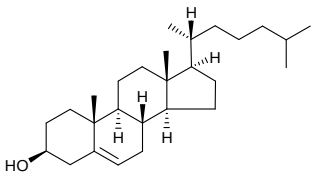
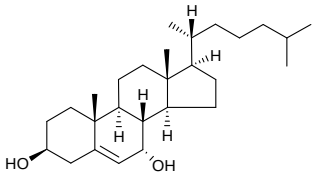
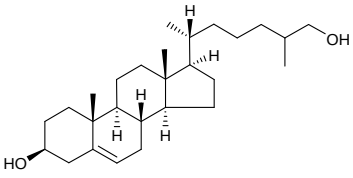
Figure S1. Sterol structures



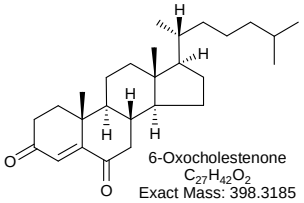
Cholesterol
$C_{27}H_{46}O$
Exact Mass: 386.3549



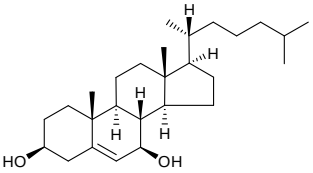
7α-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



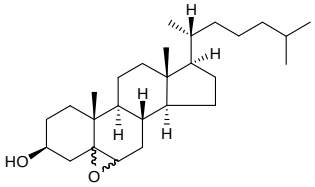
27-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



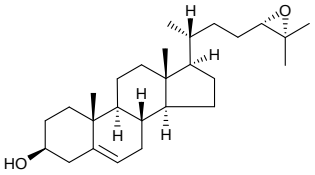
6-Oxocholestenone
$C_{27}H_{42}O_2$
Exact Mass: 398.3185



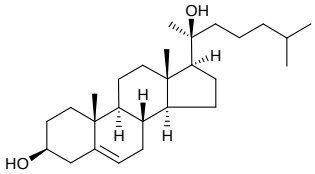
7β-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



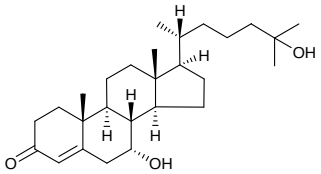
5,6-Epoxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



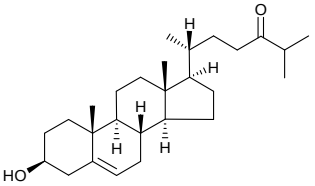
24S,25-Epoxycholesterol
$C_{27}H_{44}O_2$
Exact Mass: 400.3341



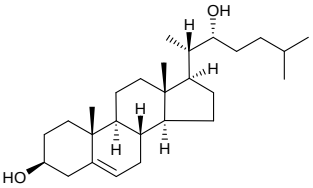
20S-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



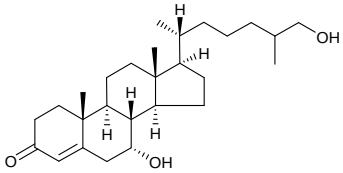
7α,25-Dihydroxycholest-4-en-3-one
$C_{27}H_{44}O_3$
Exact Mass: 416.3290



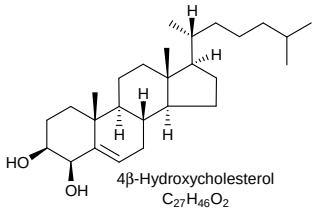
24-Oxocholesterol
$C_{27}H_{44}O_2$
Exact Mass: 400.3341



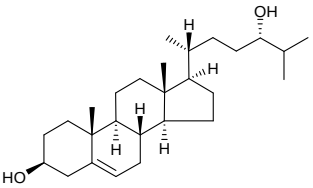
22R-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



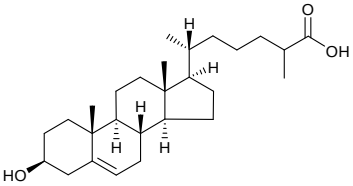
7α,27-Dihydroxycholest-4-en-3-one
$C_{27}H_{44}O_3$
Exact Mass: 416.3290



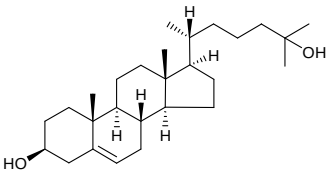
4β-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



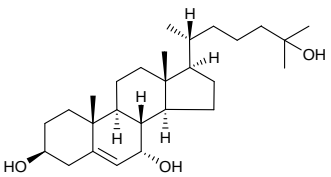
24S-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



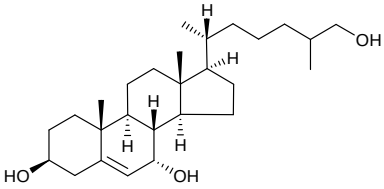
3β-Hydroxycholest-5-en-27-oic acid
$C_{27}H_{44}O_3$
Exact Mass: 416.3290



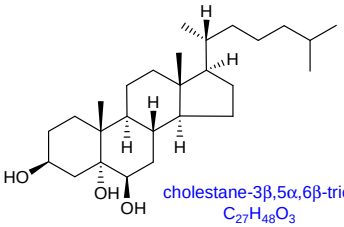
25-Hydroxycholesterol
$C_{27}H_{46}O_2$
Exact Mass: 402.3498



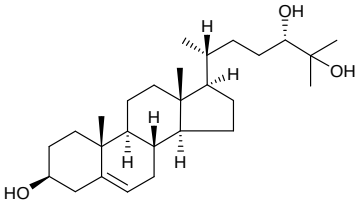
7α,25-Dihydroxycholesterol
$C_{27}H_{46}O_3$
Exact Mass: 418.3447



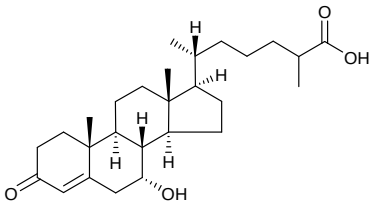
7α,27-Dihydroxycholesterol
$C_{27}H_{46}O_3$
Exact Mass: 418.3447



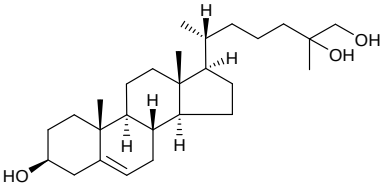
cholestane-3β,5α,6β-triol
$C_{27}H_{48}O_3$
Exact Mass: 420.3603



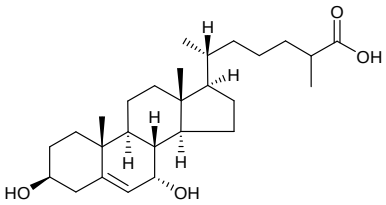
24S,25-Dihydroxycholesterol
$C_{27}H_{46}O_3$
Exact Mass: 418.3447



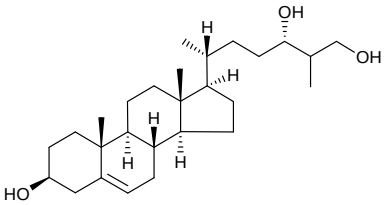
7α-Hydroxy-3-oxocholest-4-en-27-oic acid
$C_{27}H_{42}O_4$
Exact Mass: 430.3083



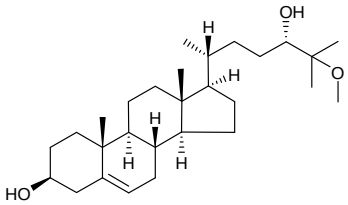
25,27-Dihydroxycholesterol
$C_{27}H_{46}O_3$
Exact Mass: 418.3447



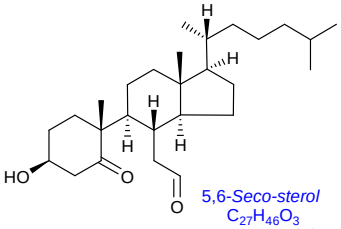
3β,7α-Dihydroxycholest-5-en-27-oic acid
$C_{27}H_{44}O_4$
Exact Mass: 432.3240



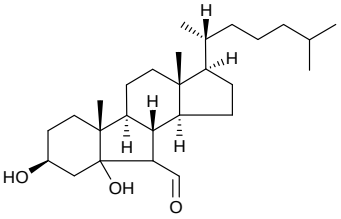
24S,27-Dihydroxycholesterol
$C_{27}H_{46}O_3$
Exact Mass: 418.3447



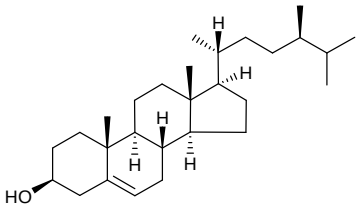
24S-Hydroxy-25-methoxycholesterol
$C_{28}H_{48}O_3$
Exact Mass: 432.3603



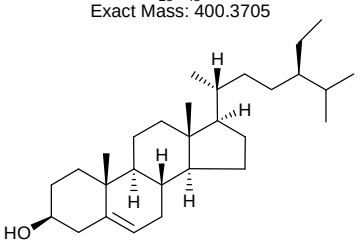
5,6-*Seco-sterol*
$C_{27}H_{46}O_3$
Exact Mass: 418.3447



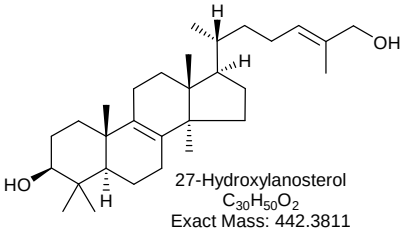
Aldol
$C_{27}H_{46}O_3$
Exact Mass: 418.3447



Campesterol
$C_{28}H_{48}O$
Exact Mass: 400.3705



Sitosterol
$C_{29}H_{50}O$
Exact Mass: 414.3862



27-Hydroxylanosterol
$C_{30}H_{50}O_2$
Exact Mass: 442.3811

Fig. S2 Analytical procedure

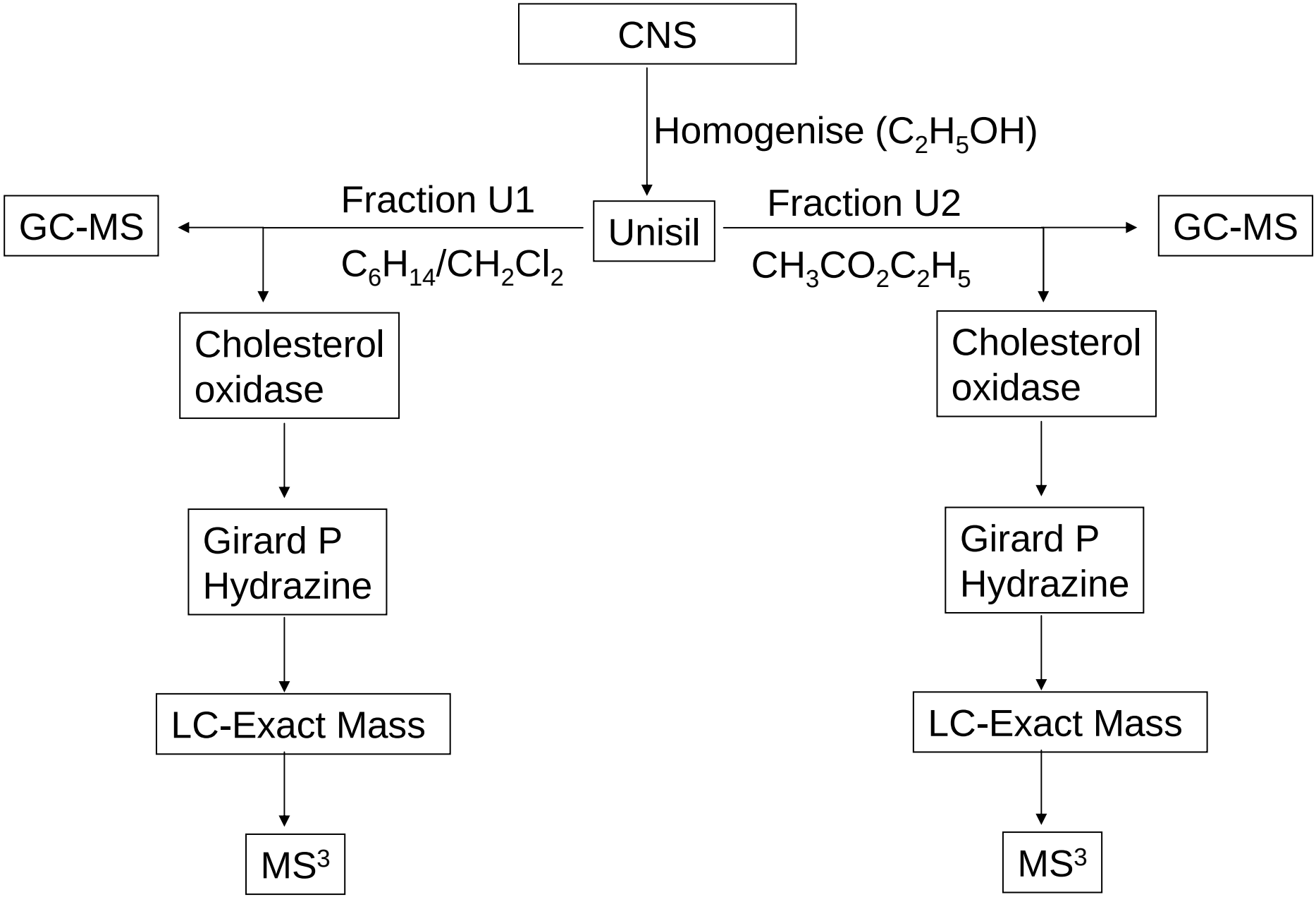


Fig. S3a Oxidation of sterols with cholesterol oxidase and derivatisation with the Girard P reagent.

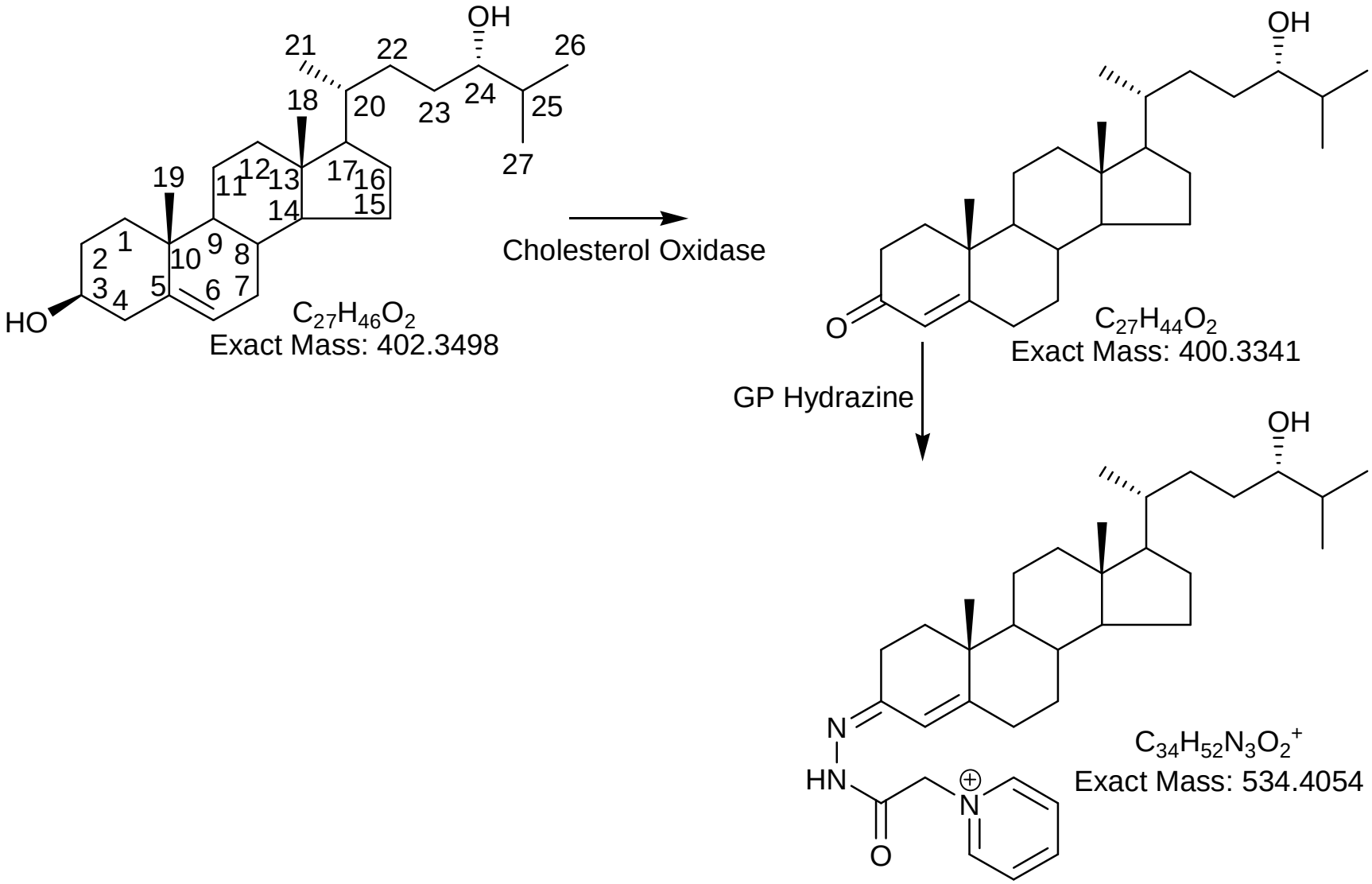


Fig. S3b Prominent fragment ions observed in MS^2 spectra of oxidised/GP-derivatised sterols.

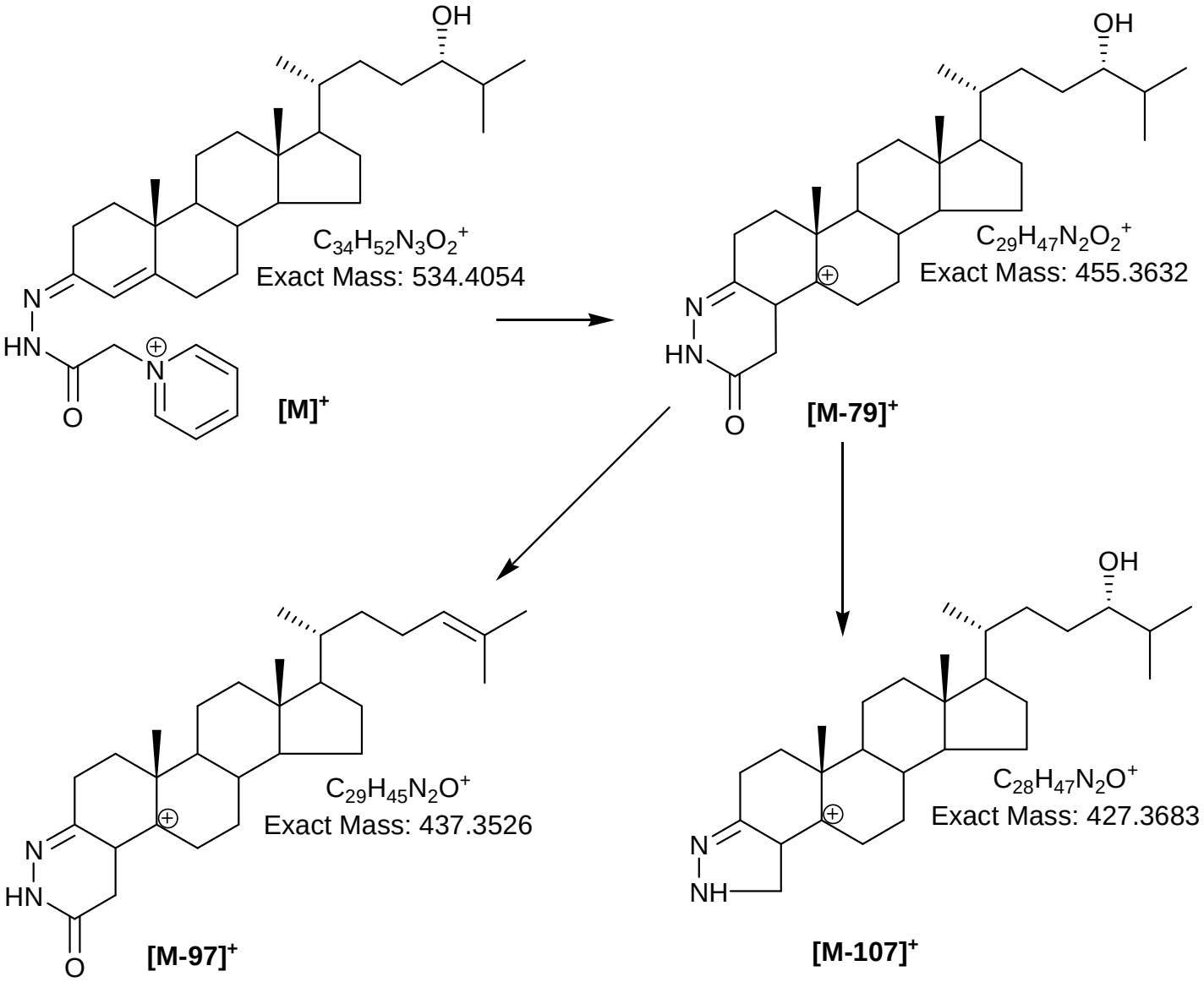
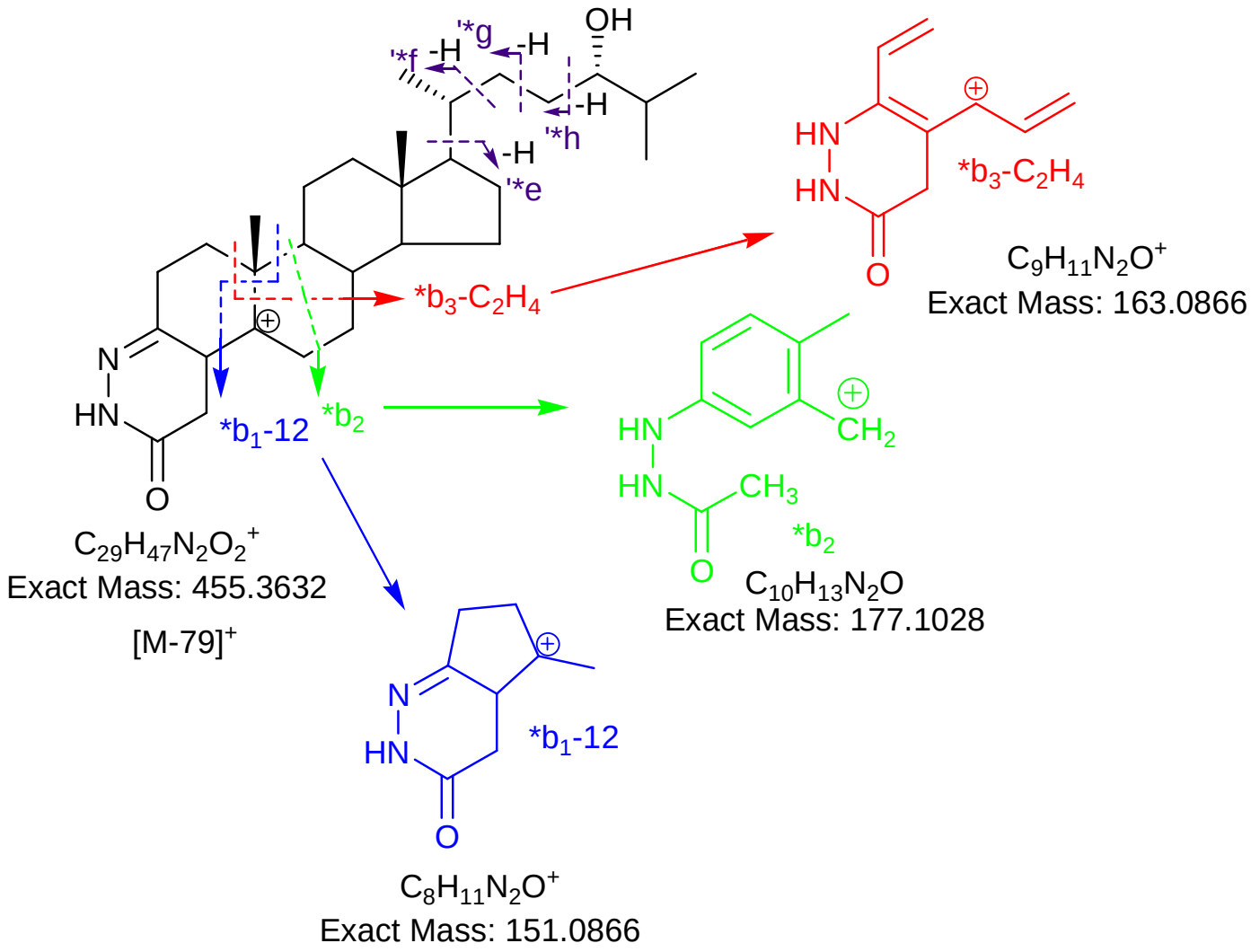


Fig. S3c Major fragment ions characteristic of 3-oxo-4-ene sterols derivatised with GP reagent.



