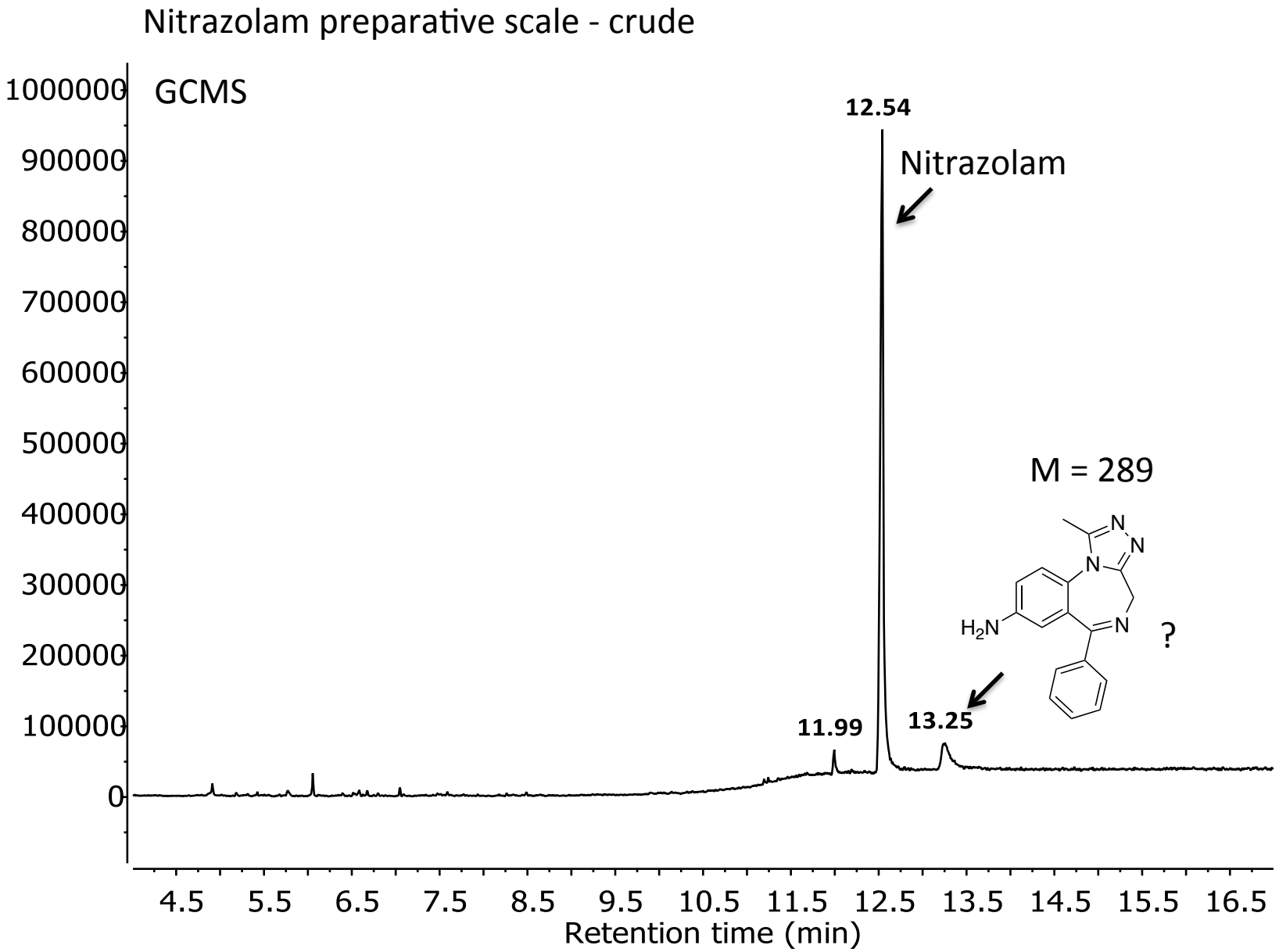

Clonazolam − crude product − preparative scale
LC-ESIMS
10.52
Clonazolam
Hydrazide
precursor
9.99
320000
280000
240000
200000
160000
120000
80000
40000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Retention time (min)

