Supplementary Information

Composition and Colors of Propolis from Vale do Jequitinhonha (Brazil): A Pilot Study Based on Untargeted Metabolomics

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Table S1. Geographical origin information for each propolis sample included in the study

Sample ID	Color category	Region	Microregion	City
AP01	other	Alto	Capelinha	Berilo
AP02	green	Alto	Capelinha	Berilo
AP03	other	Alto	Capelinha	Berilo
AP04	other	Alto	Capelinha	Berilo
AP06	green	Alto	Diamantina	São Gonçalo do Rio Preto
AP07	other	Alto	Capelinha	Minas Novas
AP08	other	Alto	Capelinha	Carbonita
AP10	green	Alto	Capelinha	Turmalina
AP11	green	Alto	Capelinha	Turmalina
AP12	other	Alto	Capelinha	Turmalina
AP14	green	Alto	Diamantina	Couto Magalhães de Minas
AP15	other	Baixo	Almenara	Felisburgo
AP16	red	Baixo	Almenara	Felisburgo
AP17	green	Baixo	Almenara	Rio do Prado
AP18	other	Alto	Capelinha	Chapada do Norte
AP19	other	Alto	Capelinha	Chapada do Norte
AP20	other	Alto	Capelinha	Chapada do Norte
AP21	red	Alto	Capelinha	Jenipapo de Minas
AP22	green	Baixo	Almenara	Felisburgo
AP23	green	Baixo	Almenara	Santa Maria do Salto
AP24	other	Baixo	Almenara	Jacinto
AP25	other	Baixo	Almenara	Almenara
AP26	other	Alto	Capelinha	Chapada do Norte

Sample ID	Color category	Region	Microregion	City
AP27	other	Baixo	Araçuaí	Araçuaí
AP28	other	Baixo	Araçuaí	Araçuai
AP30	other	Alto	Capelinha	Turmalina
AP31	other	Alto	Capelinha	Veredinha
AP32	green	Alto	Capelinha	José Gonçalves de Minas
AP33	other	Baixo	Araçuaí	Araçuai
AP34	green	Alto	Capelinha	Turmalina
AP35	red	Alto	Capelinha	Veredinha
AP36	other	Alto	Capelinha	Jenipapo de Minas
AP37	other	Alto	Capelinha	Francisco Badaró
AP38	other	Alto	Capelinha	Minas Novas
AP39	green	Alto	Capelinha	Veredinha
AP40	other	Alto	Capelinha	Veredinha
AP41	green	Alto	Diamantina	Felício dos Santos
AP42	green	Alto	Diamantina	Felício dos Santos
AP43	green	Alto	Diamantina	Felício dos Santos
AP44	green	Alto	Diamantina	Felício dos Santos
AP45	green	Alto	Diamantina	Felício dos Santos
AP46	other	Alto	Capelinha	Itamarandiba
AP47	other	Alto	Diamantina	Diamantina
AP48	red	Alto	Capelinha	Berilo/Francisco Badaró
AP49	other	Baixo	Almenara	Felisburgo
AP50	green	Alto	Capelinha	Carbonita
AP51	other	Baixo	Almenara	Rubim
AP52	other	Baixo	Almenara	Rubim

Sample ID	Color category	Region	Microregion	City
AP53	red	Baixo	Almenara	Santa Maria do Salto
AP54	green	Alto	Diamantina	Senador Modestino Gonçalves
AP57	other	Baixo	Almenara	Jacinto
AP58	other	Baixo	Almenara	Santa Maria do Salto
AP59	other	Baixo	Almenara	Rio do Prado
AP60	green	Alto	Diamantina	Diamantina

Table S2. List of compounds actively screened in the propolis samples from Vale do Jequitinhonha that are already reported in green and red propolis

Propolis type	Compound name	Molecular formula	Exact mass	*m/z*	Adduct	Δppm	t_R / min	Main fragments
Green	Caffeic acid	$C_9H_8O_4$	180.0422	179.0350	$[M-H]^-$	-0.48	7.707	150.95387; 135.04504; 99.92559; 90.99809; 78.95904
	p-Coumaric acid	$C_9H_8O_3$	164.0473	163.0401	$[M-H]^-$	-0.55	8.597	135.04504; 119.05024; 107.05012; 93.03443; 70.77375
	3,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.1268	515.1195	$[M-H]^-$	0.48	9.072	353.08783; 317.06488; 191.05602; 179.03485; 173.04535; 135.04501; 93.03448
	Rutin	$C_{27}H_{30}O_{16}$	610.1534	609.1461	$[M-H]^-$	1.16	9.474	315.05006; 300.02762; 271.02509; 299.01804; 255.03000; 151.00372
	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.1268	515.1195	$[M-H]^-$	0.51	9.774	353.08783; 299.05594; 191.05600; 179.03477; 173.04530; 135.04498
	Cinnamic acid	$C_9H_8O_2$	148.0524	147.0452	$[M-H]^-$	-0.52	8.927	129.03433; 119.04980; 109.94184; 91.93147; 70.73000
	Quercetin	$C_{15}H_{10}O_7$	302.0426	301.0354	$[M-H]^-$	0.98	10.983	286.04831; 165.99059; 151.00346; 121.02931; 110.00081; 63.02390
	Aromadendrin	$C_{15}H_{12}O_6$	288.0634	287.0561	$[M-H]^-$	0.51	9.52	259.06165; 161.02394; 125.02429; 83.01379; 69.32497; 57.03450
	Drupanin	$C_{14}H_{16}O_3$	232.1099	231.1027	$[M-H]^-$	-0.36	11.696	208.03745; 187.11278; 164.04782; 132.05788; 108.38687; 69.35880

Propolis type	Compound name	Molecular formula	Exact mass	*m/z*	Adduct	Δppm	t_R / min	Main fragments
Red	Chrysin	$C_{15}H_{10}O_4$	254.0579	253.0506	$[M – H]^–$	0.47	12.668	209.06073; 181.06583; 143.05002; 107.01376; 63.02396
	Galangin	$C_{15}H_{10}O_5$	270.0528	269.0455	$[M – H]^–$	0.48	11.771	225.05562; 201.05566; 151.00343; 117.03442; 107.01374
	Artepillin C	$C_{19}H_{24}O_3$	300.1725	299.1653	$[M – H]^–$	0.26	13.191	255.17549; 232.11035; 200.12048; 145.06572; 107.01360; 69.36283
	Liquiritigenin	$C_{15}H_{12}O_4$	256.0735	255.0663	$[M – H]^–$	0.18	10.327	213.05563; 171.04507; 151.00349; 145.06575; 107.01376; 65.00324
	Calycosin	$C_{16}H_{12}O_5$	284.0685	283.0612	$[M – H]^–$	-0.99	12.757	268.03769; 239.03494; 165.99059; 110.00088; 76.97969
	Isoliquiritigenin	$C_{15}H_{12}O_4$	256.0735	255.0663	$[M – H]^–$	0.32	12.207	213.05563; 171.04497; 151.00345; 145.06569; 107.01375; 65.00322
	Formononetin	$C_{16}H_{12}O_4$	268.0735	267.0663	$[M – H]^–$	0.37	11.812	252.04283; 223.04001; 195.04503; 135.00859; 91.01889; 69.46570
	Vestitol	$C_{16}H_{16}O_4$	272.1048	271.0976	$[M – H]^–$	0.16	11.139	256.07425; 165.01917; 149.02428; 137.02426; 119.05014
	Neovestitol	$C_{16}H_{16}O_4$	272.1048	271.0976	$[M – H]^–$	0.25	11.503	253.05074; 197.06075; 161.06061; 125.02429; 107.01376; 83.01384
	Medicarpin	$C_{16}H_{14}O_4$	270.0892	269.0819	$[M – H]^–$	0.34	13.282	226.06337; 172.88582; 165.01921;

Propolis type	Compound name	Molecular formula	Exact mass	*m/z*	Adduct	Δppm	t_R / min	Main fragments
								151.00334; 117.03445; 65.00322
	Biochanin A	$C_{16}H_{12}O_5$	284.0685	283.0612	$[M – H]^–$	-1.23	10.739	268.03775; 240.04257; 211.04008; 196.05266; 145.02956; 90.93353

t_R: retention time.

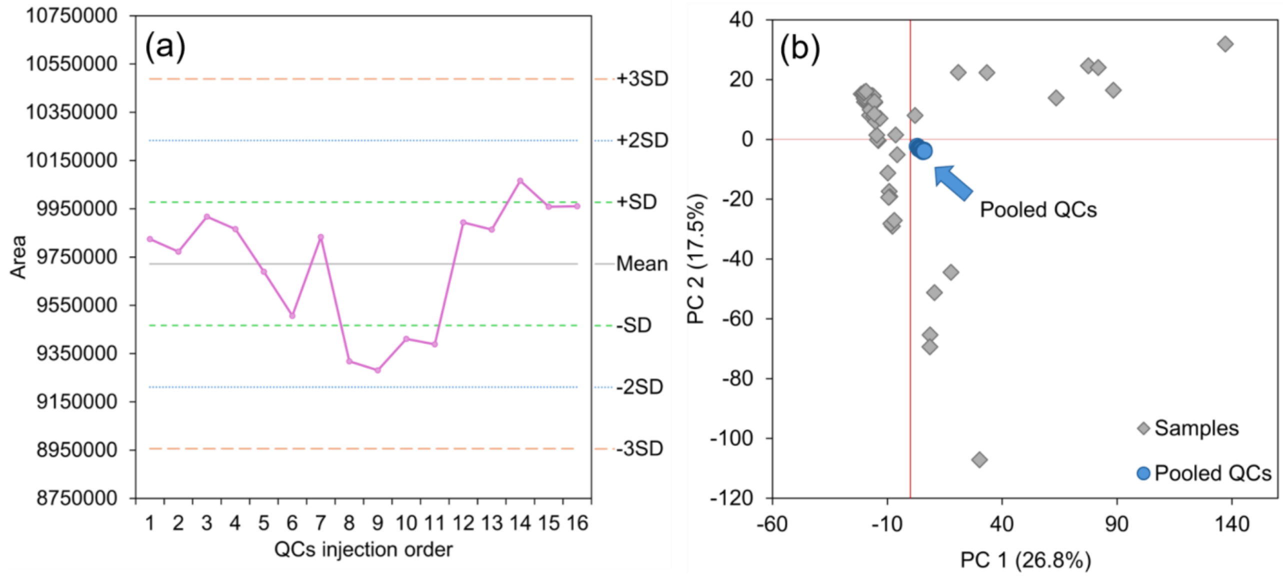

Figure S1. (a) Control chart of the internal standard, F-Phe, peak areas across QC injections in negative ionization mode. All measurements remained within ±3 standard deviations of the mean, indicating stable signal intensity and analytical performance throughout the run. (b) Principal component analysis (PCA) score plot (acquired by LC-MS in negative ionization mode). QC samples (blue) cluster tightly near the plot center, confirming high reproducibility and instrumental stability across the LC-MS sequence.

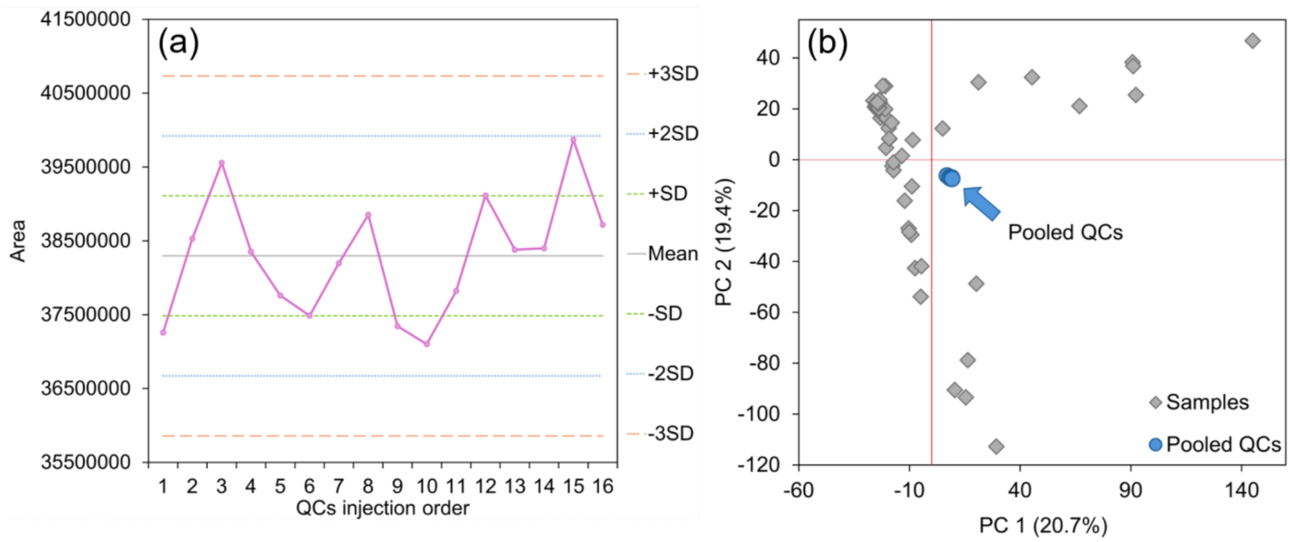

Figure S2. (a) Control chart of the internal standard, F-Phe, peak areas across QC injections in positive ionization mode. All measurements remained within ±3 standard deviations of the mean, indicating stable signal intensity and analytical performance throughout the run. (b) Principal component analysis (PCA) score plot (acquired by LC-MS in positive ionization mode). QC samples (blue) cluster tightly near the plot center, confirming high reproducibility and instrumental stability across the LC-MS sequence.

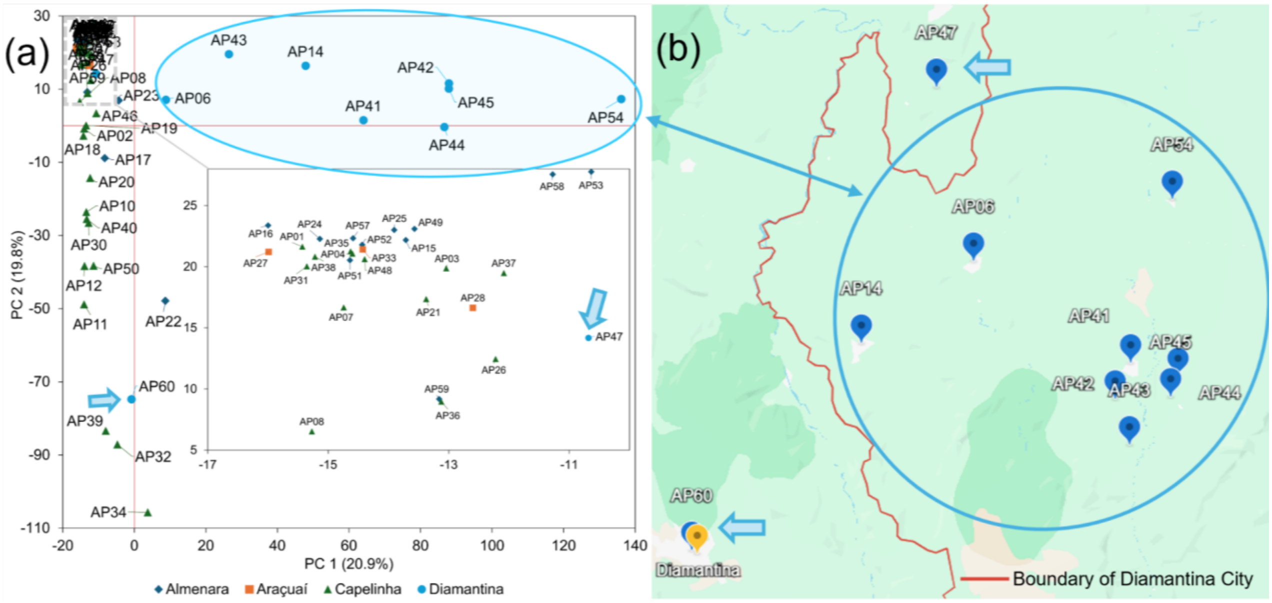

Figure S3. PCA scores plot for chemical profile data acquired by LC-MS in positive ionization mode for propolis samples categorized based on their microregion of origin (a), highlighting in light blue the samples from Diamantina microregion, with their locations of origin shown in a political map (b).