MS^n Spectra of Oxysterols from Foetal Mouse Brain

		Ctx[a]		Sc[a]				Figure	
Identified Structure after Treatment with Cholesterol Oxidase	**[M]+ of GP[a] *m/z***	**µg/g**	**Rt/min**	**µg/g**	**Rt/min**	**Inferred Structure prior to Treatment with Cholesterol Oxidase**	**Inferred Compound Trivial Name**	**MS²**	**MS³**
C-4,24-diene-3-one	516.3948	NM	10.53	NM	10.52	C-5,24-diene-3β-ol	Desmosterol	S4a	S4b
C-4,7(or8)-diene-3-one	516.3948	NM	11.00	ND		C-5,7(or 8)-diene-3β-ol	7(or 8)-Dehydrocholesterol[b]	S4c	S4d
C-4-ene-3-one	518.4105	NM	11.43	NM	11.43	C-5-ene-3β-ol	Cholesterol	S4e	S4f
24-Me-C-4-ene-3-one	532.4261	NM	12.13	NM	12.09	24-Me-C-5-ene-3β-ol	Campesterol[c]	S4g	S4h
24-Et-C-4-ene-3-one	546.4418	NM	12.84	NM	12.76	24-Et-C-5-ene-3β-ol	Sitosterol[c,d]	--	--
C-X,Y,Z-triene-x-ol-y-one	530.3741	0.008	9.1	ND		C-X,Y,Z-triene-3β,x-diol[e]	Unknown[e]	--	--
C-4-ene-24S,25-epoxide-3-one	532.3898	0.043	6.63/6.86	0.022	6.66/6.85	C-5-ene-3β-ol-24S,25-epoxide	24S,25-Epoxycholesterol	S4i	S4j
C-4-ene-24-ol,25-OMe-3-one[f]	564.416	0.122	6.05/6.42	0.069	6.06/6.43	C-5-ene-3β-ol-24S,25-epoxide	24S,25-Epoxycholesterol	S4k	S4L
		0.165		**0.091**		**C-5-ene-3β-ol-24S,25-epoxide**	**24S,25-Epoxycholesterol**		
C-4-ene-3,24-dione	532.3898	0.093	7.35	0.013	7.38	C-5-ene-3β-ol-24-one	24-Oxocholesterol	S4m	S4n
C-4-ene-3,6-dione	532.3898	0.185	9.76	0.019	9.94	C-4-ene-3,6-dione[g]	6-Oxocholestenone[g]	S4o	S4p
C-4-ene-22R-ol-3-one	534.4054	0.004	6.14	0.002	6.17	C-5-ene-3β,22R-diol	22R-Hydroxycholesterol	S4q	S4r
C-4-ene-24S-ol-3-one	534.4054	0.026	7.17/7.55	0.013	7.16/7.56	C-5-ene-3β,24S-diol	24S-Hydroxycholesterol	S4s	S4t
C-4-ene-25-ol-3-one	534.4054	0.016	7.39	0.012	7.39	C-5-ene-3β,25-diol	25-Hydroxycholesterol	S4u	S4v
C-4-ene-27-ol-3-one	534.4054	0.011	8.45/8.83	0.006	8.45/8.85	C-5-ene-3β,27-diol	27-Hydroxycholesterol	S4w	S4x
C-4-ene-7β-ol-3-one	534.4054	0.052	9.08/9.48	0.053	9.11/9.5	C-5-ene-3β,7β-diol	7β-Hydroxycholesterol	S4y	S4z
C-4-ene-7α-ol-3-one	534.4054	0.055	9.61	0.067	9.59	C-5-ene-3β,7α-diol	7α-Hydroxycholesterol[h]	S4aa	S4ab
C-7-ene-x-ol-3-one	534.4054	0.152	9.27/9.78	0.103	9.26/9.76	C-7-ene-3β,x-diol	Hydroxylathosterol	S4ac	S4ad
C-4-ene-6β-ol-3-one	534.4054	0.568	9.89	0.109	9.89	C-4-ene-6β-ol-3-one/C-3β,5α,6β-triol/C-3β-ol-5,6-epoxide[i]	6β-hydroxycholest-4-en-3-one/cholestane-3β,5α,6β-triol/5,6-epoxycholesterol[i]	S4ae	S4af

C-X,Y,Z-triene-x,y-diol-z-one	546.369	0.009	8.34	ND		C-X,Y,Z-triene-3β,x,y-triol[j]	Unknown[j]		
C-X,Y,Z-triene-x,y-diol-z-one	546.369	0.009	8.7	0.002	8.71	C-X,Y,Z-triene-3b,x,y-triol[j]	Unknown[j]		
C-X,Y,Z-triene-x,y-diol-z-one	546.369	0.026	9.29	ND		C-X,Y,Z-triene-3β,x,y-triol[j]	Unknown[j]		
C-X,Y-diene-x,y-diol-z-one	548.3847	0.004	5.45	ND		C-X,Y-diene-3β,x,y-triol[k]	Unknown[k]		
C-X,Y-diene-x,y-diol-z-one	548.3847	ND		0.001	7.31	C-X,Y-diene-3β,x,y-triol[k]	Unknown[k]		
C-X,Y-diene-x,y-diol-z-one	548.3847	0.028	9.78	0.007	9.76	C-X,Y-diene-3β,x,y-triol[k]	Unknown[k]	S4ag	S4ah
C-X,Y-diene-x,y-diol-z-one	548.3847	0.009	10.05	0.002	10.07	C-X,Y-diene-3β,x,y-triol[k]	Unknown[k]	S4ai	S4aj
C-4-ene-24,25-diol-3-one	550.4003	0.070	4.42/4.95	0.038	4.42/4.98	C-5-ene-3β,24,25-triol	24,25-Dihydroxycholesterol	S4ak	S4aL
C-4-ene-24,27-diol-3-one	550.4003	0.007	4.83	0.006	4.85	C-5-ene-3β,24,27-triol	24,27-Dihydroxycholesterol	S4am	S4an
C-x-ol-y,z-dione	550.4003	0.017	9.08	0.041	9.11	C-3β,x-diol-y-one[L]	Unknown[L]	S4ao	S4ap
C-x,y-diol-z-one	552.4160	0.002	5.67	0.001	5.68	C-3β,x,y-triol	Unknown		
C-x,y-diol-z-one	552.4160	0.018	9.44/9.89	0.002	9.47	C-3β,x,y-triol	Unknown	S4aq	S4ar
aldol	552.4160	0.066	9.59	0.075	9.59	aldol[m]	aldol[m]	S4as	

C = cholestane; NM = not measured; Rt = retention time; ND = not detected; No reference = no authentic standard available

[a] Data for GP derivatives

[b] 7- and 8-dehyrocholesterol are not resolved

[c] Possibly a contaminant

[d] Identification based on exact mass and isotopic pattern only

[e] Alternatively C-X,Y-dien-3β-ol-x-one

[f] Alternatively C-4-ene-25-ol,24-OMe-3-one, methanolysis product of C-4-ene-24S,25-epoxide-3-one

[g] C-4-ene-3,6-dione reacts with GP reagent without oxidation by cholesterol oxidase

[h] Formed enzymatically by CYP7A1 and/or by autoxidation

[i] C-4-ene-6β-ol-3-one can be formed from C-3β,5α,6β-triol and C-3β-ol-5,6-epoxides during the cholesterol oxidase/GP derivatisation reaction

[j] Alternatively C-X,Y-diene-3β,x-diol-y-one

[k] Alternatively C-X-ene-3β,x-diol-y-one

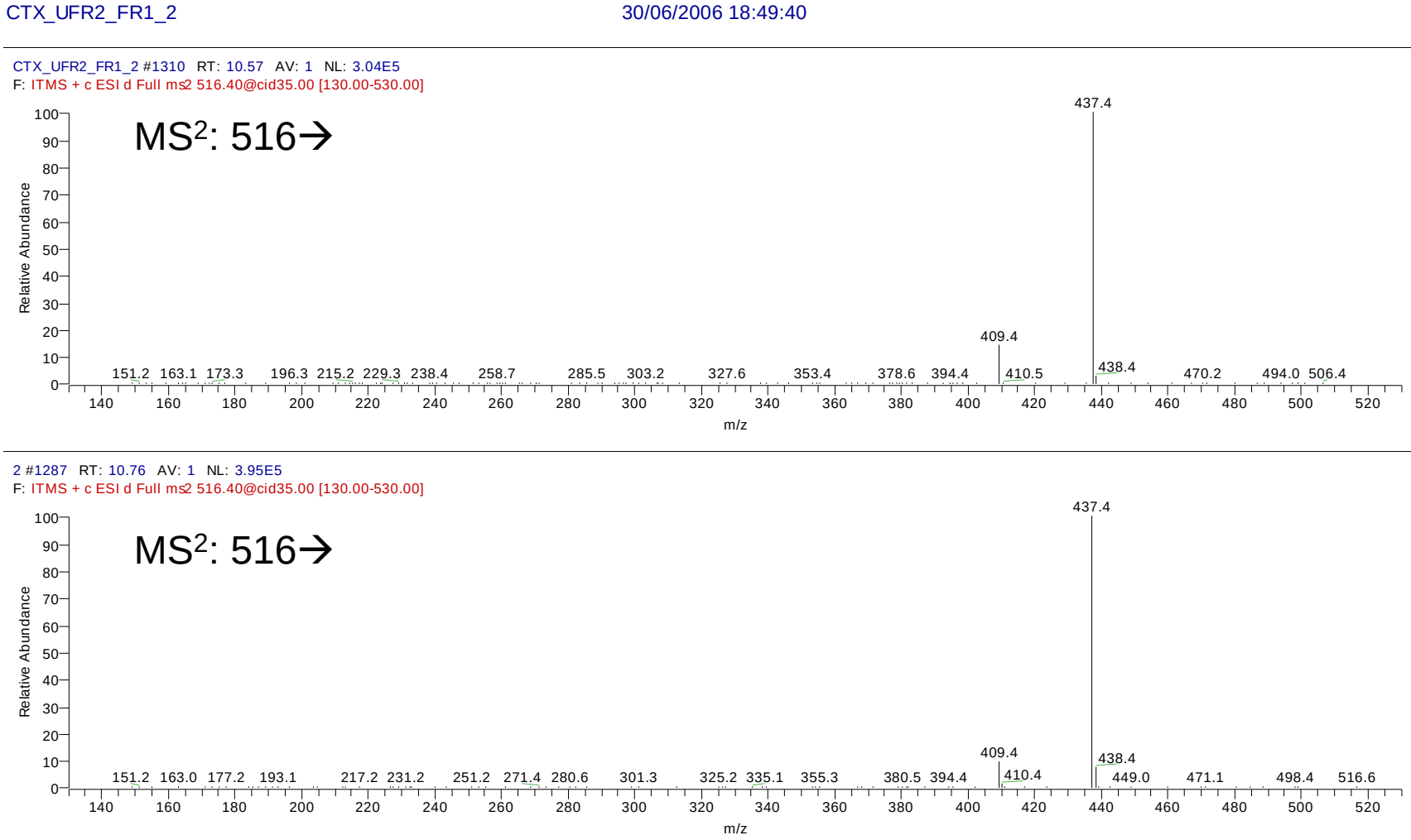
[L] Alternatively C-X-ene-3β,x,y-triol

[m] 3β,5β-dihydroxy-B-norcholestane-6β-carboxyaldehyde (aldol) reacts with GP reagent without oxidation by cholesterol oxidase

MS^2 and MS^3 Spectra of Oxysterols

- Upper panels display spectra of oxidised/derivatised oxysterols isolated from foetal mouse brain cortex
- Lower panels display spectra of authentic standards

Fig. S4a $C^{4,24}$-3-one 3-GP

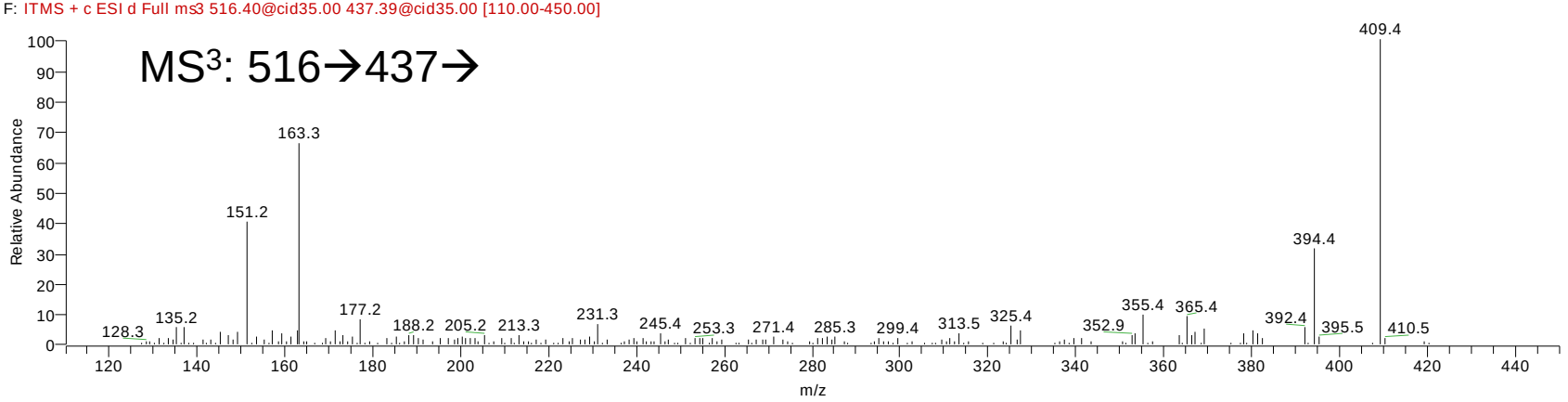


MS^2 and MS^3 spectra of sterols isolated from foetal mouse brain. Shown in the upper panels are spectra of sterols derived from the cortex and in the lower panels spectra of authentic standards. All spectra were recorded on the LIT detector of the LTQ-Orbitrap.

Fig. S4b $C^{4,24}$-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1311 RT: 10.57 AV: 1 NL: 1.75E4
F: ITMS + c ESI d Full ms3 516.40@cid35.00 437.39@cid35.00 [110.00-450.00]



2 #1288 RT: 10.77 AV: 1 NL: 2.64E4
F: ITMS + c ESI d Full ms3 516.40@cid35.00 437.34@cid35.00 [110.00-450.00]

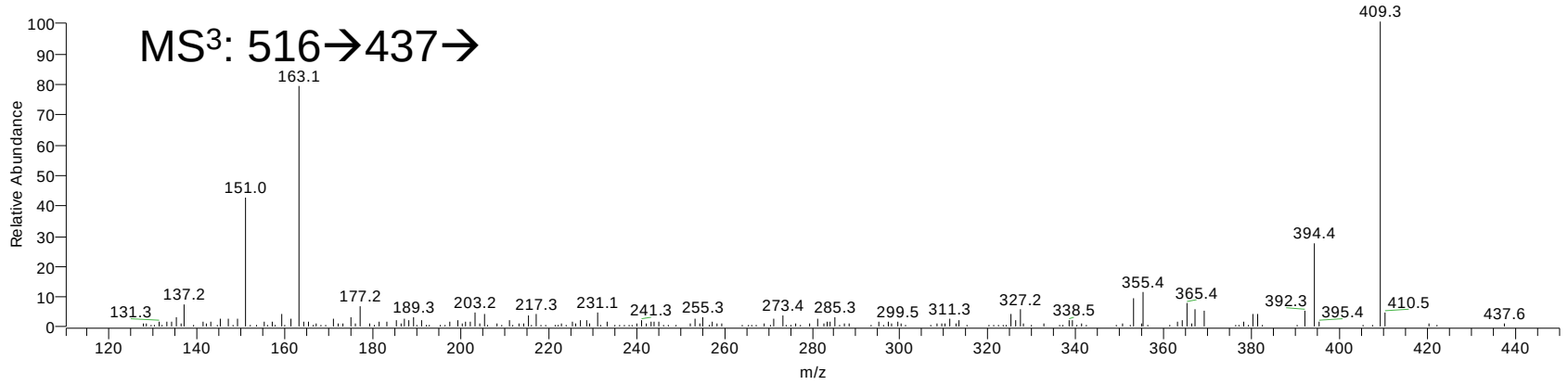


Fig. S4c $C^{4,7or8}$-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

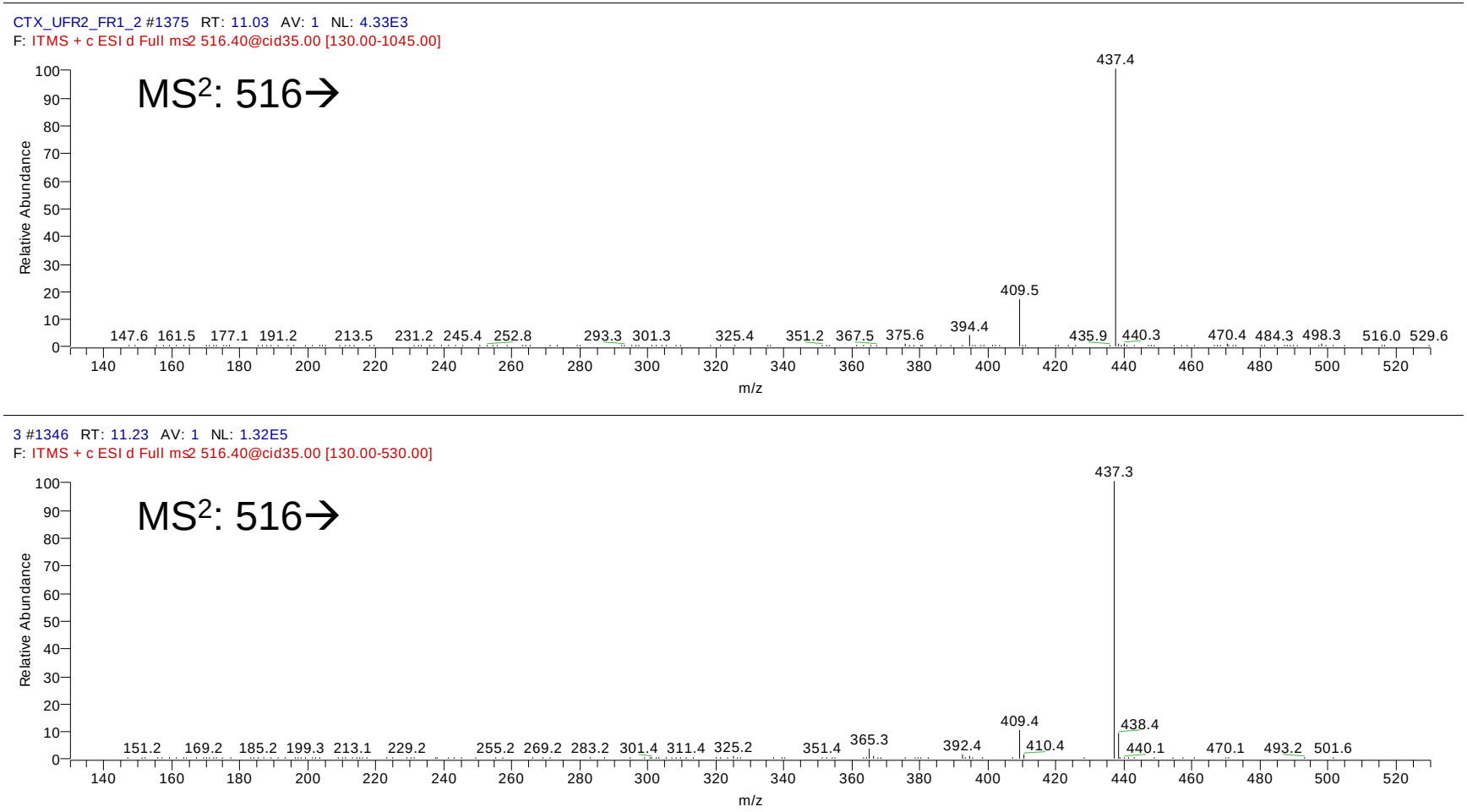
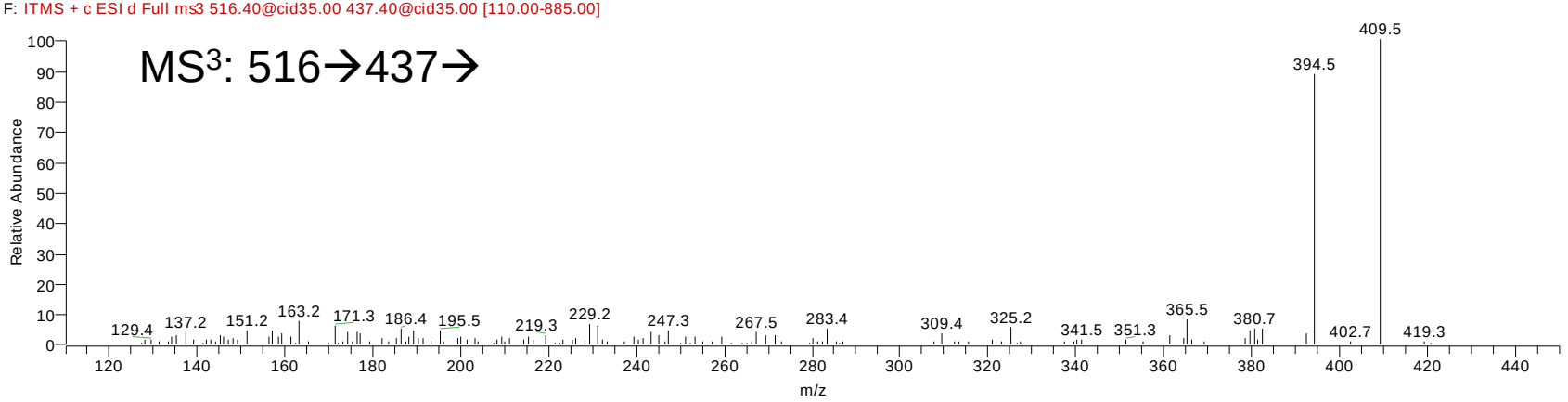


Fig. S4d $C^{4,7or8}$-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1376 RT: 11.04 AV: 1 NL: 2.66E2
F: ITMS + c ESI d Full ms3 516.40@cid35.00 437.40@cid35.00 [110.00-885.00]



3 #1347 RT: 11.23 AV: 1 NL: 9.79E3
F: ITMS + c ESI d Full ms3 516.40@cid35.00 437.33@cid35.00 [110.00-450.00]

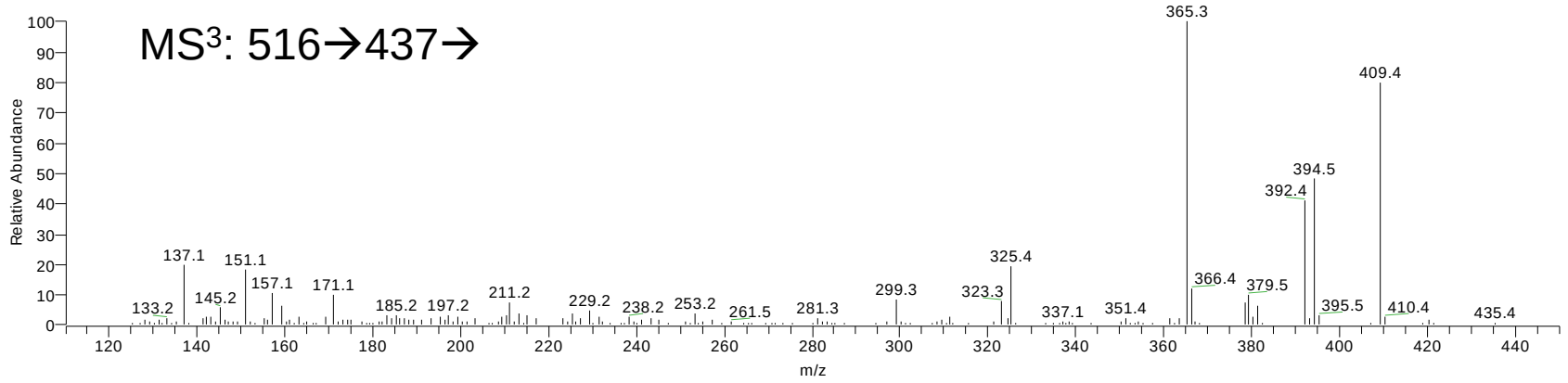
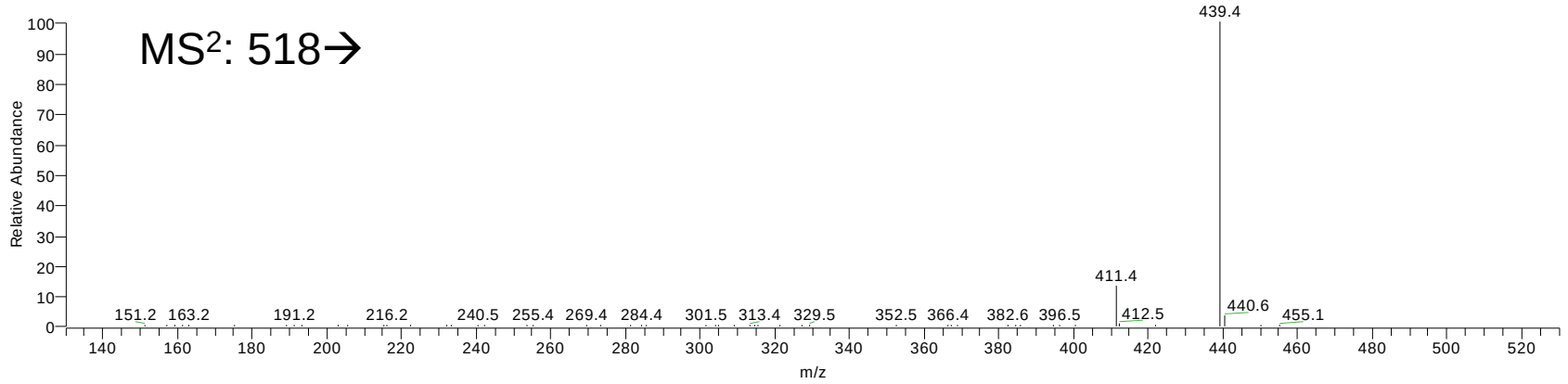


Fig. S4e C^4-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1433 RT: 11.44 AV: 1 NL: 2.93E6
F: ITMS + c ESI d Full ms2 518.41@cid35.00 [130.00-530.00]



1 #1401 RT: 11.65 AV: 1 NL: 1.08E6
F: ITMS + c ESI d Full ms2 518.41@cid35.00 [130.00-530.00]

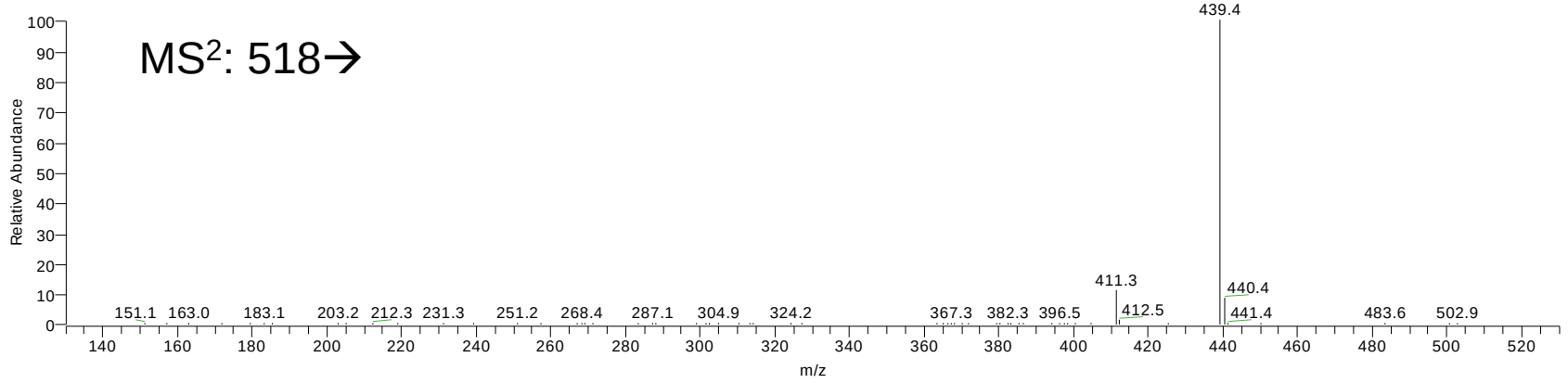
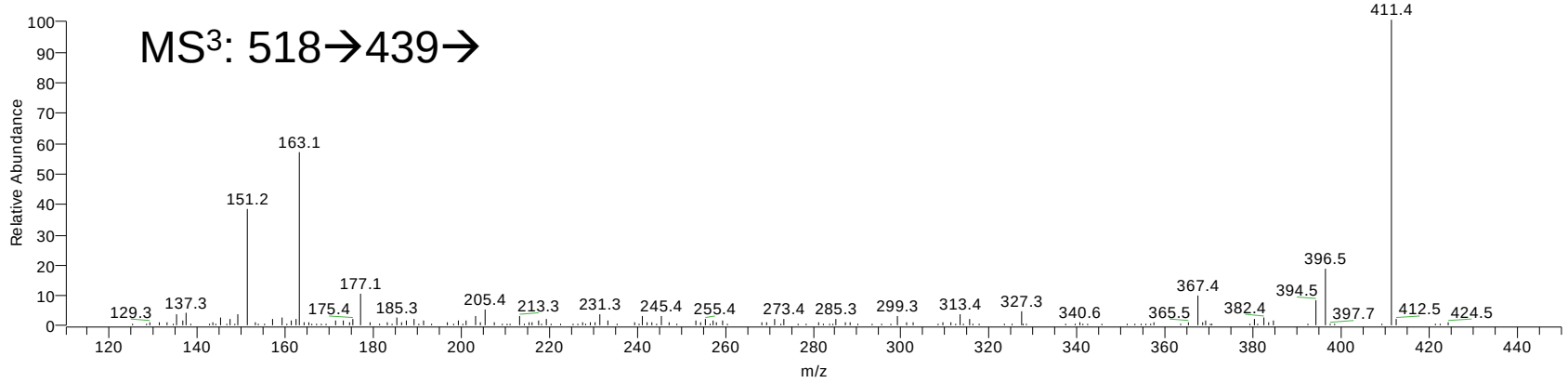


Fig. S4f C⁴-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1434 RT: 11.44 AV: 1 NL: 2.42E5
F: ITMS + c ESI d Full ms3 518.41@cid35.00 439.40@cid35.00 [110.00-450.00]



1 #1402 RT: 11.66 AV: 1 NL: 7.78E4
F: ITMS + c ESI d Full ms3 518.41@cid35.00 439.34@cid35.00 [110.00-450.00]

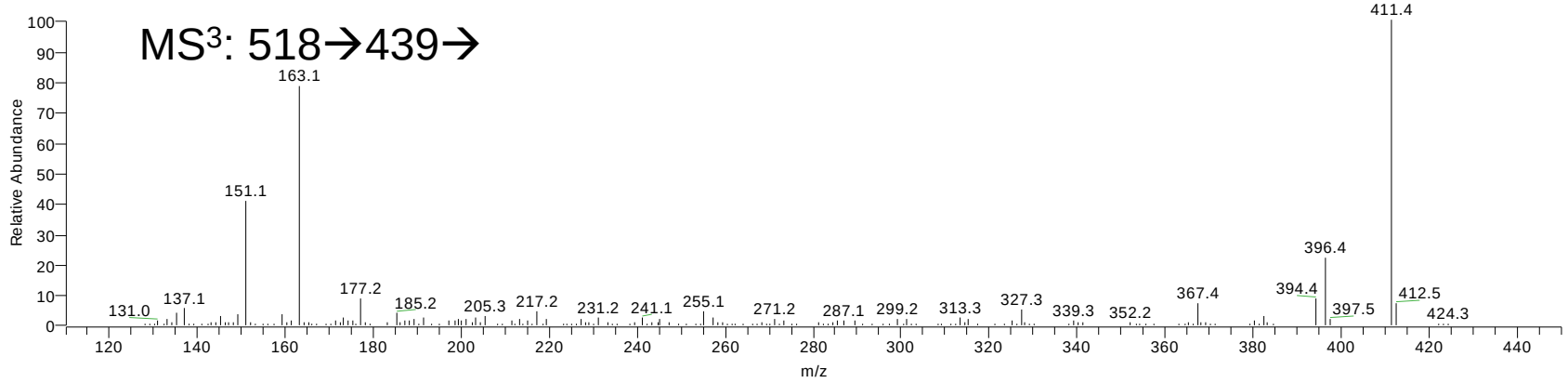


Fig. S4g 24-Me-C^4-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1525 RT: 12.15 AV: 1 NL: 2.97E4
F: ITMS + c ESI d Full ms2 532.39@cid35.00 [135.00-545.00]

