SUPPORTING INFORMATION

An adductomics pipeline for untargeted analysis of modifications to Cys34 of human serum albumin

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Table S1. Characteristics and estimated intake of dietary fat across subjects represented by 34 pooled plasma samples.

	Race			Gender			Smoking Status		
Characteristics of Pooled Samples[a]	**Black (B)**	**White (W)**	***P*-value[c]**	**Male (M)**	**Female (F)**	***P*-value[c]**	**Smoker (S)**	**Non-Smoker (NS)**	***P*-value[c]**
Number of subjects	18 (9M, 9F; 9S, 9NS)	17 (9M, 8F; 10S, 7NS)		18 (9B, 9W; 10S, 8NS)	17 (9B, 8W; 9S, 8NS)		19 (10M, 9F; 9B, 10W)	16 (8M, 8F; 9B, 7W)	
Age (y)	26 ±2	25 ± 2	0.148	26 ±3	25 ± 2	0.627	26 ±3	25 ± 2	0.516
BMI (kg/m2)	28.0±3.56	24.1±1.64	2.20E-04	25.7±2.21	26.6±4.35	0.41	26.9±3.94	25.2±2.42	0.143
Animal Fat (g/day) b	59.7±21.82	39.2±9.84	1.38E-03	54.3±22.47	45.0±15.78	0.168	57.9±22.03	40.1±11.01	5.91E-03
Vegetable Fat (g/day) b	39.2±14.5	27.3±5.67	3.77E-03	35.0±13.0	31.7±12.1	0.431	38.9±14.0	26.9±6.17	3.34E-03

[a] Between 4 and 6 individual plasma specimens were pooled by race, gender and smoking status.
[b] Dietary fats and fatty acids were compiled from standardized food frequency questionnaires applied to individual subjects and averaged for pooled-plasma specimens in the current investigation.
[c] Based on Student's *t*-test.

Table S2. Evidence used to annotate putative T3 adducts. Accurate masses for 32 adducts led to reasonable elemental compositions added to the thiol of Cys34 in the T3 peptide within three ppm of theoretical values. Annotations of adducts are based on calculated accurate masses, MS2 spectra, literature and database searches and include several classes of modifications. Monoisotopic masses and chromatographic elution times of eleven adducts were confirmed by their synthetic standards. Twenty one adducts were previously reported in the literature as adducts of Cys34, glutathione or other thiols. Database references contain accession numbers from Unimod (www.unimod.org), UniProt (www.uniprot.org) and PubChem (https://pubchem.ncbi.nlm.nih.gov).

Adduct	Annotation	Synthetic Standard	Database references	Published references *	Proteins reported modified	Notes
A1	Cys34→Gly		UNIMOD #552	PMID: 15063314, PMID: 21075673	serum transthyretin, papain	
A2	Cys34→Dehydroalanine		UNIMOD #368	PMID: 15063314, PMID: 18265430, 1	serum transthyretin, serum albumin, synthetic peptides	
A3	Cys34→Oxoalanine/Formylglycine		UNIMOD #402	PMID: 18722427, PMID: 17450134, PMID: 25514000, PMID: 21075673, PMID: 19575405	recombinant protiens, papain, serum albumin	
A4	Not Cys34 adduct					$y7^{2+}$ or/and $y7^{+}$ were not detected in MS2. $b14^{+}$ was detected in MS2
A5	Not Cys34 adduct					$y7^{2+}$ or/and $y7^{+}$ were not detected in MS2. $b14^{+}$ was detected in MS2
A6	T3 Labile adduct			1	synthetic peptids	
A7	Unmodified T3	+				
A8	T3 Dimer	+				
A9	Cys34-Gln crosslink	+	UniProt: KW-0882	PMID: 22591159, PMID: 20729215, PMID: 24220033	serum albumin, pilus adhesin from *Streptococcus pyogenes*	
A10	Methylation (not Cys34)		UNIMOD #34			
A11	Cyanide adduct		UNIMOD #438	PMID: 24916017, PMID: 21366342, PMID: 21148632	Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH), serum albumin	

A12	Cys34 Sulfinic acid	+	UNIMOD #425	PMID: 24916017, PMID: 21148632, PMID: 24416365	serum albumin, Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)	
A13	Not Cys34 adduct					$y7^{2+}$ or/and $y7^{+}$ were not detected in MS2. $b14^{+}$ was detected in MS2
A14	Ethylene oxide adduct			PMID: 25407640	recombinant human serum albumin	
A15	Cys34 Sulfonic acid	+	UNIMOD #345	PMID: 24916017, PMID: 21148632, PMID: 24416365	serum albumin, Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)	
A16	Not Cys34 adduct					$y7^{2+}$ or/and $y7^{+}$ were not detected in MS2. $b14^{+}$ was detected in MS2
A17	Not Cys34 adduct					$y7^{2+}$ or/and $y7^{+}$ were not detected in MS2. $b14^{+}$ was detected in MS2
A18	Acrylonitrile adduct			PMID: 10563837, PMID: 22231508, PMID: 5656374	Glutathione, wheat gluten, bovine serum albumin	
A19	Na adduct of A12		UNIMOD #30	PMID: 16444685	synthetic peptides	
A20	Methylisocyanate adduct			PMID: 2302207	glutathione	
A21	Methylvinylketone adduct			PMID: 25087629, PMID: 1897958, PMID: 22084934	Glutathione, Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)	
A22	S-Mercaptoacetamide		CID: 12961			
A23	S-Mercaptoacetic acid		CID: 1133			
A24	S-Cys (-H2O)	+	UNIMOD #23	PMID: 25800200	synthetic peptides	
A25	Unknown					
A26	Unknown					
A27	Unknown					
A28	S-hCys (-H2O)		UNIMOD #23	PMID: 25800200	synthetic peptides	
A29	S-Cys	+	UNIMOD #312	PMID: 24416365, PMID: 23215783, PMID: 15791656, PMID: 18624771 2	human serum albumin	
A30	S-Cys (NH2→OH)					
A31	Unknown					
A32	S-hCys	+		PMID: 17285228,	serum albumin,	

A33	S-hCys	+	UNIMOD #1271	PMID: 17760510, PMID: 16395265, PMID: 18624771, PMID: 26658763	metallothionein, serum	
A34	Unknown					
A35	Na adduct of A29			PMID: 16444685		
A36	Not Cys34 adduct					$y7^{2+}$ or/and $y7^{+}$ were not detected in MS2. $b14^{+}$ was detected in MS2
A37	K adduct of A29					
A38	S-Cys-Gly (-H_2O)		UNIMOD #23	PMID: 25800200	synthetic peptides	
A39	S-(*N* -acetyl)Cys			PMID: 15499198	human serum albumin	
A40	S-Cys-Gly	+		PMID: 18624771, PMID: 1632485	human serum albumin	
A41	Not Cys34 adduct					$y7^{2+}$ or/and $y7^{+}$ were not detected in MS2. $b14^{+}$ was detected in MS2
A42	S-γ-Gln-Cys			PMID: 24738021	human serum albumin	
A43	S-Glutathione	+	UNIMOD #55	PMID: 18624771, PMID: 1632485	suman serum albumin	

* Citations:

1 Jianyong Wu-PhD thesis 2009/ Tandem Mass Spectrometric Analysis of Protein and Peptide Adducts of Lipid Peroxidation-Derived Aldehydes

2 http://www.ionsource.com/Card/Deamidation/mono0001.htm

Table S3. Covariance parameters for mixed models
(performed with natural logarithms of peak-area ratios) and estimated CVs.

CovParm' refers to the covariance parameter for the mixed model.
ID' refers to the between-specimen variance component
Residual' refers to the within-specimen variance component (error variance)
CV' refers to the coefficient variation estimated from the within-specimen variance component

Variable is ln(peak area adduct*1000/peak area housekeeping peptide)

Adduct	CovParm	Subject	Estimate	CV
1	ID	ID	0.007431	
1	Residual		0.1788	0.442472
2	ID	ID	0	
2	Residual		0.1288	0.37076
3	ID	ID	0.2064	
3	Residual		0.2041	0.475837
4	ID	ID	0	
4	Residual		0.3211	0.61534
5	ID	ID	0.01576	
5	Residual		0.1574	0.412873
6	ID	ID	0.02108	
6	Residual		0.08073	0.289962
7	ID	ID	0.09397	
7	Residual		0.1066	0.335394
8	ID	ID	0.09089	
8	Residual		1.0419	1.354473
9	ID	ID	0.02888	
9	Residual		0.07329	0.275758
10	ID	ID	0.2159	
10	Residual		0.2709	0.557803
11	ID	ID	0.2552	
11	Residual		0.1288	0.37076
12	ID	ID	0.02255	
12	Residual		0.08476	0.297415
13	ID	ID	0	
13	Residual		0.101	0.326001
14	ID	ID	0.1335	
14	Residual		0.07254	0.274291
15	ID	ID	0.08514	
15	Residual		0.09789	0.320689
16	ID	ID	0.3442	
16	Residual		0.396	0.697043
17	ID	ID	0.007284	
17	Residual		0.2534	0.537027
18	ID	ID	0.2221	
18	Residual		0.09907	0.322713

19	ID	ID	0.1866	
19	Residual		0.05752	0.243324
20	ID	ID	0.000045	
20	Residual		0.006651	0.081689
21	ID	ID	0.01044	
21	Residual		0.1809	0.445304
22	ID	ID	0.01105	
22	Residual		0.09973	0.32384
23	ID	ID	0.05202	
23	Residual		0.07186	0.272956
24	ID	ID	0	
24	Residual		0.1009	0.325831
25	ID	ID	0.3888	
25	Residual		0.3133	0.606574
26	ID	ID	0.144	
26	Residual		0.08373	0.295525
27	ID	ID	0.1256	
27	Residual		0.07608	0.281156
28	ID	ID	0.06713	
28	Residual		0.1579	0.413581
29	ID	ID	0.00438	
29	Residual		0.05193	0.230872
30	ID	ID	0.04683	
30	Residual		0.06135	0.251537
31	ID	ID	0.07844	
31	Residual		0.1426	0.391495
32	ID	ID	0	
32	Residual		0.6284	0.935205
33	ID	ID	0.0389	
33	Residual		0.06382	0.256711
34	ID	ID	0.1894	
34	Residual		0.1172	0.352625
35	ID	ID	0.02206	
35	Residual		0.2132	0.487475
36	ID	ID	0.1587	
36	Residual		0.3216	0.6159
37	ID	ID	0.01711	
37	Residual		0.2098	0.483147
38	ID	ID	0.09685	
38	Residual		0.2378	0.518127
39	ID	ID	0.1692	
39	Residual		0.1002	0.324641
40	ID	ID	0.01503	
40	Residual		0.05735	0.242954
41	ID	ID	0.05769	
41	Residual		0.1852	0.451064
42	ID	ID	0	

42	Residual		0.07828	0.285351
43	ID	ID	0.05674	
43	Residual		0.09891	0.322439

Table S4. P-values for 2-sided Wilcoxon (exact) tests for smoke, race, gender

smoke		Bonferroni adjusted P-value (0.05/34=0.00147)
Adduct	P-value	0=Not Sig,1=Sig
A1	0.08286	0
A3	0.97082	0
A5	0.22808	0
A6	0.25613	0
A7	0.86416	0
A8	0.16747	0
A9	0.01375	0
A10	0.01354	0
A11	0.02502	0
A12+A19	0.00058	1
A14	0	1
A15	0.00503	0
A16	0.10119	0
A17	0.17666	0
A18	0	1
A20	1	0
A21	0.60121	0
A22	0.46658	0
A23	0.58361	0
A25	0.95189	0
A26	0.28442	0
A27	0.80984	0
A28	0.5601	0
A29+A35+A37	0.0012	1
A30	0.65622	0
A31	0.06044	0
A33	0.31896	0
A34	0.03956	0
A36	0.09281	0
A38	0.60741	0
A39	0.0063	0
A40	0.28641	0
A41	0.68065	0
A43	0.73213	0

race		
Adduct	P-value	
A1	0.9188	0
A3	0.56038	0
A5	0.27429	0

A6	0.04103	0
A7	0.20538	0
A8	0.94581	0
A9	0.97289	0
A10	0.65157	0
A11	0.51772	0
A12+A19	0.37528	0
A14	0.88672	0
A15	0.25941	0
A16	0.09604	0
A17	0.60227	0
A18	0.66206	0
A20	0.72495	0
A21	0.75323	0
A22	0.86706	0
A23	0.13089	0
A25	0.97924	0
A26	0.19656	0
A27	0.16865	0
A28	0.03434	0
A29+A35+37	0.78566	0
A30	0.89187	0
A31	0.20538	0
A33	0.47446	0
A34	0.00539	0
A36	0.25506	0
A38	0.07886	0
A39	0.03413	0
A40	0.56289	0
A41	0.39323	0
A43	0.20538	0

gender

Adduct	P-value	
A1	0.29683	0
A3	0.15742	0
A5	0.22382	0
A6	0.77225	0
A7	0.67023	0
A8	0.57382	0
A9	0.42243	0
A10	0.91272	0
A11	0.93218	0
A12+A19	0.85147	0
A14	0.57991	0
A15	0.4632	0
A16	0.32941	0

A17	0.23015	0
A18	0.62231	0
A20	1	0
A21	0.87173	0
A22	0.40176	0
A23	0.52815	0
A25	0.73816	0
A26	0.30581	0
A27	0.31587	0
A28	0.23716	0
A29+A35+A37	0.98642	0
A30	0.22382	0
A31	0.87825	0
A33	0.82486	0
A34	0.40284	0
A36	0.74854	0
A38	0.93218	0
A39	0.52742	0
A40	0.38379	0
A41	0.31228	0
A43	0.28112	0

Table S5
Multivariable regression of adduct PARs (adduct peak area*1000/housekeeping-peptide peak area, in natural scale).
Reference groups for dichotomous covariates :smoke (0=NS), race (0=W), gender (0=F)
Units for continuous covariates (mean value for each pooled specimen): mnbmi (kg/m^2), mnafat (g/d), mnvfat (g/d)

Adduct 1
The REG Procedure
Model: MODEL1
Dependent Variable: A1

Number of Observations Read	34
Number of Observations Used	34

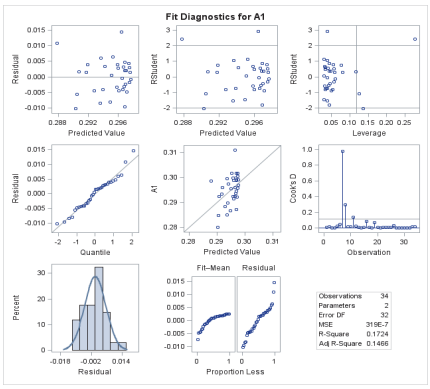
Analysis of Variance

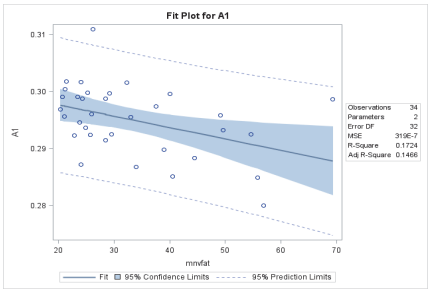
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.000213	0.000213	6.67	0.0146
Error	32	0.00102	3.19E-05		
Corrected Total	33	0.00124			

Root MSE	0.00565	R-Square	0.1724
Dependent Mean	0.29506	Adj R-Sq	0.1466
Coeff Var	1.91541		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.30172	0.00276	109.38	<.0001	.	0.29611	0.30734
mnvfat	1	-0.0002	7.79E-05	-2.58	0.0146	0.17244	-0.00036	-4.2E-05





Adduct 5
The REG Procedure
Model: MODEL1
Dependent Variable: A5

Number of Observations Read	34
Number of Observations Used	34

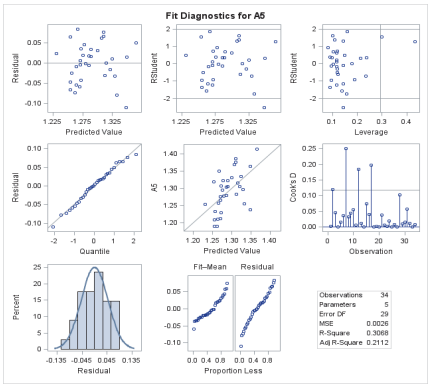
Analysis of Variance

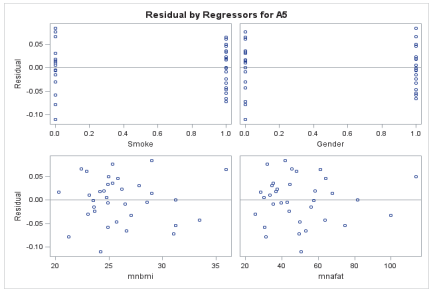
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.03357	0.00839	3.21	0.0268
Error	29	0.07584	0.00262		
Corrected Total	33	0.10941			

Root MSE	0.05114	R-Square	0.3068
Dependent Mean	1.29029	Adj R-Sq	0.2112
Coeff Var	3.9633		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	1.402	0.07153	19.6	<.0001	.	1.25569	1.5483
Smoke	1	-0.04392	0.02003	-2.19	0.0365	0.04502	-0.08488	-0.00296
Gender	1	-0.03932	0.01877	-2.09	0.045	0.02712	-0.07771	-0.00093
mnbmi	1	-0.00573	0.00291	-1.97	0.059	0.02043	-0.01168	0.000232
mnafat	1	0.00166	0.000555	2.99	0.0056	0.21427	0.000527	0.0028





Adduct 6
The REG Procedure
Model: MODEL1
Dependent Variable: A6

Number of Observations Read	34
Number of Observations Used	34

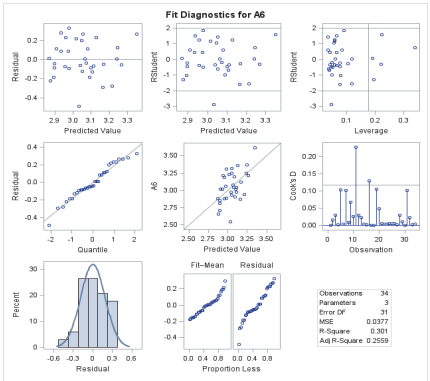
Analysis of Variance

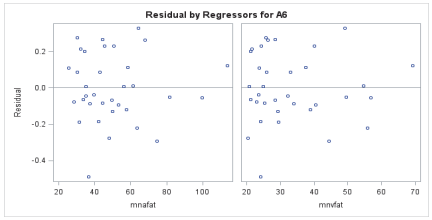
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.50267	0.25134	6.67	0.0039
Error	31	1.1676	0.03766		
Corrected Total	33	1.67027			

Root MSE	0.19407	R-Square	0.301
Dependent Mean	3.05003	Adj R-Sq	0.2559
Coeff Var	6.363		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	2.96724	0.09647	30.76	<.0001	.	2.77049	3.16399
mnafat	1	0.01127	0.00309	3.65	0.001	0.10369	0.00497	0.01757
mnvfat	1	-0.0145	0.0049	-2.96	0.0059	0.19726	-0.02449	-0.0045





Adduct 9
The REG Procedure
Model: MODEL1
Dependent Variable: A9

Number of Observations Read	34
Number of Observations Used	34

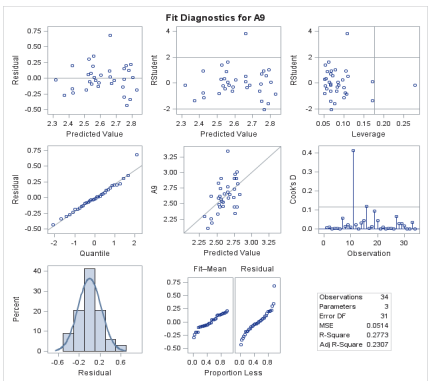
Analysis of Variance

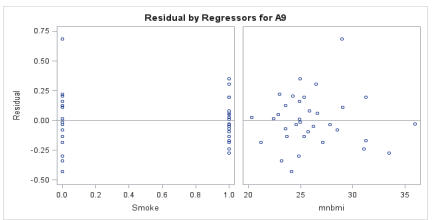
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.61165	0.30583	5.95	0.0065
Error	31	1.59424	0.05143		
Corrected Total	33	2.2059			

Root MSE	0.22678	R-Square	0.2773
Dependent Mean	2.61768	Adj R-Sq	0.2307
Coeff Var	8.66322		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	3.29391	0.30373	10.84	<.0001	.	2.67444	3.91337
Smoke	1	-0.1885	0.08128	-2.32	0.0271	0.1983	-0.35426	-0.02273
mnbmi	1	-0.02187	0.01188	-1.84	0.0753	0.07898	-0.04611	0.00236





Adduct 10

The REG Procedure
Model: MODEL1
Dependent Variable: A10

Number of Observations Read	34
Number of Observations Used	34

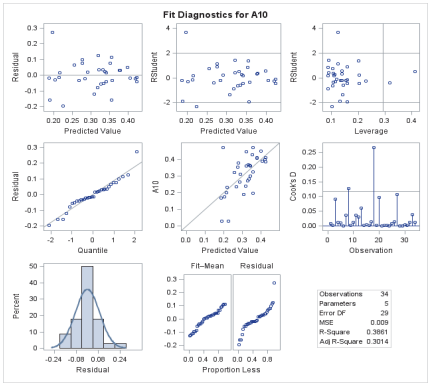
Analysis of Variance

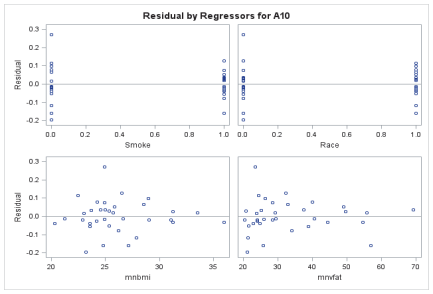
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.16352	0.04088	4.56	0.0056
Error	29	0.26001	0.00897		
Corrected Total	33	0.42353			

Root MSE	0.09469	R-Square	0.3861
Dependent Mean	0.3119	Adj R-Sq	0.3014
Coeff Var	30.35886		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.55768	0.1504	3.71	0.0009	.	0.25008	0.86527
Smoke	1	0.17449	0.04305	4.05	0.0003	0.18807	0.08644	0.26254
Race	1	0.13507	0.04687	2.88	0.0074	0.03612	0.03921	0.23093
mnbmi	1	-0.01092	0.00639	-1.71	0.0981	0.07672	-0.02398	0.00215
mnvfat	1	-0.0038	0.00189	-2.01	0.0543	0.08518	-0.00767	7.44E-05





Adduct 11

The REG Procedure
Model: MODEL1
Dependent Variable: A11

Number of Observations Read	34
Number of Observations Used	34

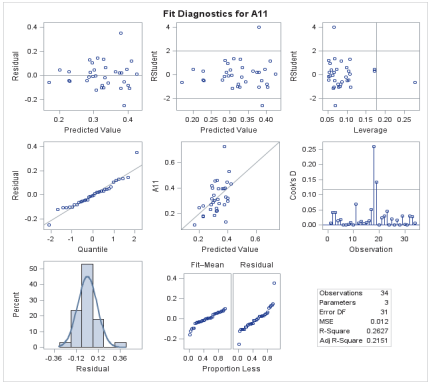
Analysis of Variance

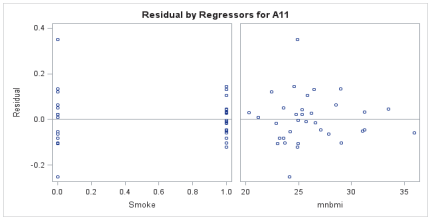
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.13292	0.06646	5.52	0.0089
Error	31	0.3731	0.01204		
Corrected Total	33	0.50602			

Root MSE	0.10971	R-Square	0.2627
Dependent Mean	0.32374	Adj R-Sq	0.2151
Coeff Var	33.88719		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.68971	0.14693	4.69	<.0001	.	0.39003	0.98938
Smoke	1	-0.07266	0.03932	-1.85	0.0742	0.15085	-0.15285	0.00753
mnbmi	1	-0.01247	0.00575	-2.17	0.0379	0.11184	-0.02419	-0.00074





Adduct (A44=A12+A19)

The REG Procedure
Model: MODEL1
Dependent Variable: A12+A19

Number of Observations Read	34
Number of Observations Used	34

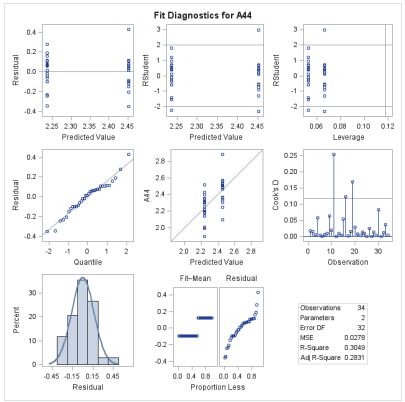
Analysis of Variance

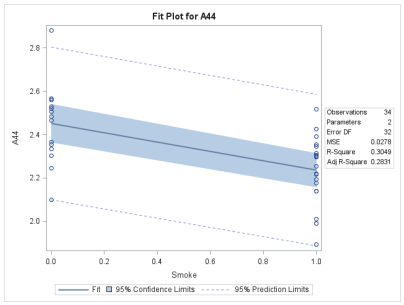
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.39003	0.39003	14.03	0.0007
Error	32	0.88935	0.02779		
Corrected Total	33	1.27938			

Root MSE	0.16671	R-Square	0.3049
Dependent Mean	2.3314	Adj R-Sq	0.2831
Coeff Var	7.15063		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	2.45194	0.04304	56.96	<.0001	.	2.36426	2.53962
Smoke	1	-0.21571	0.05758	-3.75	0.0007	0.30486	-0.333	-0.09842





Adduct A14

The REG Procedure
Model: MODEL1
Dependent Variable: A14

Number of Observations Read	34
Number of Observations Used	34

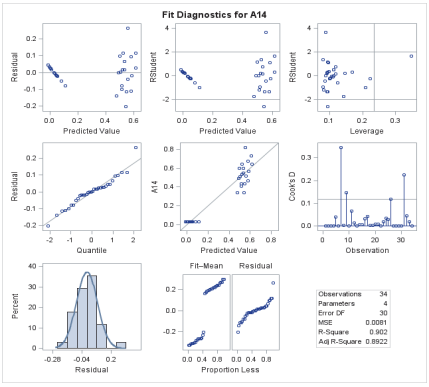
Analysis of Variance

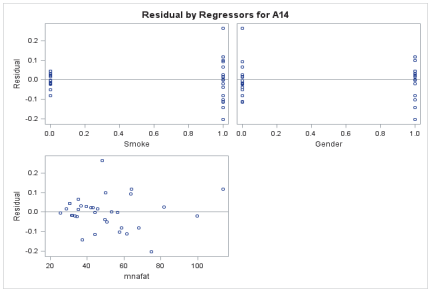
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2.23817	0.74606	92.04	<.0001
Error	30	0.24318	0.00811		
Corrected Total	33	2.48135			

Root MSE	0.09003	R-Square	0.902
Dependent Mean	0.31581	Adj R-Sq	0.8922
Coeff Var	28.50913		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	-0.00878	0.04272	-0.21	0.8385	.	-0.09603	0.07846
Smoke	1	0.47951	0.03515	13.64	<.0001	0.88363	0.40773	0.55128
Gender	1	-0.05872	0.03205	-1.83	0.0769	0.00618	-0.12418	0.00673
mnafat	1	0.00175	0.000908	1.93	0.0629	0.01219	-0.0001	0.00361





Adduct 15
The REG Procedure
Model: MODEL1
Dependent Variable: A15

Number of Observations Read 34
Number of Observations Used 34

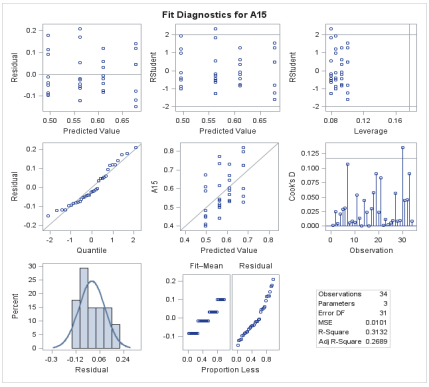
Analysis of Variance

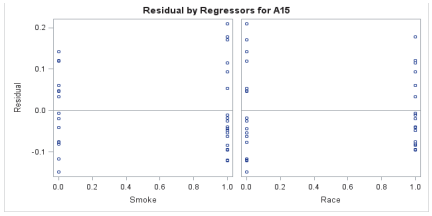
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.14228	0.07114	7.07	0.003
Error	31	0.31201	0.01006		
Corrected Total	33	0.4543			

Root MSE	0.10032	R-Square	0.3132
Dependent Mean	0.57945	Adj R-Sq	0.2689
Coeff Var	17.31361		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.6775	0.03176	21.33	<.0001	.	0.61272	0.74229
Smoke	1	-0.11554	0.03471	-3.33	0.0023	0.22958	-0.18634	-0.04474
Race	1	-0.06697	0.03447	-1.94	0.0612	0.08361	-0.13727	0.00334