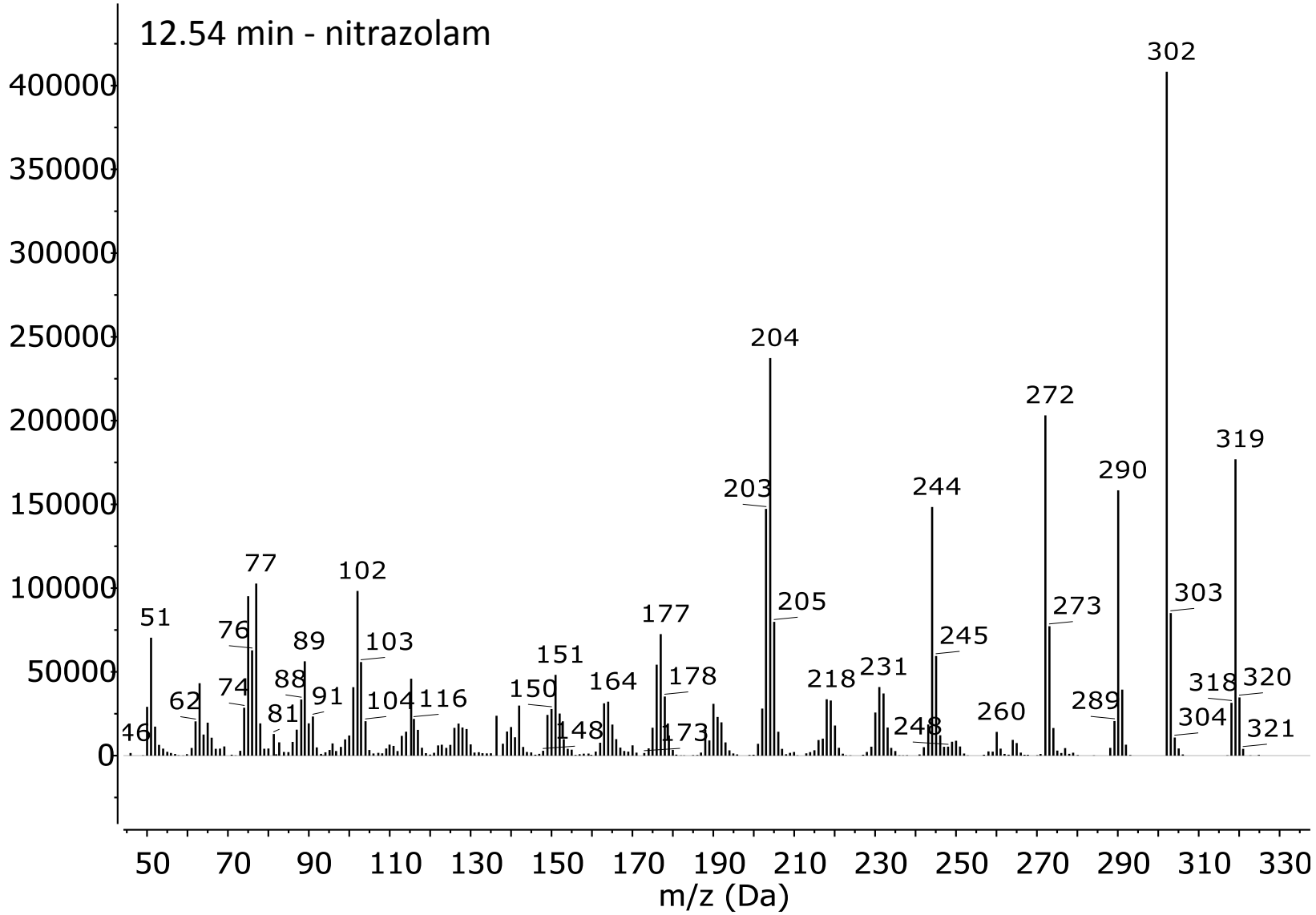

TIC
200000
0
10.52
9.99
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Retention time (min)
m/z 354
100000
0
10.52
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Retention time (min)
m/z 372
4000
0
9.99
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Retention time (min)
m/z 324
500
0
9.02
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Retention time (min)