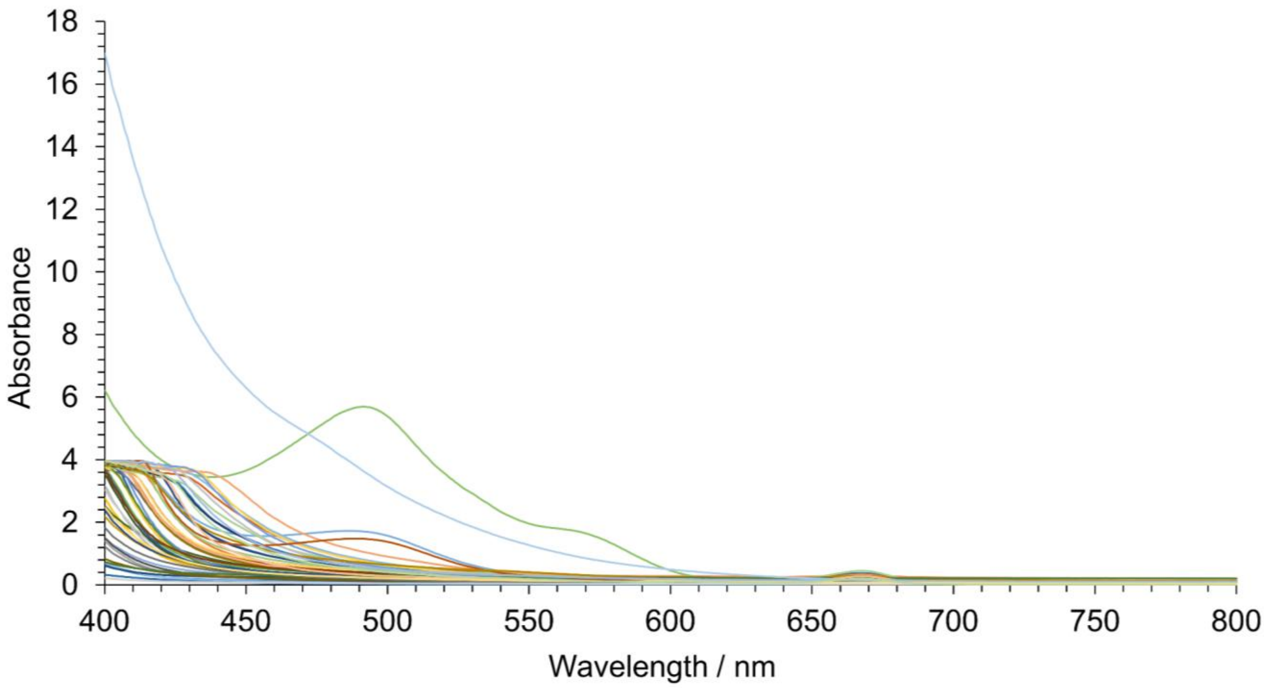

Figure S4. Overlaid UV-Vis spectra of all propolis extracts across the visible region (400-800 nm). Samples AP21 and AP53 were corrected for dilution effects.

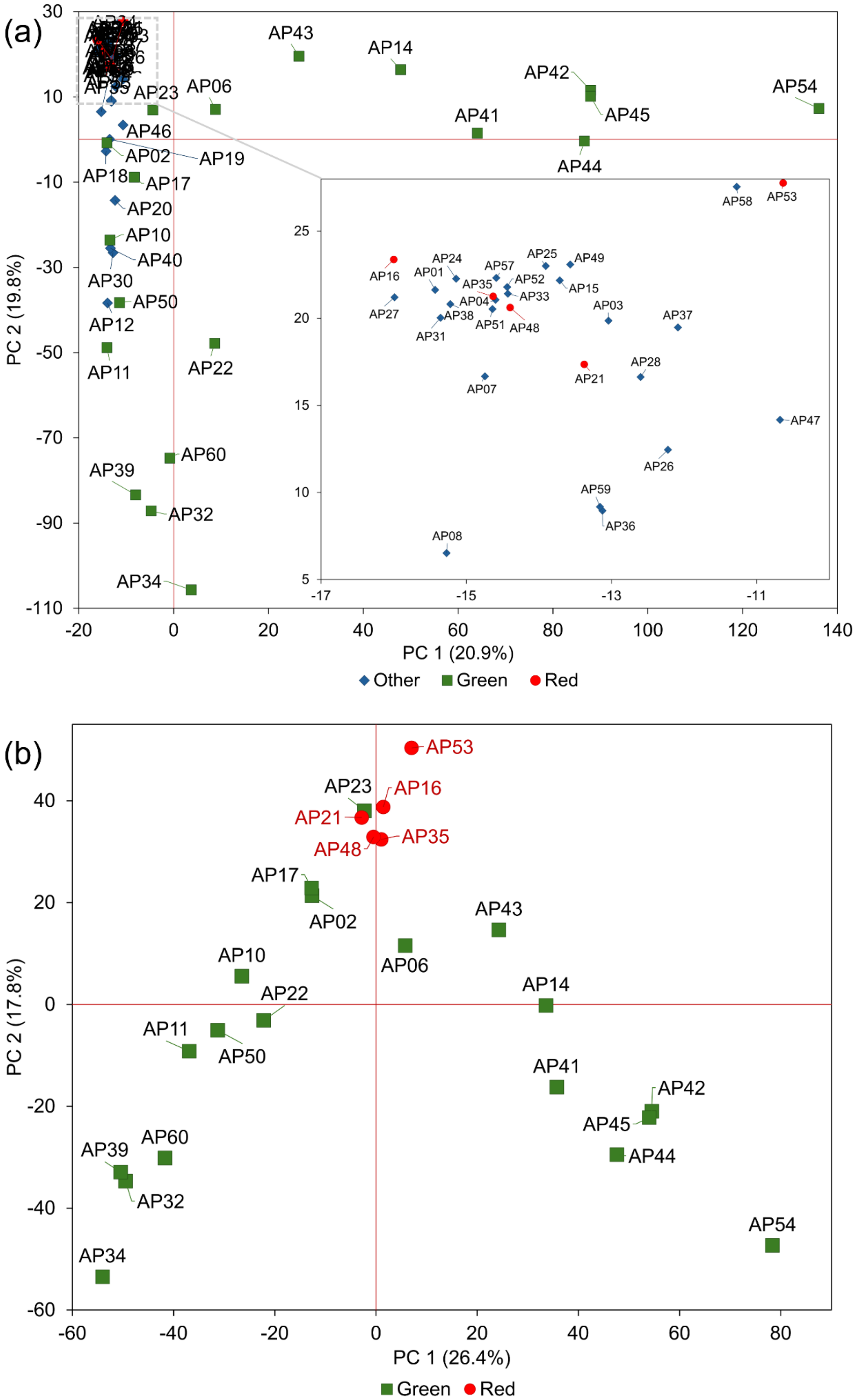

Figure S5. PCA scores plot for chemical profile data acquired by LC-MS. (a) PCA acquired in positive ionization mode for propolis samples color-coded based on the presence of absorption bands in the regions of 630-750 nm ("green" group), 450-600 nm ("red" group), and the samples that had no absorption bands in these regions ("others" group). (b) PCA of samples from the "green" and "red" groups only, acquired in positive ionization mode.

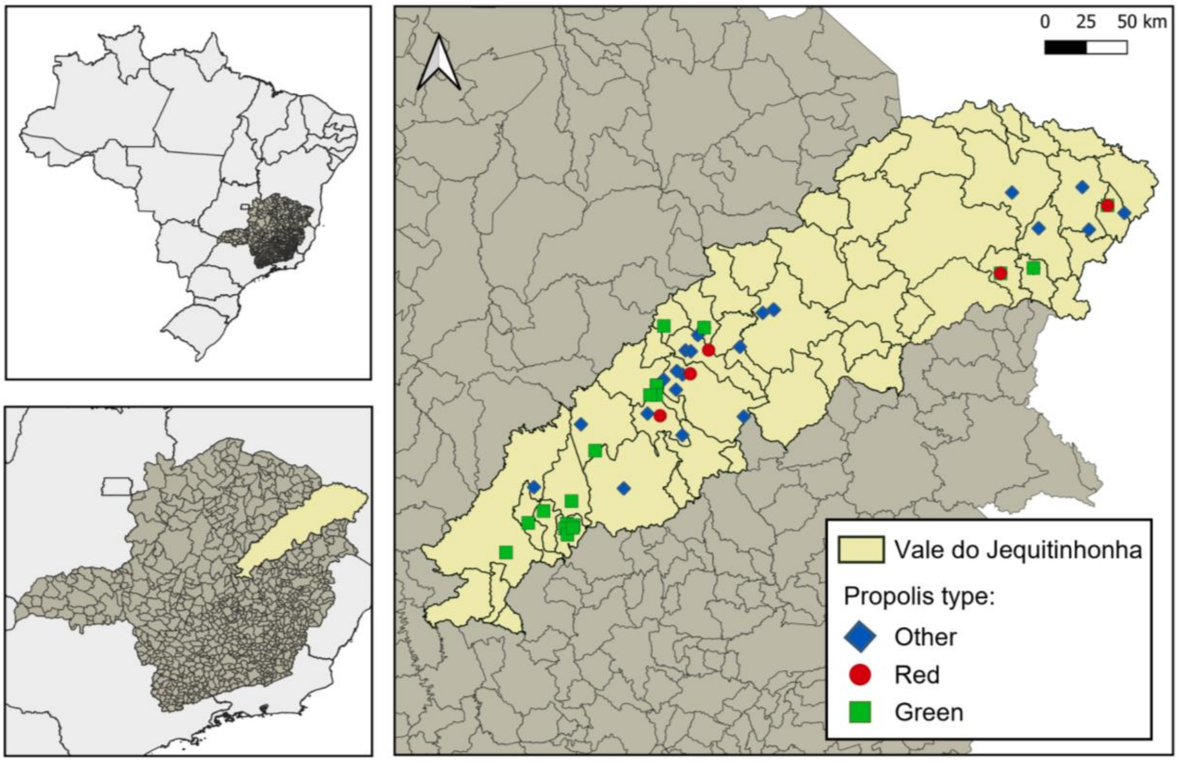

Figure S6. Geographic distribution of the propolis samples by color groups in the Vale do Jequitinhonha mesoregion, Minas Gerais, Brazil. The map highlights the location of the sampling sites by each sample group: green, red and other (in blue) within the Vale do Jequitinhonha area (shown in yellow) and the municipal boundaries of Minas Gerais state (shown in gray).

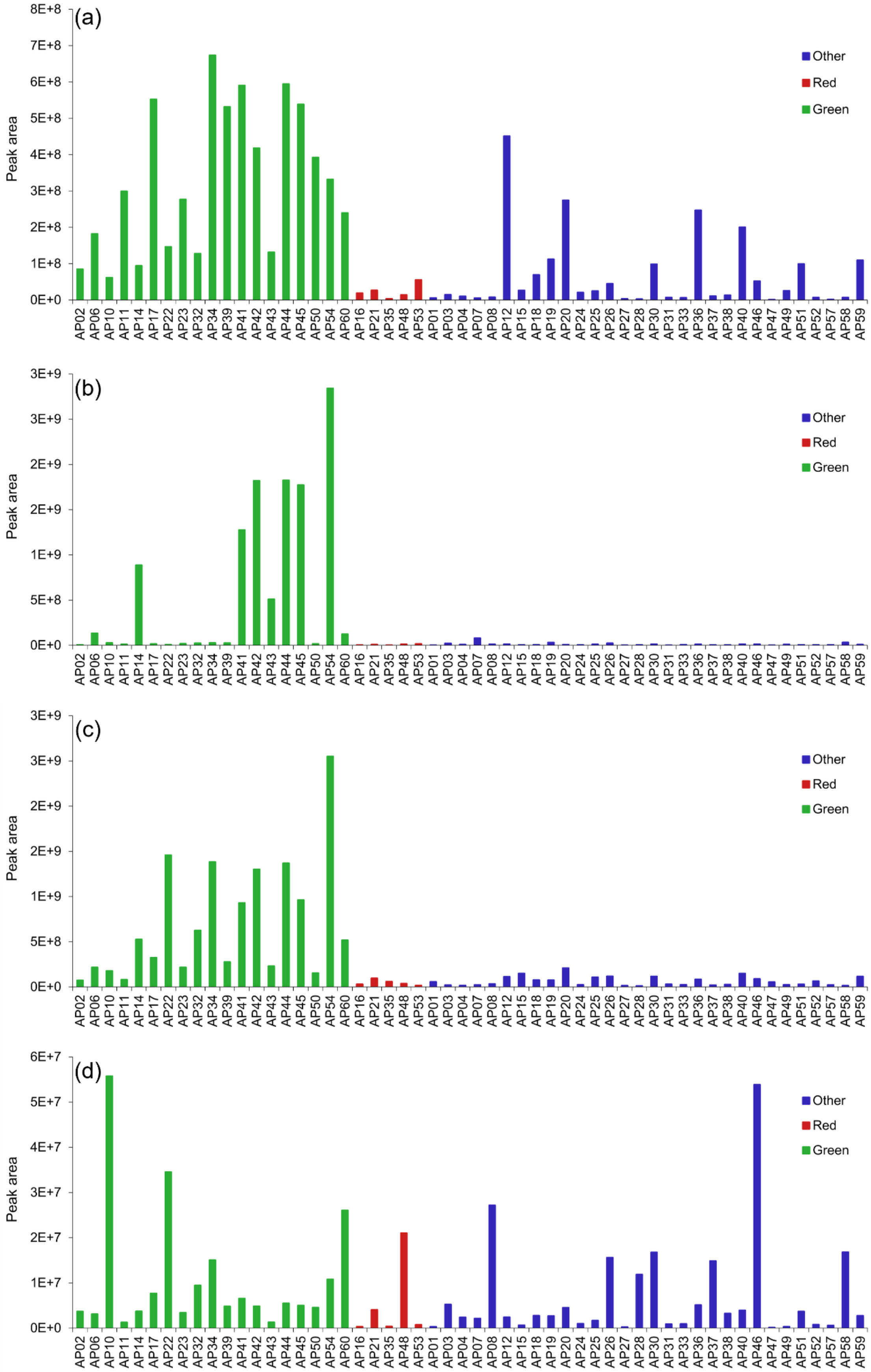

Figure S7. Peak areas of caffeic acid (a), *p*-coumaric acid (b), 3,5-dicaffeoylquinic acid (c), and rutin (d) obtained in negative ionization mode for all propolis samples, grouped according to extract color (green, red, and others).