Adduct 16
The REG Procedure
Model: MODEL1
Dependent Variable: A16

Number of Observations Read 34
Number of Observations Used 34

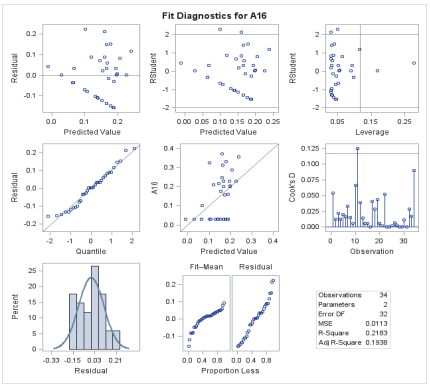
Analysis of Variance

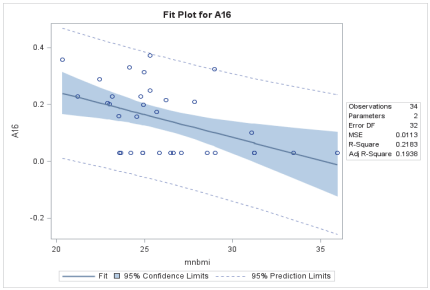
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.10132	0.10132	8.93	0.0053
Error	32	0.36291	0.01134		
Corrected Total	33	0.46423			

Root MSE	0.10649	R-Square	0.2183
Dependent Mean	0.14675	Adj R-Sq	0.1938
Coeff Var	72.56817		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.5663	0.14155	4	0.0003	.	0.27797	0.85462
mnbmi	1	-0.01607	0.00538	-2.99	0.0053	0.21825	-0.02703	-0.00512





Adduct 18
The REG Procedure
Model: MODEL1
Dependent Variable: A18

Number of Observations Read 34
Number of Observations Used 34

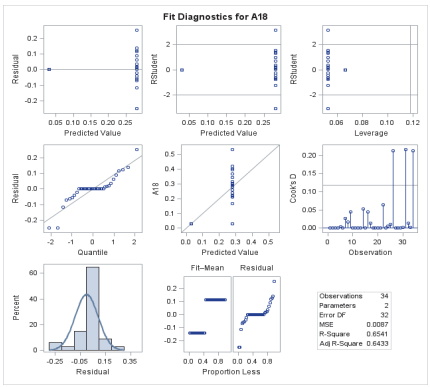
Analysis of Variance

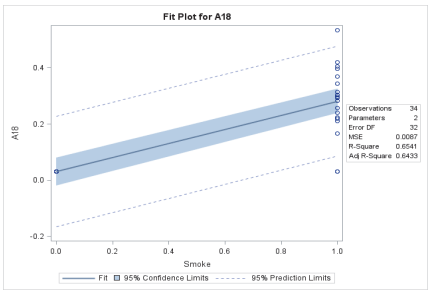
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.5281	0.5281	60.5	<.0001
Error	32	0.27931	0.00873		
Corrected Total	33	0.80741			

Root MSE	0.09343	R-Square	0.6541
Dependent Mean	0.17027	Adj R-Sq	0.6433
Coeff Var	54.87067		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.03	0.02412	1.24	0.2227	.	-0.01914	0.07914
Smoke	1	0.251	0.03227	7.78	<.0001	0.65407	0.18527	0.31673





Adduct 23
The REG Procedure
Model: MODEL1
Dependent Variable: A23

Number of Observations Read 34
Number of Observations Used 34

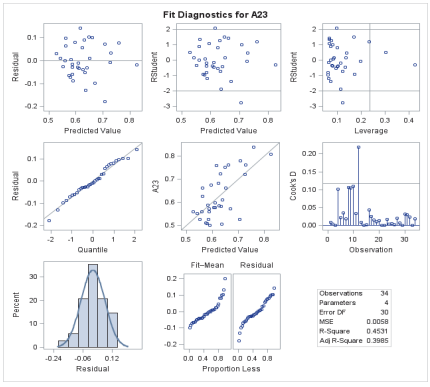
Analysis of Variance

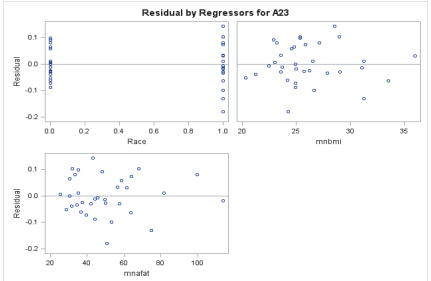
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.14486	0.04829	8.29	0.0004
Error	30	0.17482	0.00583		
Corrected Total	33	0.31969			

Root MSE	0.07634	R-Square	0.4531
Dependent Mean	0.62652	Adj R-Sq	0.3985
Coeff Var	12.18444		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.92356	0.11772	7.85	<.0001	.	0.68315	1.16397
Race	1	0.09256	0.03569	2.59	0.0145	0.12743	0.01968	0.16543
mnbmi	1	-0.01714	0.00478	-3.58	0.0012	0.20112	-0.02691	-0.00737
mnafat	1	0.00208	0.000796	2.61	0.0138	0.12459	0.000456	0.00371





Adduct 28
The REG Procedure
Model: MODEL1
Dependent Variable: A28

Number of Observations Read	34
Number of Observations Used	34

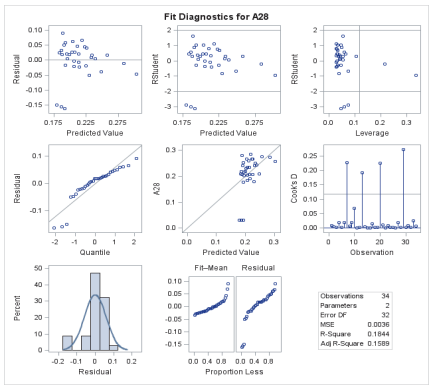
Analysis of Variance

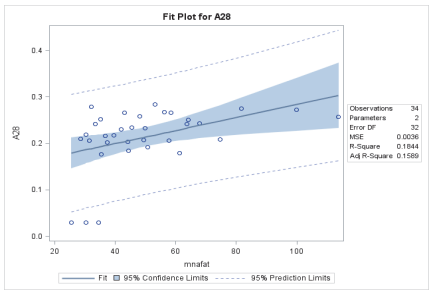
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.02593	0.02593	7.23	0.0113
Error	32	0.11471	0.00358		
Corrected Total	33	0.14064			

Root MSE	0.05987	R-Square	0.1844
Dependent Mean	0.21381	Adj R-Sq	0.1589
Coeff Var	28.00217		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.14388	0.02795	5.15	<.0001	.	0.08694	0.20082
mnafat	1	0.0014	0.00052	2.69	0.0113	0.1844	0.00034	0.00246





Adduct (A45=A29+A35+A37)
The REG Procedure
Model: MODEL1
Dependent Variable: A45

Number of Observations Read	34
Number of Observations Used	34

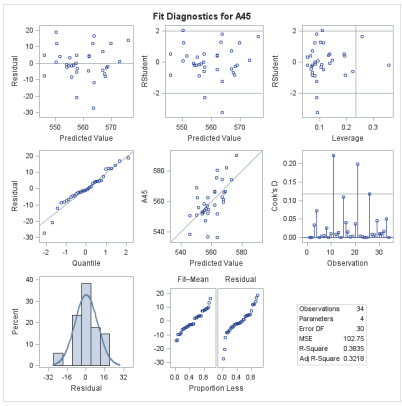
Analysis of Variance

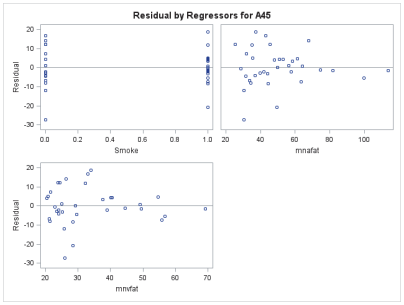
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1917.374	639.1248	6.22	0.0021
Error	30	3082.586	102.7529		
Corrected Total	33	4999.96			

Root MSE	10.13671	R-Square	0.3835
Dependent Mean	559.77482	Adj R-Sq	0.3218
Coeff Var	1.81085		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	569.0614	5.06798	112.29	<.0001	.	558.7112	579.4116
Smoke	1	-11.0416	4.08882	-2.7	0.0113	0.2731	-19.3921	-2.69114
mnafat	1	0.33821	0.16149	2.09	0.0448	0.00502	0.0084	0.66802
mnvfat	1	-0.60386	0.2667	-2.26	0.031	0.10535	-1.14853	-0.05918





Adduct A31
Dependent Variable: A31

Number of Observations Read	34
Number of Observations Used	34

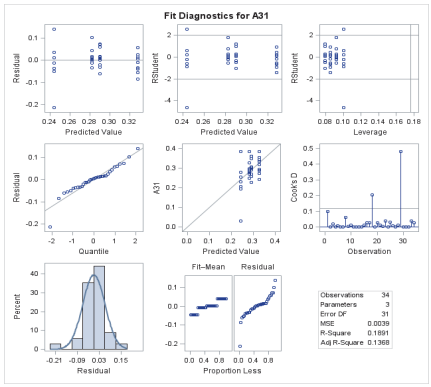
Analysis of Variance

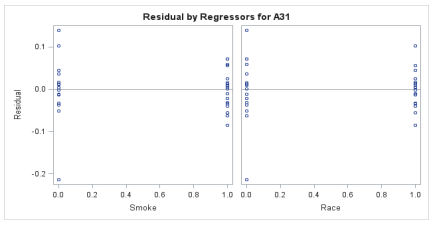
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02835	0.01417	3.61	0.0388
Error	31	0.12157	0.00392		
Corrected Total	33	0.14991			

Root MSE	0.06262	R-Square	0.1891
Dependent Mean	0.28888	Adj R-Sq	0.1368
Coeff Var	21.67765		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.2441	0.01983	12.31	<.0001	.	0.20366	0.28454
Smoke	1	0.04591	0.02167	2.12	0.0422	0.10644	0.00172	0.0901
Race	1	0.03825	0.02152	1.78	0.0853	0.08265	-0.00564	0.08213





Adduct 34
The REG Procedure
Model: MODEL1
Dependent Variable: A34

Number of Observations Read	34
Number of Observations Used	34

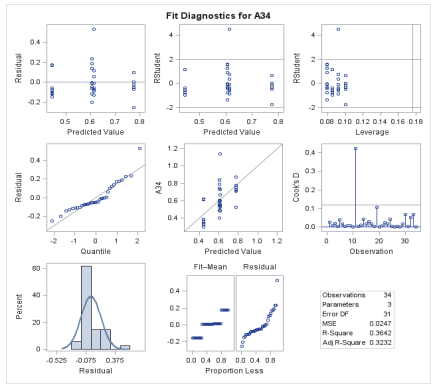
Analysis of Variance

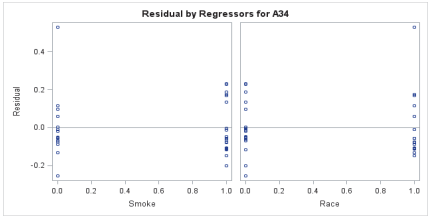
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.43927	0.21964	8.88	0.0009
Error	31	0.76685	0.02474		
Corrected Total	33	1.20612			

Root MSE	0.15728	R-Square	0.3642
Dependent Mean	0.59907	Adj R-Sq	0.3232
Coeff Var	26.25401		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.77534	0.0498	15.57	<.0001	.	0.67378	0.87691
Smoke	1	-0.16962	0.05442	-3.12	0.0039	0.1777	-0.28061	-0.05863
Race	1	-0.16297	0.05404	-3.02	0.0051	0.18651	-0.27319	-0.05275





Adduct 38
The REG Procedure
Model: MODEL1
Dependent Variable: A38

Number of Observations Read 34
Number of Observations Used 34

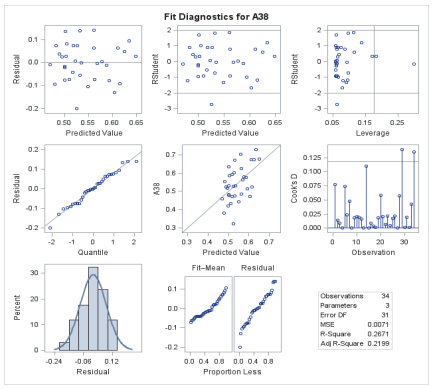
Analysis of Variance

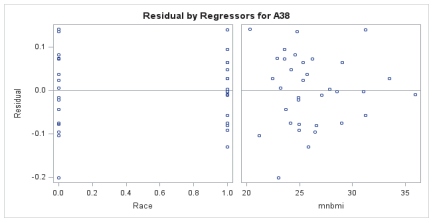
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.07986	0.03993	5.65	0.0081
Error	31	0.21907	0.00707		
Corrected Total	33	0.29892			

Root MSE	0.08406	R-Square	0.2671
Dependent Mean	0.5423	Adj R-Sq	0.2199
Coeff Var	15.50149		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.83171	0.12795	6.5	<.0001	.	0.57075	1.09267
Race	1	0.11643	0.03559	3.27	0.0026	0.11436	0.04386	0.18901
mnbmi	1	-0.01332	0.00524	-2.54	0.0162	0.15278	-0.024	-0.00263





Adduct 39
The REG Procedure
Model: MODEL1
Dependent Variable: A39

Number of Observations Read 33
Number of Observations Used 33

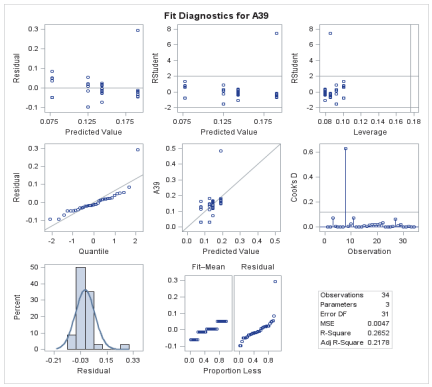
Analysis of Variance

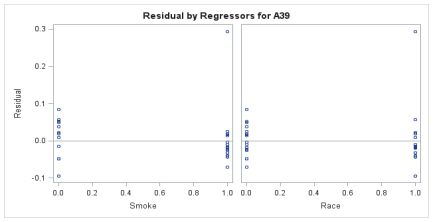
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02282	0.01141	6.8	0.0037
Error	30	0.05034	0.00168		
Corrected Total	32	0.07316			

Root MSE	0.04096	R-Square	0.3119
Dependent Mean	0.12784	Adj R-Sq	0.266
Coeff Var	32.04275		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	0.08858	0.01305	6.79	<.0001	.	0.06194	0.11523
Smoke	1	0.04754	0.01438	3.31	0.0025	0.22746	0.01818	0.0769
Race	1	0.02748	0.01432	1.92	0.0646	0.08444	-0.00177	0.05674





Adduct 40
The REG Procedure
Model: MODEL1
Dependent Variable: A40

Number of Observations Read 34
Number of Observations Used 34

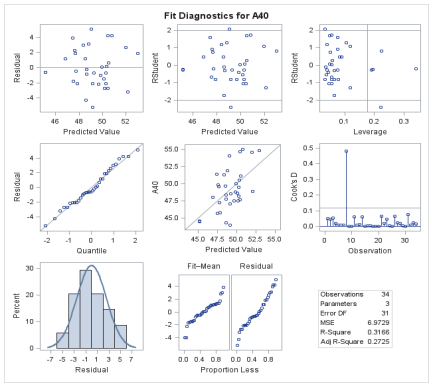
Analysis of Variance

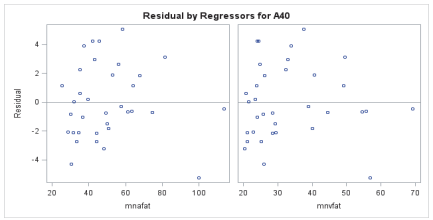
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	100.1216	50.0608	7.18	0.0027
Error	31	216.1606	6.97292		
Corrected Total	33	316.2822			

Root MSE	2.64063	R-Square	0.3166
Dependent Mean	49.14659	Adj R-Sq	0.2725
Coeff Var	5.37297		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	51.4759	1.31261	39.22	<.0001	.	48.79881	54.15299
mnafat	1	0.11918	0.04203	2.84	0.008	0.00689	0.03346	0.2049
mnvfat	1	-0.24992	0.06668	-3.75	0.0007	0.30967	-0.38592	-0.11392





Adduct 43
The REG Procedure
Model: MODEL1
Dependent Variable: A43

Number of Observations Read 34
Number of Observations Used 34

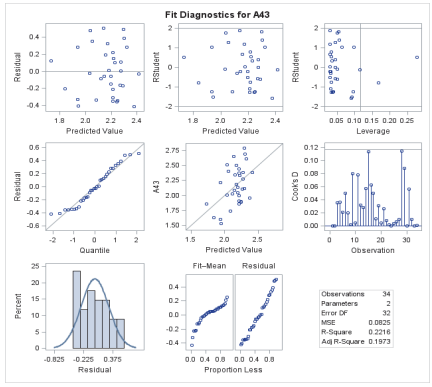
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.75137	0.75137	9.11	0.005
Error	32	2.63901	0.08247		
Corrected Total	33	3.39038			

Root MSE	0.28717	R-Square	0.2216
Dependent Mean	2.16099	Adj R-Sq	0.1973
Coeff Var	13.28904		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Squared Semi-partial Corr Type I	95% Confidence Limits	
Intercept	1	3.30349	0.3817	8.65	<.0001	.	2.52599	4.08099
mnbmi	1	-0.04377	0.0145	-3.02	0.005	0.22162	-0.07331	-0.01423



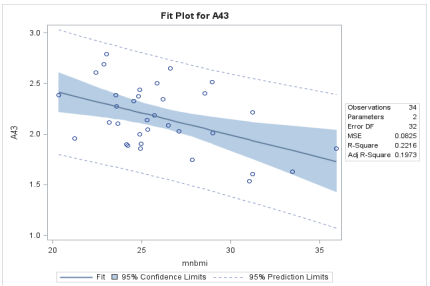
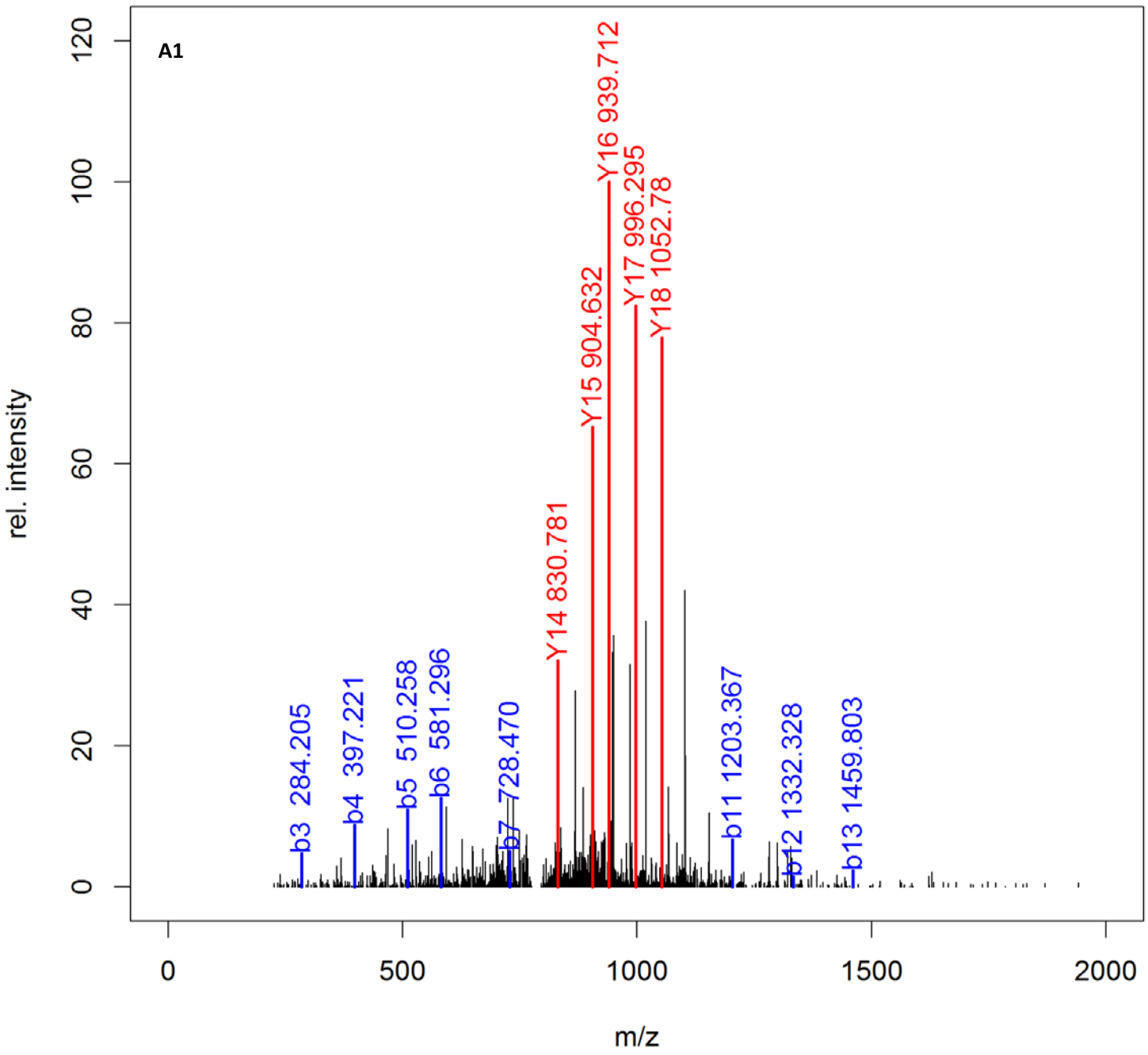
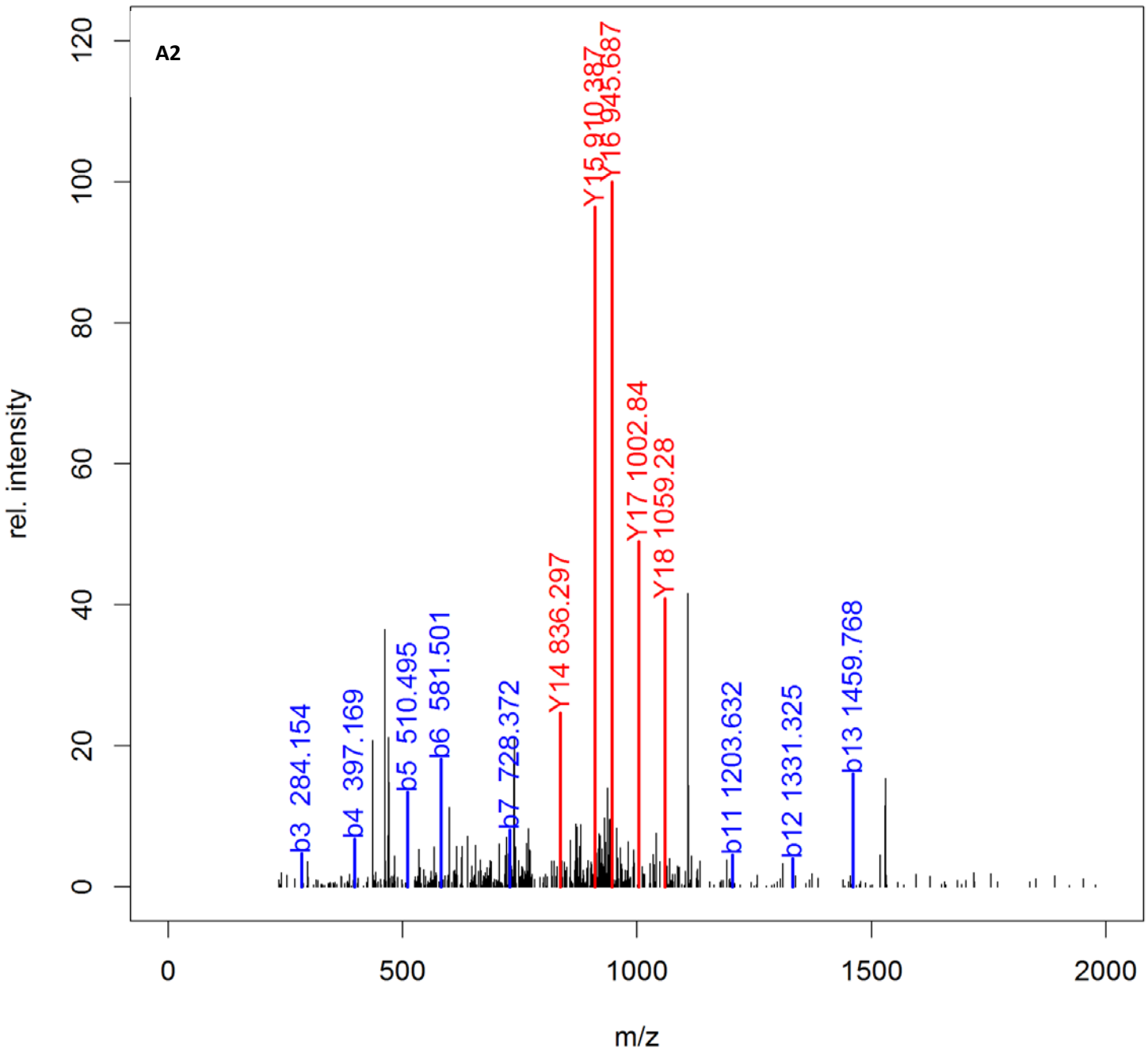


Figure S1 – The MS2 spectra of T3 and its related adducts coded from A1 to A43. The headline in each figure indicates the raw file name_sample ID_Precursor scan #_MIM_RT_Tandem scan #.

