

In vitro digests of meat products reveal specific metabolic signature of processed meat products in an untargeted metabolomics analysis

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TABLE S2: Metabolic features with significantly higher levels in processed meat digests compared to non-processed pork and beef digests. A Welch's t-test revealed 178 metabolic features that were significantly (FDR-adjusted p-value <0.05) and with at least 2-fold increase in processed meat products (n = 63) compared to non-processed pork and beef (n = 36).

m/z	RT	FDR-adjusted p-value	Fold- change ^a	Cluster ^b	Adduct	HMDB ID ^c	Annotation	Annotation level of confidence ^d
359.2448	7.13	3.71E-06	2.31	1				
523.3828	7.14	2.35E-06	2.77	1				
241.1416	5.48	0.000339	2.10	1				
265.1778	6.66	0.00011	2.24	1				
570.8279	4.92	2.35E-06	2.01	1				
669.3921	3.93	2.33E-06	2.19	1				
581.317	4.92	9.77E-07	2.26	1				
631.4697	9.75	3.36E-06	2.19	1				
652.5865	9.75	6.41E-06	2.22	1				
685.5394	7.87	0.000228	2.04	1				
522.8854	7.13	9.28E-05	2.04	1				
575.502	9.75	1.01E-05	2.27	1				
531.4017	8.15	0.000601	2.03	1	[M+Na] ⁺	HMDB0007038	Unknown 1: Possibly diglyceride	4

Online supporting material

347.2192	6.69	0.000182	2.14	1				
353.2662	7.26	0.007331	2.21	1	[M+Na] ⁺	HMDB0011533	Unknown 2: Possibly monoglyceride	4
302.1623	5.92	0.002954	2.34	1				
325.2363	6.18	0.002154	2.03	1	[M+Na] ⁺	HMDB0011530	Unknown 3: Possibly monoglyceride	4
793.5126	7.04	0.000432	2.12	1				
811.4872	7.04	0.002099	2.06	1				
337.272	9.75	0.000136	2.72	1				
263.2366	7.78	0.000116	2.77	1				
608.524	9.30	0.013896	2.17	1				
364.254	6.92	0.001657	2.57	1				
361.1687	6.76	0.002712	2.19	1				
271.2271	6.48	0.000799	3.45	1				
312.2536	6.69	0.018251	2.09	1				
615.4575	6.75	0.005894	2.18	1				
317.1556	6.12	0.016059	2.08	1				
409.2028	7.78	0.004203	2.43	1				
315.1643	6.12	0.006405	2.12	1				
421.2532	7.21	0.002053	2.93	1				
353.2669	7.68	0.019551	2.30	1	[M+Na] ⁺	HMDB0011533	Unknown 4 : Possible monoglyceride	4

Online supporting material

369.2406	7.18	0.01056	2.07	1				
279.1712	3.36	0.015885	3.13	2	[M+H] ⁺	HMDB0028998	Phe-Ile dipeptide	2
245.1381	4.32	0.019551	2.34	2				
523.383	7.17	0.007815	3.80	2				
524.3847	7.17	1.65E-05	501.94	2				
332.2184	3.63	0.02459	3.50	2				
360.2896	6.78	0.01454	4.36	2				
145.0496	0.86	3.67E-05	415.11	2				
244.9731	1.86	0.00029	14.42	2				
285.1313	4.17	0.000112	327.15	2				
261.0284	1.86	0.000513	306.60	2				
336.1922	3.22	0.000807	295.48	2				
127.0383	3.03	0.001373	262.82	2				
167.0312	1.53	0.004018	216.55	2				
379.2782	7.04	0.000233	2.89	3				
280.2356	6.72	0.010854	2.06	3				
365.2294	6.18	0.000737	2.56	3				
260.1949	0.85	0.000147	3.53	3				
581.8191	4.92	2.35E-06	2.48	3				

Online supporting material

615.349	3.76	8.40E-05	2.70	3				
213.1105	4.45	2.94E-08	7.23	3				
172.0058	3.07	6.93E-06	2.69	3				
130.0323	2.42	8.12E-05	2.71	3				
176.0364	0.89	1.73E-06	3.35	3				
539.319	6.91	5.74E-05	4.34	3				
637.4798	8.99	7.39E-05	3.10	3				
632.5247	8.99	2.17E-06	3.93	3				
217.0851	5.22	5.29E-05	5.03	3				
402.2352	3.30	1.86E-05	5.67	3				
754.4483	4.62	7.64E-07	9.90	4				
308.1262	6.01	3.66E-05	3.80	4	[M+Na] ⁺	HMDB0029377	Piperine	1
393.3841	7.34	7.94E-05	2.52	4				
345.2181	5.99	9.03E-08	8.61	4				
272.1287	5.74	7.35E-05	3.47	4	[M+H] ⁺	HMDB0029374	Piperyline	2
312.1597	6.29	6.85E-07	10.83	4	[M+H] ⁺	HMDB0034371	Piperettine	2
288.1601	5.93	7.99E-08	13.03	4	[M+H] ⁺	HMDB0033874	Piperanine	2
340.1909	6.57	0.000624	3.09	4	[M+H] ⁺	HMDB0040811	Dehydropipernonaline (Pepper compound 5)	3
344.2236	6.78	4.43E-06	7.17	4	[M+H] ⁺	HMDB0030340	Piperolein B (Pepper compound 7)	3

Online supporting material

403.2953	6.78	6.71E-09	681.21	4				
347.2317	5.93	2.29E-08	29.92	4				
224.2012	6.36	4.79E-07	12.10	4	[M+H] ⁺	HMDB0030951	Pellitorine	1
722.3292	6.01	1.50E-08	628.89	4				
700.3479	6.02	1.55E-08	601.29	4				
314.1751	6.28	1.61E-07	23.66	4				
371.2331	6.28	3.46E-08	686.62	4			Compound in pepper	3
331.2016	5.74	3.46E-08	606.90	4				
316.192	6.41	1.52E-07	586.71	4	[M+H] ⁺	HMDB0030185	(E)-Piperolein A (Pepper compound 1)	3
310.1422	5.93	1.58E-07	554.04	4	[M+Na] ⁺	HMDB0033874	Piperanine, also as [M+H]⁺	2
755.4229	5.98	6.85E-07	23.03	4				
334.1411	6.28	2.08E-06	20.10	4	[M+Na] ⁺	HMDB0034371	Piperettine	2
285.1363	5.99	6.47E-07	524.09	4	[M+Na] ⁺		Comp from pepper	3
314.1394	4.46	7.49E-08	607.34	4	[M+H] ⁺	PubChem CID : 10086948	Piperdardine	3
362.3409	7.60	3.69E-07	531.77	4	[M+H] ⁺	HMDB0032032	2,4,14-Eicosatrienoic acid isobutylamide	3
421.4146	7.60	2.32E-07	23.64	4				
406.2351	6.97	0.001903	2.35	4	[M+Na] ⁺	HMDB0030186	(E,E,E)-Sylvatine	3
593.2622	5.98	5.65E-05	4.37	4	[M+Na] ⁺	HMDB0036360	Pipercyclobutanamide A	3
401.2776	6.67	0.001417	3.91	4				

Online supporting material

274.1444	5.79	5.35E-06	443.90	4	[M+H] ⁺	HMDB0030187	Pepper compound	4
330.2072	6.62	1.26E-06	494.56	4	[M+H] ⁺	HMDB0039808	Pipertipine (Pepper compound 4)	3
366.2044	6.78	2.54E-06	472.13	4	[M+Na] ⁺	HMDB0030340	Piperolein B (Pepper compound 7)	3
399.2626	6.56	4.70E-06	454.50	4				
326.1768	6.40	4.97E-06	437.90	4	[M+H] ⁺	HMDB0038644	(2E,4E,8E)-Piperamide-C9:3 (Pepper compound 2)	3
328.1913	6.38	2.56E-06	442.84	4	[M+H] ⁺	HMDB0038645	(2E,8E)-Piperamide-C9:2 (Pepper compound 3)	3
356.222	6.70	9.28E-06	421.51	4	[M+H] ⁺	HMDB0033449	Pipercide	2
389.2802	6.61	1.71E-05	405.30	4				
443.3264	6.97	1.78E-05	402.80	4				
342.2088	6.67	0.000208	11.43	4	[M+H] ⁺	HMDB0030339	Pipernonaline (Pepper compound 6)	2
337.2684	7.68	0.00142	2.64	5				
436.285	7.25	0.015124	2.53	5				
328.25	6.47	0.019999	2.95	5				
185.0753	3.45	0.000652	14.15	5				
362.3153	6.74	0.023118	7.40	5				
311.2942	7.23	0.022502	2.45	5				
651.424	6.76	0.000425	2.93	5				
193.1585	6.75	0.002837	2.72	5				
225.1845	6.75	0.003458	3.54	5				

Online supporting material

179.1432	6.75	0.00031	3.39	5				
171.1382	6.75	0.001009	3.96	5				
341.2684	7.14	0.001067	3.51	5				
617.5135	9.38	0.003503	3.14	5	[M+H] ⁺	HMDB0007112	Unknown 5 : Possibly diglyceride	4
676.5864	9.38	0.033529	2.20	5				
693.5442	7.67	0.002254	4.21	5	[M+H] ⁺	HMDB0007776	Unknown 6 : Possibly diglyceride	4
721.047	7.04	0.031479	2.56	5				
869.5439	7.03	0.03205	2.80	5				
273.2595	7.27	0.035203	2.80	5				
391.2467	6.45	0.021811	3.39	5				
655.4702	9.38	0.000152	7.60	5	[M+H] ⁺	HMDB0112171	Unknown 7 : Possibly lipid	4
351.2534	6.44	0.00908	7.40	5	[M+H] ⁺	HMDB0011541	Unknown 8 : Possibly monoglyceride	4
291.1935	6.52	9.83E-05	402.30	5				
269.2103	6.52	0.00286	8.77	5				
635.4554	6.75	0.000198	316.28	5				
619.4827	6.76	0.000194	310.22	5				
384.1969	6.76	0.033007	2.43	5				
231.1	5.67	0.00119	12.83	5				
175.0271	1.62	0.021831	2.93	5				

Online supporting material

654.4484	6.89	0.014143	2.67	5				
124.0394	1.08	0.002177	4.04	5	[M+H] ⁺	HMDB0001488	nicotinic acid	3
350.1542	6.61	0.001745	3.92	5				
266.9938	3.85	0.001026	7.12	5				
189.0501	3.85	0.001118	12.42	5				
199.1694	6.89	0.006623	195.35	5				
274.0747	3.26	0.036974	4.38	5				
263.2369	6.26	0.00427	211.63	5				
261.0949	0.76	0.007683	175.05	5				
348.2149	6.92	1.56E-13	35.35	6				
343.2605	6.92	3.68E-08	617.52	6				
113.0597	2.76	0.002122	2.70	6				
239.0234	4.01	0.002993	2.46	6				
254.9955	4.01	3.42E-05	3.95	6				
185.0421	0.74	7.24E-10	706.89	6				
127.0388	2.65	9.68E-06	455.62	6				
191.0686	4.57	1.88E-07	643.75	6	[M+Na] ⁺	HMDB0029680	4-methylsyringol	1
197.0089	2.77	7.46E-08	574.52	6				
183.1018	5.08	0.000111	8.28	6	[M+H] ⁺	HMDB0033394	4-ethylsyringol	1