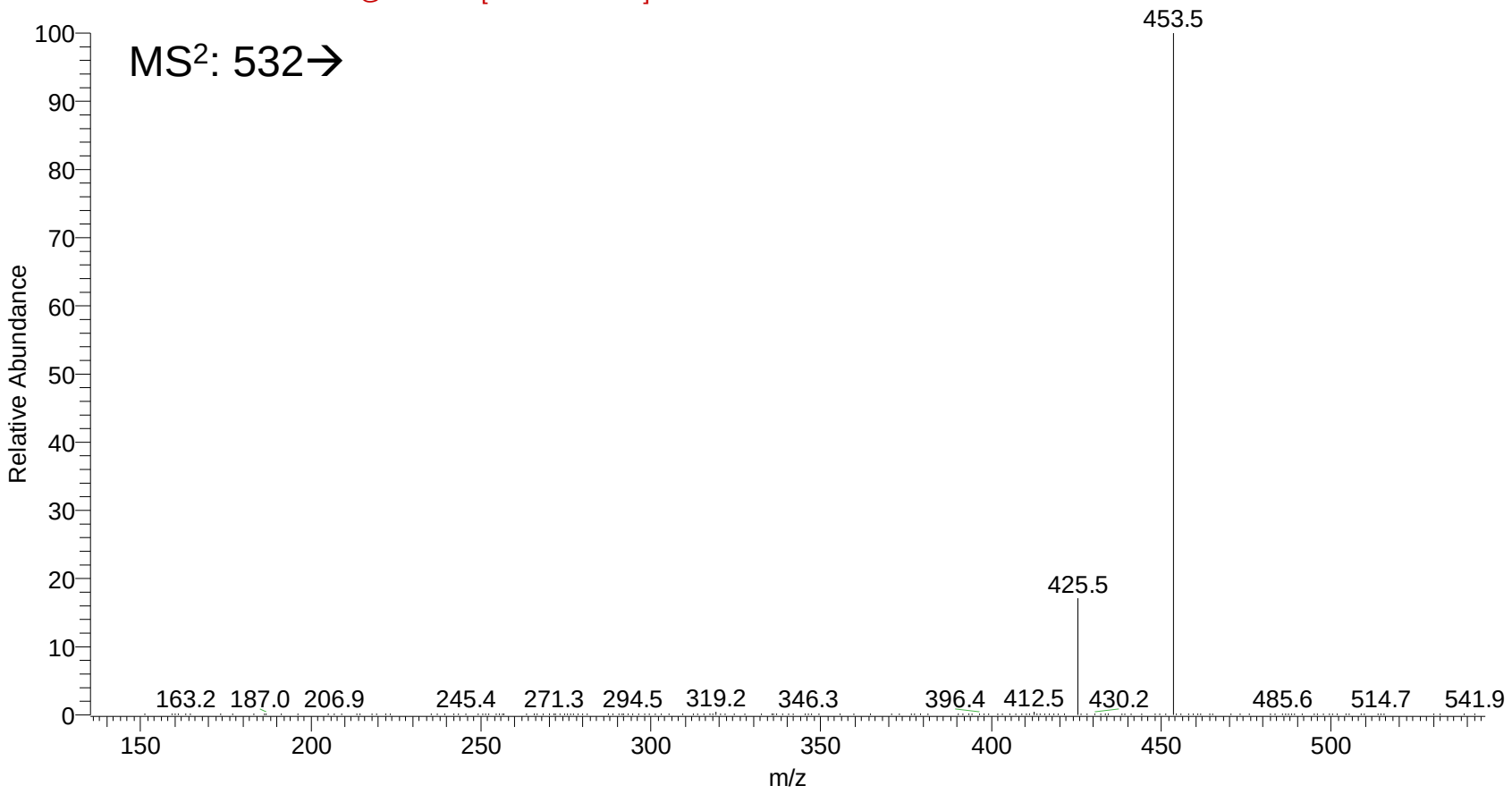


Fig. S4h 24-Me-C^4-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1526 RT: 12.16 AV: 1 NL: 2.30E3
F: ITMS + c ESI d Full ms3 532.39@cid35.00 453.38@cid35.00 [110.00-465.00]

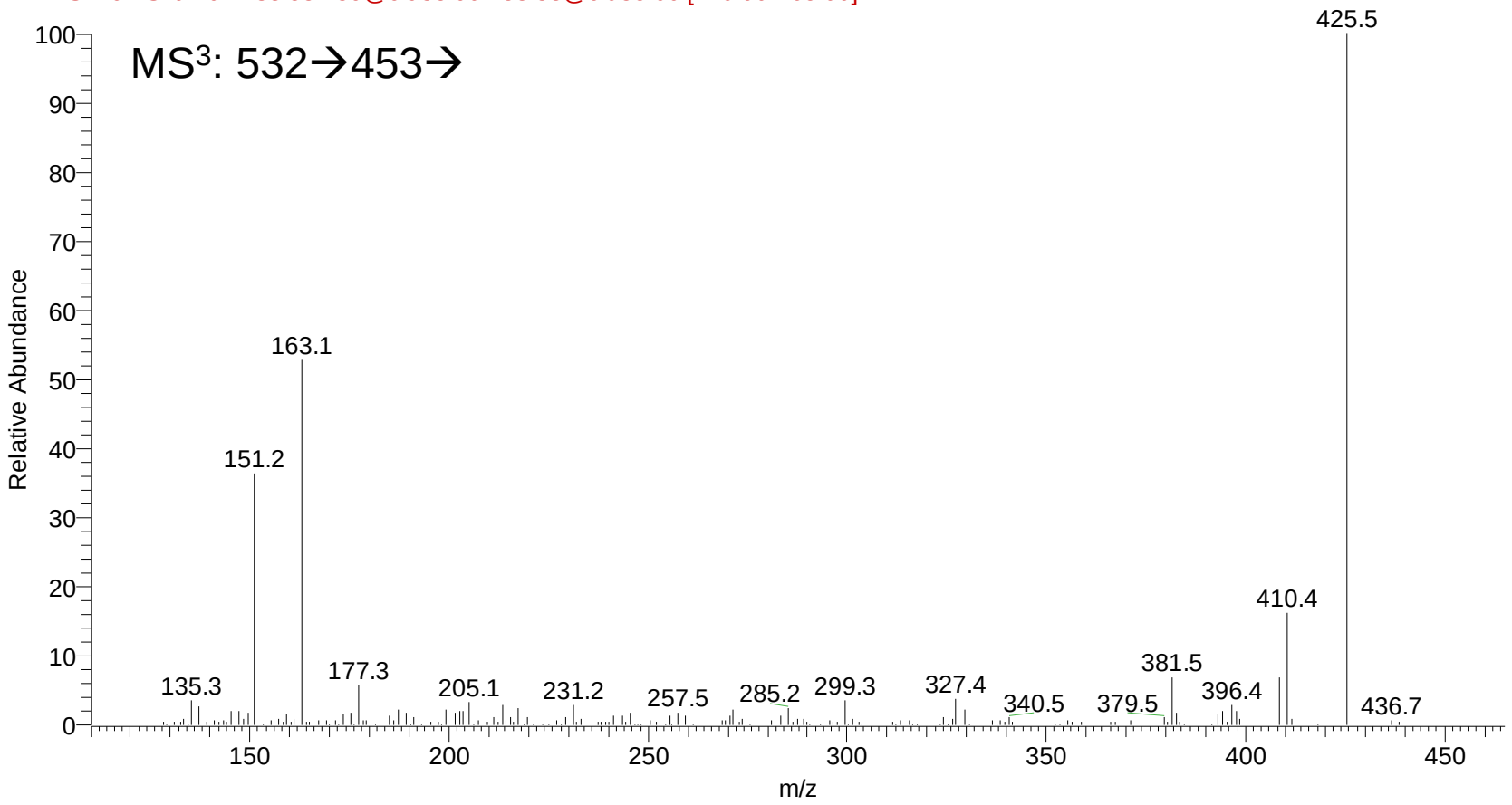


Fig. S4i C4-24S,25-epoxide-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

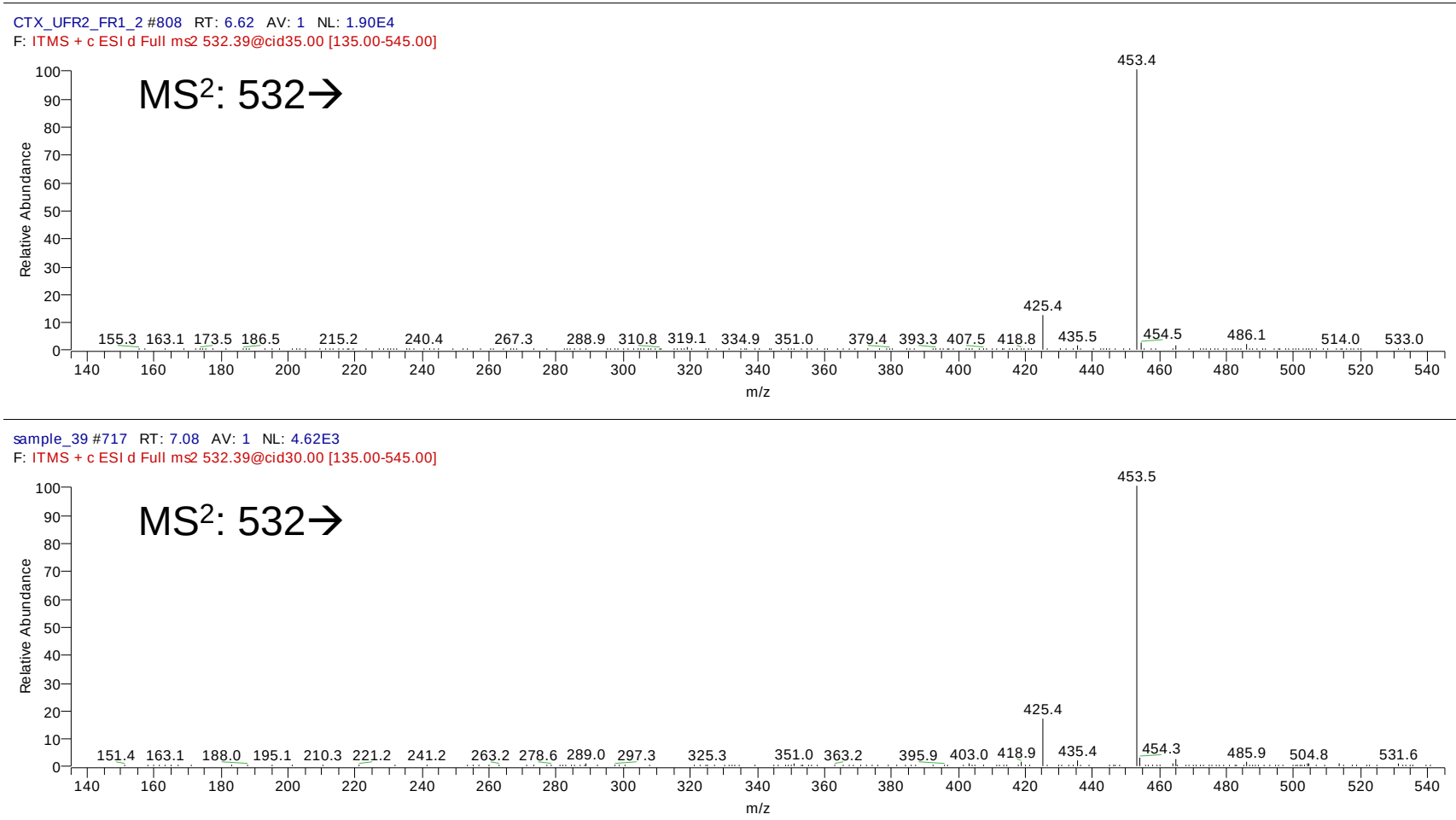
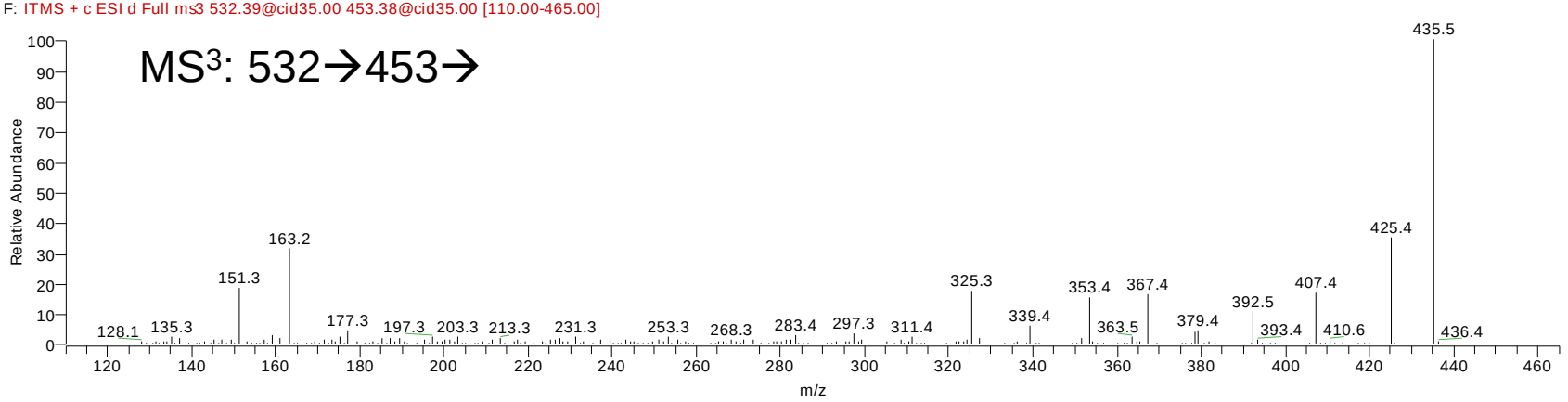


Fig. S4j C⁴-24S,25-epoxide-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #809 RT: 6.62 AV: 1 NL: 2.24E3
F: ITMS + c ESI d Full ms3 532.39@cid35.00 453.38@cid35.00 [110.00-465.00]



sample_39 #718 RT: 7.09 AV: 1 NL: 3.31E2
F: ITMS + c ESI d Full ms3 532.39@cid30.00 453.46@cid35.00 [110.00-465.00]

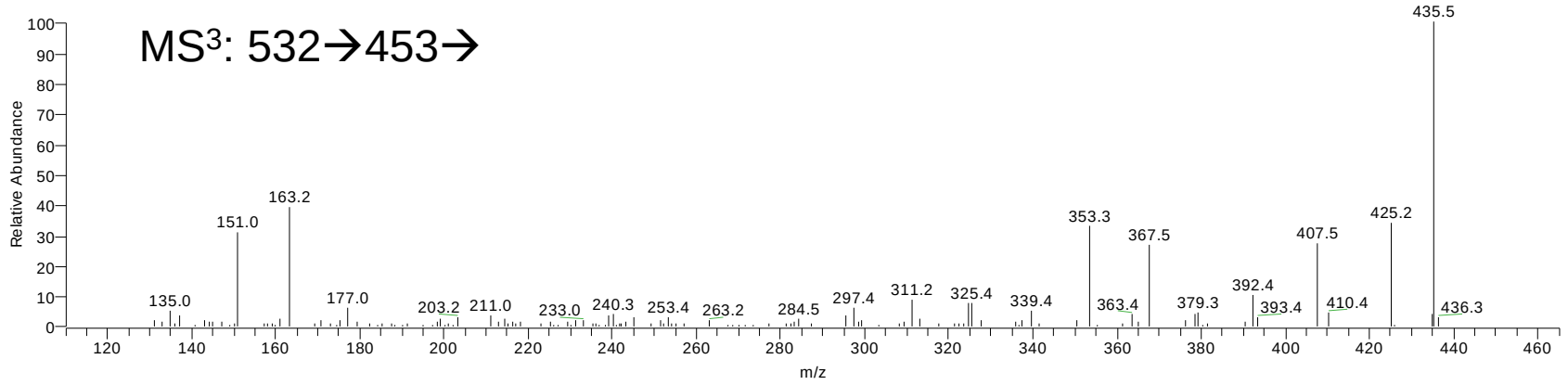
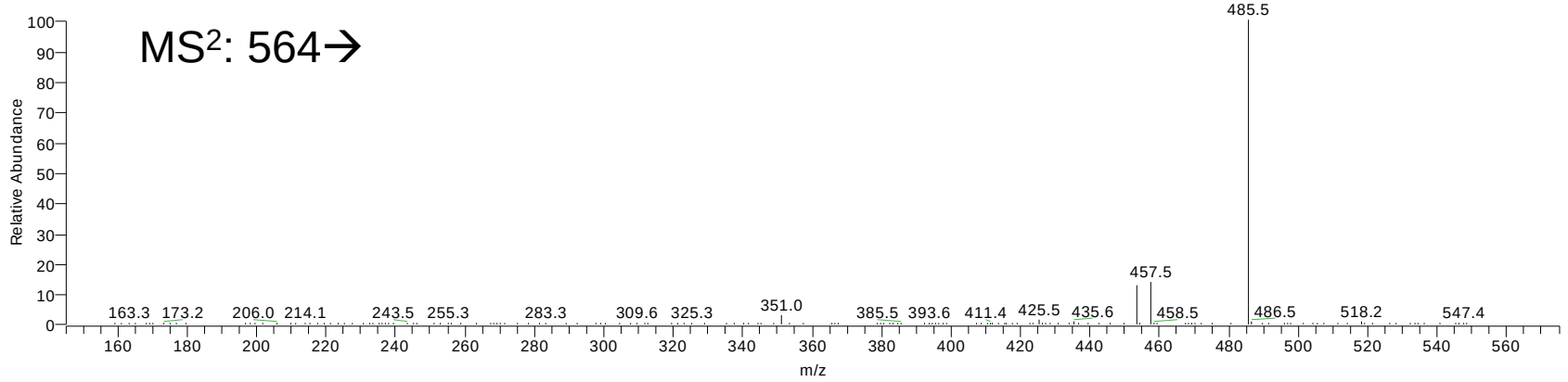


Fig. S4k C^4-24-ol,25-OMe-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #740 RT: 6.07 AV: 1 NL: 3.39E4
F: ITMS + c ESI d Full ms2 564.38@cid35.00 [145.00-575.00]



sample_39 #646 RT: 6.43 AV: 1 NL: 9.65E4
F: ITMS + c ESI d Full ms2 564.42@cid30.00 [145.00-575.00]

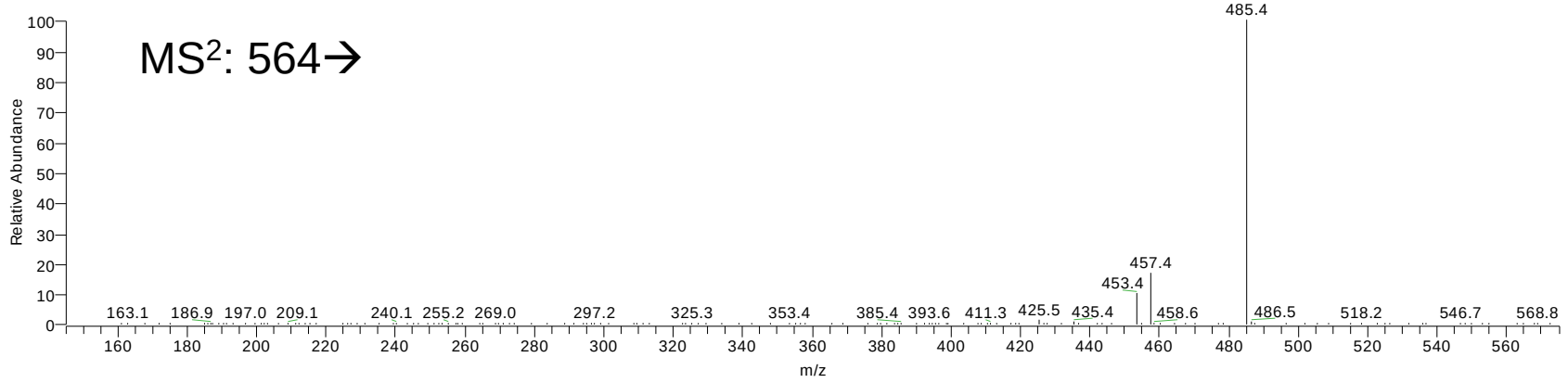


Fig. S4l C^4-24-ol,25-OMe-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

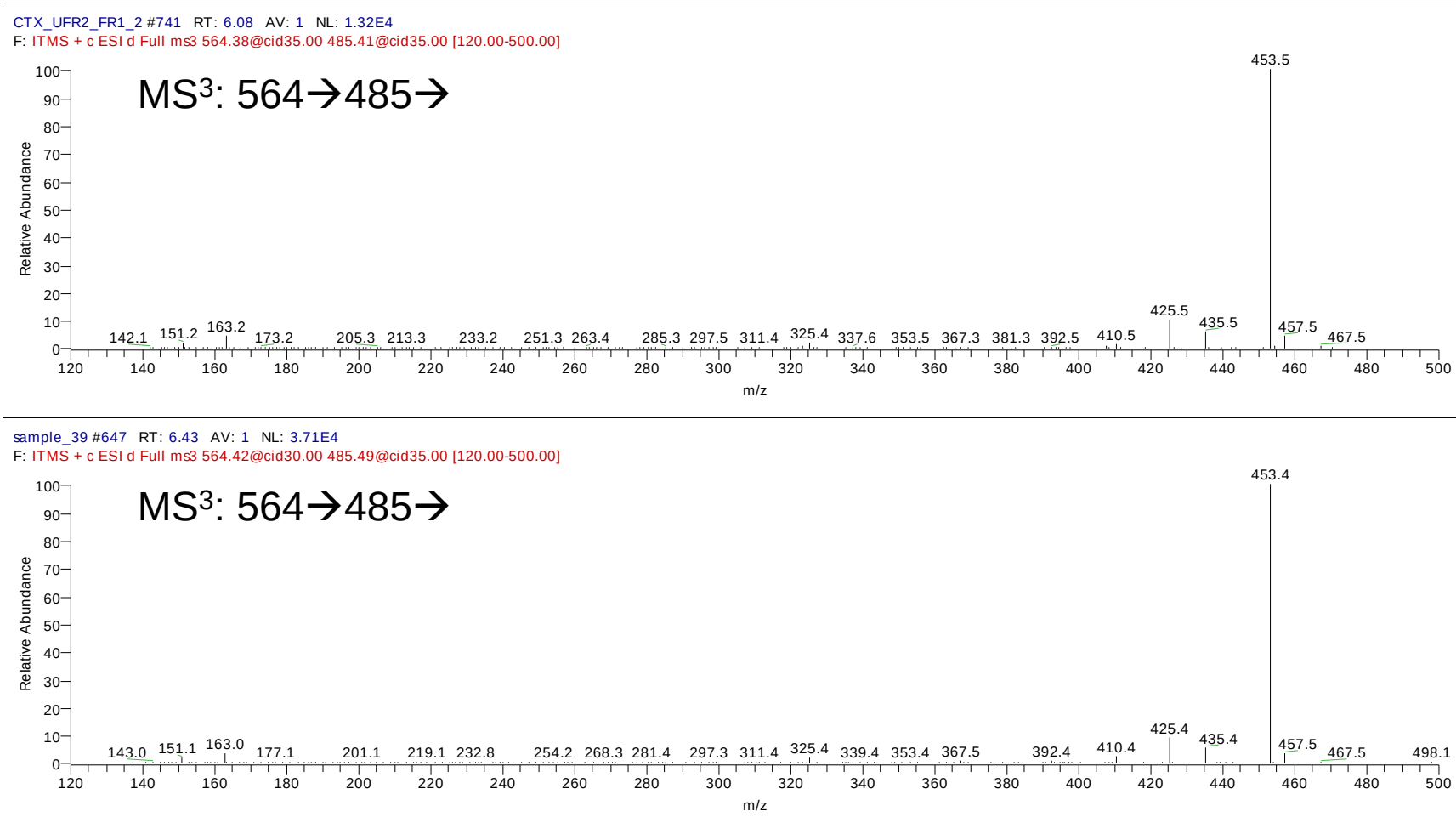
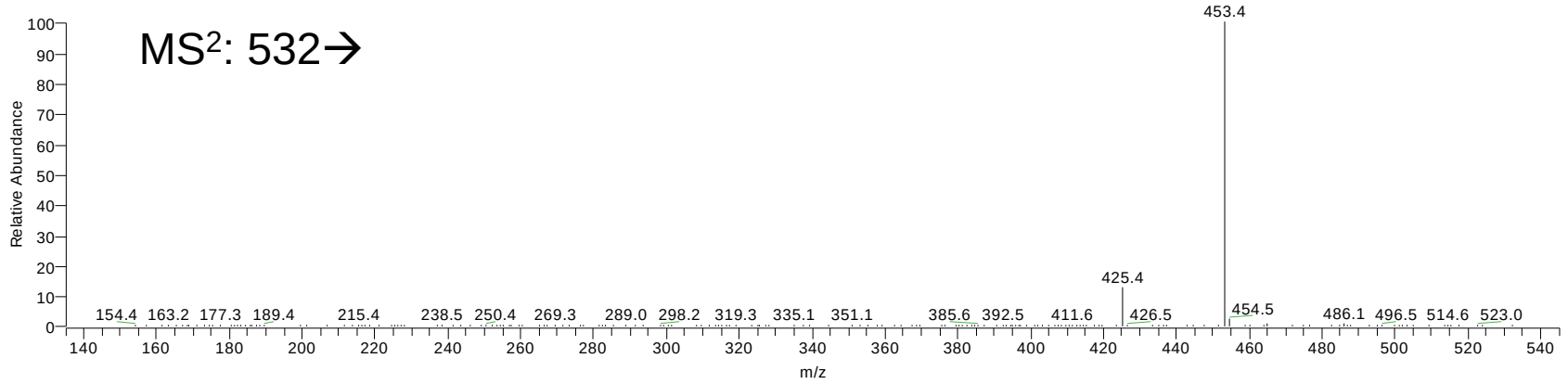


Fig. S4m C⁴-3,24-dione 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #901 RT: 7.38 AV: 1 NL: 3.53E4
F: ITMS + c ESI d Full ms2 532.39@cid35.00 [135.00-545.00]



23 #890 RT: 7.51 AV: 1 NL: 6.32E5
F: ITMS + c ESI d Full ms2 532.39@cid35.00 [135.00-545.00]