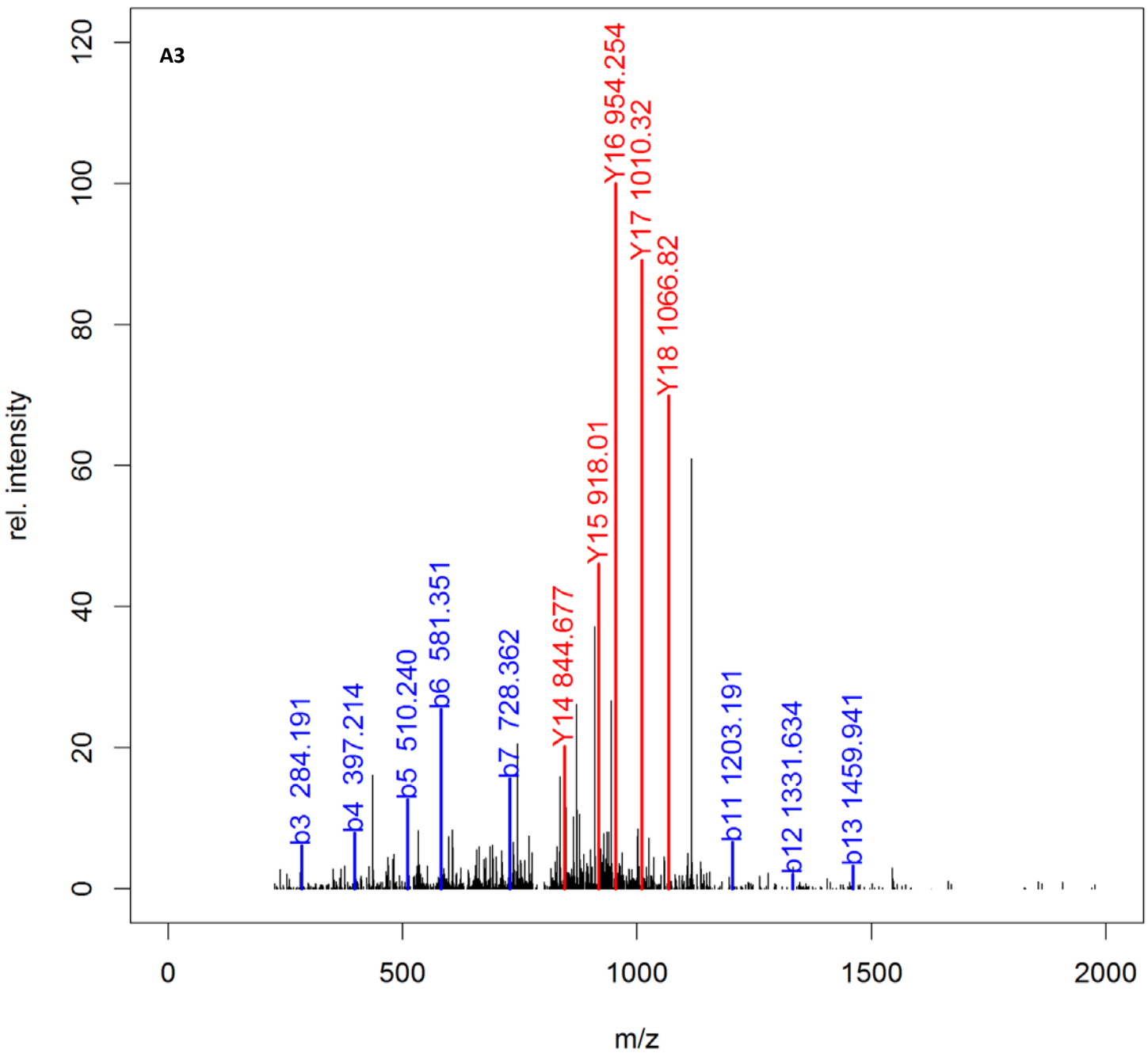
ORB35104_28_PrecursorScan_1557_MZ_796.4294_RT_27.66_MS.MS.Scan_1559



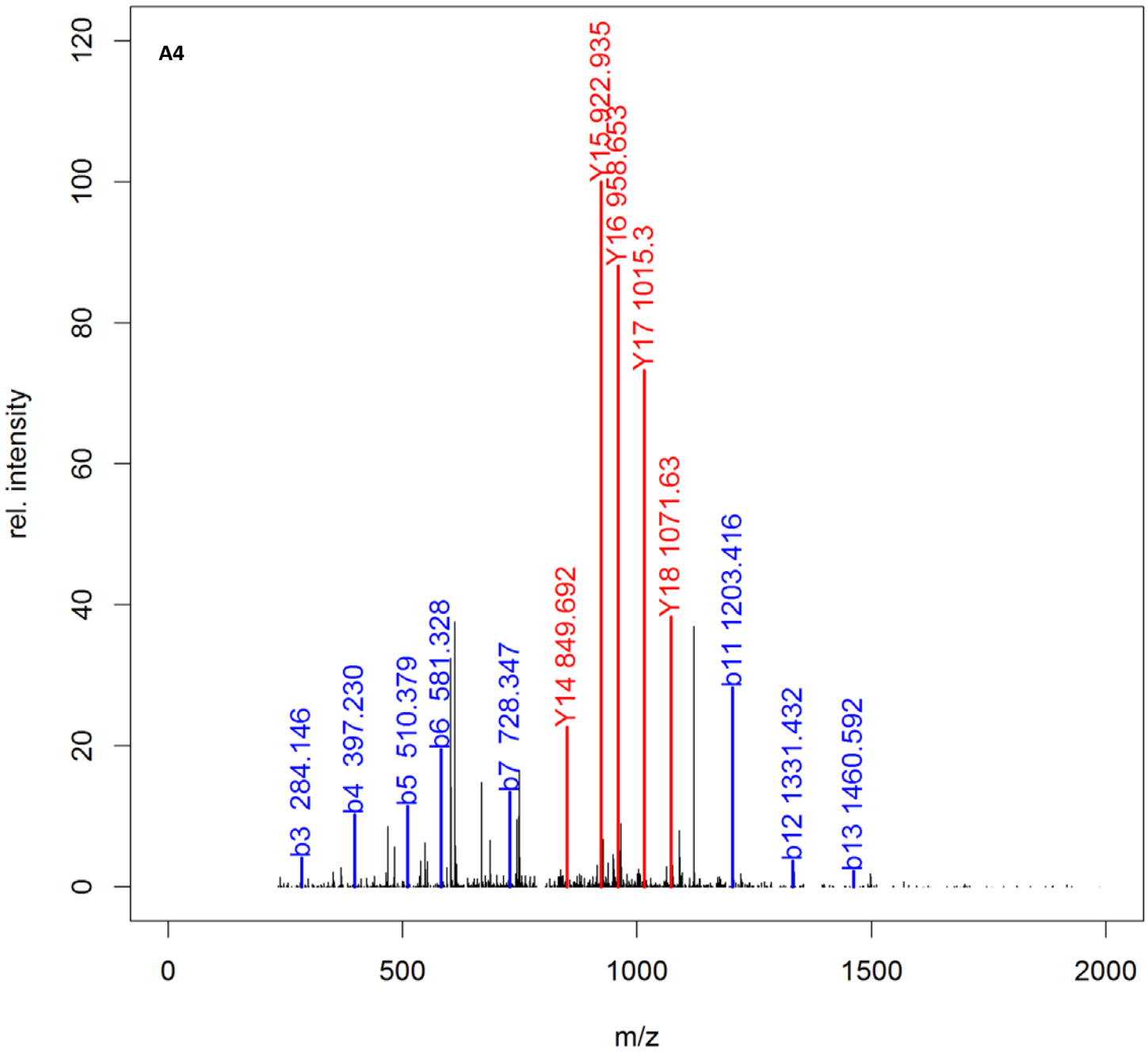
ORB35142_37_PrecursorScan_1645_MZ_800.4315_RT_28.92_MS.MS.Scan_1646



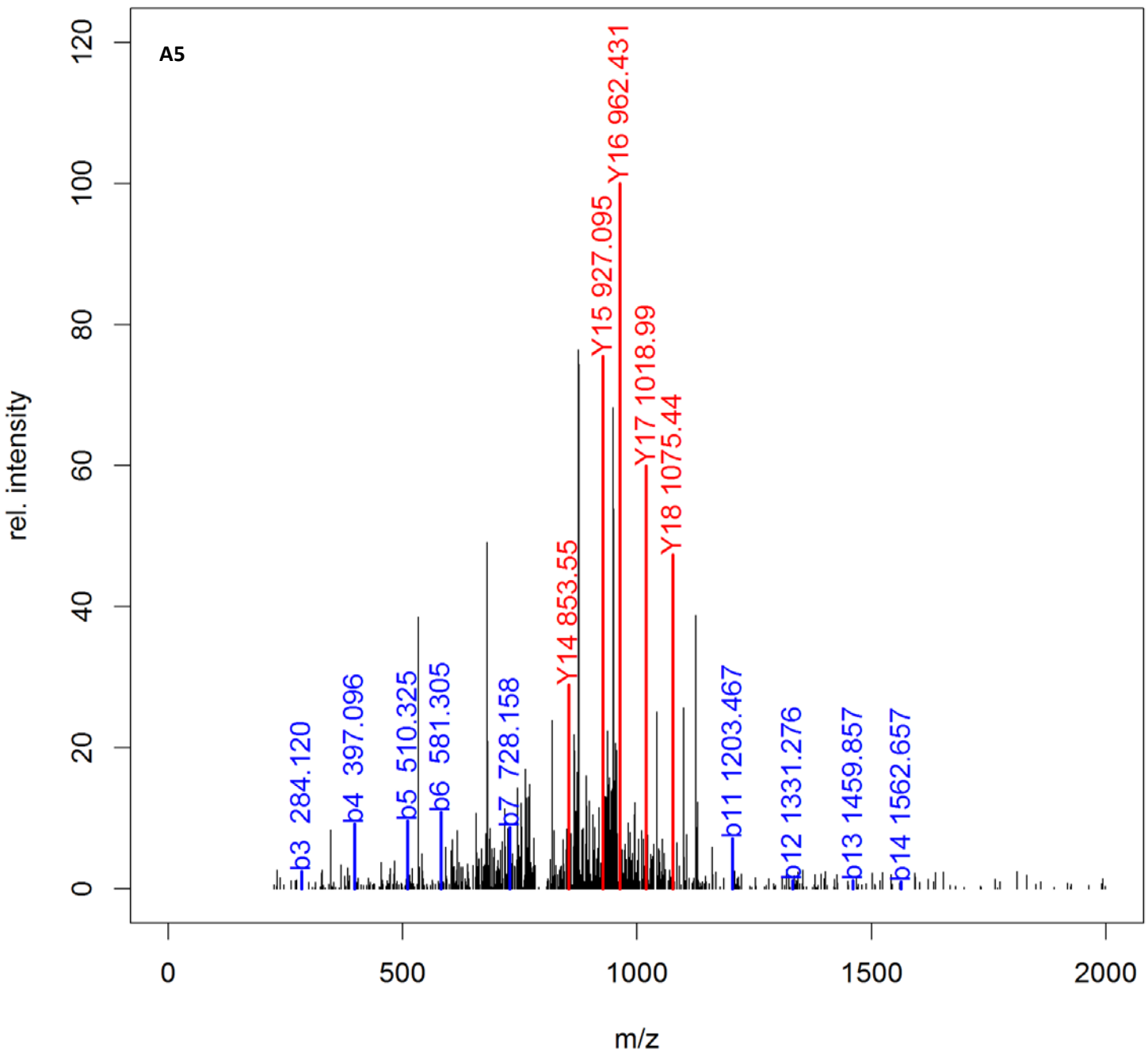
ORB35195_17_PrecursorScan_1616_MZ_805.764_RT_27.54_MS.MS.Scan_1619



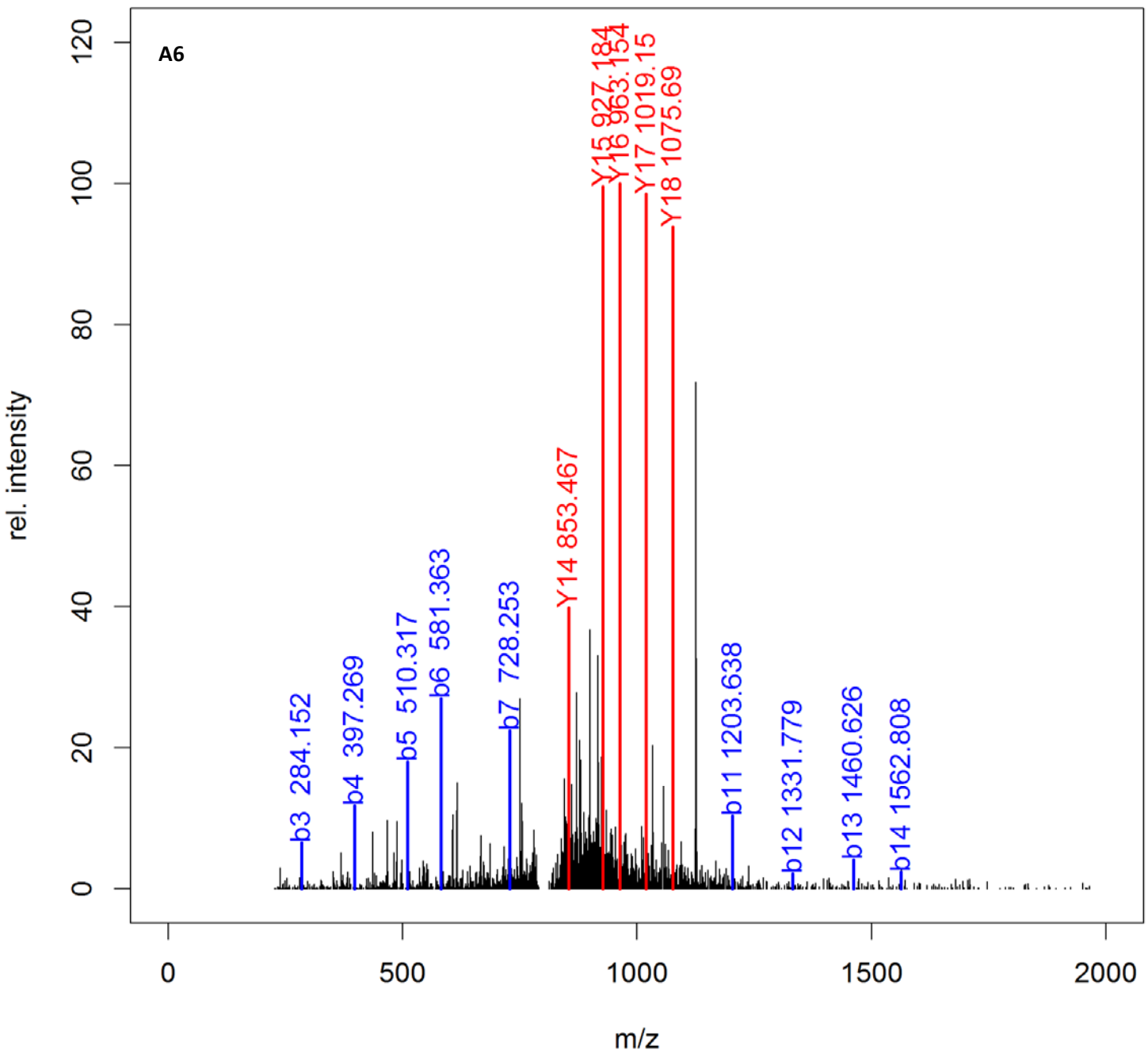
ORB35161_25_PrecursorScan_1615_MZ_808.731_RT_28.04_MS.MS.Scan_1617



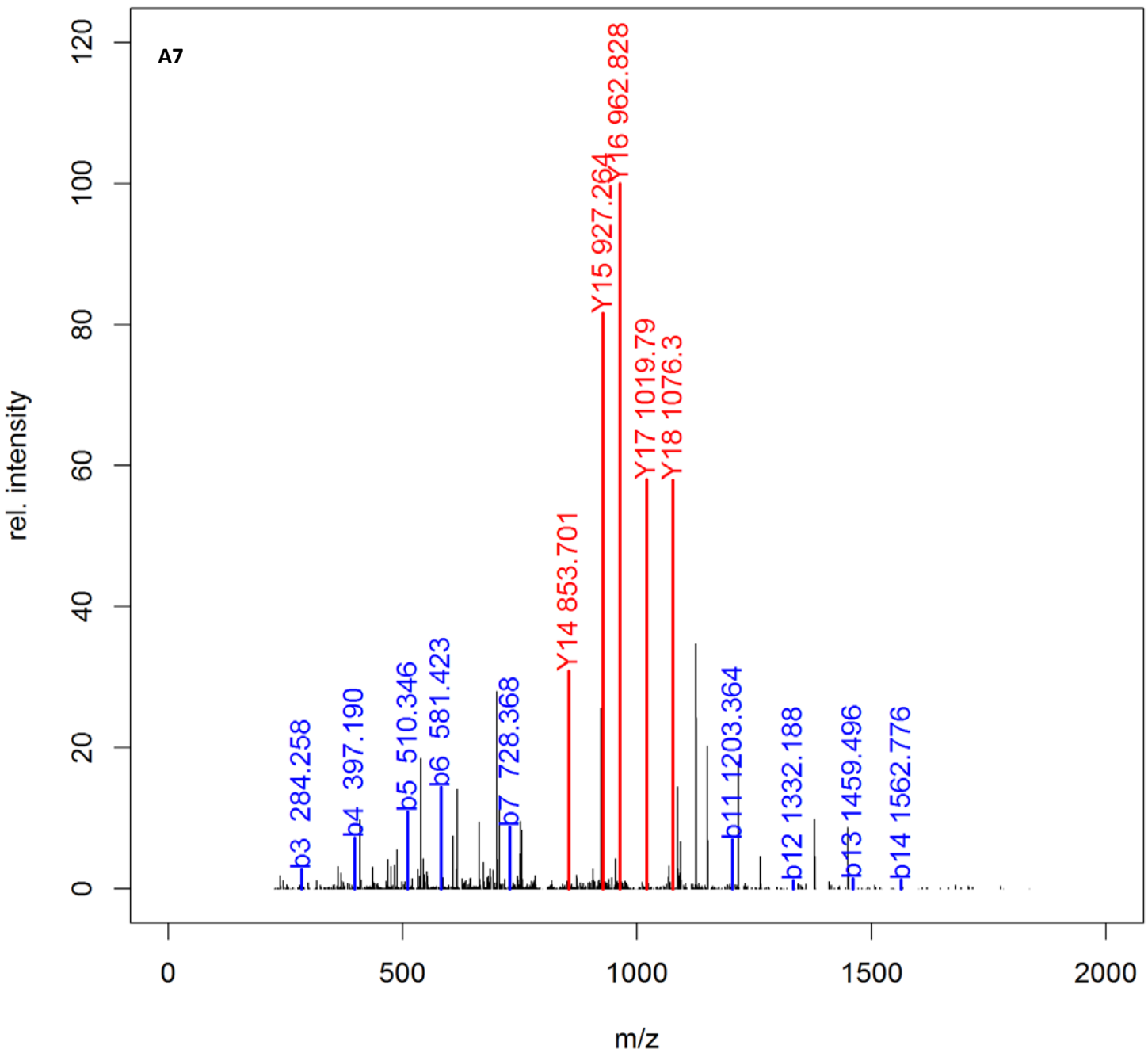
ORB35220_20_PrecursorScan_1647_MZ_810.4524_RT_28.43_MS.MS.Scan_1648



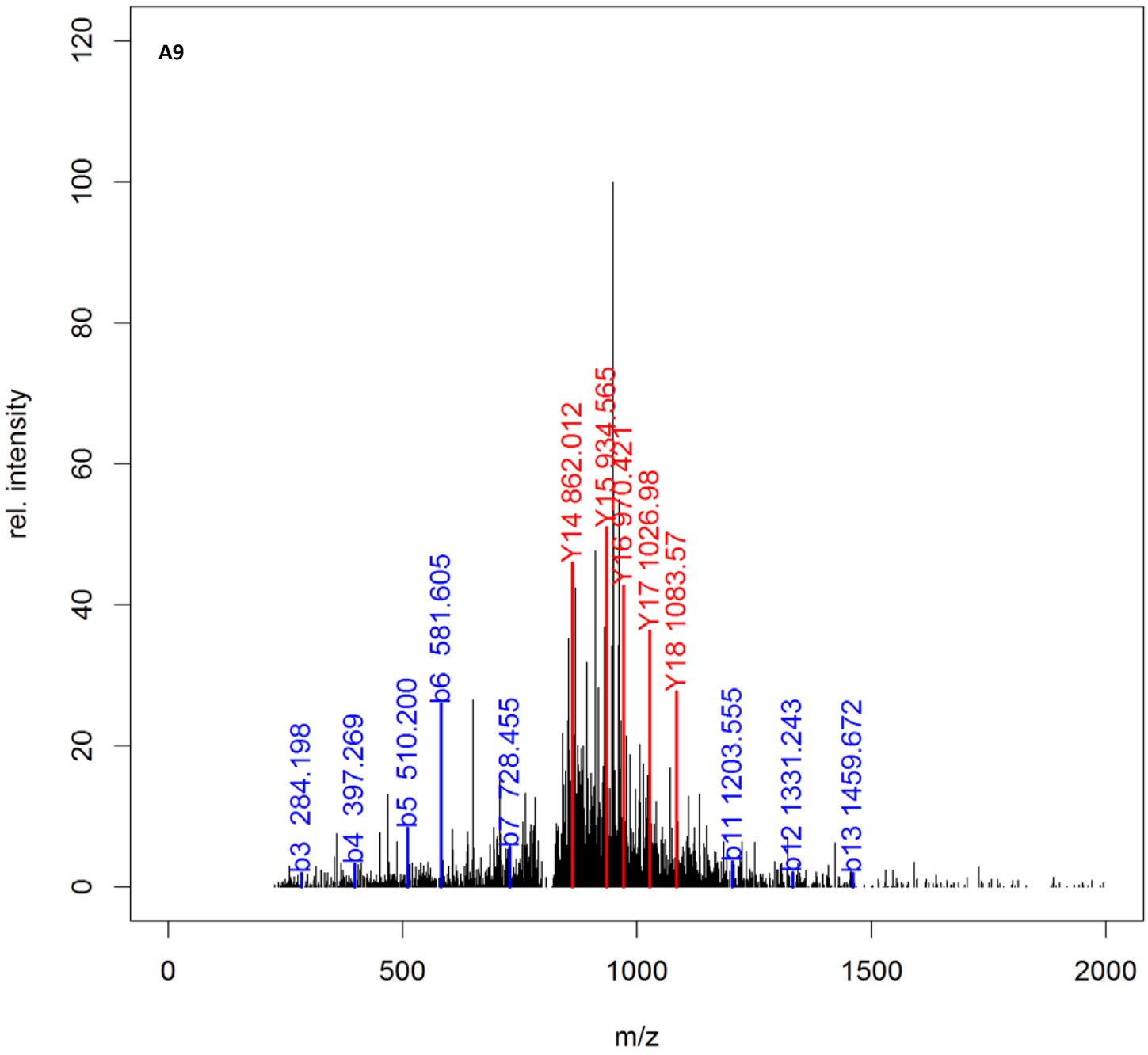
ORB35166_8_PrecursorScan_1424_MZ_811.7604_RT_26.04_MS.MS.Scan_1425



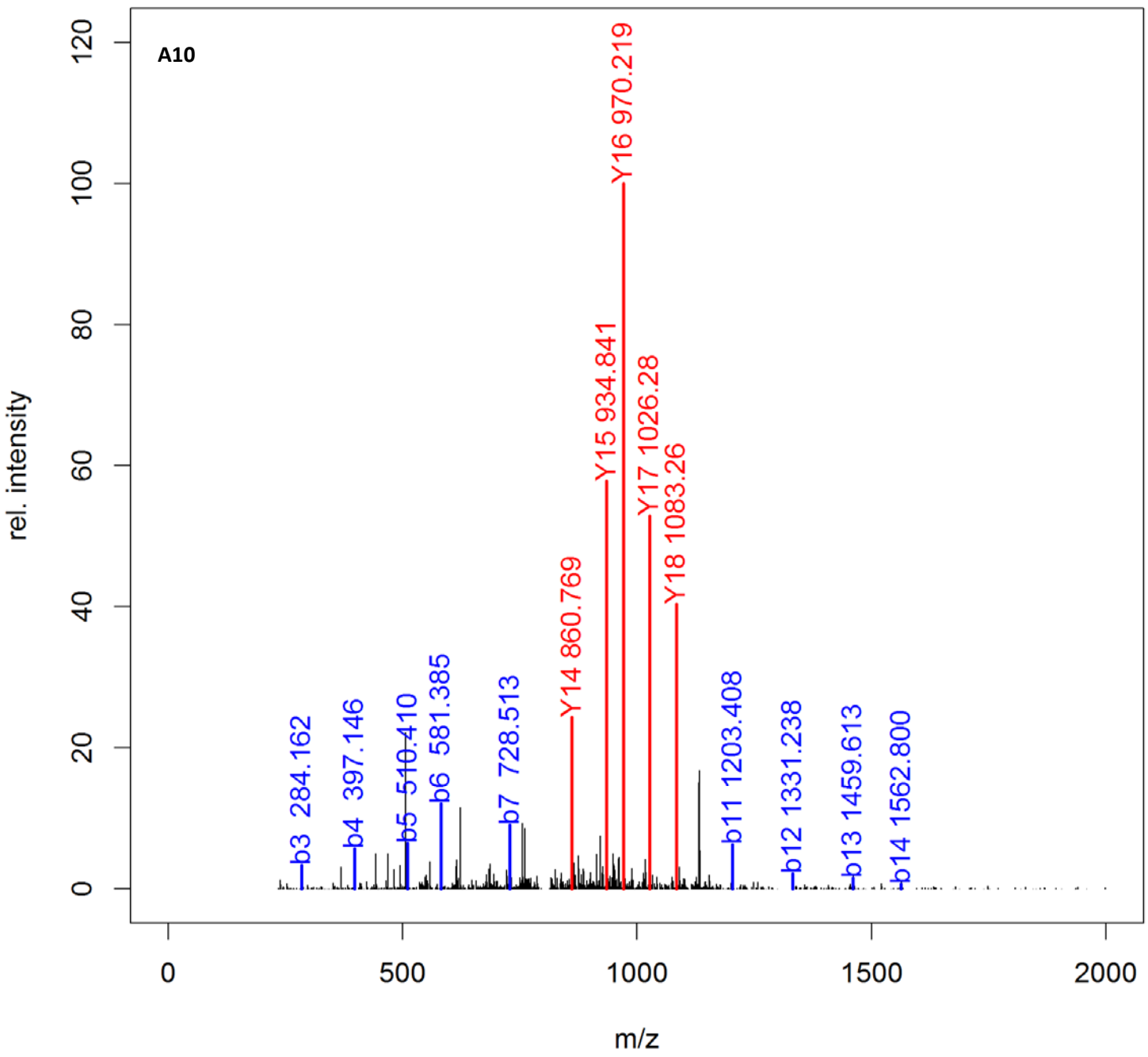
ORB35060_34_PrecursorScan_1605_MZ_811.7607_RT_27.89_MS.MS.Scan_1606



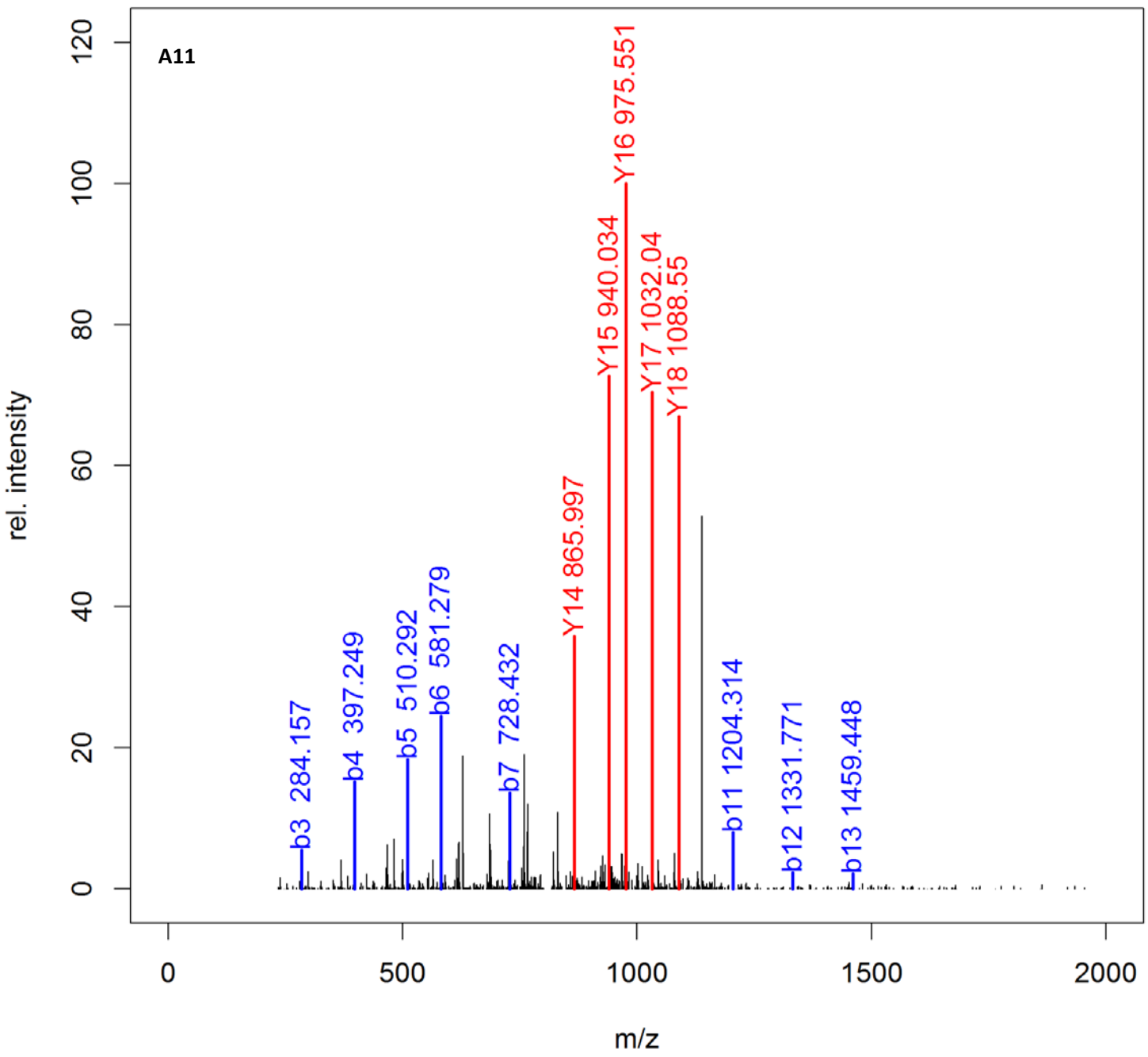
ORB35185_17_PrecursorScan_1548_MZ_816.4196_RT_27.2_MS.MS.Scan_1549