Online supporting material

169.086	4.57	5.34E-06	23.07	6	[M+H] ⁺	HMDB0029680	4-methylsyringol	1
205.0841	5.08	3.20E-06	513.53	6	[M+Na] ⁺	HMDB0033394	4-ethylsyringol	1
257.061	4.01	2.71E-06	447.57	6				
269.0107	4.57	5.43E-06	408.09	6				
190.0607	4.57	5.57E-06	409.16	6				
168.0782	4.57	5.70E-06	408.34	6				
268.0026	4.57	1.02E-05	404.13	6				
253.0391	4.57	1.85E-05	395.38	6				
211.0959	3.60	0.013716	2.97	6				
183.0642	3.63	0.017322	3.09	6				
409.2559	6.12	0.000492	260.44	6				
537.3002	6.73	0.000477	268.54	6				
397.3824	8.19	0.001554	175.01	6				
217.0846	5.33	0.001599	282.41	6	[M+Na] ⁺	HMDB0037271	4-allylsyringol	1
219.1001	5.54	0.025749	7.05	6	[M+Na] ⁺	HMDB0036226	4-propylsyringol	1
562.2922	3.70	0.018079	5.37	6				
627.3506	4.23	0.035012	7.22	6				
445.245	4.70	0.007605	221.20	6				
345.2213	4.18	0.007582	220.80	6				

Online supporting material

445.7474	4.70	0.021726	10.58	6				
456.2355	4.70	0.043219	7.68	6				
472.2045	4.69	0.02006	159.52	6				
607.3806	3.63	0.012317	185.67	6				
195.102	5.32	0.019201	178.91	6	[M+H] ⁺	HMDB0037271	4-allylsyringol	1
197.0813	3.84	0.049335	4.16	6				
194.0948	5.33	0.005164	197.66	6				
479.2973	3.24	0.006717	194.73	6				
271.0771	4.57	0.011452	163.49	6				
197.1173	5.54	0.020426	141.68	6	[M+H] ⁺	HMDB0036226	4-propylsyringol, [M+Na]⁺ above	1
464.2185	4.70	0.032136	142.30	6				
216.0774	5.33	0.032155	138.03	6				
327.2068	3.64	0.032156	132.57	6				

Abbreviations: RT, Retention time; ppm: parts per million; ^a mean intensity of metabolite in processed meat products divided by mean intensity in non-processed meat products, ^b clusters of metabolites in figure 2, ^c identifier of the Human Metabolome Database (www.hmdb.ca), ^d level of confidence as described in Sumner et al. [1]

TABLE S3 : Features significantly elevated in processed meat digests compared to non-processed meat products and found in pepper extracts. Pepper origin of all compounds is confirmed by comparison of MS/MS spectra with corresponding compounds in pepper extracts. Some could be annotated based on comparison with chemical standards or comparison of their MS/MS spectra with those curated in public databases. References are found at the end of the Supplement.

m/z	RT ^a (min)	Adjusted p-value ^b	Adduct	Mass accuracy ^c (ppm)	HMDB ID/ PubChem CID	Annotation (in brackets annotation in Figure 2)	Level of confidence ^d	Detection (Pepper type)	Reference ^e
224.2012	6.34	4.79E-07	H	1	HMDB0030951	Pellitorine	1	A ^f	[2]
272.1287	5.74	7.35E-05	H	2	HMDB0029374	Piperyline	2	A	[3,4]
288.1601	5.93	7.99E-08	H	2	HMDB0033874	Piperanine	2	A	[3–5]
308.1262	6.01	3.66E-05	Na	2	HMDB0029377	Piperine	1	A	
312.1597	6.29	6.85E-07	H	1	HMDB0034371	Piperettine	2	A	[3]
314.1751	6.28	1.61E-07	H	0	PubChem CID 10086948	Piperdardine	2	A	[3]
316.192	6.41	1.52E-07	H	4	HMDB0030185	(E)-Piperolein A (Pepper compound 1)	3	A	
326.1768	6.40	4.97E-06	H	5	HMDB0038644	(2E,4E,8E)-Piperamide-C9:3 (Pepper compound 2)	3	A	
328.1913	6.38	2.56E-06	H	2	HMDB0038645	(2E,8E)-Piperamide-C9:2 (Pepper compound 3)	3	A	
330.2072	6.62	1.26E-06	H	2	HMDB0039808	Pipertipine OR retrofractamide C OR 8E-piperamideC9:1 (Pepper compound 4)	3	B, G	
340.1909	6.57	0.000624	H	0	HMDB0040811	Dehydropipernonaline (Pepper compound 5)	3	A	
342.2088	6.67	0.000208	H	7	HMDB0030339	Pipernonaline OR Retrofractamide D (Pepper compound 6)	3	A	
344.2236	6.78	4.43E-06	H	5	HMDB0030340	Piperolein B (Pepper compound 7)	3	A	
356.222	6.70	9.28E-06	H	4	HMDB0033449	Pipercide	2	A	[4]

^a RT, Retention time; ^b FDR-adjusted p-value of a Welch's t-test comparing metabolite intensities in processed meat digests to non-processed meat digests; ^c accuracy of measured mass compared to the theoretical mass of the annotation; ^d annotation level of confidence: Identity confirmed by comparison of RT and MS/MS fragmentation pattern with chemical standard (level 1), Annotation based on match of RT and MS/MS fragmentation pattern with compound present in black pepper extract AND matching MSMS fragmentation pattern with literature spectra (level 2), Annotation based on match of RT and MS/MS fragmentation pattern

Online supporting material

with compound present in black pepper extract (level 3); ^e literature references for MS/MS spectra of pepper compounds: see last page of supplement ^f Detection in extracts of ground pepper (n = 6): A, all (black, white, green); B, G (black, green)

TABLE S4 : Details on the recursive feature finding used to create the feature table from the HRMS raw data. All steps were carried out using the Agilent MassHunter software package (Mass Profiler Professional version B14.9.1, Qualitative Analysis version B06.00 and DA Reprocessor version B.05.00; Agilent technologies).

First step: Molecular feature extraction	
Mass range	50-1000 m/z
Peak height threshold	1500 counts
Ions	Limited to [M+H] ⁺
Isotope grouping	Common organic molecules (no halogens), single-charged ions
Alignment	15 ppm, +/- 0.1 min
Second step: Recursive analysis	
Creation of targets	features detected in at least 3 samples
Chromatogram extraction	+/- 25 ppm and +/- 1.0 min around target
Alignment of features	15 ppm, +/- 0.1 min

FIGURE S1 : Annotation evidence for Pellironine in fried sausage digest, comparison with compound in black pepper extract.

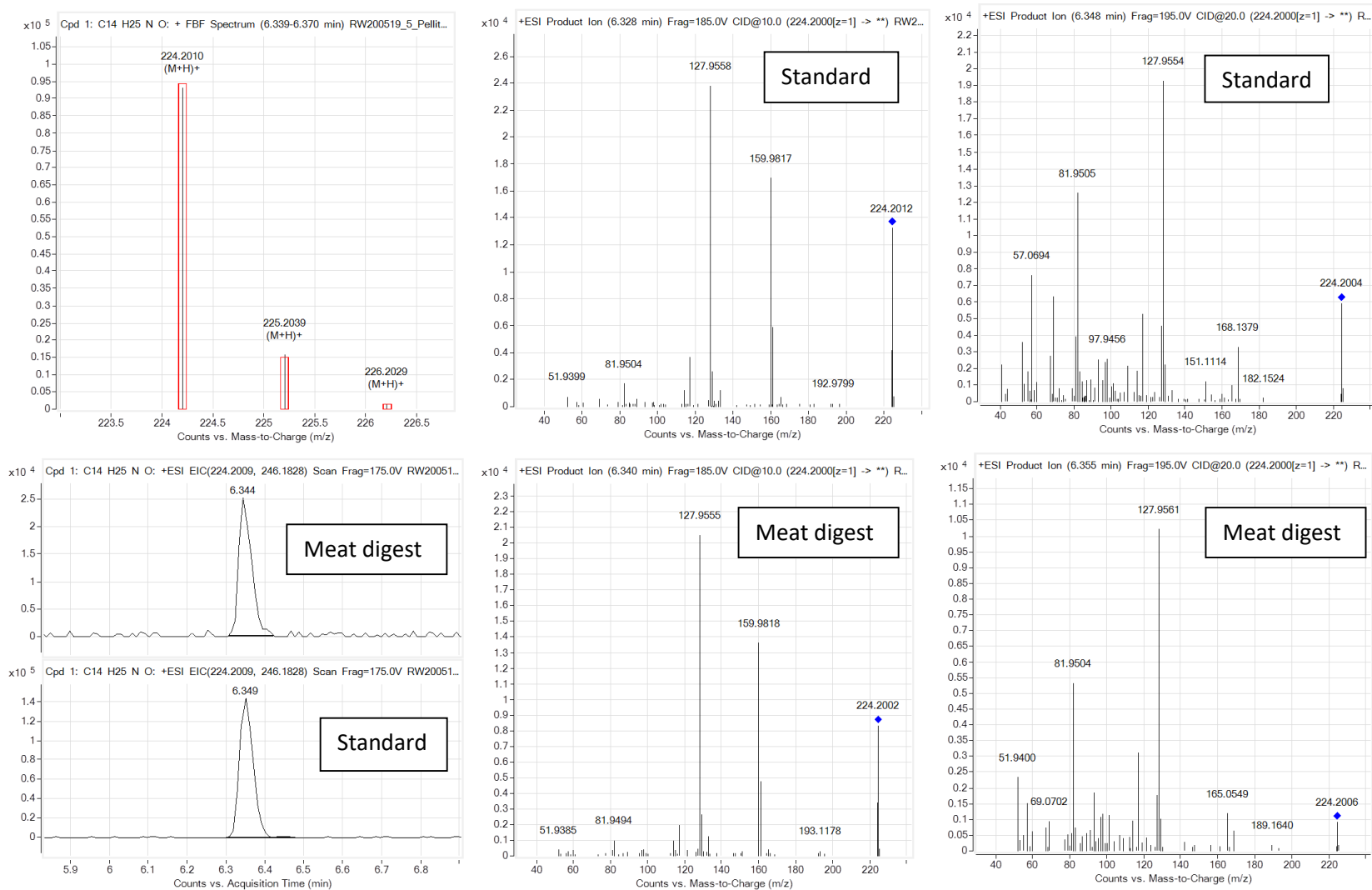


FIGURE S2 : Annotation evidence for Piperine in fried sausage digest, comparison with compound in black pepper extract.

