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

CTX_UFR2_FR1_2 30/06/2006 18:49:40

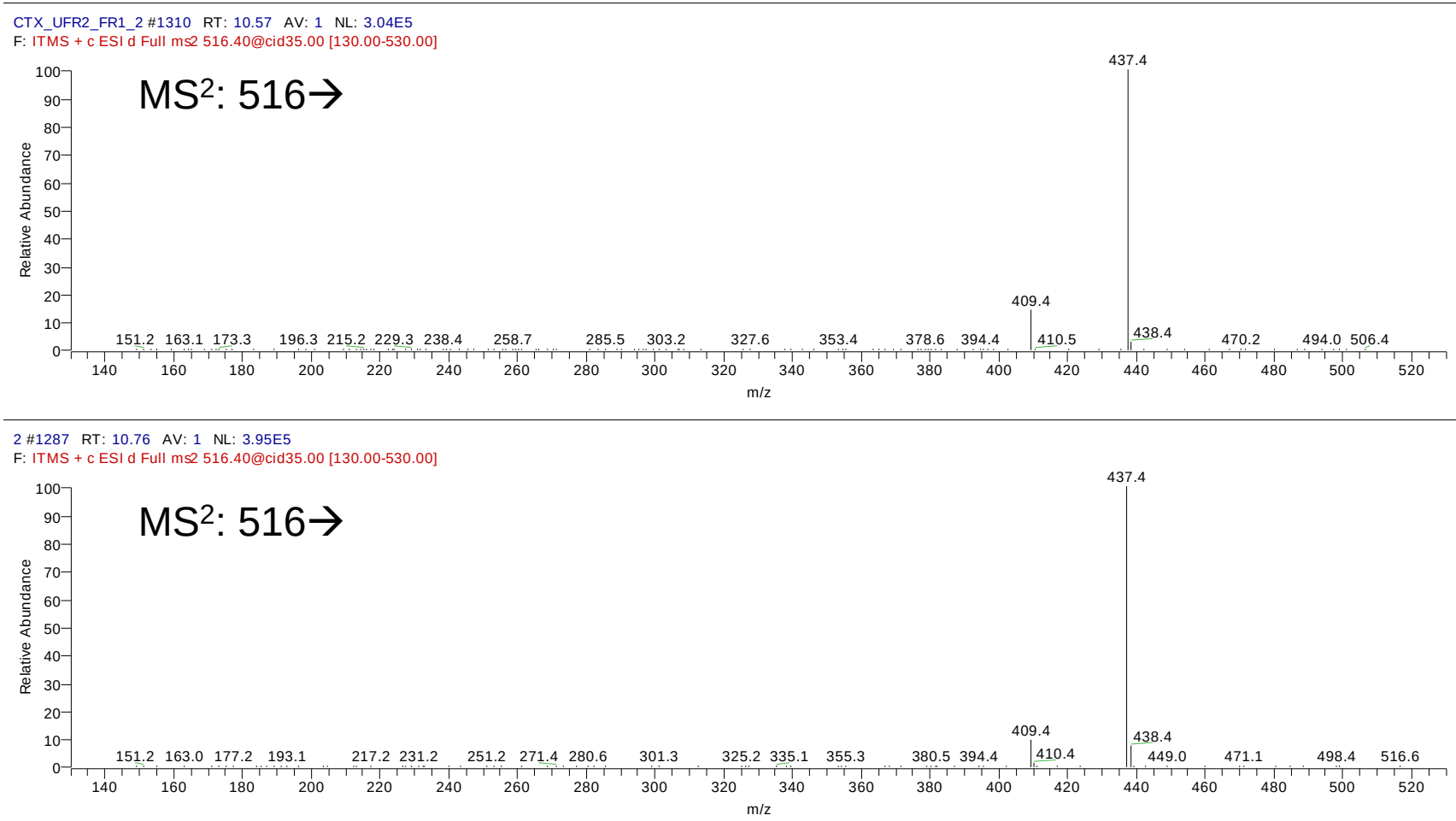
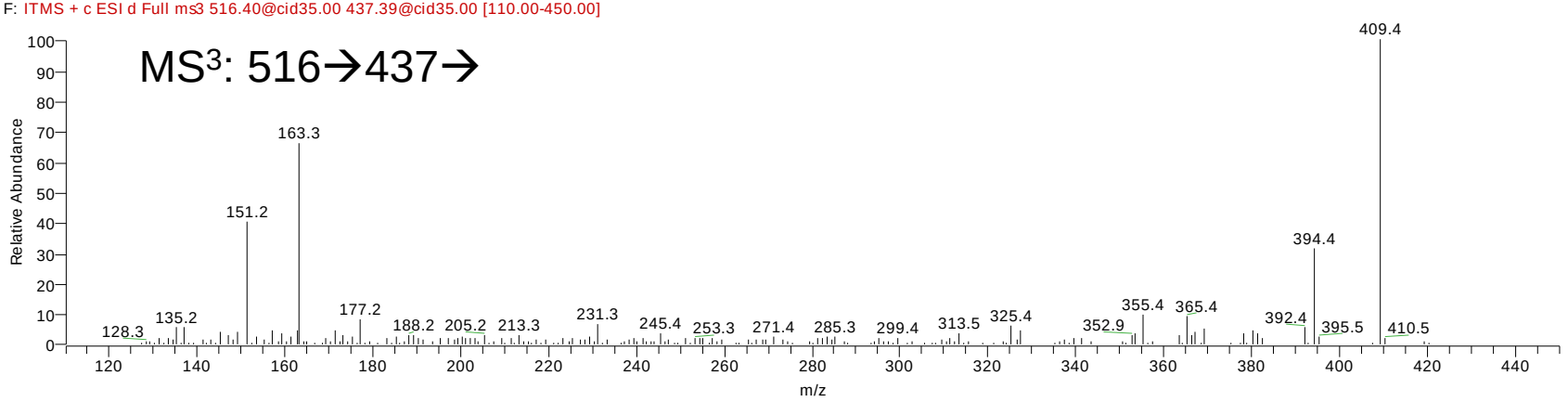


Fig. S4b $C^{4,24}$-3-one 3-GP

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]

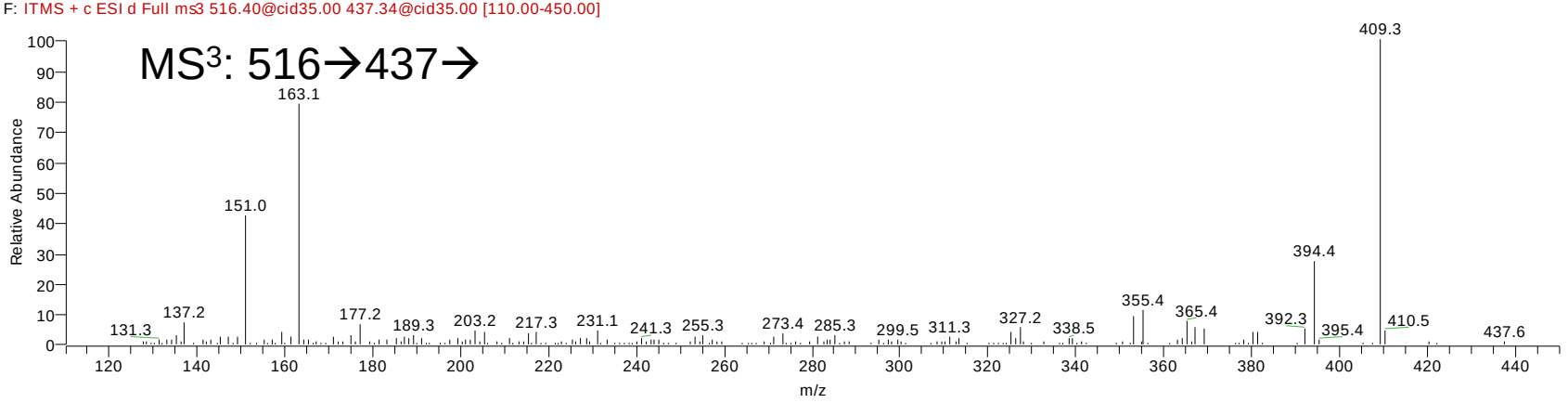
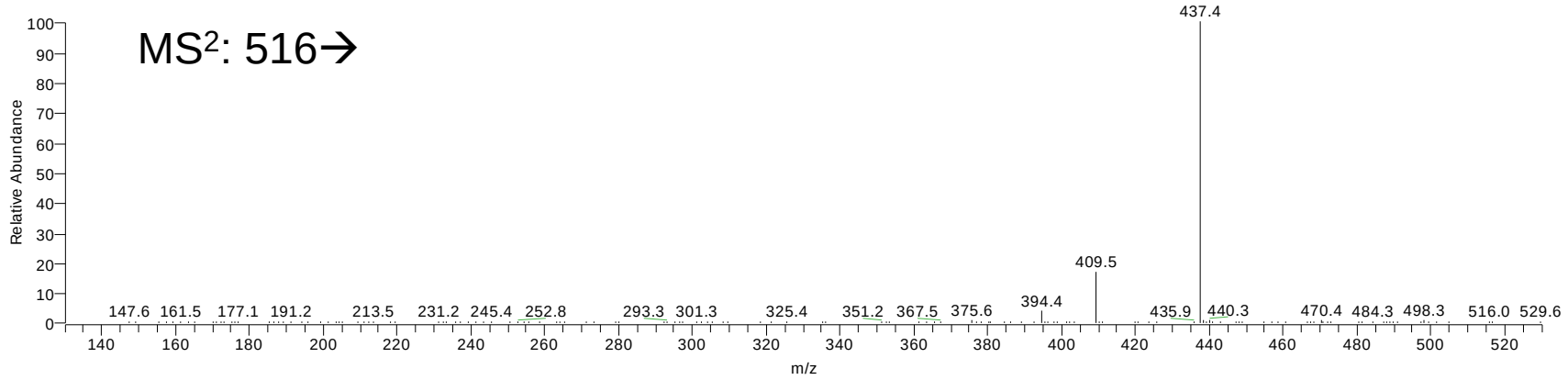


Fig. S4c $C^{4,7or8}$-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

CTX_UFR2_FR1_2 #1375 RT: 11.03 AV: 1 NL: 4.33E3
F: ITMS + c ESI d Full ms2 516.40@cid35.00 [130.00-1045.00]



3 #1346 RT: 11.23 AV: 1 NL: 1.32E5
F: ITMS + c ESI d Full ms2 516.40@cid35.00 [130.00-530.00]

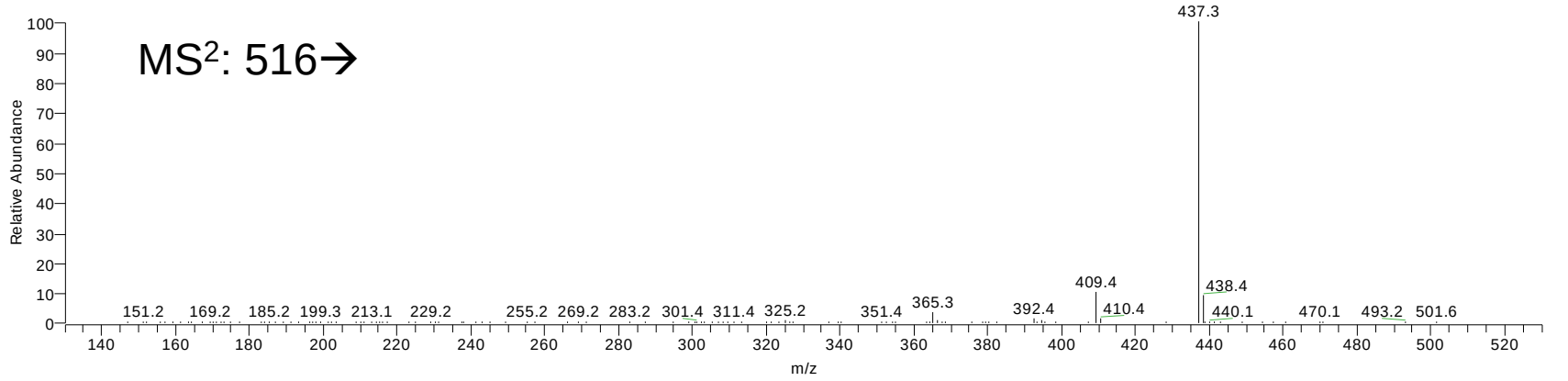
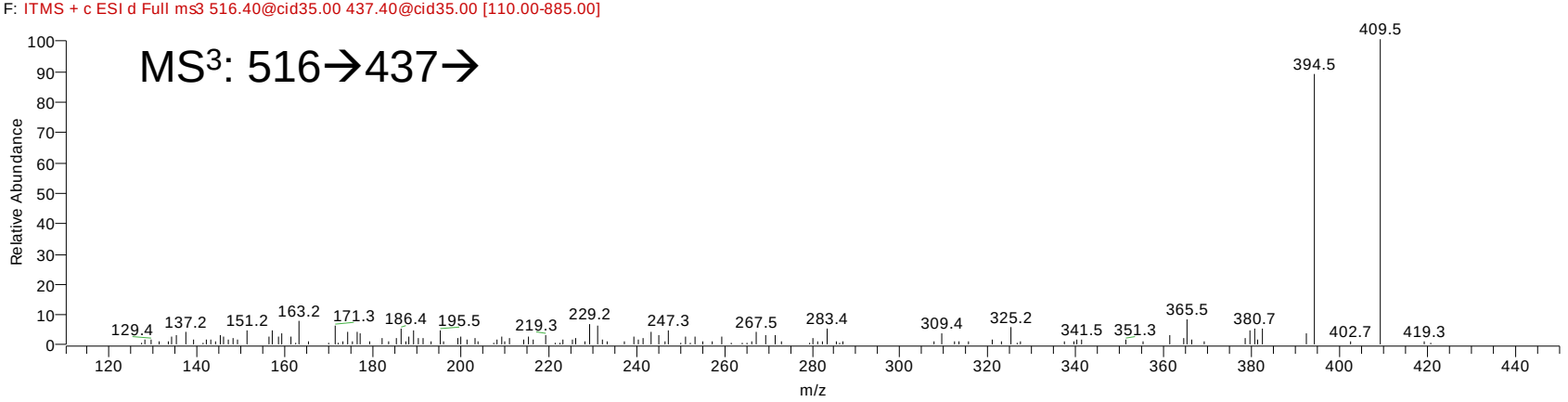


Fig. S4d $C^{4,7or8}$-3-one 3-GP

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]

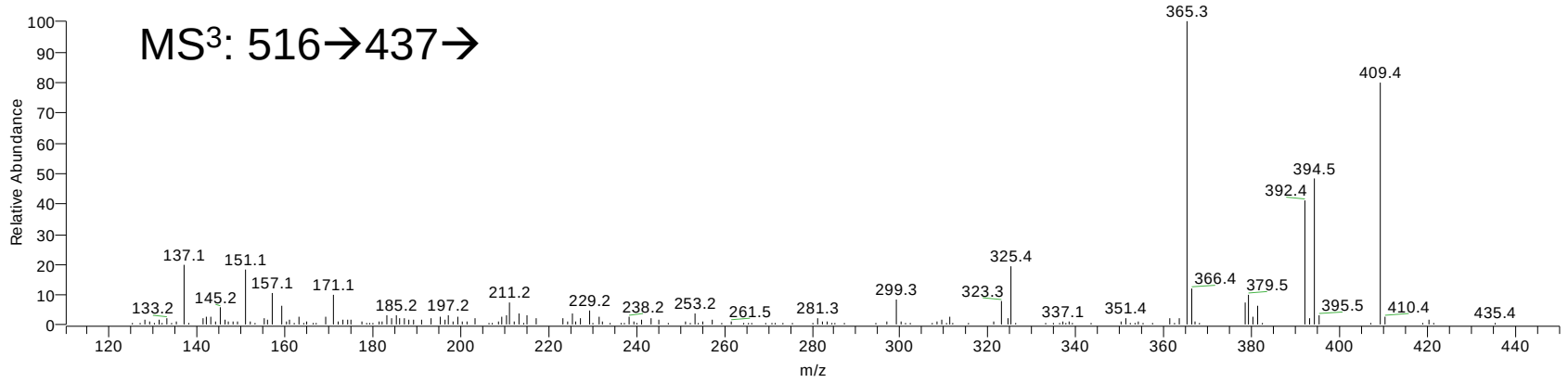
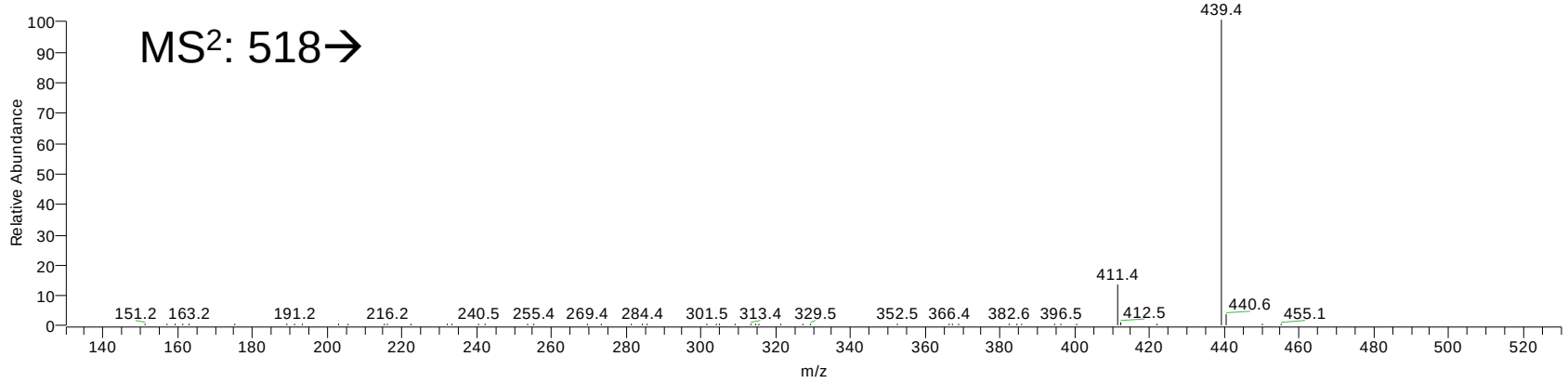


Fig. S4e C^4-3-one 3-GP

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]

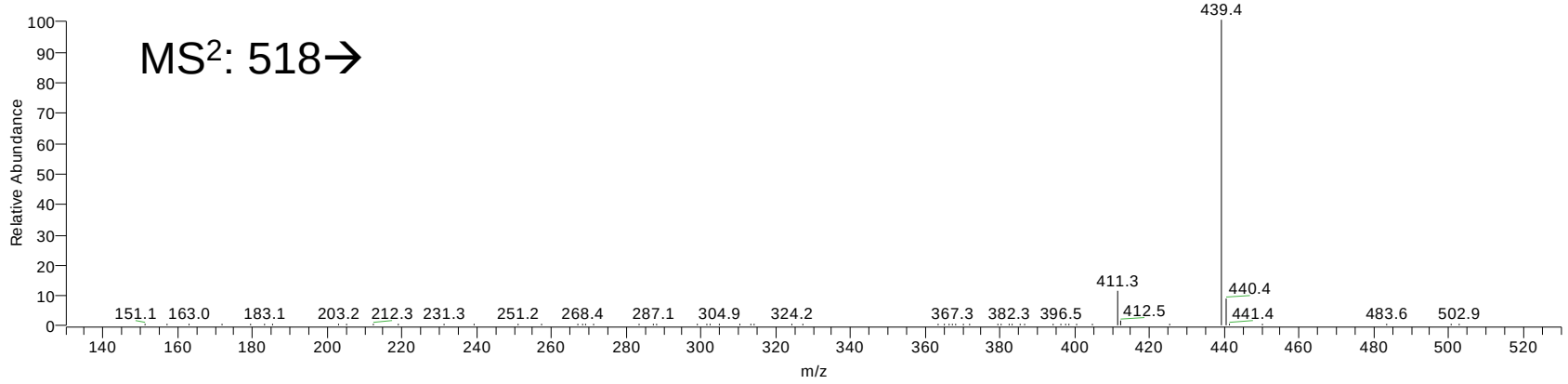
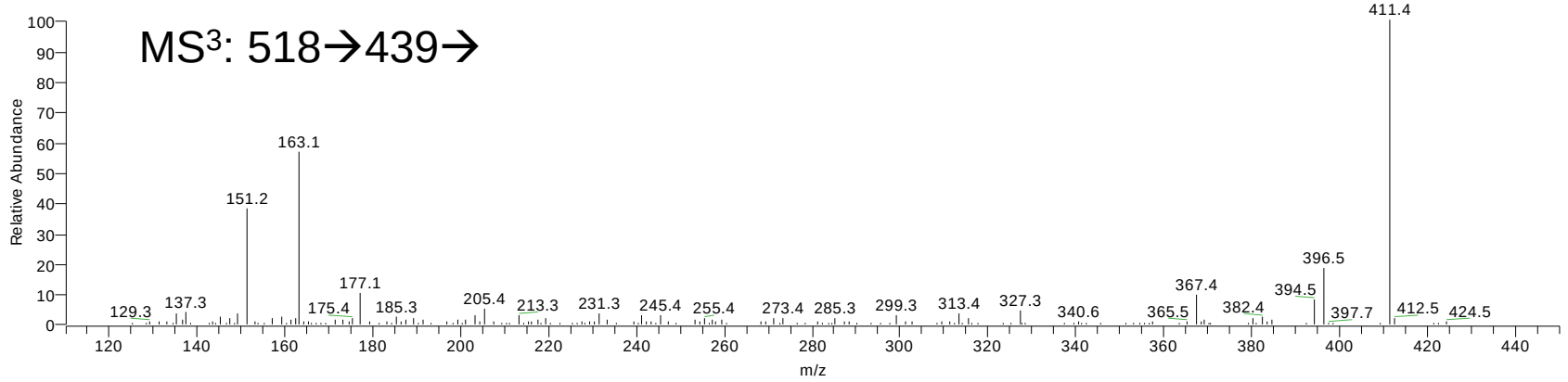