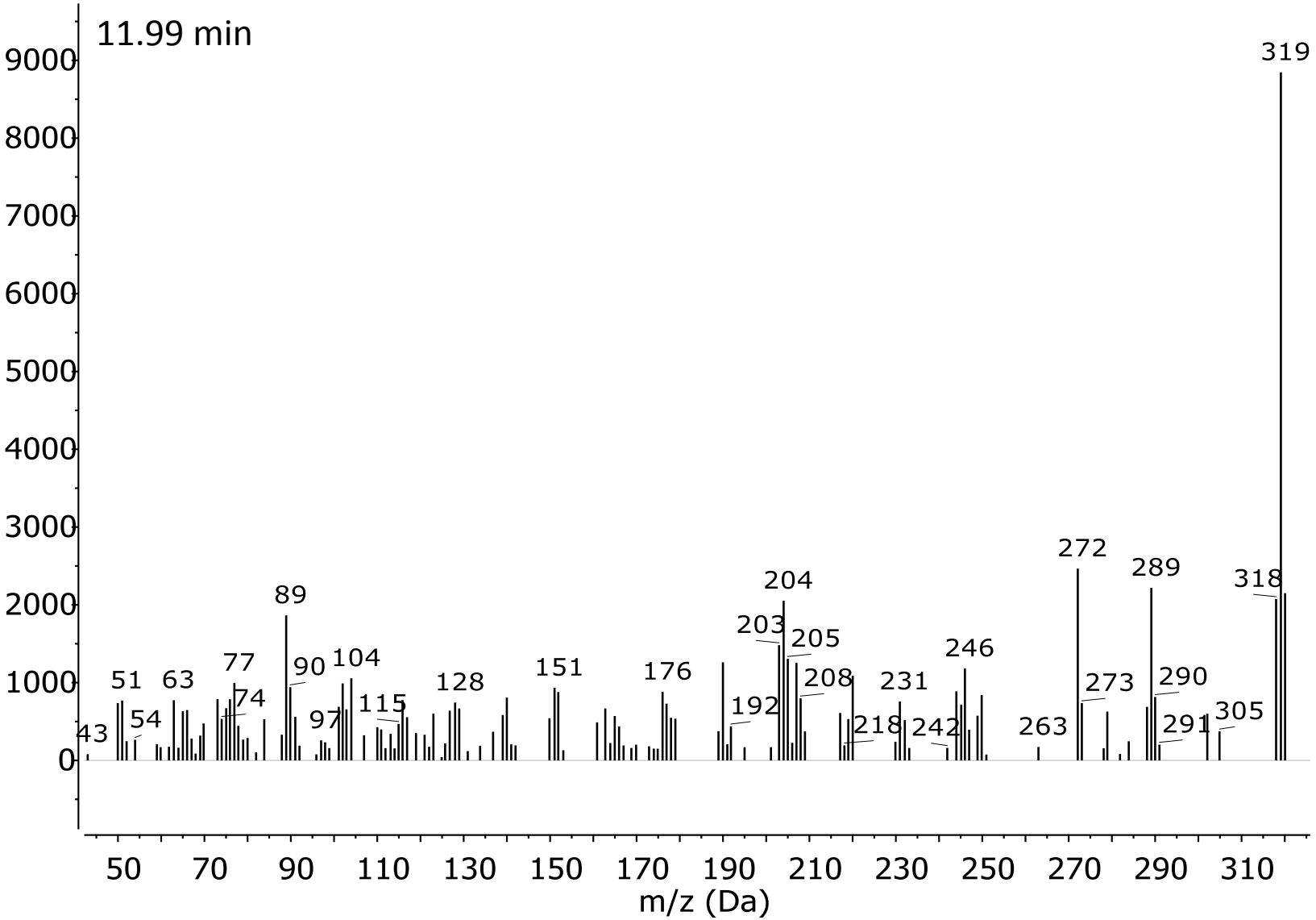

10.52 min.
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
354
356
355
376
377
392
88
237
70 90 110 130 150 170 190 210 230 250 270 290 310 330 350 370 390 41
m/z (Da)


9.99 min.
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
372
374
410
214
394
89
373
82
91
236
396
412
395
79
98
114
140
169
187
210
234
252
269
298
315
333
365
390
413
138
153
70
100
130
160
190
220
250
280
310
340
370
400
m/z (Da)