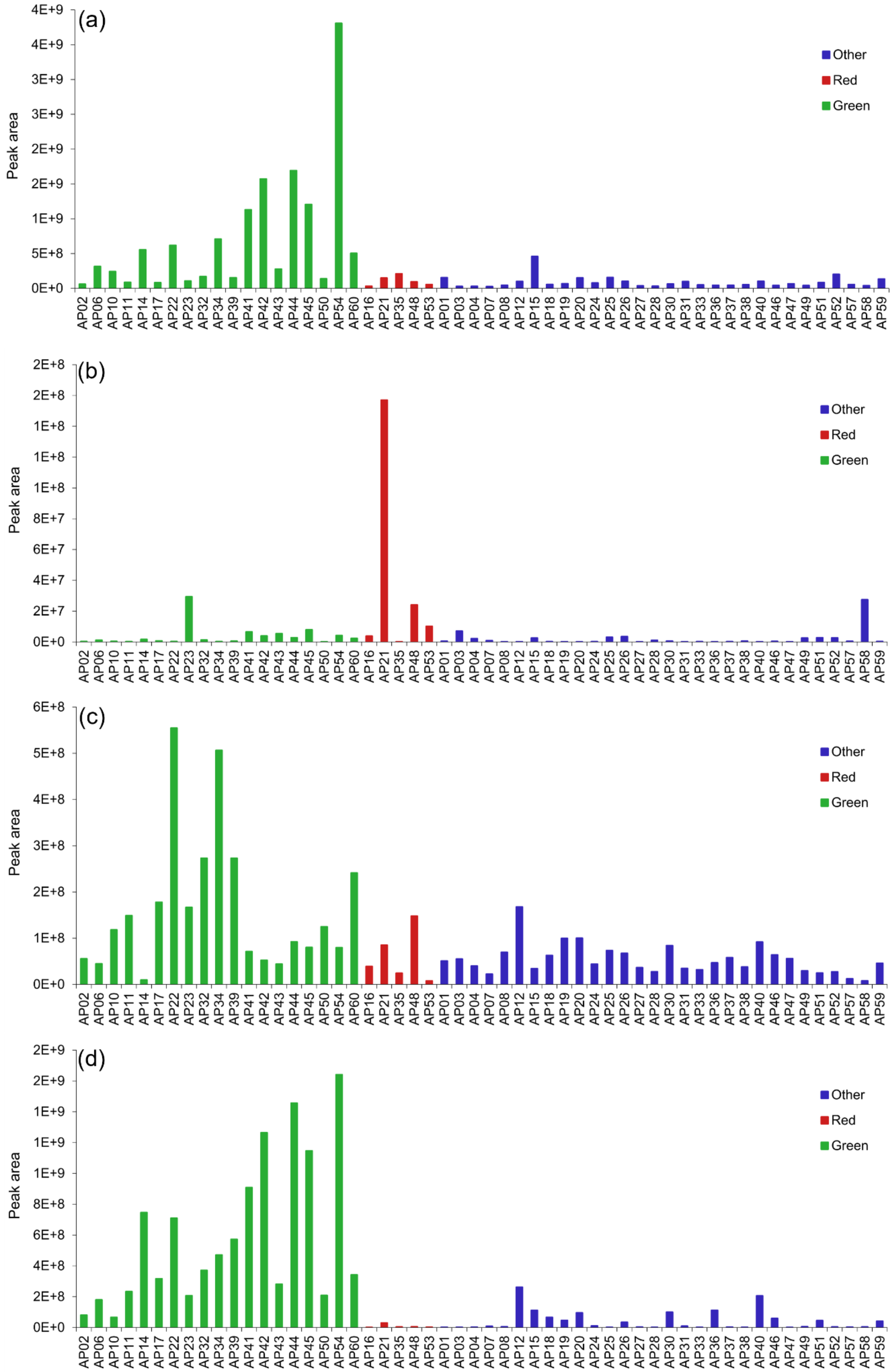

Figure S8. Peak areas of 4,5-dicaffeoylquinic acid (a), cinnamic acid (b), quercetin (c), and aromadendrin (d) obtained in negative ionization mode for all propolis samples, grouped according to extract color (green, red, and others).

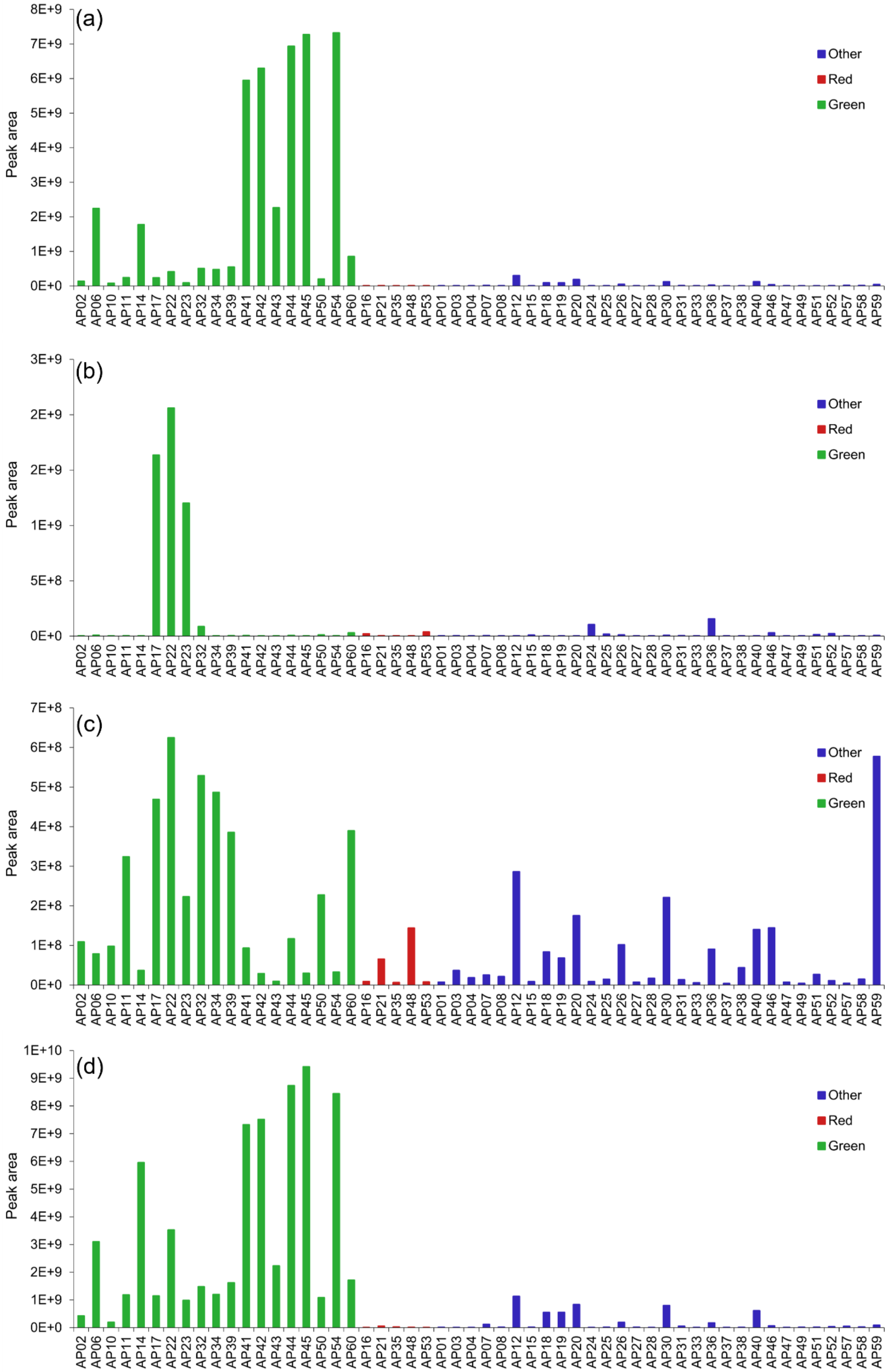

Figure S9. Peak areas of drupanin (a), chrysin (b), galangin (c), and artepillin C (d) obtained in negative ionization mode for all propolis samples, grouped according to extract color (green, red, and others).

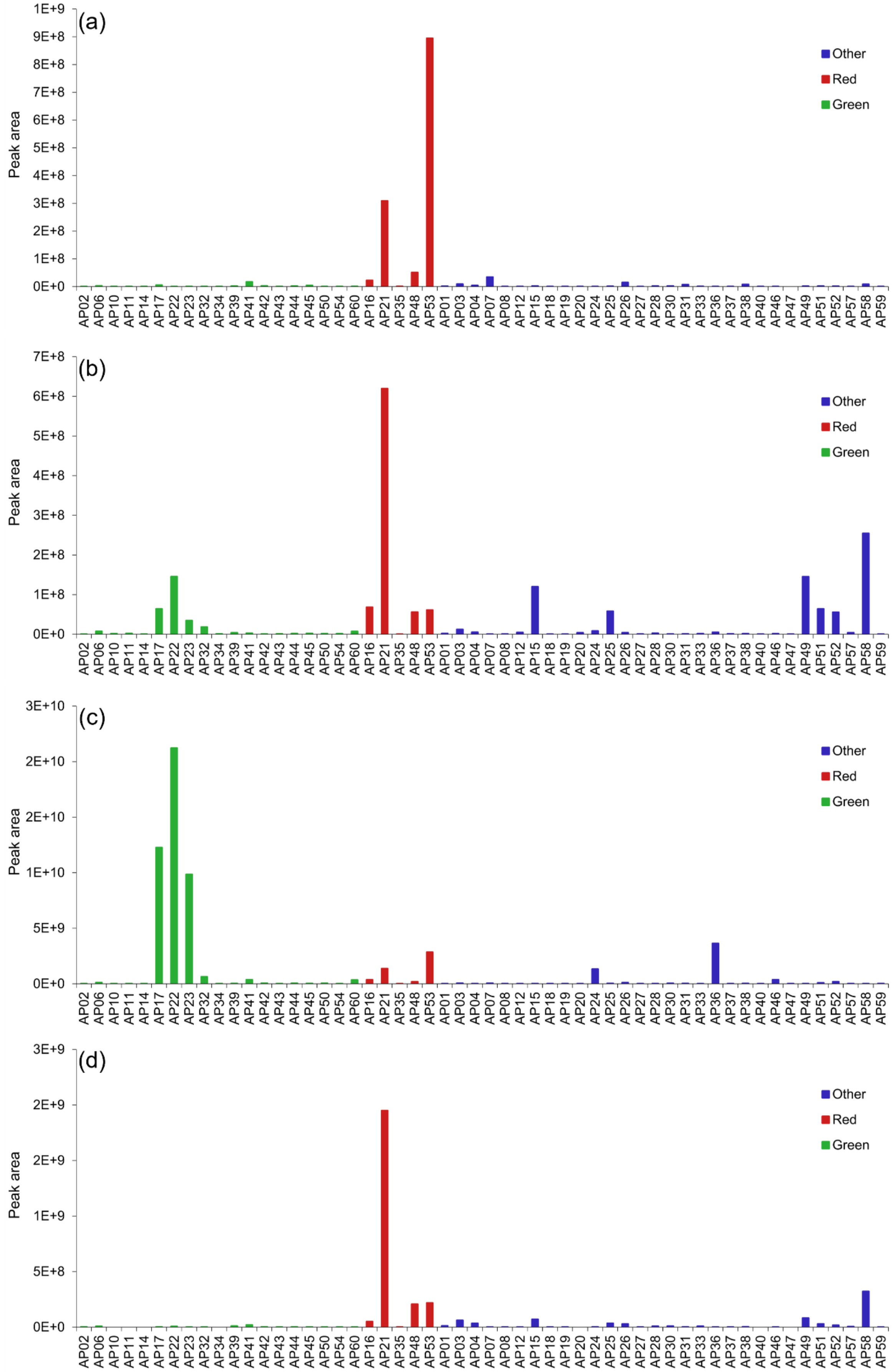

Figure S10. Peak areas of liquiritigenin (a), calycosin (b), isoliquiritigenin (c), and formononetin (d) obtained in negative ionization mode for all propolis samples, grouped according to extract color (green, red, and others).

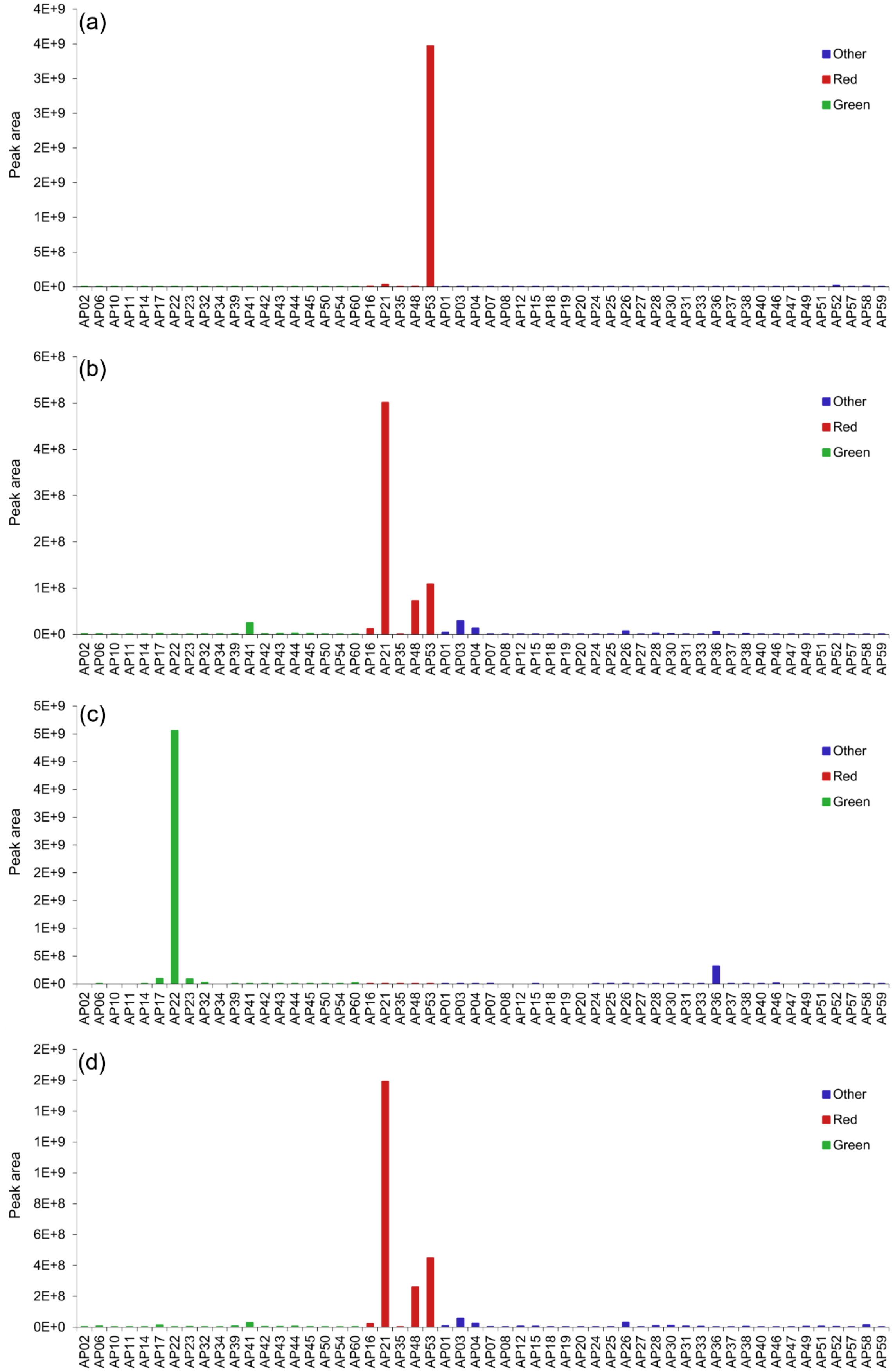

Figure S11. Peak areas of vestitol (a), neovestitol (b), medicarpin (c), and biochanin A (d) obtained in negative ionization mode for all propolis samples, grouped according to extract color (green, red, and others).