Fig. S4f C⁴-3-one 3-GP

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]

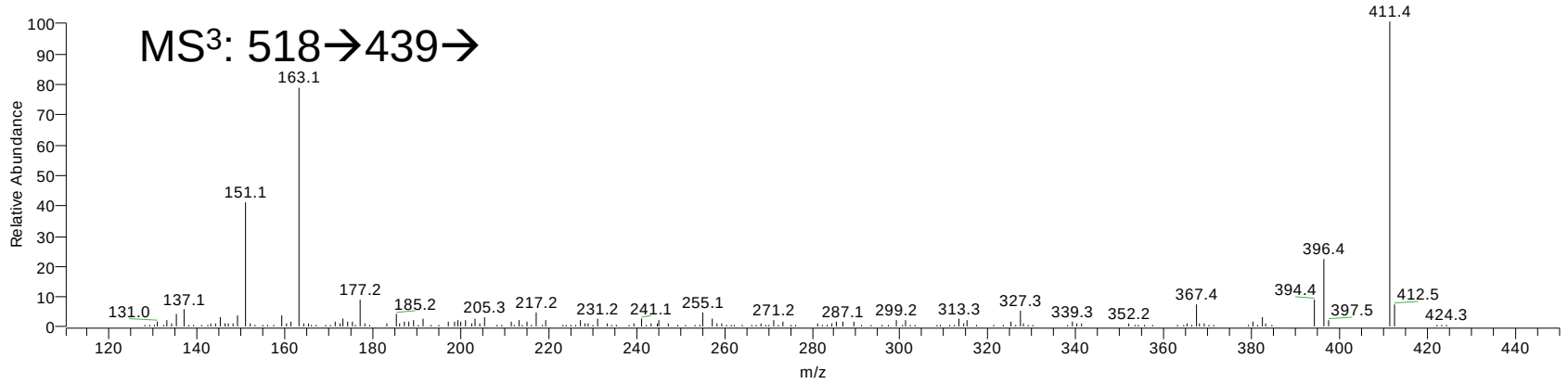


Fig. S4g 24-Me-C^4-3-one 3-GP

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]

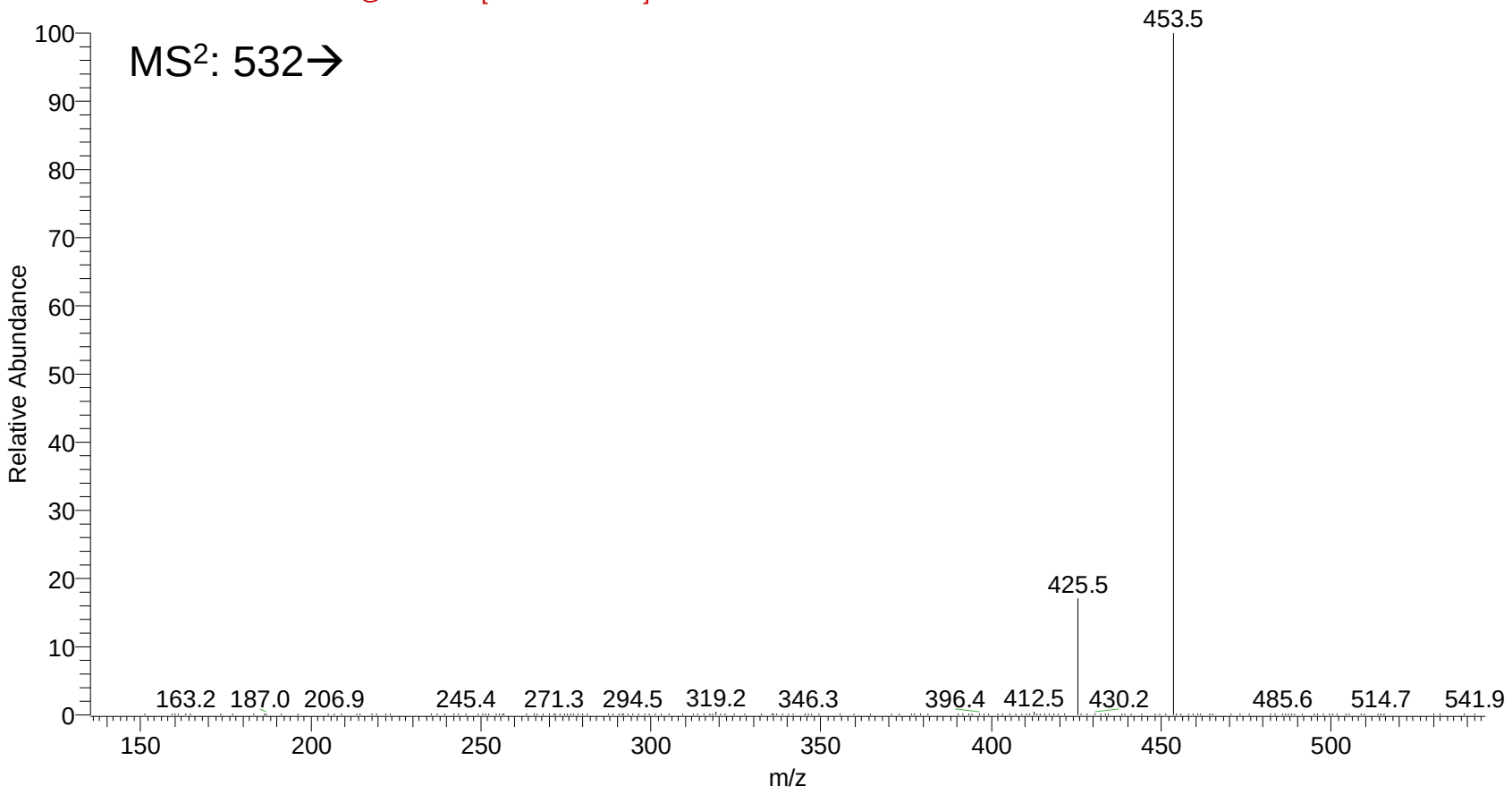


Fig. S4h 24-Me-C^4-3-one 3-GP

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]

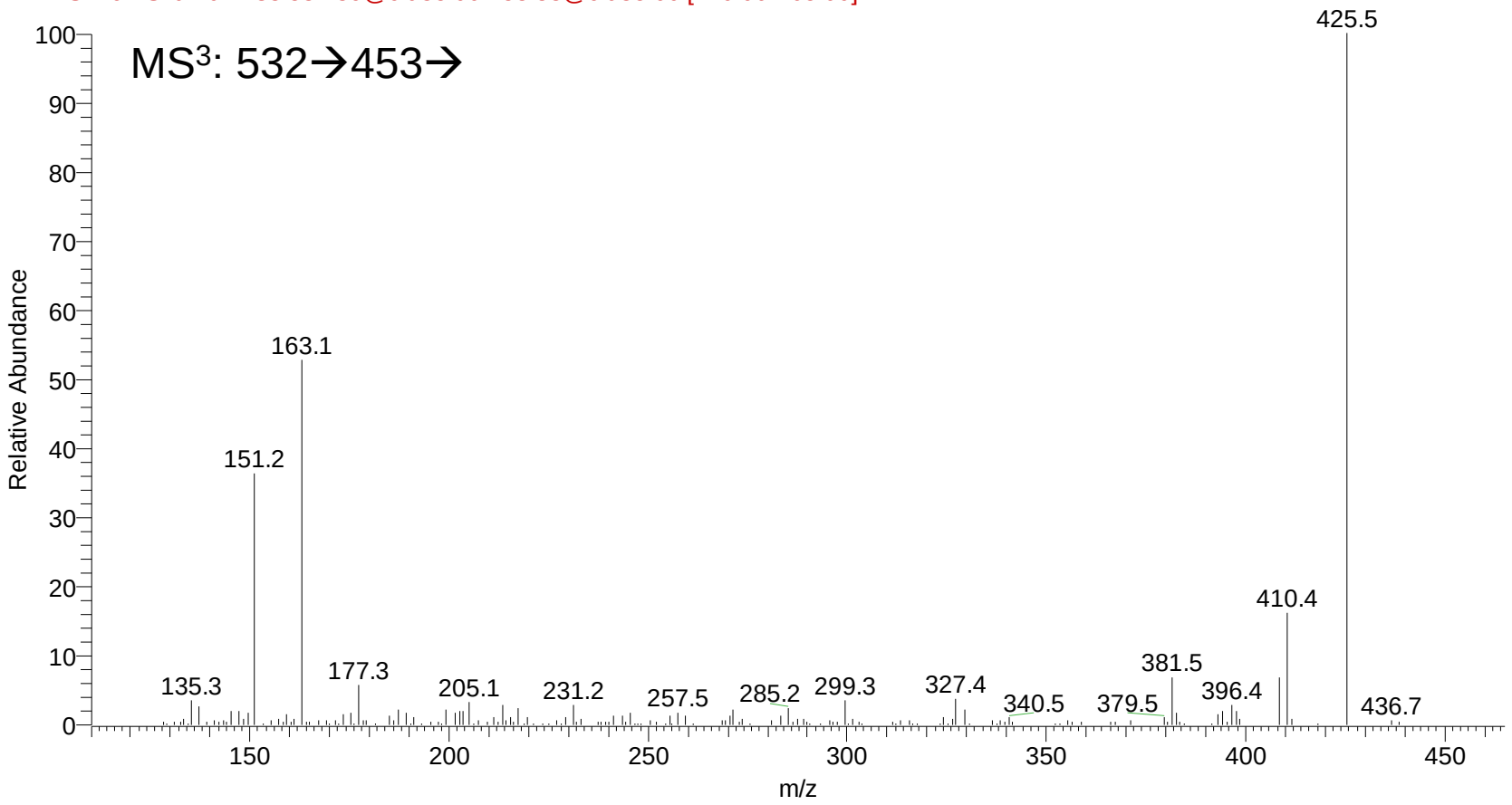


Fig. S4i C4-24S,25-epoxide-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

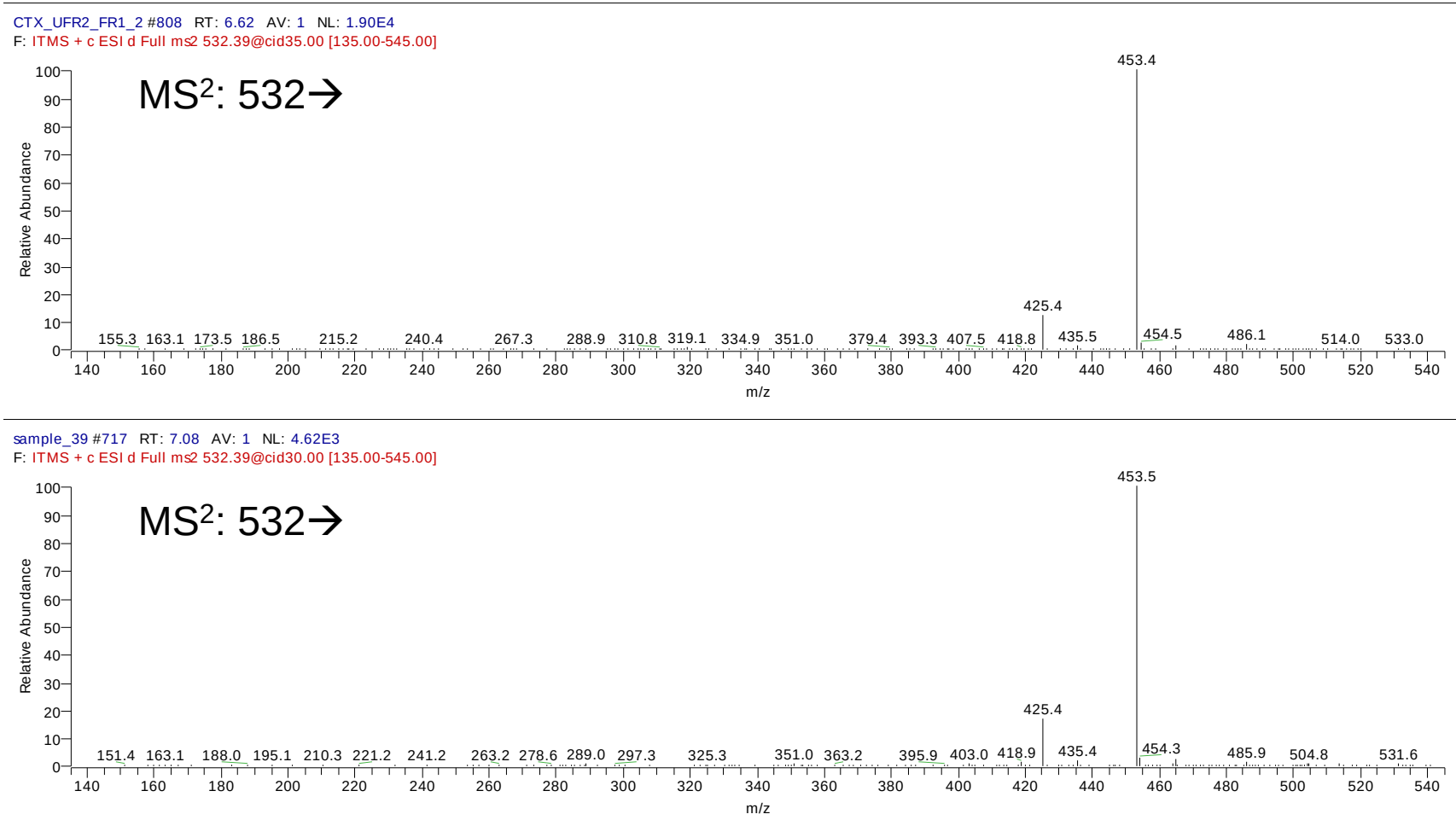
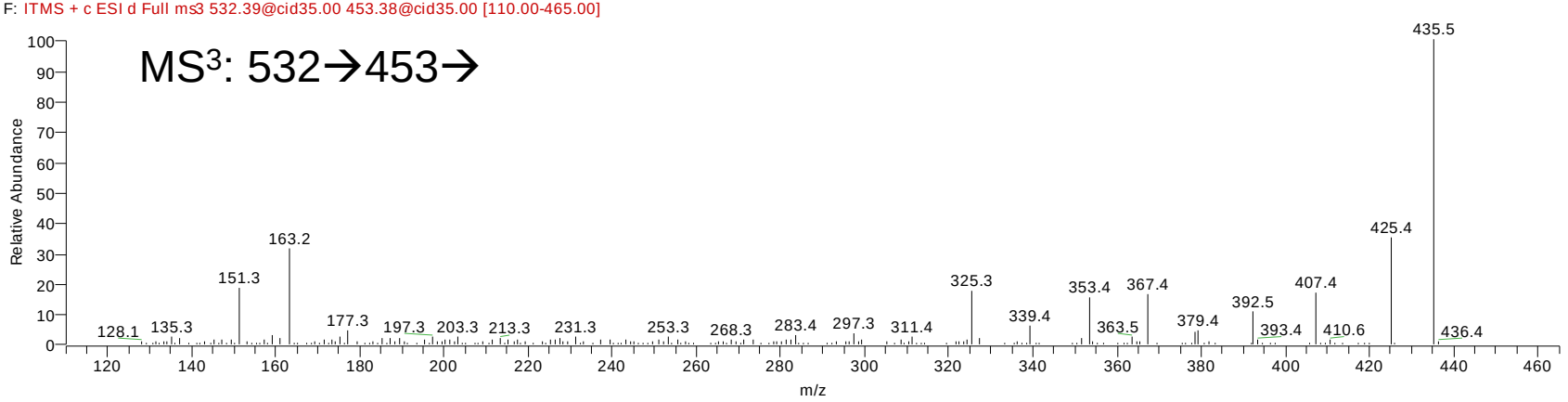


Fig. S4j C4-24S,25-epoxide-3-one 3-GP

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]

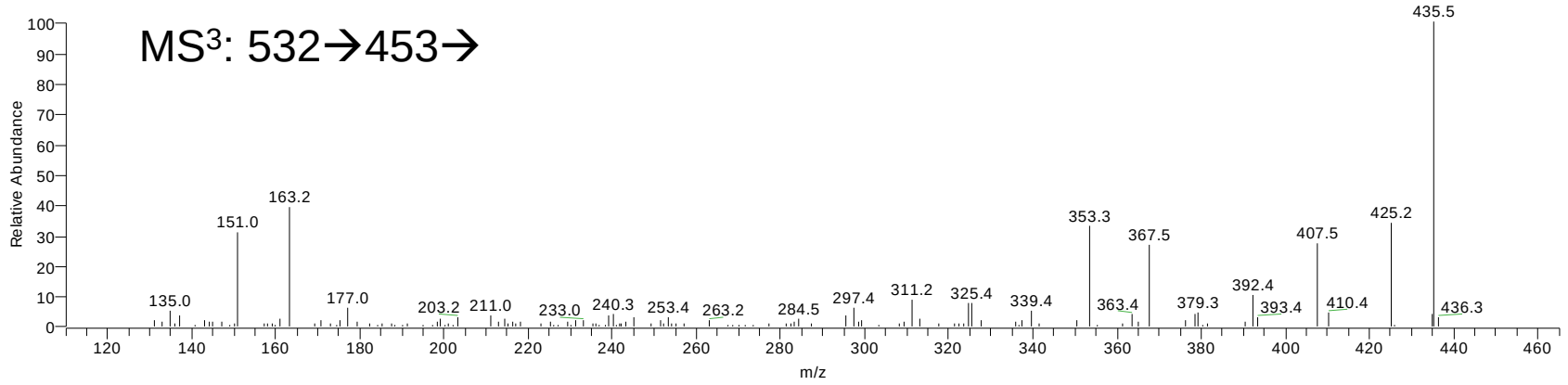
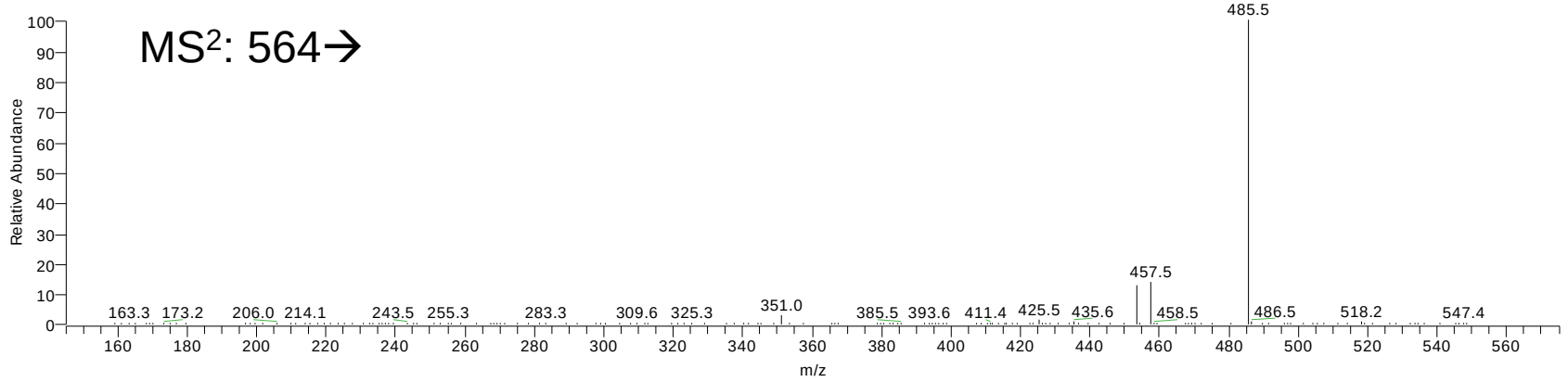


Fig. S4k C4-24-ol,25-OMe-3-one 3-GP

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]