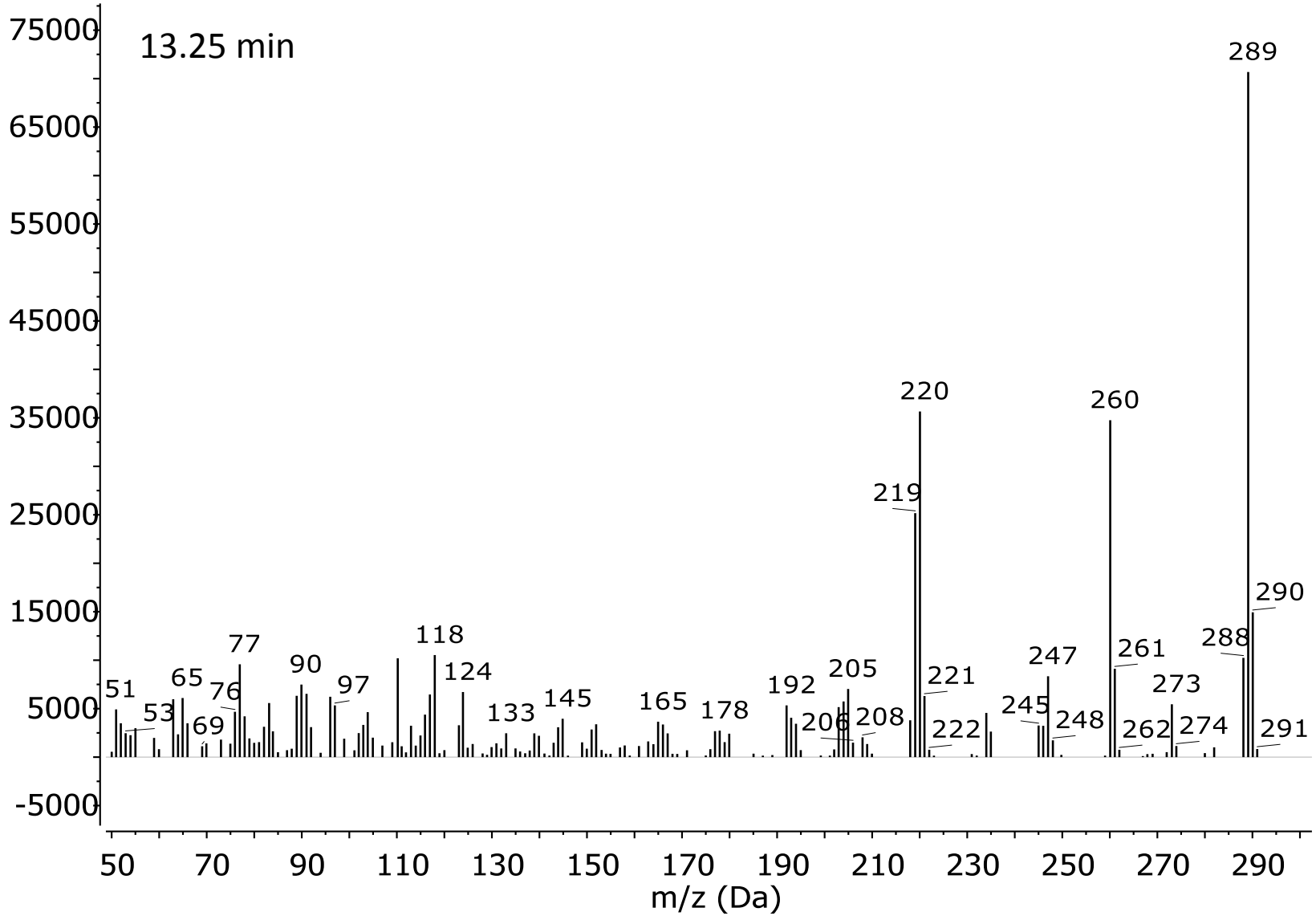

9.02 min.
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
70
78
91
99
102
135
140
142
143
147
157
167
180
191
196
214
235
237
255
256
298
324
325
328
346
348
362
363
70
90
110
130
150
170
190
210
230
250
270
290
310
330
350
370
m/z (Da)


Nitrazolam preparative scale - crude
GCMS
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
12.54
Nitrazolam
M = 289
H2N
?
11.99
13.25
4.5
5.5
6.5
7.5
8.5
9.5
10.5
11.5
12.5
13.5
14.5
15.5
16.5
Retention time (min)


12.54 min - nitrazolam
400000
350000
300000
250000
200000
150000
100000
50000
0
50
70
90
110
130
150
170
190
210
230
250
270
290
310
330
m/z (Da)
302
204
272
319
290
203
244
77
102
205
303
51
177
273
76
89
103
245
151
231
178
218
318
320
88
164
62
74
91
104
116
150
289
260
304
81
148
173
248
321
46