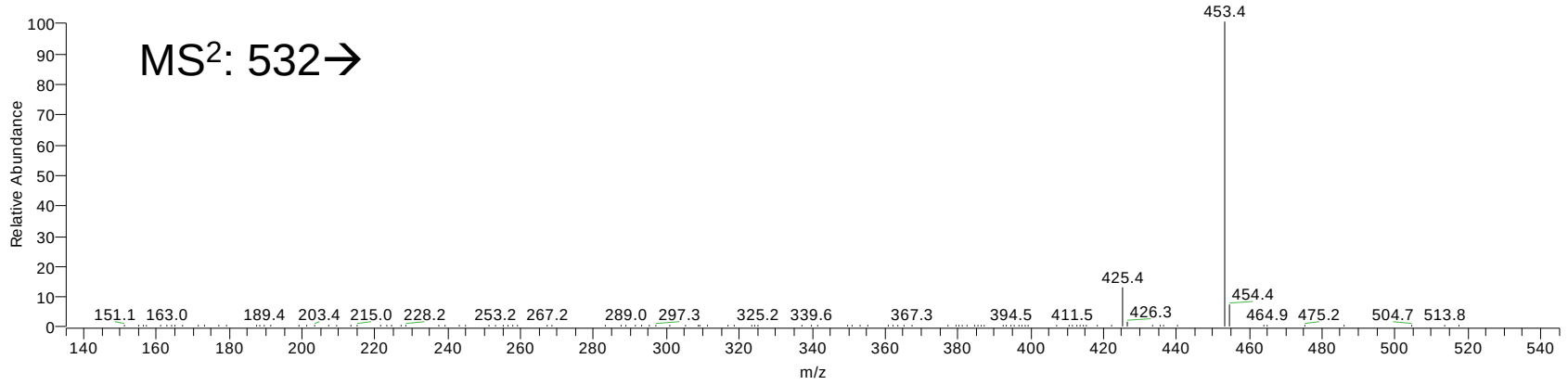
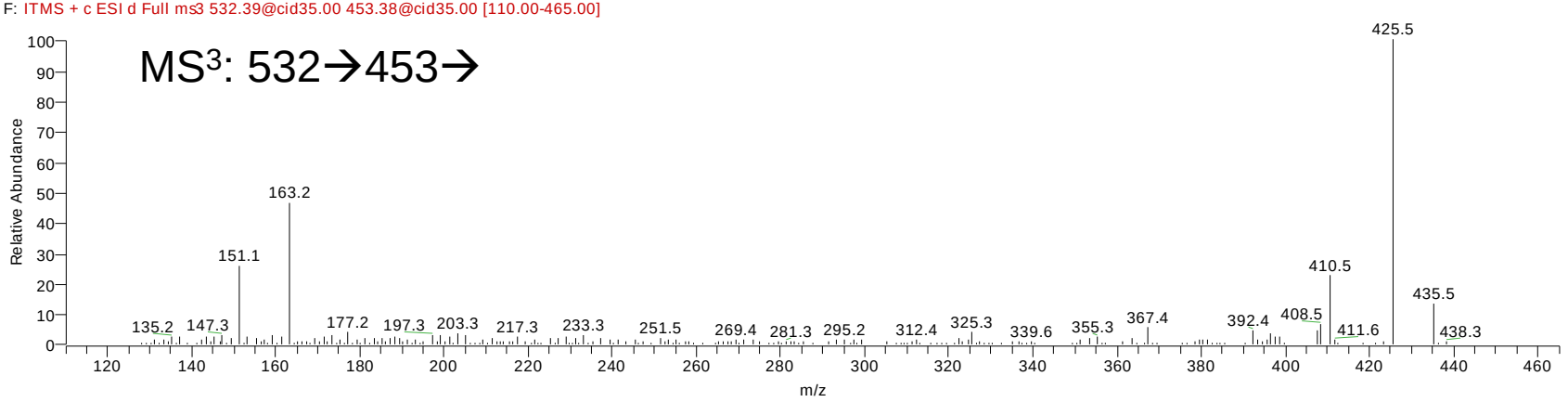


Fig. S4n C⁴-3,24-dione 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #902 RT: 7.38 AV: 1 NL: 2.52E3
F: ITMS + c ESI d Full ms3 532.39@cid35.00 453.38@cid35.00 [110.00-465.00]



23 #891 RT: 7.51 AV: 1 NL: 5.26E4
F: ITMS + c ESI d Full ms3 532.39@cid35.00 453.31@cid35.00 [110.00-465.00]

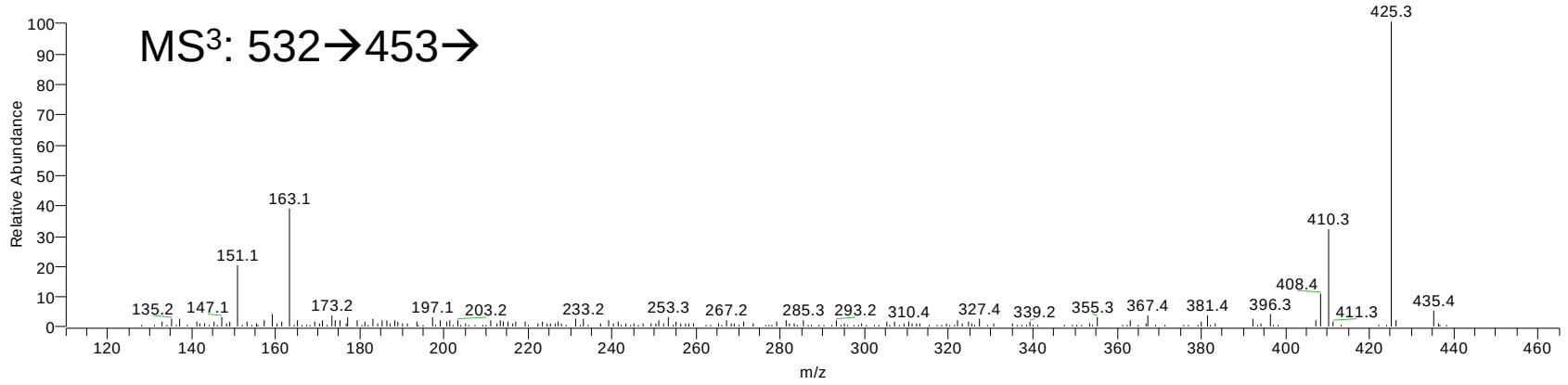
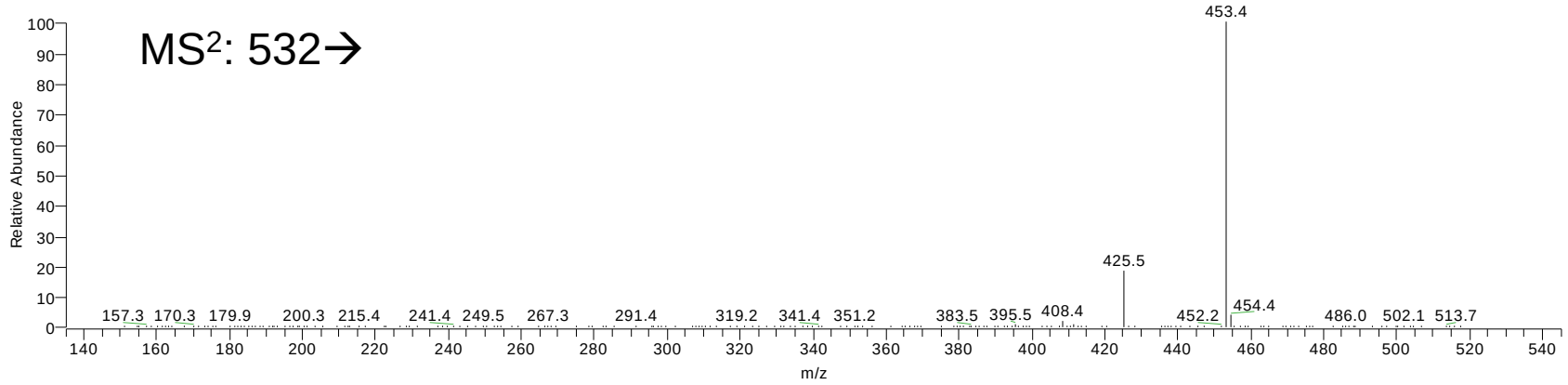


Fig. S4o C4-3,6-dione 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1204 RT: 9.79 AV: 1 NL: 6.86E4
F: ITMS + c ESI d Full ms2 532.39@cid35.00 [135.00-545.00]



17 #1134 RT: 9.53 AV: 1 NL: 1.59E5
F: ITMS + c ESI d Full ms2 532.39@cid35.00 [135.00-545.00]

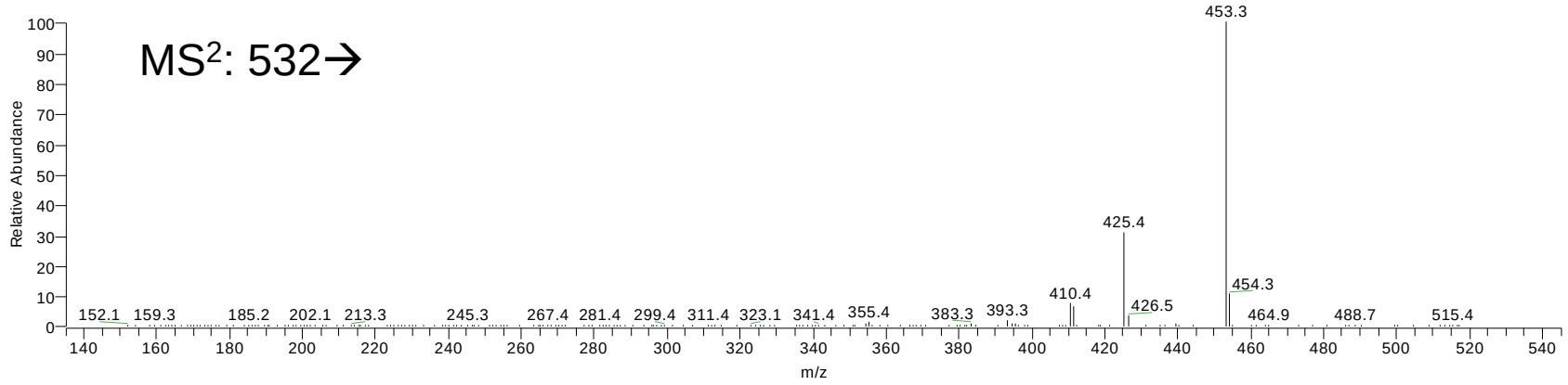
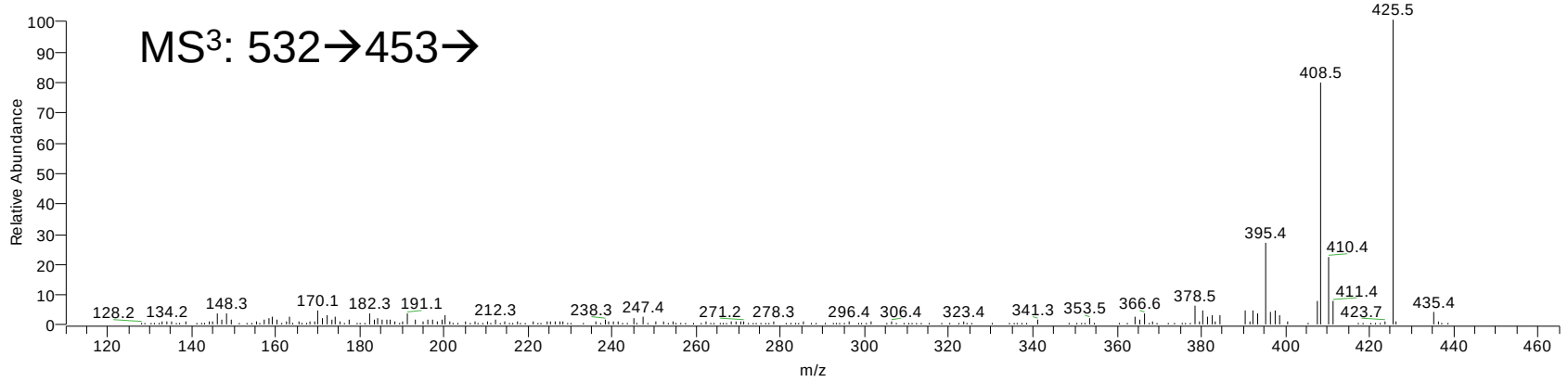


Fig. S4p C^4-3,6-dione 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1205 RT: 9.79 AV: 1 NL: 7.82E3
F: ITMS + c ESI d Full ms3 532.39@cid35.00 453.38@cid35.00 [110.00-465.00]



17 #1135 RT: 9.54 AV: 1 NL: 7.54E4
F: ITMS + c ESI d Full ms3 532.39@cid35.00 453.30@cid35.00 [110.00-465.00]

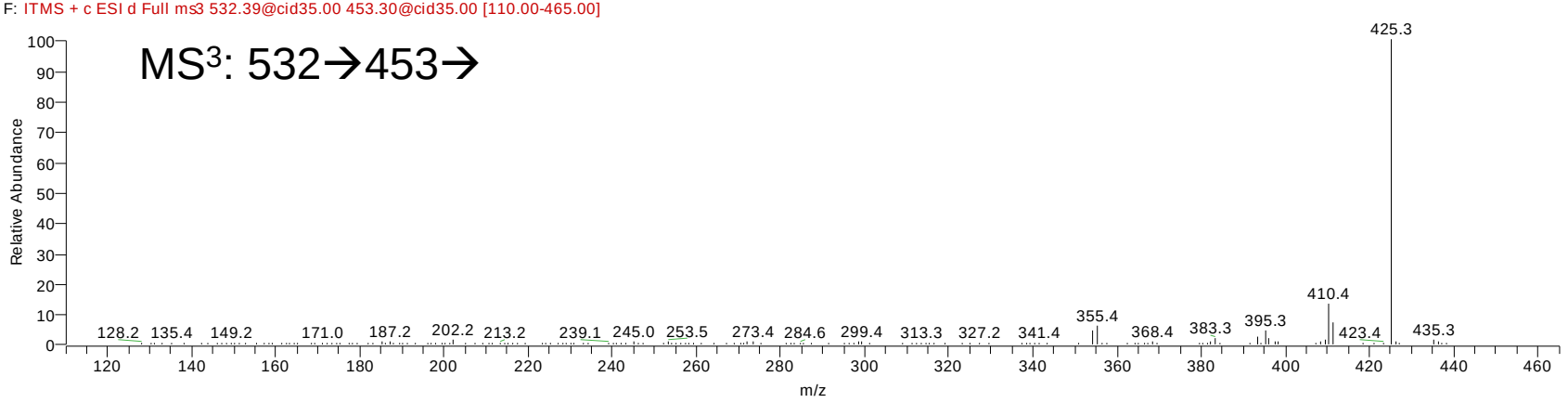
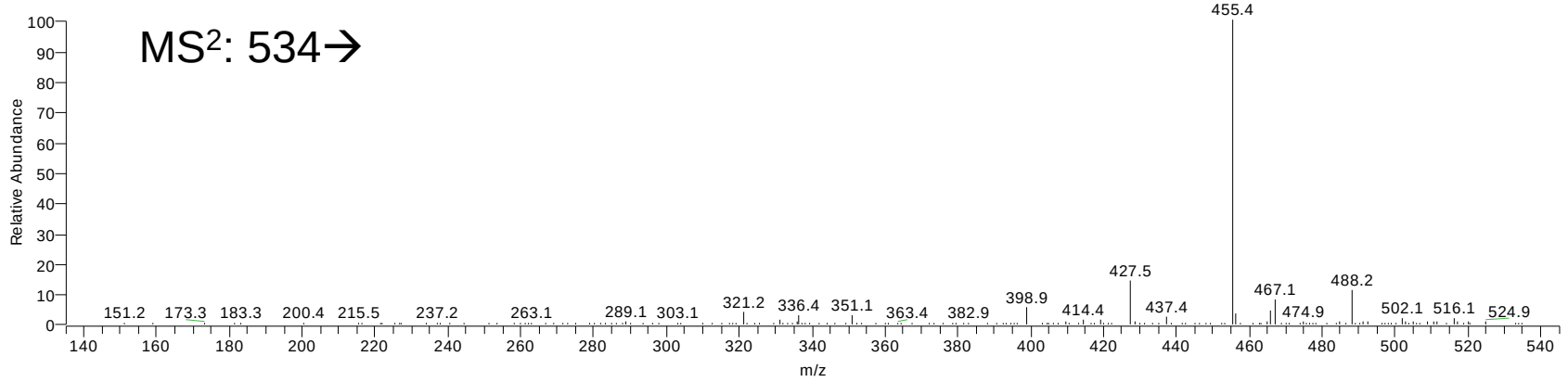


Fig. S4q C4-22R-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #753 RT: 6.17 AV: 1 NL: 2.54E3
F: ITMS + c ESI d Full ms2 534.41@cid35.00 [135.00-1080.00]



9 #752 RT: 6.24 AV: 1 NL: 5.08E5
F: ITMS + c ESI d Full ms2 534.41@cid35.00 [135.00-545.00]

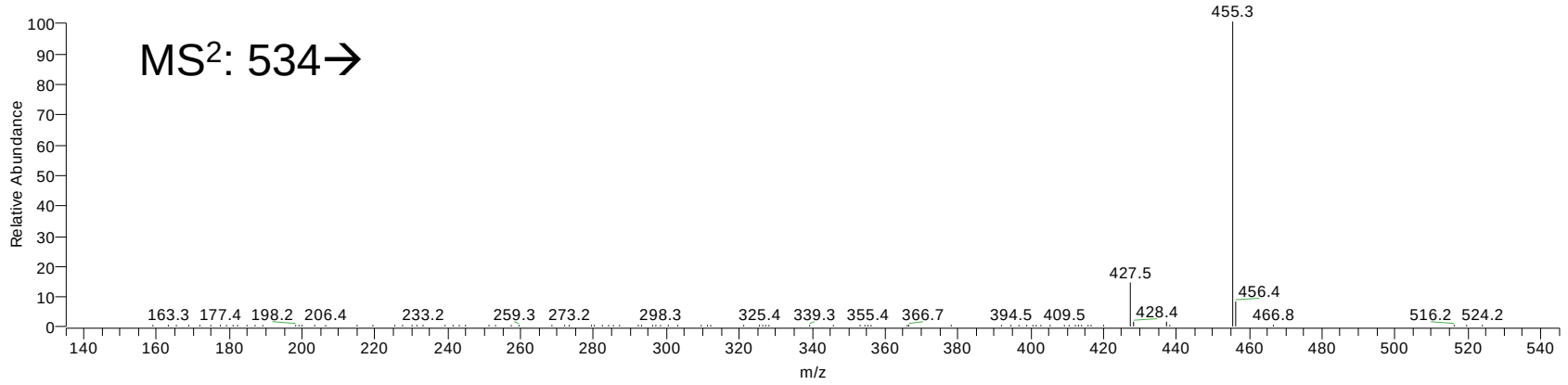
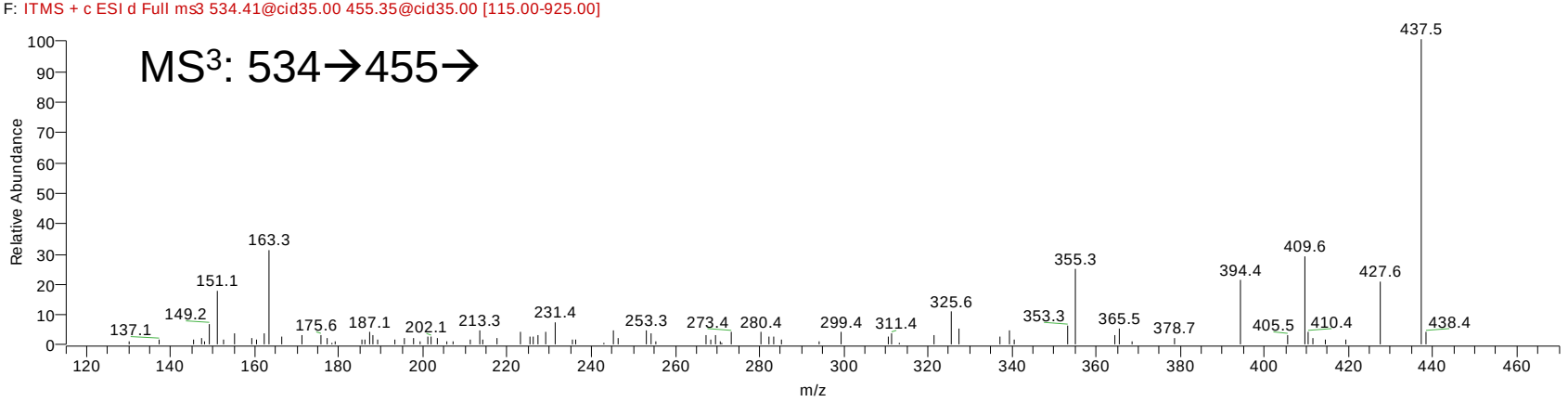


Fig. S4r C^4-22R-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #754 RT: 6.18 AV: 1 NL: 1.94E2
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.35@cid35.00 [115.00-925.00]



9 #753 RT: 6.24 AV: 1 NL: 6.08E4
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.34@cid35.00 [115.00-470.00]

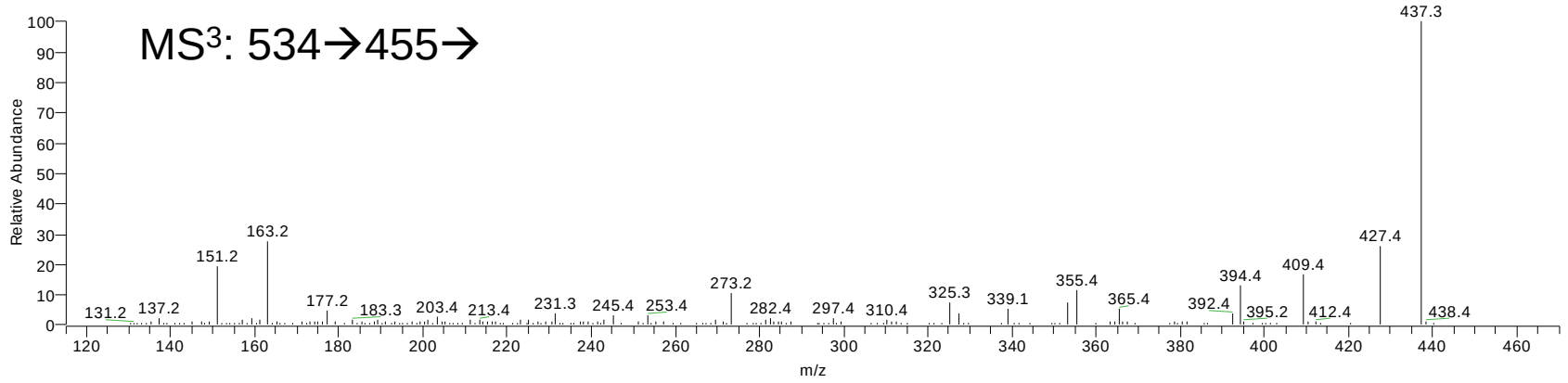


Fig. S4s C4-24S-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

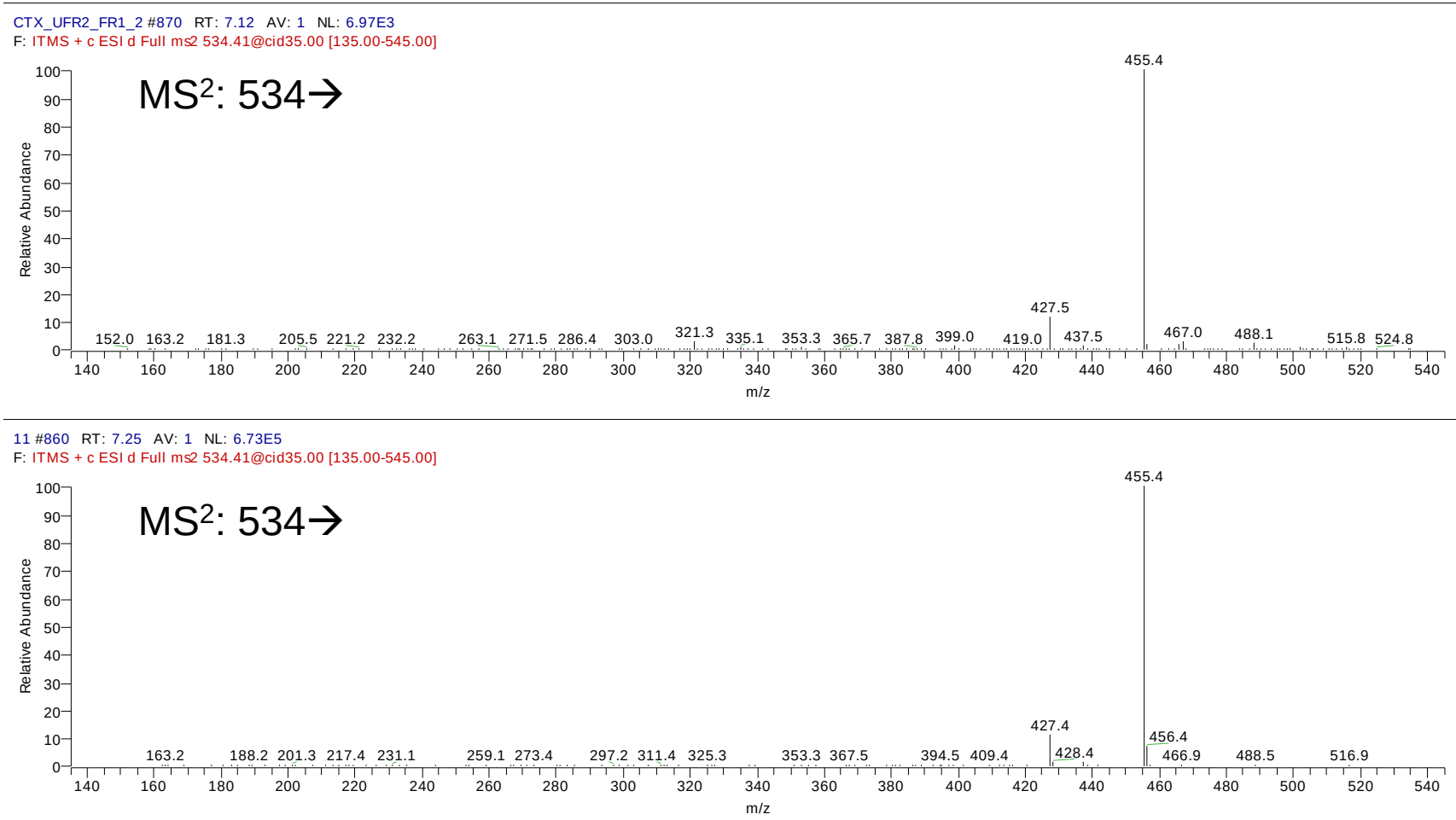
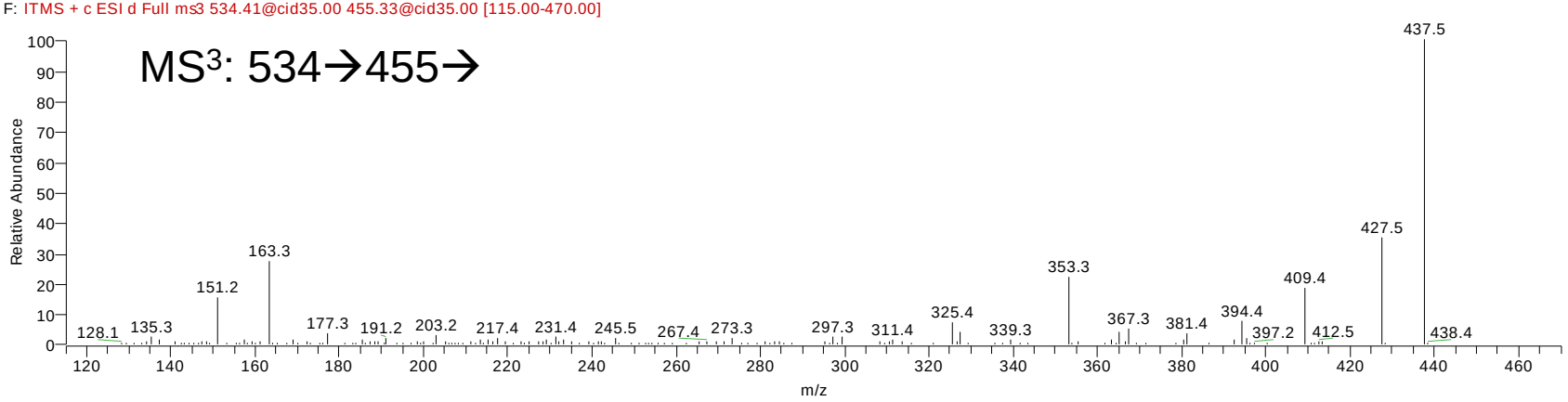


Fig. S4t C^4-24S-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #871 RT: 7.13 AV: 1 NL: 1.21E3
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.33@cid35.00 [115.00-470.00]



11 #861 RT: 7.26 AV: 1 NL: 9.18E4
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.29@cid35.00 [115.00-470.00]

