

Supplementary File

Multi-targeted MS-based metabolomics fingerprinting of black and white pepper coupled with molecular networking in relation to their *in vitro* antioxidant and antidiabetic effects

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Table S1. Major metabolites detected in black and white pepper samples. Values are expressed as mean $\pm$ S.D (n = 3). Statistical analysis is carried out by t-Test: Paired Two Sample for Means where significance difference at $p \leq 0.05$ and non-significance at $p > 0.05$.

Metabolite	BP (mg/g) Mean $\pm$ SD	WP (mg/g) Mean $\pm$ SD	t-Test (p-value)
Oxalic acid (2TMS)	2.44 $\pm$ 0.50	2.78 $\pm$ 0.17	0.45
γ -Aminobutyric acid, (3TMS) isomer	0.63 $\pm$ 0.10	0.06 $\pm$ 0.02	0.01
α -Hydroxybehenic acid methyl ester, (TMS)	21.29 $\pm$ 21.49	21.06 $\pm$ 21.21	0.99
Piperine	3.44 $\pm$ 1.45	5.95 $\pm$ 0.49	0.13
Glycerol, (3TMS)	0.18 $\pm$ 0.02	0.25 $\pm$ 0.01	0.07
Myoinositol (TMS)	0.12 $\pm$ 0.03	0.02 $\pm$ 0.01	0.03

Table S2. Results of antioxidant activity, total phenolic and total flavonoids of black and white pepper, values are expressed as mean $\pm$ S.D (n = 3). Statistical analysis is carried out by t-Test: Paired Two Sample for Means where significance difference at $p < 0.05$.

Sample ID	BP	WP		p-value (t-Test)
DPPH	52.0692	31.7324		
	48.2317	26.6145		
	49.0845	28.6617		
avg	49.7951*	29.0028*	mgTE/g w	0.0004
stdev $\pm$	2.01507	2.57594		
ABTS	21.088	11.386		
	20.1344	11.1057		
	20.4977	11.9467		
avg	20.5733*	11.4795*	mgTE/g w	0.001
stdev $\pm$	0.4813	0.42818		
FRAP	104.806	74.6267		
	105.725	79.1665		
	103.151	78.7881		
avg	104.561*	77.5271*	mgTE/g w	0.004
stdev $\pm$	1.30443	2.51895		
TPC Folin	44.8976	35.2252		
	44.3379	37.4737		
	47.5094	39.7221		
avg	45.5816*	37.4737*	mgGAE/g w	0.01
stdev $\pm$	1.6928	2.24847		
TFC AlCl ₃	10.0482	7.71191		
	8.76865	7.60223		
	9.4084	10.081		
avg	9.4084**	8.46504**	mgRE/g w	0.39
stdev $\pm$	0.63975	1.40052		
* $p < 0.05$ ** $p > 0.05$				

Table S3. Enzyme inhibition activity of black and white pepper, values are expressed as mean $\pm$ S.D (n = 3). Statistical analysis is carried out by t-Test: Paired Two Sample for Means where significance difference at $p < 0.05$ and non-significance at $p > 0.05$.

Sample ID		IC50 (mg/mL) - replicates			Final	±	SD
		1	2	3			
BP	Alpha-glucosidase	0.7858	0.7637	0.7688	0.7726**	±	0.012
WP		0.7213	0.4654	0.6821	0.6179**	±	0.138
Acarbose		0.4891					
BP	Lipase	NA	NA	NA	NA	±	
WP		NA	NA	NA	NA	±	