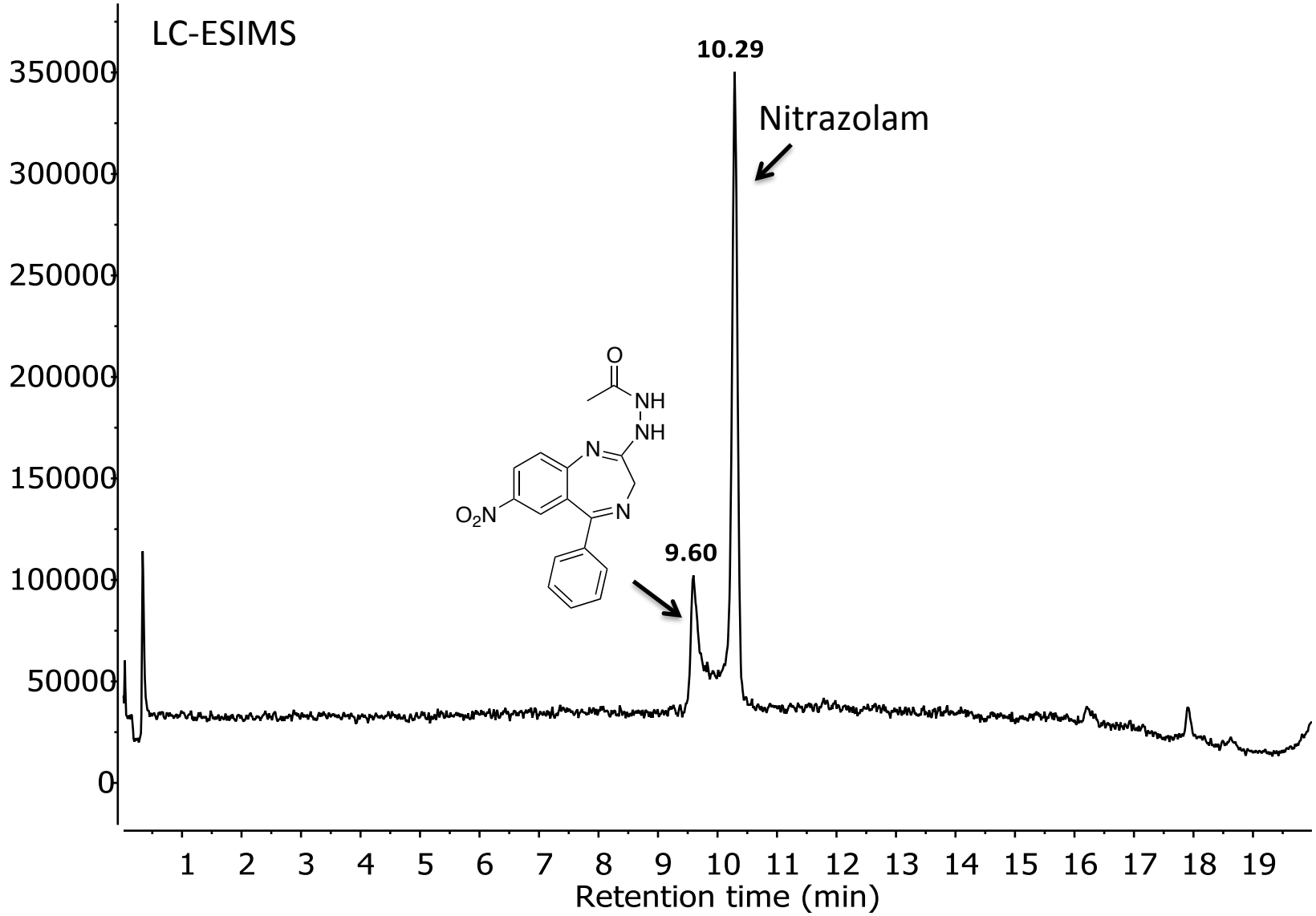


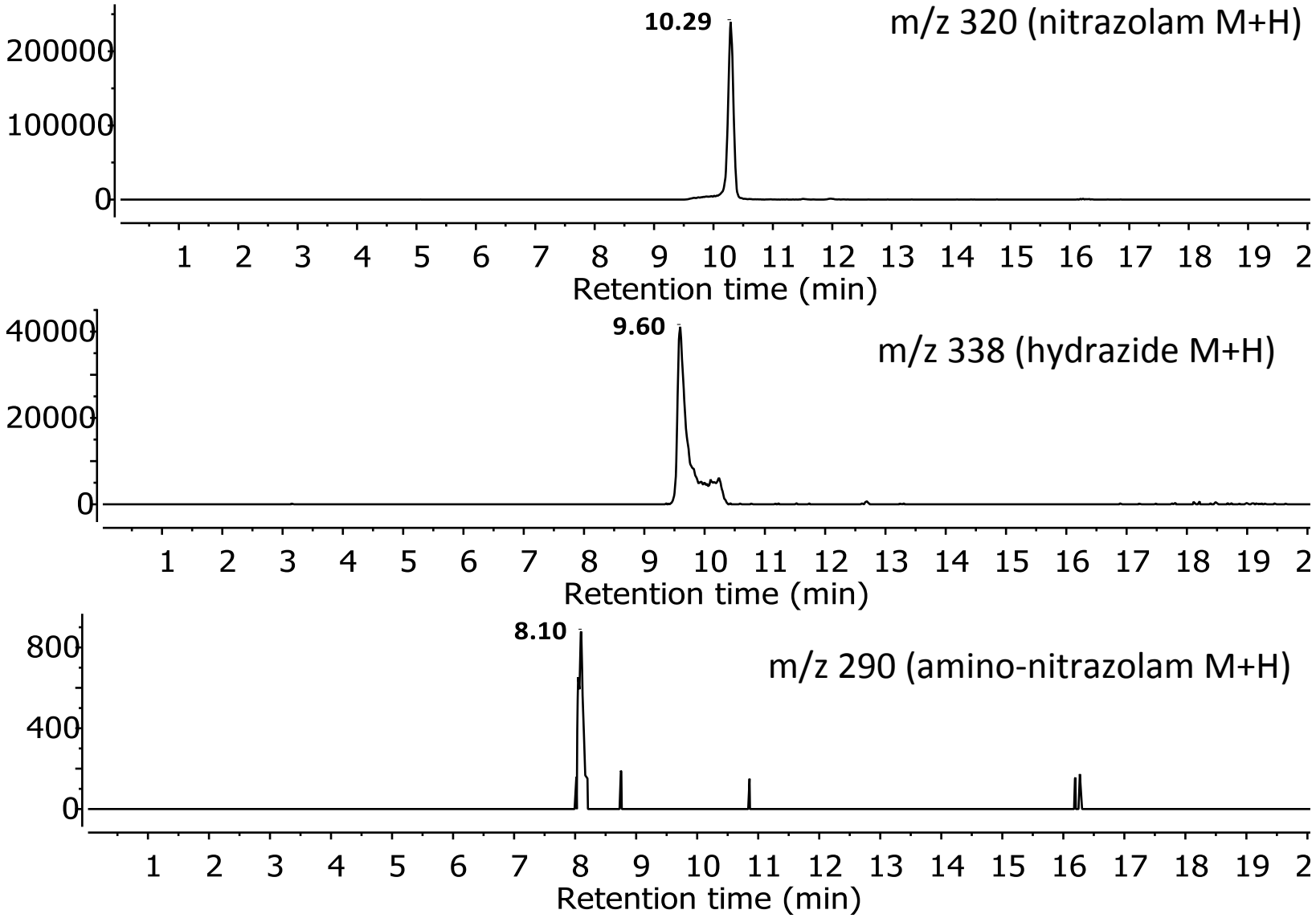
11.99 min
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
319
272
289
318
204
89
203
205
246
77
90
104
151
176
51
63
128
208
231
273
290
74
115
192
305
54
97
218
242
263
291
43
50
70
90
110
130
150
170
190
210
230
250
270
290
310
m/z (Da)