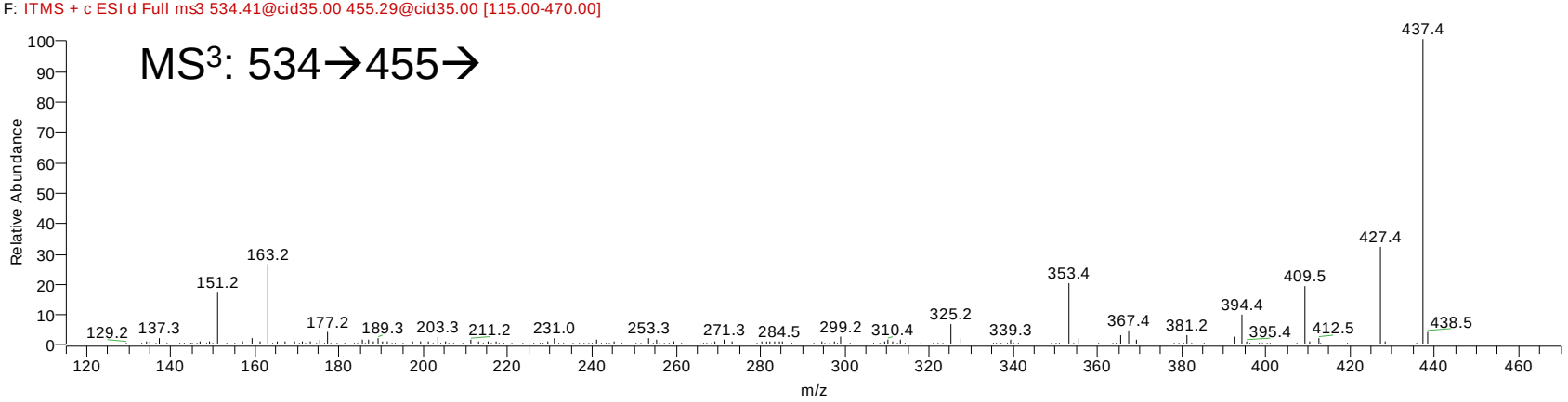


Fig. S4u C^4-25-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

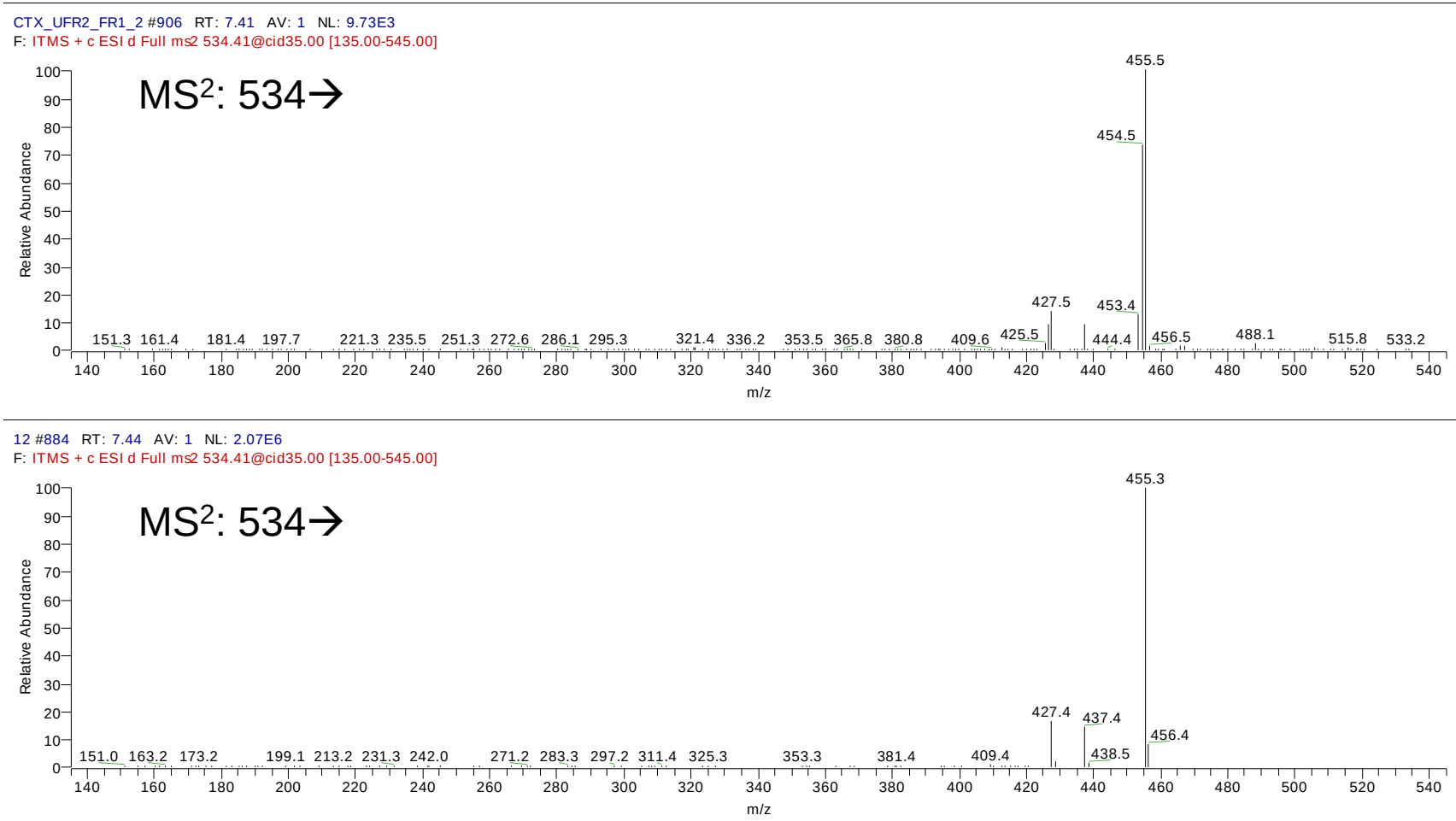
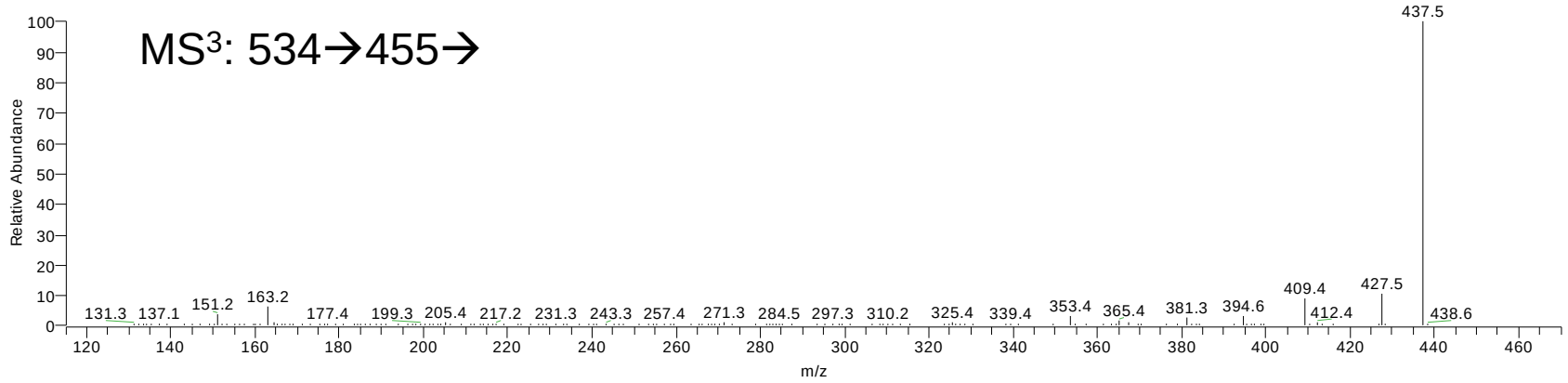


Fig. S4v C^4-25-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #907 RT: 7.42 AV: 1 NL: 3.51E3
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.33@cid35.00 [115.00-470.00]



12 #885 RT: 7.44 AV: 1 NL: 1.43E6
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.30@cid35.00 [115.00-470.00]

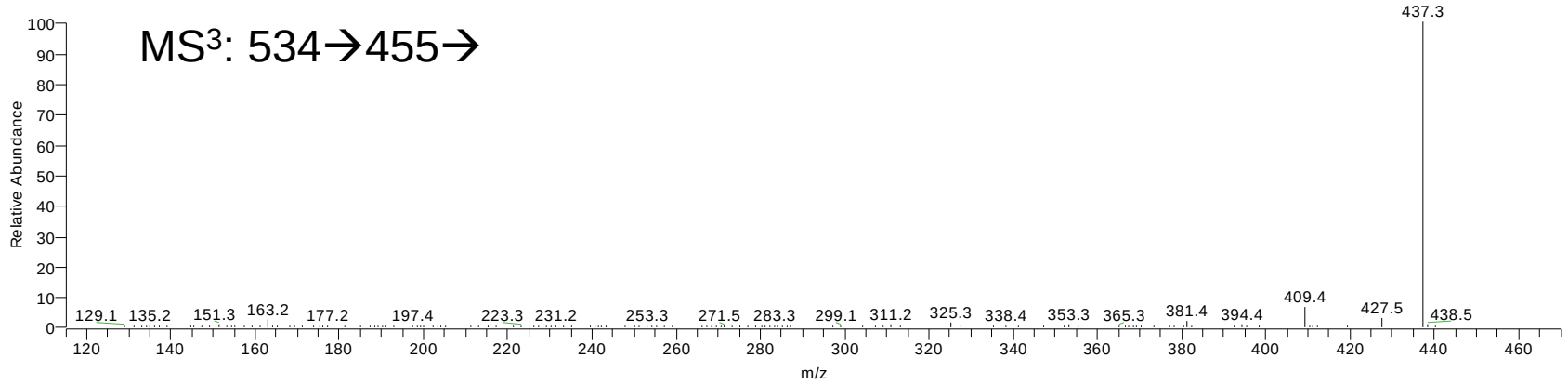
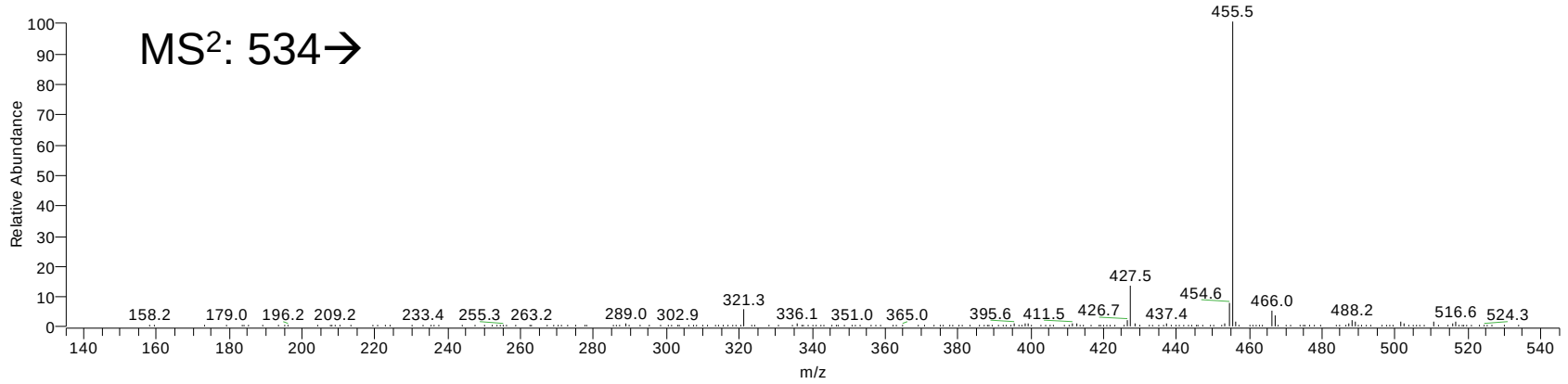


Fig. S4w C^4-27-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1028 RT: 8.41 AV: 1 NL: 4.48E3
F: ITMS + c ESI d Full ms2 534.41@cid35.00 [135.00-1080.00]



sample_14 #828 RT: 8.13 AV: 1 NL: 3.77E5
F: ITMS + c ESI d Full ms2 534.40@cid30.00 [135.00-545.00]

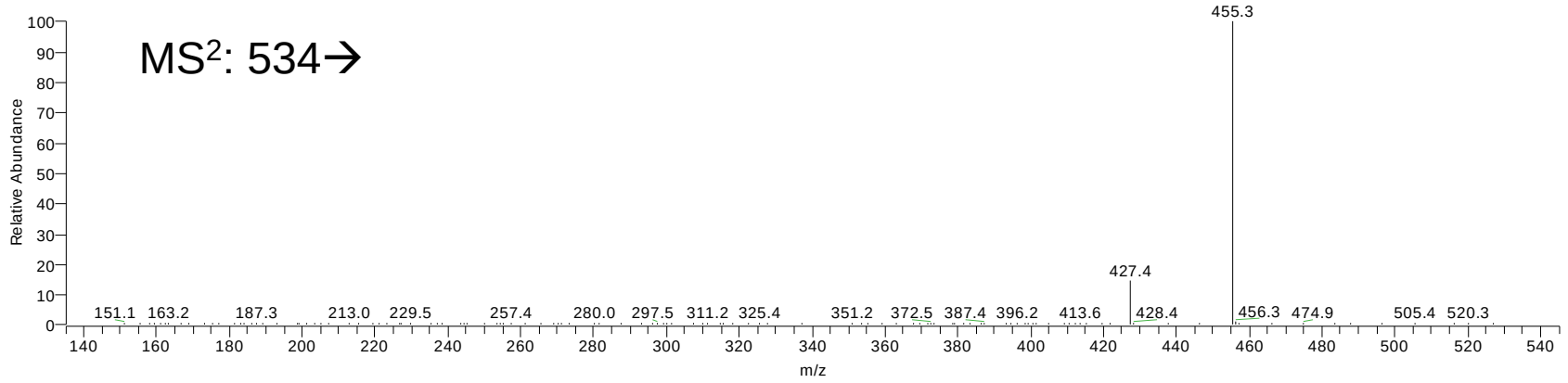
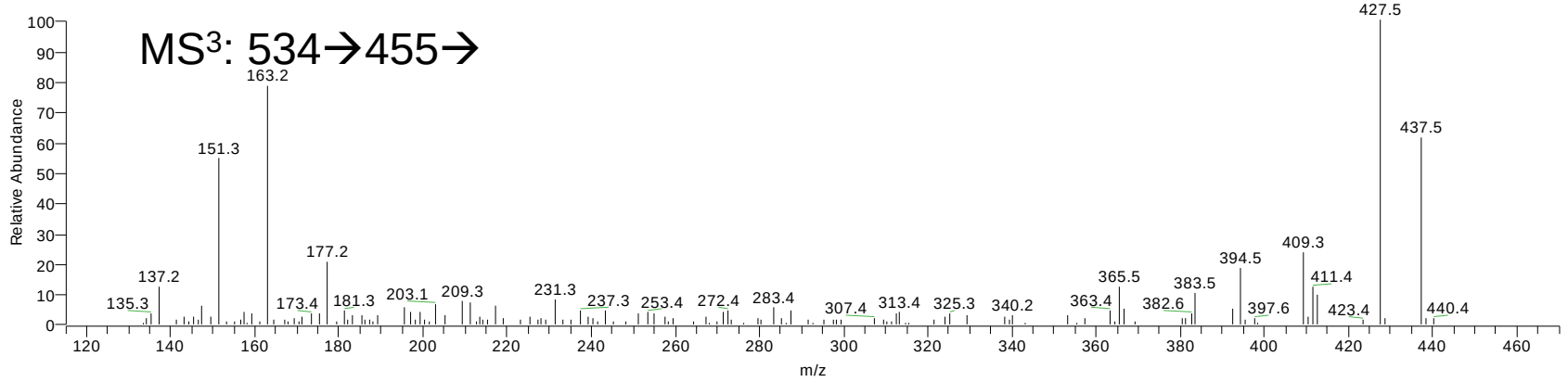


Fig. S4x C^4-27-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1029 RT: 8.42 AV: 1 NL: 2.73E2
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.35@cid35.00 [115.00-925.00]



sample_14 #829 RT: 8.13 AV: 1 NL: 3.36E4
F: ITMS + c ESI d Full ms3 534.40@cid30.00 455.44@cid35.00 [115.00-470.00]

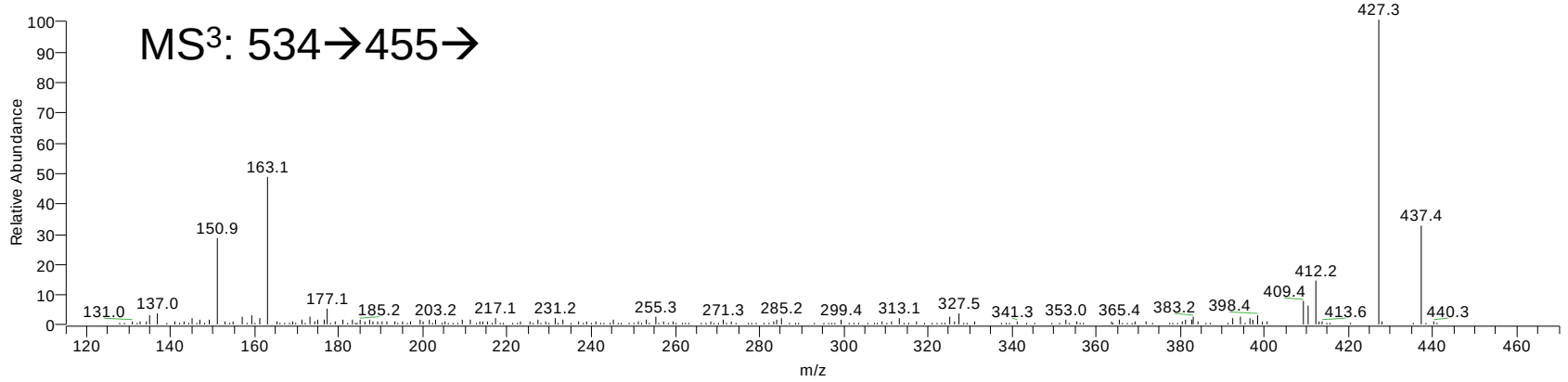
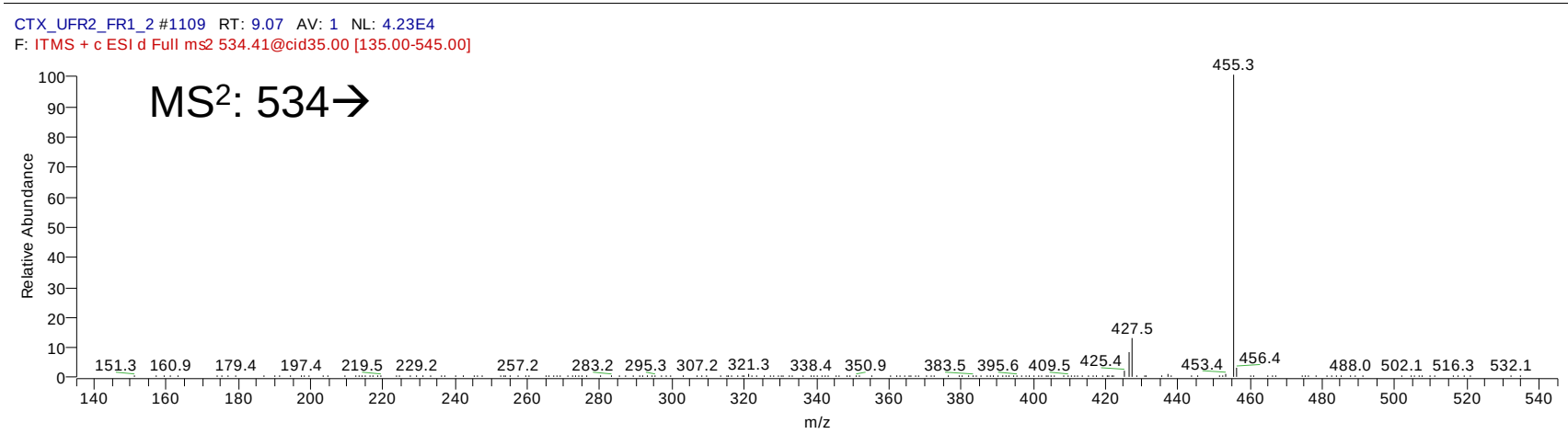


Fig. S4y C^4-7β-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40



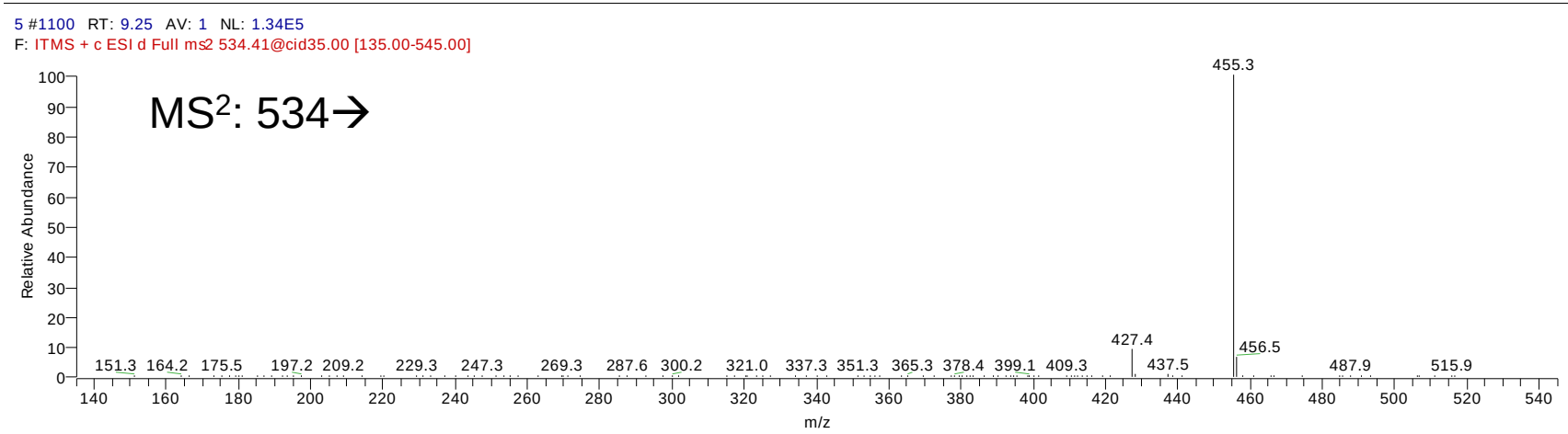
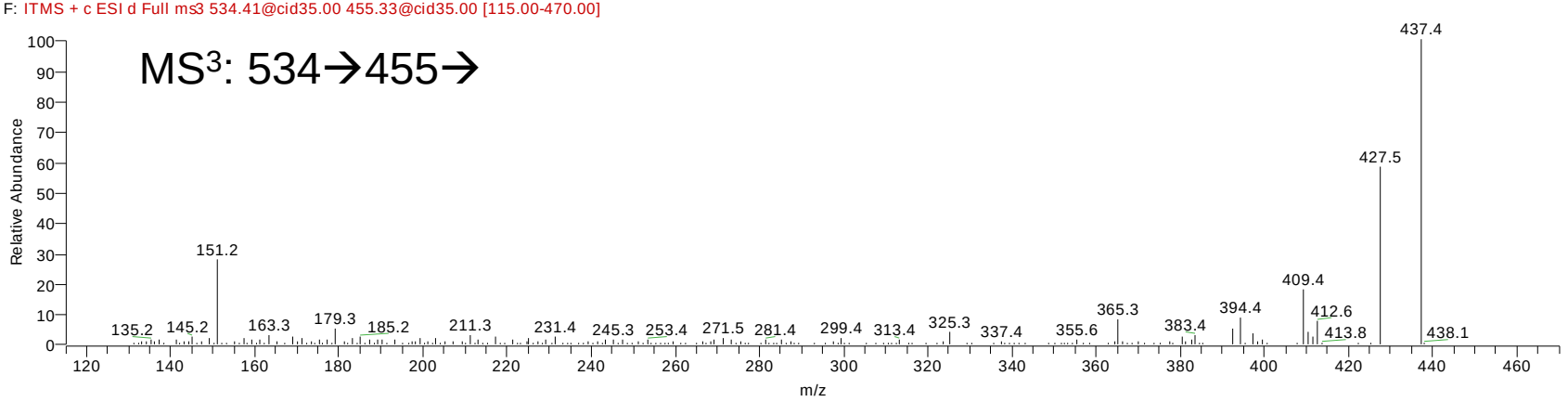


Fig. S4z C^4-7β-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1110 RT: 9.08 AV: 1 NL: 4.69E3
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.33@cid35.00 [115.00-470.00]



5 #1101 RT: 9.25 AV: 1 NL: 1.31E4
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.34@cid35.00 [115.00-470.00]

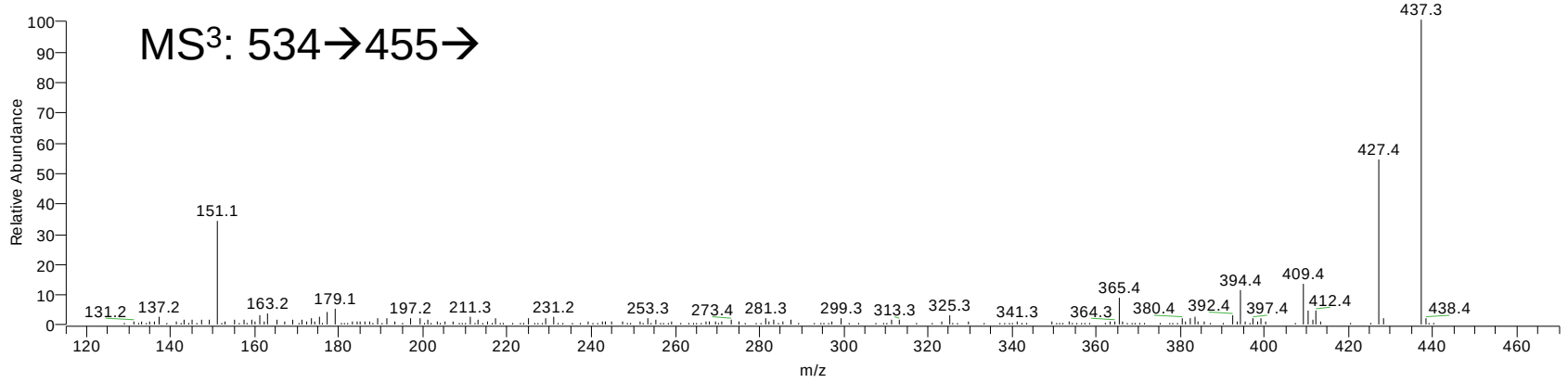
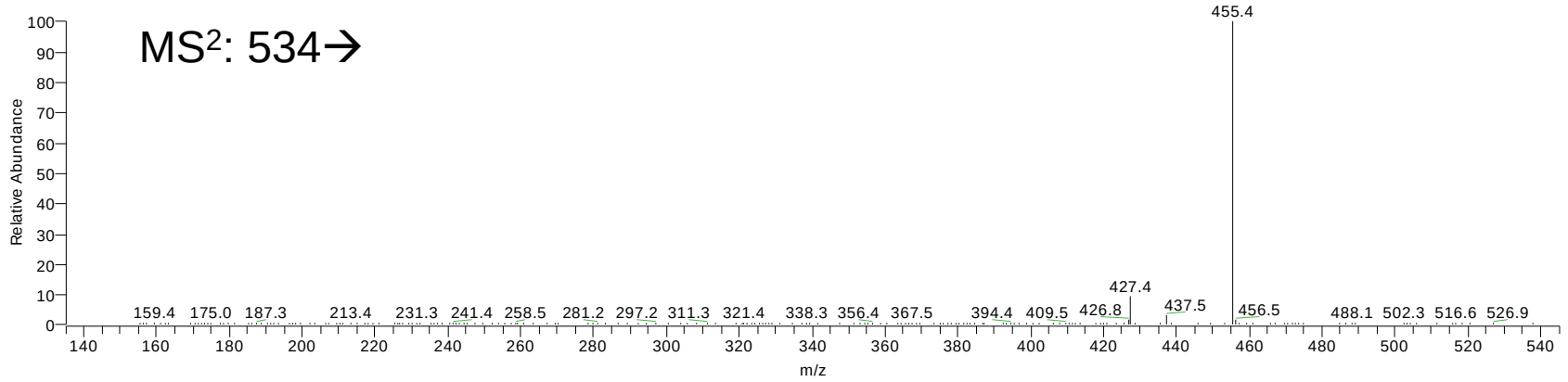


Fig. S4aa C^4-7α-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1178 RT: 9.60 AV: 1 NL: 7.25E4
F: ITMS + c ESI d Full ms2 534.41@cid35.00 [135.00-545.00]



sample_20 #1015 RT: 10.06 AV: 1 NL: 2.85E5
F: ITMS + c ESI d Full ms2 534.41@cid30.00 [135.00-545.00]