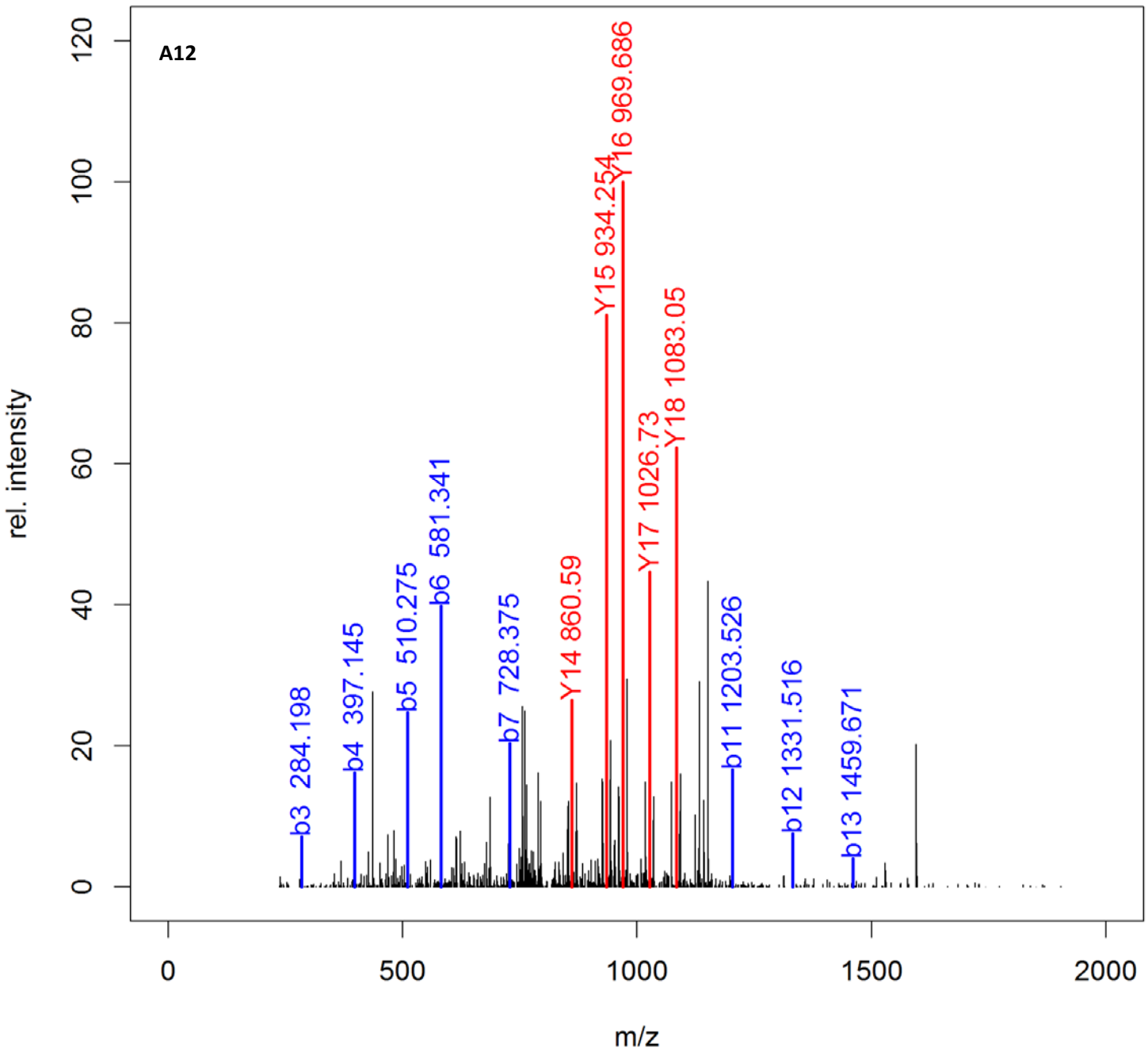
ORB35148_39_PrecursorScan_1658_MZ_816.4327_RT_28.82_MS.MS.Scan_1659



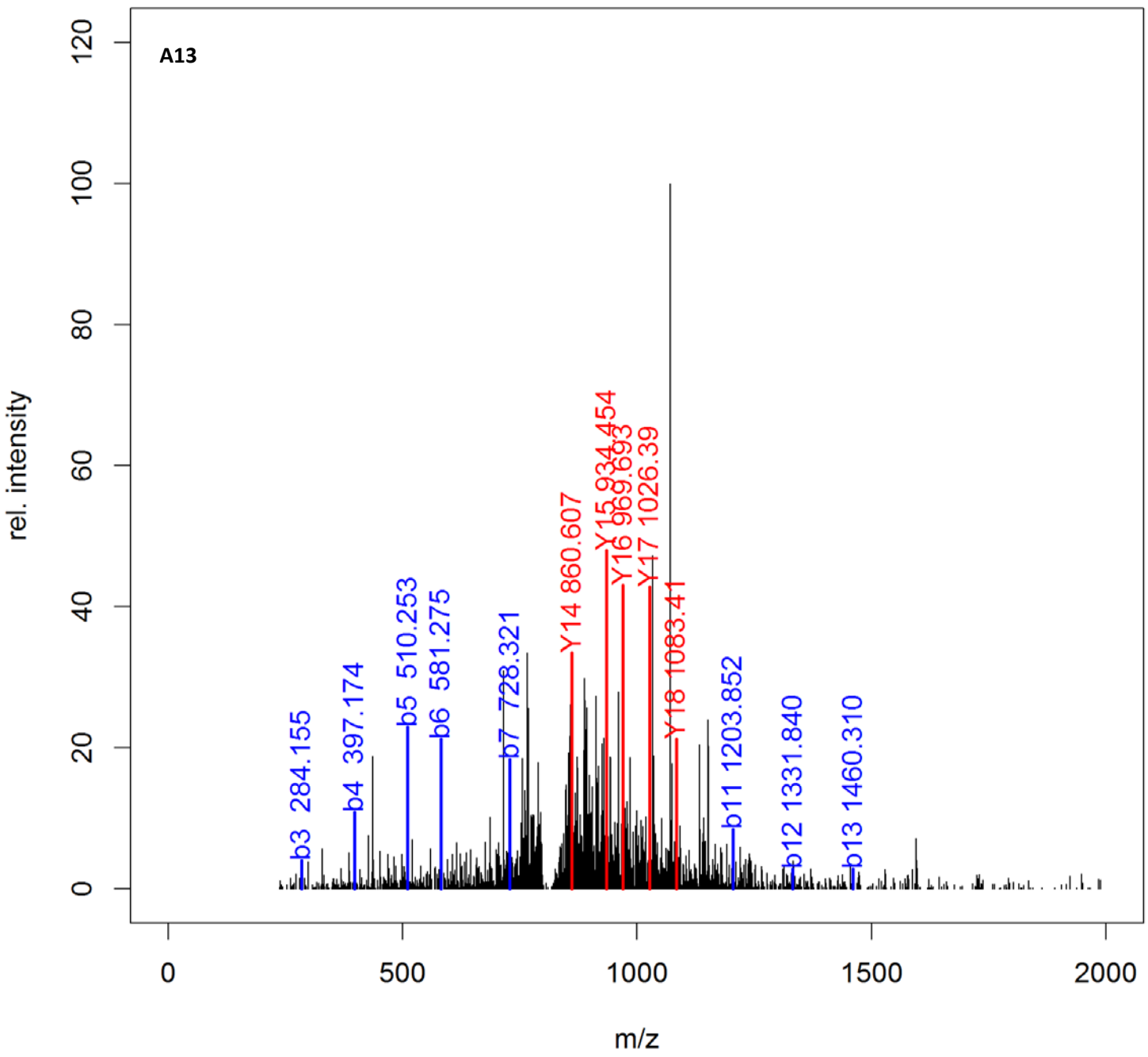
ORB35022_29_PrecursorScan_1756_MZ_820.0912_RT_28.72_MS.MS.Scan_1757



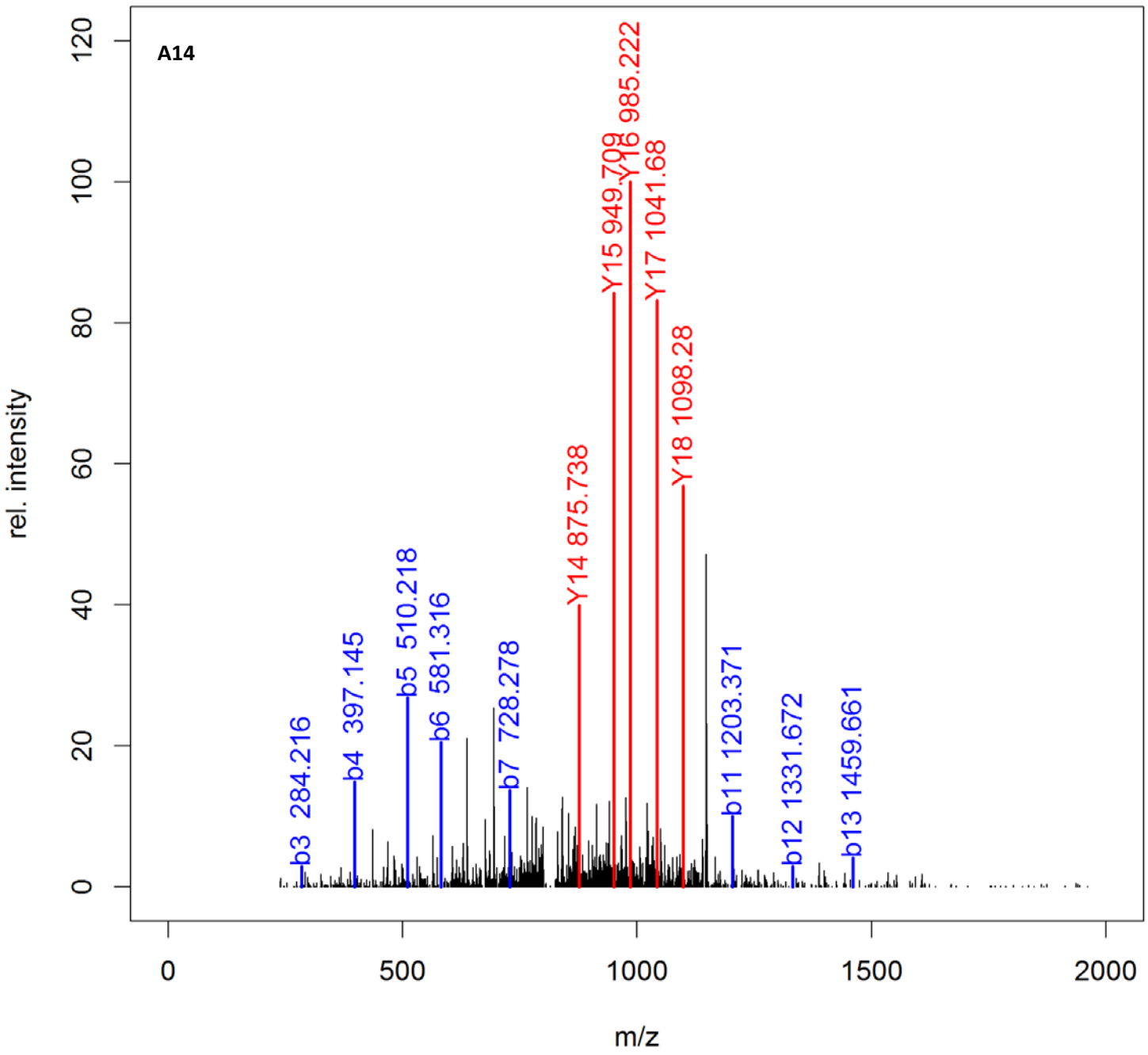
ORB35080_20_PrecursorScan_1574_MZ_822.4235_RT_27.1_MS.MS.Scan_1575



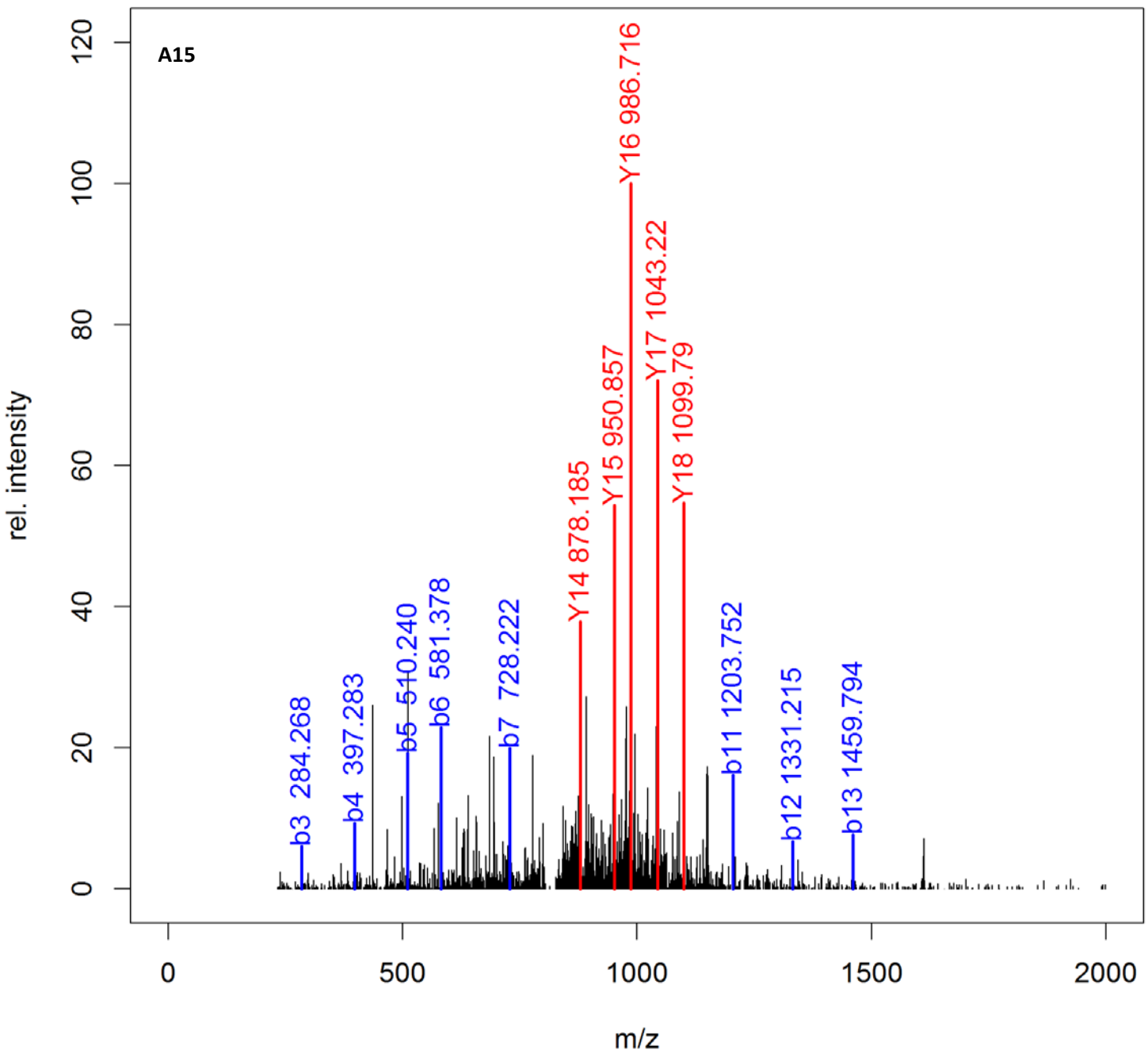
ORB35073_11_PrecursorScan_1615_MZ_823.3971_RT_27.17_MS.MS.Scan_1618



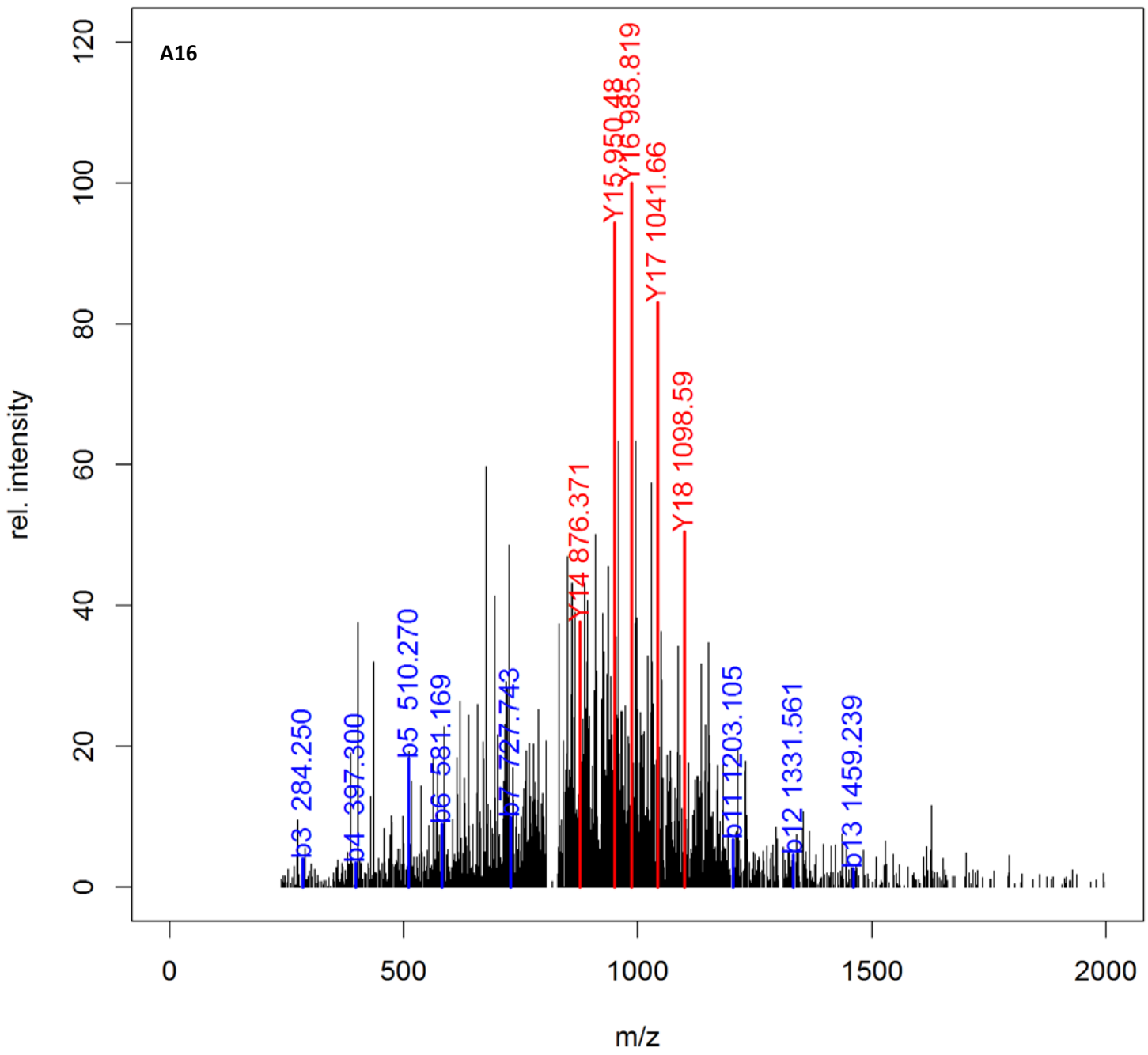
ORB35029_26_PrecursorScan_1657_MZ_826.4355_RT_27.88_MS.MS.Scan_1659



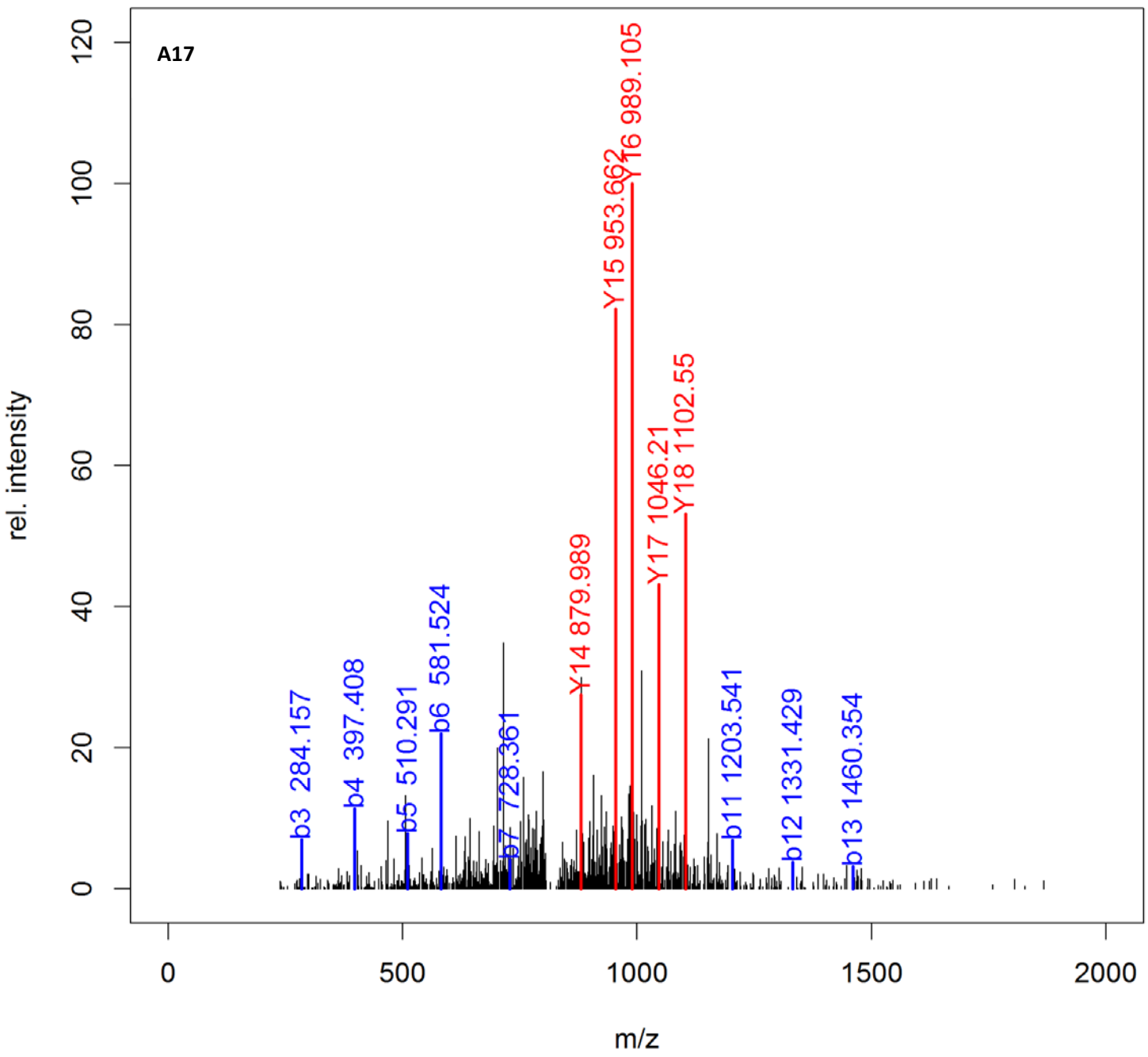
ORB35152_19_PrecursorScan_1621_MZ_827.7555_RT_27.78_MS.MS.Scan_1622



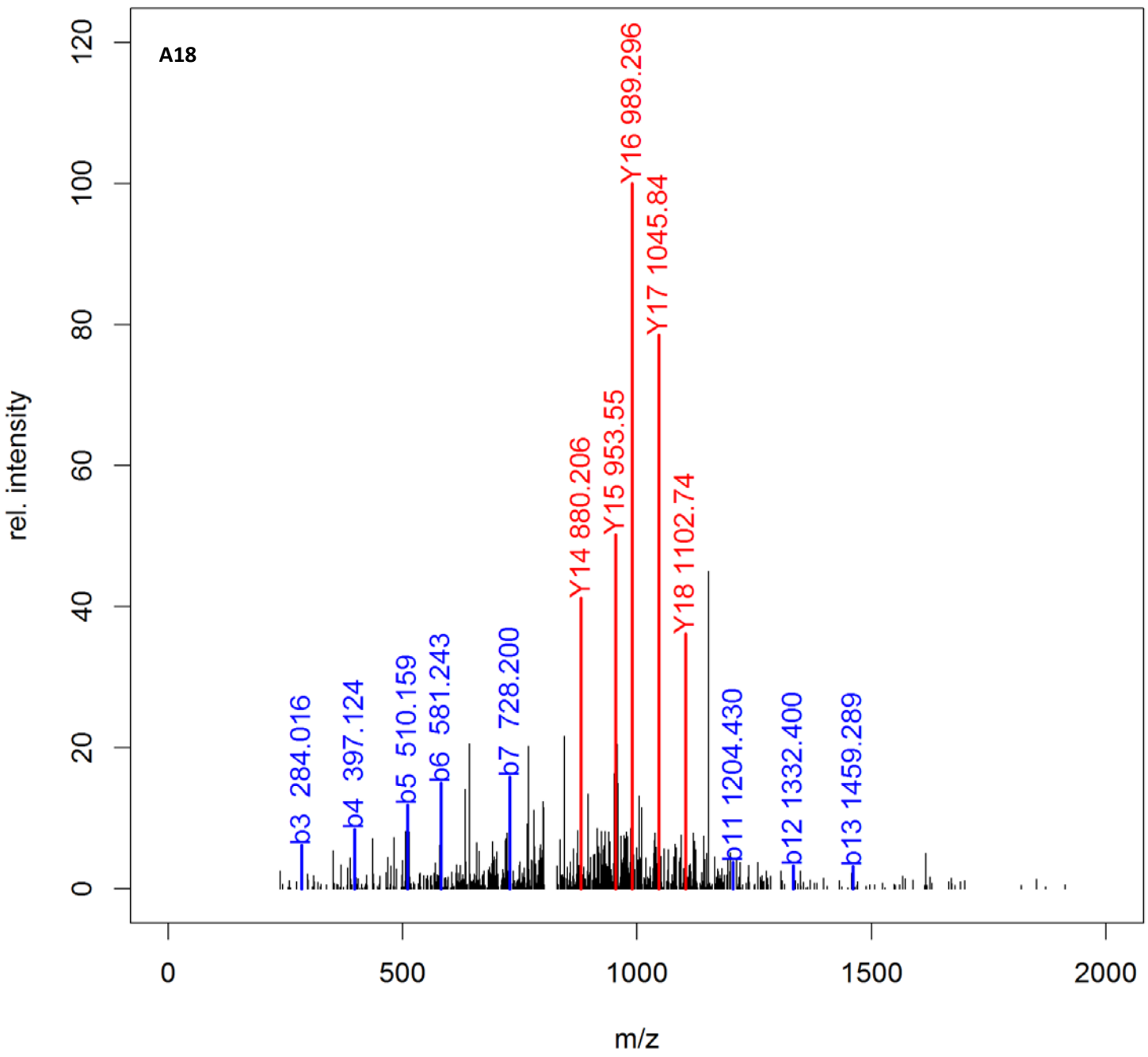
ORB35186_22_PrecursorScan_1584_MZ_829.1238_RT_26.9_MS.MS.Scan_1586



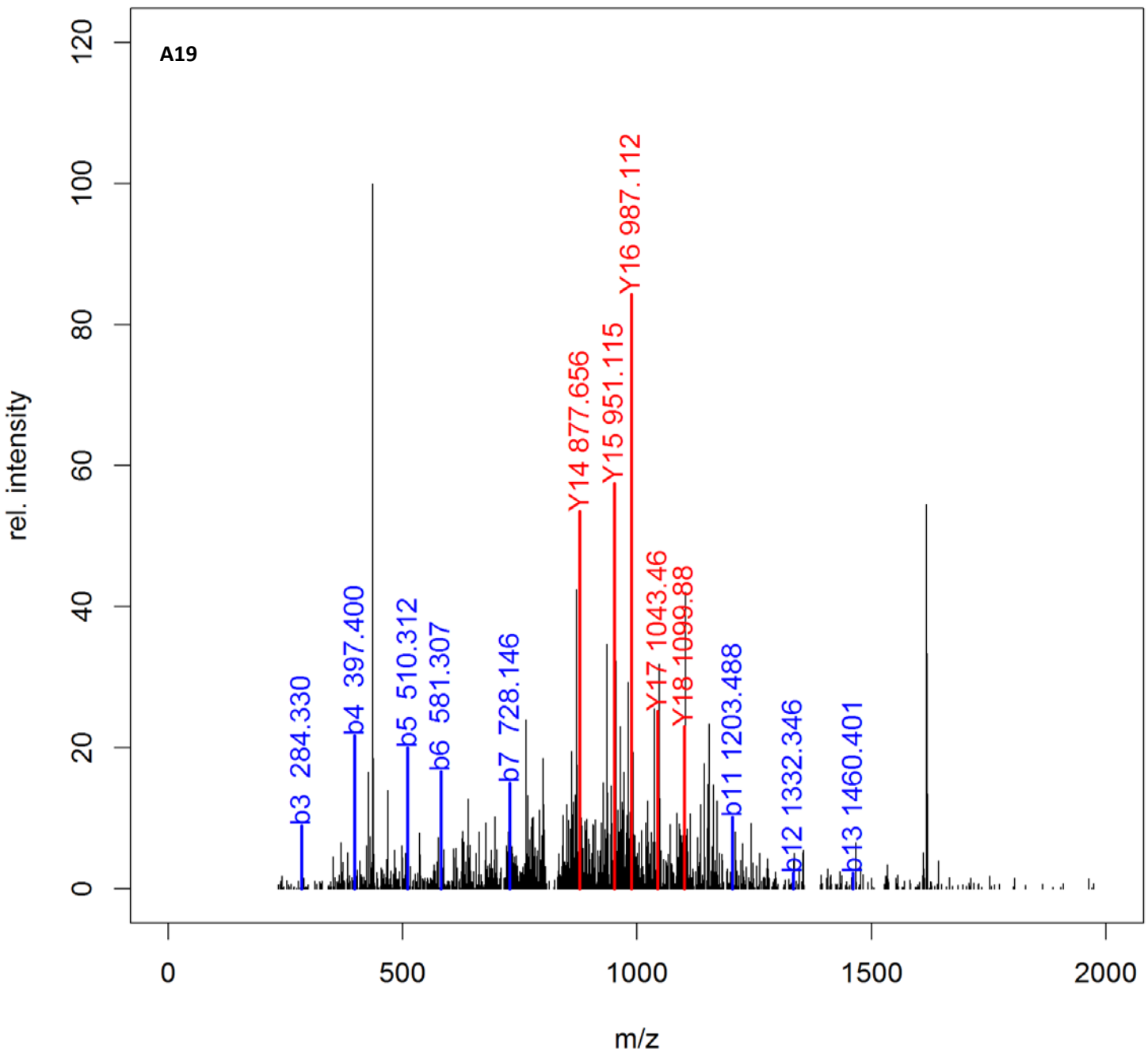
ORB35102_26_PrecursorScan_1623_MZ_829.3959_RT_28.64_MS.MS.Scan_1624