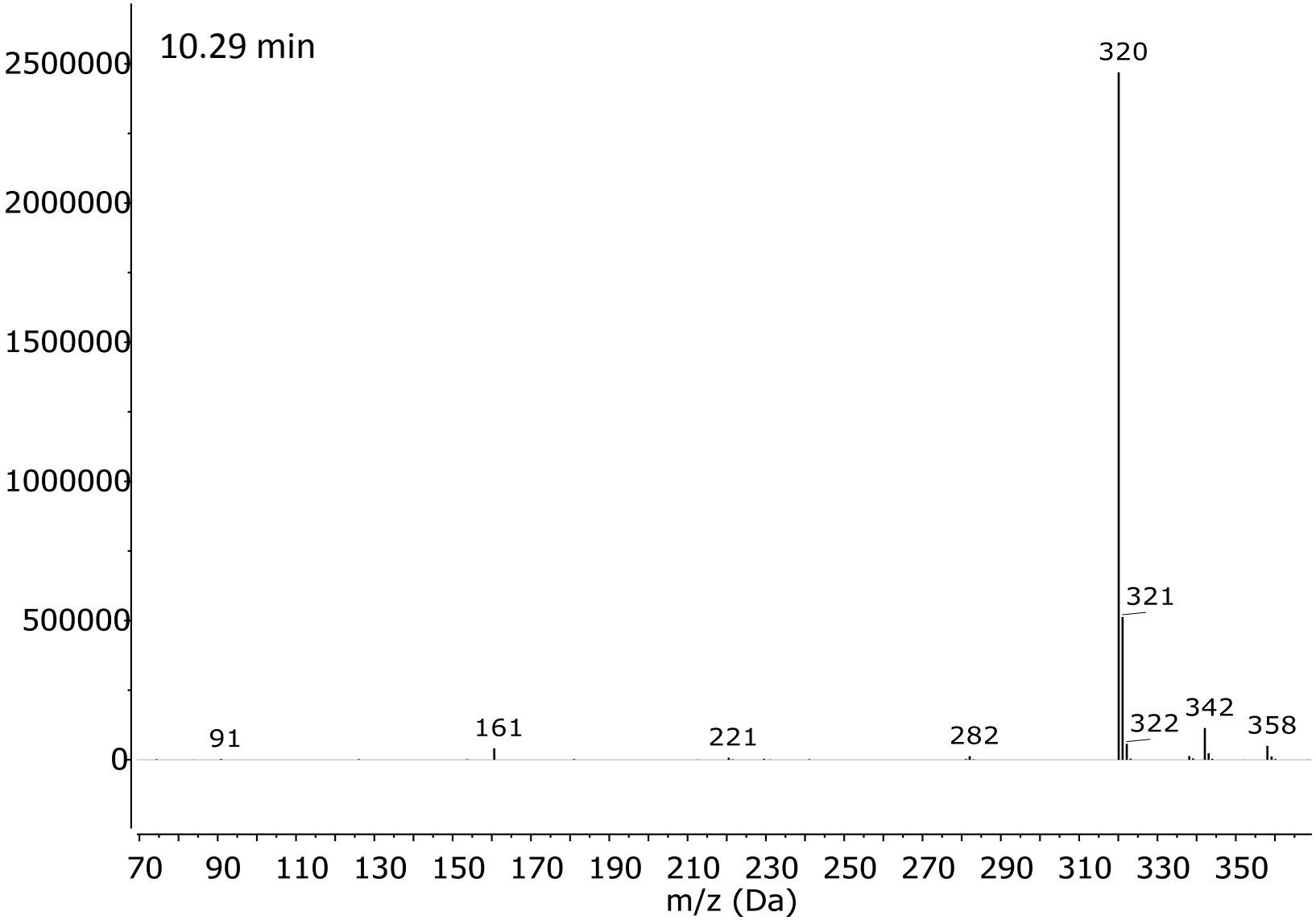


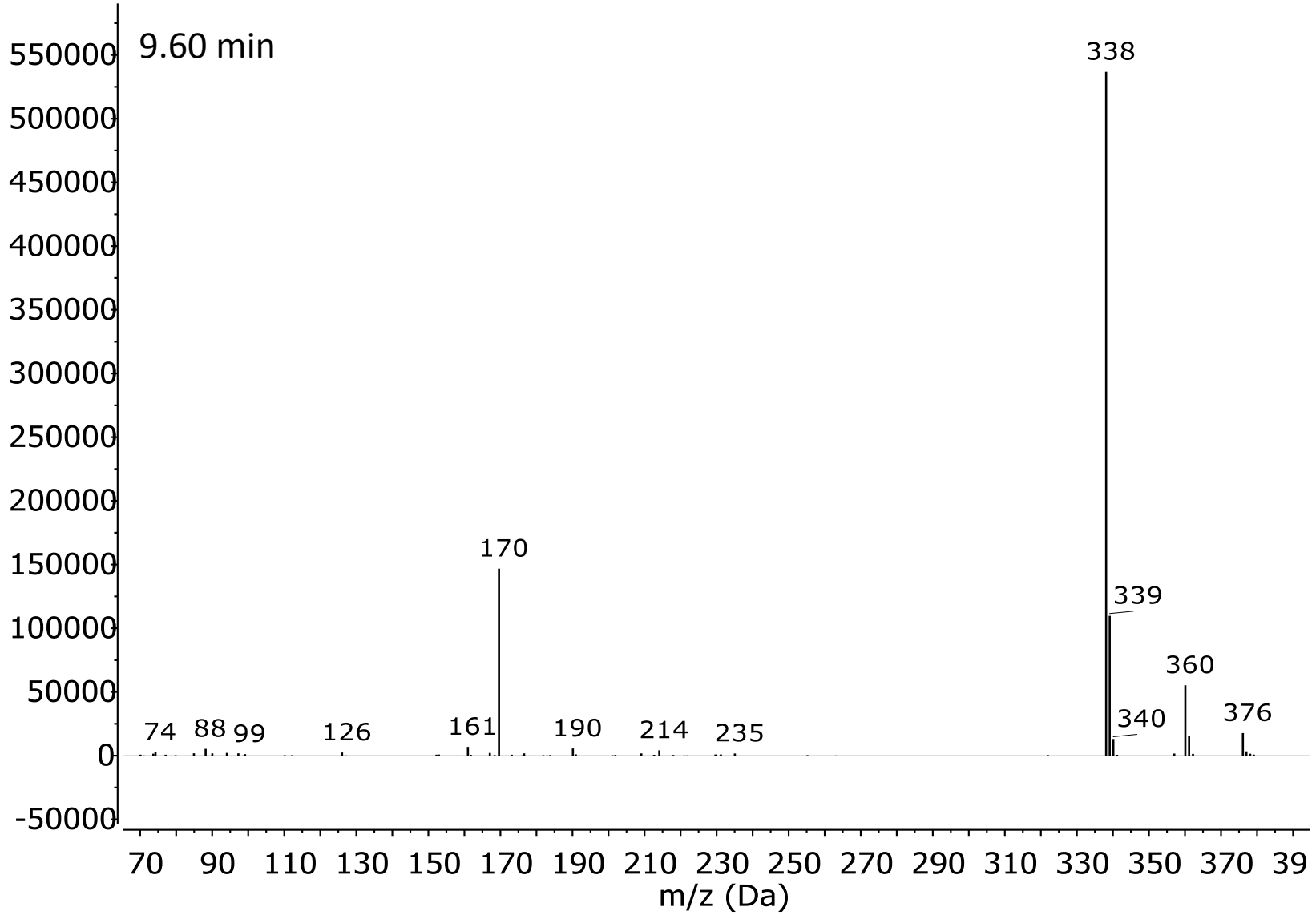
13.25 min
75000
65000
55000
45000
35000
25000
15000
5000
-5000
289
220
260
219
290
118
77
90
124
205
247
261
288
51
65
76
97
133
145
165
178
192
221
273
53
69
206
208
222
245
248
262
274
291
50
70
90
110
130
150
170
190
210
230
250
270
290
m/z (Da)