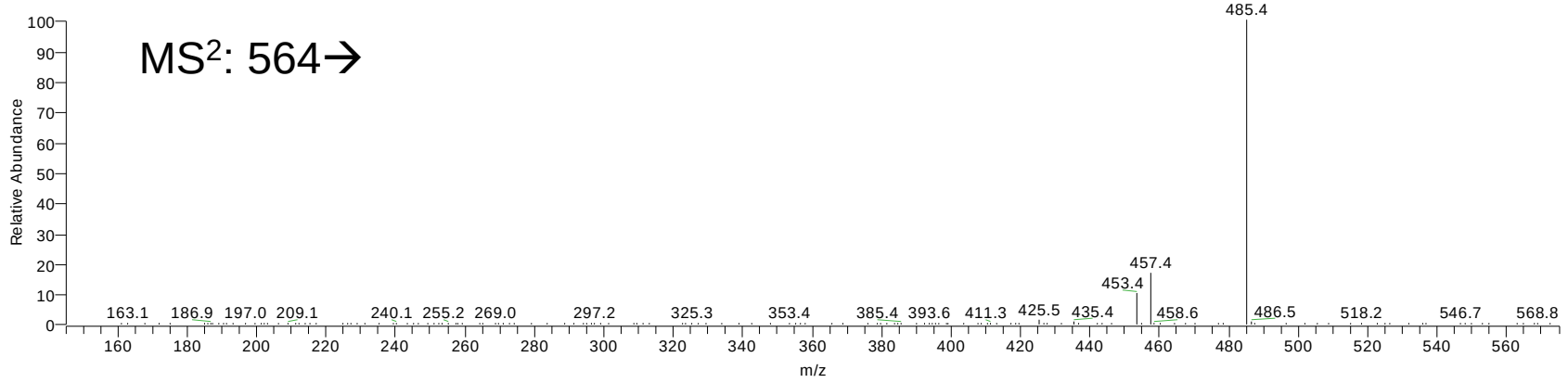


Fig. S4l C^4-24-ol,25-OMe-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

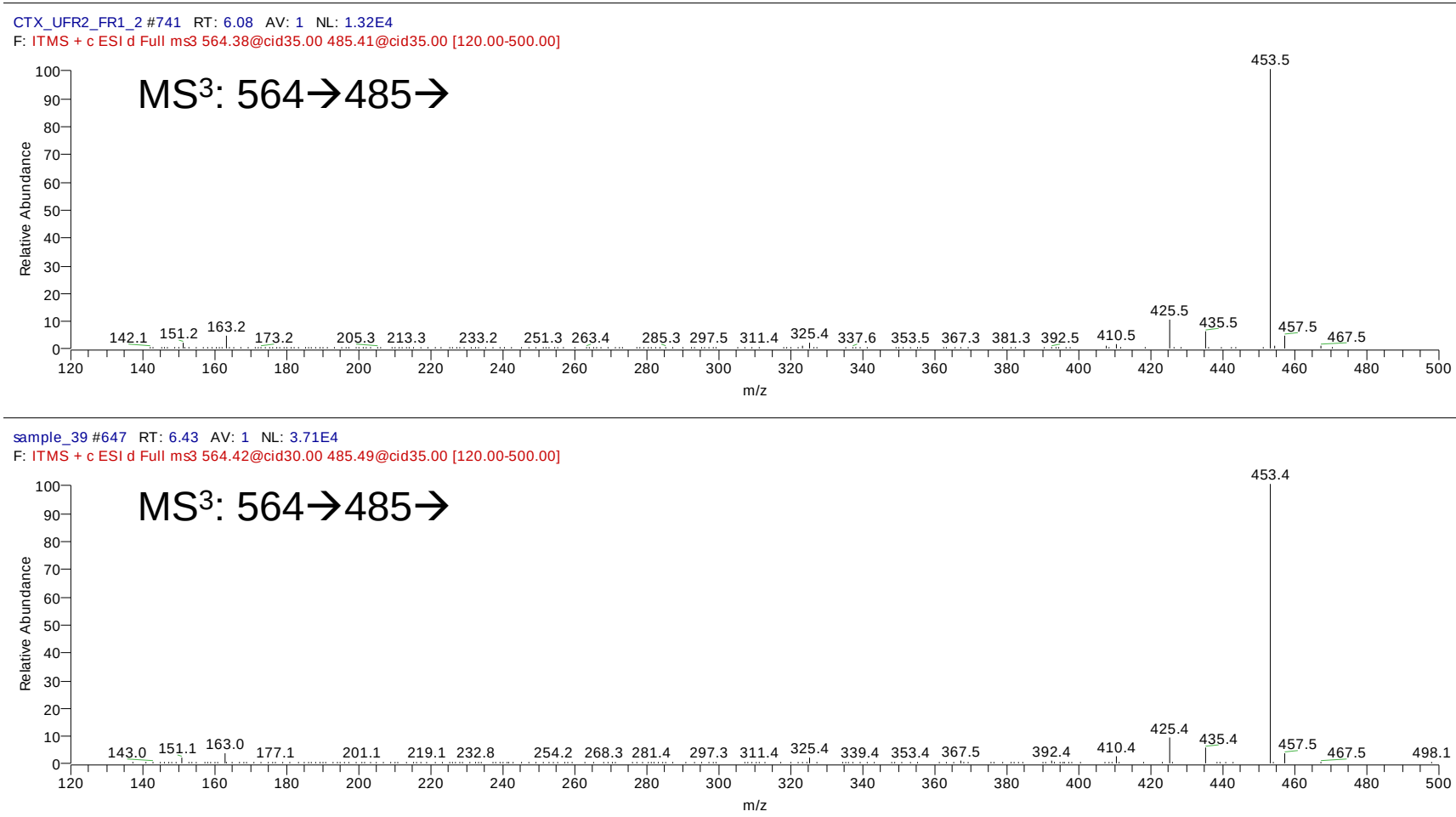
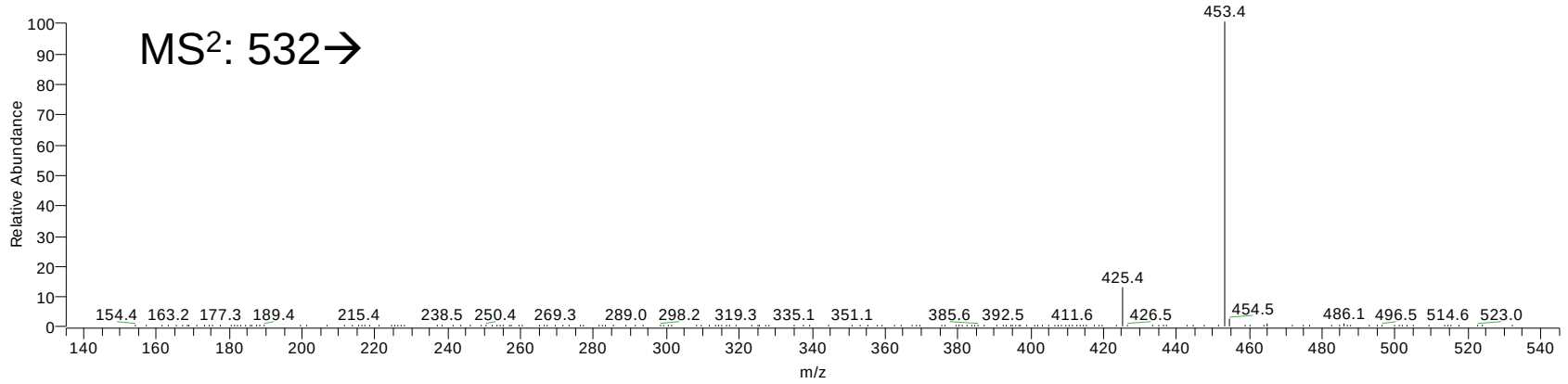


Fig. S4m C⁴-3,24-dione 3-GP

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]

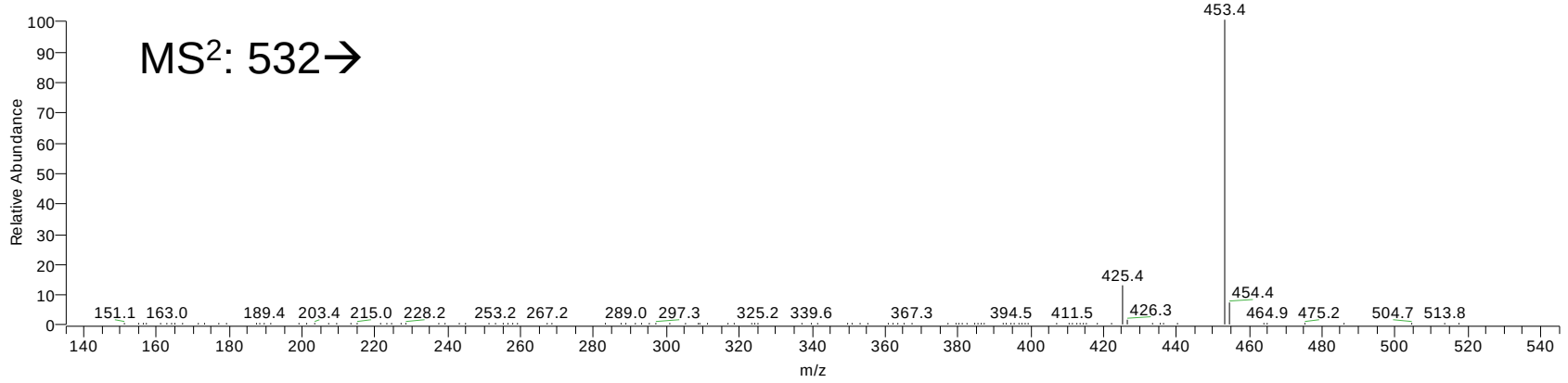
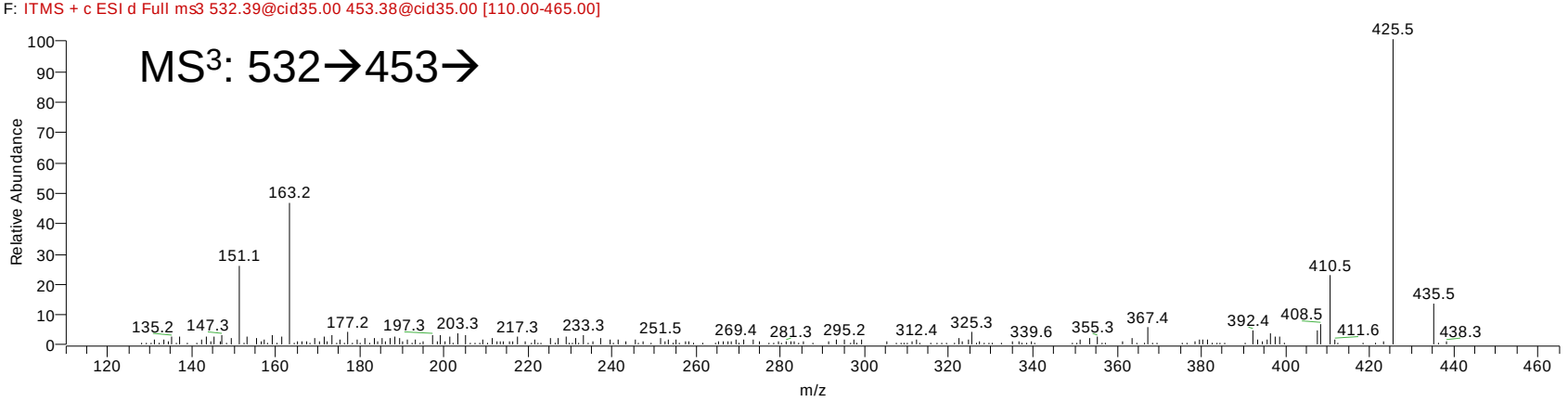


Fig. S4n C⁴-3,24-dione 3-GP

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]

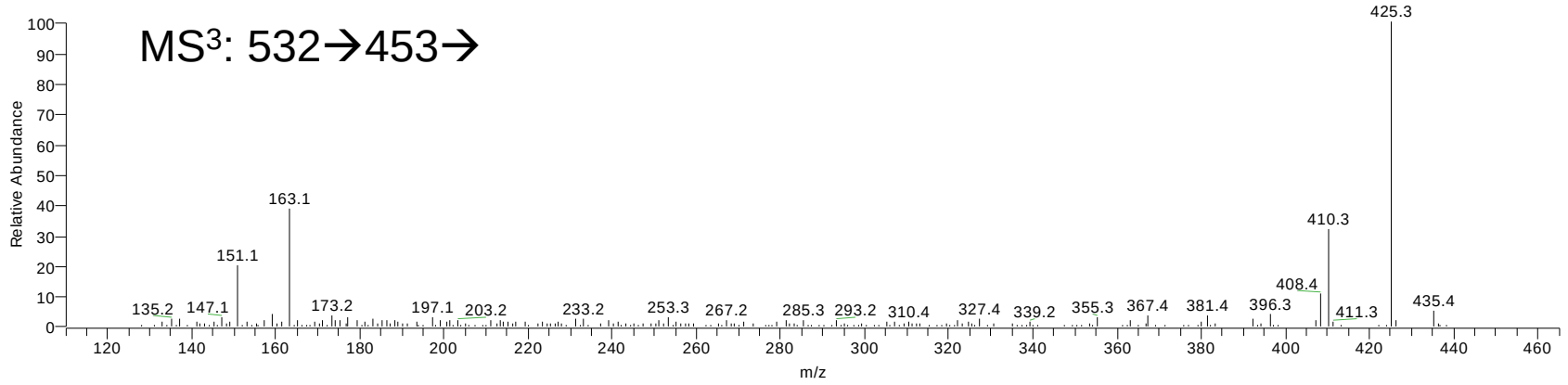
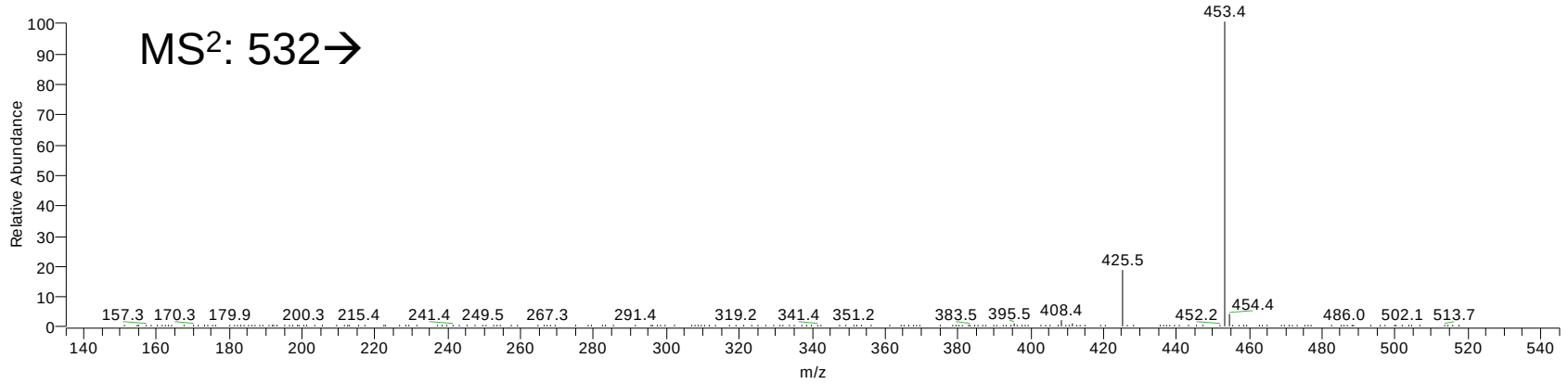


Fig. S4o C⁴-3,6-dione 3-GP

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]

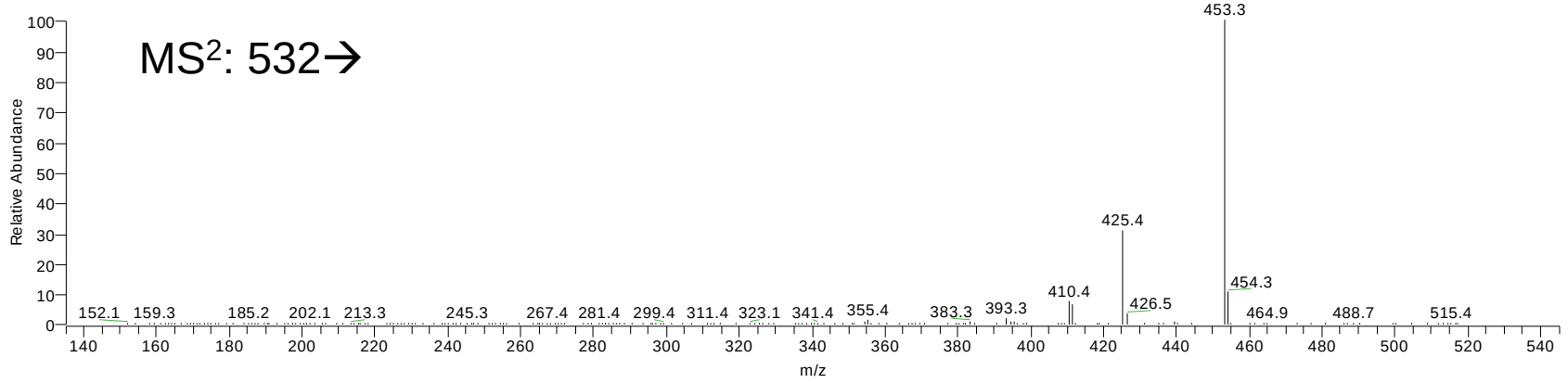
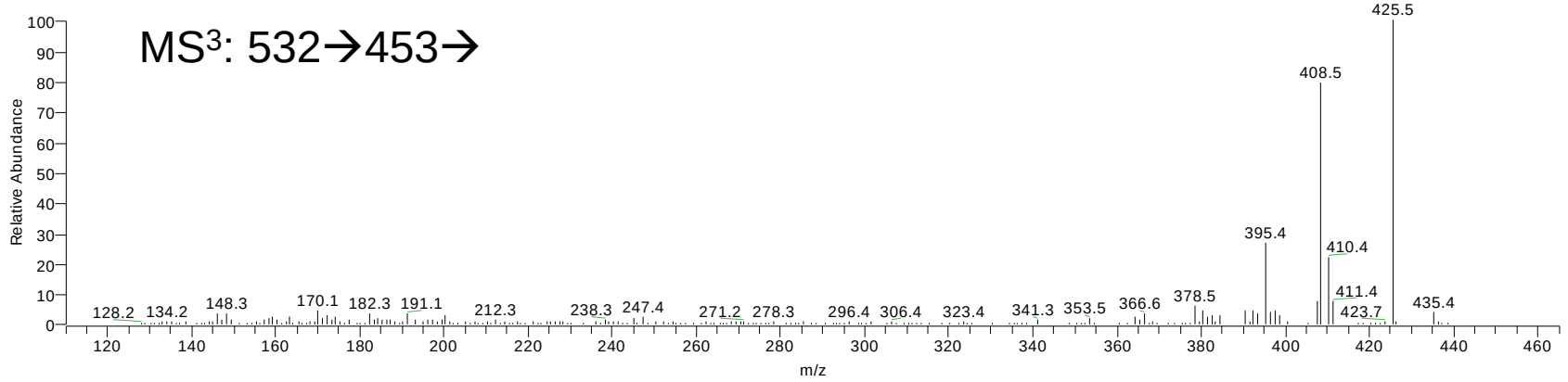


Fig. S4p C^4-3,6-dione 3-GP

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]

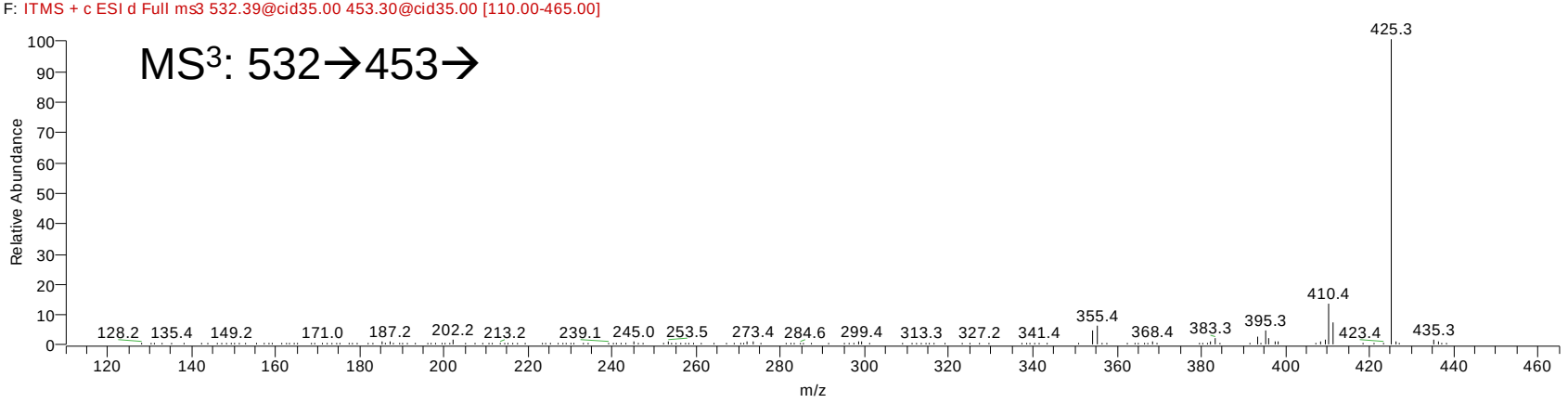
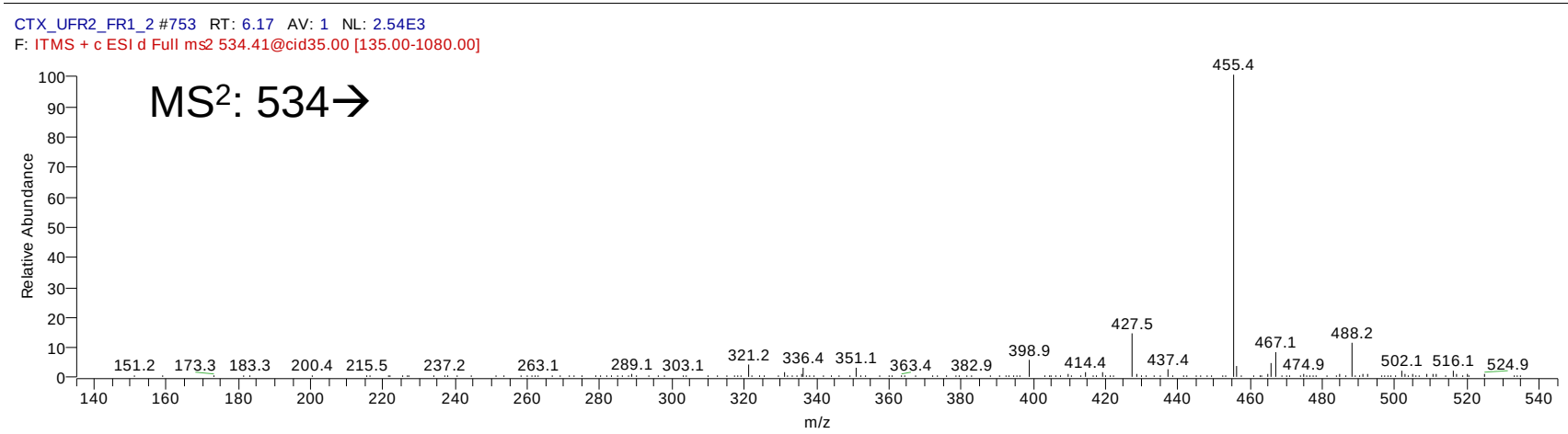