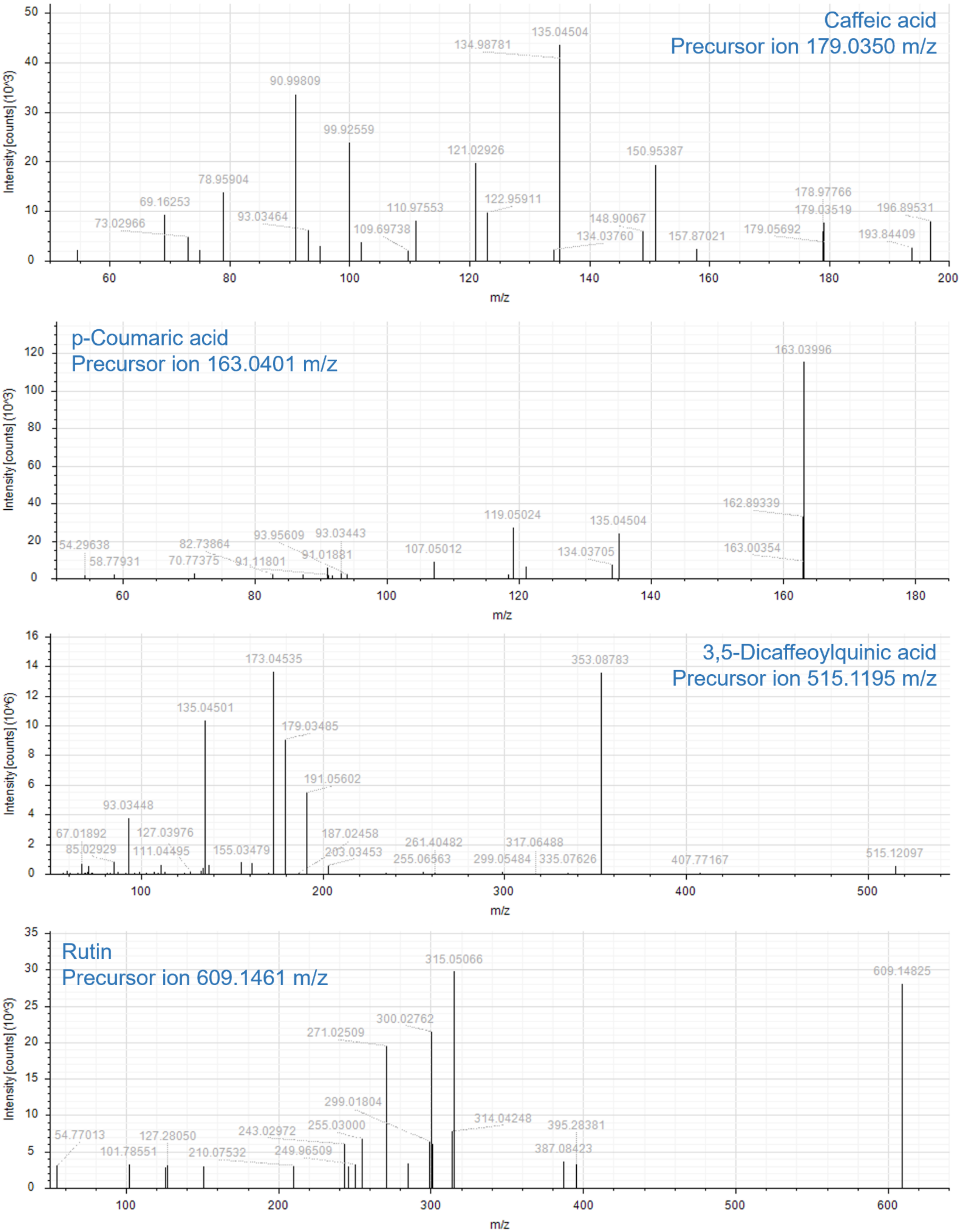

Figure S12. MS/MS spectra of caffeic acid, *p*-coumaric acid, 3,5-dicaffeoylquinic acid, and rutin.

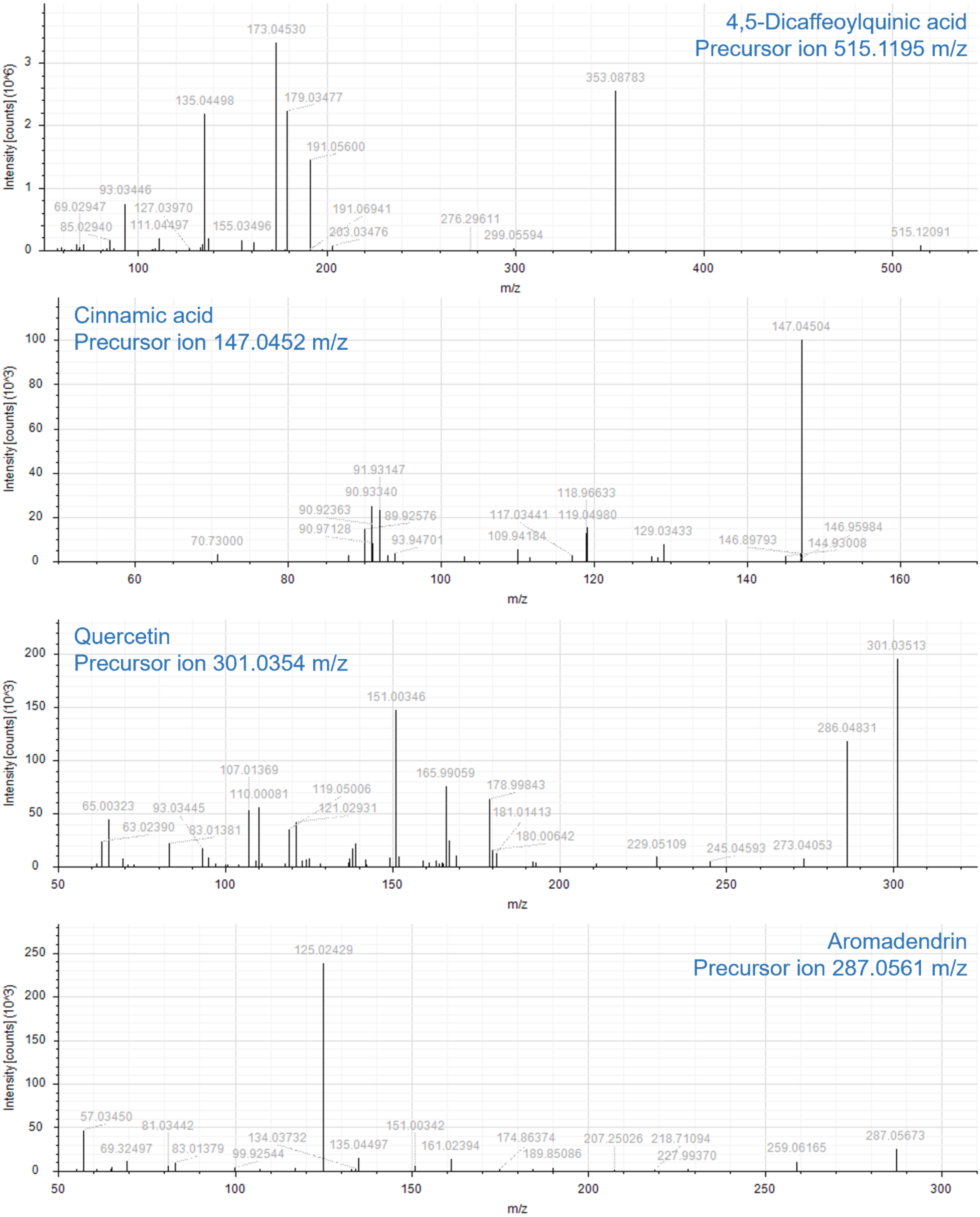

Figure S13. MS/MS spectra of 4,5-dicaffeoylquinic acid, cinnamic acid, quercetin, and aromadendrin.

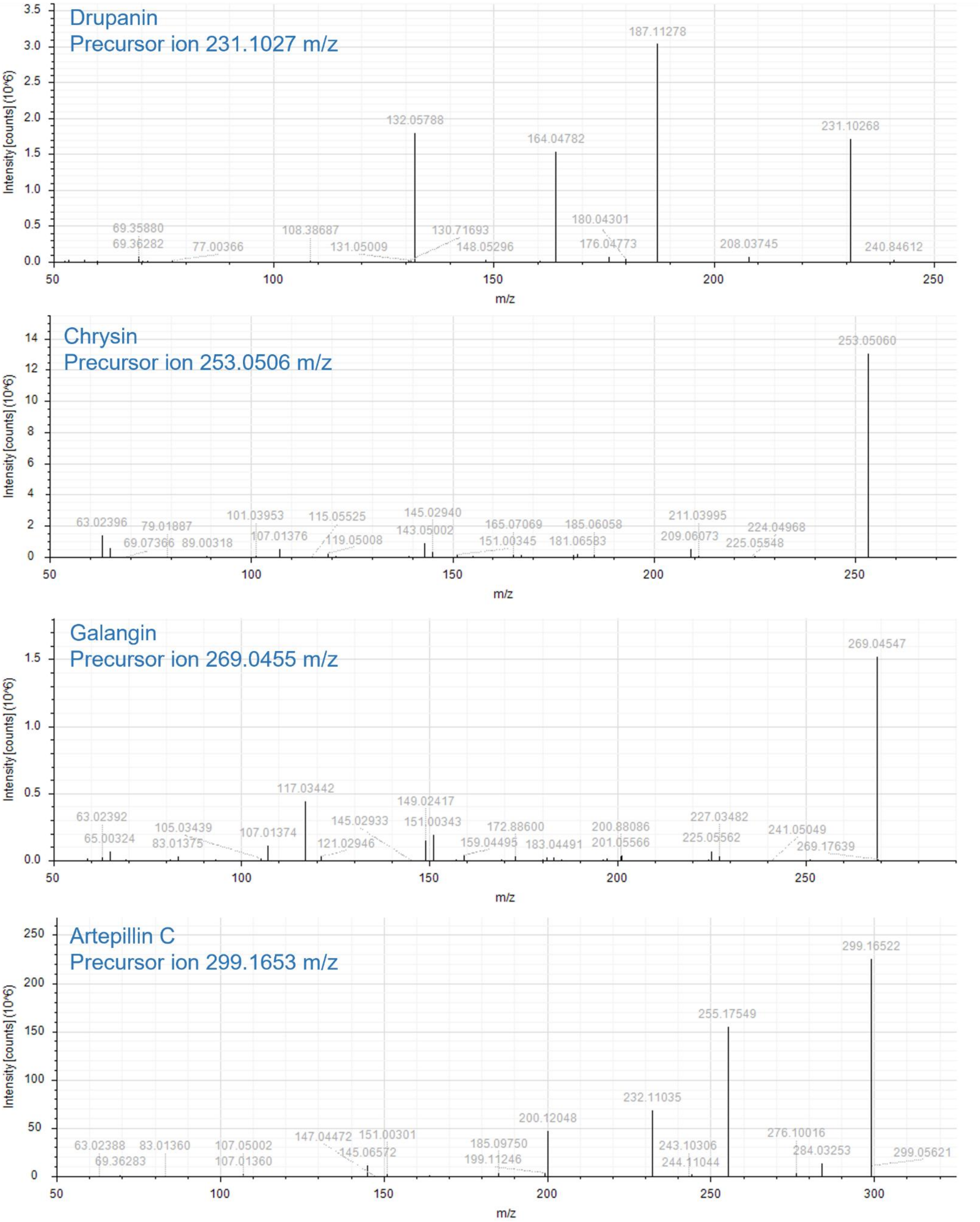

Figure S14. MS/MS spectra of drupanin, chrysin, galangin, and artepillin C.

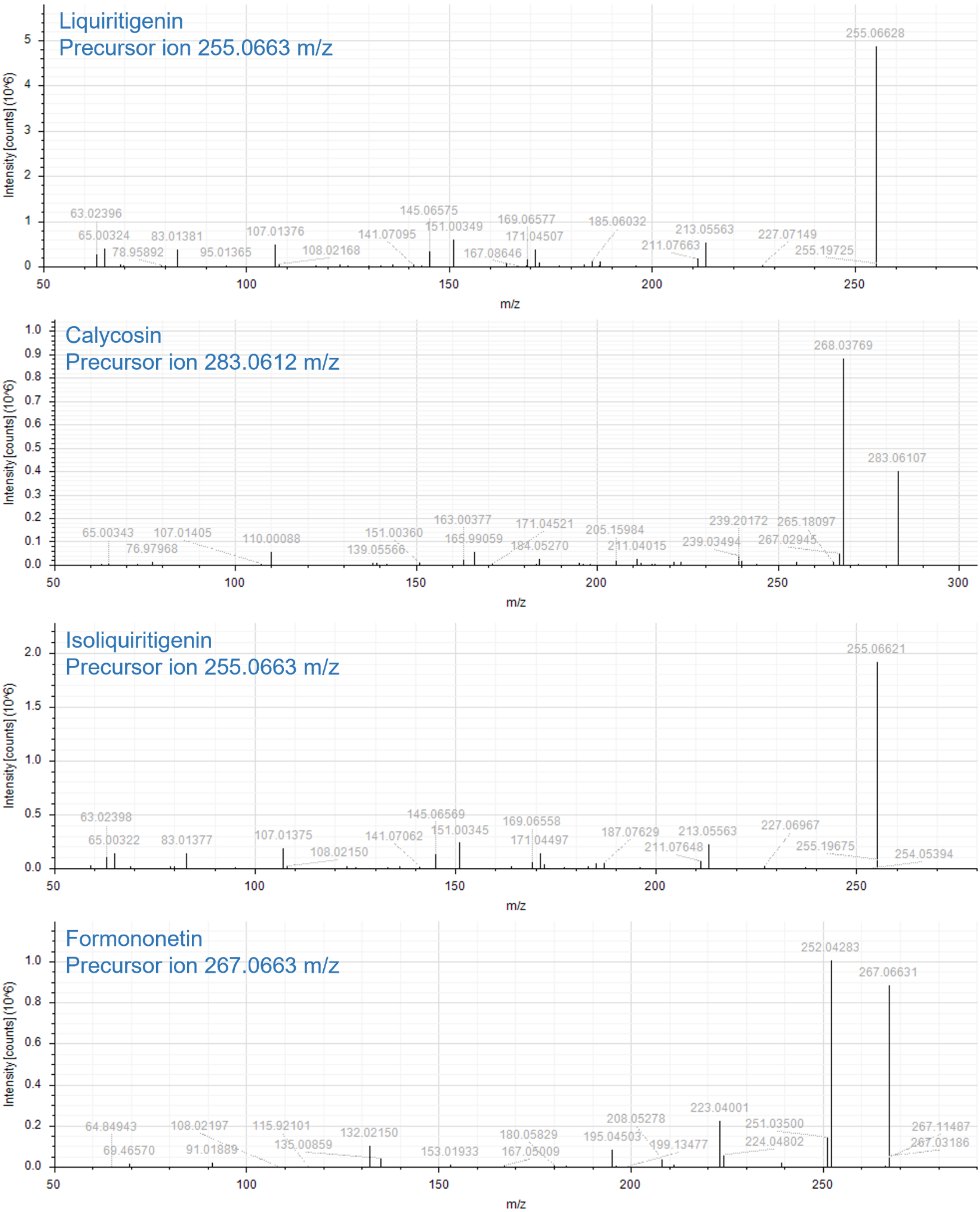

Figure S15. MS/MS spectra of liquiritigenin, calycosin, isoliquiritigenin, and formononetin.