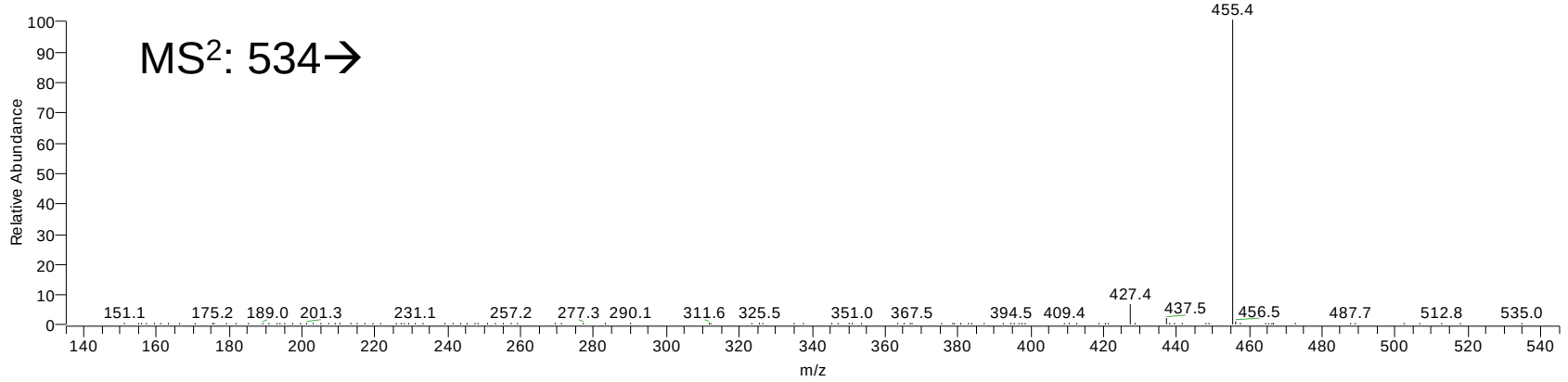
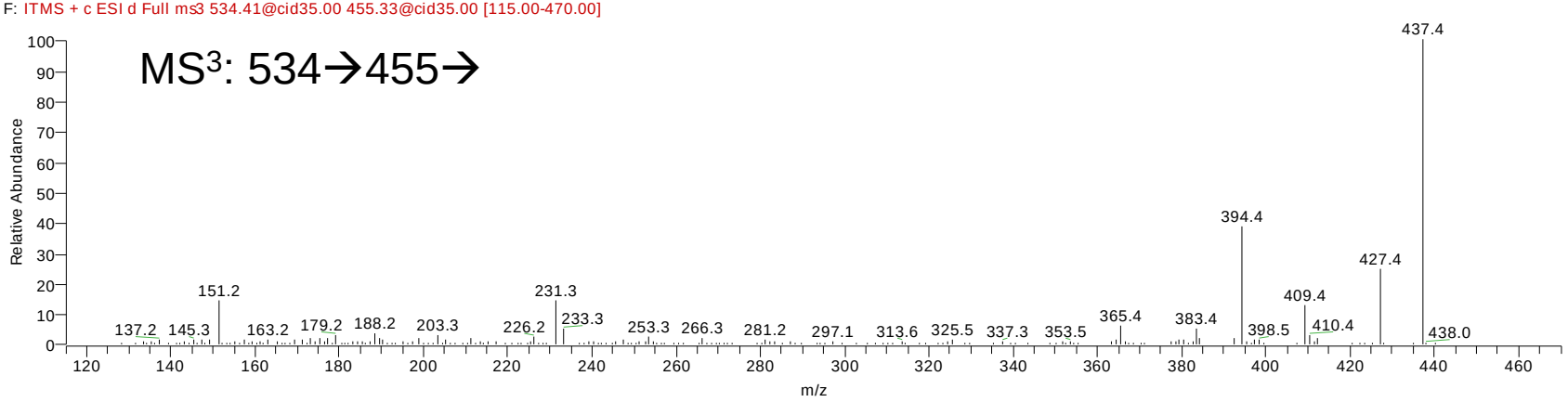


Fig. S4ab C^4-7α-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1179 RT: 9.60 AV: 1 NL: 9.59E3
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.33@cid35.00 [115.00-470.00]



sample_20 #1016 RT: 10.06 AV: 1 NL: 4.06E4
F: ITMS + c ESI d Full ms3 534.41@cid30.00 455.29@cid35.00 [115.00-470.00]

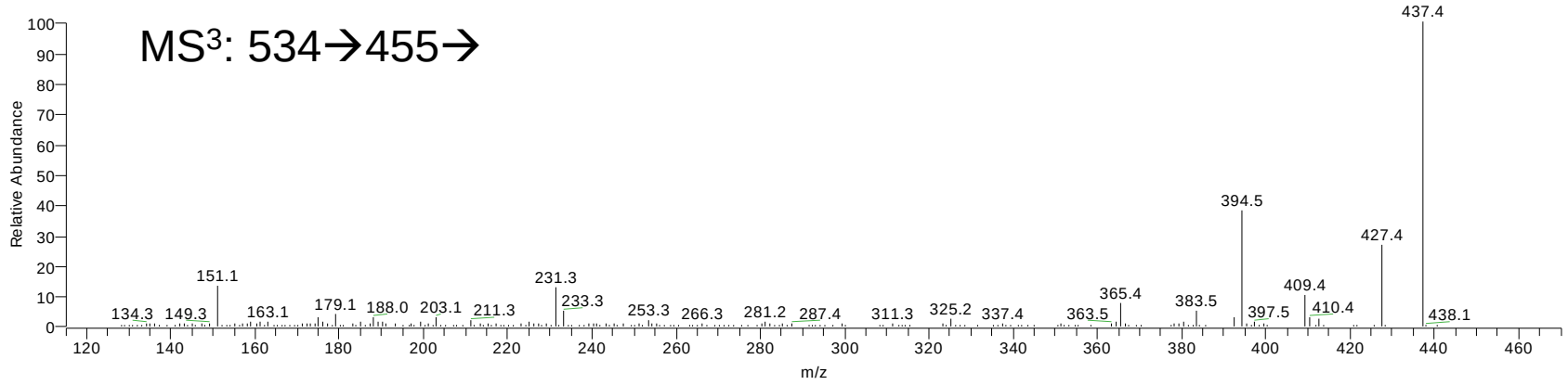


Fig. S4ac C^7-x-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1198 RT: 9.75 AV: 1 NL: 4.31E4
F: ITMS + c ESI d Full ms2 534.41@cid35.00 [135.00-545.00]

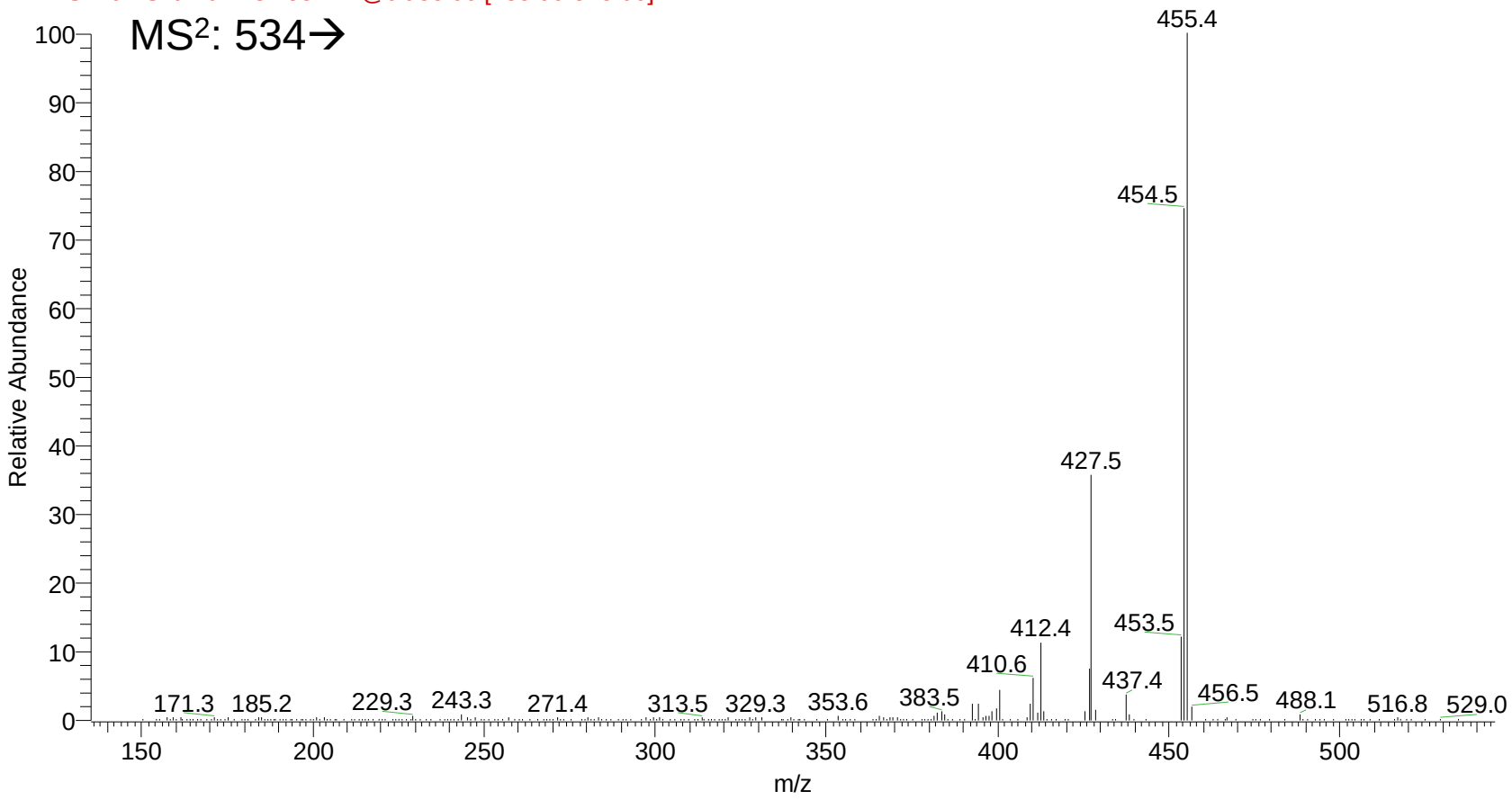


Fig. S4ad C^7-x-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1199 RT: 9.75 AV: 1 NL: 1.41E4
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.33@cid35.00 [115.00-470.00]

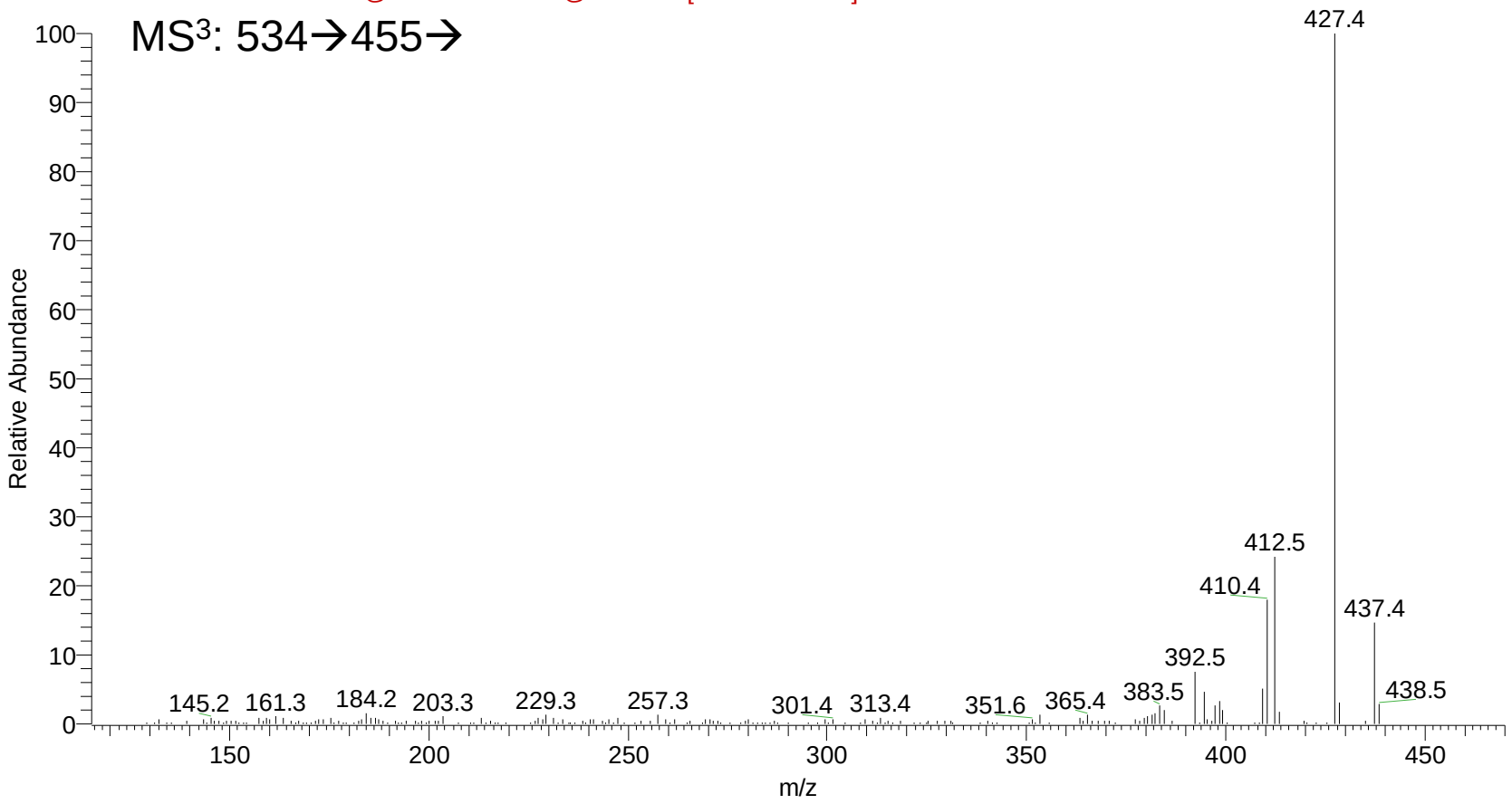


Fig. S4ae C^4-6β-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

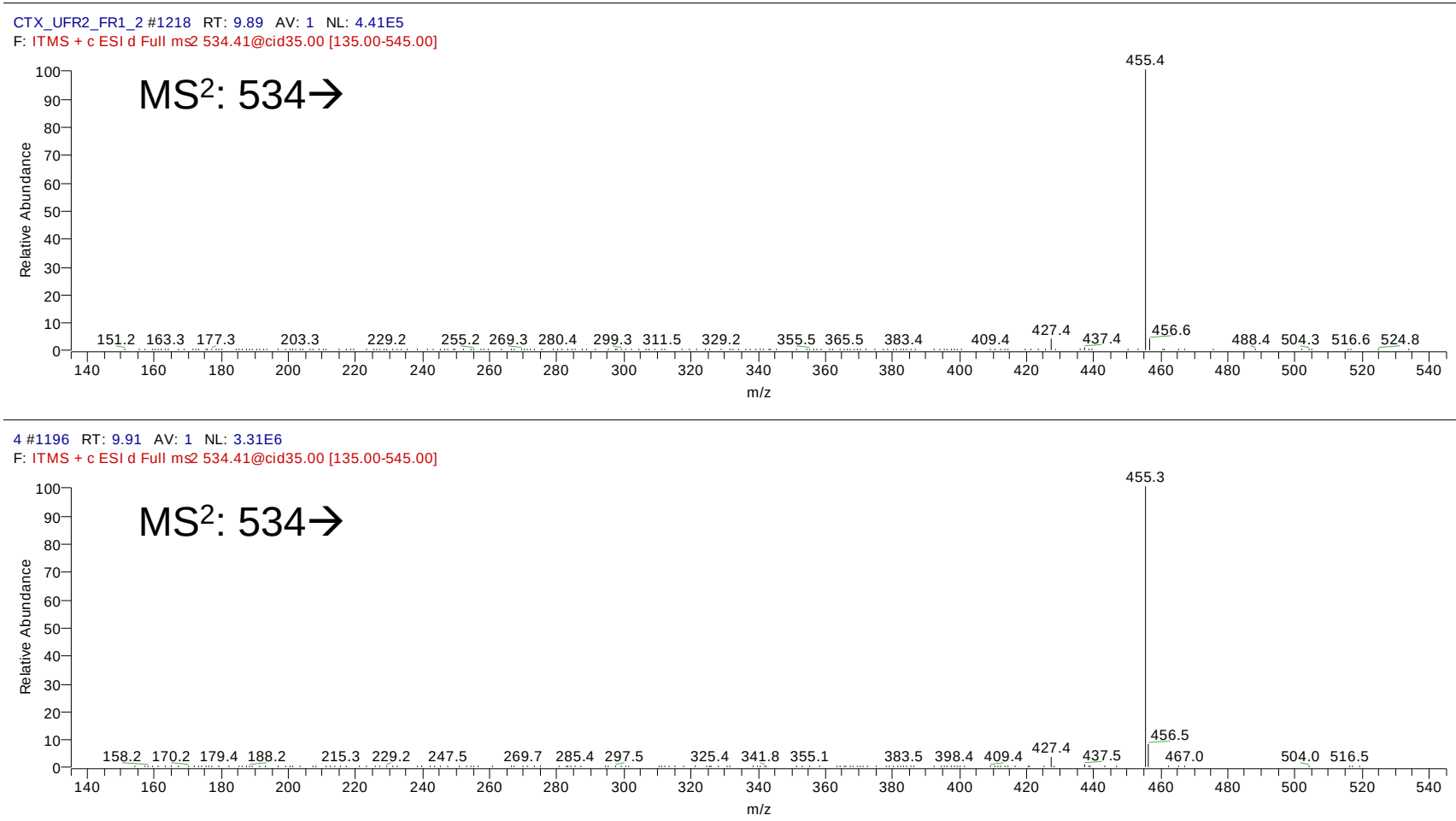
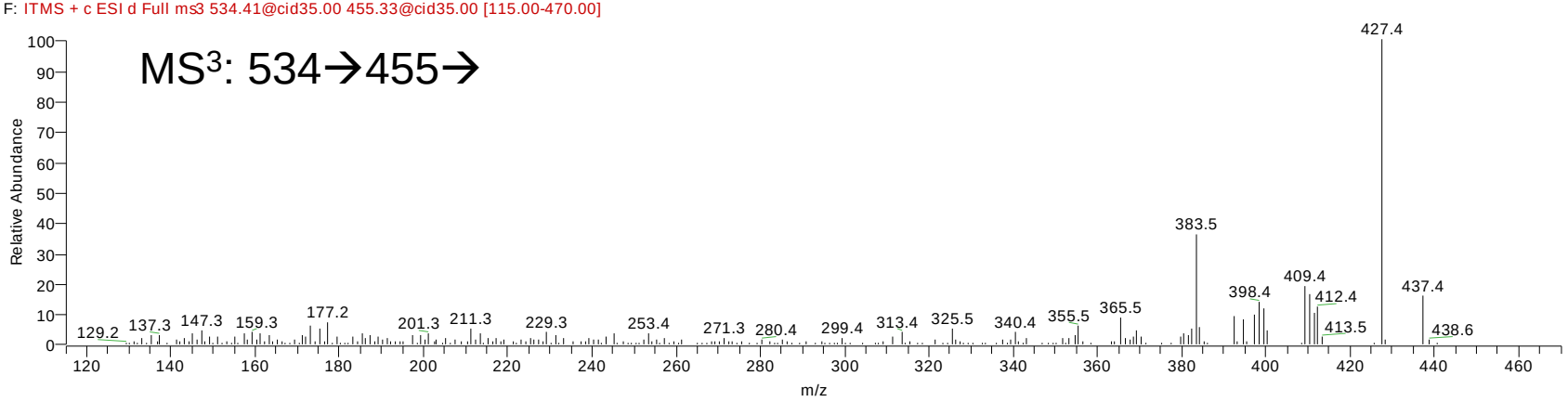


Fig. S4af C^4-6β-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1219 RT: 9.89 AV: 1 NL: 3.74E4
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.33@cid35.00 [115.00-470.00]



4 #1197 RT: 9.91 AV: 1 NL: 2.49E5
F: ITMS + c ESI d Full ms3 534.41@cid35.00 455.32@cid35.00 [115.00-470.00]

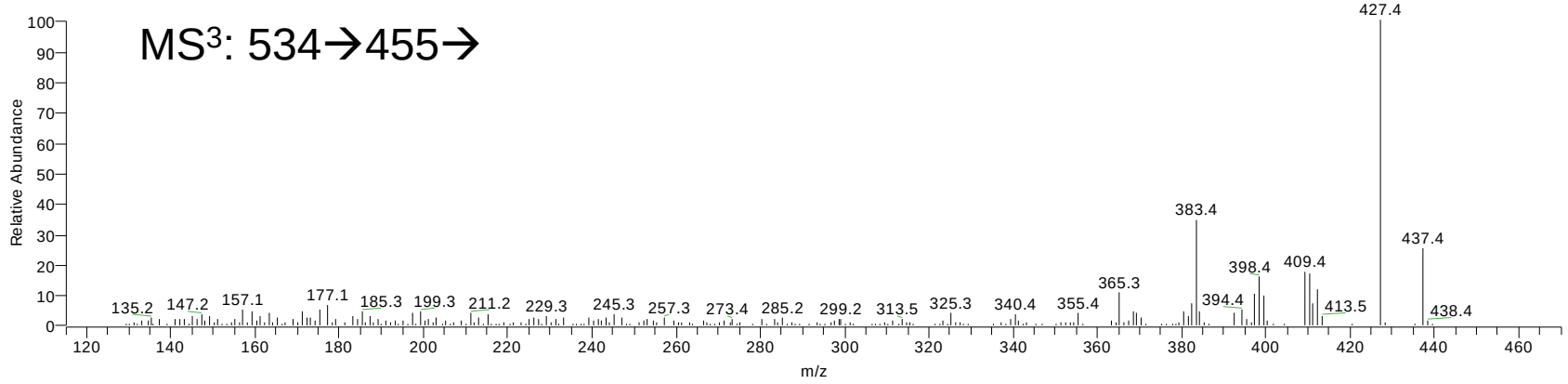


Fig. S4ag $C^{X,Y}$-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1201 RT: 9.76 AV: 1 NL: 1.80E3
F: ITMS + c ESI d Full ms2 548.38@cid35.00 [140.00-560.00]

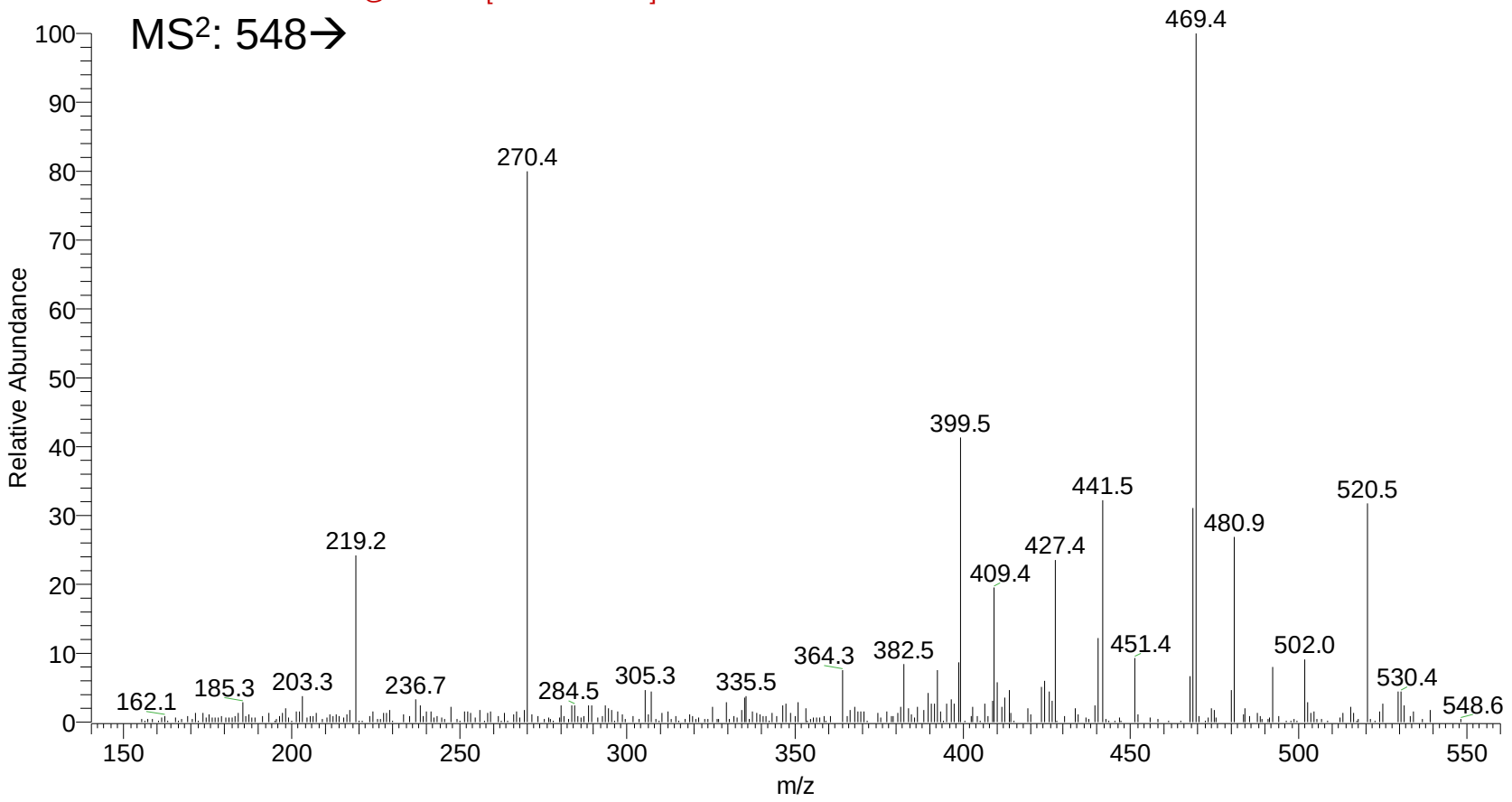


Fig. S4ah $C^{X,Y}$-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1202 RT: 9.77 AV: 1 NL: 2.59E2
F: ITMS + c ESI d Full ms3 548.38@cid35.00 469.37@cid35.00 [115.00-480.00]