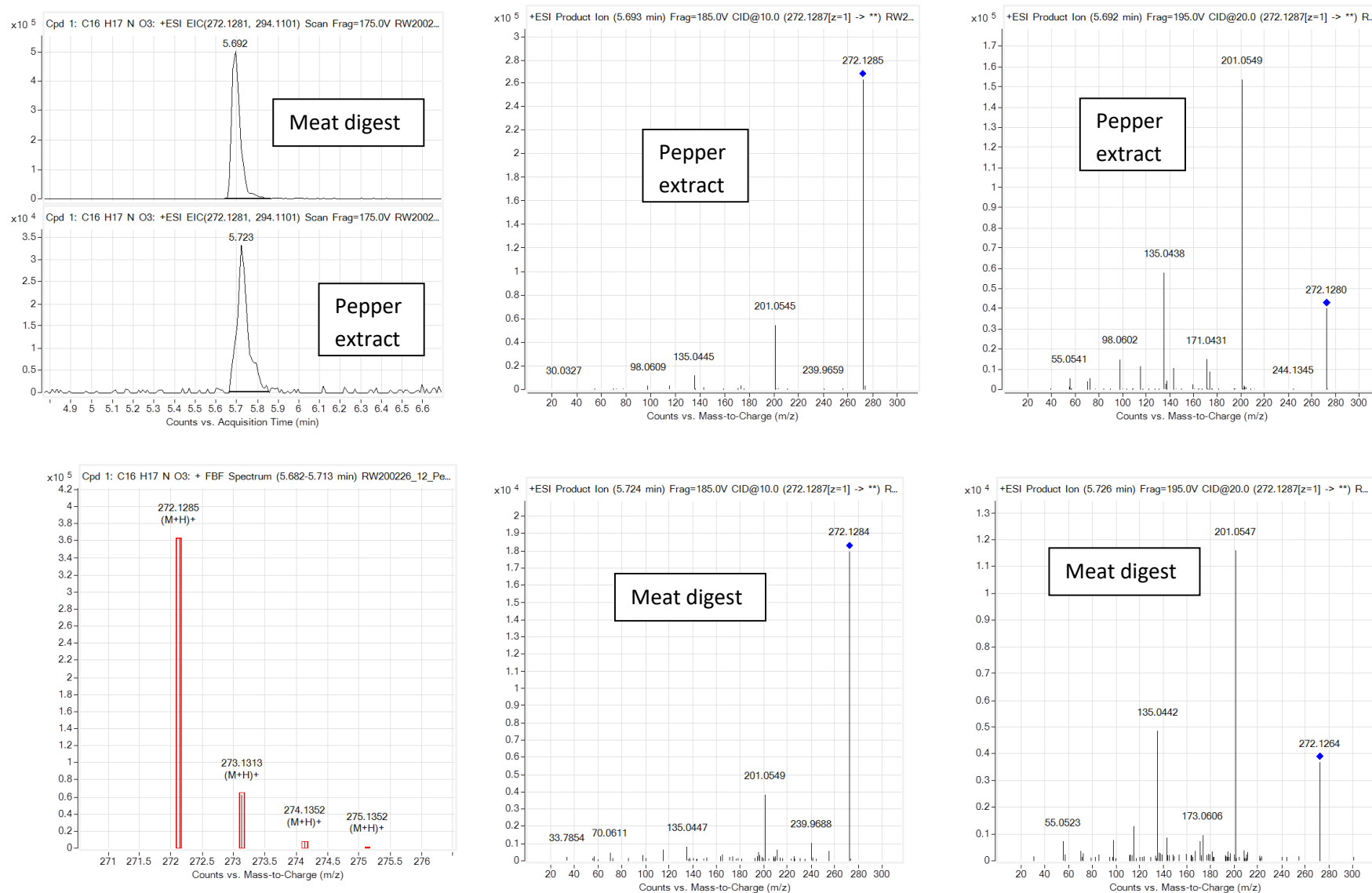


FIGURE S3 : Annotation evidence for Piperanine in fried sausage digest, comparison with compound in black pepper extract.

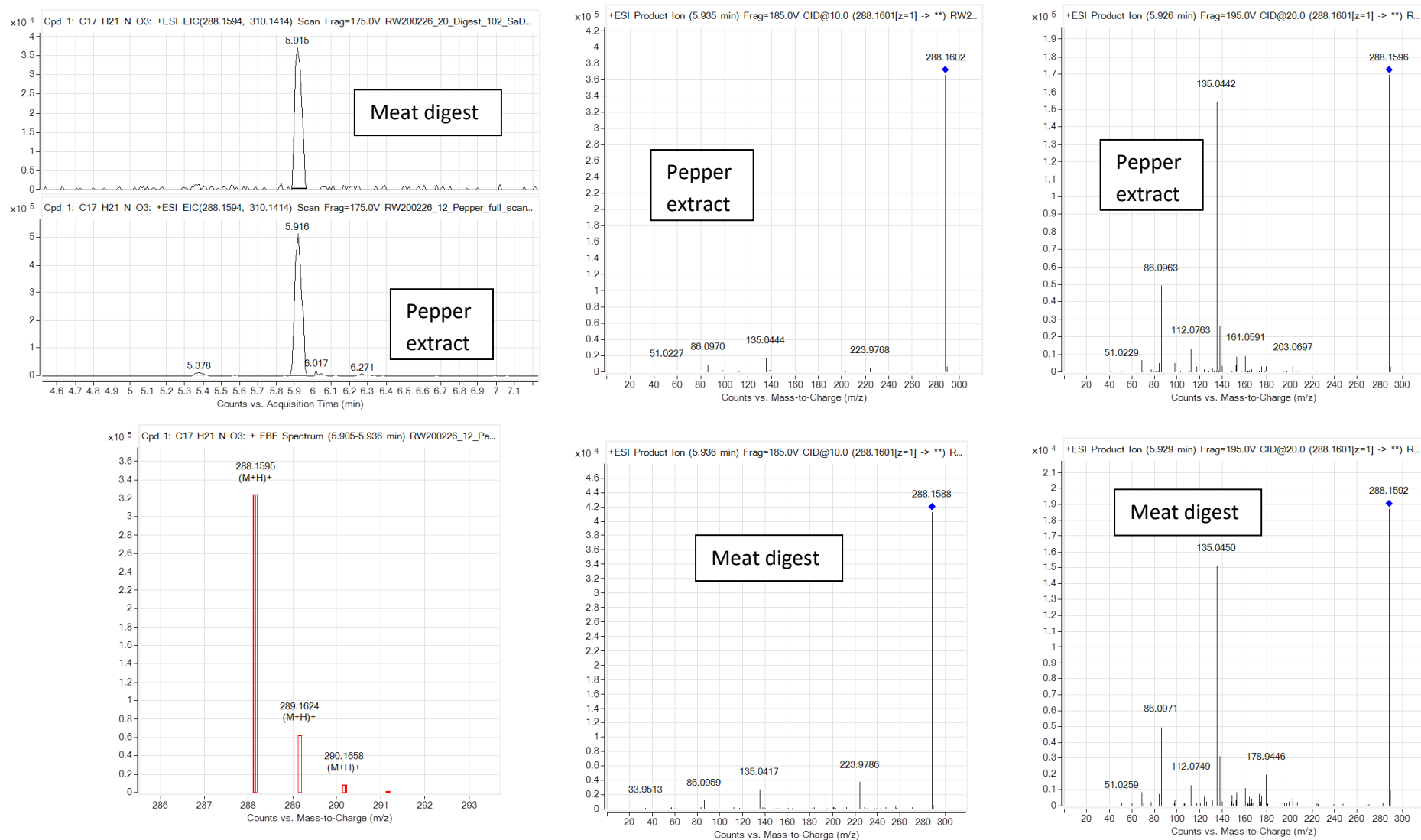


FIGURE S4 : Annotation evidence for Piperine in fried sausage digest, comparison with chemical standard and compound in black pepper extract.

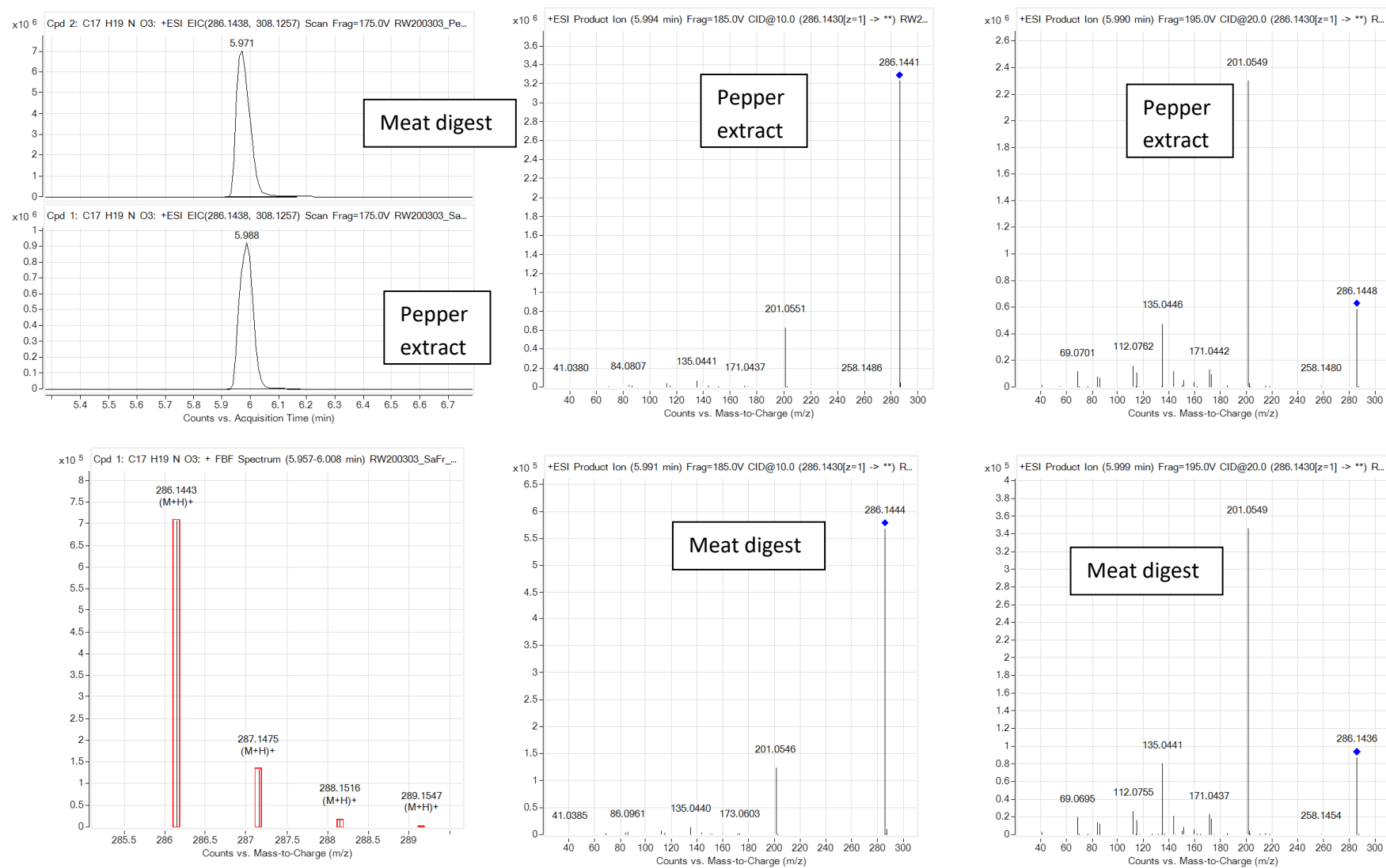


FIGURE S5 : Annotation evidence for Piperettine in fried sausage digest, comparison with compound in black pepper extract.