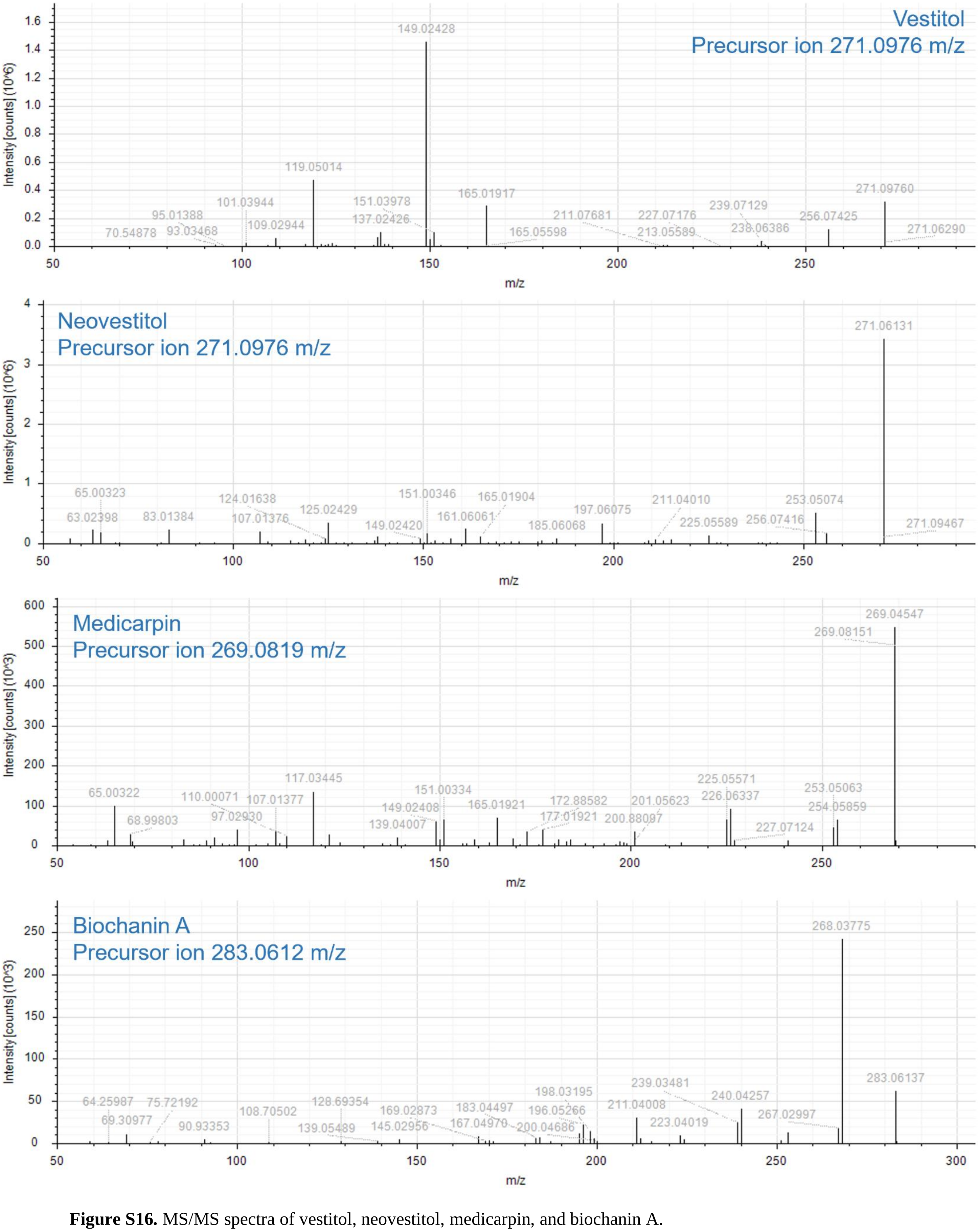

Figure S16. MS/MS spectra of vestitol, neovestitol, medicarpin, and biochanin A.

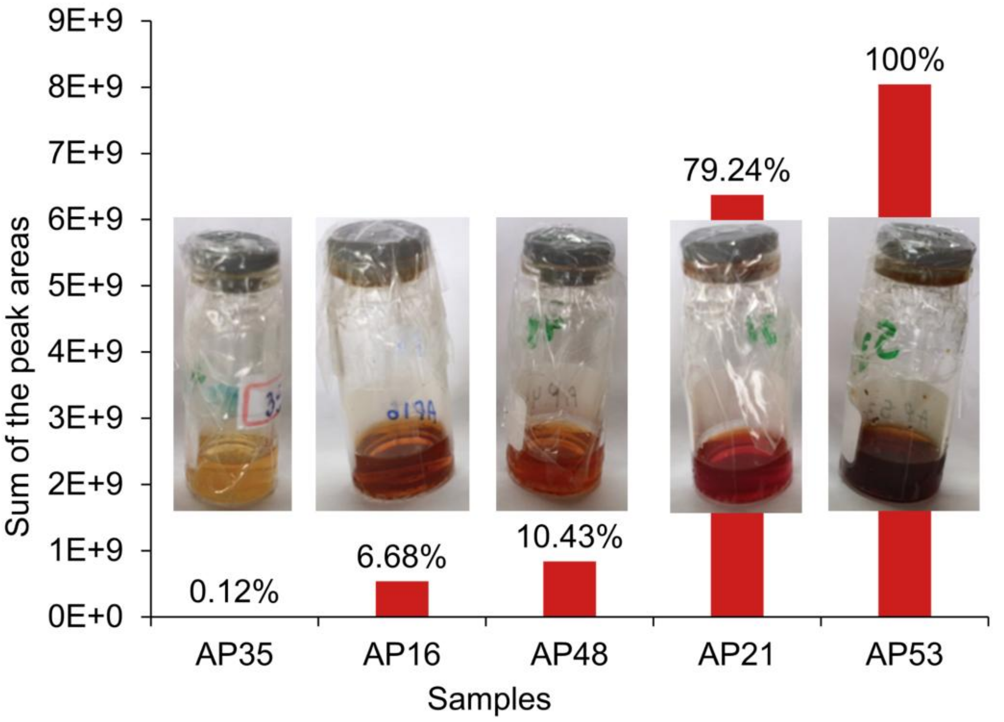

Figure S17. Bar graphs showing the sum of peak areas of characteristic compounds of red propolis (i.e., liquiritigenin, isoliquiritigenin, calycosin, formononetin, vestitol, neovestitol, biochanin A, and medicarpin) that were detected in the propolis samples of the red class analyzed in the present study (samples AP35, AP16, AP48, AP21, and AP53), including images of each propolis extract for color visualization. The percentages indicate the proportion of each sample relative to the AP53, which presented the highest sum of peak areas for these compounds.

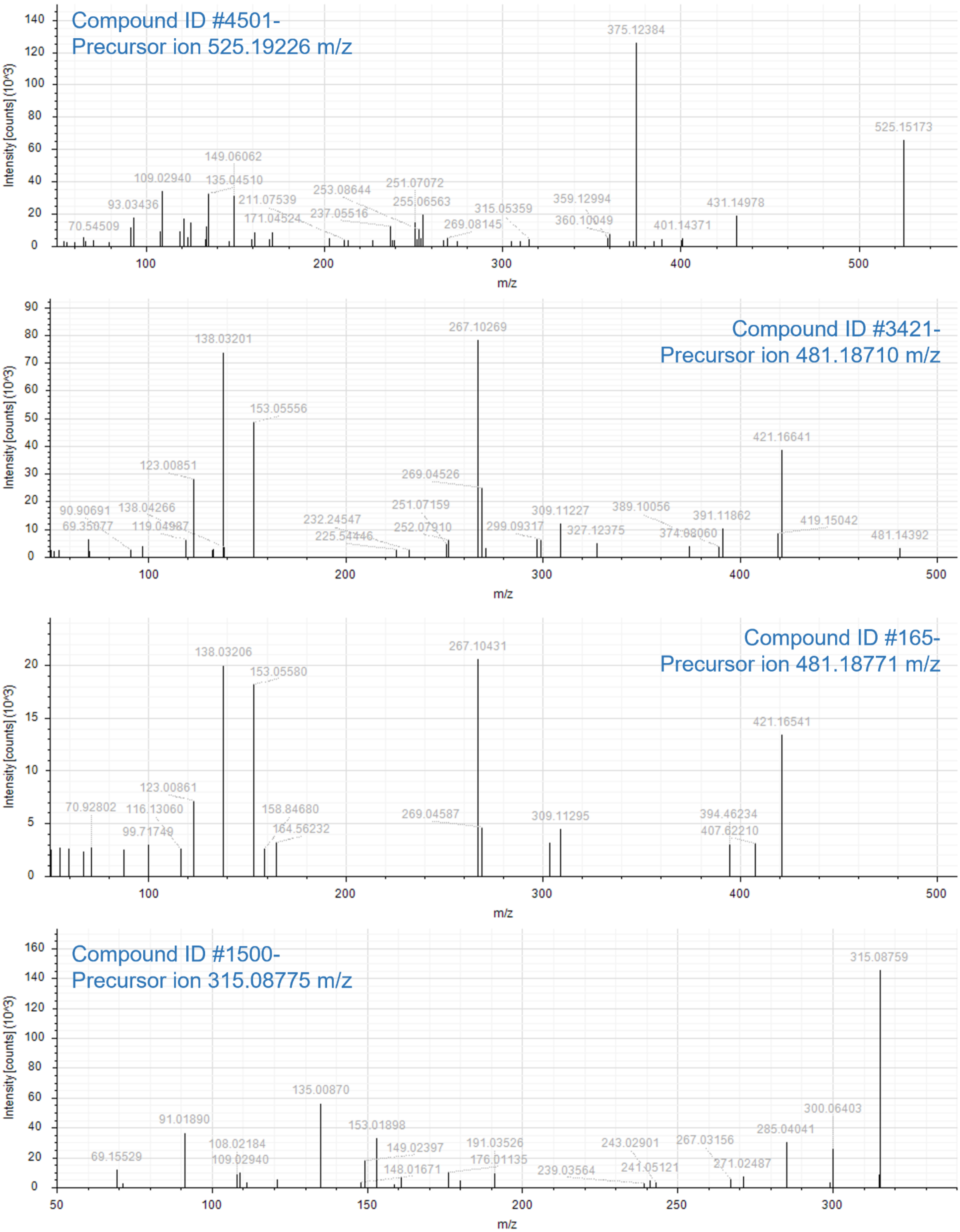

Figure S18. MS/MS spectra of compounds with IDs 4501-, 3421-, 165-, and 1500-.

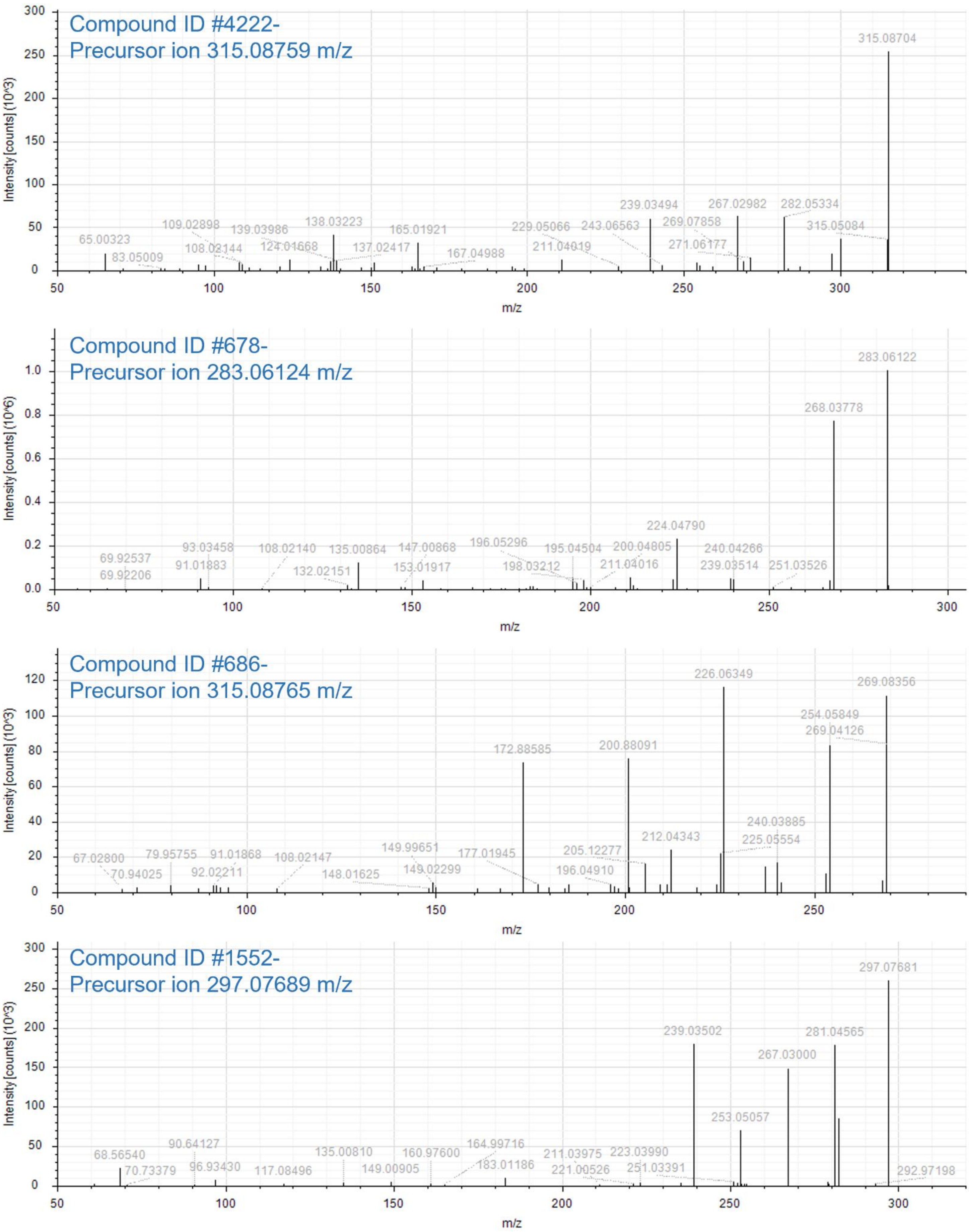

Figure S19. MS/MS spectra of compounds with IDs 4222-, 678-, 686-, and 1552-.