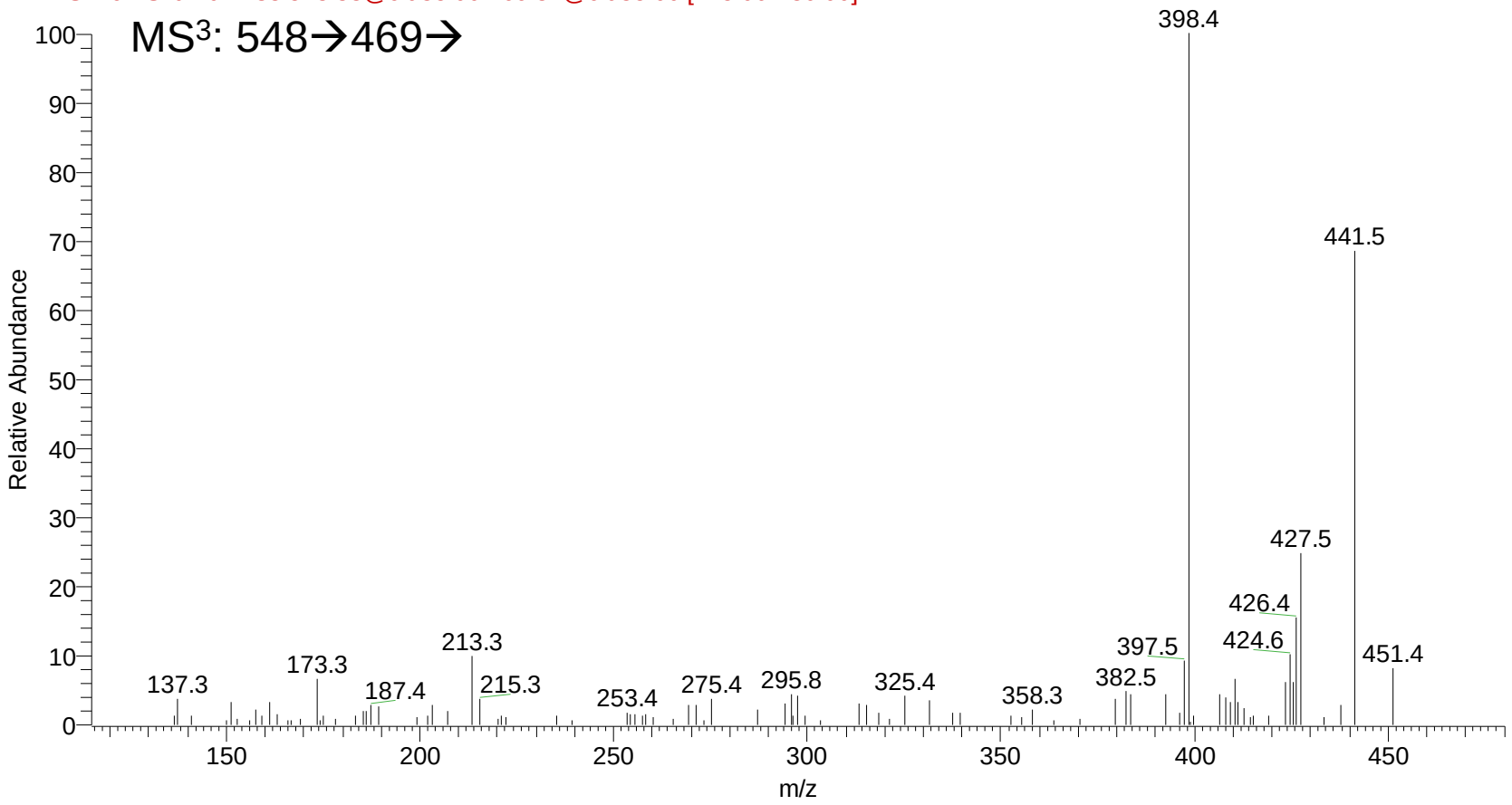


Fig. S4ai $C^{X,Y}$-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1242 RT: 10.06 AV: 1 NL: 5.20E2
F: ITMS + c ESI d Full ms2 548.38@cid35.00 [140.00-1110.00]

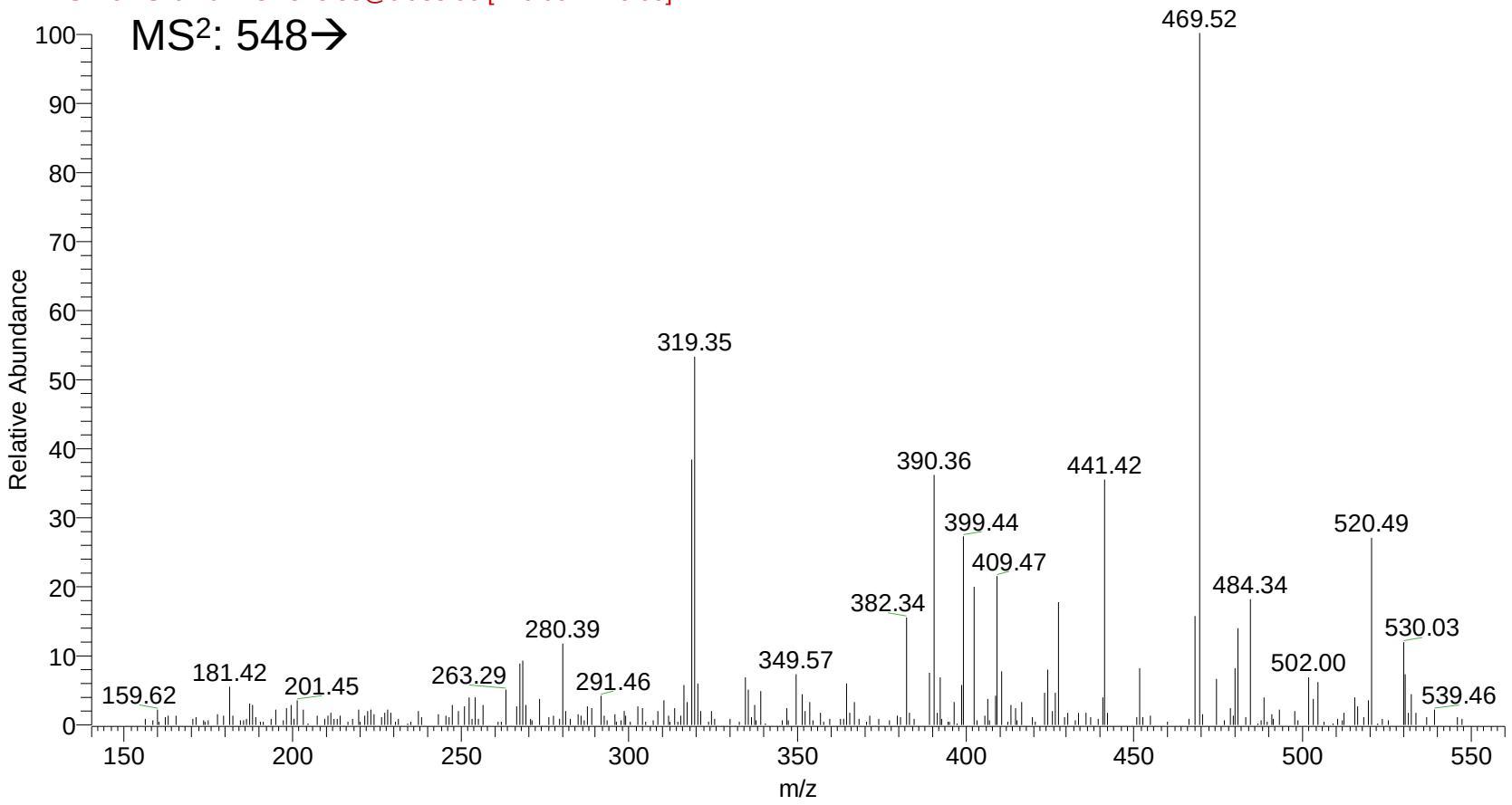


Fig. S4aj $C^{X,Y}$-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1243 RT: 10.07 AV: 1 NL: 5.82E1
F: ITMS + c ESI d Full ms3 548.38@cid35.00 469.37@cid35.00 [115.00-950.00]

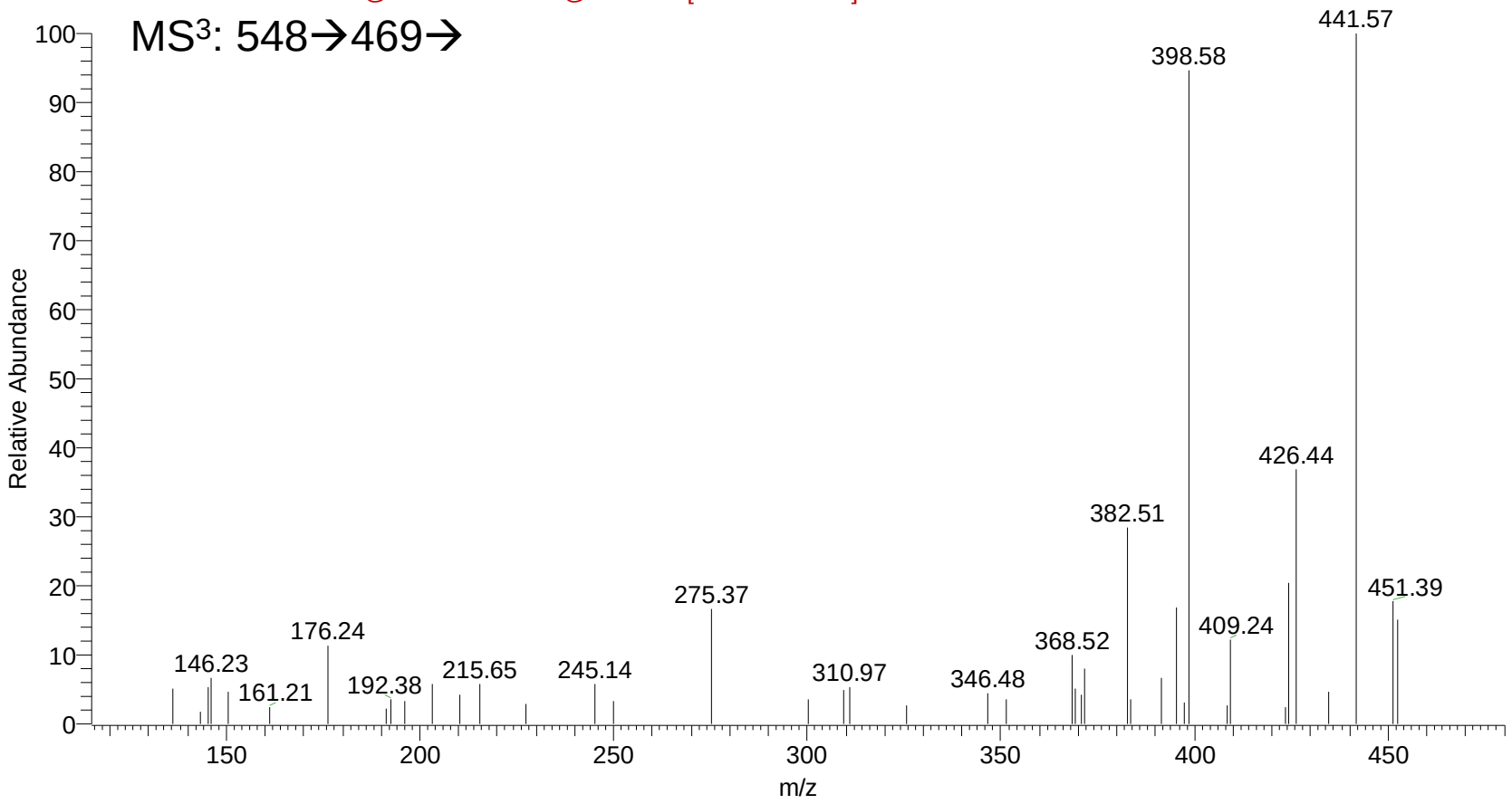
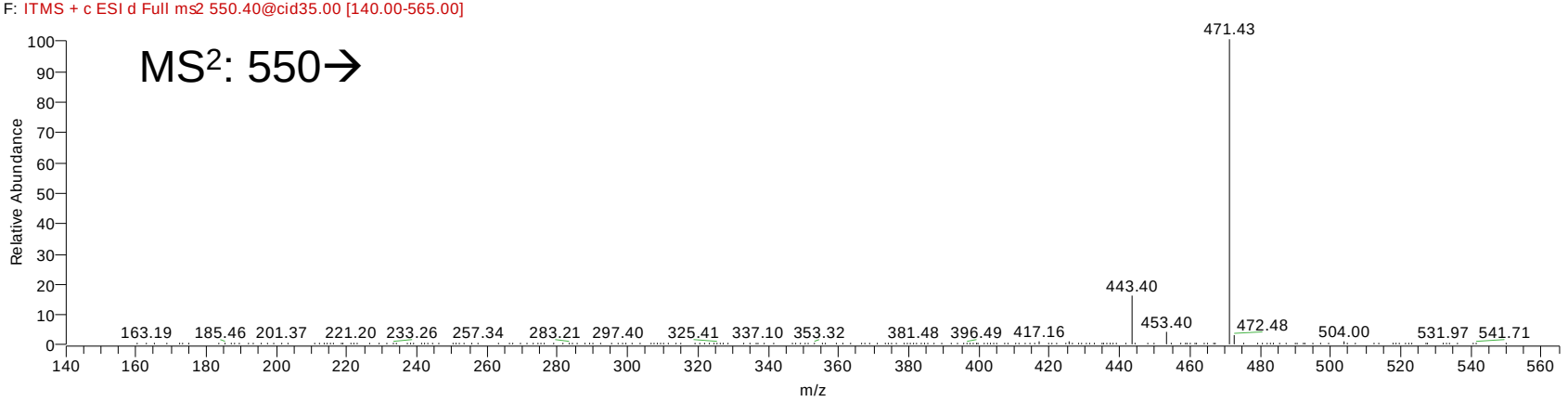


Fig. S4ak C⁴-24,25-diol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #540 RT: 4.43 AV: 1 NL: 2.50E4
F: ITMS + c ESI d Full ms2 550.40@cid35.00 [140.00-565.00]



sample_39 #467 RT: 4.72 AV: 1 NL: 8.53E4
F: ITMS + c ESI d Full ms2 550.40@cid30.00 [140.00-565.00]

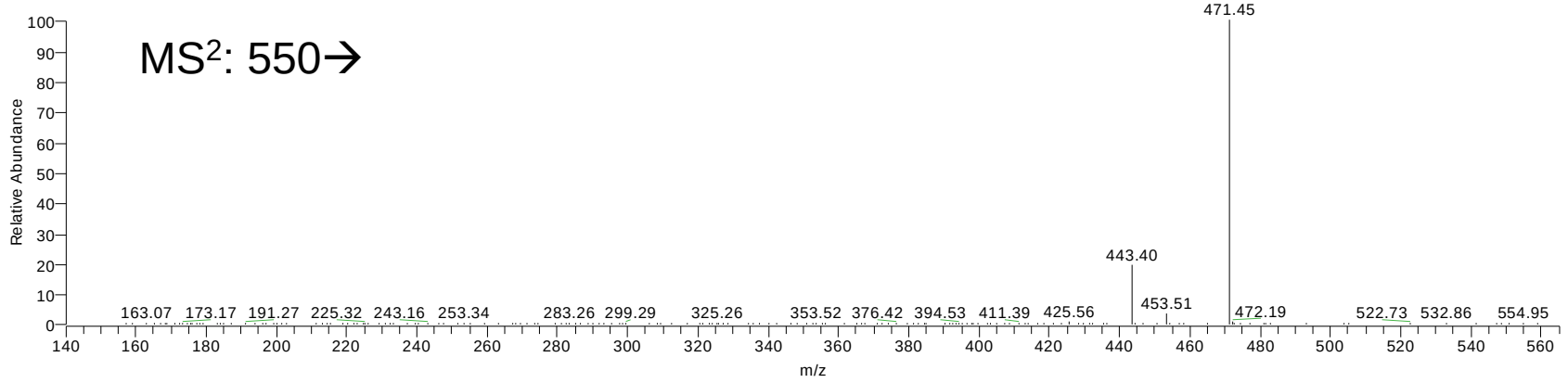
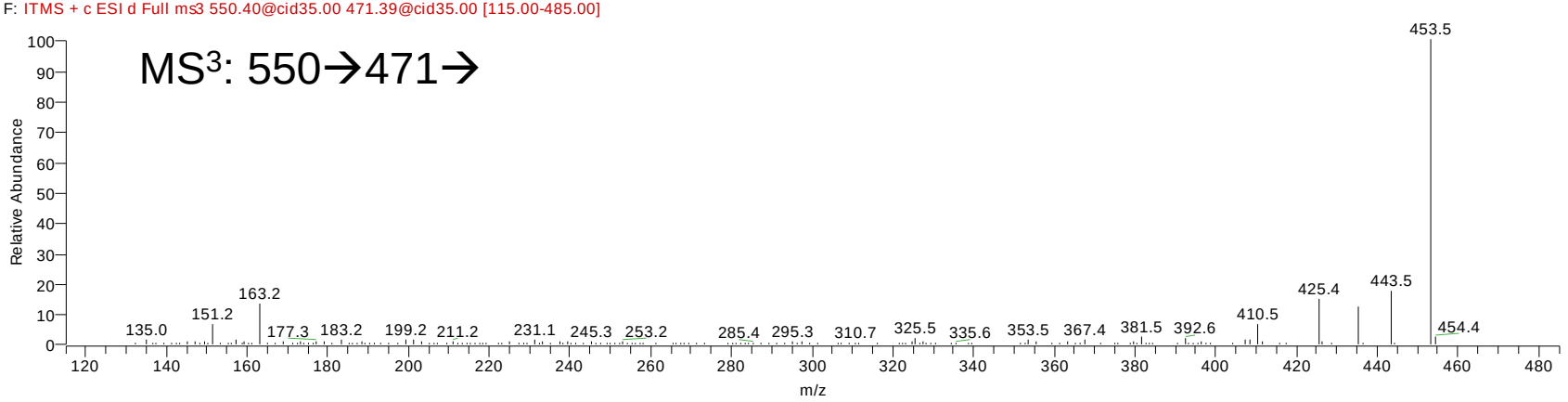


Fig. S4al C⁴-24,25-diol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #541 RT: 4.44 AV: 1 NL: 4.76E3
F: ITMS + c ESI d Full ms3 550.40@cid35.00 471.39@cid35.00 [115.00-485.00]



sample_39 #468 RT: 4.72 AV: 1 NL: 1.88E4
F: ITMS + c ESI d Full ms3 550.40@cid30.00 471.47@cid35.00 [115.00-485.00]

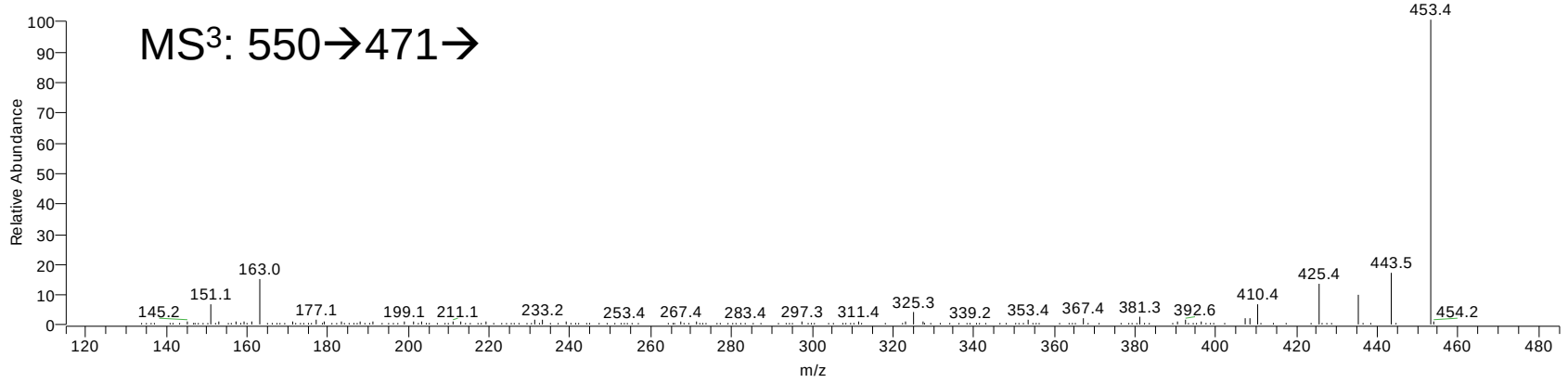


Fig. S4am C^4-24,27-diol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #589 RT: 4.84 AV: 1 NL: 1.08E4
F: ITMS + c ESI d Full ms2 550.40@cid35.00 [140.00-565.00]

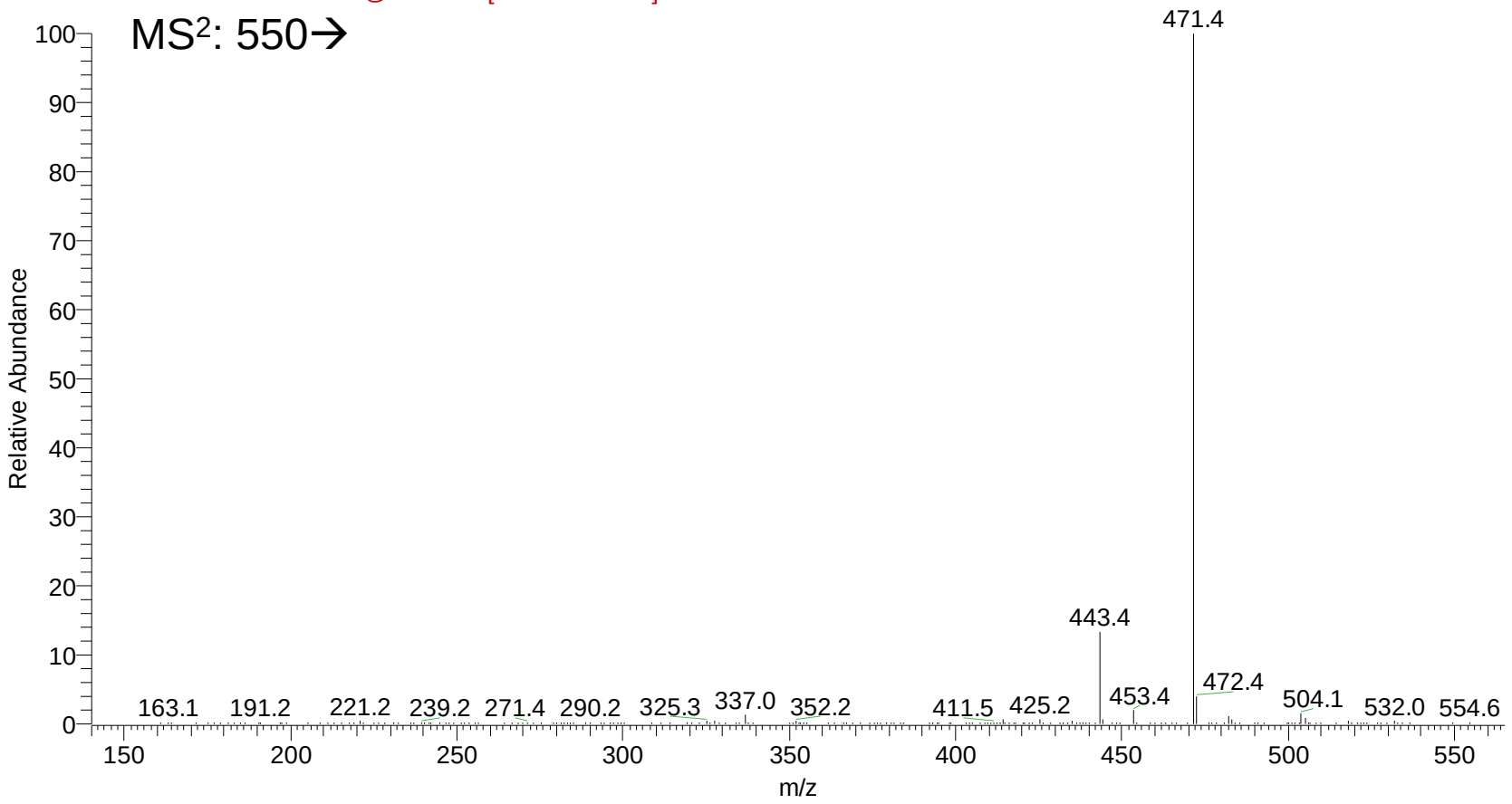


Fig. S4an C^4-24,27-diol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #590 RT: 4.84 AV: 1 NL: 1.03E3
F: ITMS + c ESI d Full ms3 550.40@cid35.00 471.39@cid35.00 [115.00-485.00]

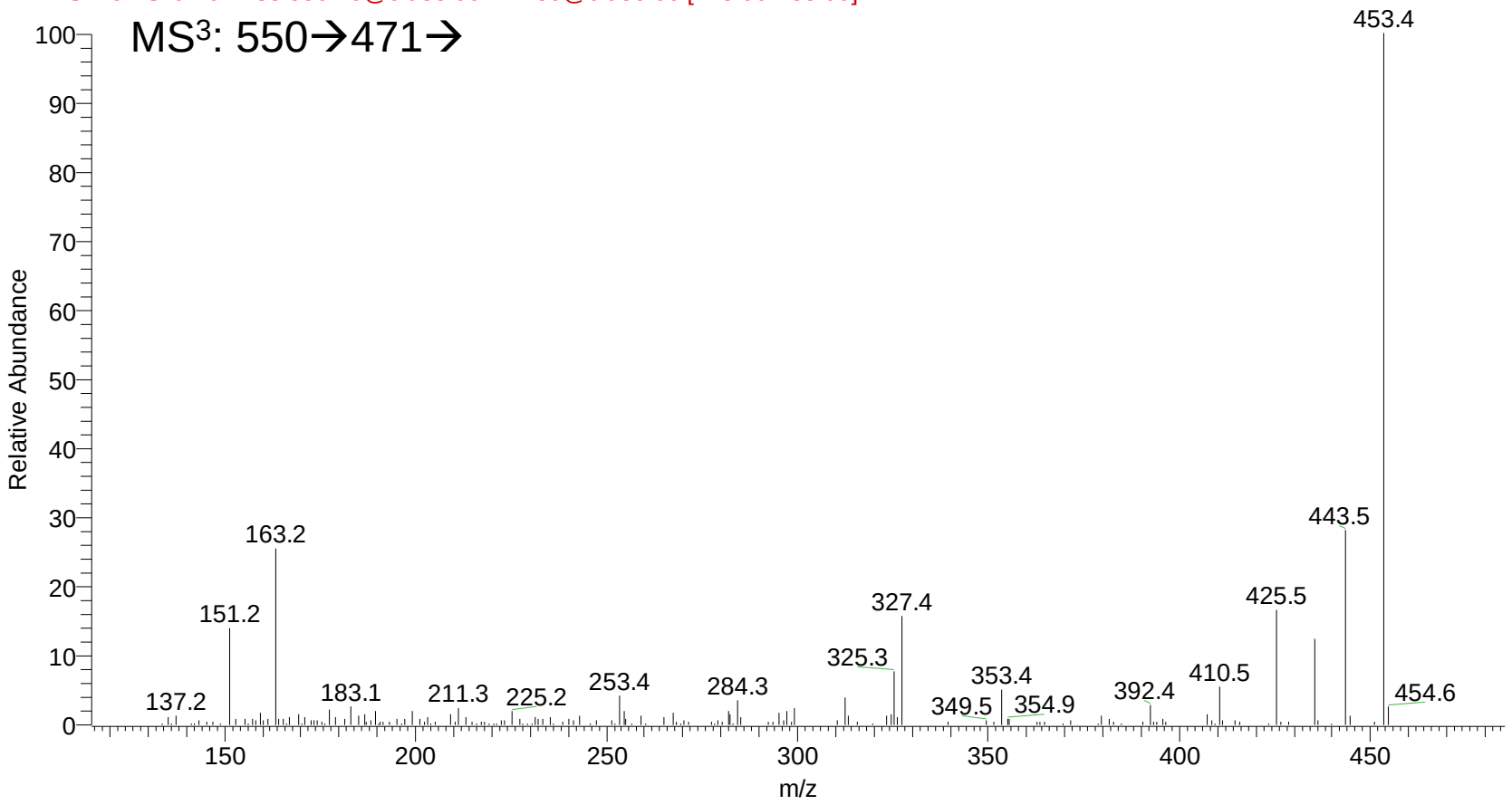


Fig. S4ao C-x-ol-y,z-dione y-GP / C^{X}-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1112 RT: 9.09 AV: 1 NL: 2.98E3
F: ITMS + c ESI d Full ms2 550.40@cid35.00 [140.00-565.00]