Fig. S4q C^4-22R-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40



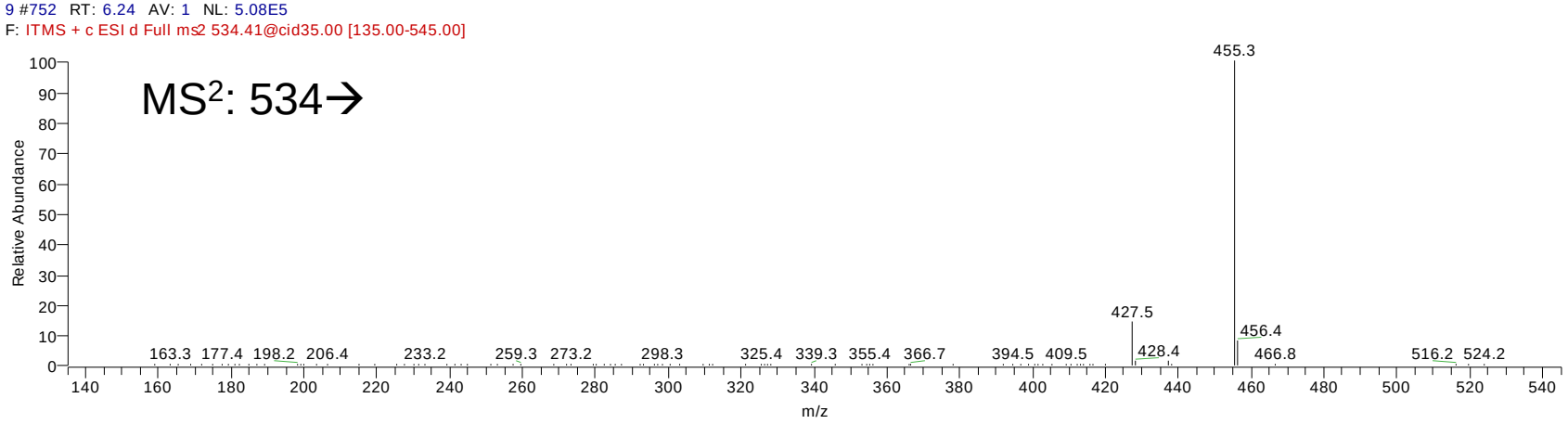
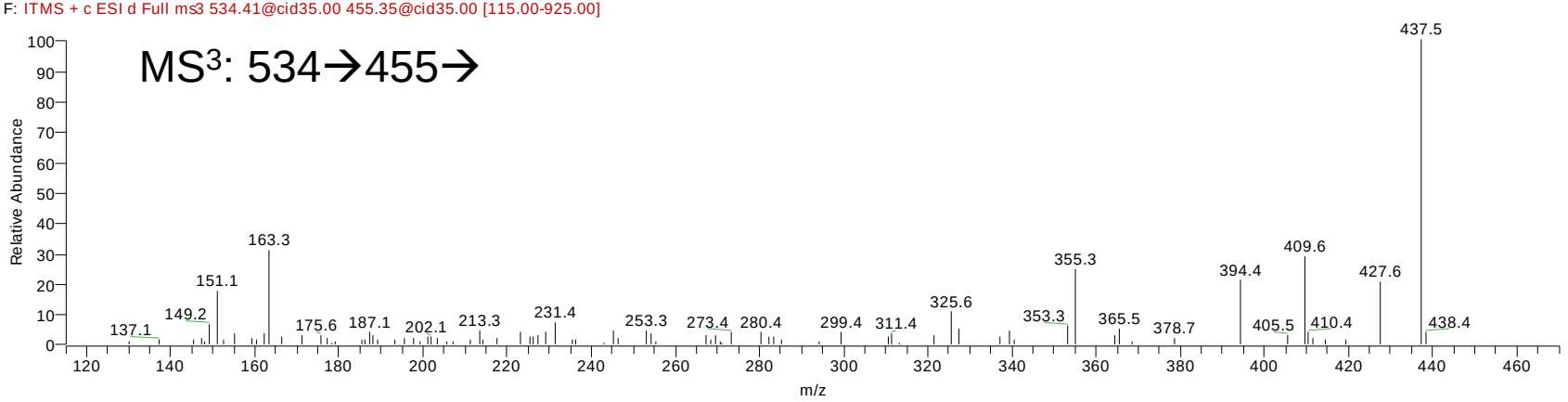


Fig. S4r C^4-22R-ol-3-one 3-GP

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]

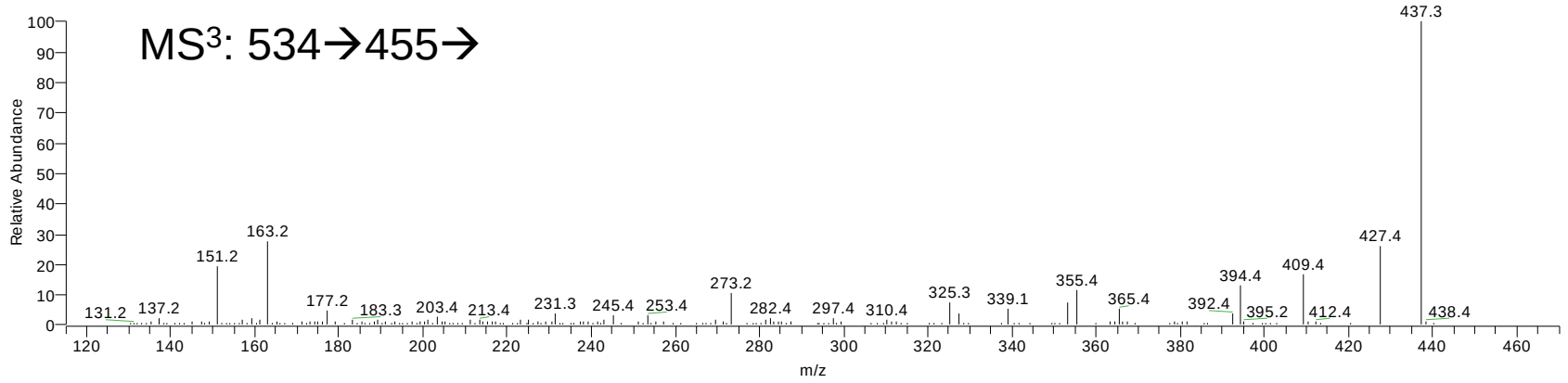


Fig. S4s C4-24S-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

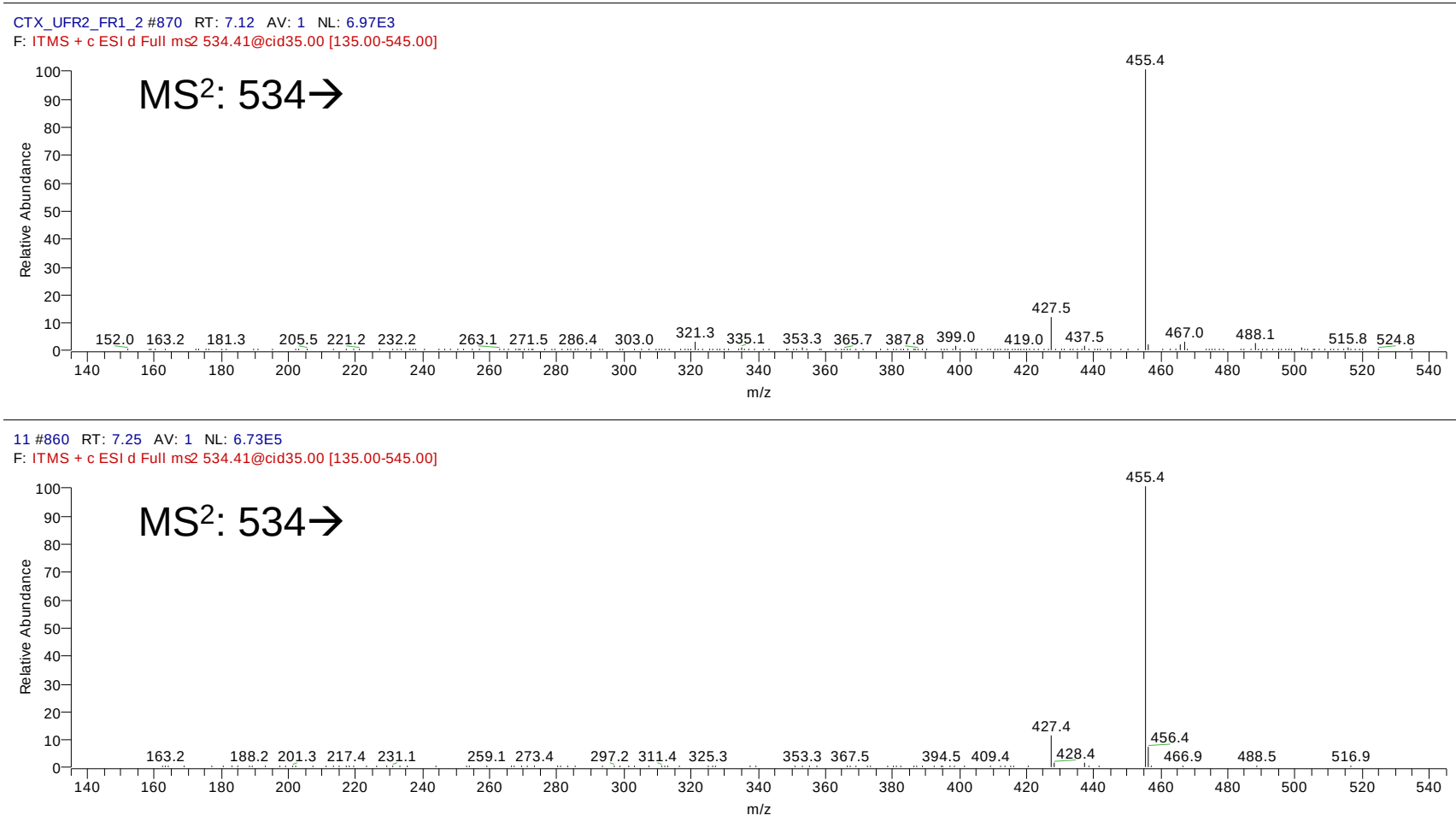
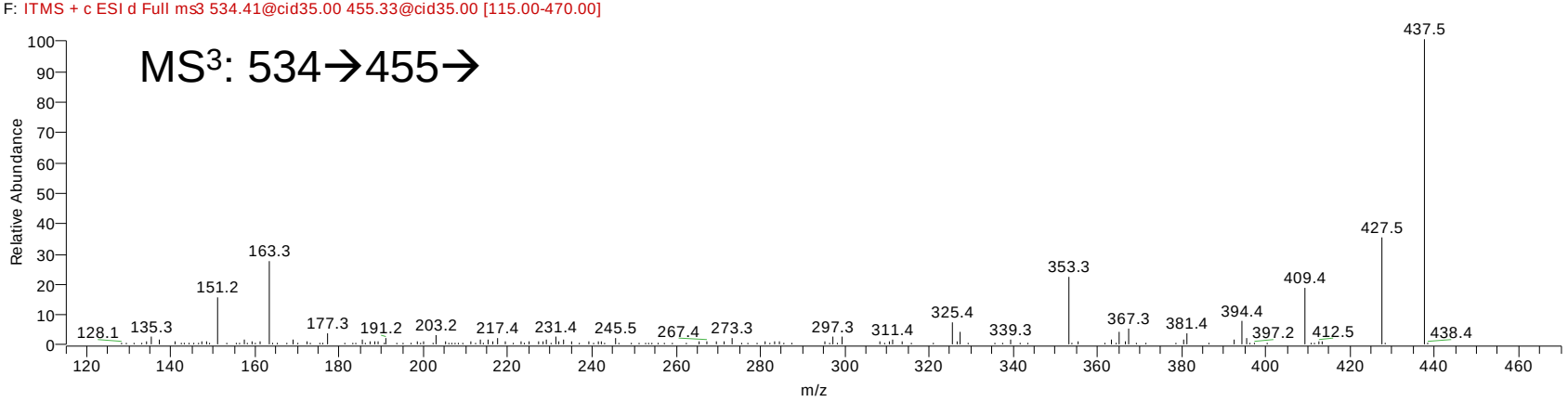


Fig. S4t C^4-24S-ol-3-one 3-GP

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]

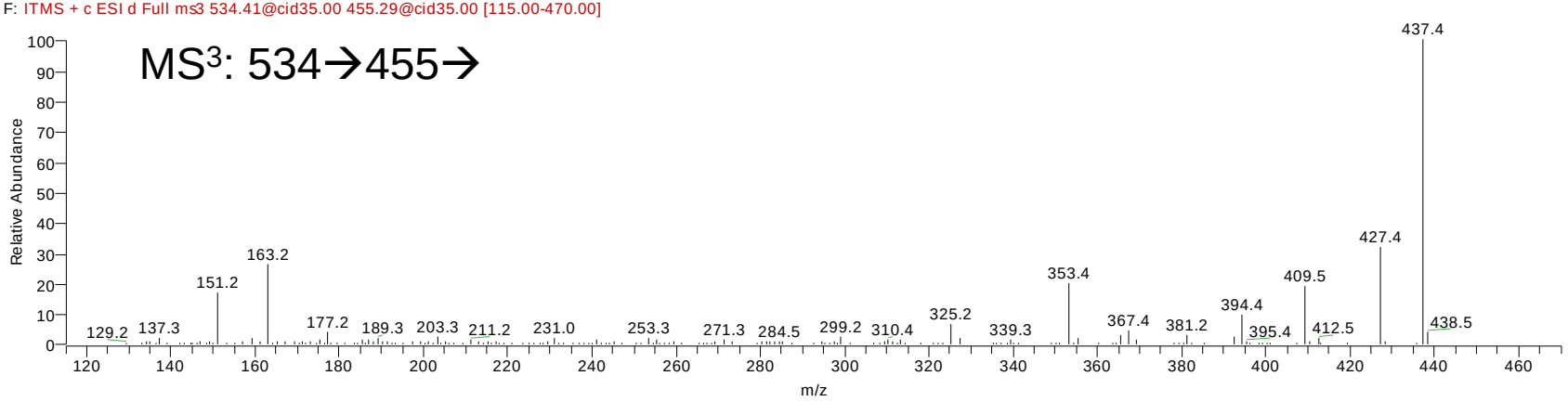


Fig. S4u C^4-25-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

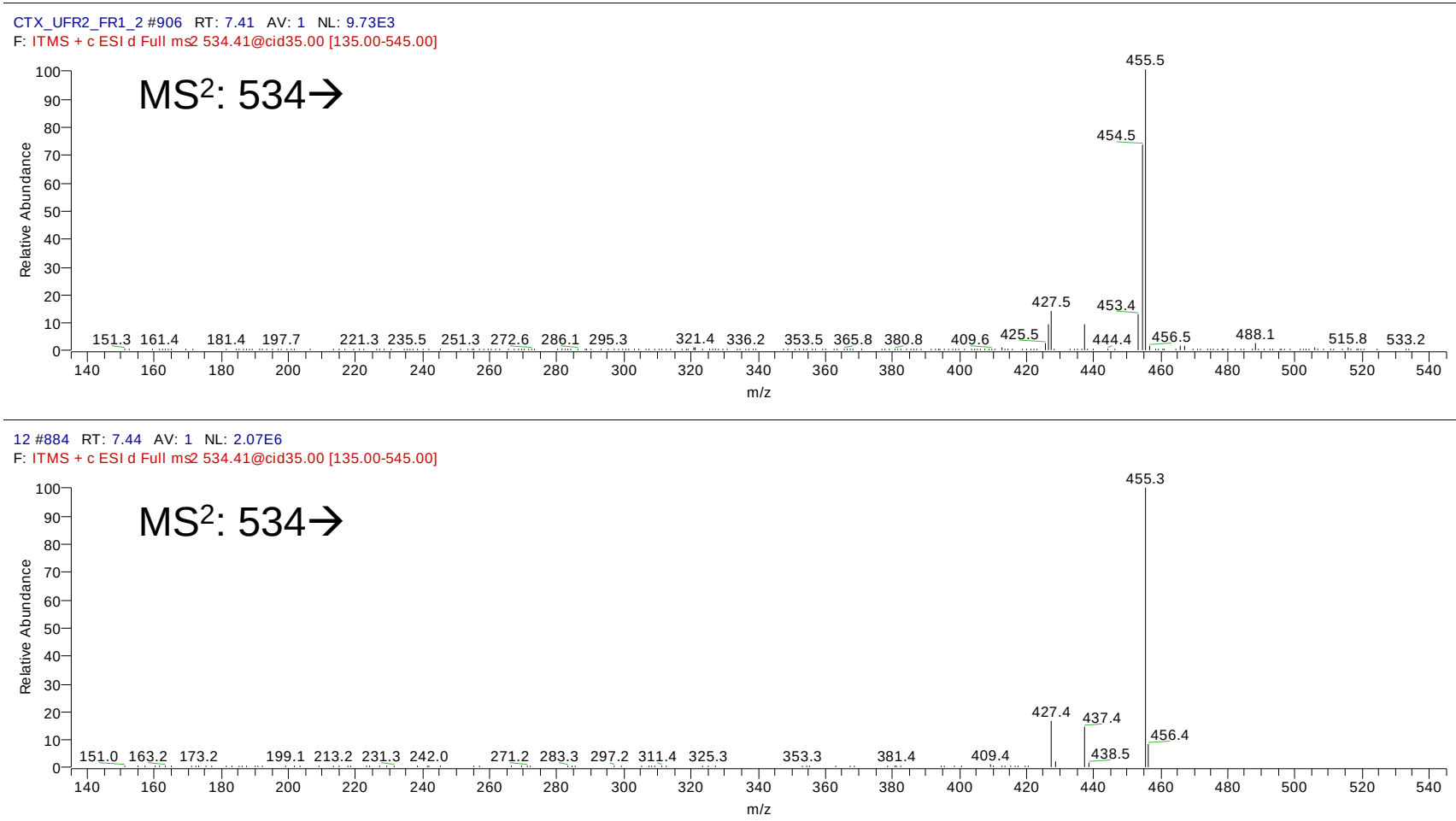
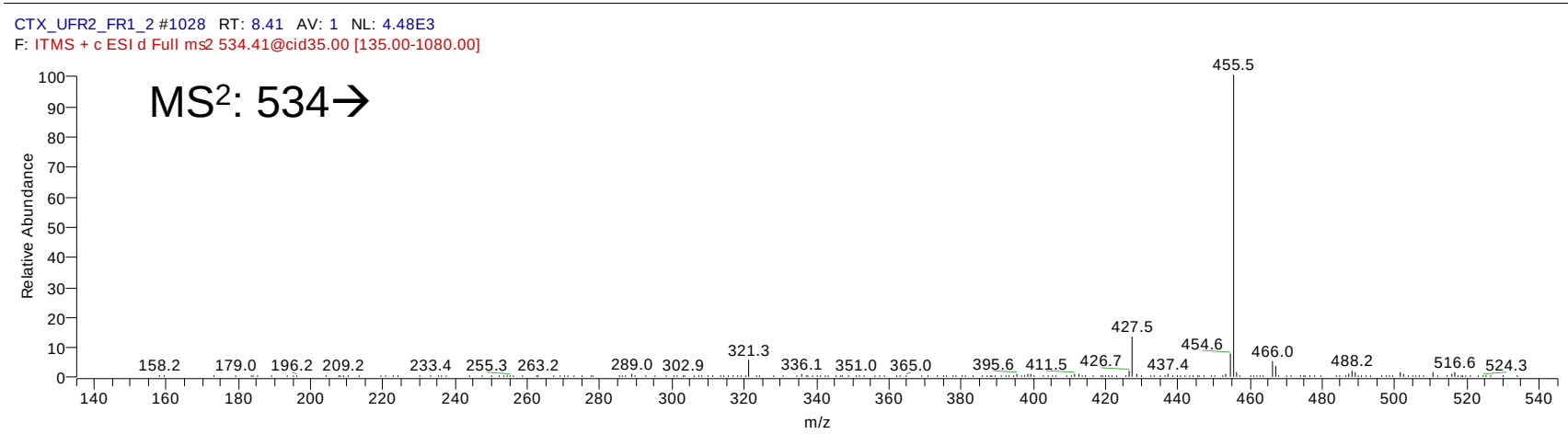