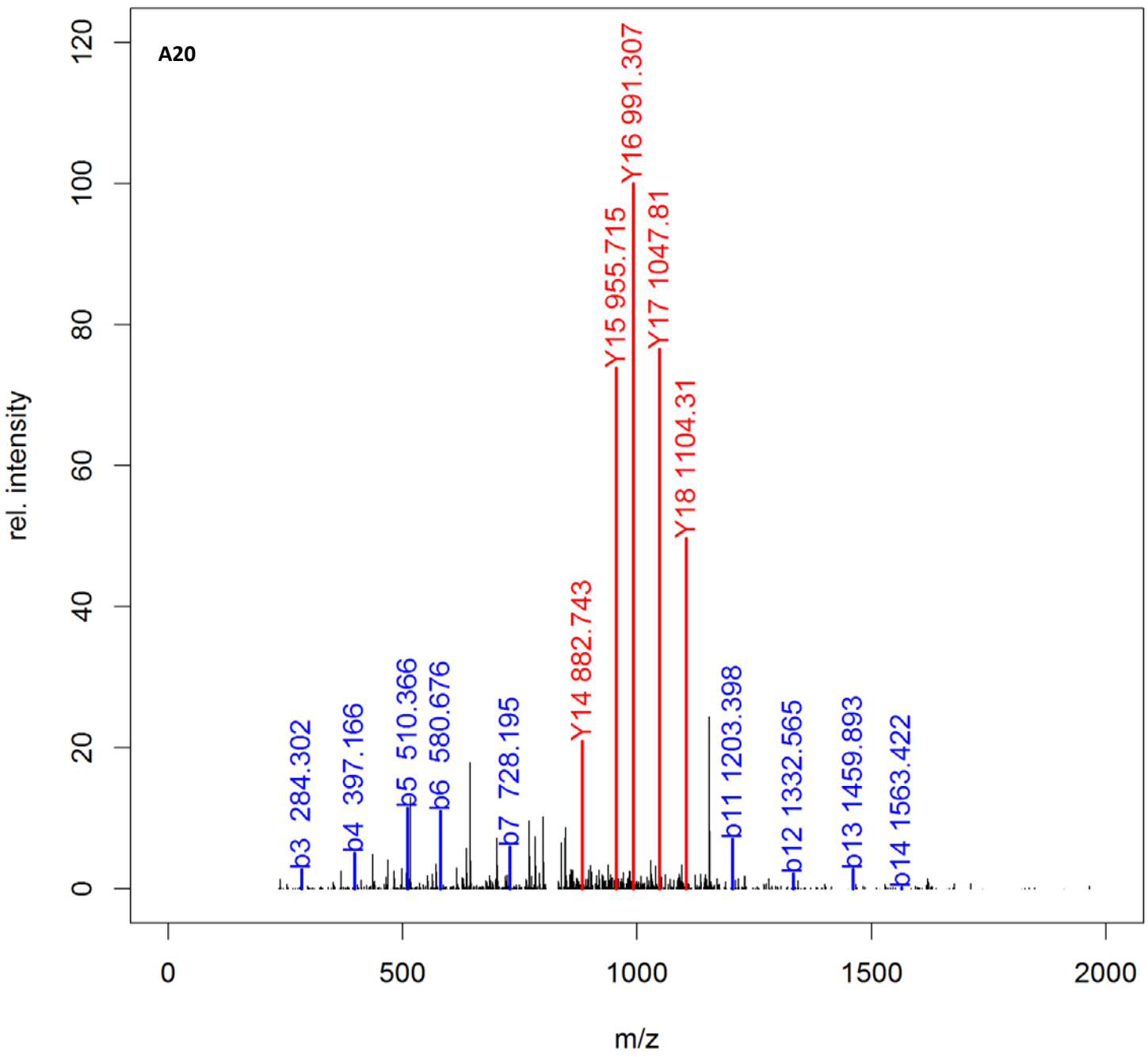
ORB35027_29_PrecursorScan_1745_MZ_829.4359_RT_29.01_MS.MS.Scan_1749



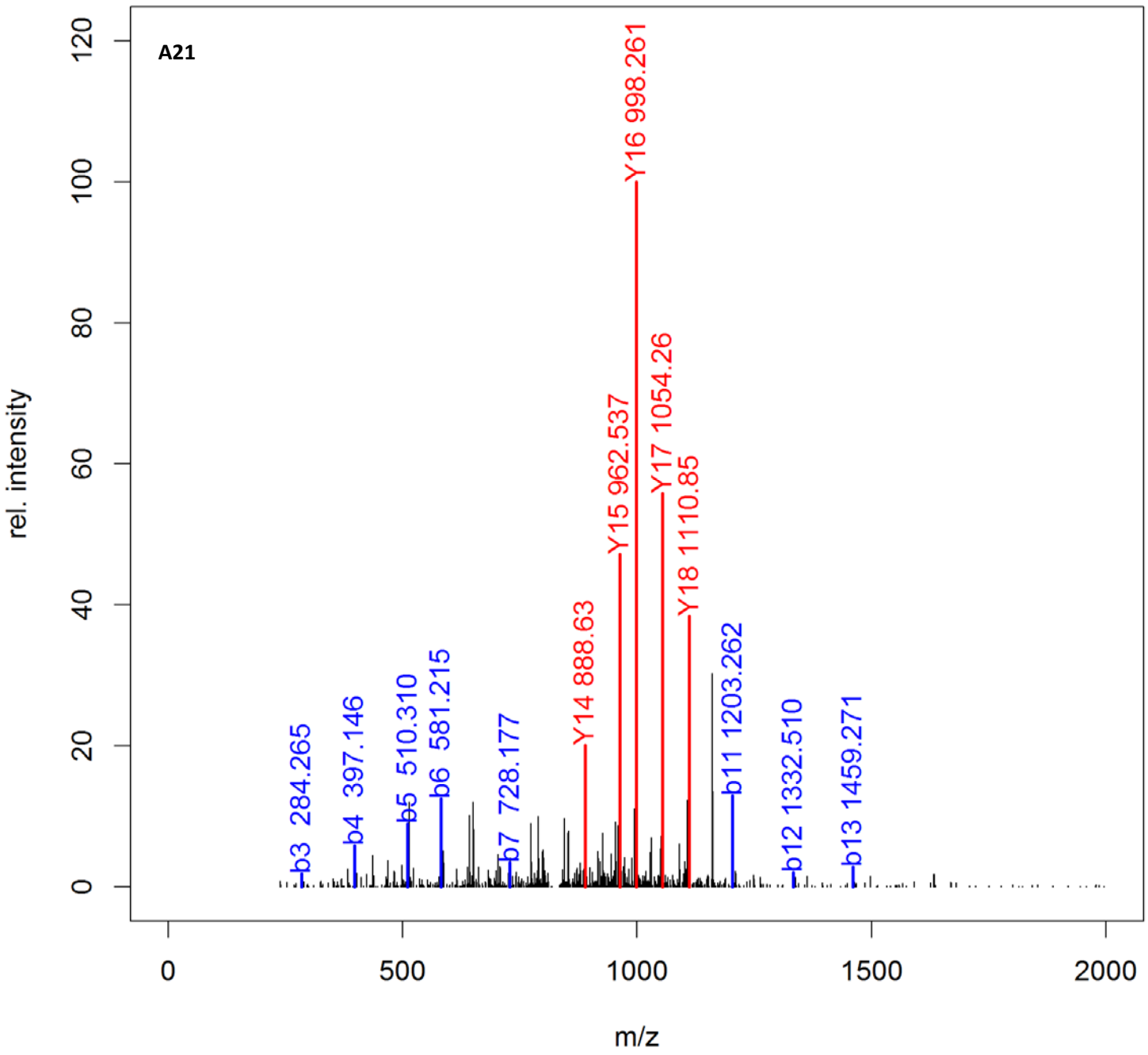
ORB35142_24_PrecursorScan_1540_MZ_829.7502_RT_27.39_MS.MS.Scan_1543



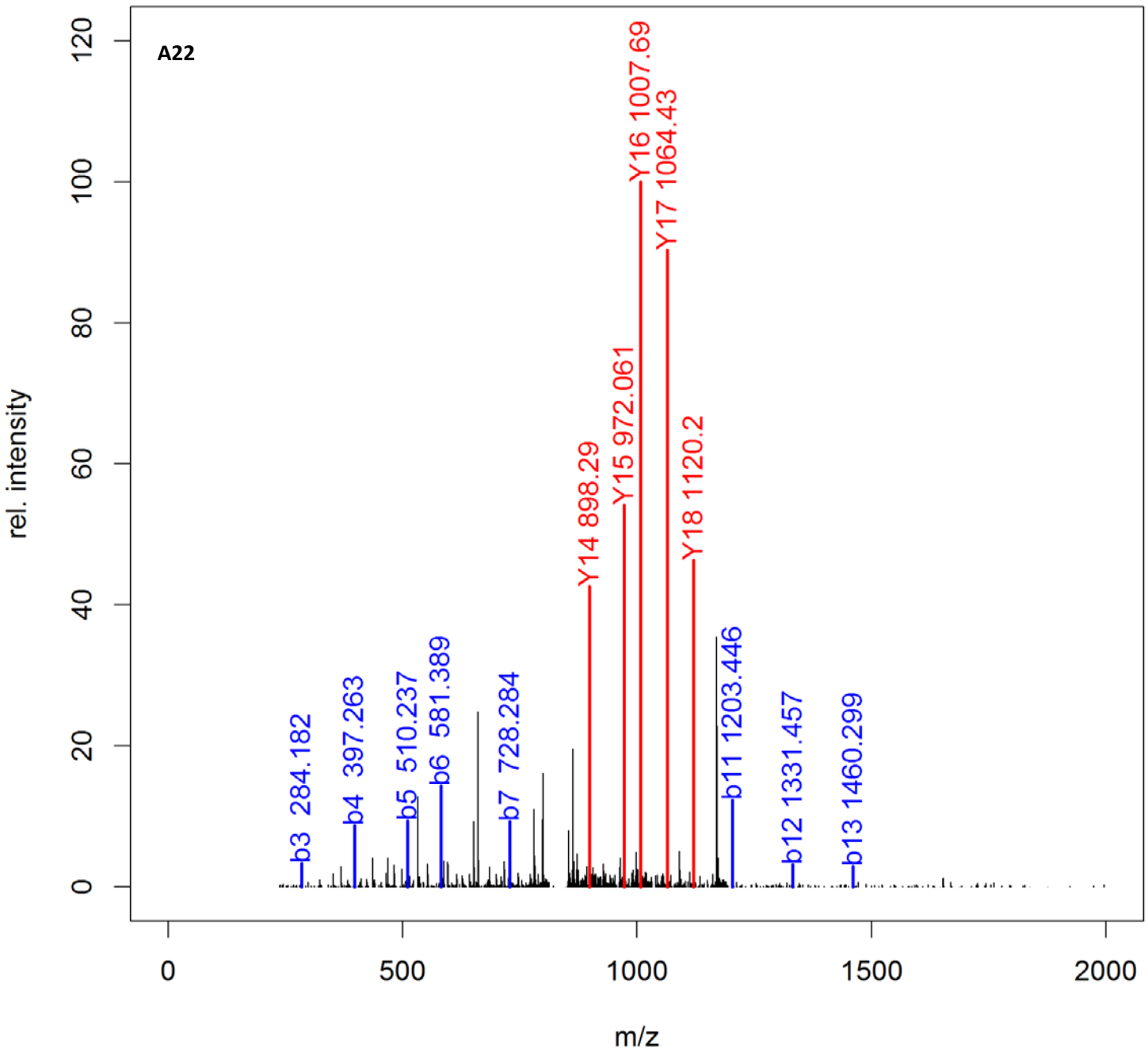
ORB35095_17_PrecursorScan_1586_MZ_830.7711_RT_27.38_MS.MS.Scan_1588



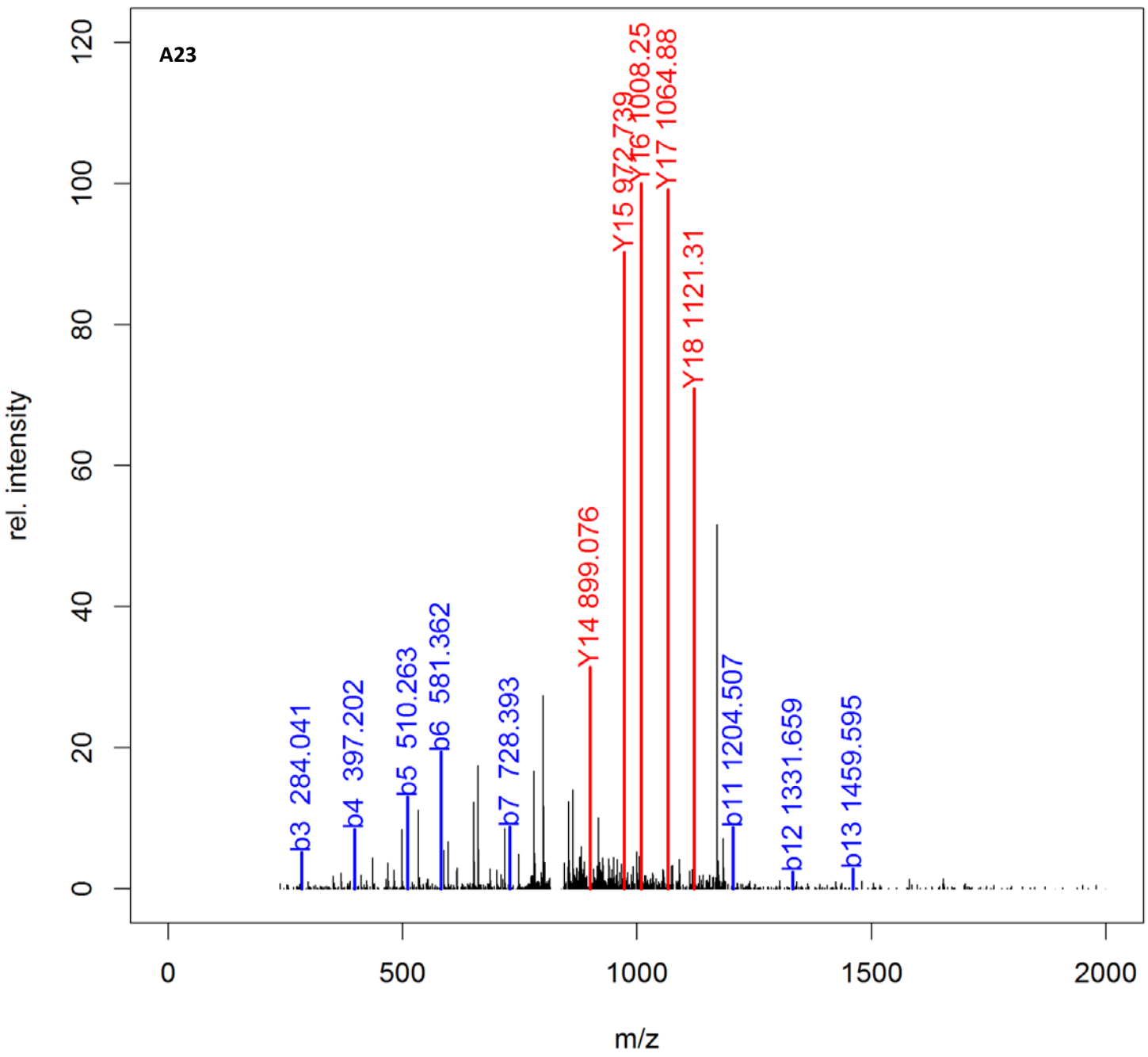
ORB35027_30_PrecursorScan_1750_MZ_835.1066_RT_29.04_MS.MS.Scan_1751



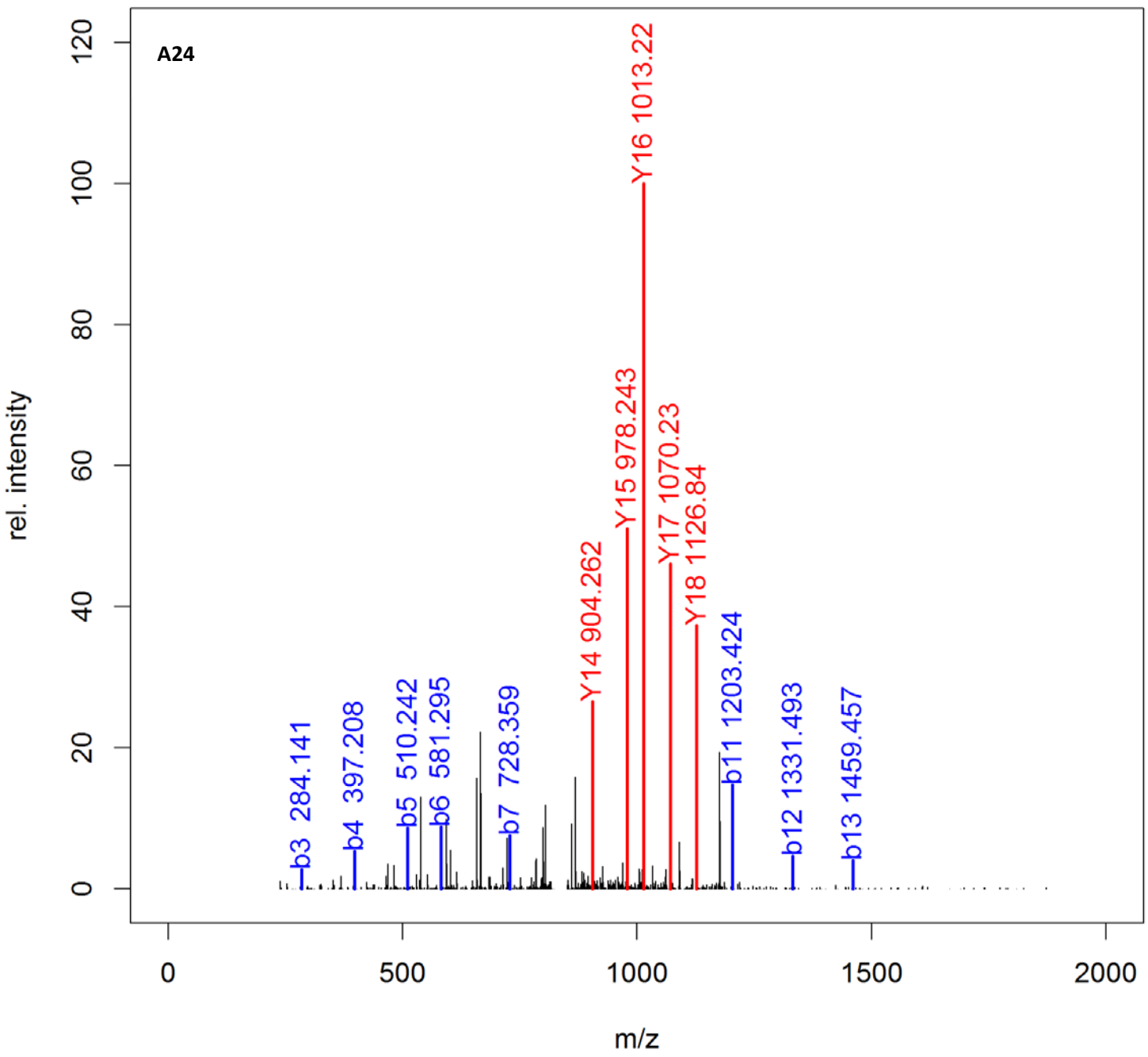
ORB35095_25_PrecursorScan_1608_MZ_841.4253_RT_27.72_MS.MS.Scan_1610



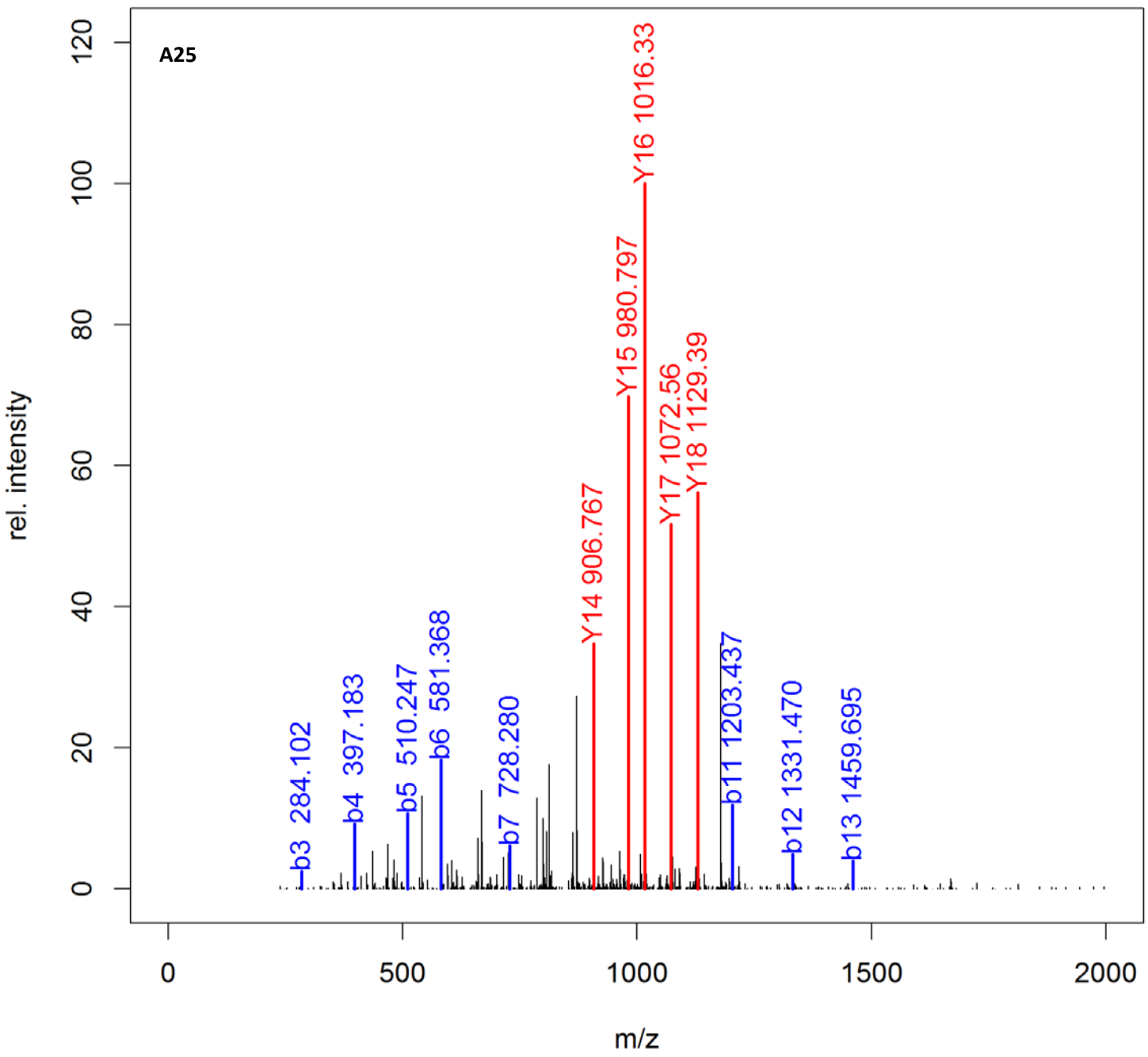
ORB35083_31_PrecursorScan_1639_MZ_841.7532_RT_27.98_MS.MS.Scan_1641