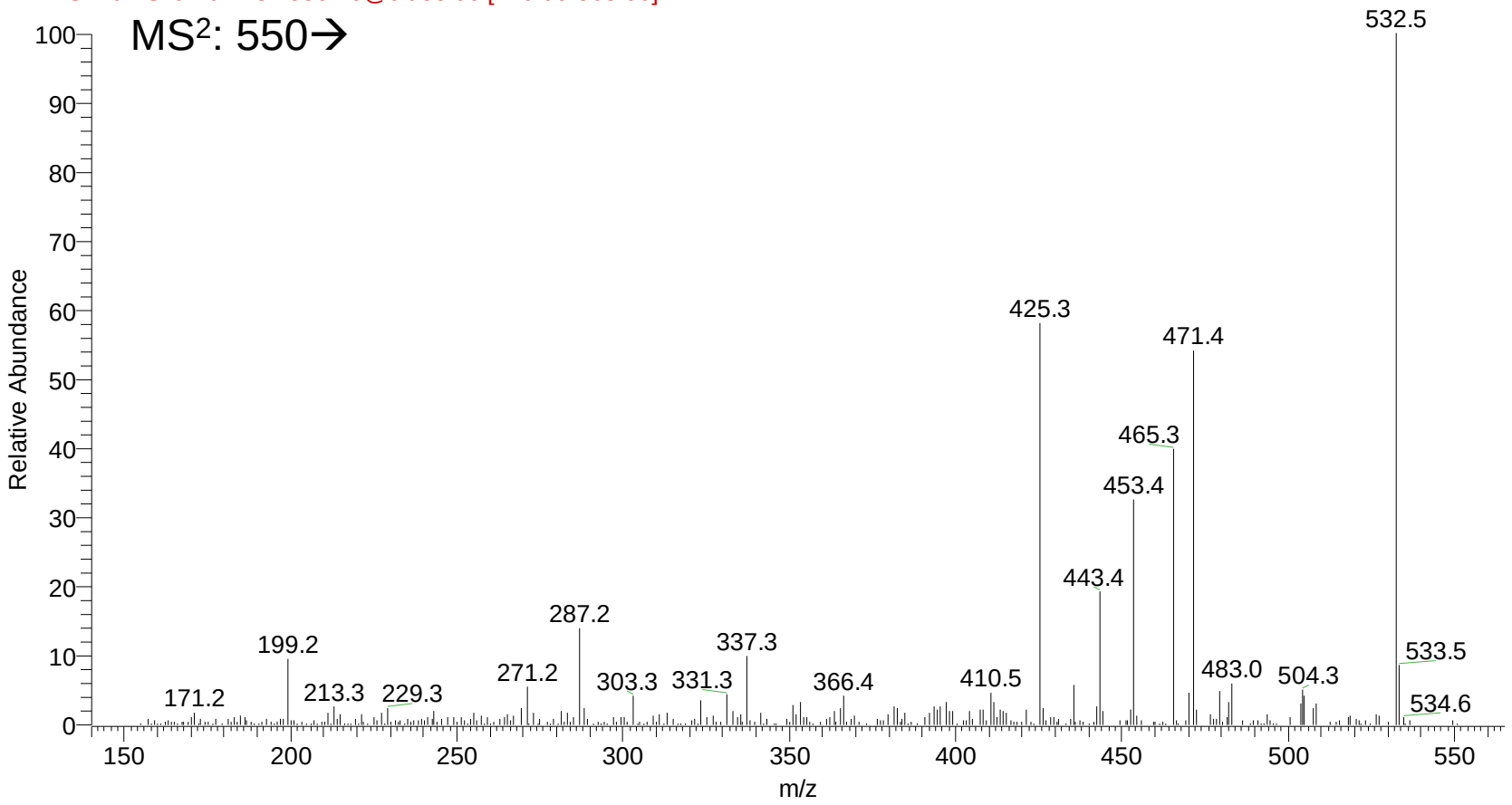


Fig. S4ap C-x-ol-y,z-dione y-GP / C^{X}-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1113 RT: 9.10 AV: 1 NL: 1.33E2
F: ITMS + c ESI d Full ms3 550.40@cid35.00 471.39@cid35.00 [115.00-485.00]

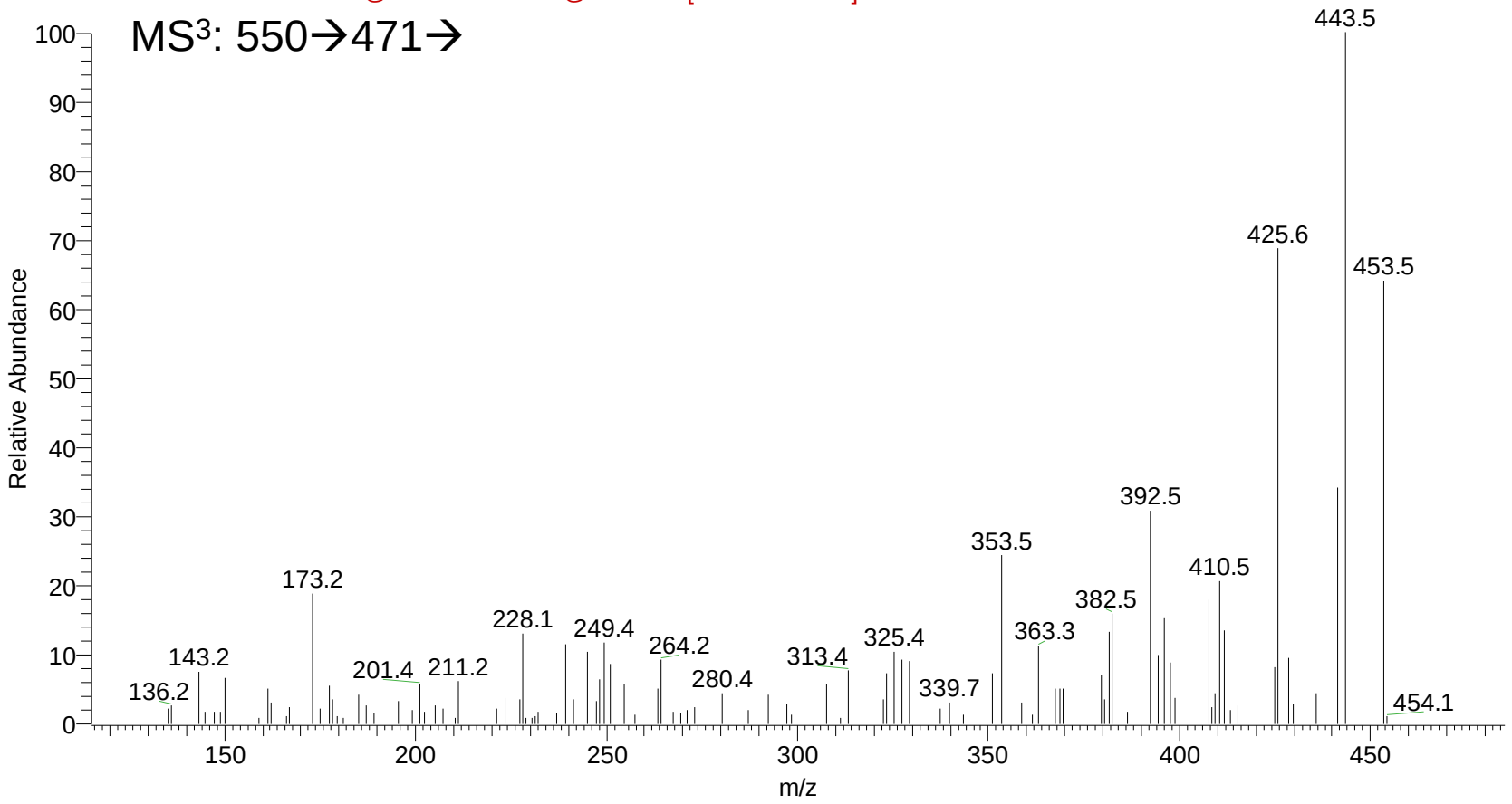


Fig. S4aq C-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1161 RT: 9.47 AV: 1 NL: 3.58E3
F: ITMS + c ESI d Full ms2 552.41@cid35.00 [140.00-565.00]

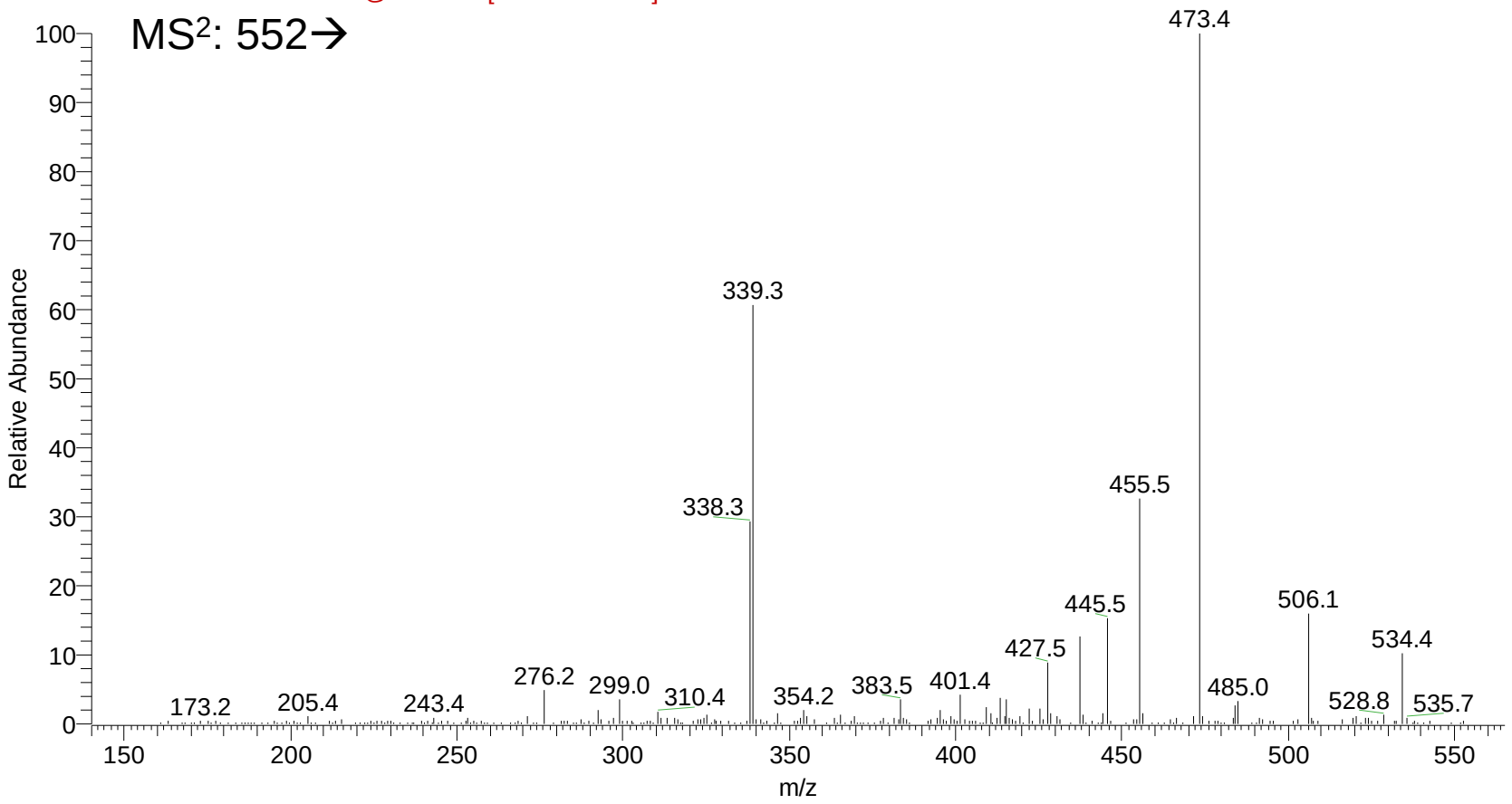


Fig. S4ar C-x,y-diol-z-one z-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1162 RT: 9.48 AV: 1 NL: 5.63E2
F: ITMS + c ESI d Full ms3 552.41@cid35.00 473.44@cid35.00 [120.00-485.00]

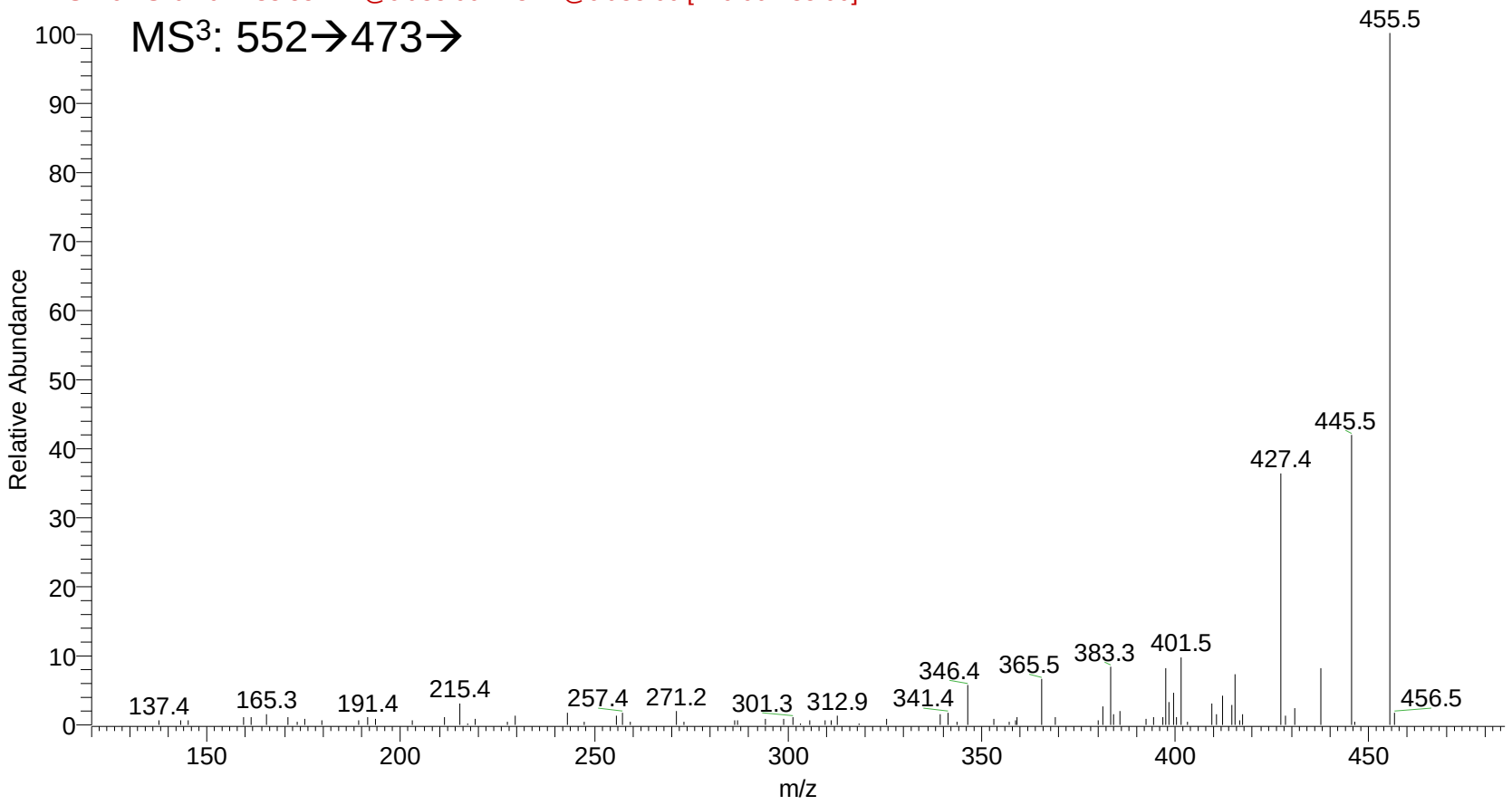


Fig. S4as aldol

CTX_UFR2_FR1_2 30/06/2006 18:49:40

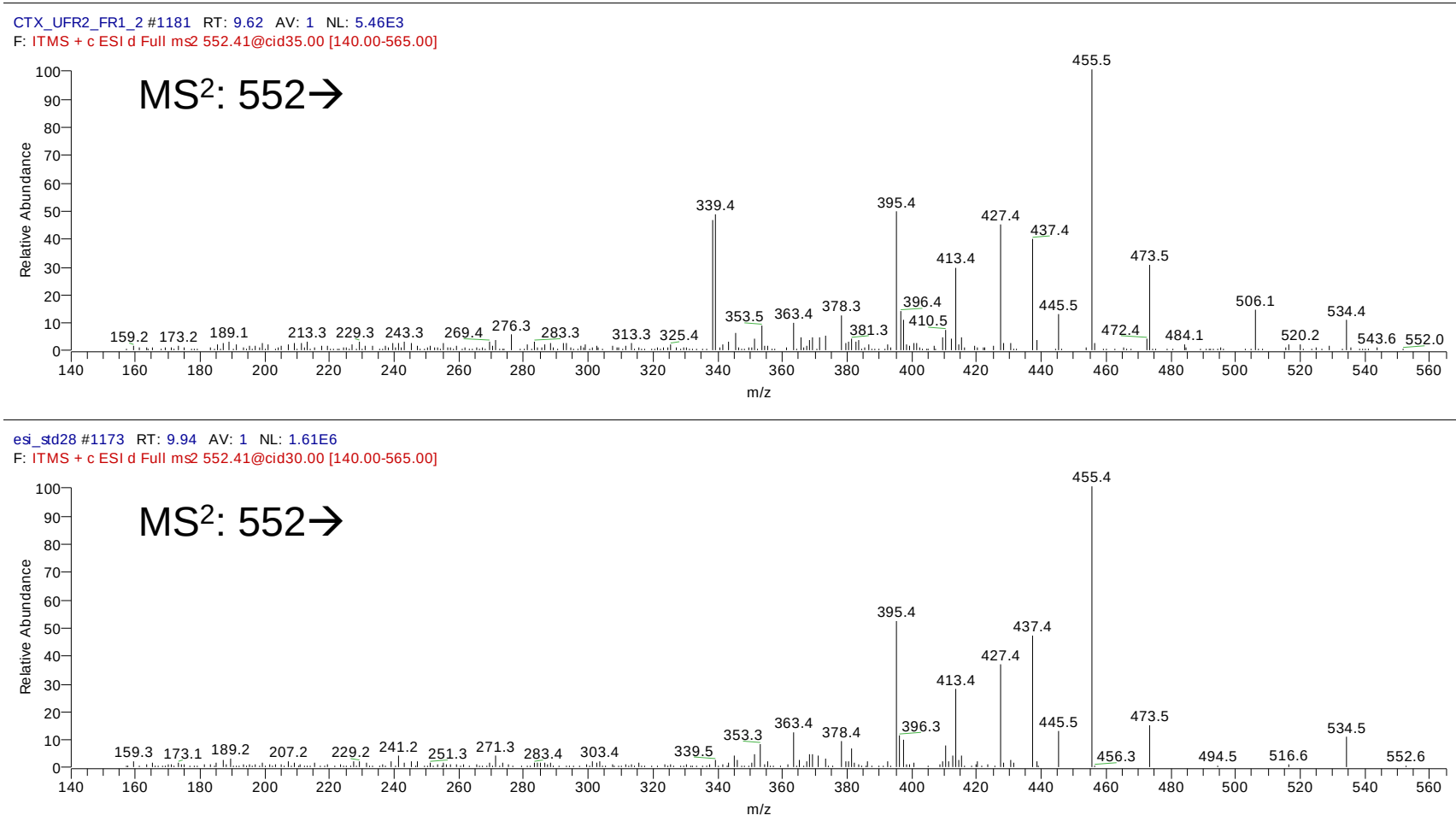
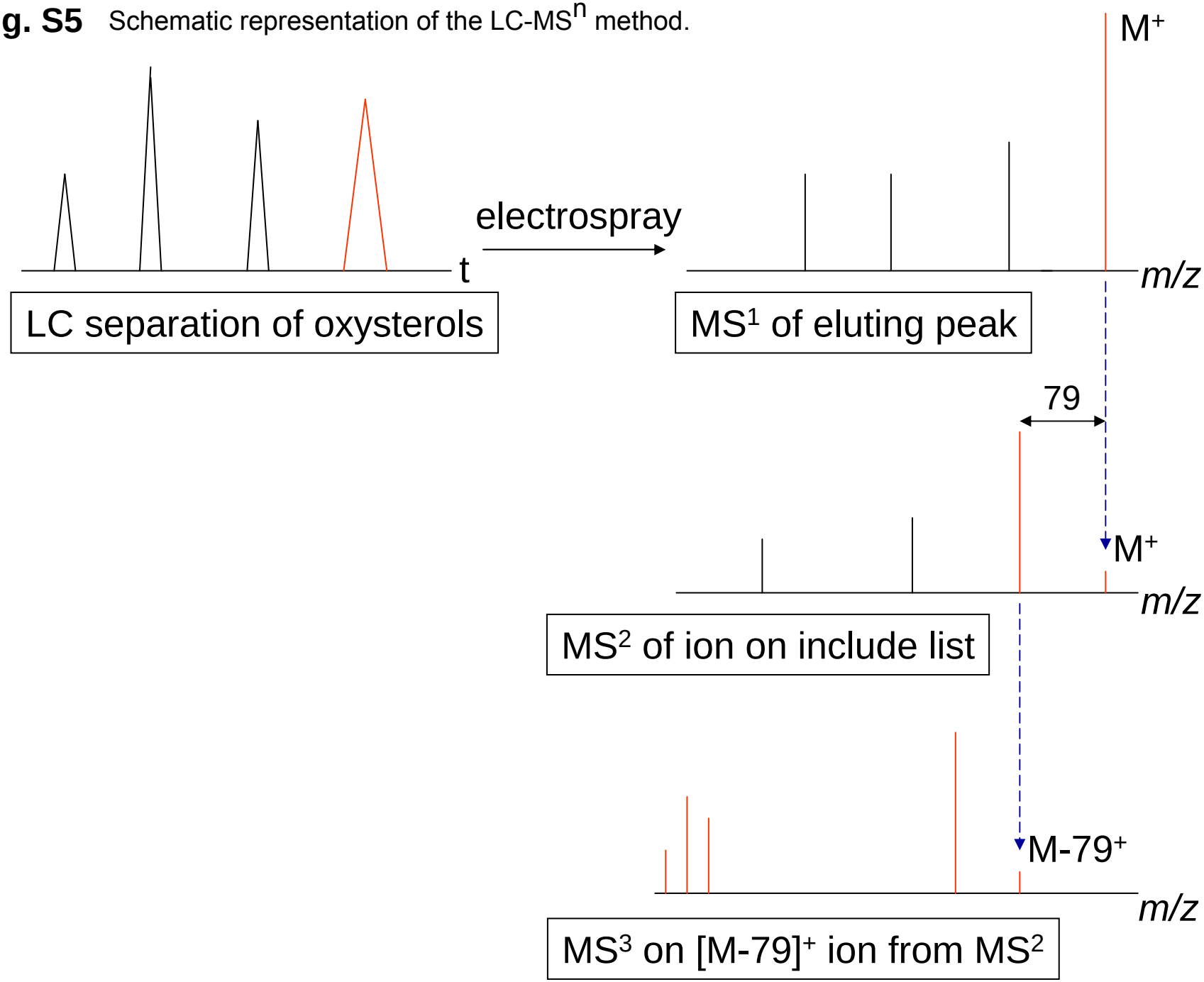


Fig. S5 Schematic representation of the LC-MS^n method.



Supplementary Table S1. Unesterified Oxysterol Content of Foetal CNS Regions Cortex (Ctx) and Spinal Cord (Sc) as Determined by LC-MS^n using the LCQ Ion-trap

			Ctx	Sc			
Identified Structure after Treatment with Cholesterol Oxidase	**[M]+ of GP[a] *m/z***	**Rt/min[b]**	**µg/g**	**µg/g**	**Inferred Structure prior to Treatment with Cholesterol Oxidase**	**Inferred Compound Trivial Name**	**Comment/ Parameters for Identification**
C-4,24-diene-3-one	516.4	23.95	NM	NM	C-5,24-diene-3β-ol	Desmosterol	Rt, MS^n
C-4-ene-3-one	518.4	25.59	NM	NM	C-5-ene-3β-ol	Cholesterol	Rt, MS^n
24-Me-C-4-ene-3-one	532.4	26.74	NM	NM	24-Me-C-5-ene-3β-ol	Campesterol	Contaminant[c], MS^n
24-Et-C-4-ene-3-one	546.4	28.15	NM	NM	24-Et-C-5-ene-3β-ol	Sitosterol	Contaminant[c], MS^n
C-4-ene-24S,25-epoxide-3-one	532.4	17.80	0.010	0.005	C-5-ene-3β-ol-24S,25-epoxide	24S,25-Epoxycholesterol	Rt, MS^n
C-4-ene-24-ol,25-OMe-3-one[d,e]	564.4	16.73	0.165	0.90	C-5-ene-3β-ol-24S,25-epoxide	24S,25-Epoxycholesterol	Rt, MS^n
			0.175	**0.095**	**C-5-ene-3β-ol-24S,25-epoxide**	**24S,25-Epoxycholesterol**	**Total 24S,25-Epoxycholesterol[f]**
C-4-ene-3,24-dione	532.4	19.10	0.035	0.015	C-5-ene-3β-ol-24-one	24-Oxocholesterol	Rt, MS^n
C-4-ene-3,6-dione	532.4	21.89	0.185	0.020	C-4-ene-3,6-dione[g]	6-Oxocholestenone[g]	Autoxidation, Rt, MS^n
C-4-ene-24S-ol-3-one	534.4	18.68	0.025	0.015	C-5-ene-3β,24S-diol	24S-Hydroxycholesterol	Rt, MS^n
C-4-ene-7β-ol-3-one	534.4	21.08	0.025	0.030	C-5-ene-3β,7β-diol	7β-Hydroxycholesterol	Autoxidation, Rt, MS^n
C-4-ene-7α-ol-3-one	534.4	21.75	0.025	0.025	C-5-ene-3β,7α-diol	7α-Hydroxycholesterol[h]	Rt, MS^n
C-4-ene-6β-ol-3-one	534.4	22.60	0.570	0.110	C-4-ene-6β-ol-3-one/C-3β,5α,6β-triol/C-3β-ol-5,6-epoxide[i]	6β-hydroxycholest-4-en-3-one/cholestane-3β,5α,6β-triol/5,6-epoxycholesterol[i]	Autoxidation, Rt, MS^n

C-4-ene-24,25-diol-3-one	550.4	12.02	0.070	0.040	C-5-ene-3β,24,25-triol	24,25-Dihydroxycholesterol	Rt, MS^n
C-4-ene-24,27-diol-3-one	550.4	11.91	0.005	0.005	C-5-ene-3β,24,27-triol	24,27-Dihydroxycholesterol	No reference, MS^n
Aldol	552.4	22.53	0.030	0.020	Aldol[j]	Aldol[j]	MS^n

C = cholestane; NM = not measured; Rt = retention time; ND = not detected; No reference = no authentic standard available
[a] Data for GP derivatives from replicate analysis
[b] LC-MS^n performed using capillary column-LC interfaced to an LCQ ion-trap mass spectrometer
[c] Possibly a contaminant
[d] Alternatively C-4-ene-25-ol,24-OMe-3-one
[e] Methanolysis product of C-4-ene-24S,25-epoxide-3-one
[f] Sum of values for C-4-ene-24S,25-epoxide-3-one and C-4-ene-24-ol,25-OMe-3-one (or C-4-ene-25-ol,24-OMe-3-one)
[g] C-4-ene-3,6-dione reacts with GP reagent without oxidation by cholesterol oxidase
[h] Formed enzymatically by CYP7A1 and/or by autoxidation
[i] C-4-ene-6β-ol-3-one can be formed from C-3β,5α,6β-triol and C-3β-ol-5,6-epoxides during the cholesterol oxidase/GP derivatisation reaction
[j] 3β,5β-dihydroxy-B-norcholestane-6β-carboxyaldehyde (aldol) reacts with GP reagent without oxidation by cholesterol oxidase
[n] MS/MS or MS/MS/MS

Supplementary Table S2. Data dependent settings for analysis on the LTQ-Orbitrap

Parent–ion mass	Neutral loss	Parent-ion structure	Parent molecule before oxidation/derivatisation
516.3948	79.04	$C^{4,x}$-3-one 3-GP	Desmosterol/7-Dehydrocholesterol
518.4105	79.04	C^{4}-3-one 3-GP	Cholesterol
520.4261	79.04	C-3-one 3-GP	3β-Hydroxycholestane
532.3898	79.04	C^{4}-3,x-dione 3-GP/ C^{4}-3-one-x,y-epoxide 3-GP	Oxocholesterol/ Epoxycholesterol
534.4054	79.04	C^{4}-x-ol-3-one 3-GP	Hydroxycholesterol
536.4211	79.04	C-x-ol-3-one 3-GP/ C-3-one-5,6-epoxide 3-GP	Cholestanediol/ 5,6-Epoxycholesterol
546.3690	79.04	$CA^{4,x}$-3-one 3-GP	3β-Hydroxycholestadienoic acid
548.3847	79.04	CA^{4}-3-one 3-GP	3β-Hydroxycholestenoic acid
550.4003	79.04	C^{4}-x,y-diol-3-one 3-GP	Dihydroxycholesterol
552.4160	79.04	C-x,y-diol-3-one 3-GP 5,6-*seco*-sterol/aldol 3-GP	Cholestanetriol 3β-hydroxy-5-oxo-5,6-*seco*cholestan-6-al
560.3483	79.04	$CA^{4,x,y}$-x-ol-3-one 3-GP	Dihydroxycholestatrienoic acid
562.3639	79.04	$CA^{4,x}$-x-ol-3-one 3-GP	Dihydroxycholestadienoic acid
564.3796	79.04	CA^{4}-x-ol-3-one 3-GP	Dihydroxycholestenoic acid
566.3592	79.04	C^{4}-x,y,z-triol-3-one 3-GP	Trihydroxycholesterol
568.4109	79.04	C-x,y,z-triol-3-one 3-GP	Cholestanetetraol