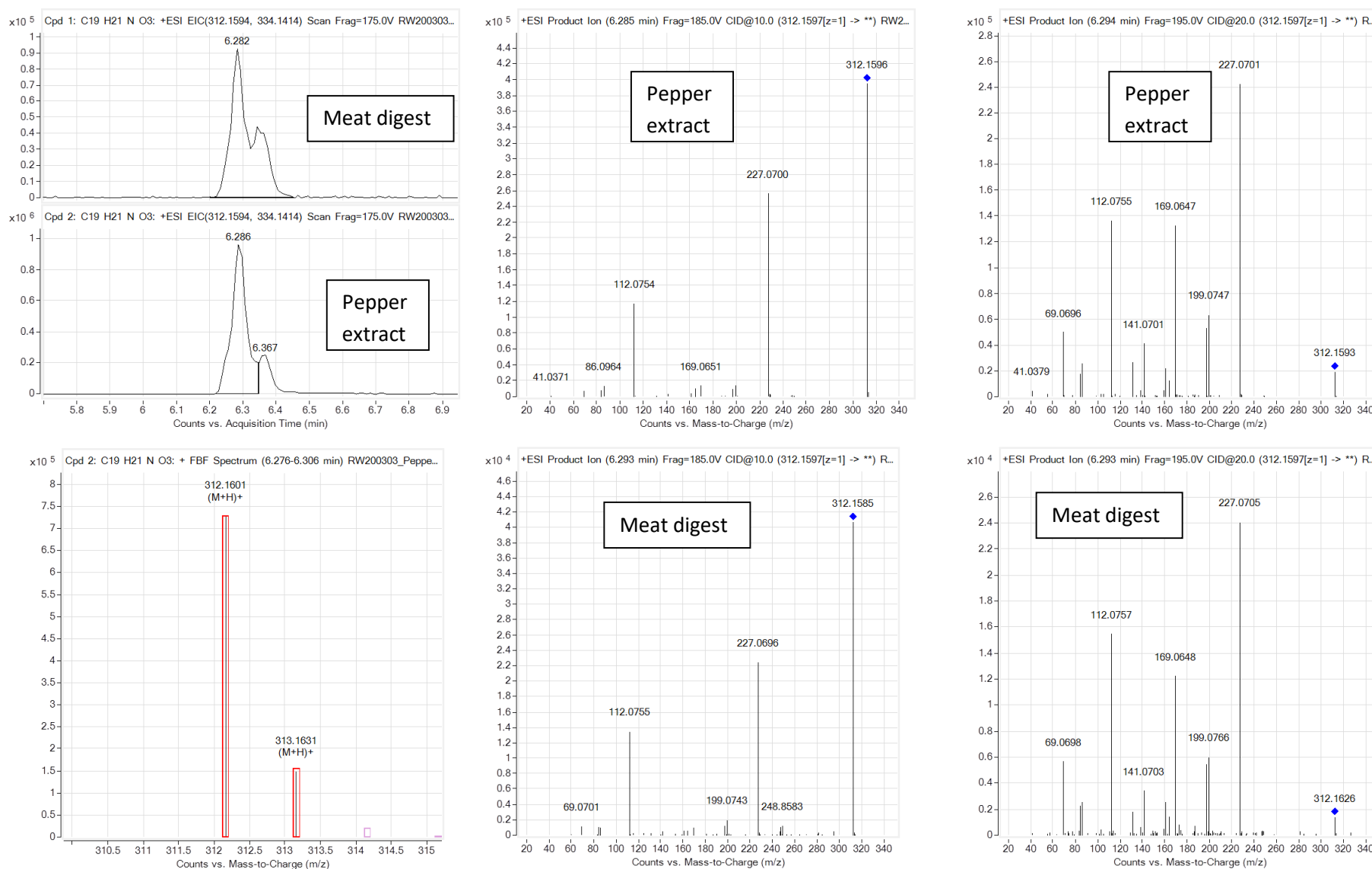


FIGURE S6 : Annotation evidence for Piperdardine in fried sausage digest, comparison with compound in black pepper extract.

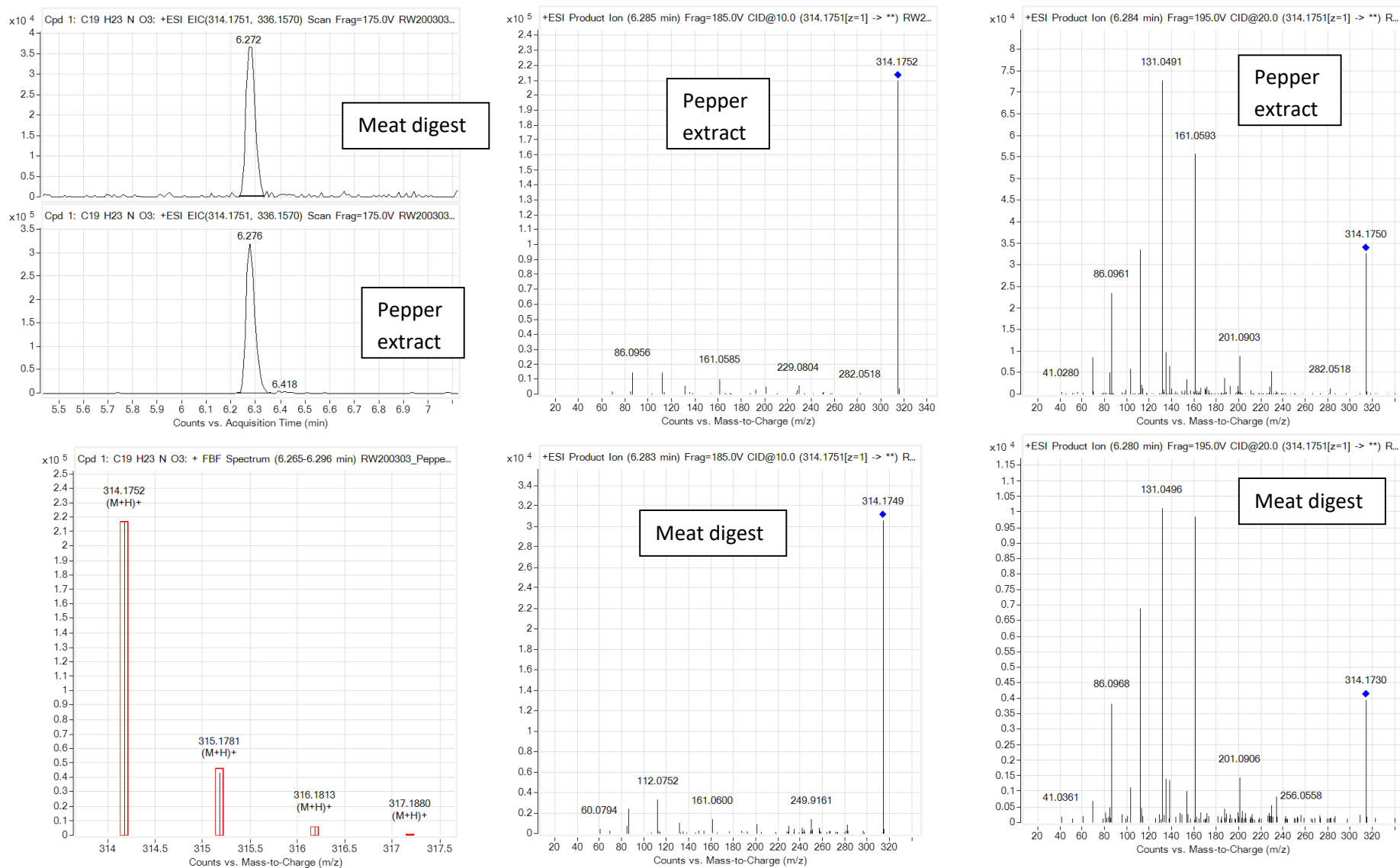


FIGURE S7 : Annotation evidence for pepper compound 2 in fried sausage digest, comparison with compound in black pepper extract.

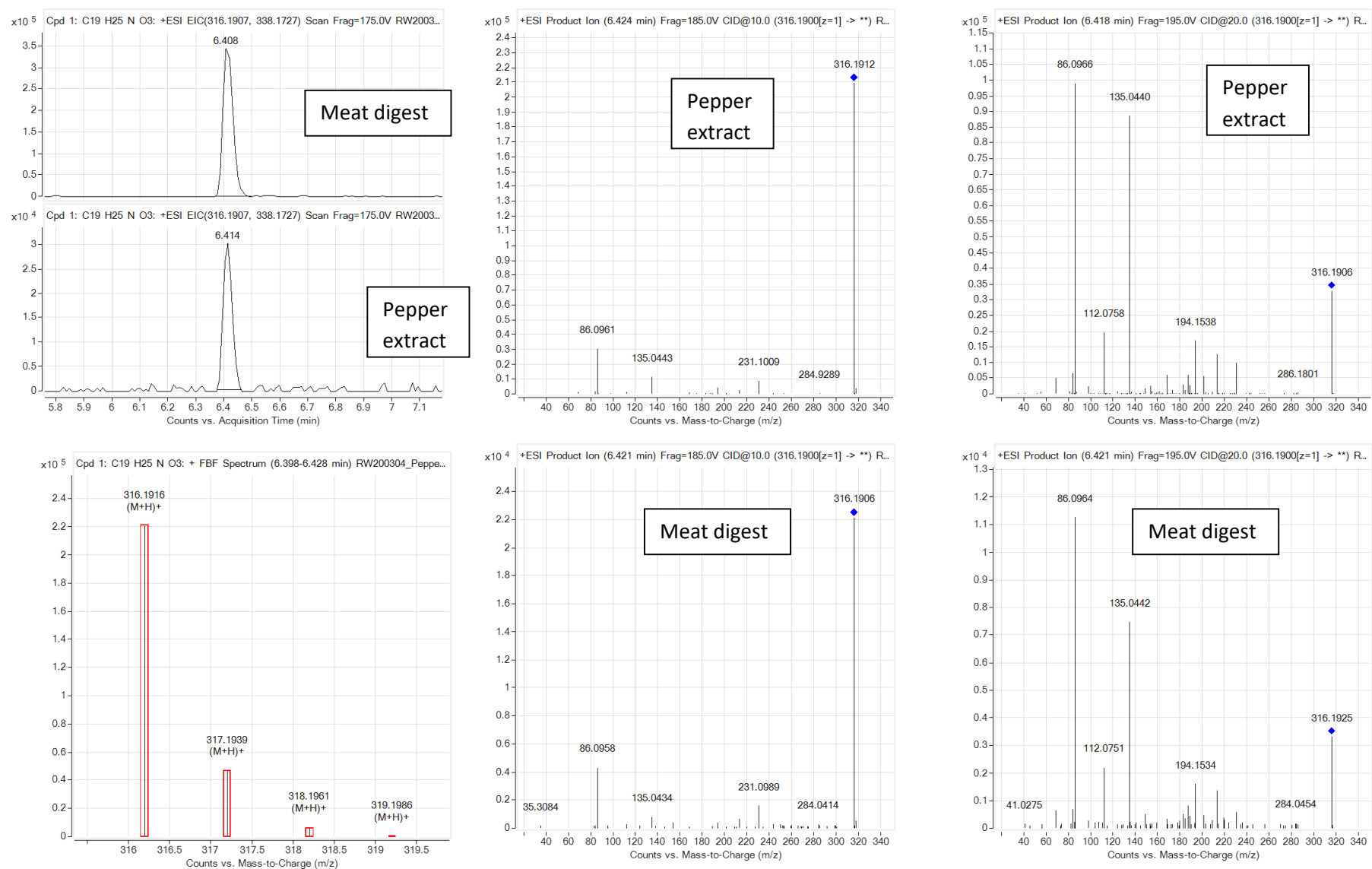
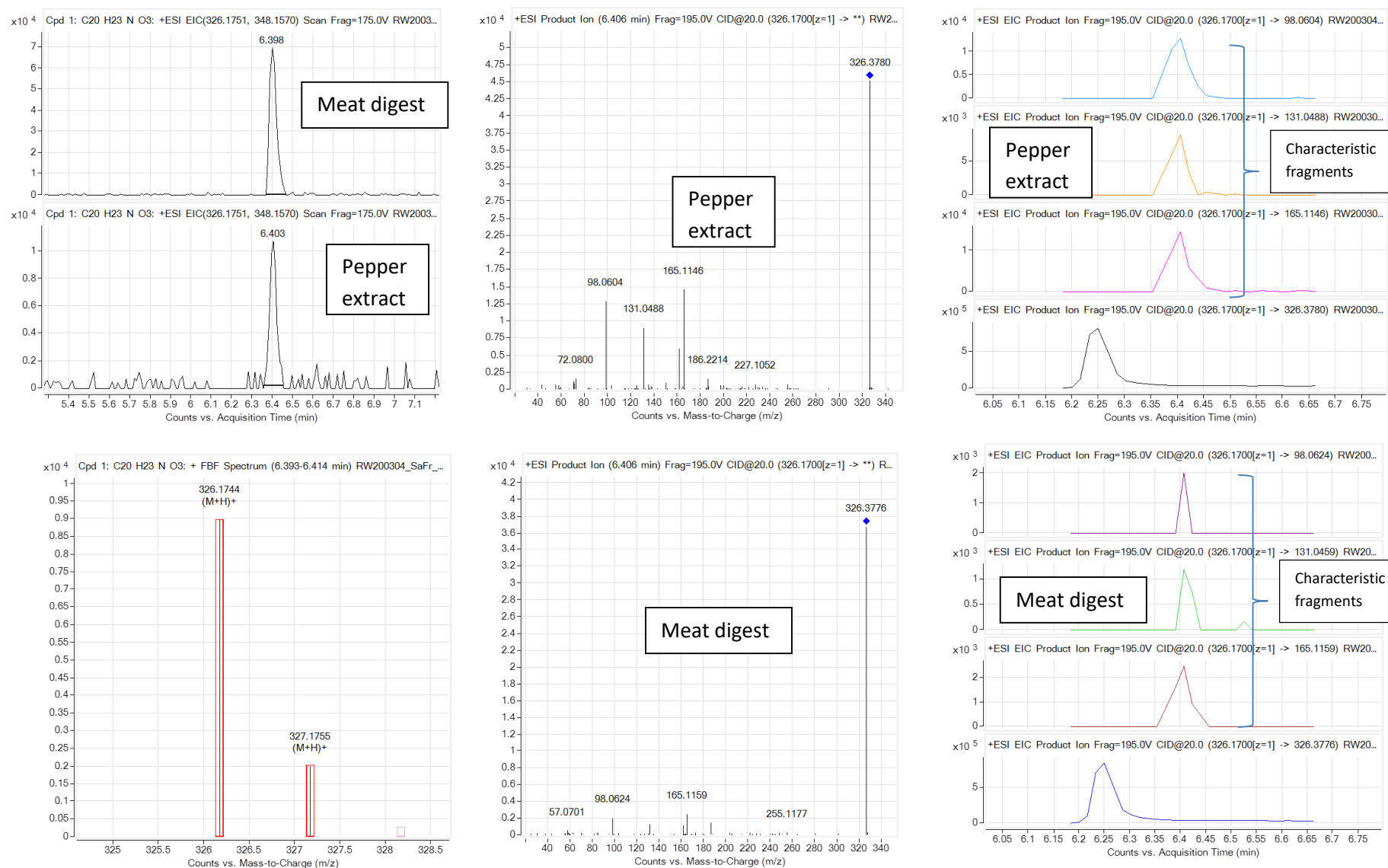