Fig. S4v C^4-25-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

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

CTX_UFR2_FR1_2 30/06/2006 18:49:40



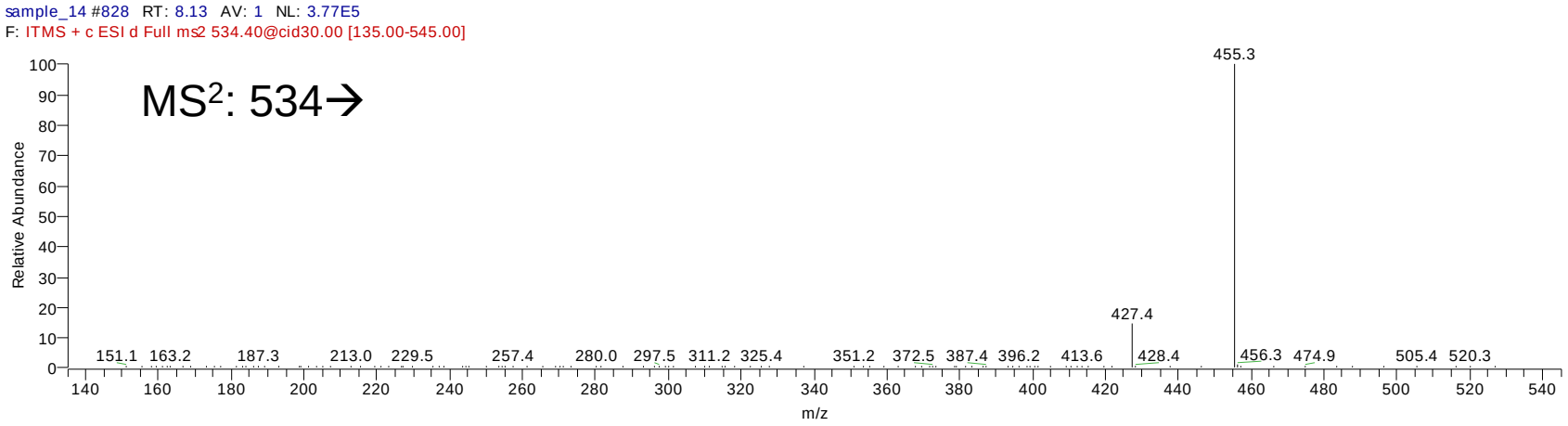
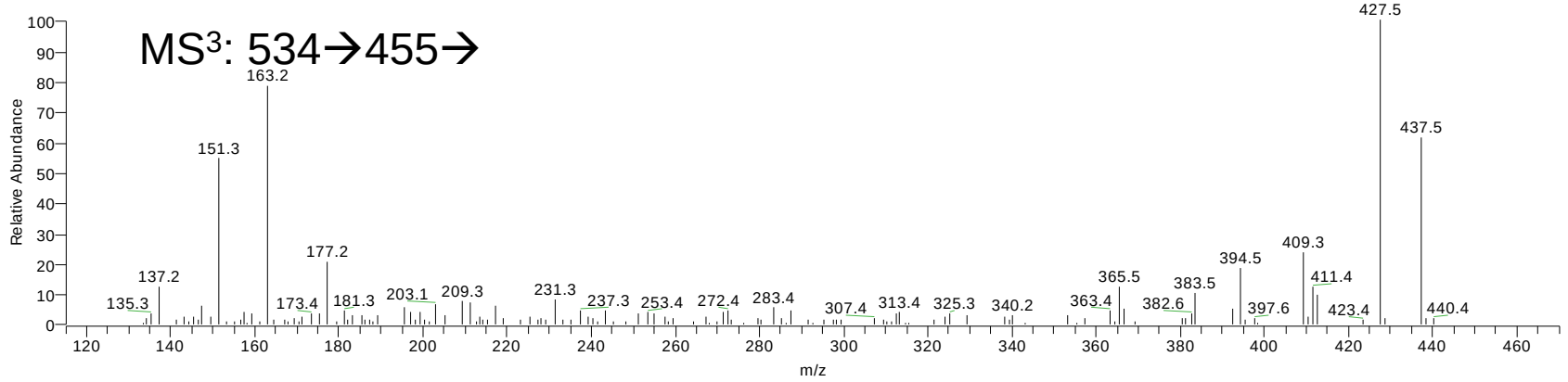


Fig. S4x C^4-27-ol-3-one 3-GP

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]

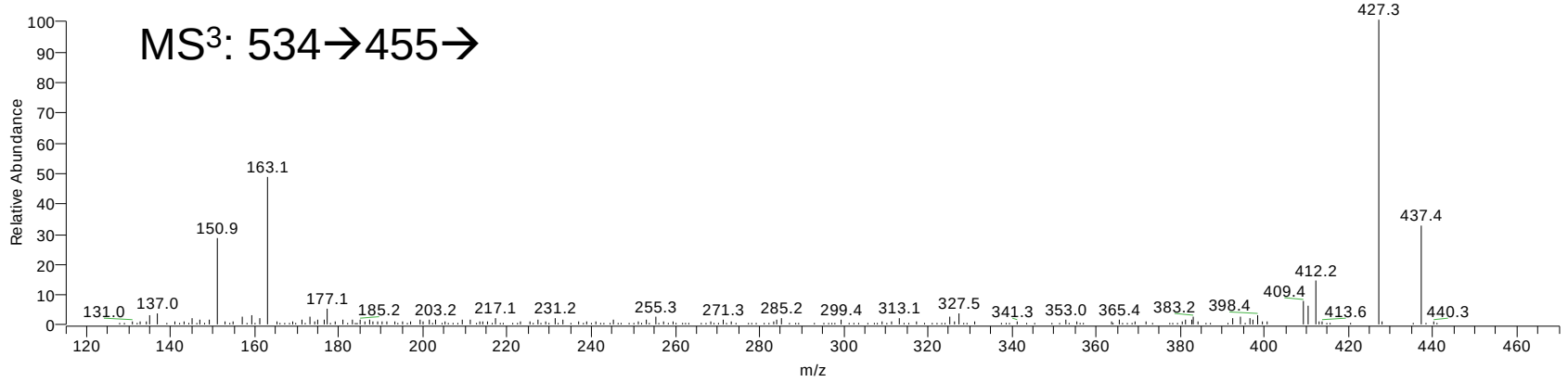


Fig. S4y C^4-7β-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

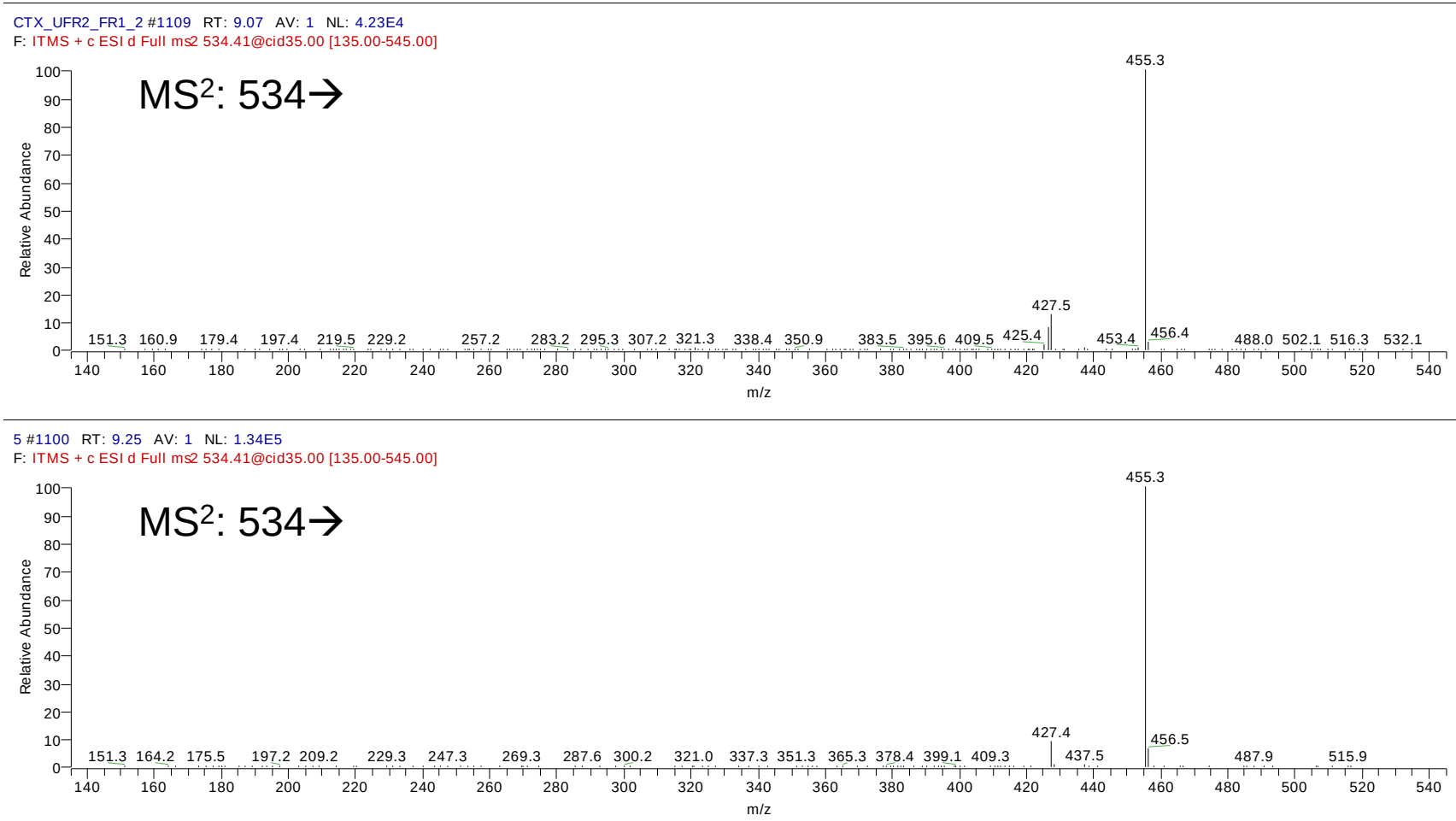
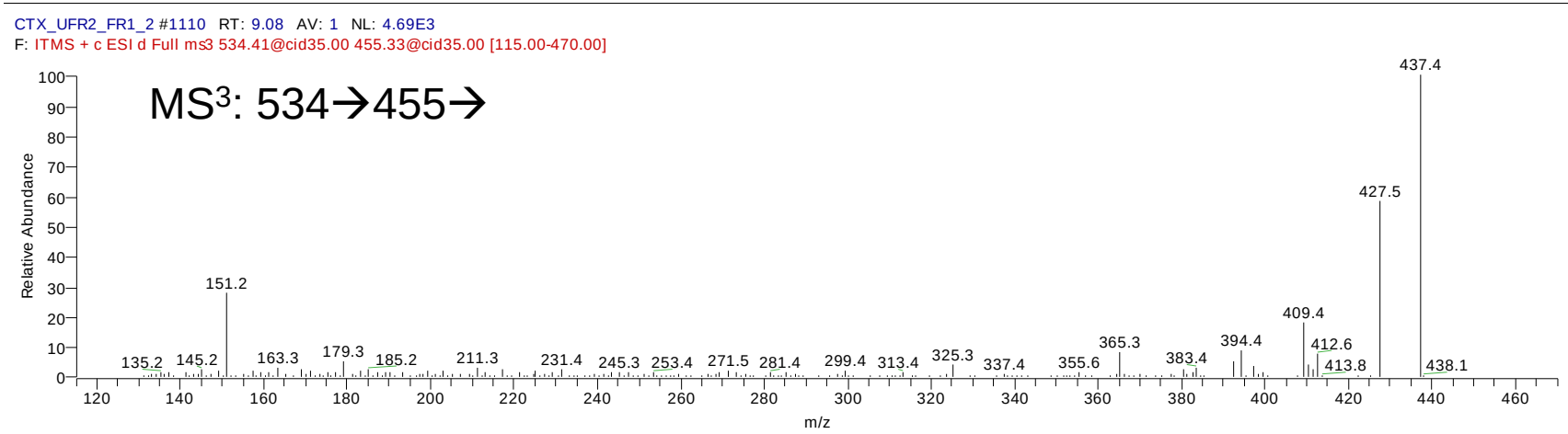


Fig. S4z C^4-7β-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40



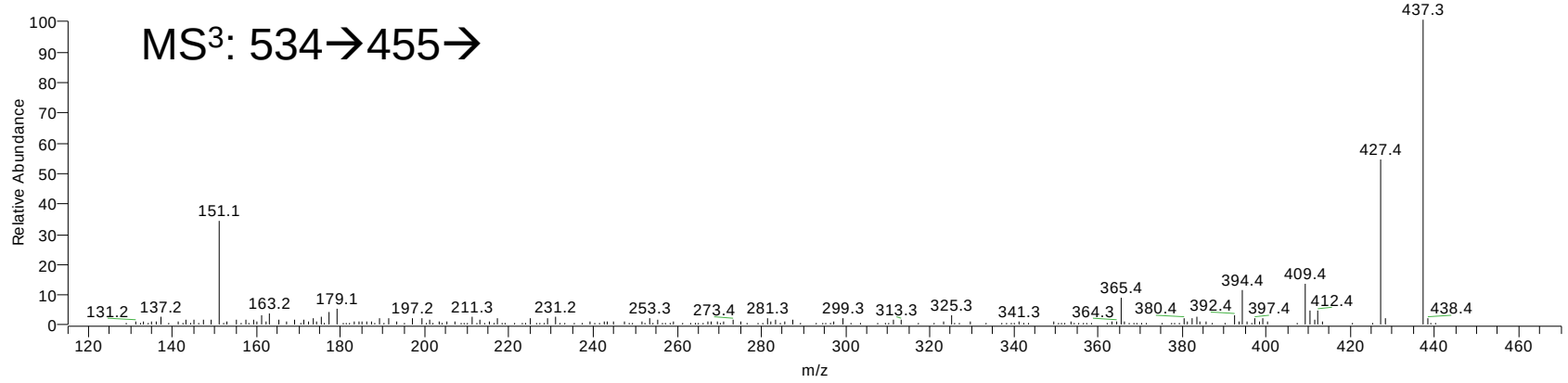


Fig. S4aa C^4-7α-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

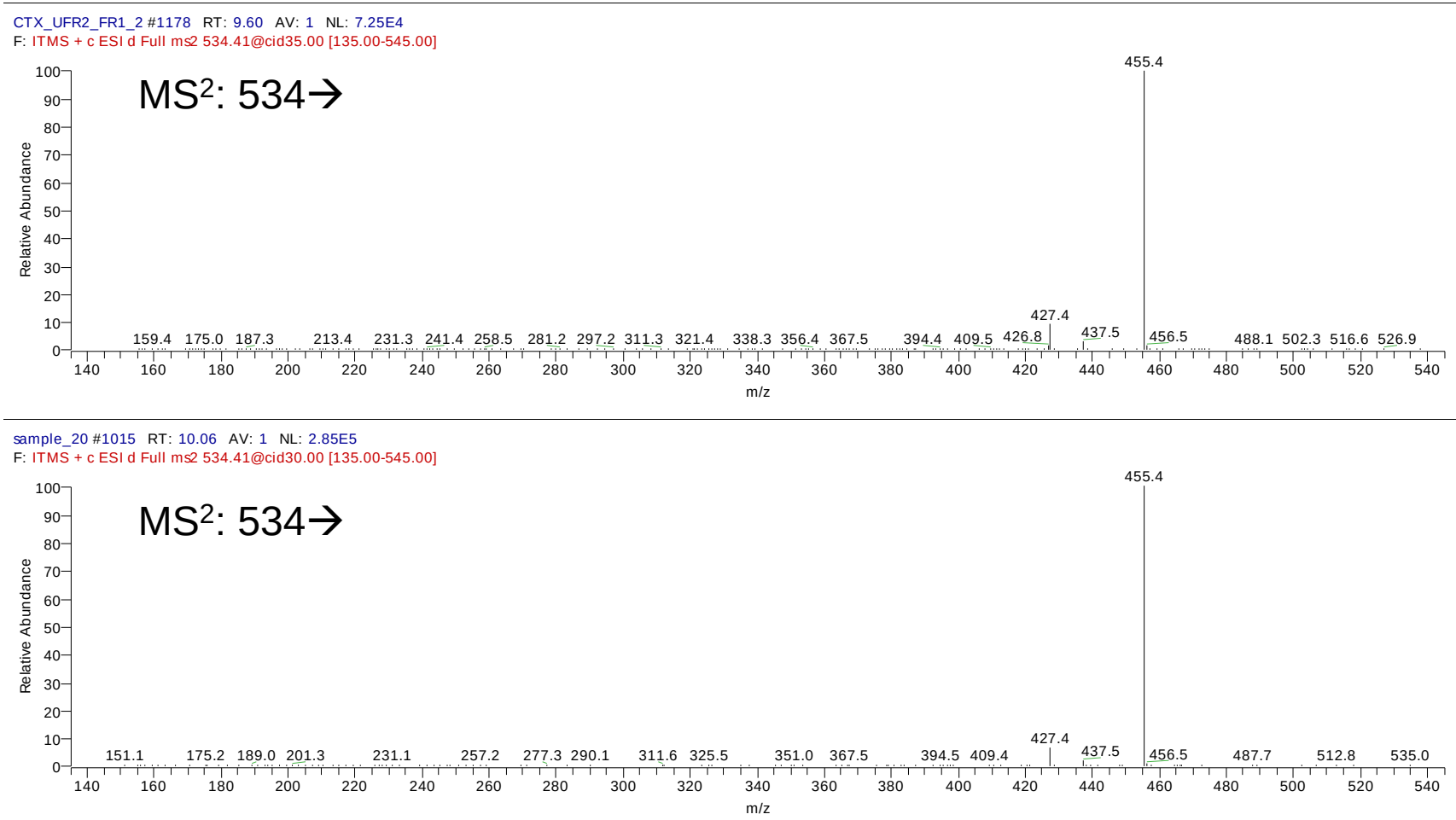
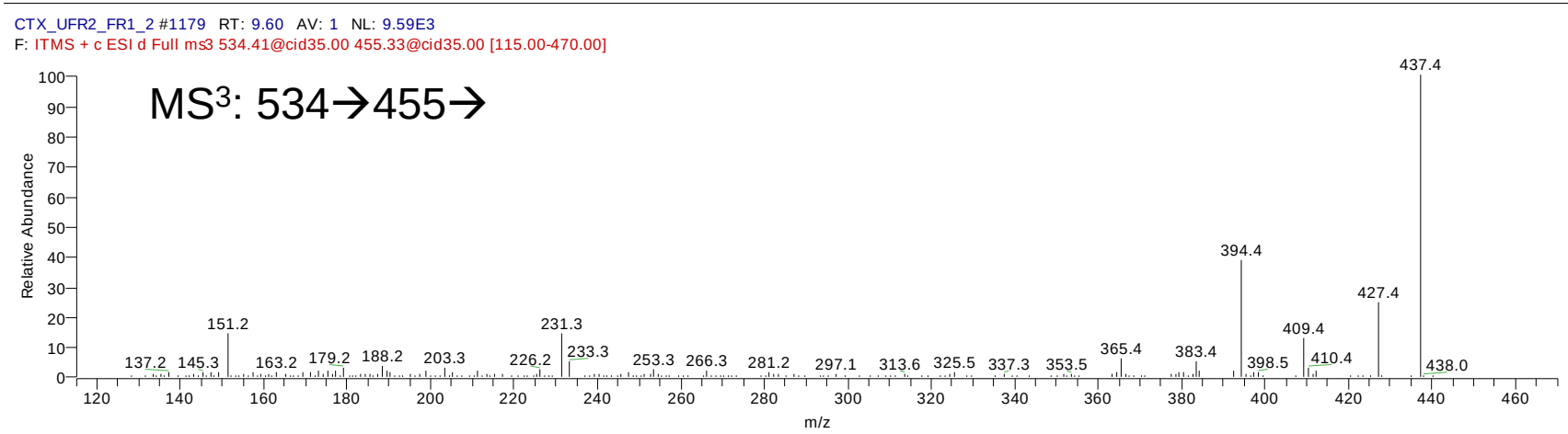