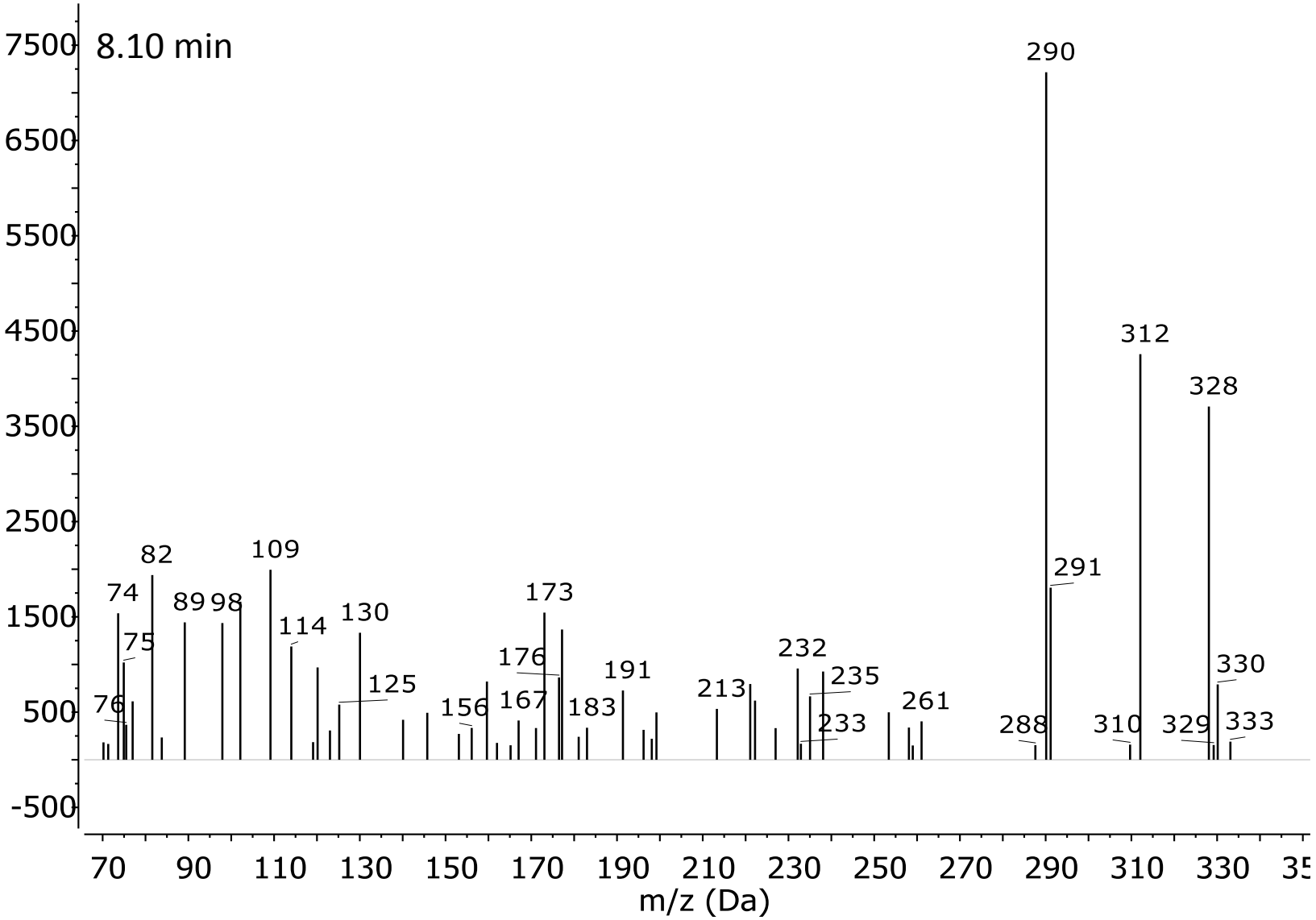
Nitrazolam preparative scale - crude




10.29
m/z 320 (nitrazolam M+H)
200000
100000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 2
Retention time (min)
9.60
m/z 338 (hydrazide M+H)
40000
20000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 2
Retention time (min)
8.10
m/z 290 (amino-nitrazolam M+H)
800
400
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 2
Retention time (min)


10.29 min
2500000
2000000
1500000
1000000
500000
0
320
321
322
342
358
91
161
221
282
70
90
110
130
150
170
190
210
230
250
270
290
310
330
350
m/z (Da)


9.60 min
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
-50000
338
170
339
360
340
376
74
88
99
126
161
190
214
235
70
90
110
130
150
170
190
210
230
250
270
290
310
330
350
370
m/z (Da)


8.10 min
7500
6500
5500
4500
3500
2500
1500
500
-500
290
312
328
291
330
82
109
74
89
98
173
114
130
75
176
232
235
191
125
213
76
156
167
183
233
261
288
310
329
333
70
90
110
130
150
170
190
210
230
250
270
290
310
330
m/z (Da)