FIGURE S8 : Annotation evidence for pepper compound 3 in fried sausage digest, comparison with compound in black pepper extract.



The same characteristic fragments ($m/z = 98.06$, $m/z = 131.05$, $m/z = 165.12$, $RT = 6.4$ min) are present in both pepper extract and meat digest, but a second compound has been selected for fragmentation ($m/z = 326.38$, $RT = 6.25$ min) which shows higher intensity and adds noise to the MS/MS spectrum.

FIGURE S9 : Annotation evidence for pepper compound 4 in fried sausage digest, comparison with compound in black pepper extract.

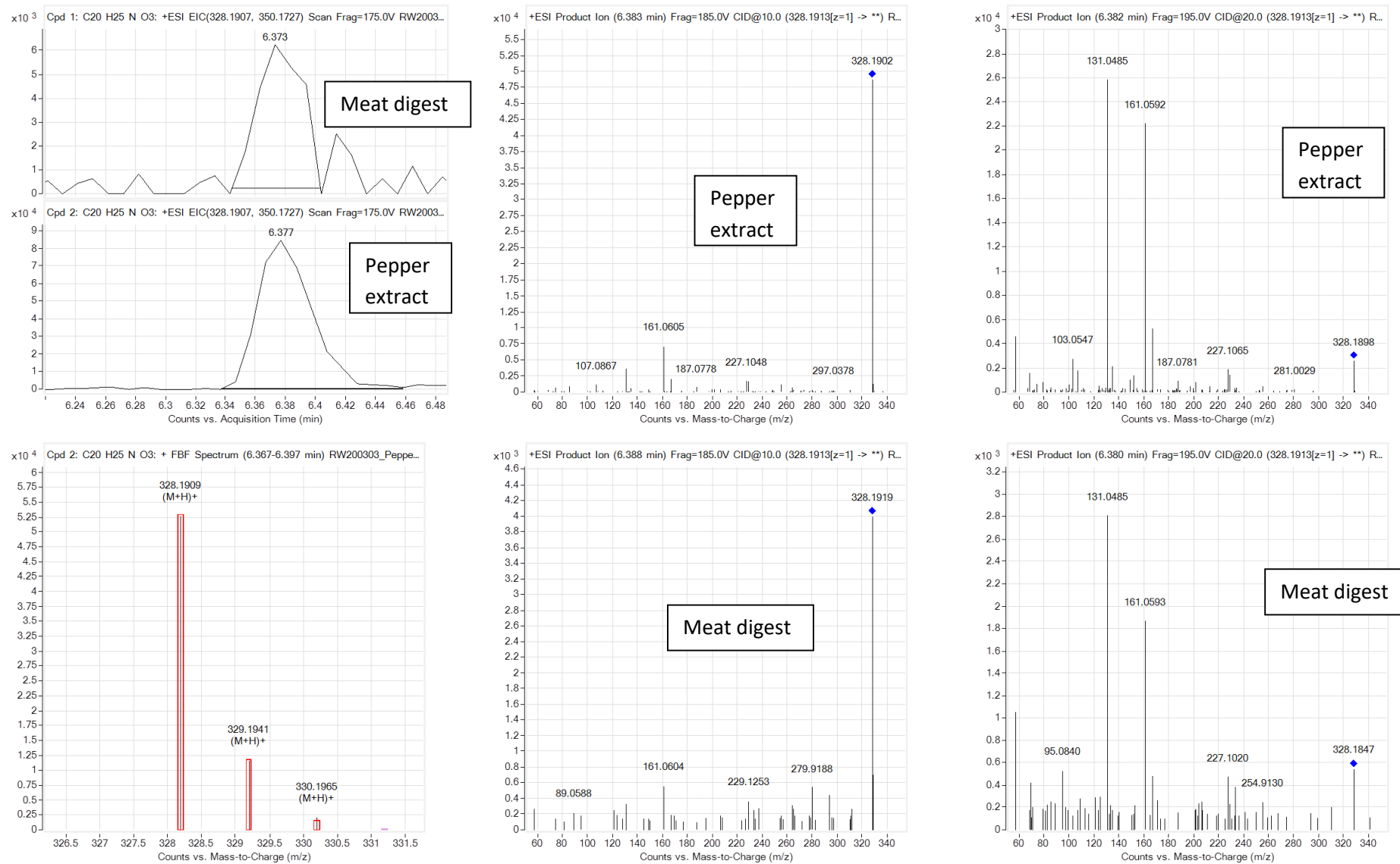


FIGURE S10 : Annotation evidence for pepper compound 5 in fried sausage digest, comparison with compound in black pepper extract.

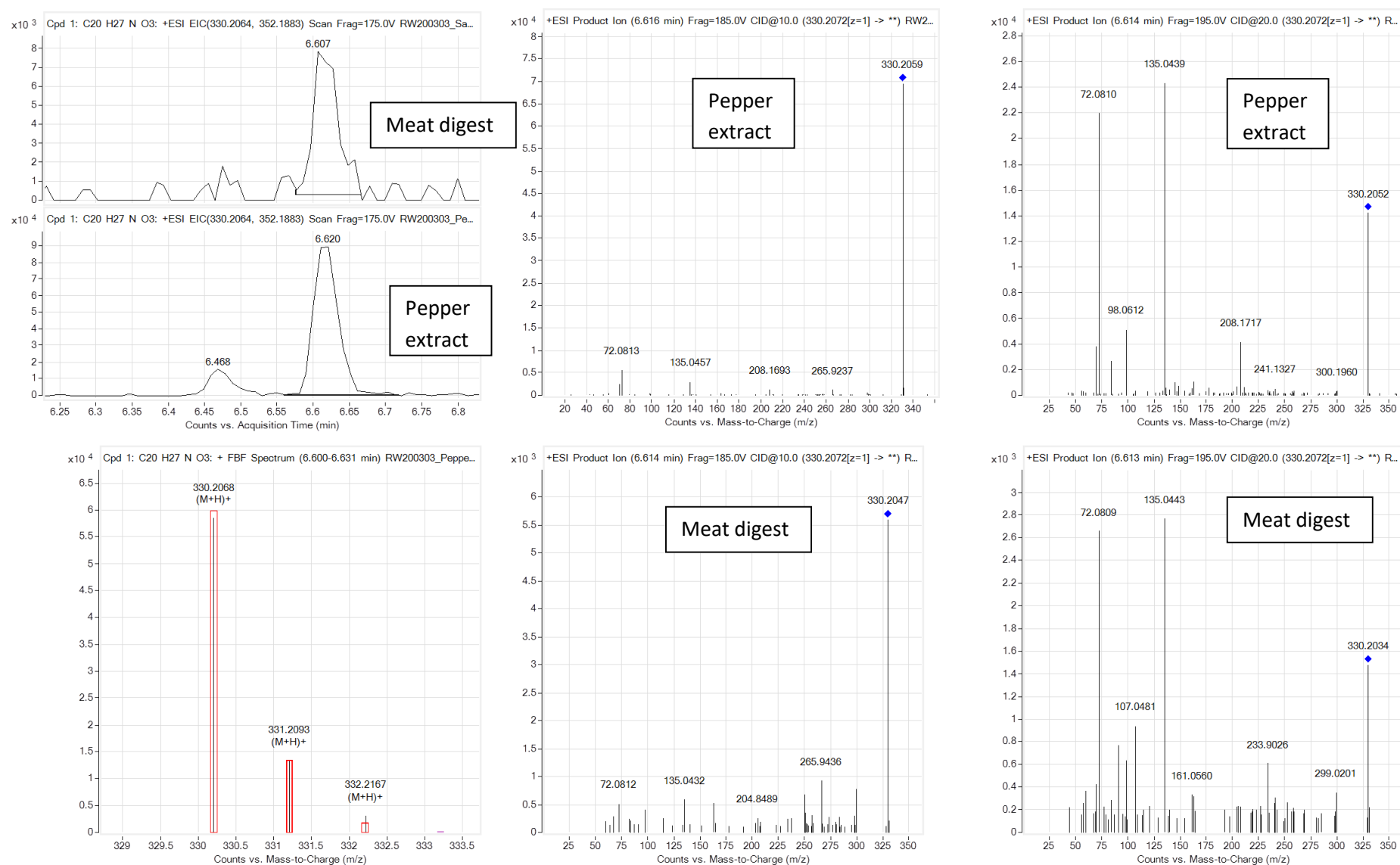


FIGURE S11 : Annotation evidence for pepper compound 6 in fried sausage digest, comparison with compound in black pepper extract.