Fig. S4ab C^4-7α-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40



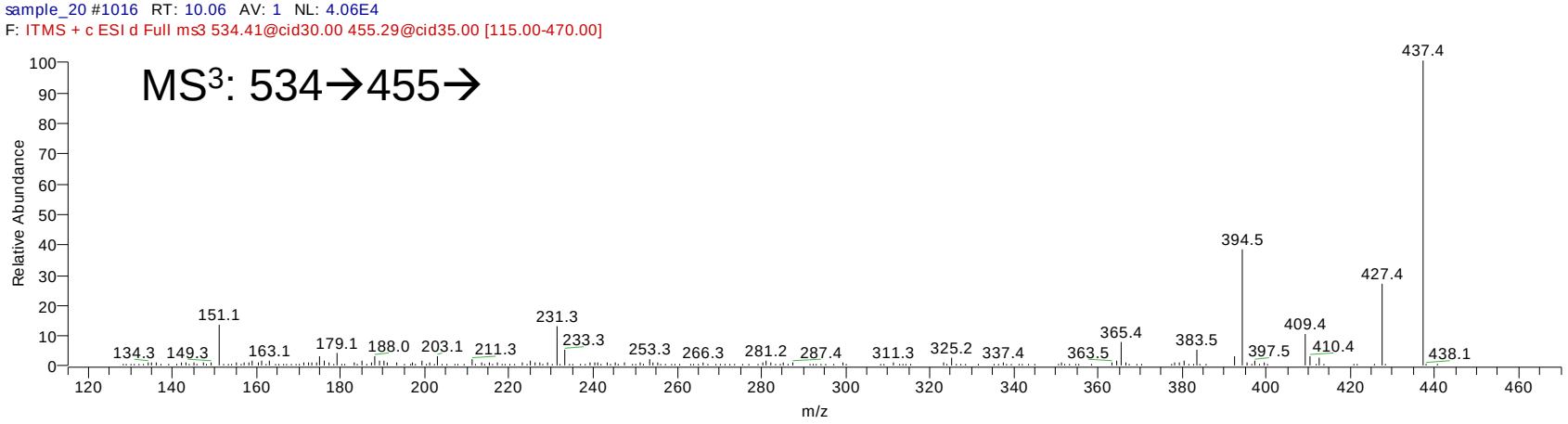


Fig. S4ac C^7-x-ol-3-one 3-GP

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]

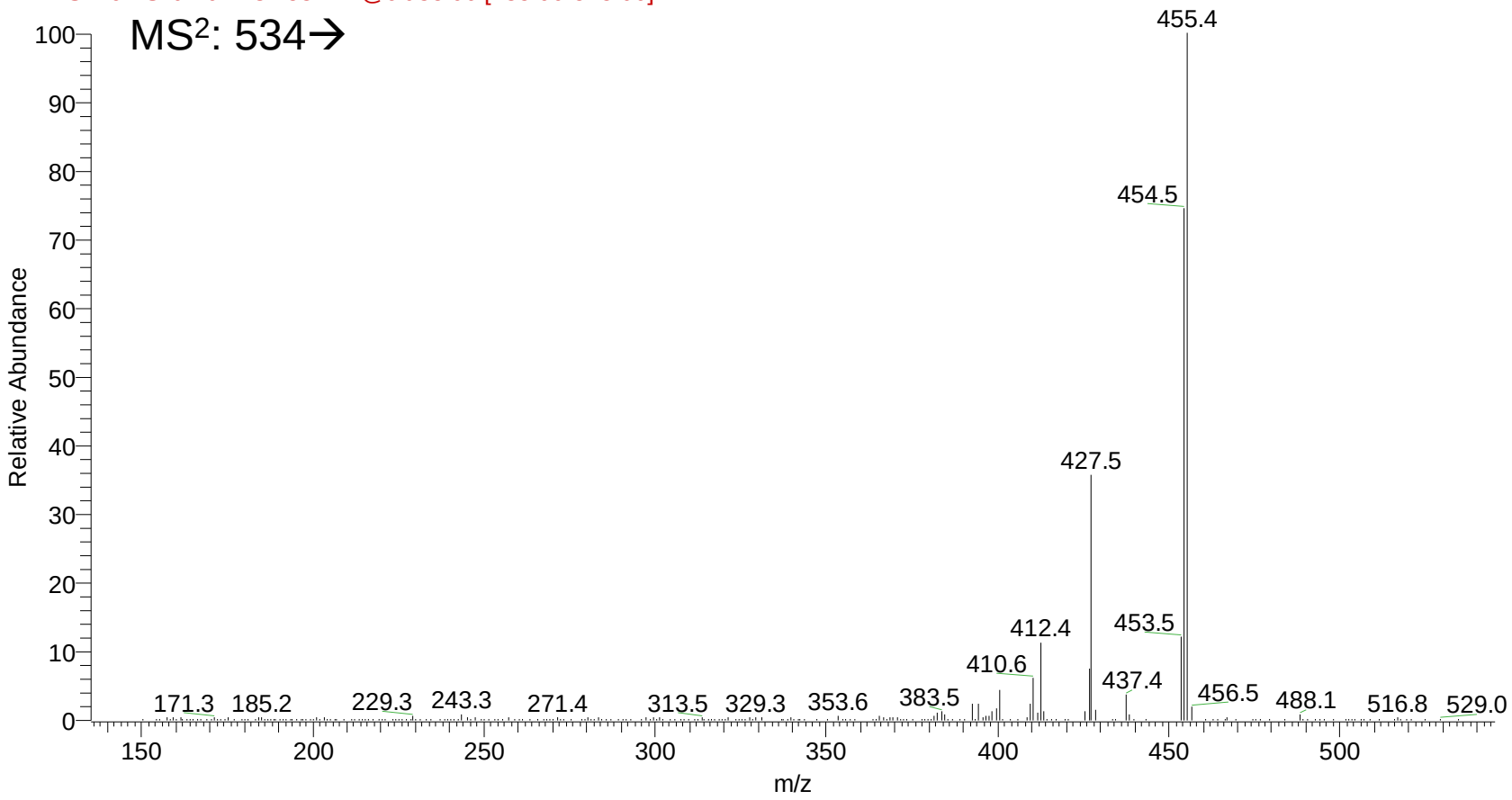


Fig. S4ad C^7-x-ol-3-one 3-GP

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]

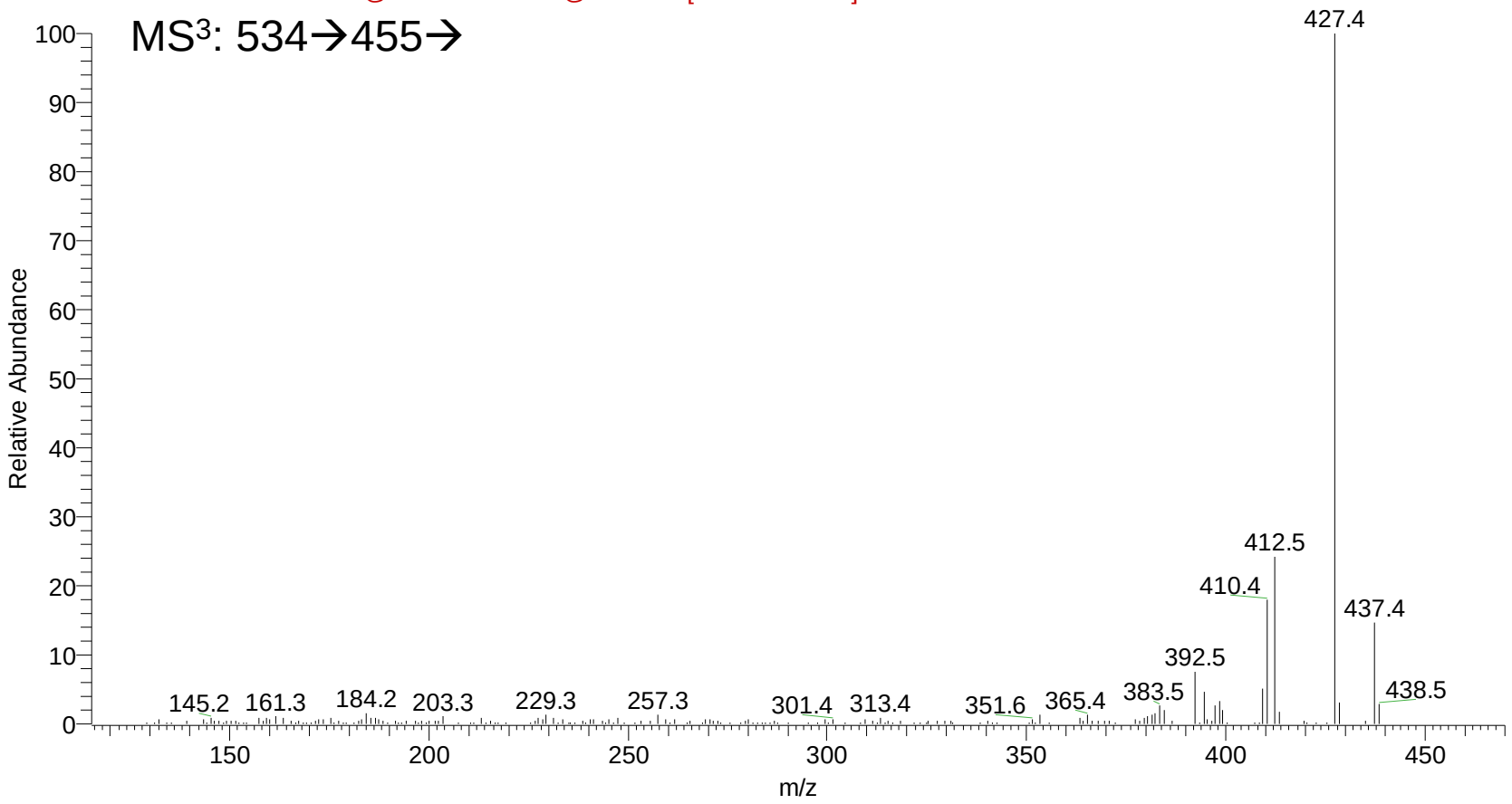


Fig. S4ae C^4-6β-ol-3-one 3-GP

CTX_UFR2_FR1_2 30/06/2006 18:49:40

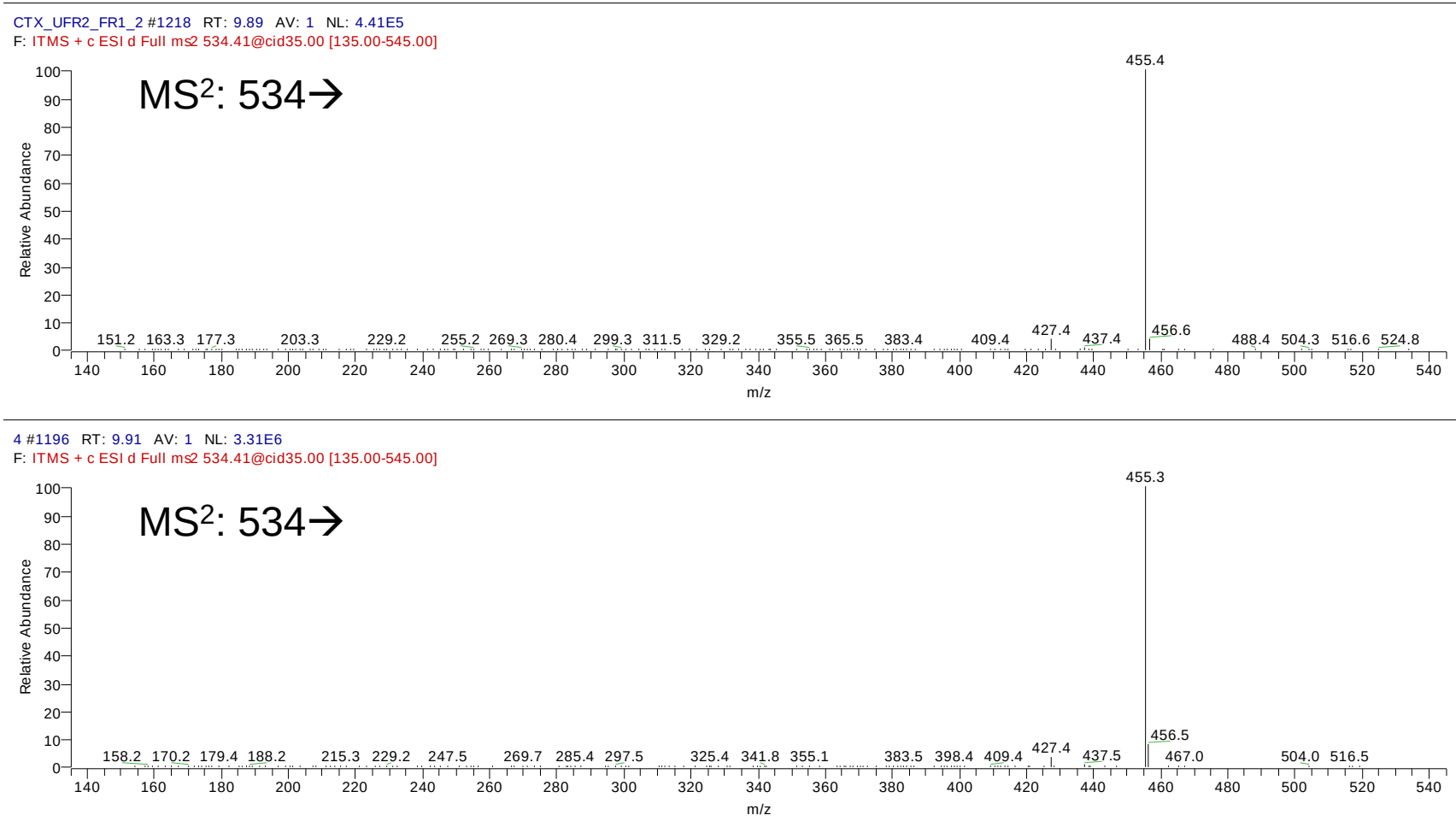
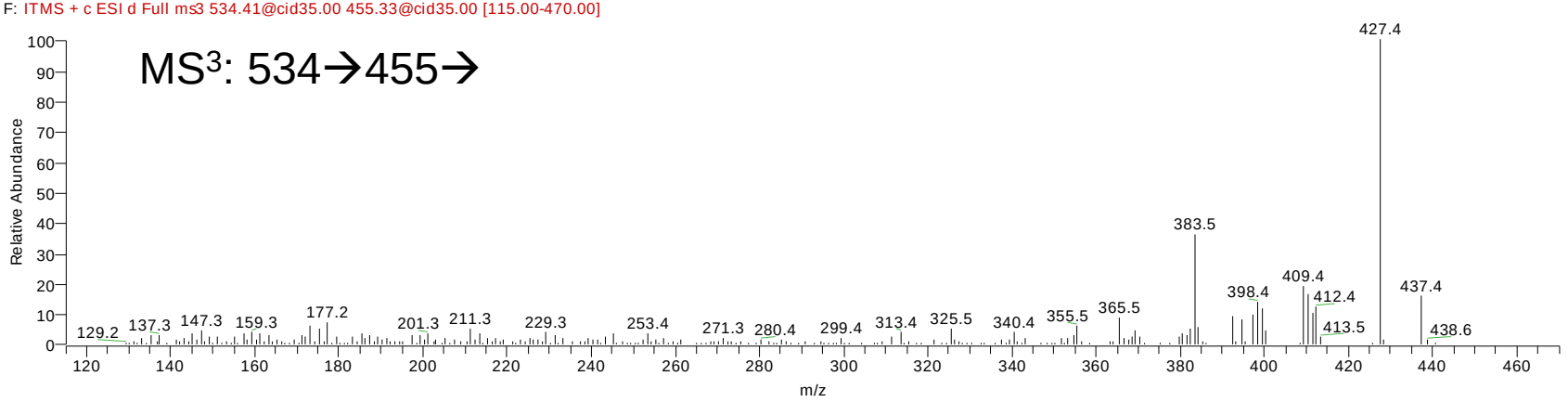


Fig. S4af C^4-6β-ol-3-one 3-GP

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]

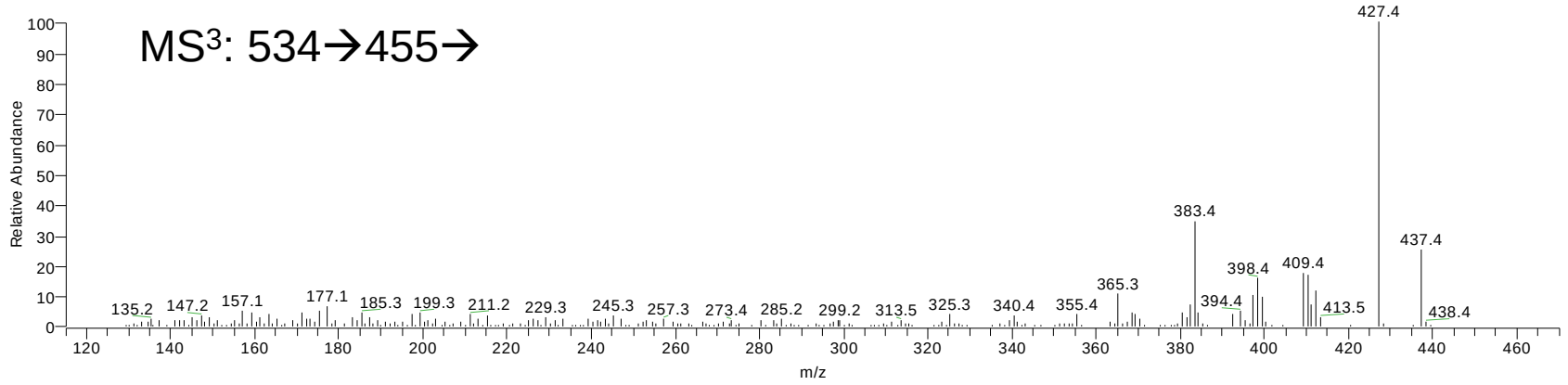


Fig. S4ag $C^{X,Y}$-x,y-diol-z-one z-GP

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]

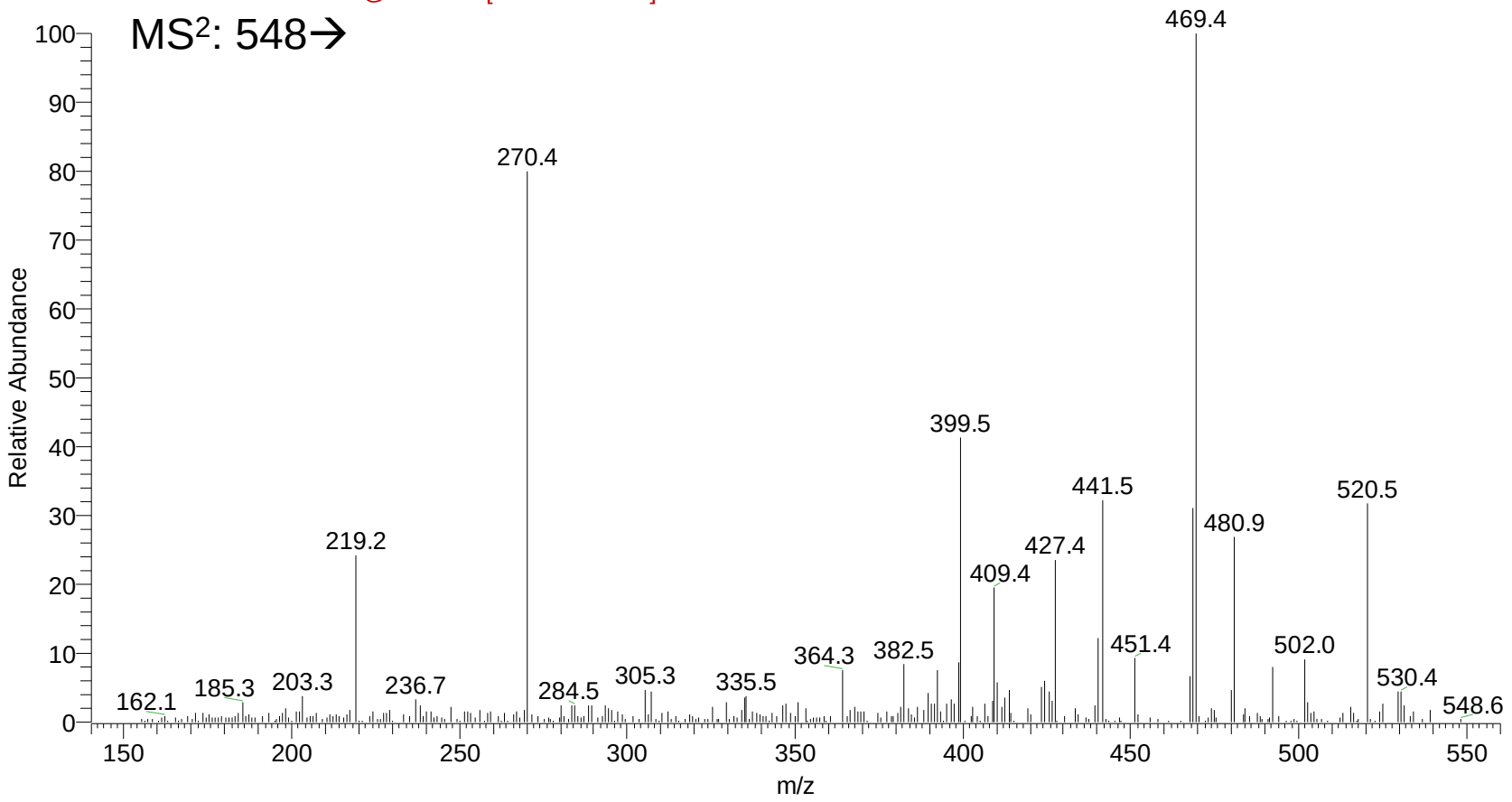


Fig. S4ah $C^{X,Y}$-x,y-diol-z-one z-GP

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]