ORB35127_17_PrecursorScan_1521_MZ_845.4249_RT_27.14_MS.MS.Scan_1522



ORB35083_37_PrecursorScan_1719_MZ_847.1097_RT_29.1_MS.MS.Scan_1720



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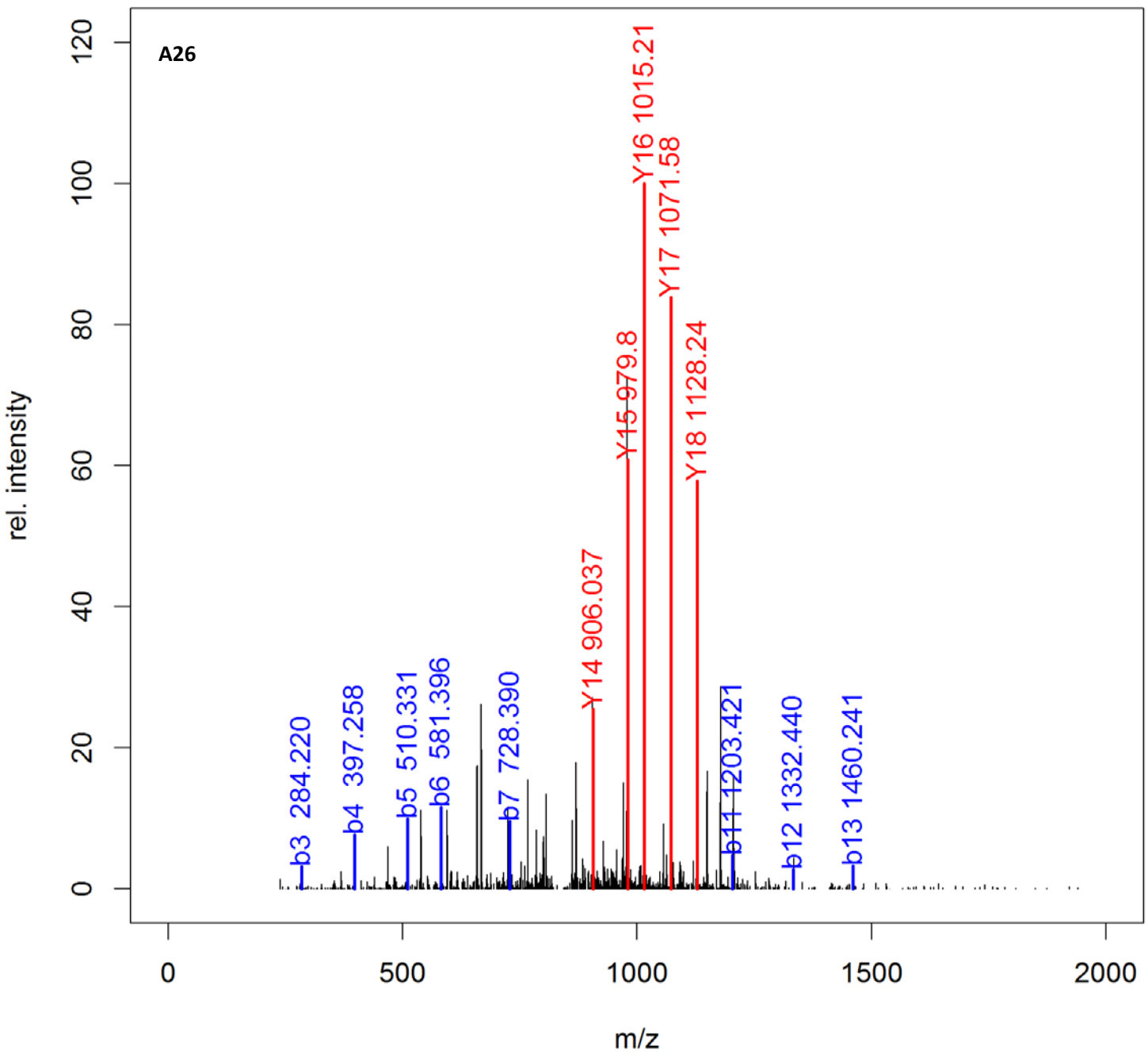
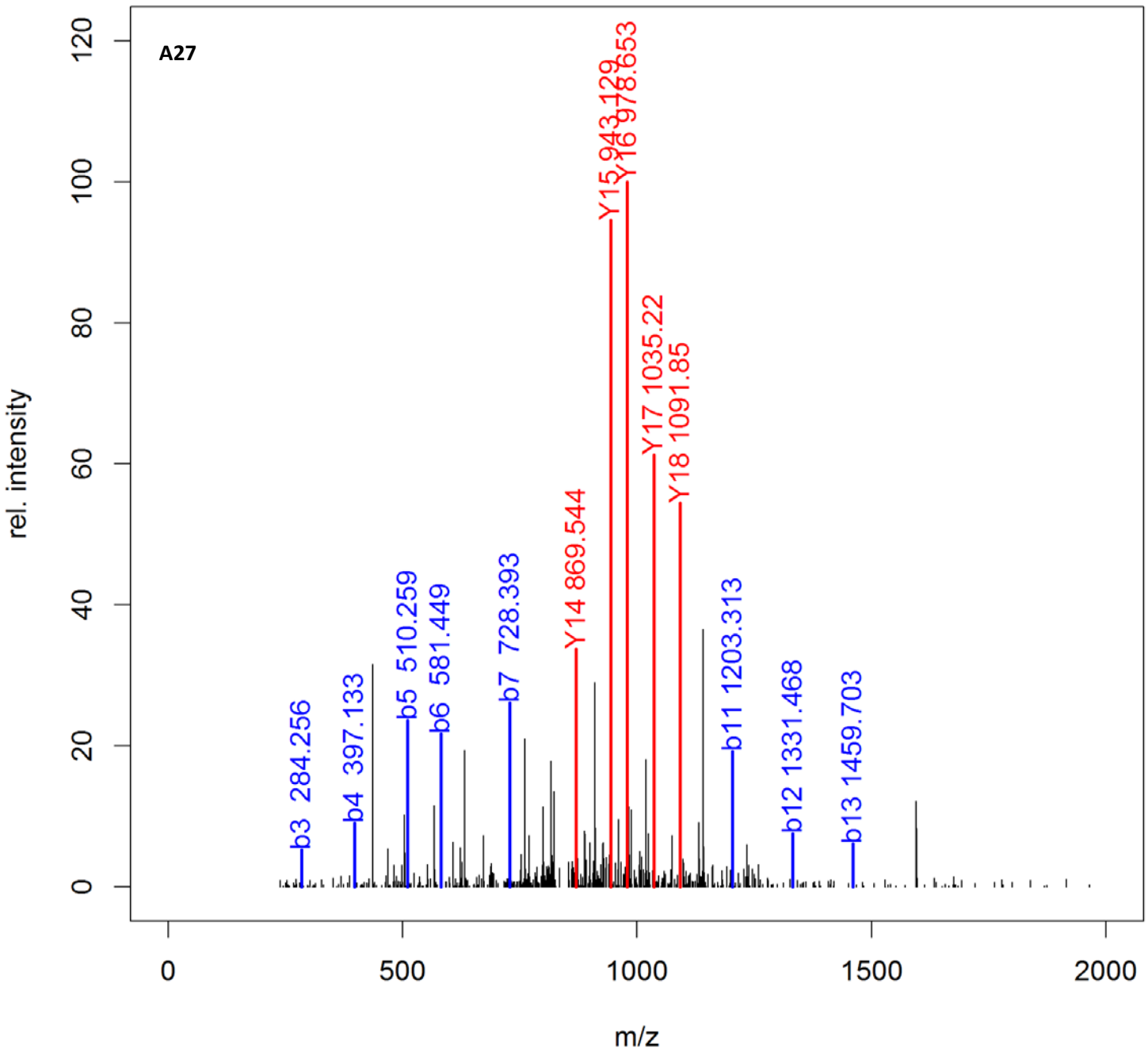
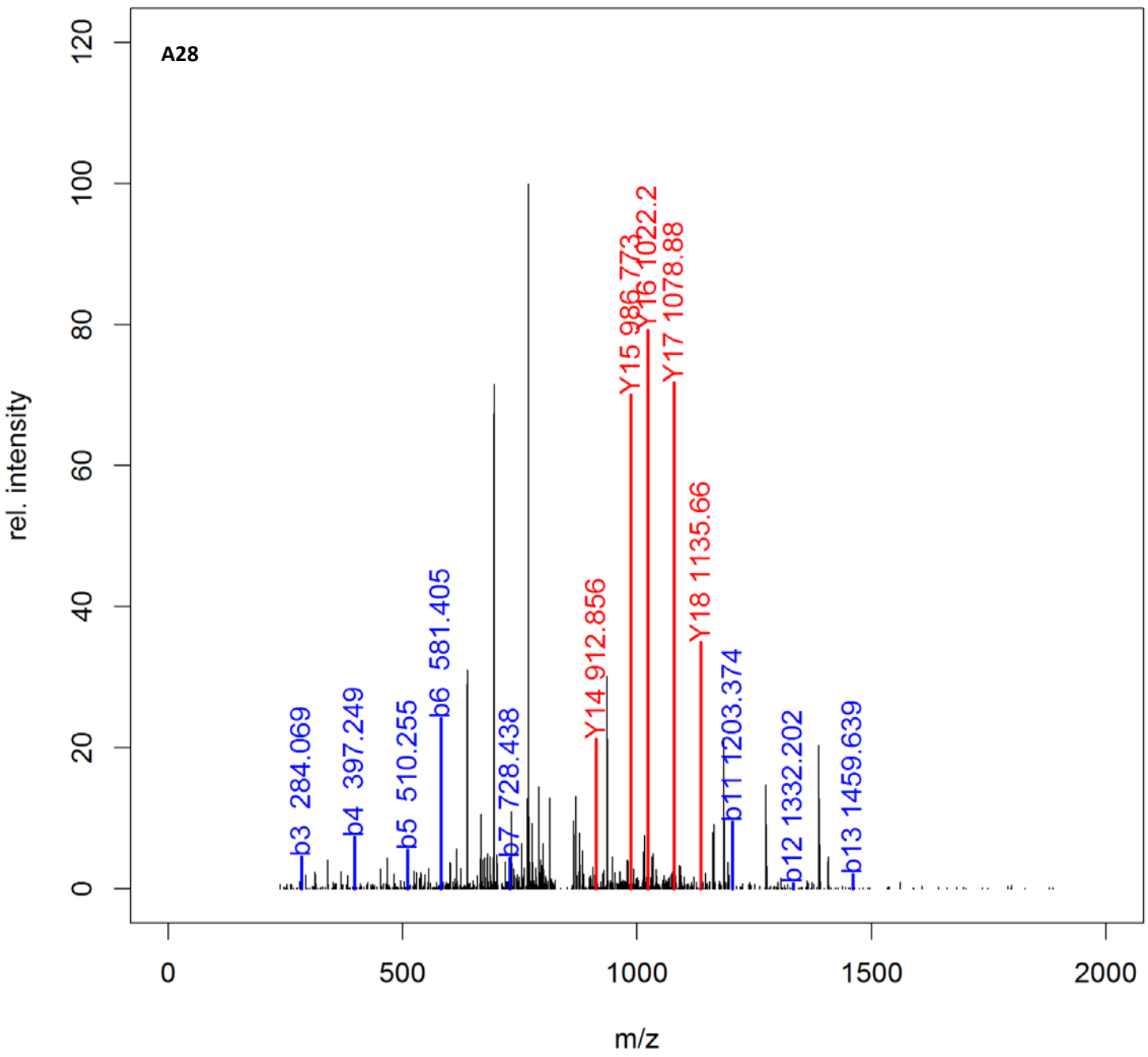


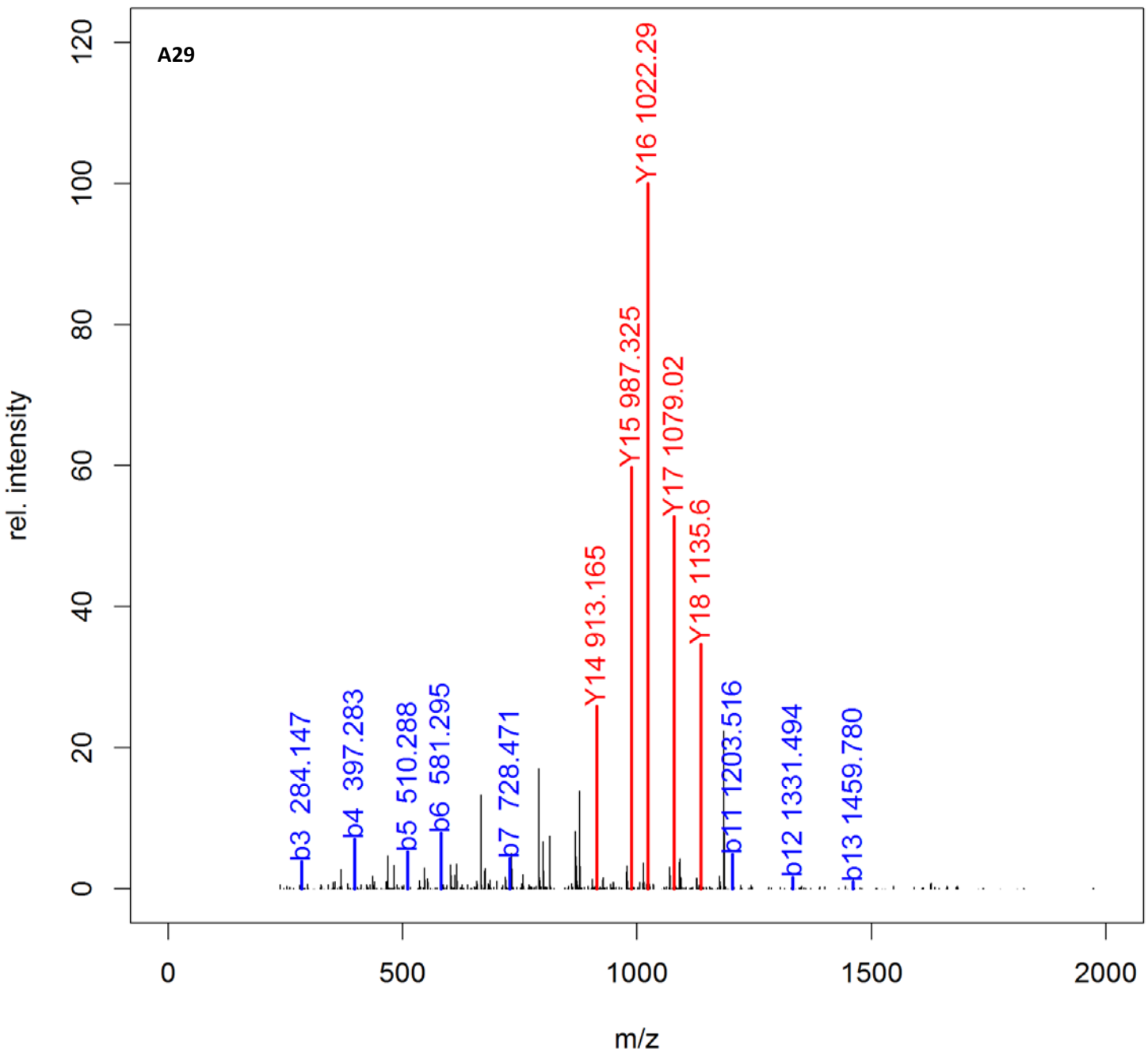
ORB35024_29_PrecursorScan_1707_MZ_849.0703_RT_28.47_MS.MS.Scan_1708



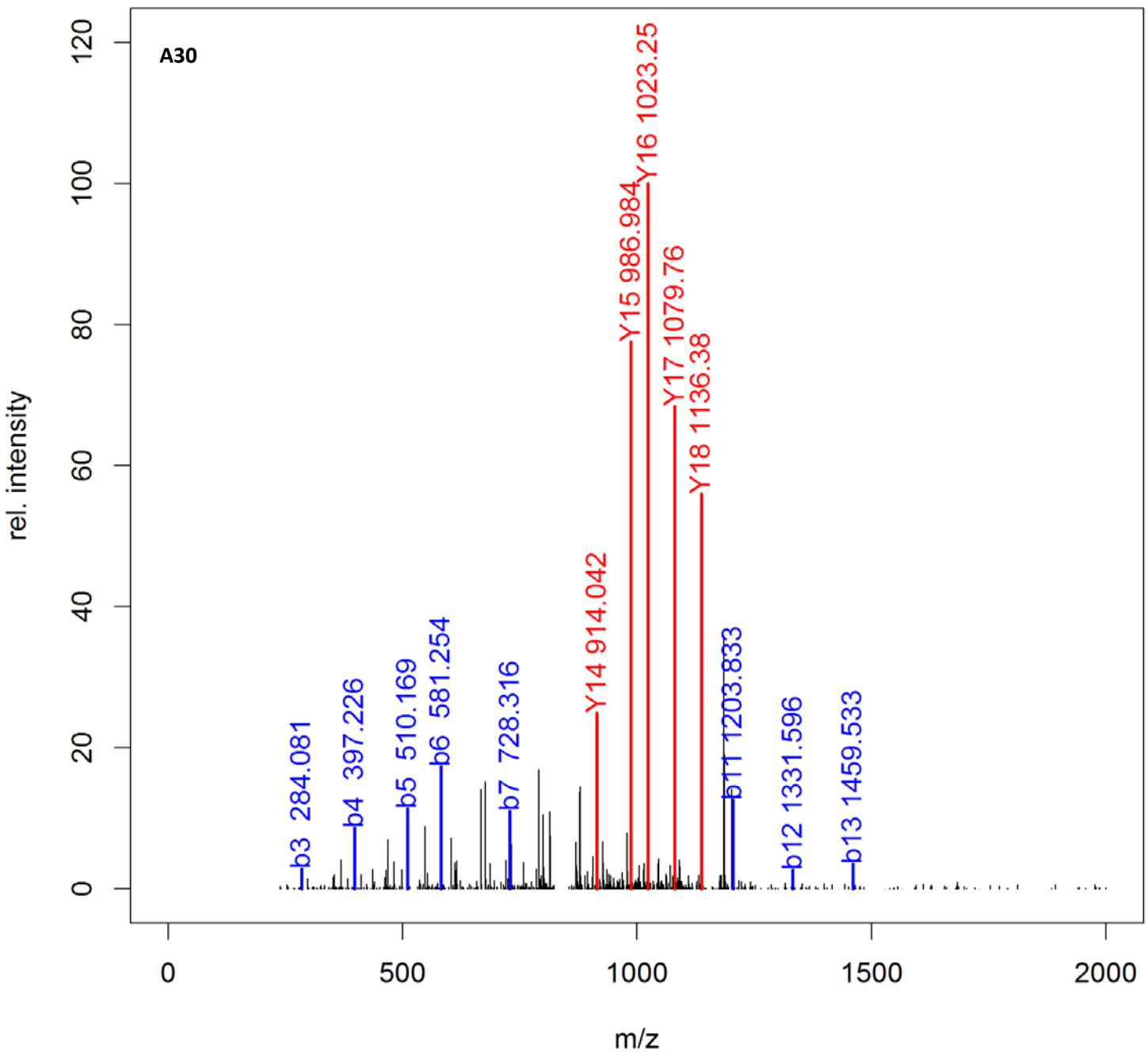
ORB35027_12_PrecursorScan_1619_MZ_850.4359_RT_27.21_MS.MS.Scan_1620



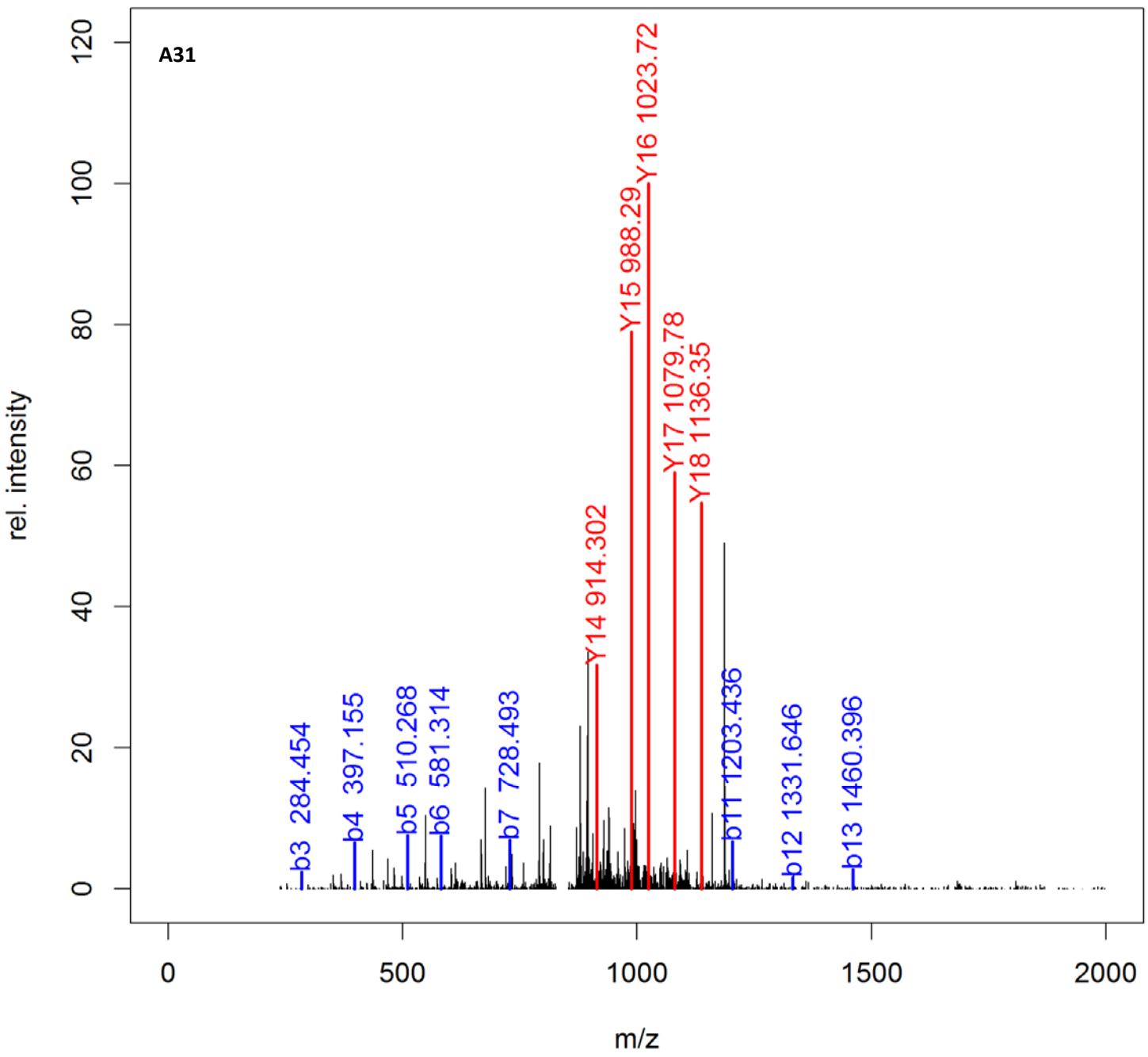
ORB35080_10_PrecursorScan_1506_MZ_851.4293_RT_26.04_MS.MS.Scan_1507



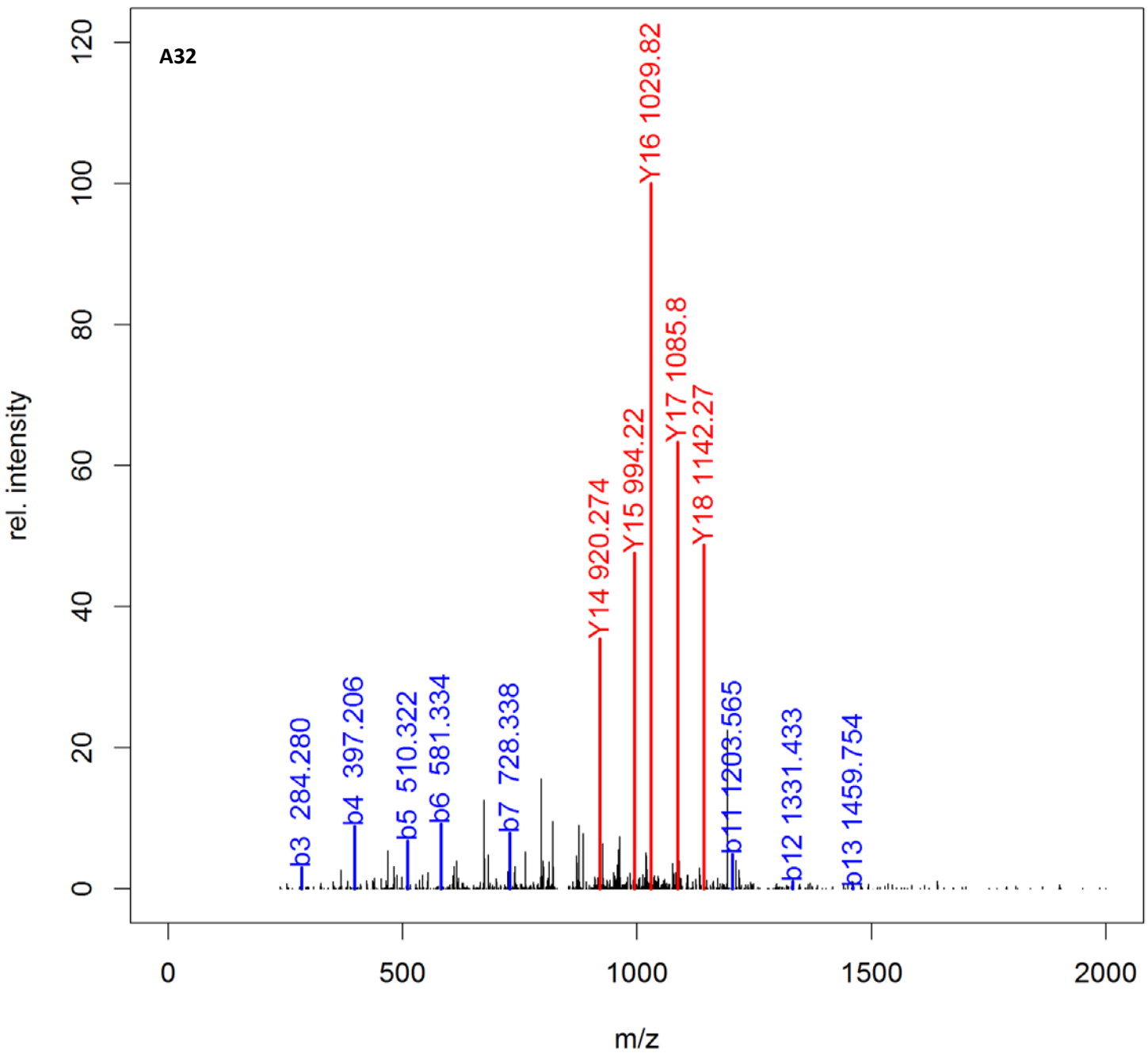
ORB35163_29_PrecursorScan_1619_MZ_851.7569_RT_27.64_MS.MS.Scan_1620



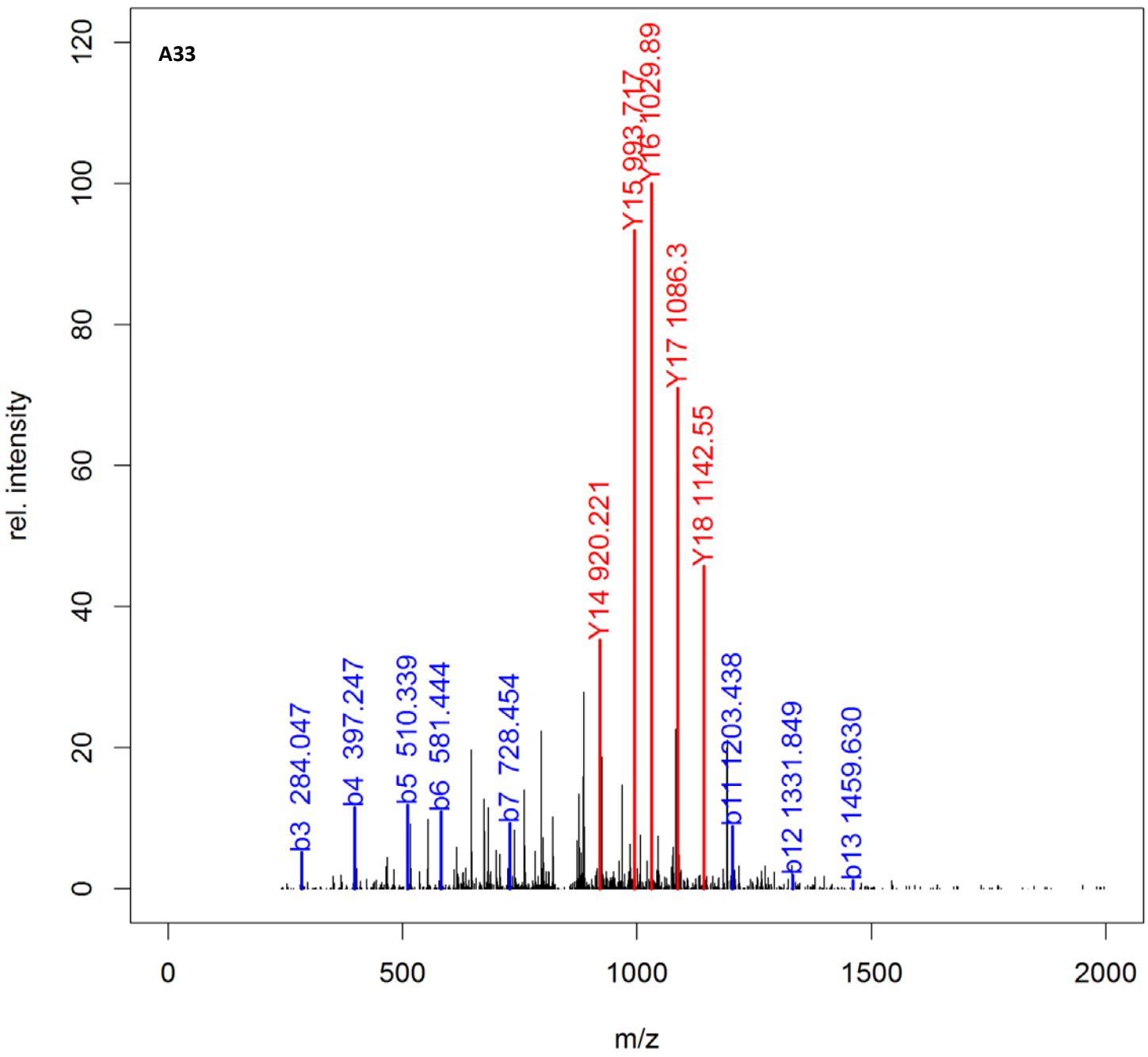
ORB35224_20_PrecursorScan_1635_MZ_853.7839_RT_27.76_MS.MS.Scan_1636