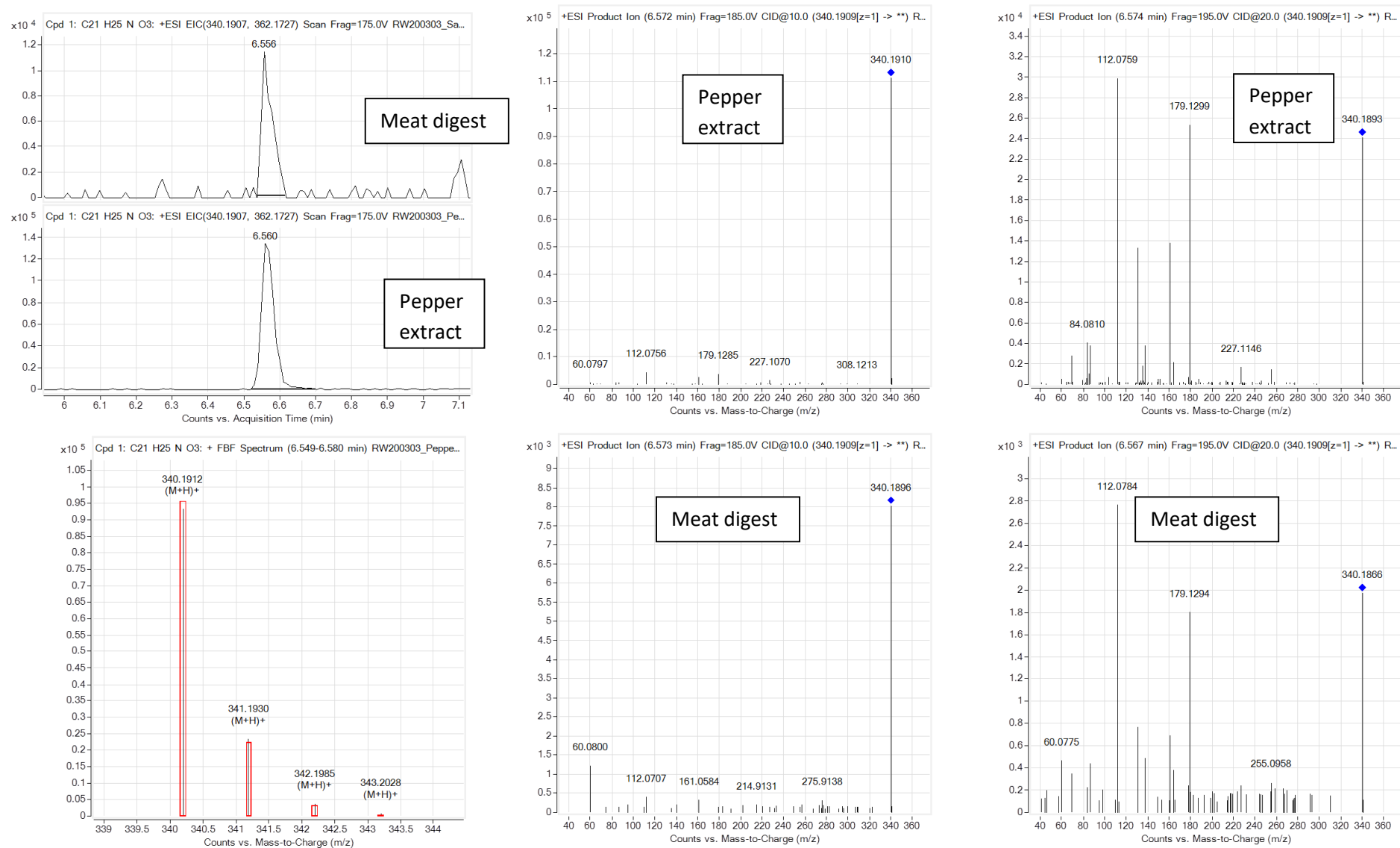


FIGURE S12 : Annotation evidence for pepper compound 7 in fried sausage digest, comparison with compound in black pepper extract.

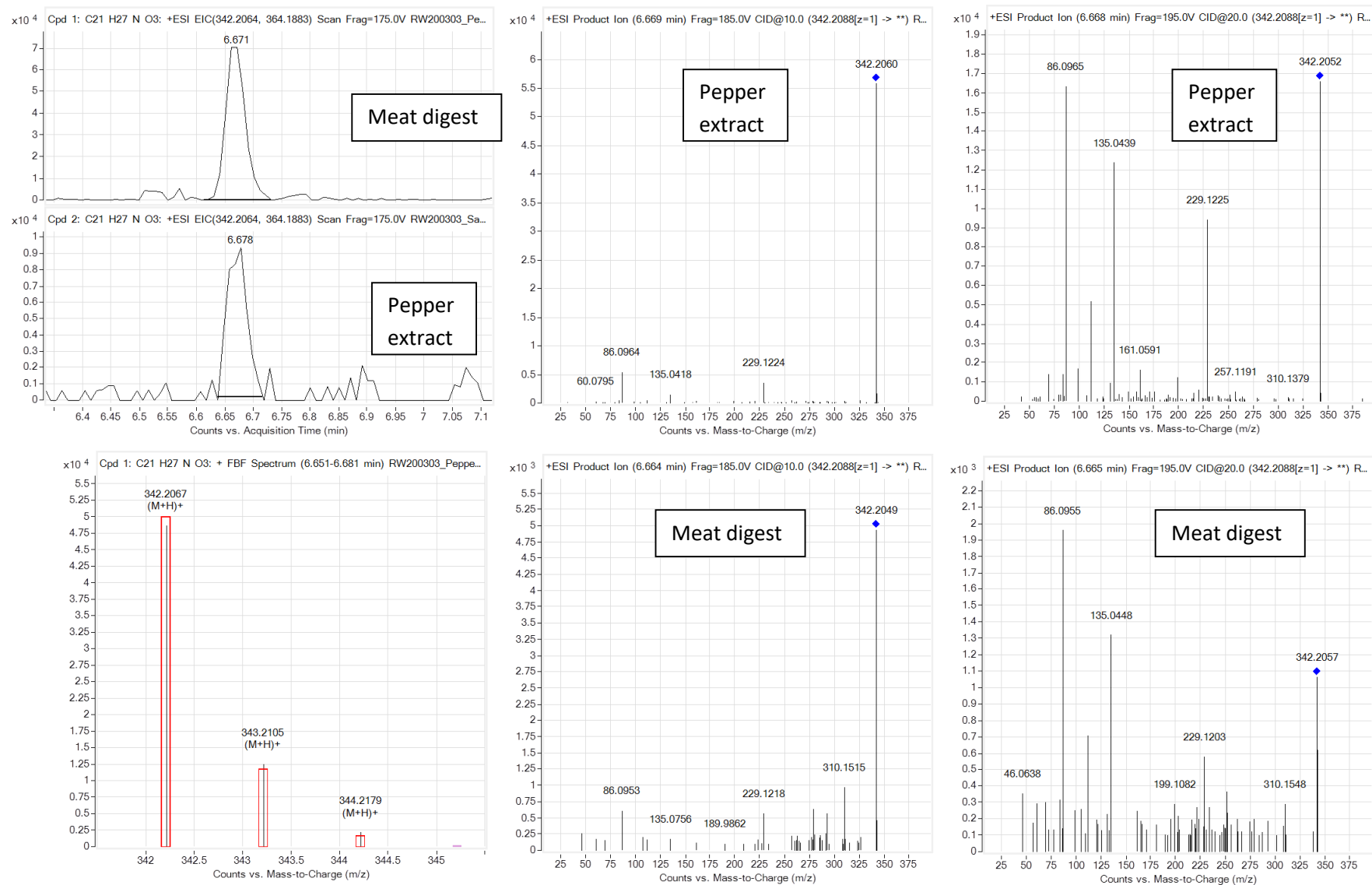


FIGURE S13 : Annotation evidence for pepper compound 8 in fried sausage digest, comparison with compound in black pepper extract.

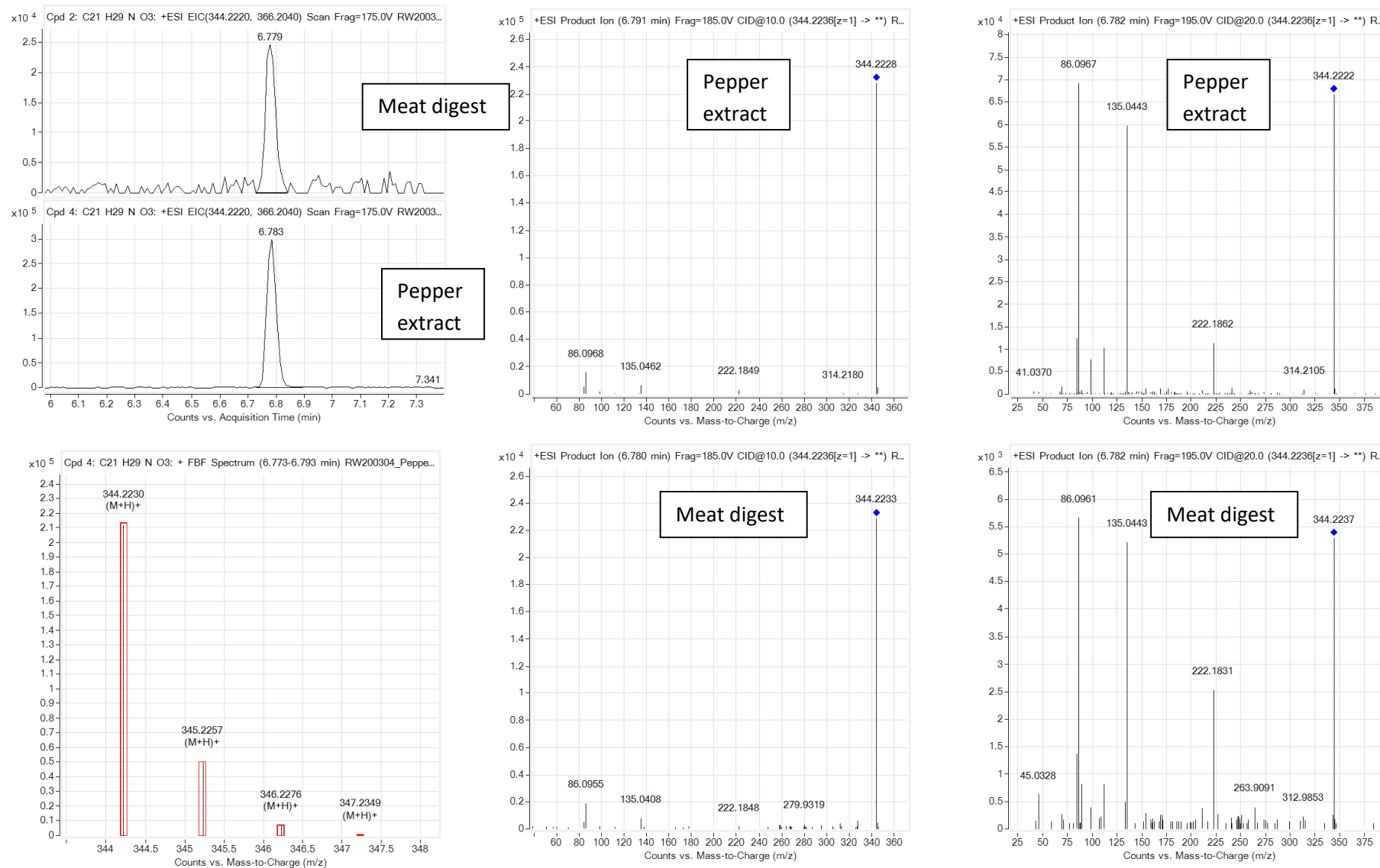


FIGURE S14 : Annotation evidence for piperidine in fried sausage digest, comparison with compound in black pepper extract.