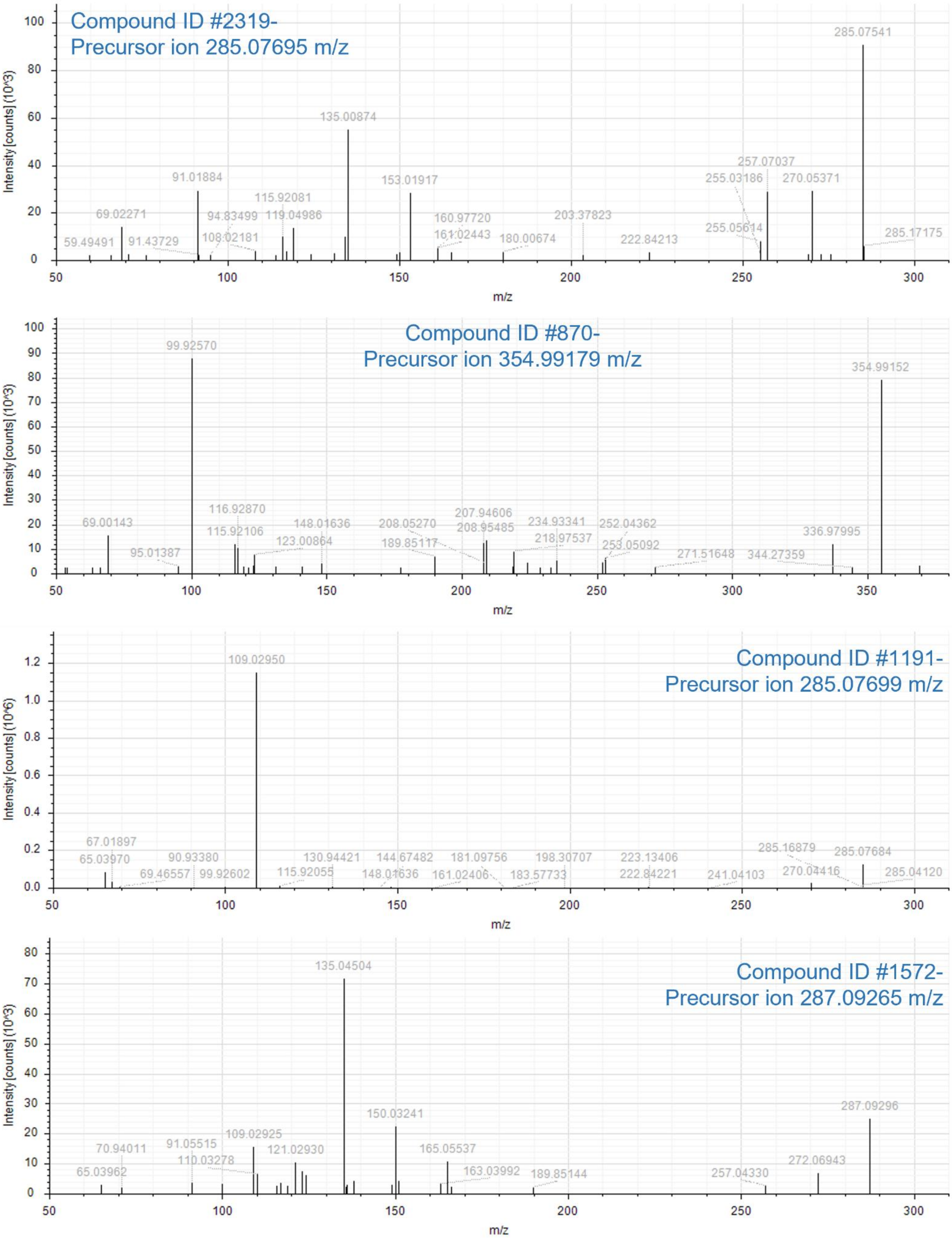

Figure S20. MS/MS spectra of compounds with IDs 2319-, 870-, 1191-, and 1572-.

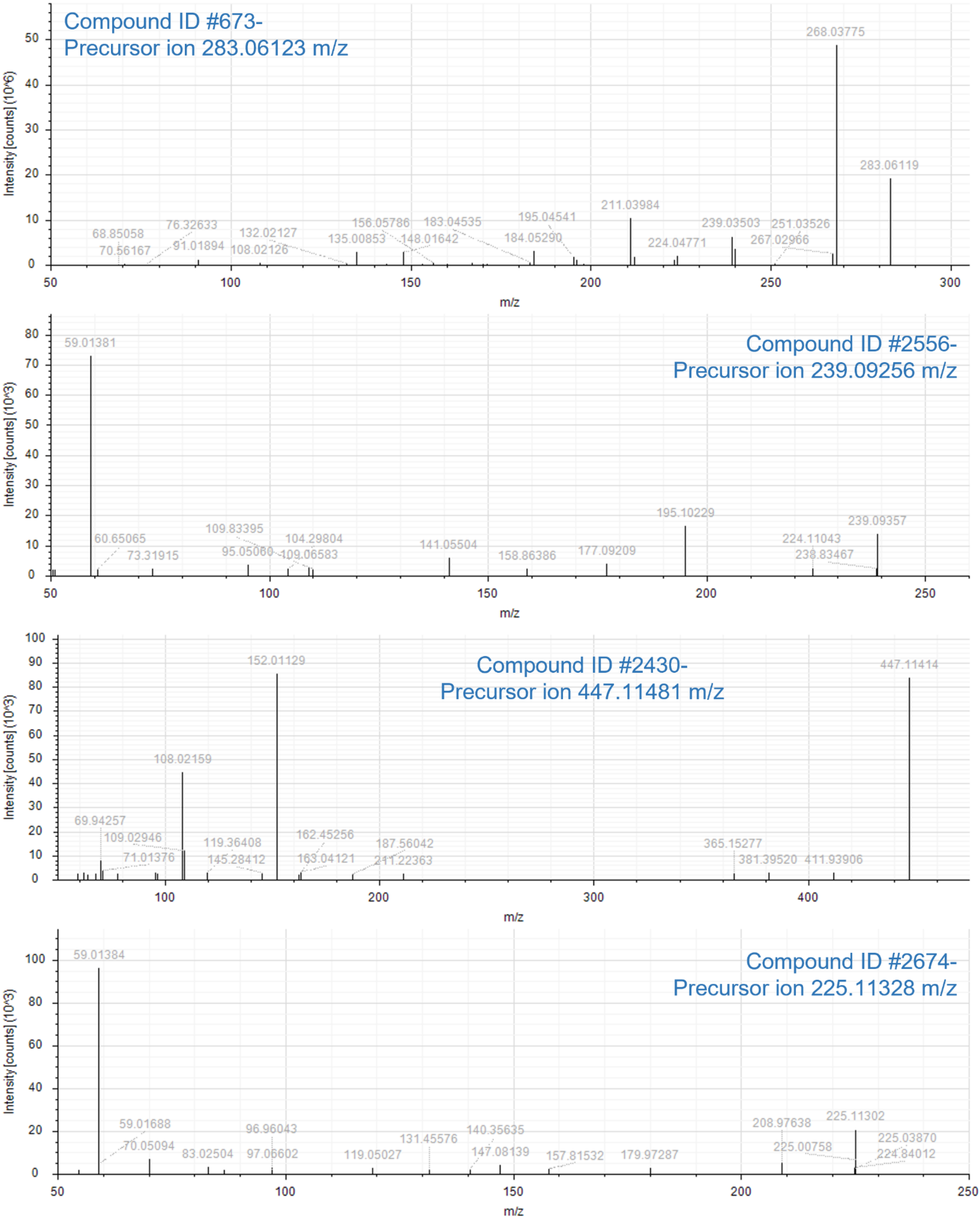

Figure S21. MS/MS spectra of compounds with IDs 673-, 2556-, 2430-, and 2674-.

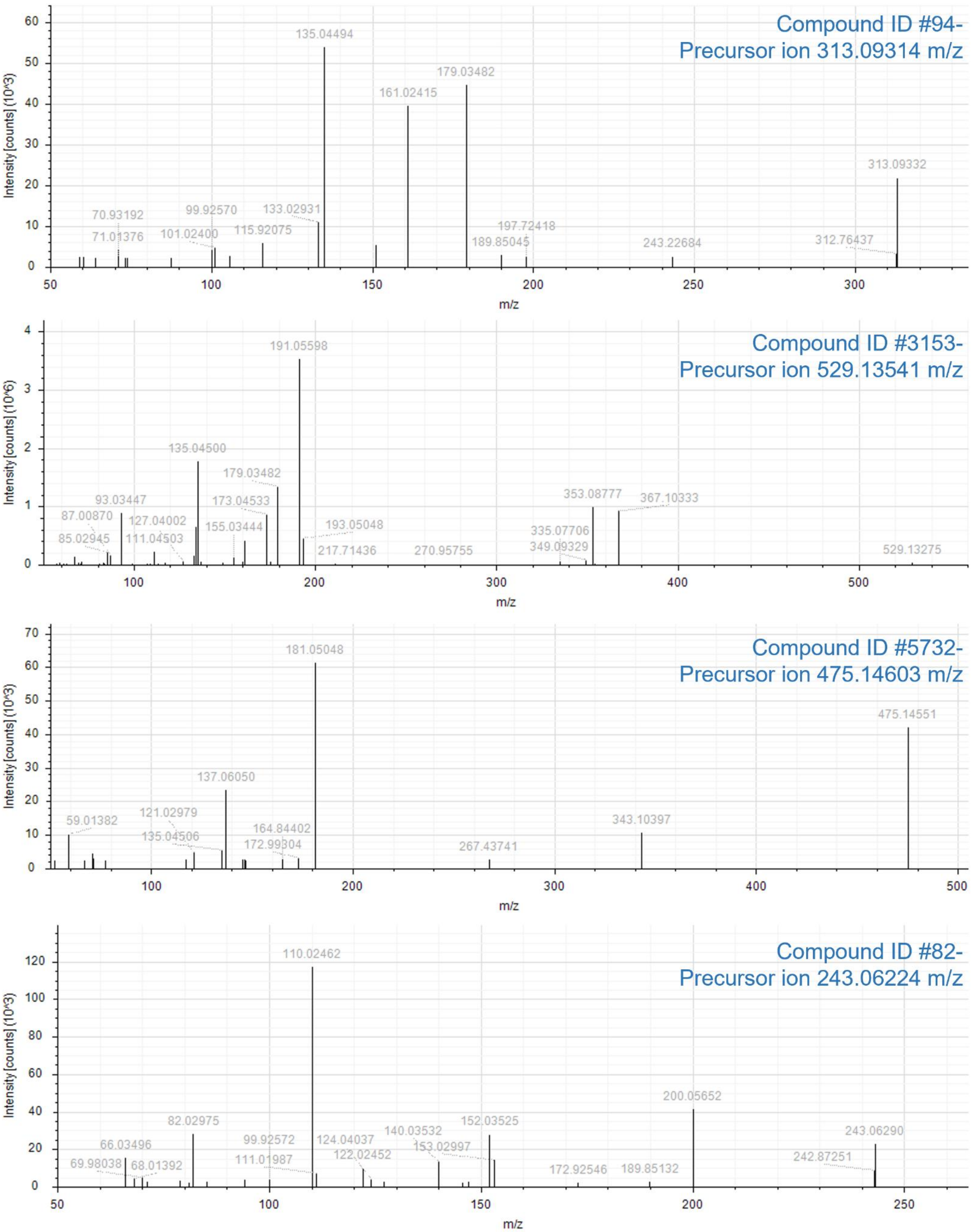

Figure S22. MS/MS spectra of compounds with IDs 94-, 3153-, 5732-, and 82-.

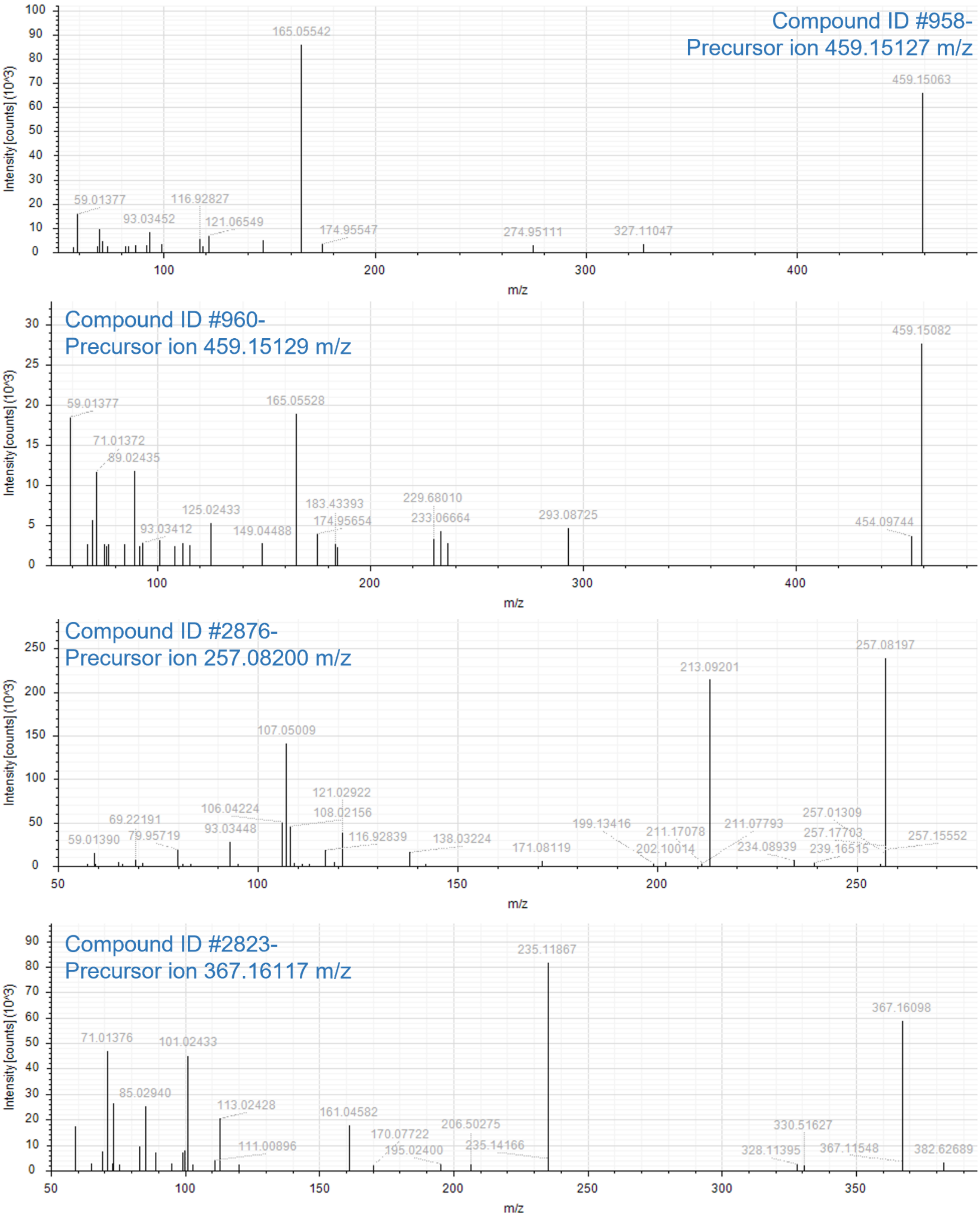

Figure S23. MS/MS spectra of compounds with IDs 958-, 960-, 2876-, and 2823-.