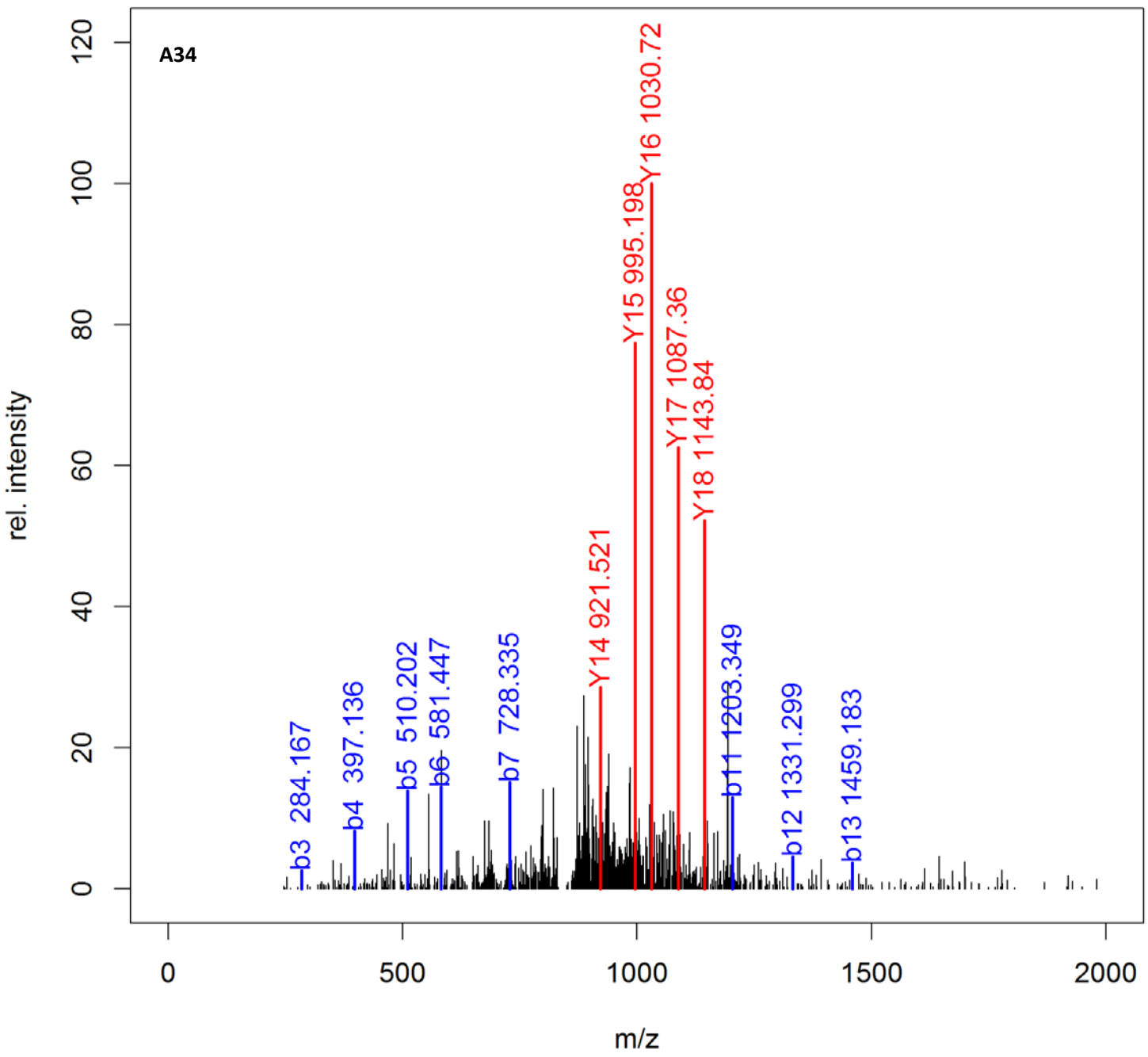
ORB35080_13_PrecursorScan_1533_MZ_856.1001_RT_26.47_MS.MS.Scan_1534



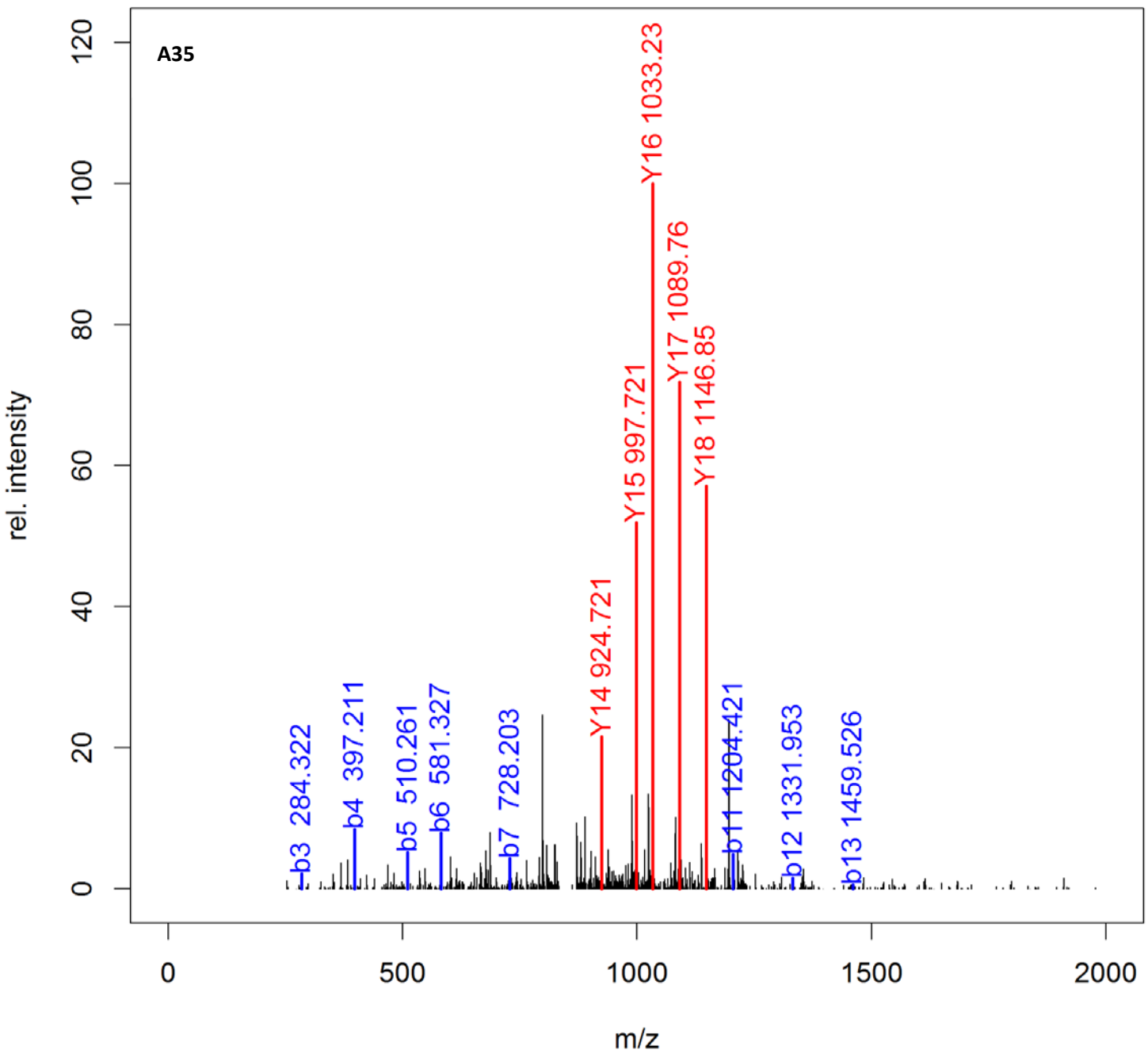
ORB35080_17_PrecursorScan_1553_MZ_856.0998_RT_26.79_MS.MS.Scan_1554



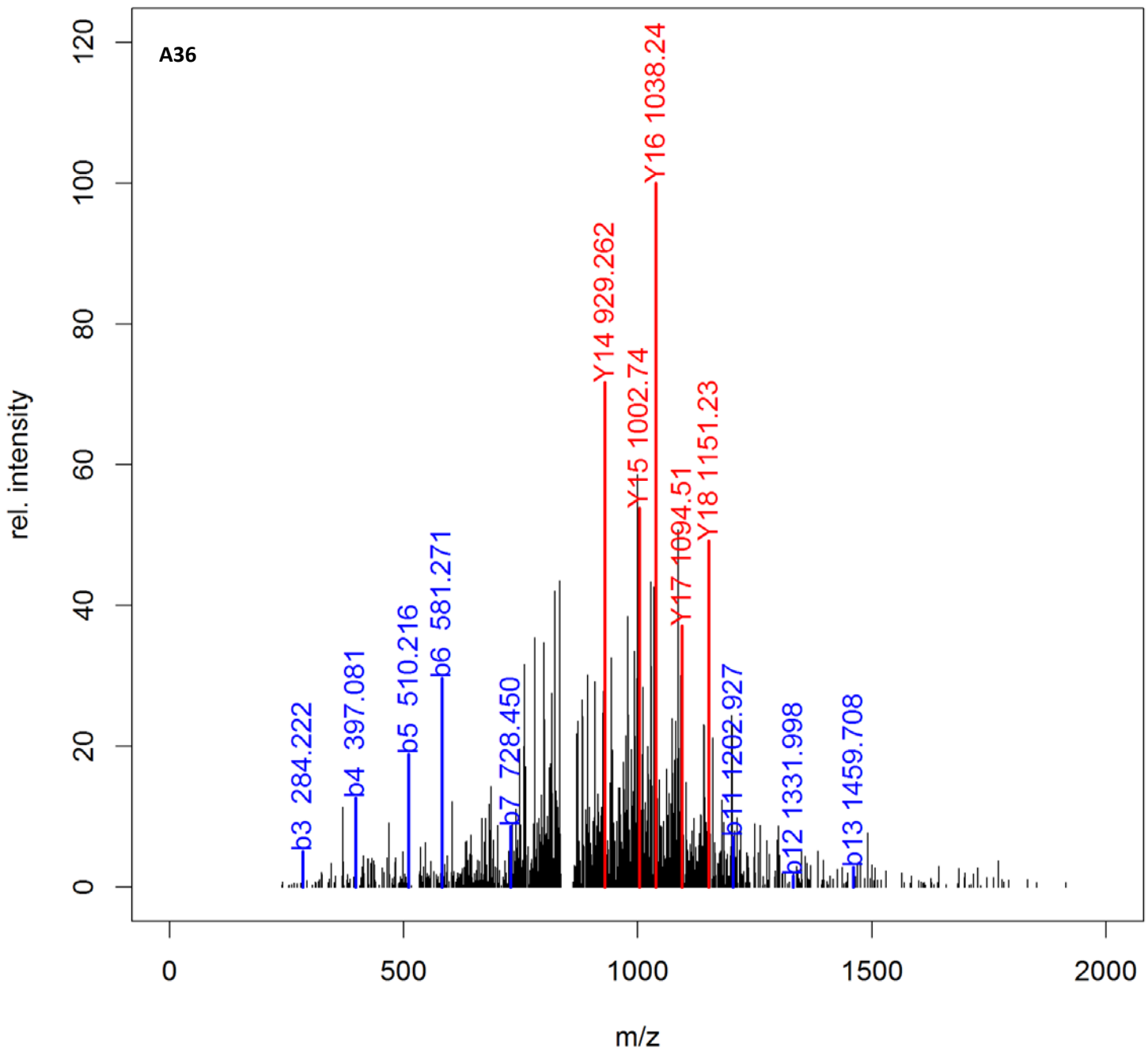
ORB35028_25_PrecursorScan_1696_MZ_857.0991_RT_27.9_MS.MS.Scan_1698



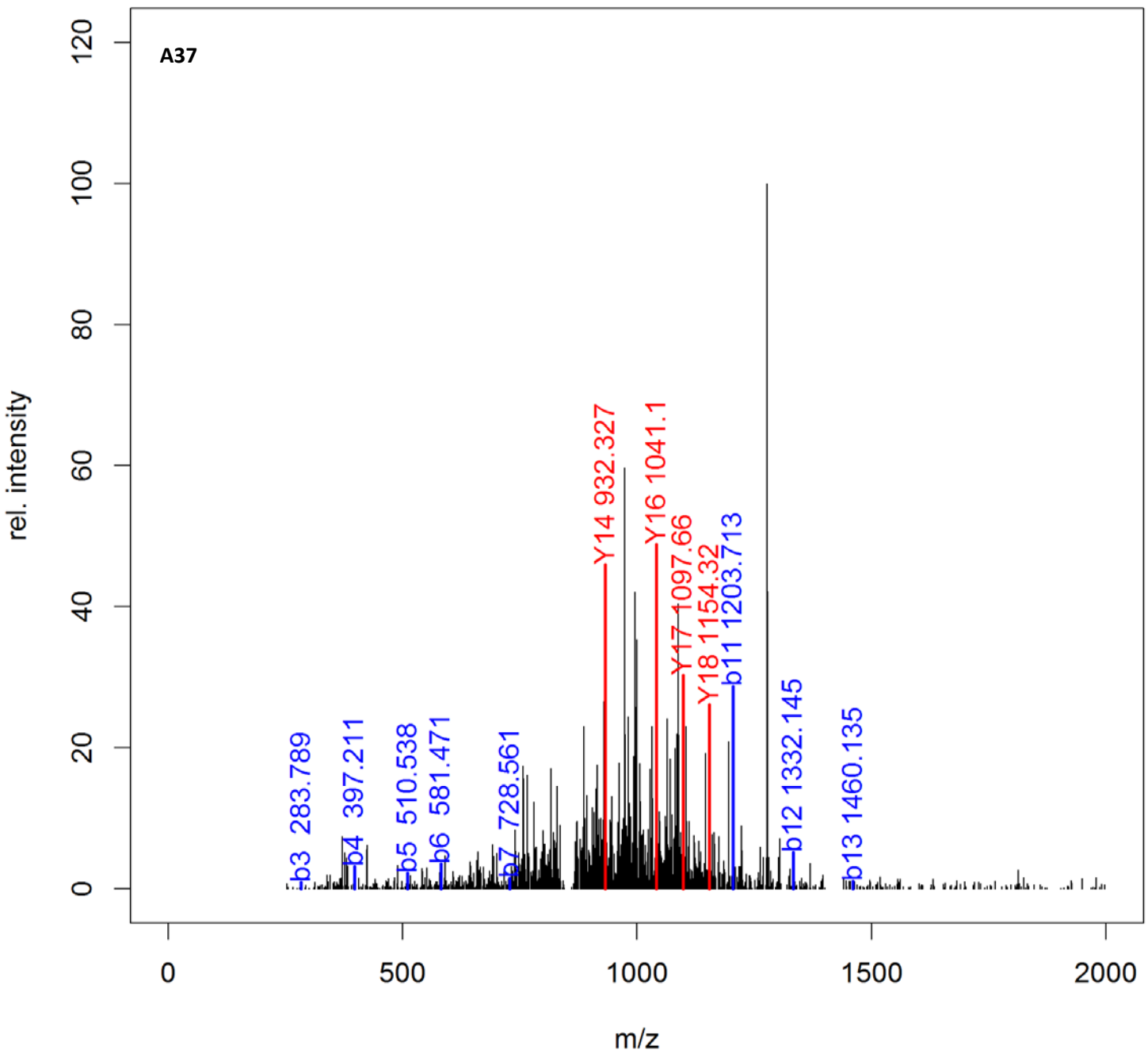
ORB35230_5_PrecursorScan_1514_MZ_858.7535_RT_26.21_MS.MS.Scan_1515



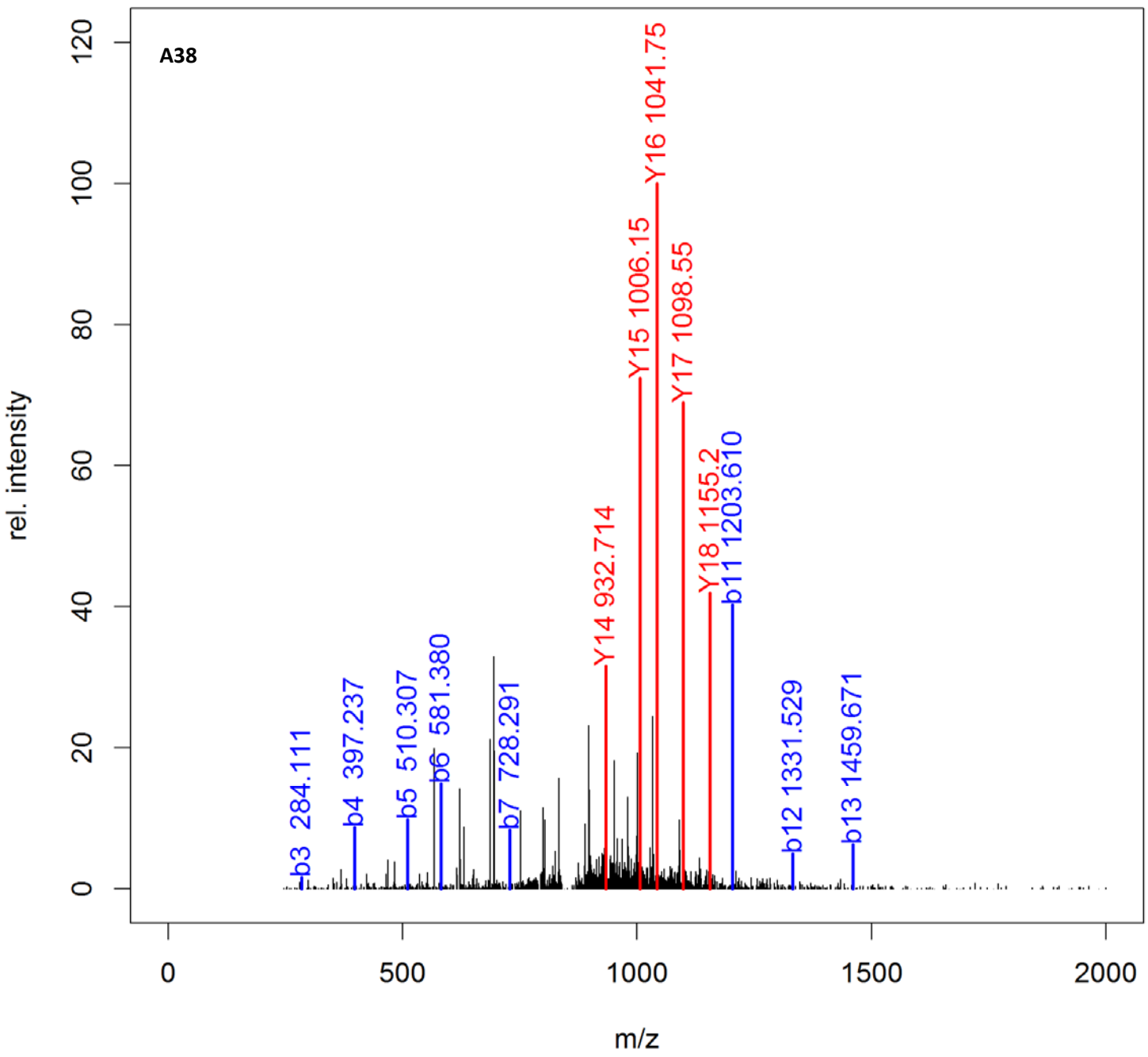
ORB35118_5_PrecursorScan_1527_MZ_862.0903_RT_26.59_MS.MS.Scan_1528



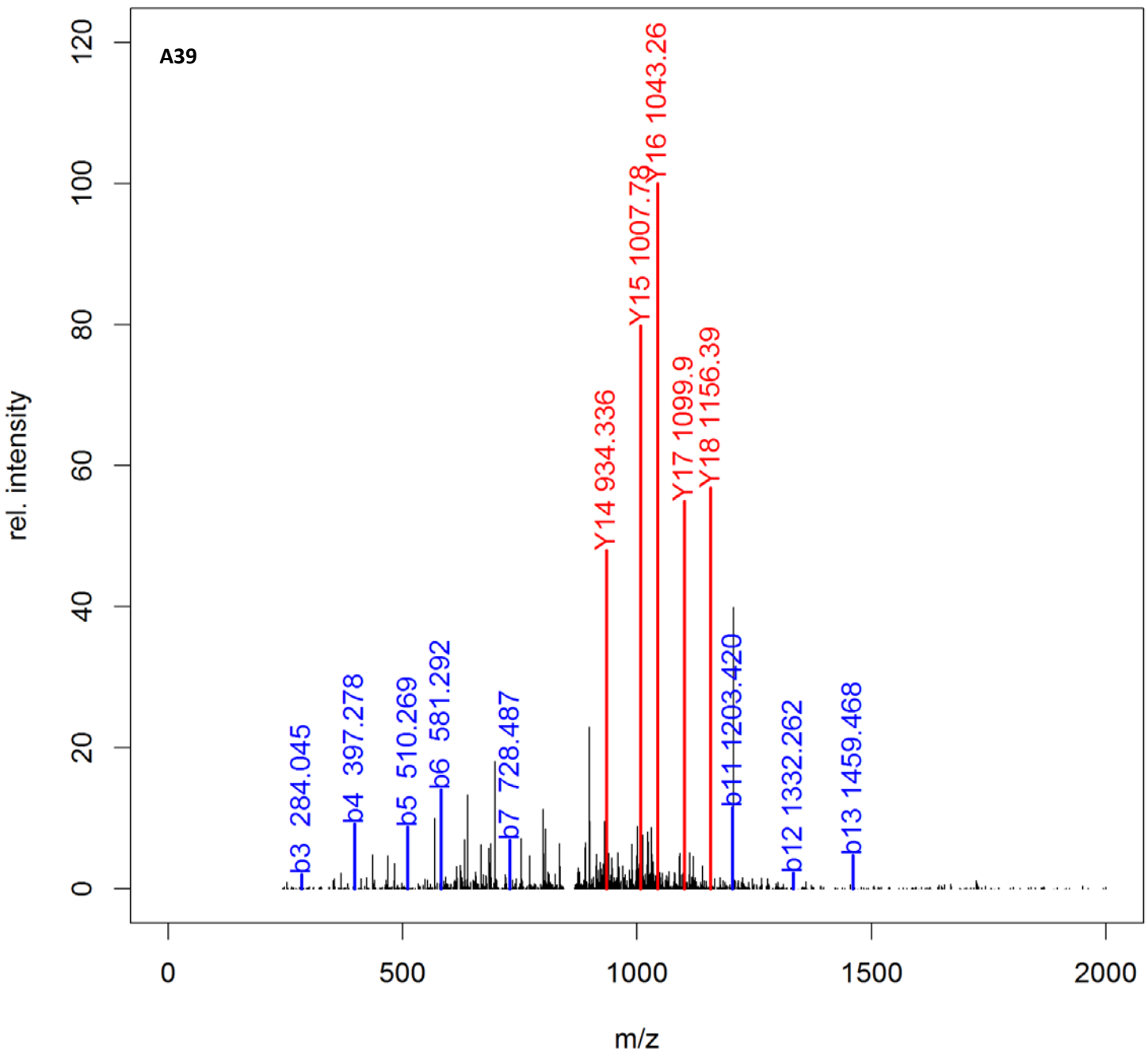
ORB35029_7_PrecursorScan_1568_MZ_864.0787_RT_26.66_MS.MS.Scan_1570



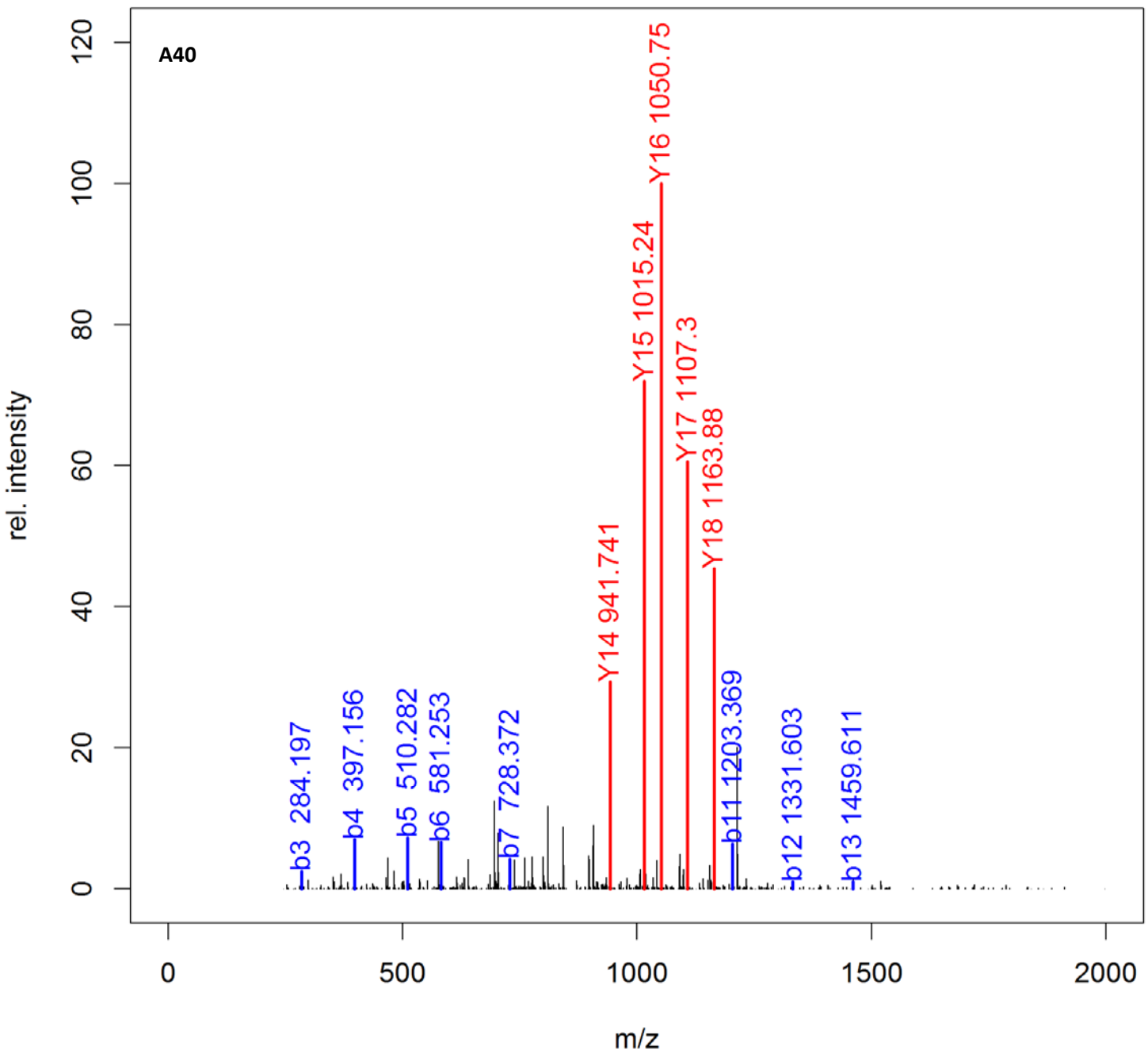
ORB35145_17_PrecursorScan_1518_MZ_864.4328_RT_26.98_MS.MS.Scan_1520



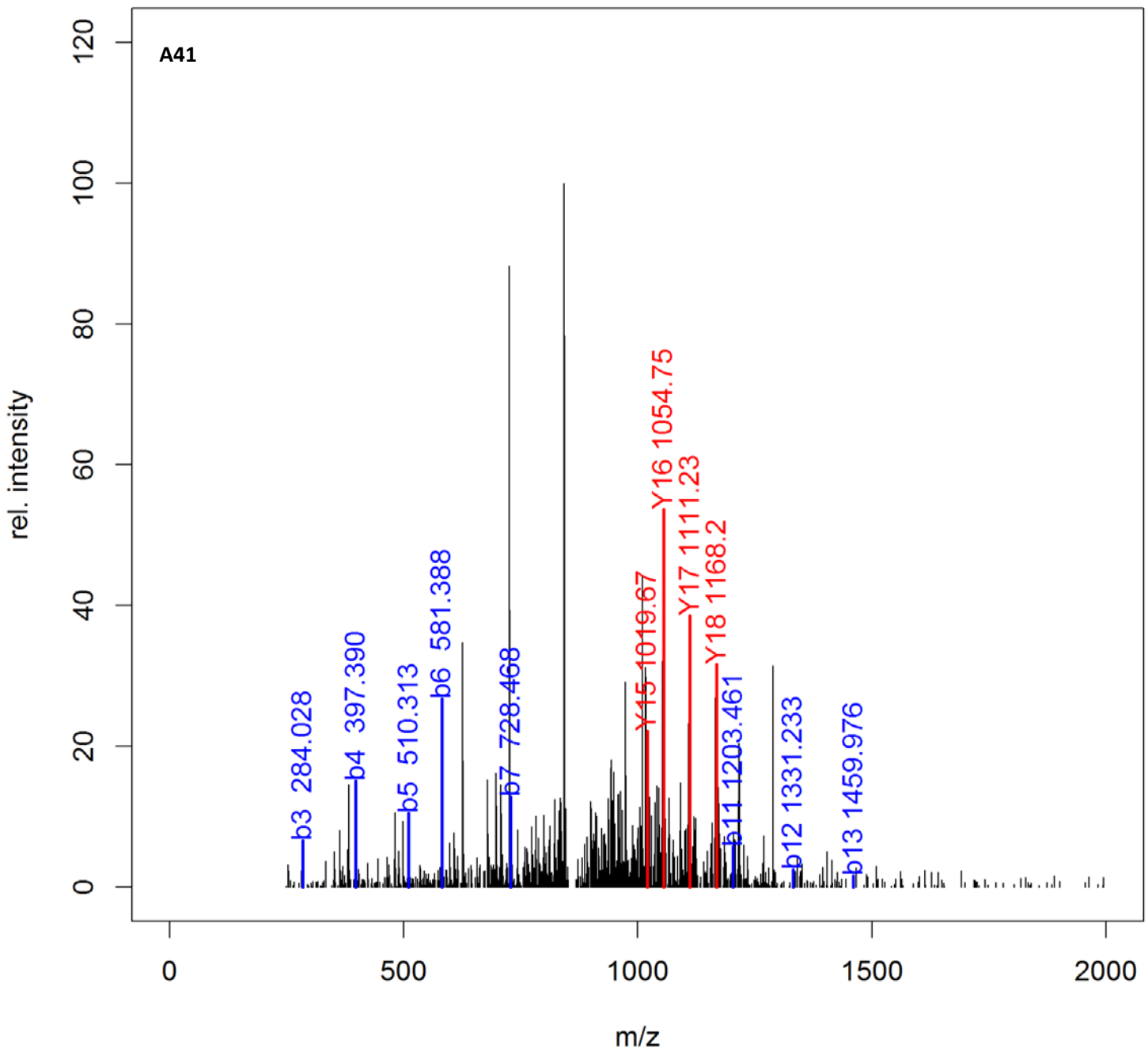
ORB35032_17_PrecursorScan_1657_MZ_865.4313_RT_27.72_MS.MS.Scan_1658