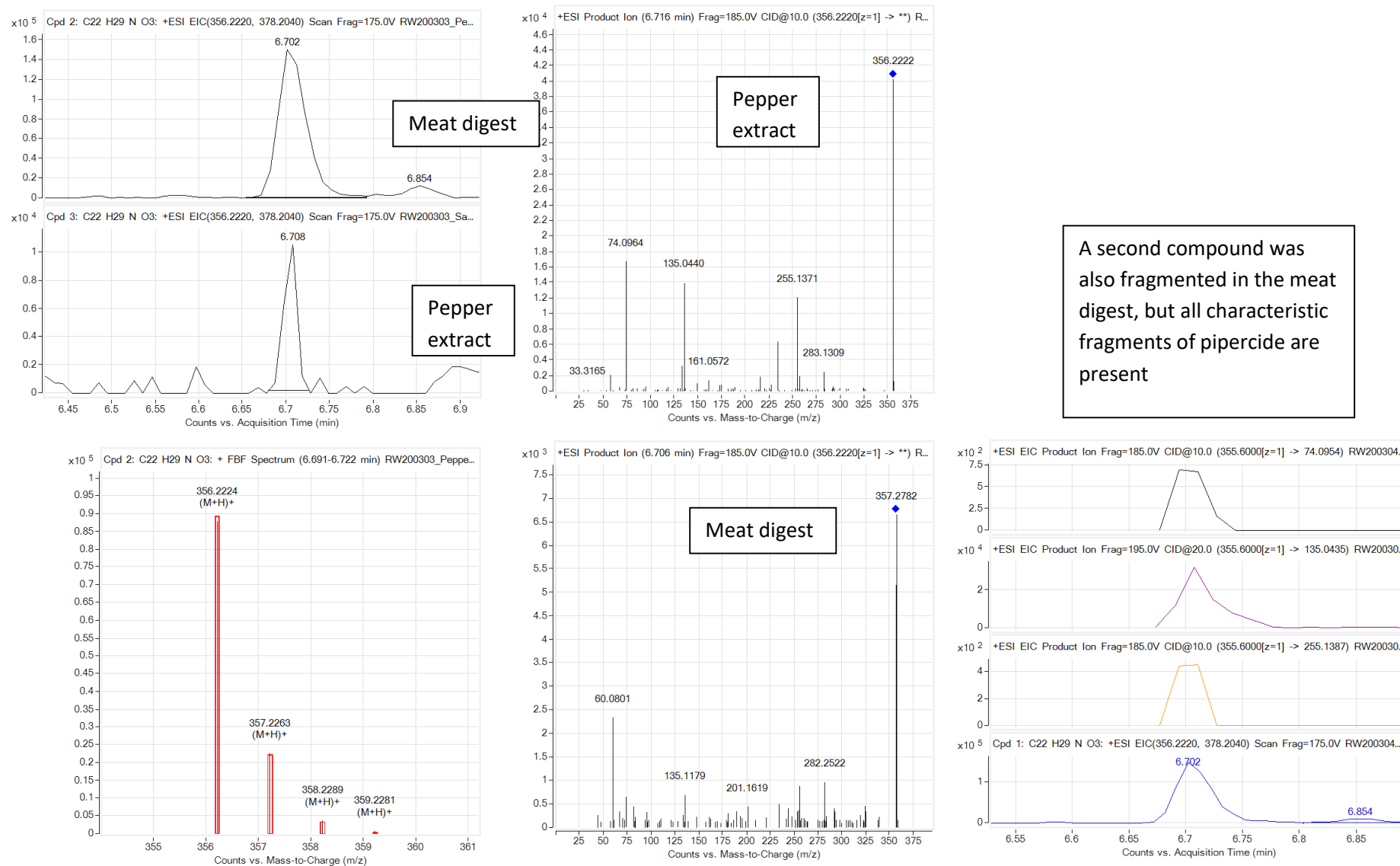


FIGURE S15 : Annotation evidence for phenylalanylisoleucine.

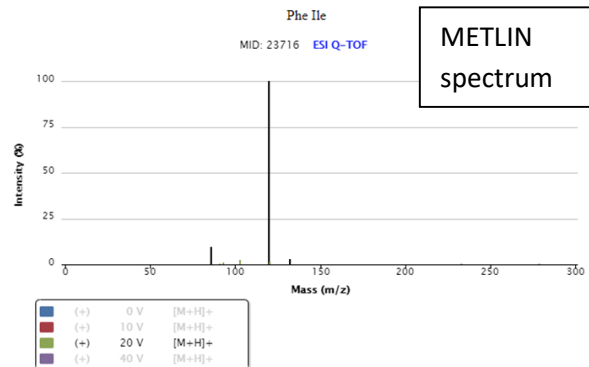
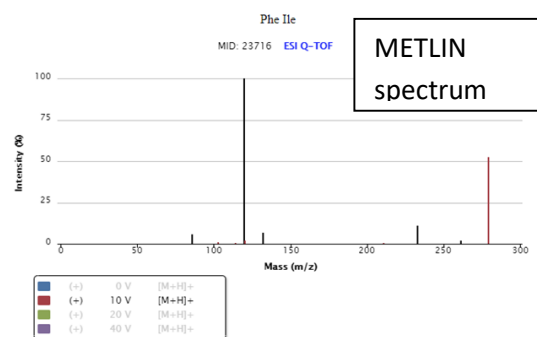
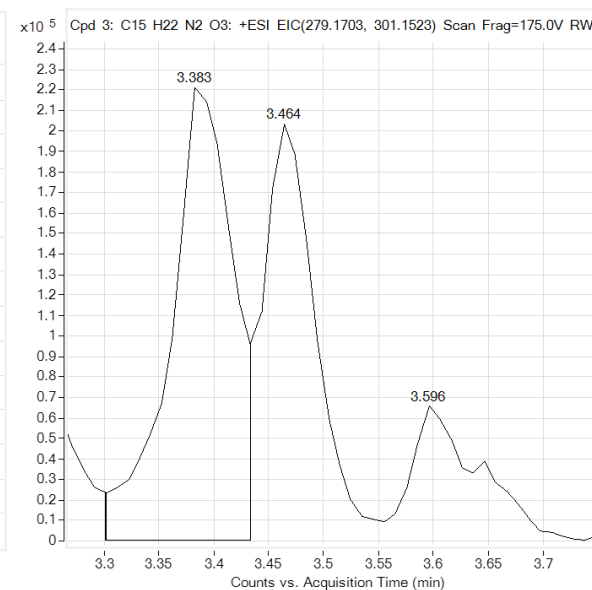
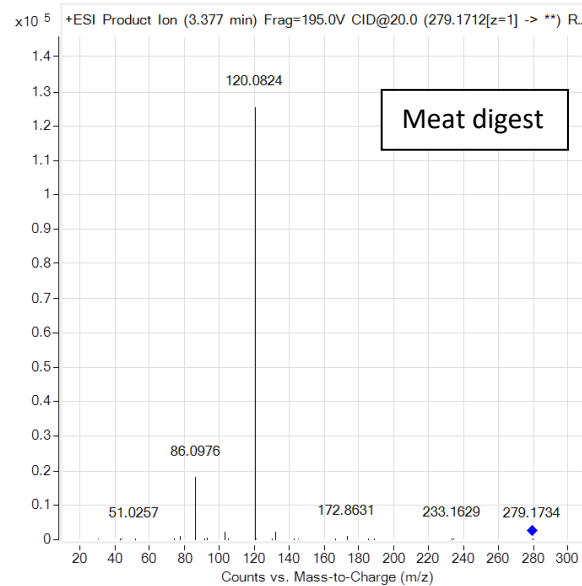
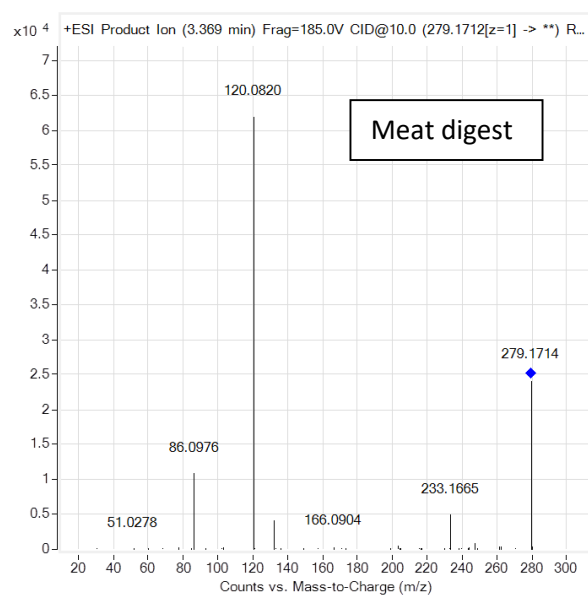
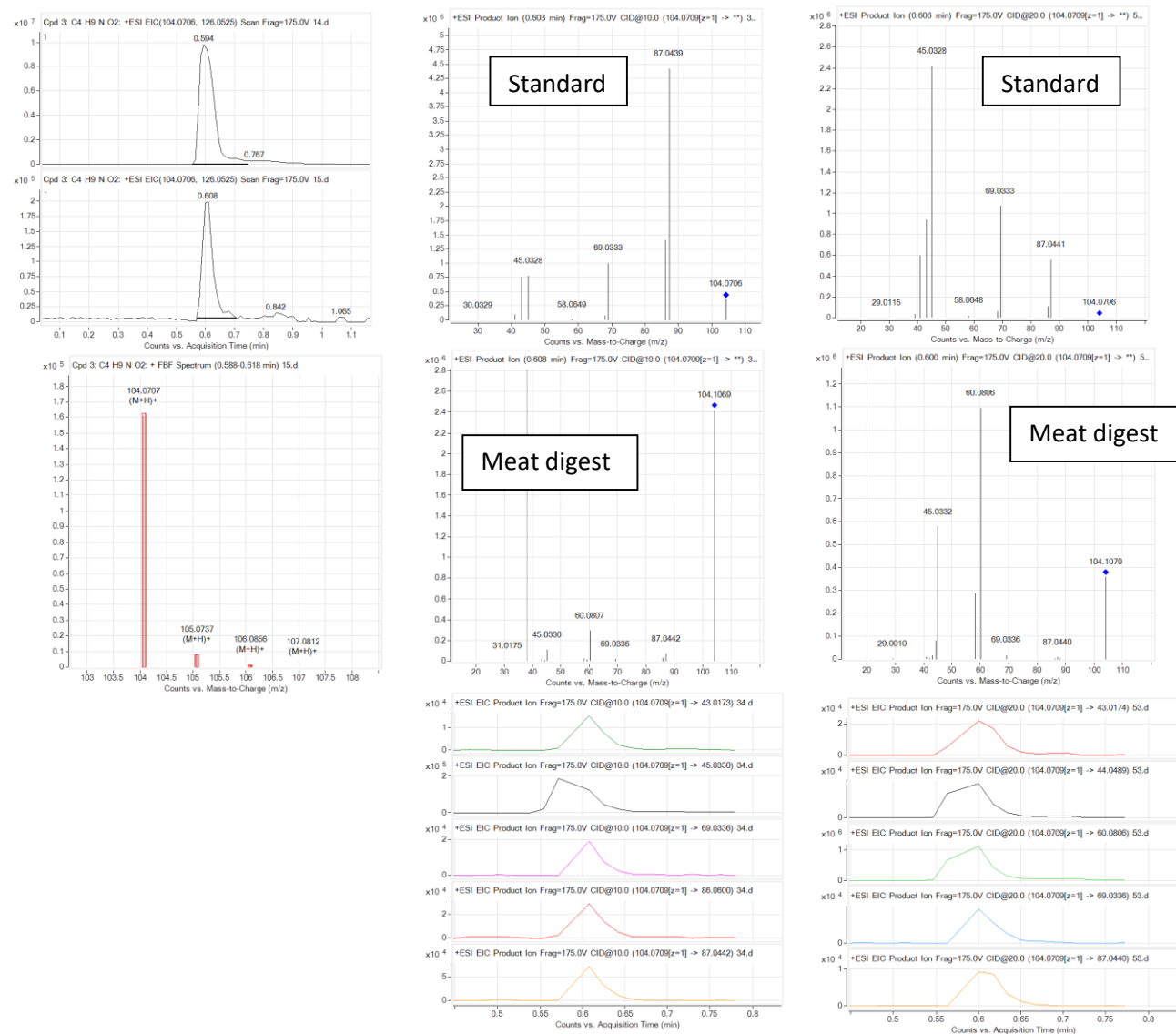


FIGURE S16 : Annotation evidence for γ -aminobutyric acid in fermented sausage digest, comparison with chemical standard.



Another ion (m/z = 104.107) with higher intensity as GABA is also fragmented

All characteristic fragments of GABA are present in low intensities in the meat digest samples

FIGURE S17 : Annotation evidence for histamine in fermented sausage digest, comparison with chemical standard.