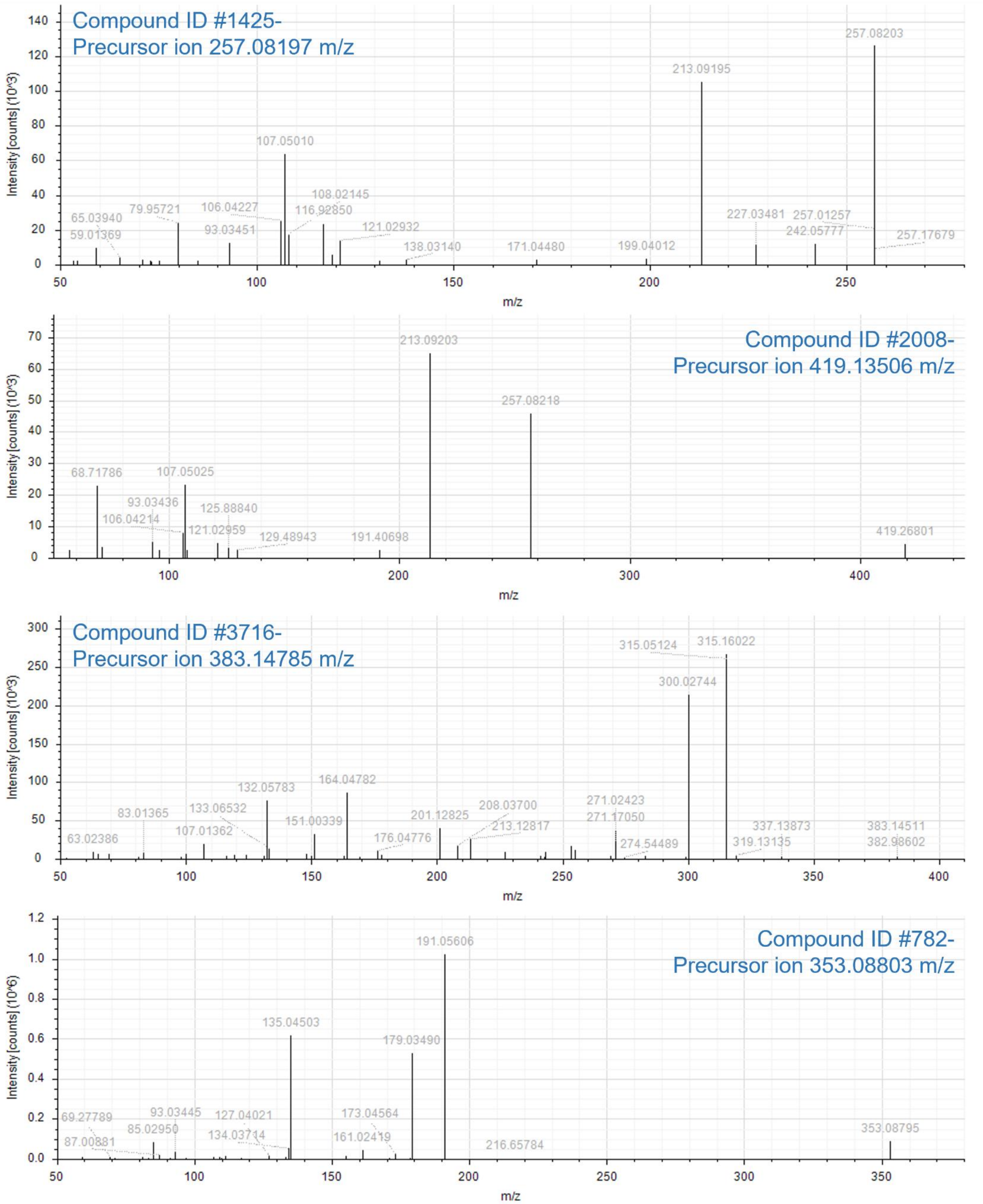

Figure S24. MS/MS spectra of compounds with IDs 1425-, 2008-, 3716-, and 782-.

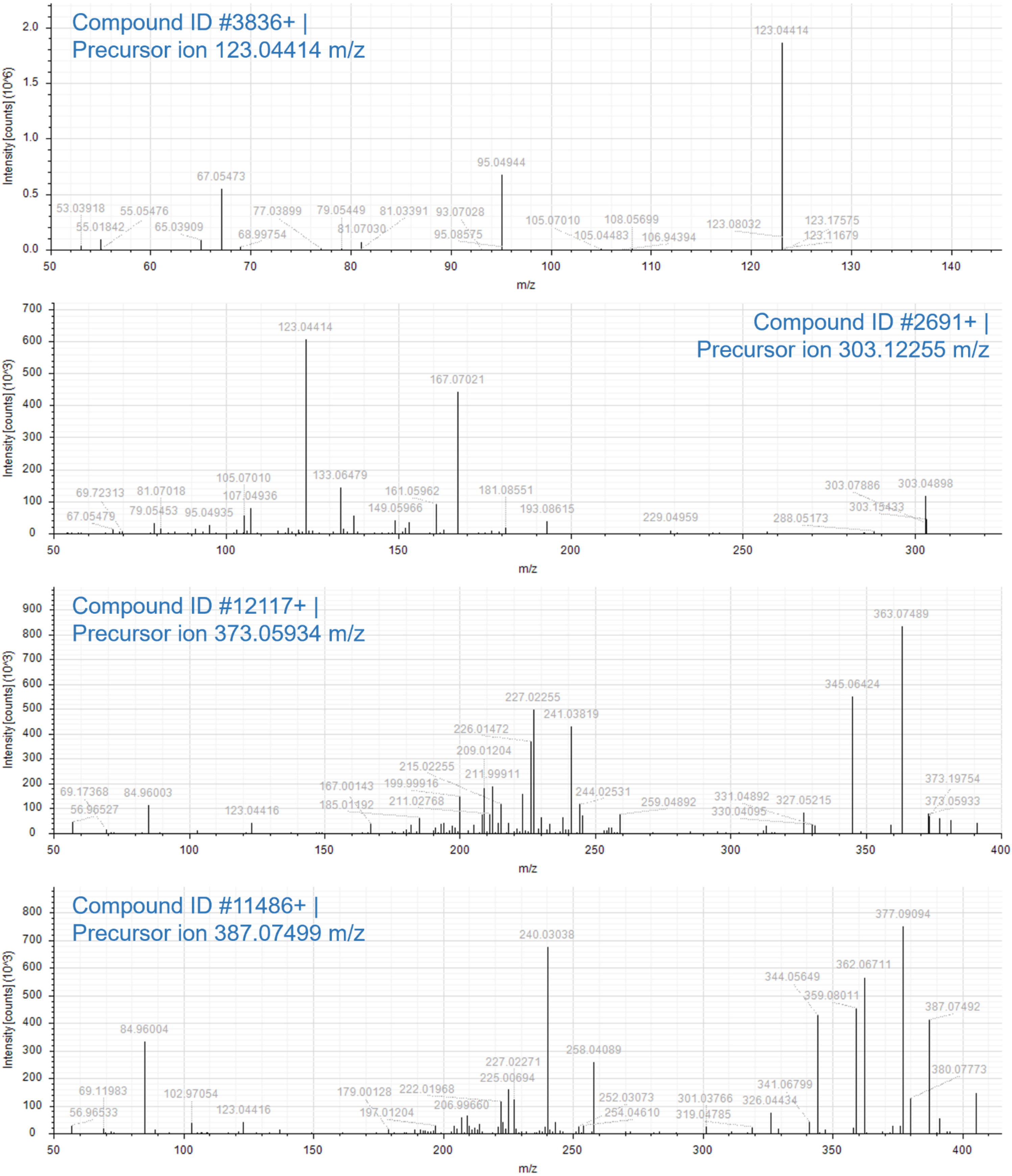

Figure S25. MS/MS spectra of compounds with IDs 3836+, 2691+, 12117+, and 11486+.

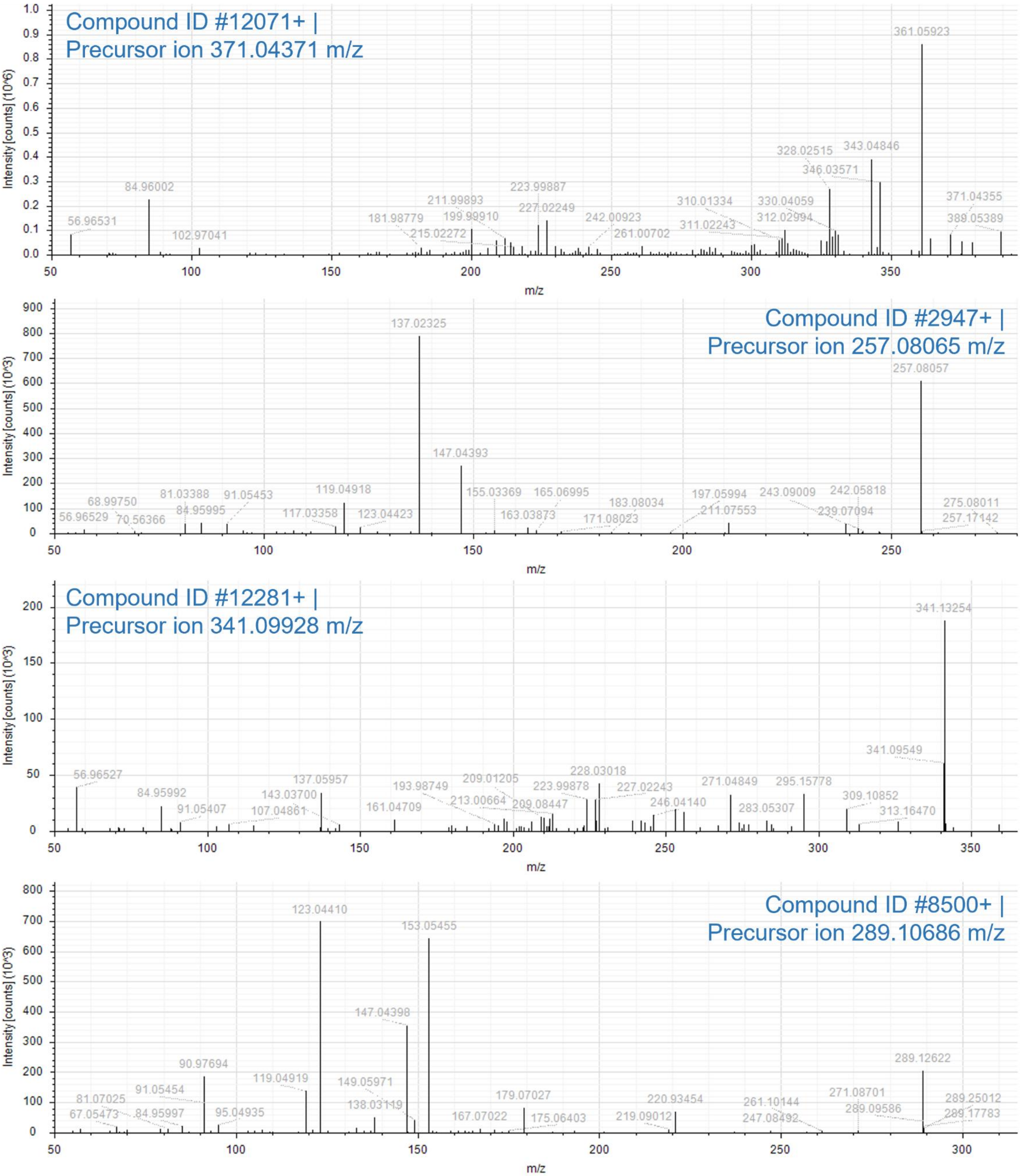

Figure S26. MS/MS spectra of compounds with IDs 12071+, 2947+, 12281+, and 8500+.

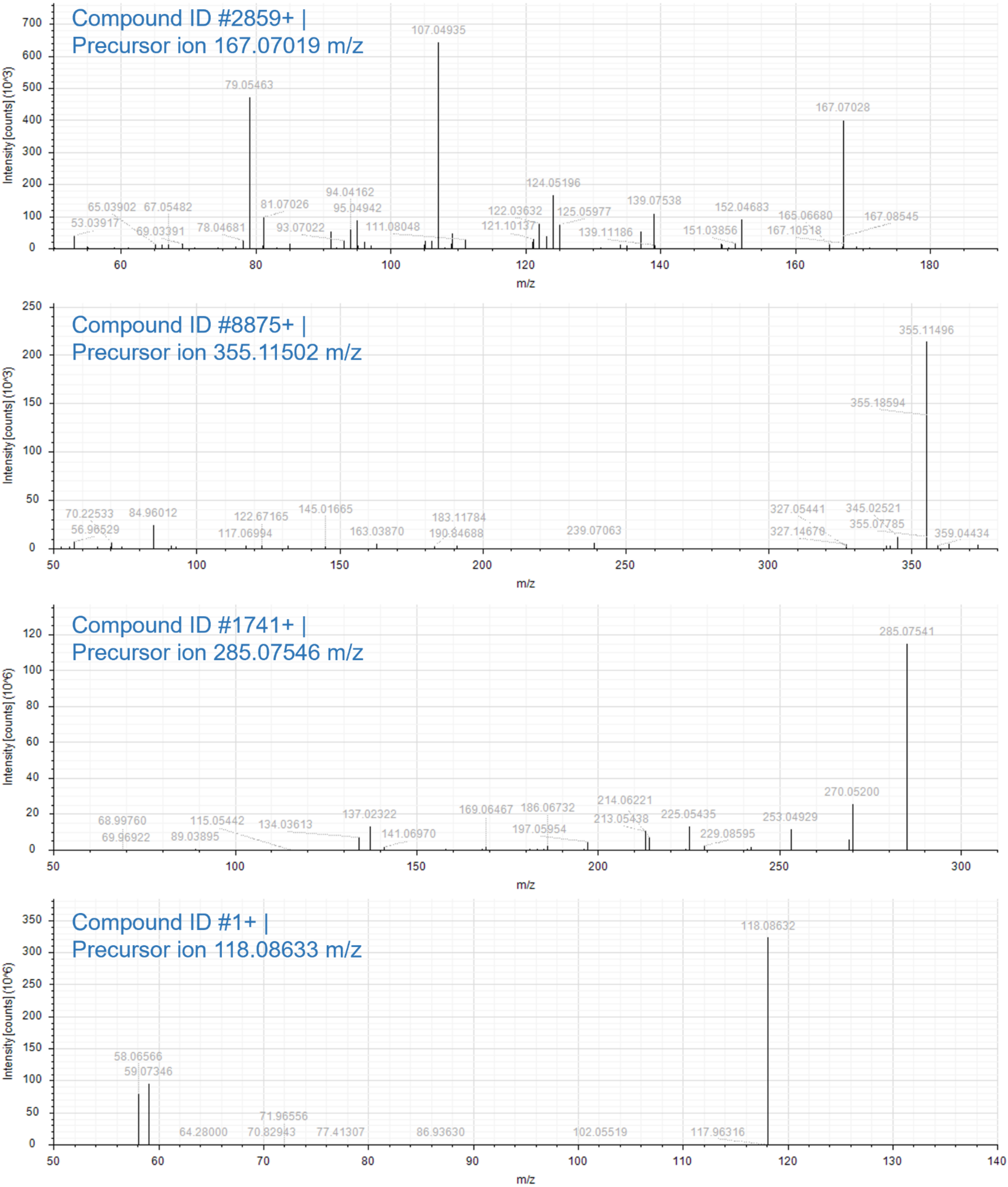

Figure S27. MS/MS spectra of compounds with IDs 2859+, 8875+, 1741+, and 1+.