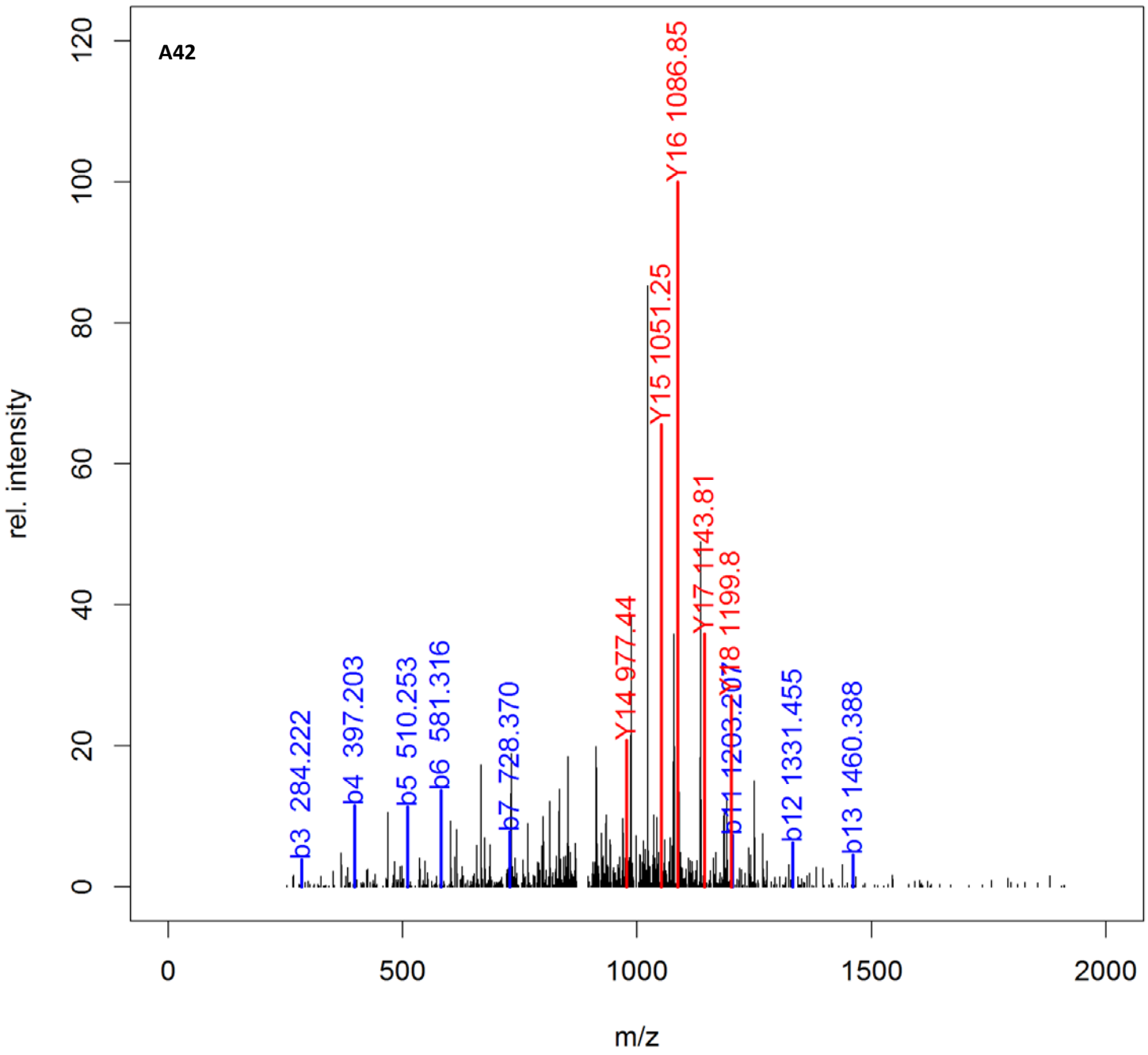
ORB35080_6_PrecursorScan_1483_MZ_870.4359_RT_25.79_MS.MS.Scan_1484



ORB35024_8_PrecursorScan_1578_MZ_872.7306_RT_26.78_MS.MS.Scan_1579



ORB35159_18_PrecursorScan_1526_MZ_894.4422_RT_26.73_MS.MS.Scan_1527



ORB35060_15_PrecursorScan_1507_MZ_913.4497_RT_26.49_MS.MS.Scan_1508

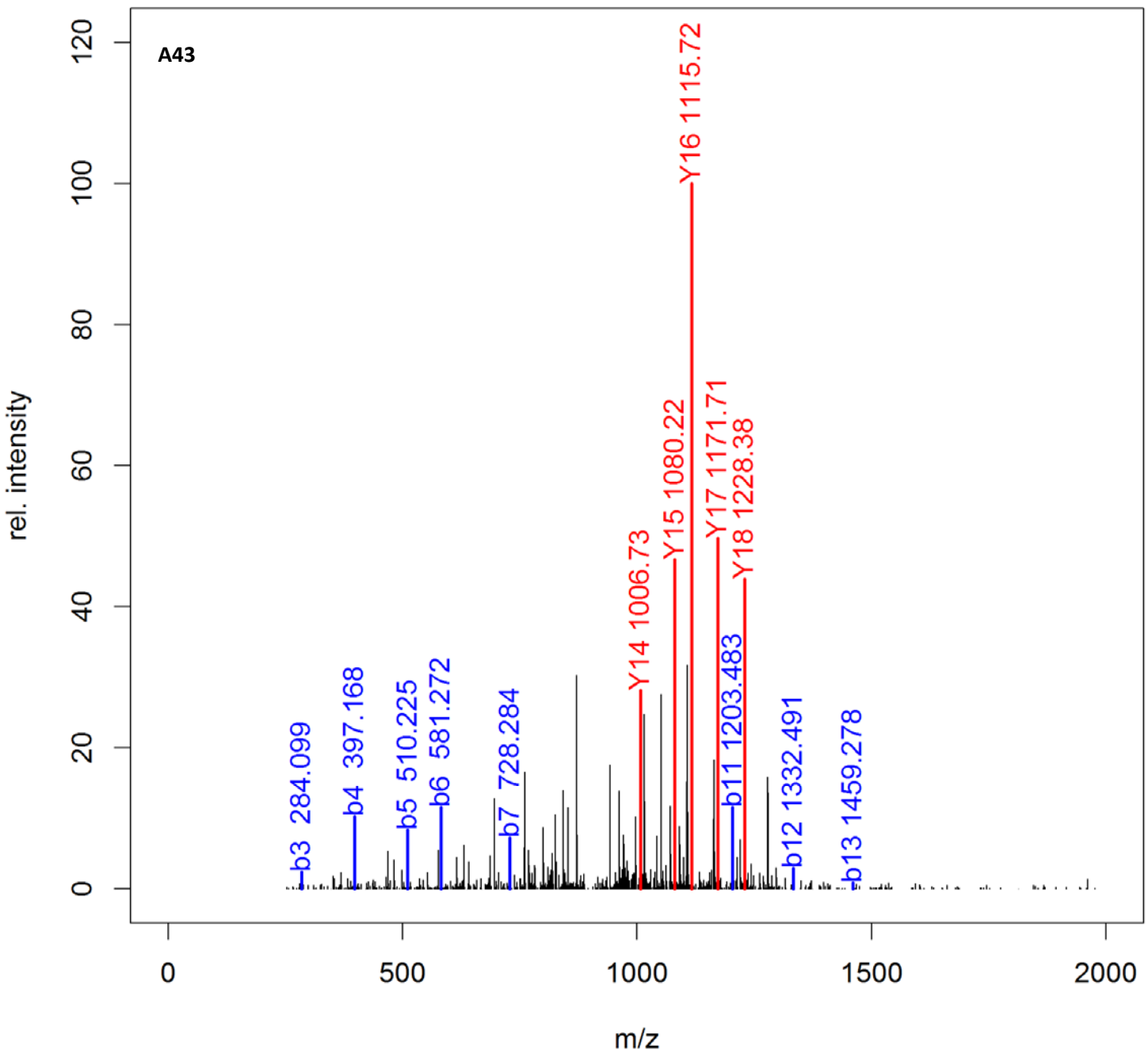
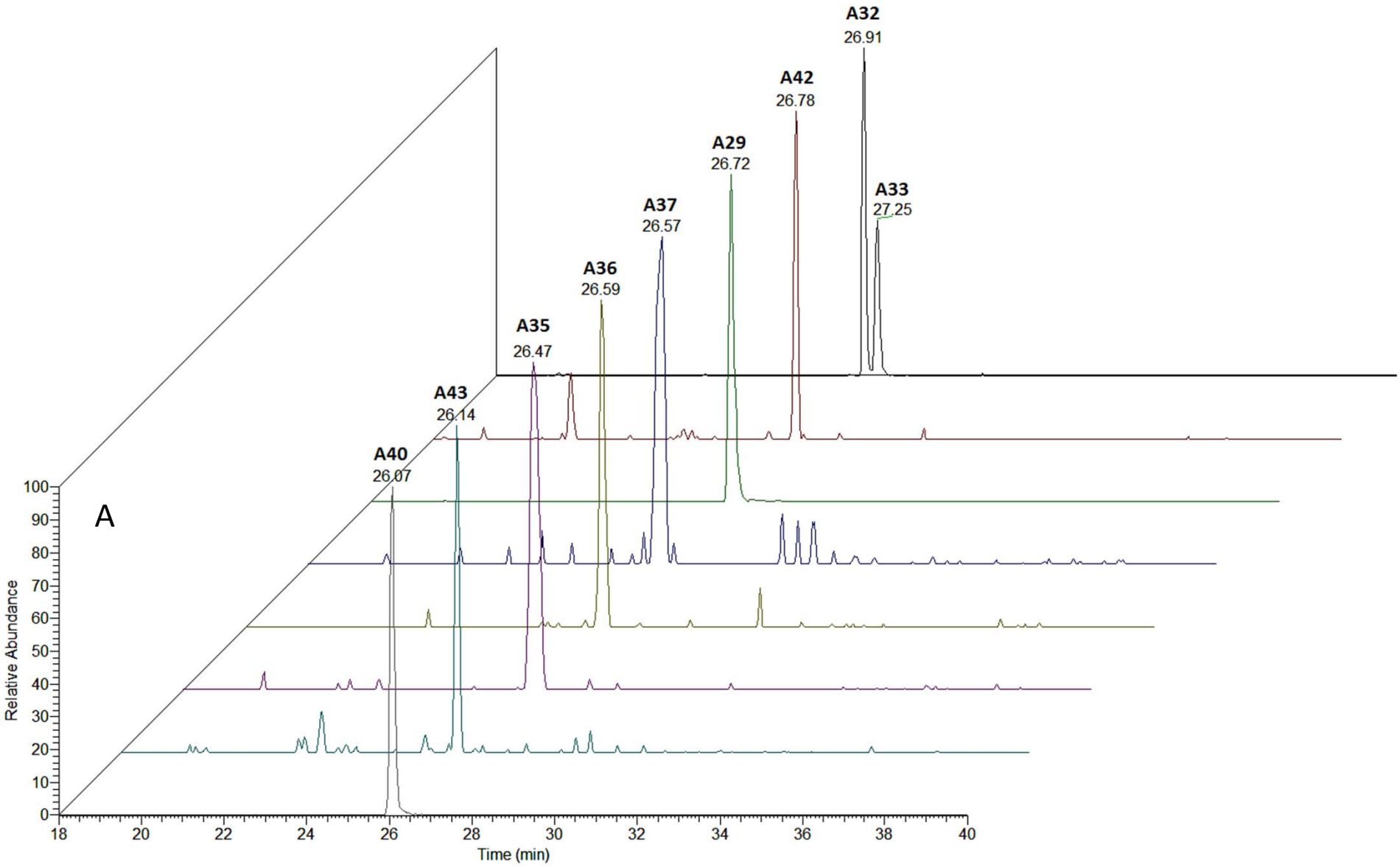
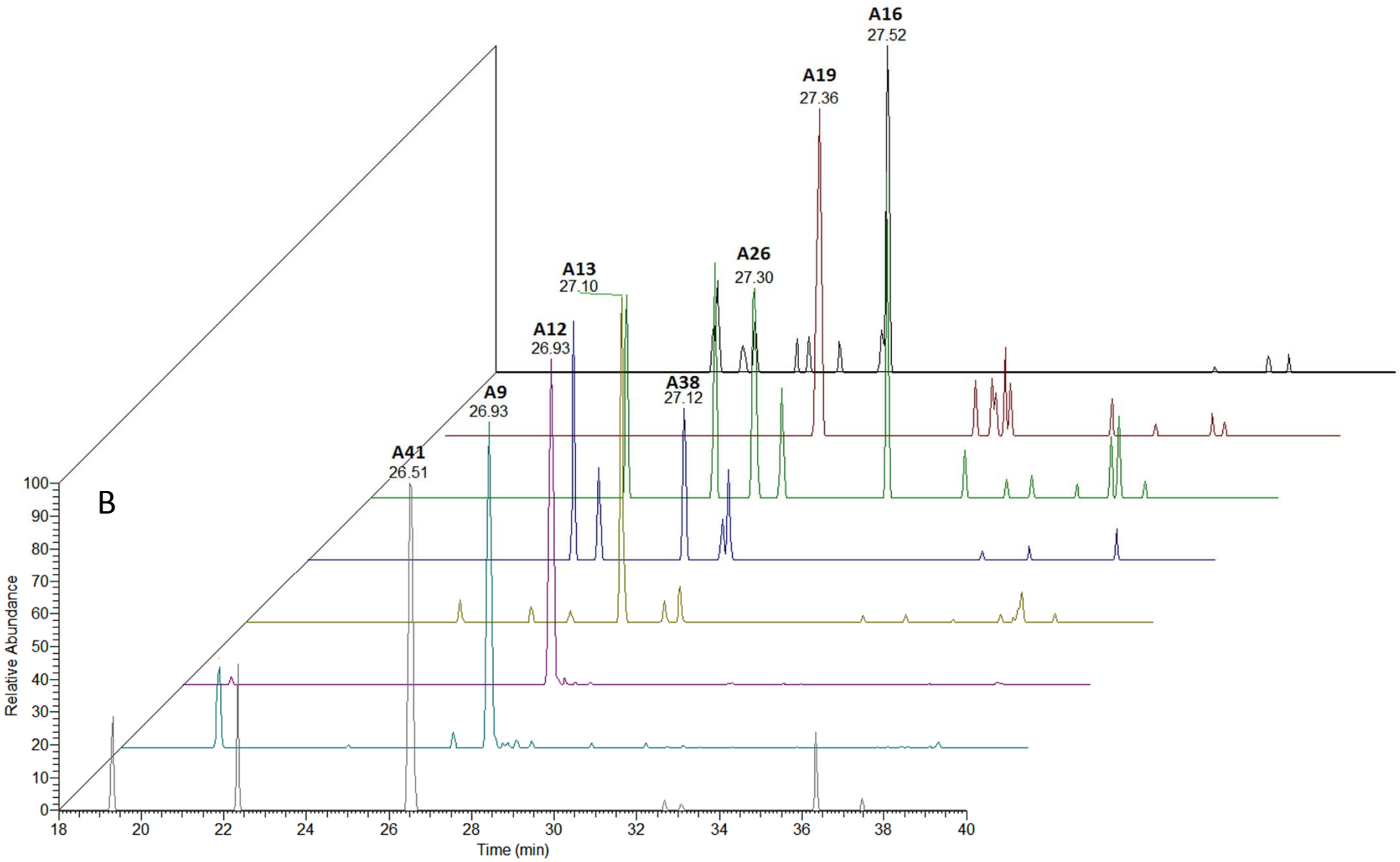
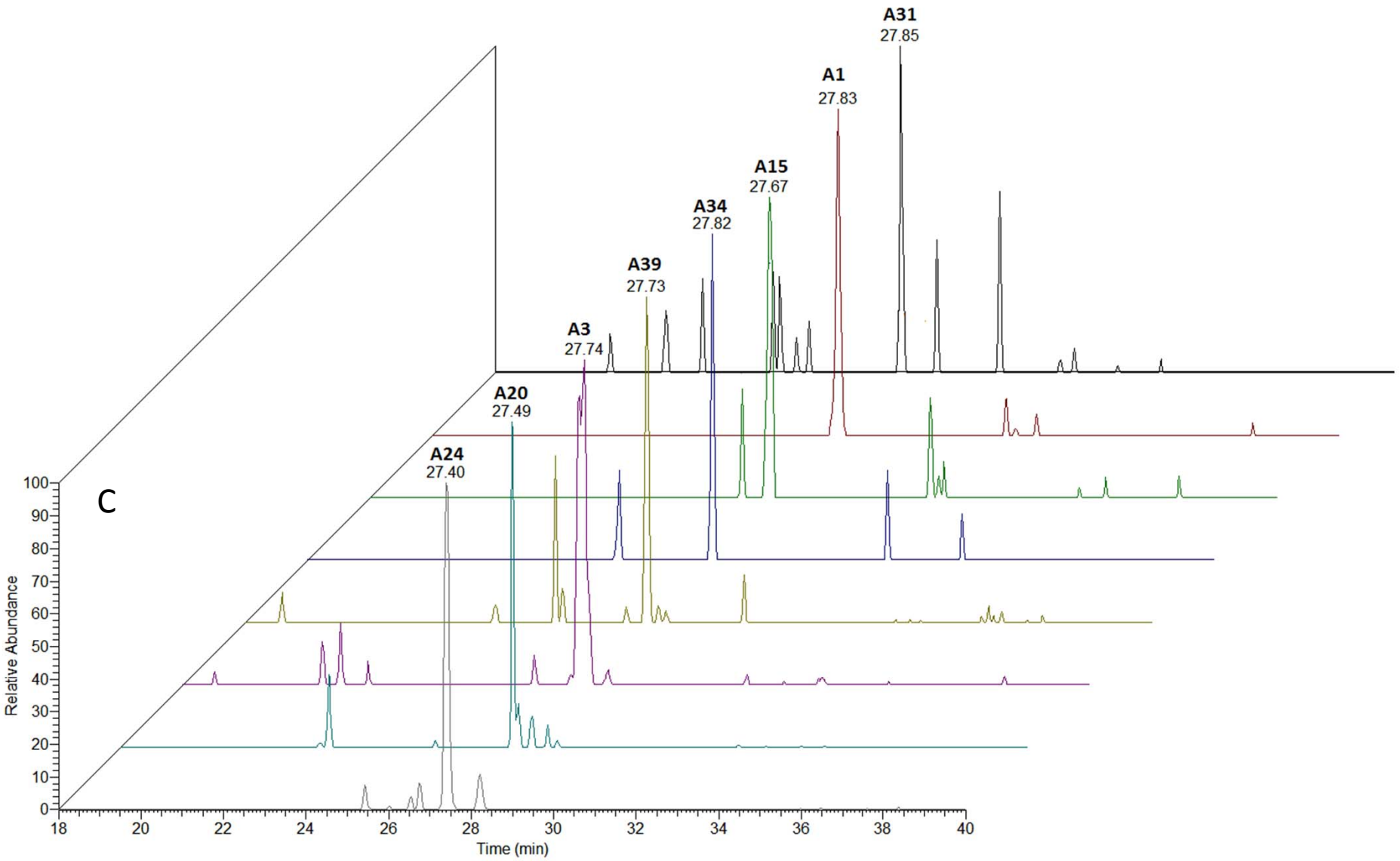
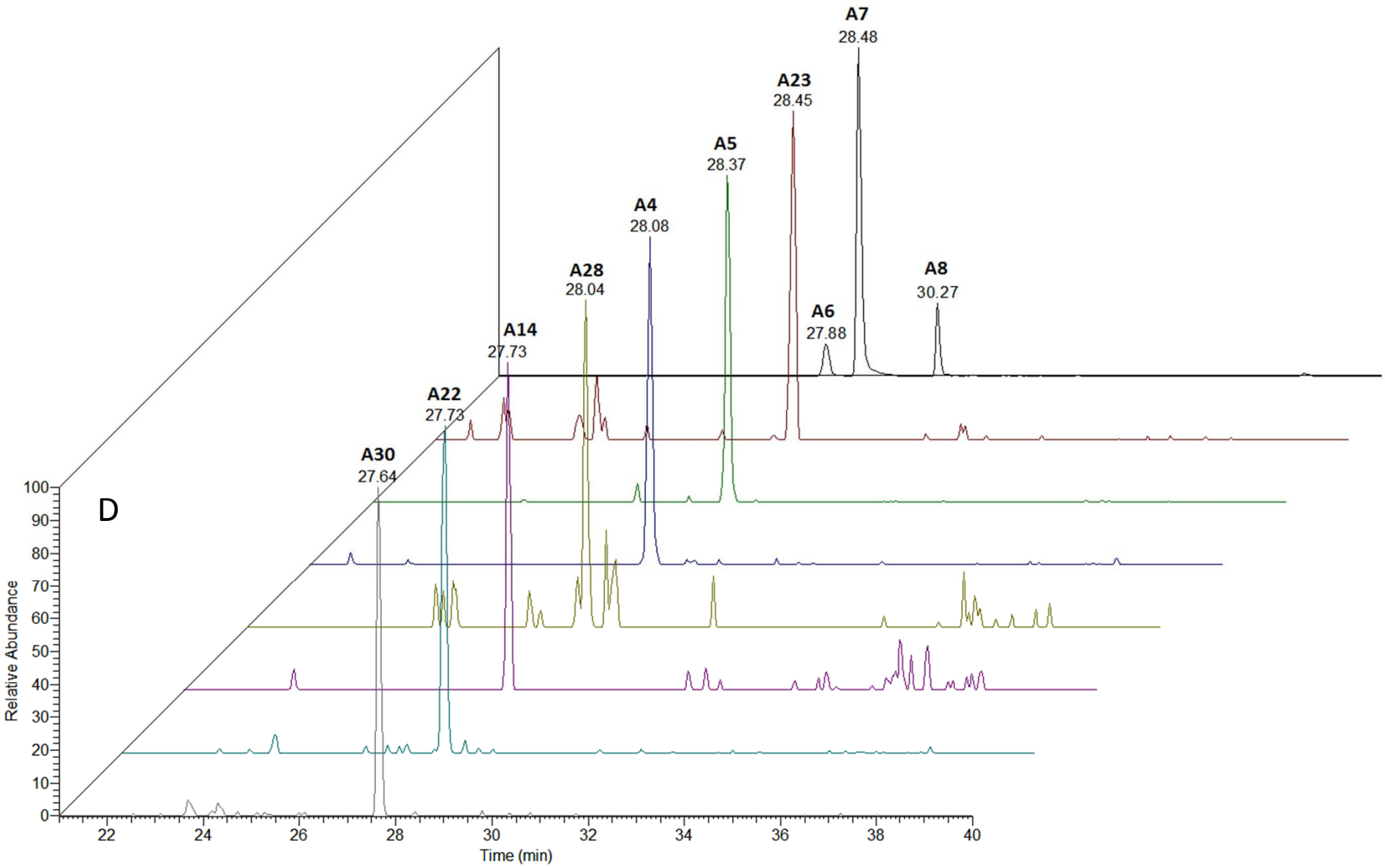


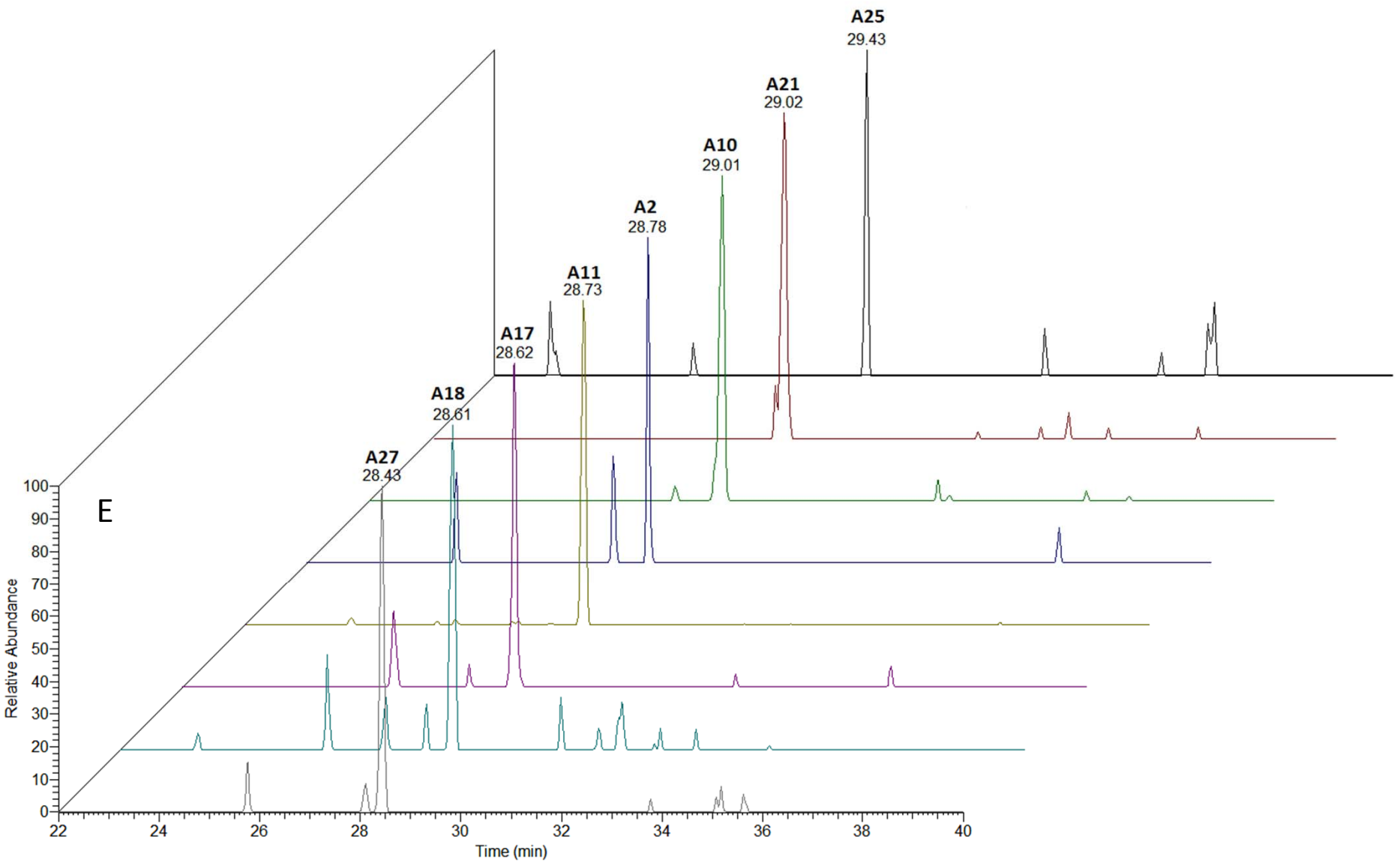
Figure S2. Selected ion chromatograms (SICs) of T3 peptides detected in validation samples. SICs are shown in order of increasing retention time. For clarity, each SIC was selected from a plasma sample with a large S/N. Annotations are given in Table 1 of the text.


A32
26.91
A42
26.78
A29
26.72
A33
27.25
A37
26.57
A36
26.59
A35
26.47
A43
26.14
A40
26.07
A
Relative Abundance
Time (min)
0
10
20
30
40
50
60
70
80
90
100
18
20
22
24
26
28
30
32
34
36
38
40


B
A16
27.52
A19
27.36
A26
27.30
A13
27.10
A12
26.93
A38
27.12
A9
26.93
A41
26.51
Relative Abundance
Time (min)
0
10
20
30
40
50
60
70
80
90
100
18
20
22
24
26
28
30
32
34
36
38
40


A31
27.85
A1
27.83
A15
27.67
A34
27.82
A39
27.73
A3
27.74
A20
27.49
A24
27.40
C
Relative Abundance
Time (min)
0
10
20
30
40
50
60
70
80
90
100
18
20
22
24
26
28
30
32
34
36
38
40


D
A7
28.48
A23
28.45
A5
28.37
A4
28.08
A28
28.04
A8
30.27
A6
27.88
A14
27.73
A22
27.73
A30
27.64
Relative Abundance
Time (min)
0
10
20
30
40
50
60
70
80
90
100
22
24
26
28
30
32
34
36
38
40


E
A25
29.43
A21
29.02
A10
29.01
A2
28.78
A11
28.73
A17
28.62
A18
28.61
A27
28.43
Relative Abundance
Time (min)
0
10
20
30
40
50
60
70
80
90
100
22
24
26
28
30
32
34
36
38
40