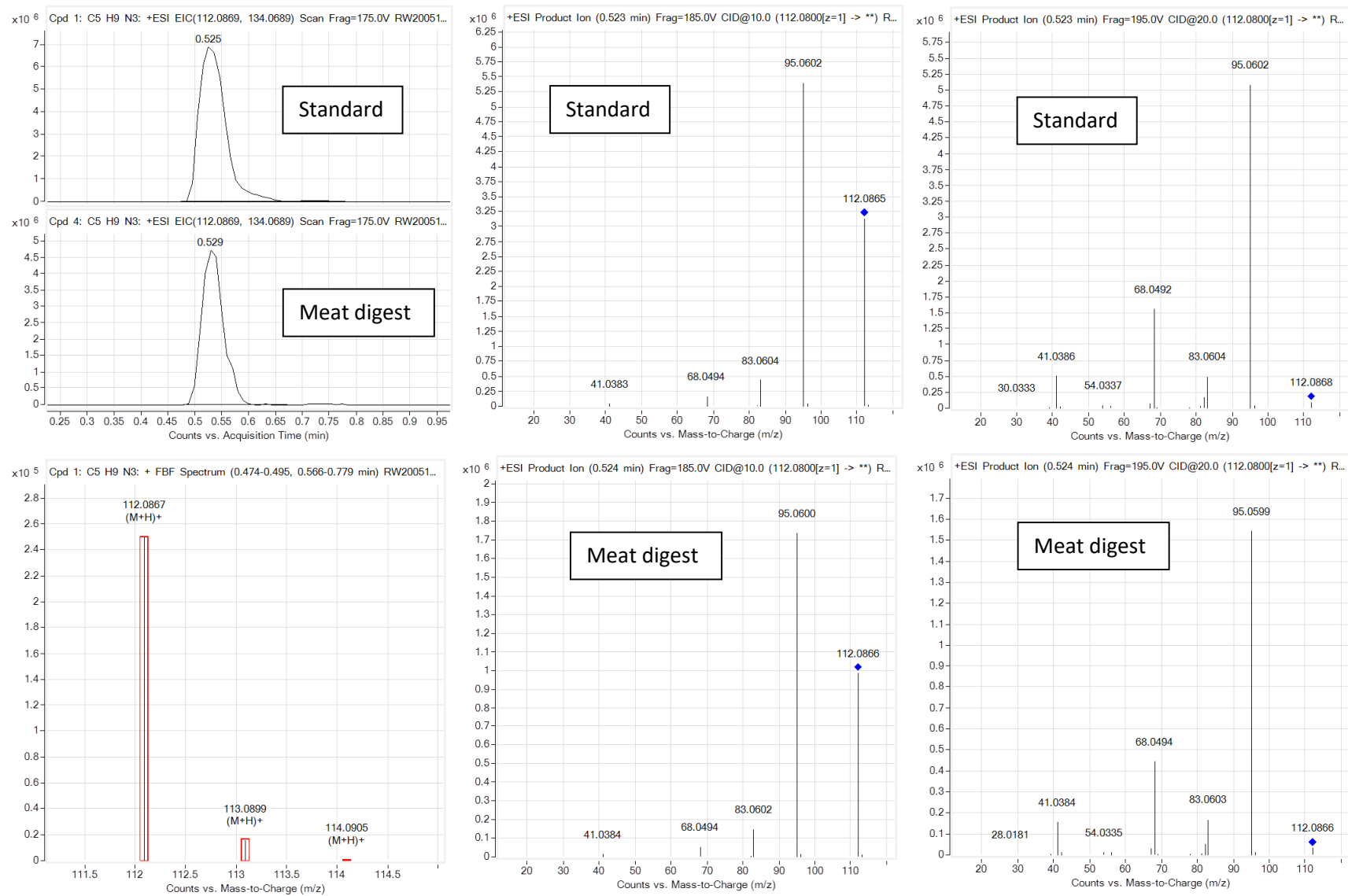


FIGURE S18: Annotation evidence for tyramine in fermented sausage digest, comparison with chemical standard.

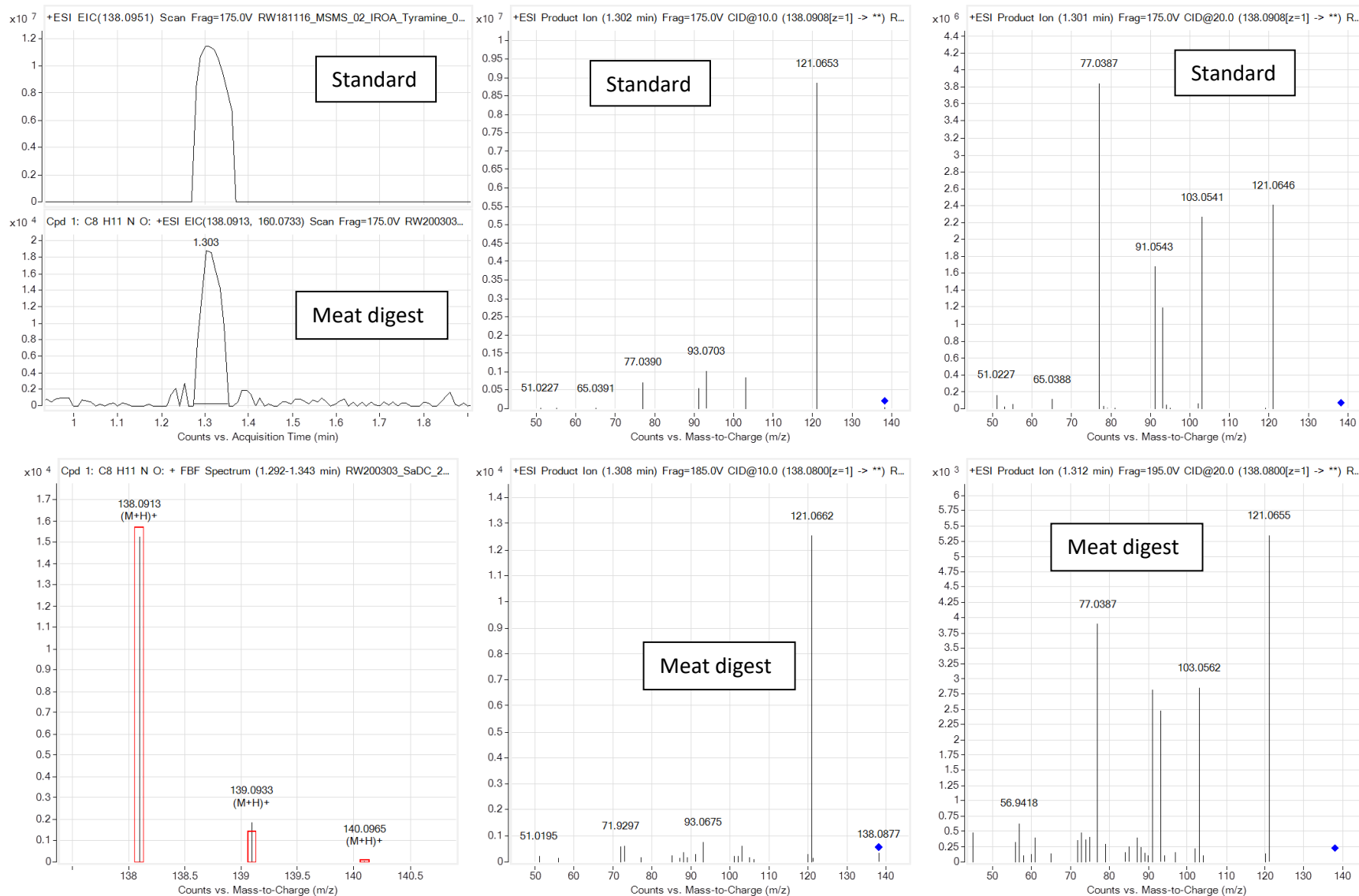
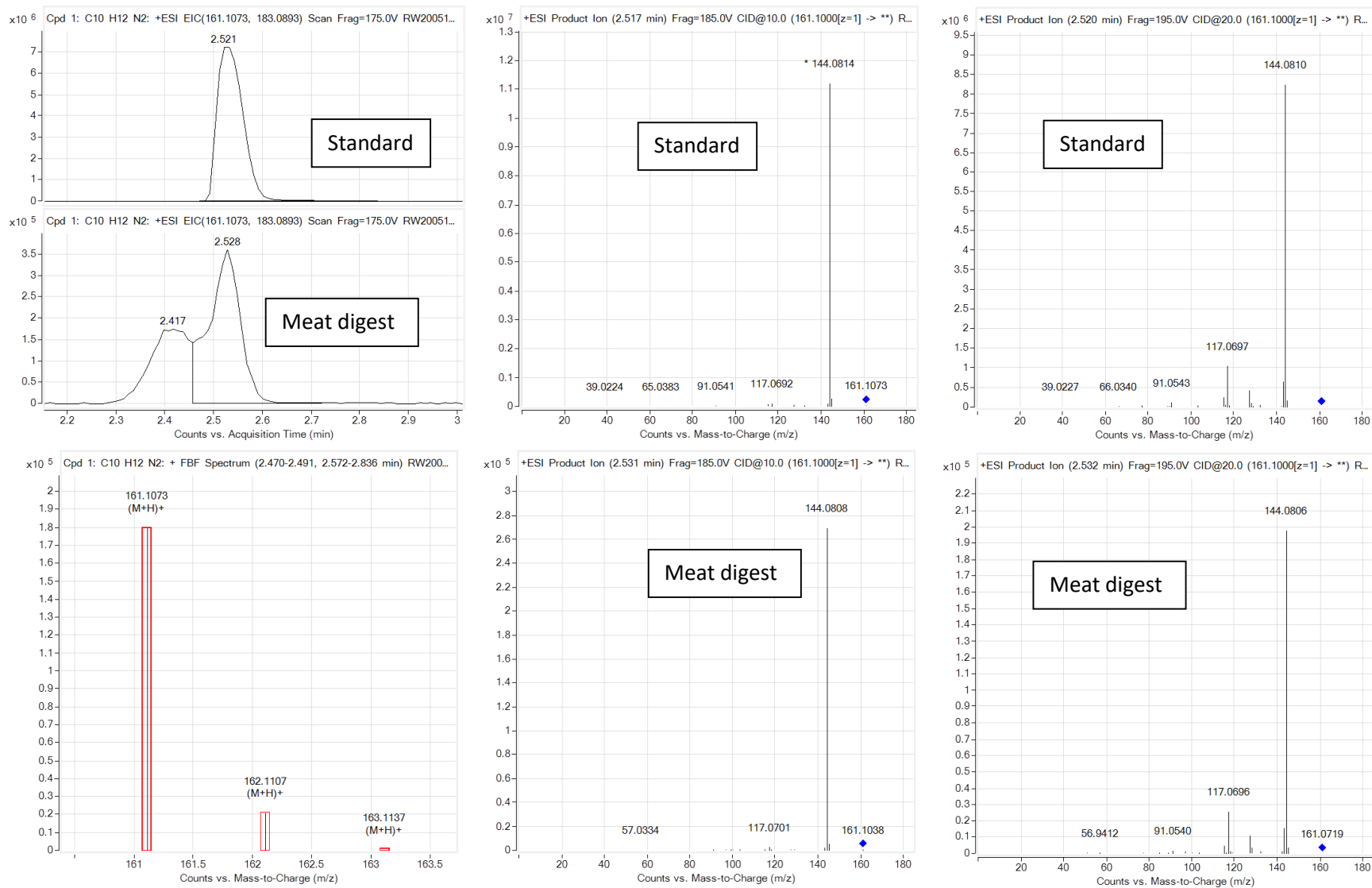


FIGURE S19 : Annotation evidence for tryptamine in fermented sausage digest, comparison with chemical standard.



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