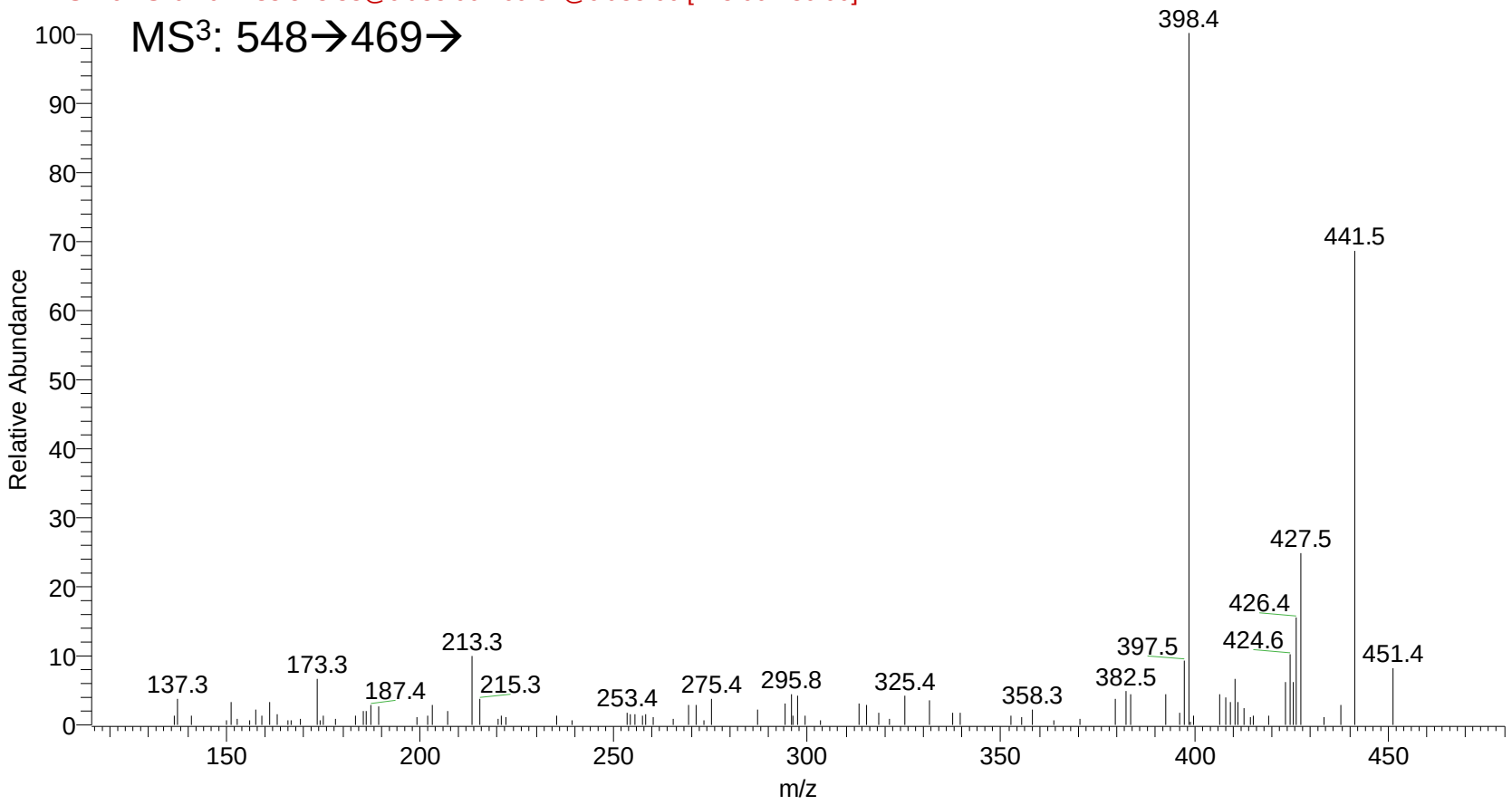


Fig. S4ai $C^{X,Y}$-x,y-diol-z-one z-GP

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]

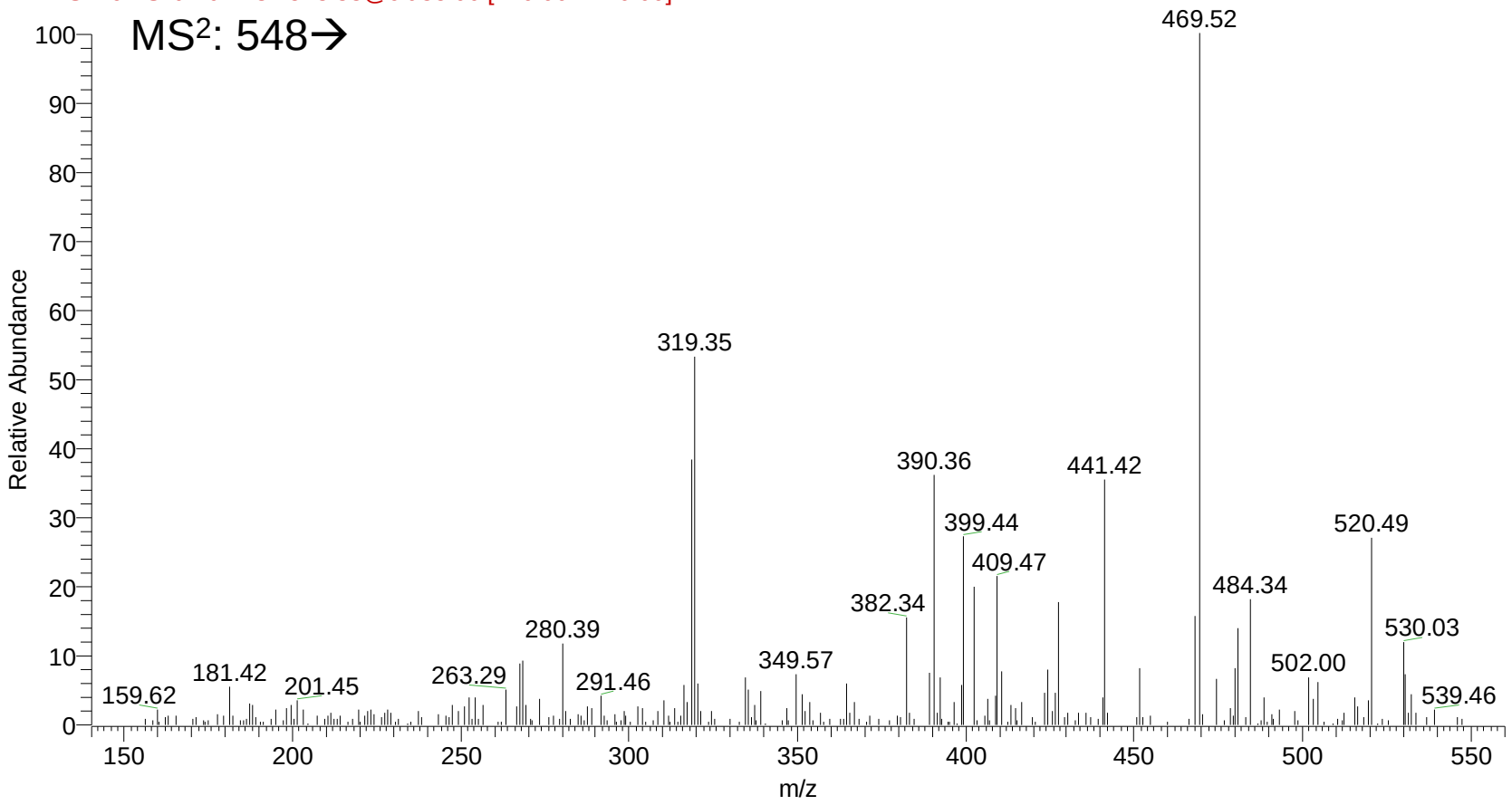


Fig. S4aj $C^{X,Y}$-x,y-diol-z-one z-GP

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]

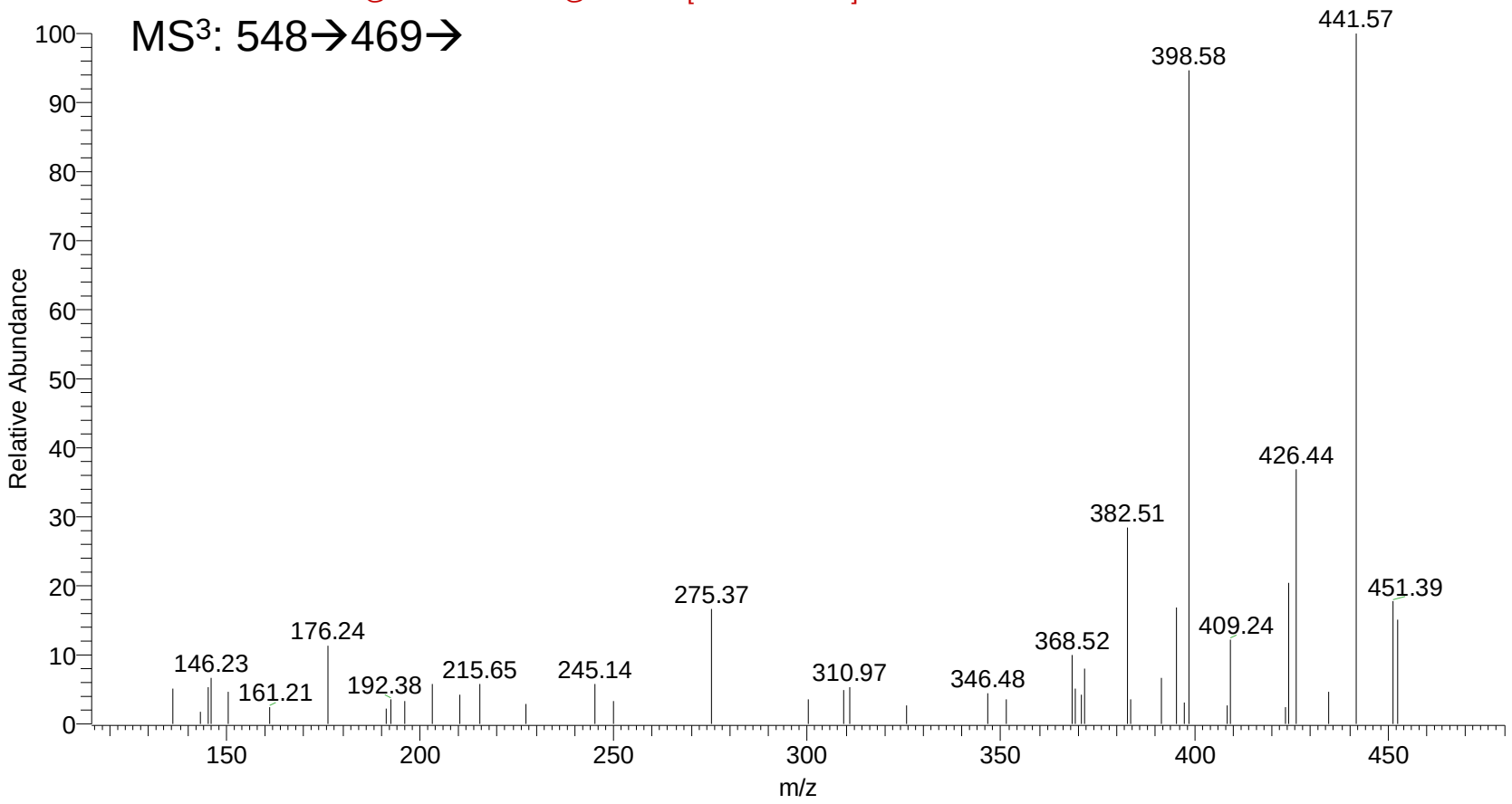
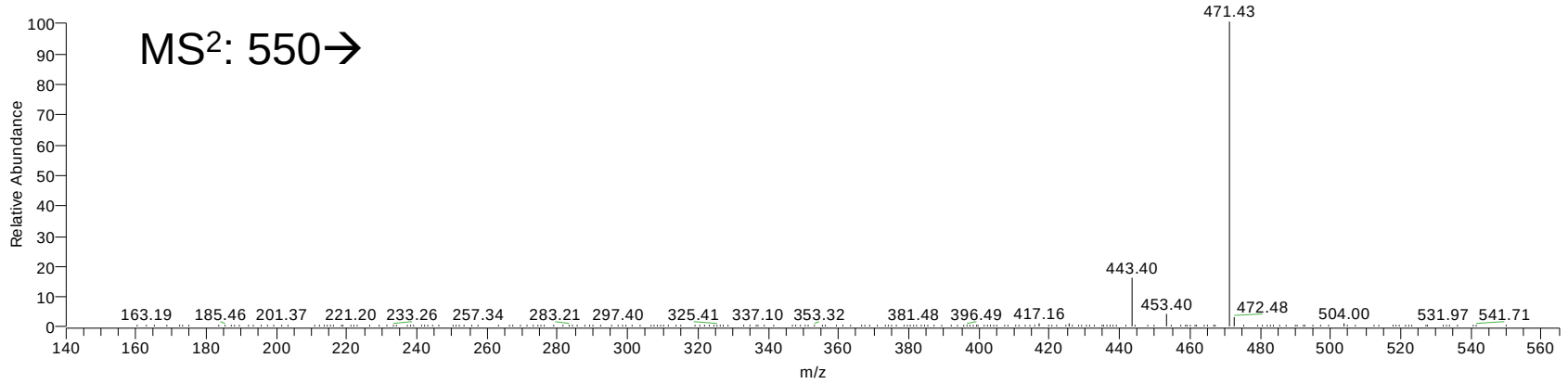


Fig. S4ak C⁴-24,25-diol-3-one 3-GP

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]

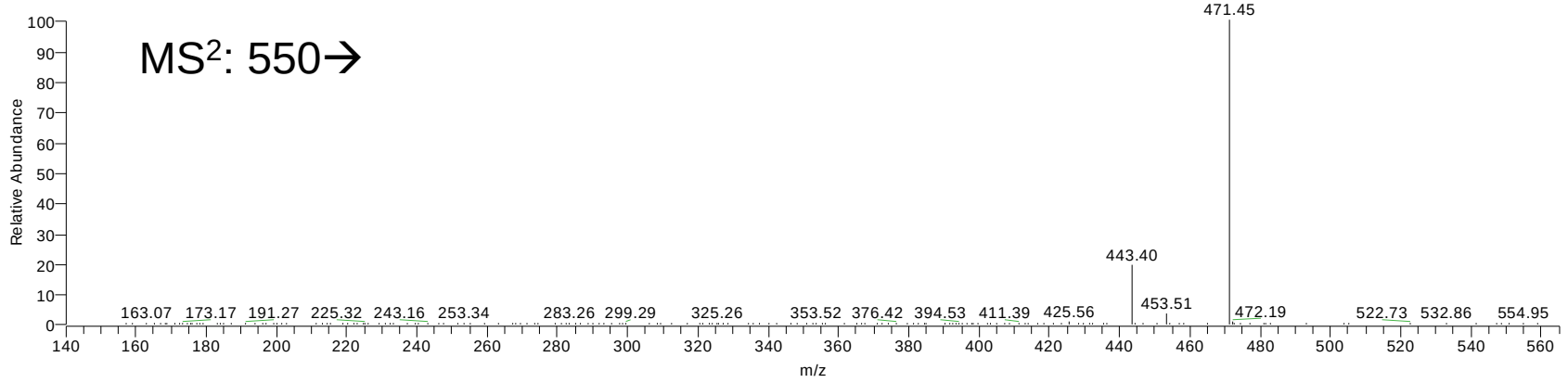
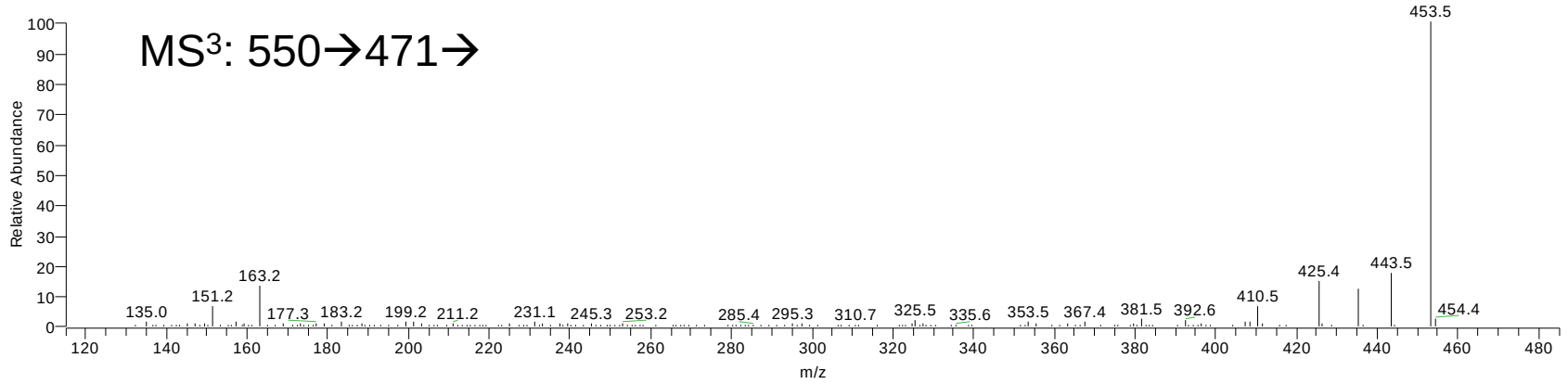


Fig. S4al C⁴-24,25-diol-3-one 3-GP

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]

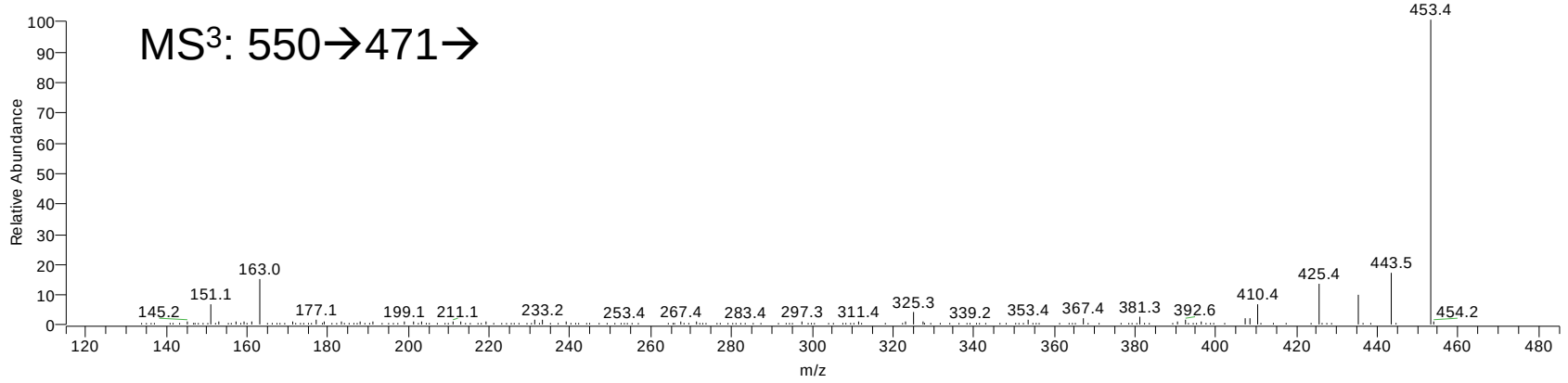


Fig. S4am C^4-24,27-diol-3-one 3-GP

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]

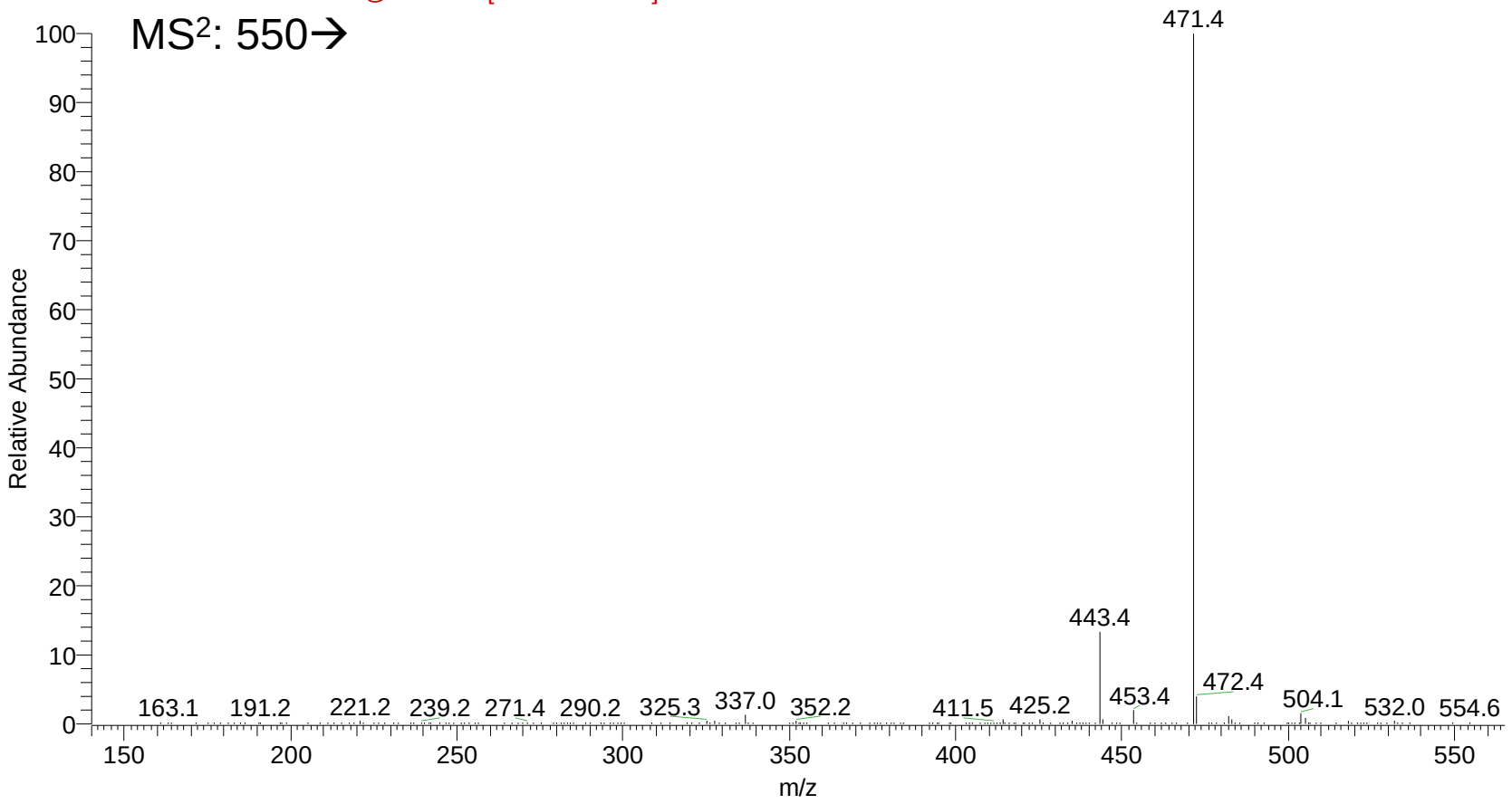


Fig. S4an C^4-24,27-diol-3-one 3-GP

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]