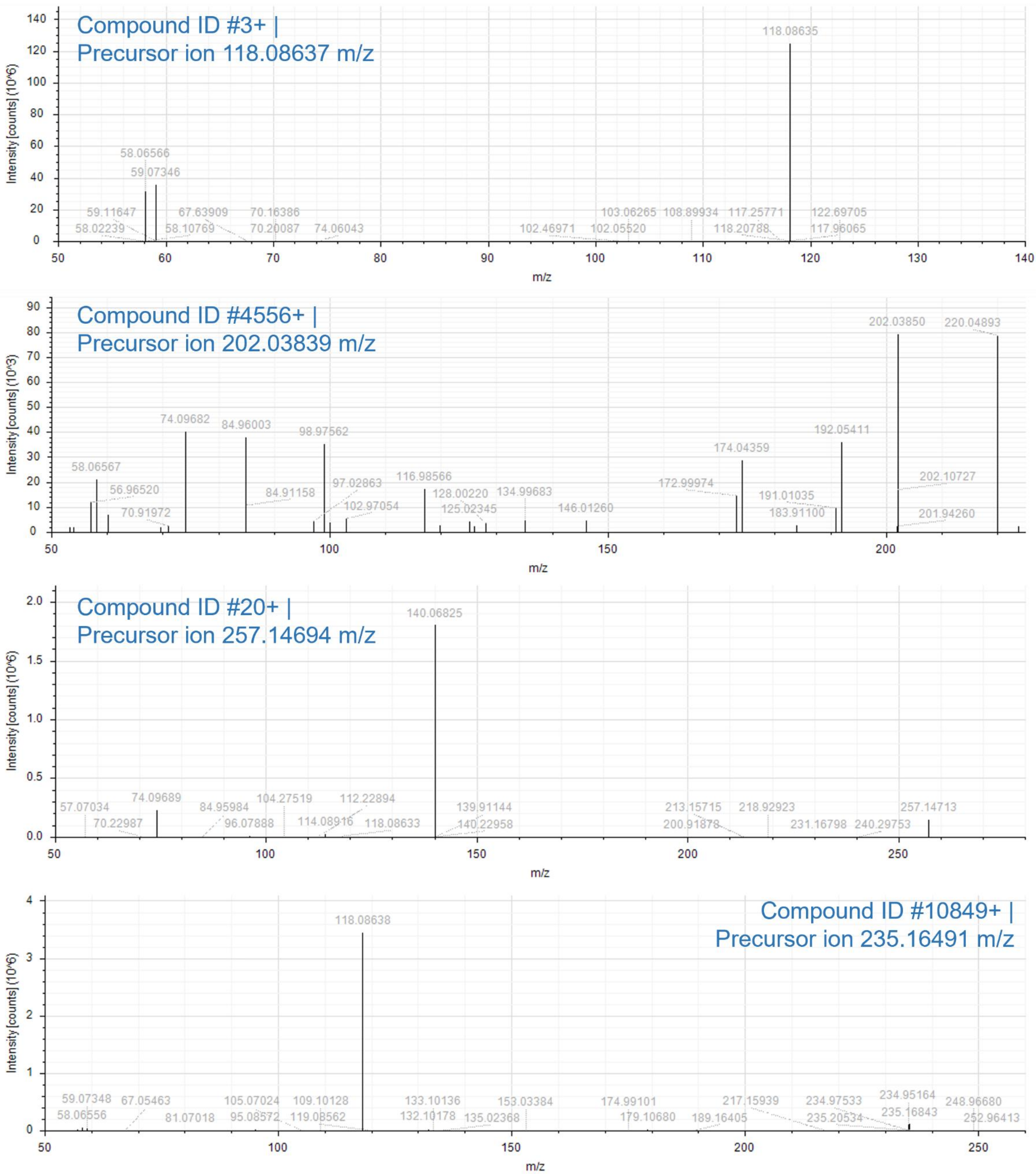

Figure S28. MS/MS spectra of compounds with IDs 3+, 4556+, 20+, and 10849+.

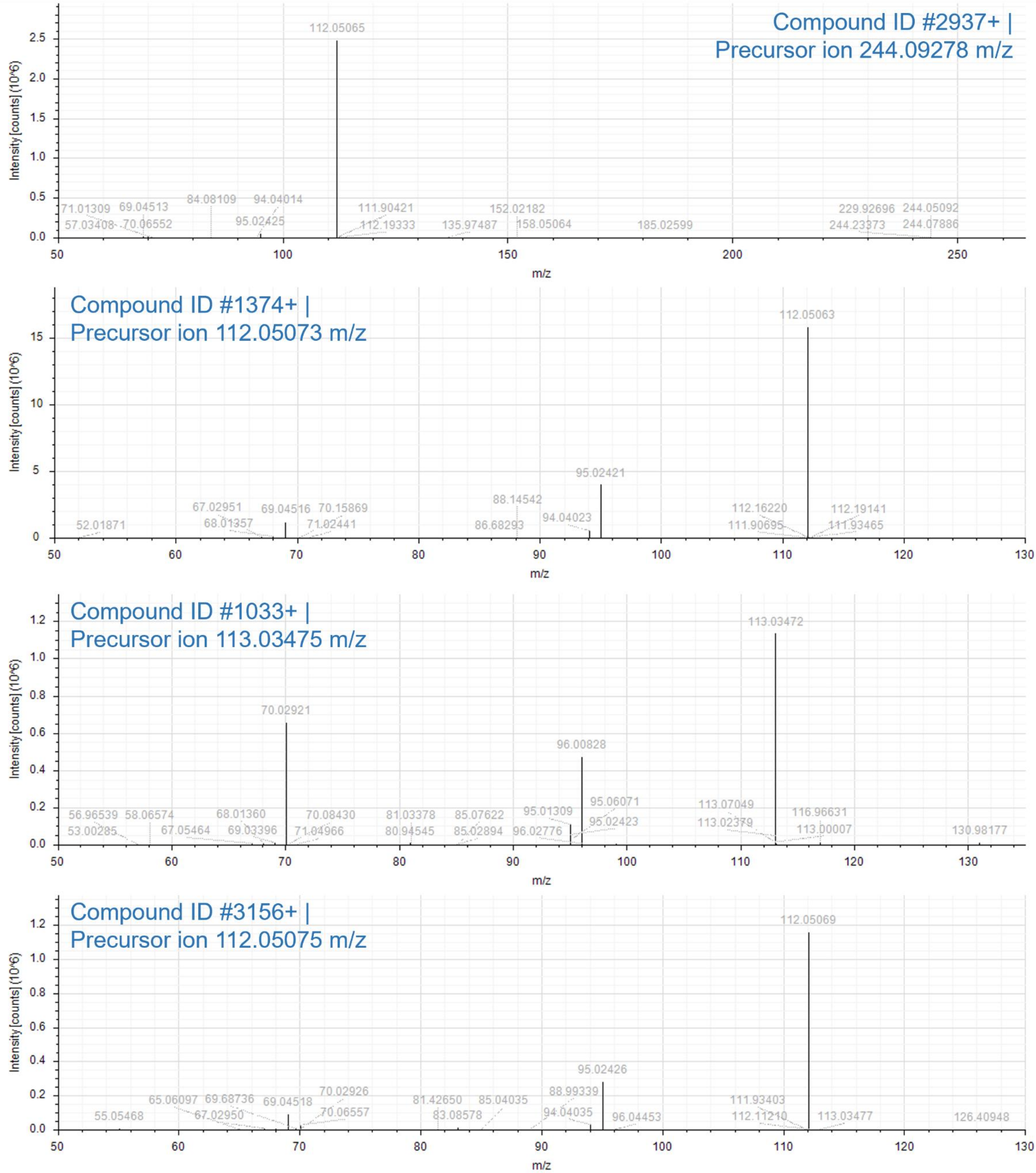

Figure S29. MS/MS spectra of compounds with IDs 2937+, 1374+, 1033+, and 3156+.

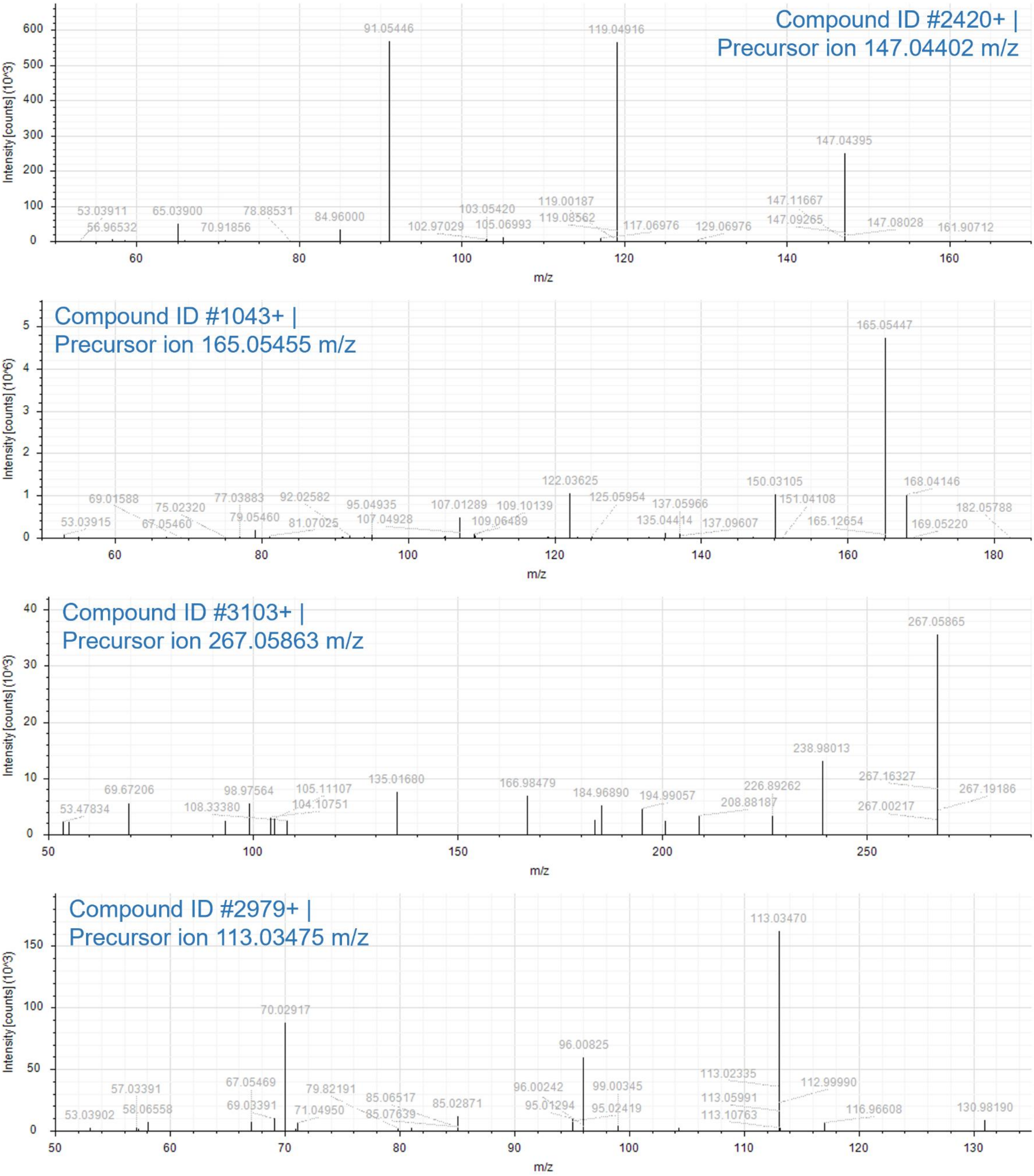

Figure S30. MS/MS spectra of compounds with IDs 2420+, 1043+, 3103+, and 2979+.

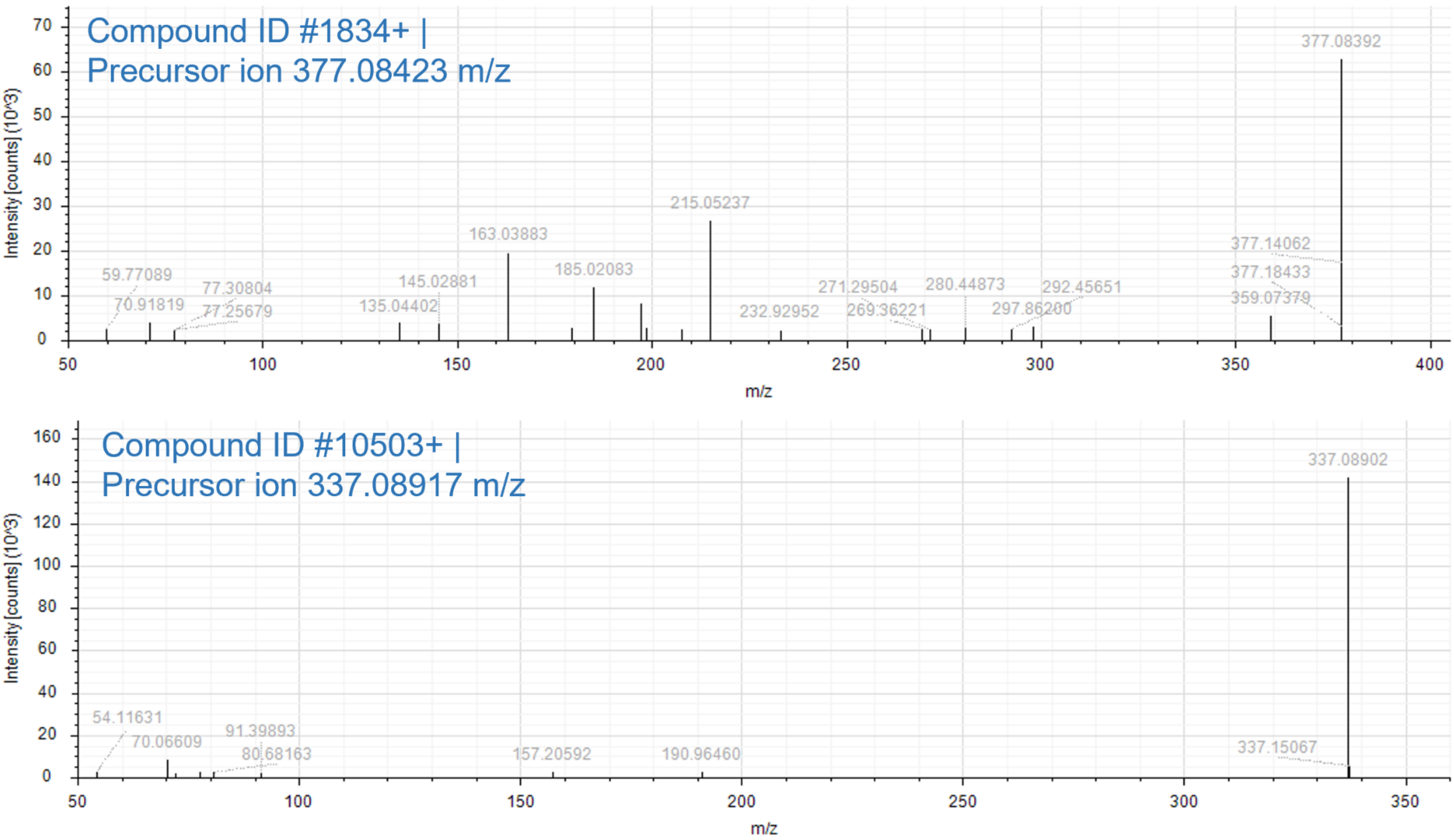

Figure S31. MS/MS spectra of compounds with IDs 1834+ and 10503+.