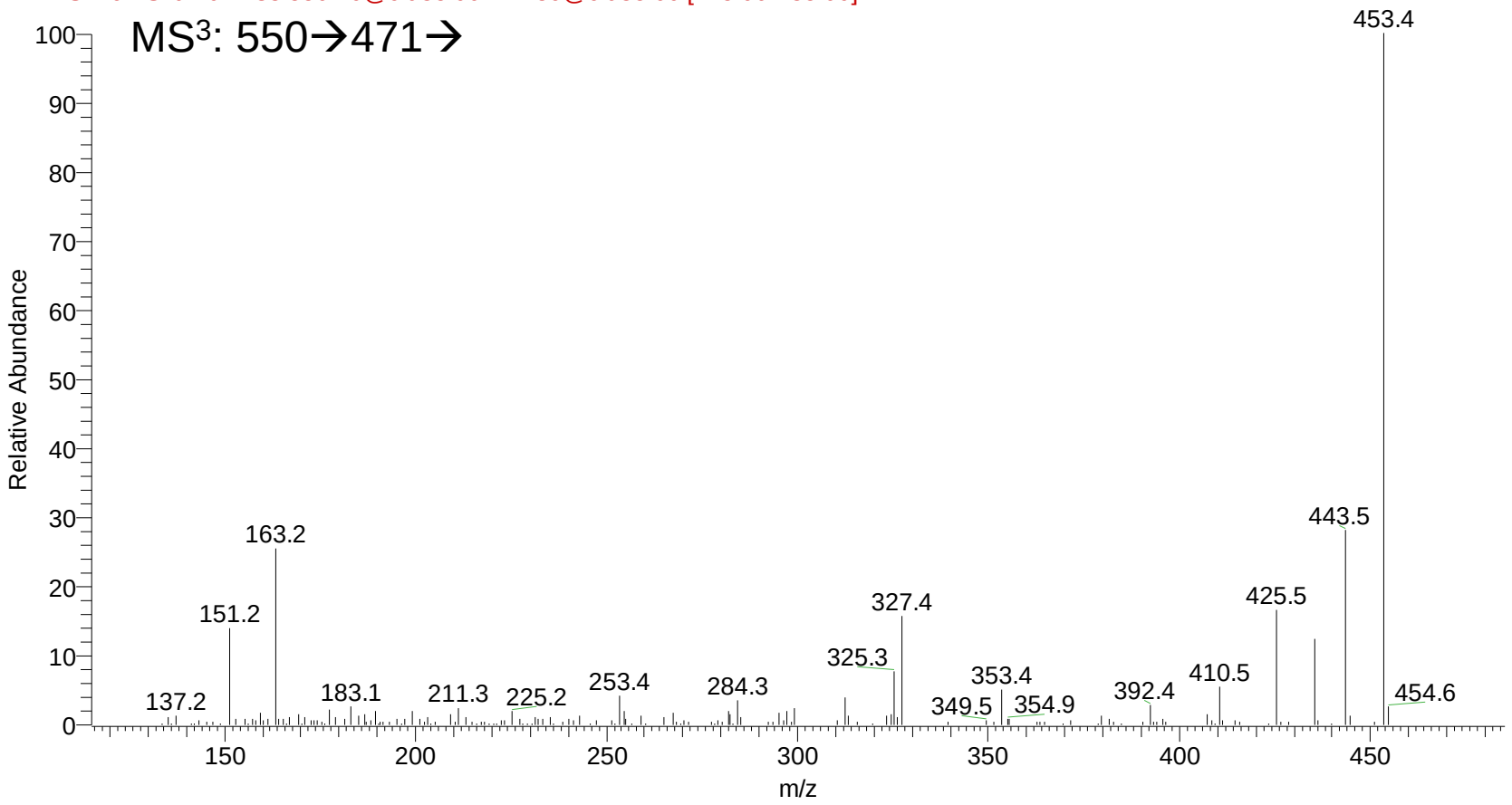


Fig. S4ao C-x-ol-y,z-dione y-GP / C^{X}-x,y-diol-z-one z-GP

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]

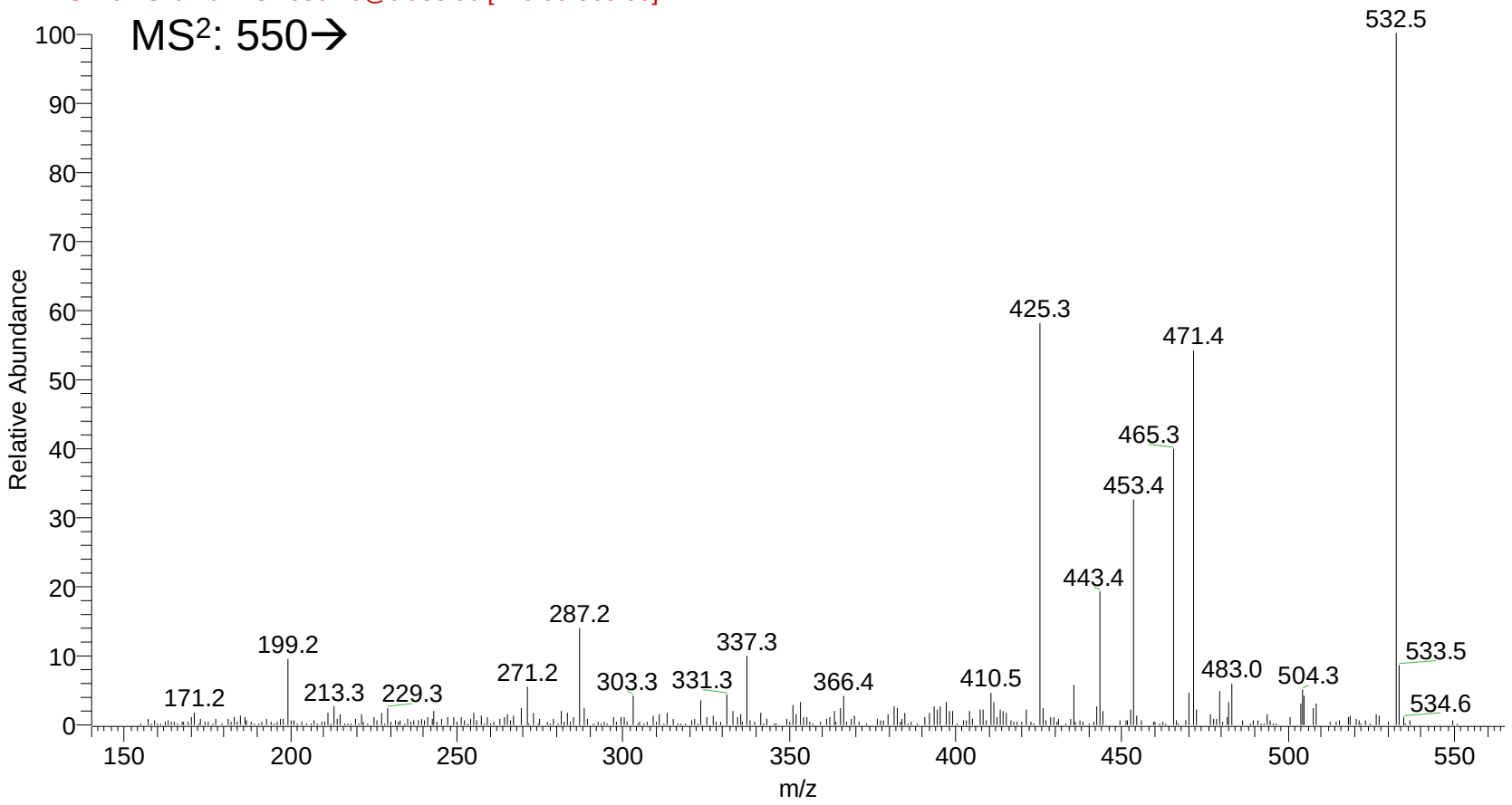


Fig. S4ap C-x-ol-y,z-dione y-GP / C^{X}-x,y-diol-z-one z-GP

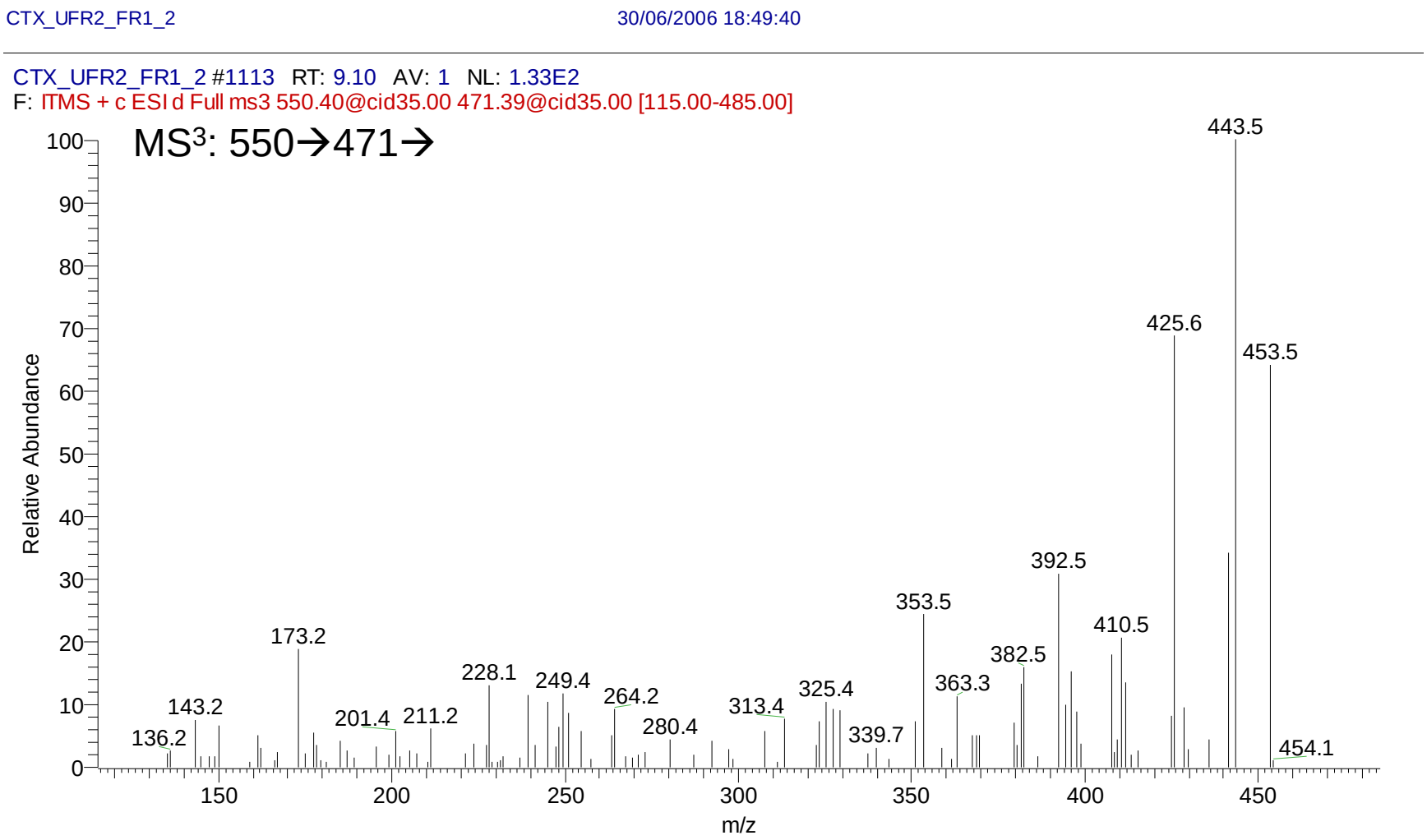


Fig. S4aq C-x,y-diol-z-one z-GP

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]

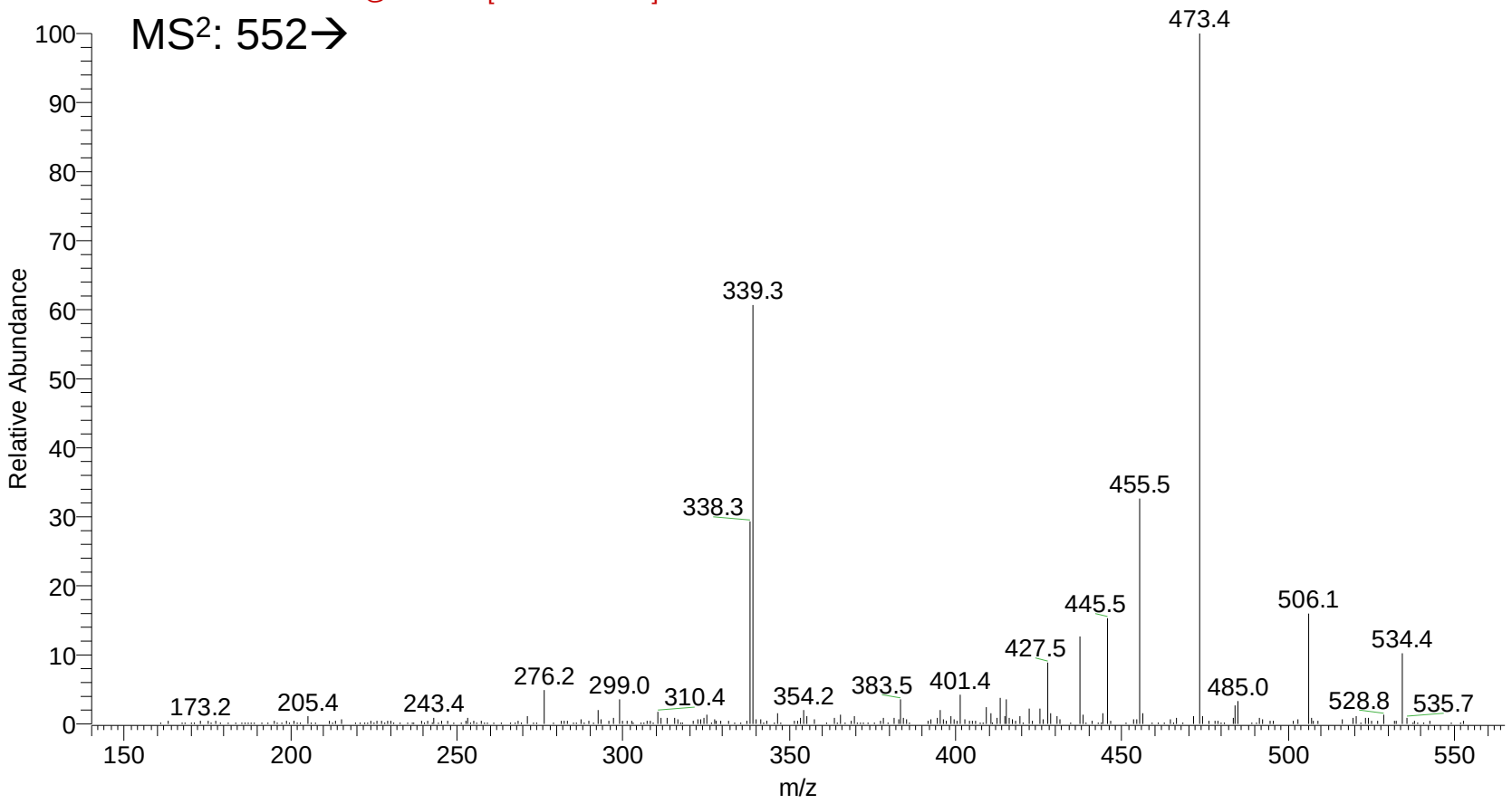


Fig. S4ar C-x,y-diol-z-one z-GP

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]

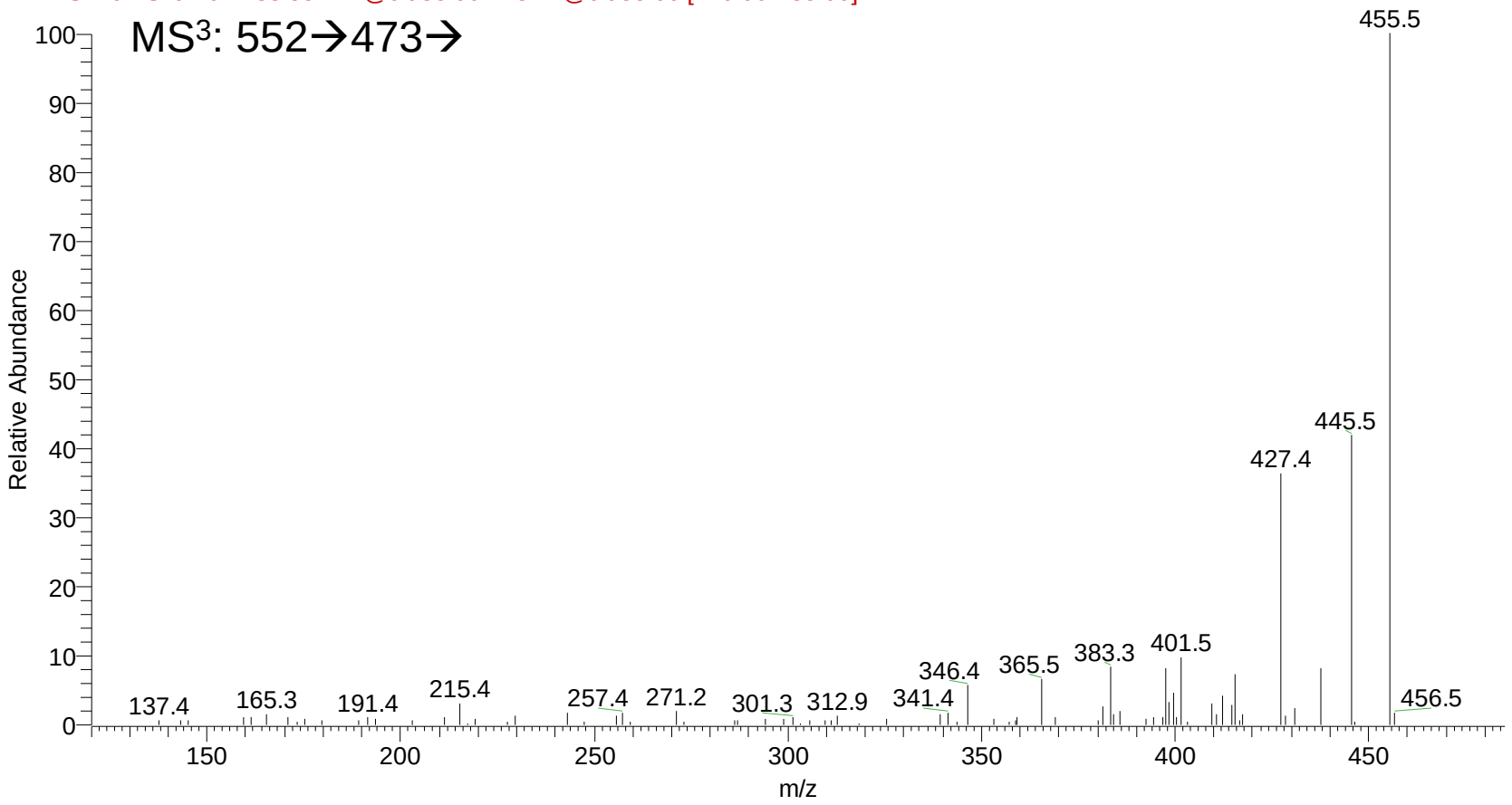


Fig. S4as aldol

CTX_UFR2_FR1_2 30/06/2006 18:49:40