** p > 0.05

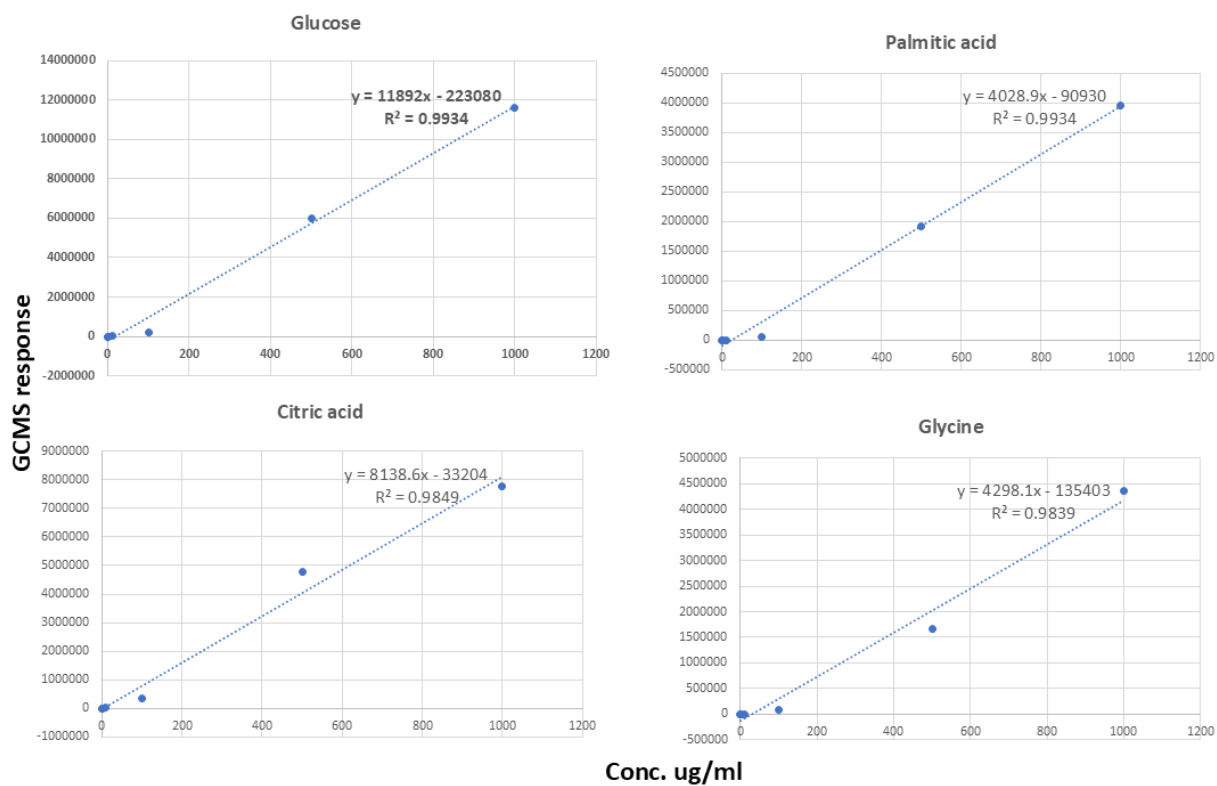


Figure S1. Calibration curves for glucose, glycine, citric acid and palmitic acid displayed 0.9948 correlation coefficient.

FAM283_1(1) #4644 RT: 15.01 AV: 1 NL: 2.91E6
F: FTMS + c ESI d Full ms2 597.30@cid35.00 [150.00-610.00]

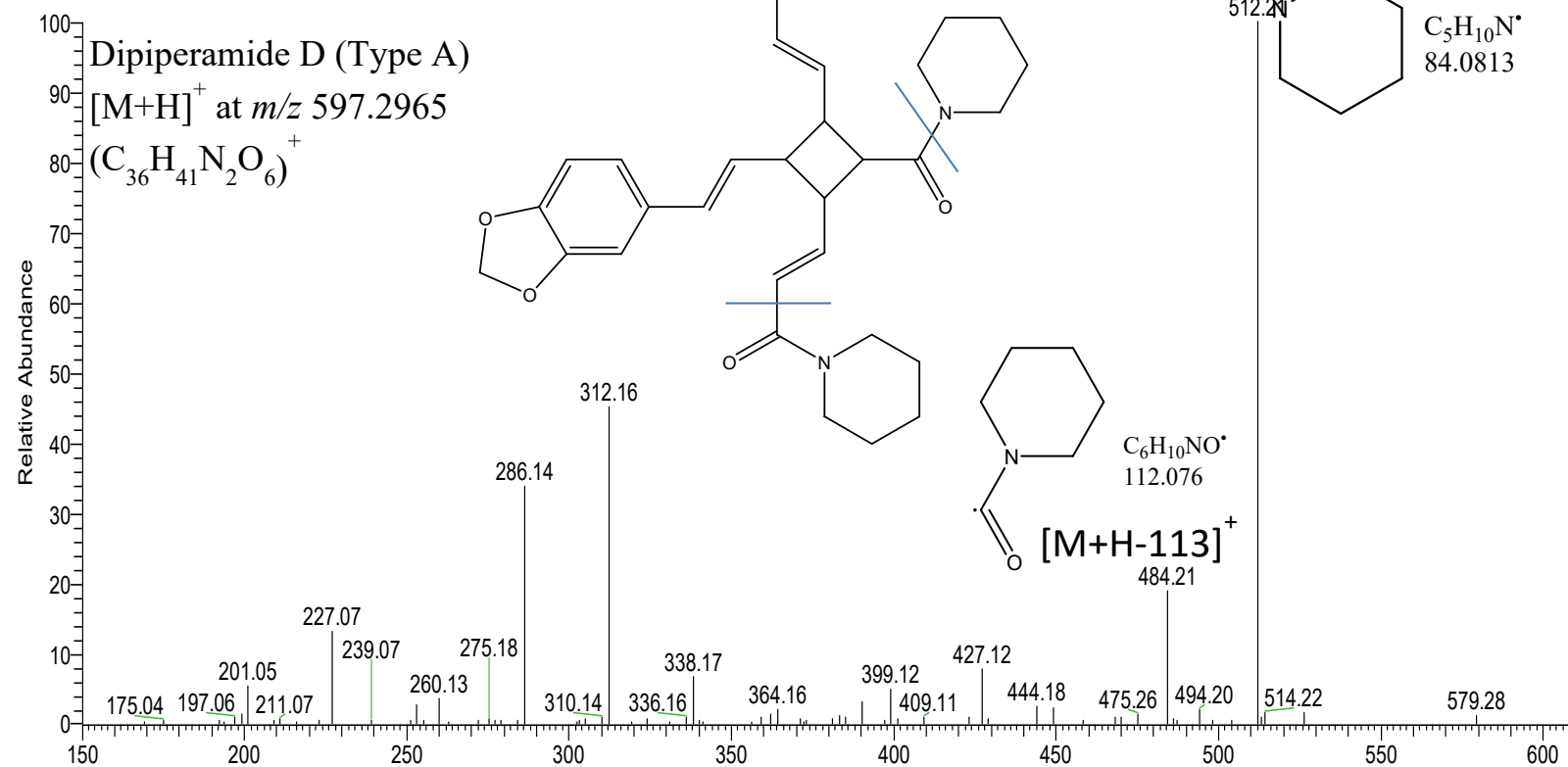


Figure S2. MS₂ spectrum of dipiperamide D (Type A piperamide).

FAM283_2(2) #5650 RT: 18.40 AV: 1 NL: 5.19E6
F: FTMS + c ESI d Full ms2 362.34@cid35.00 [85.00-375.00]

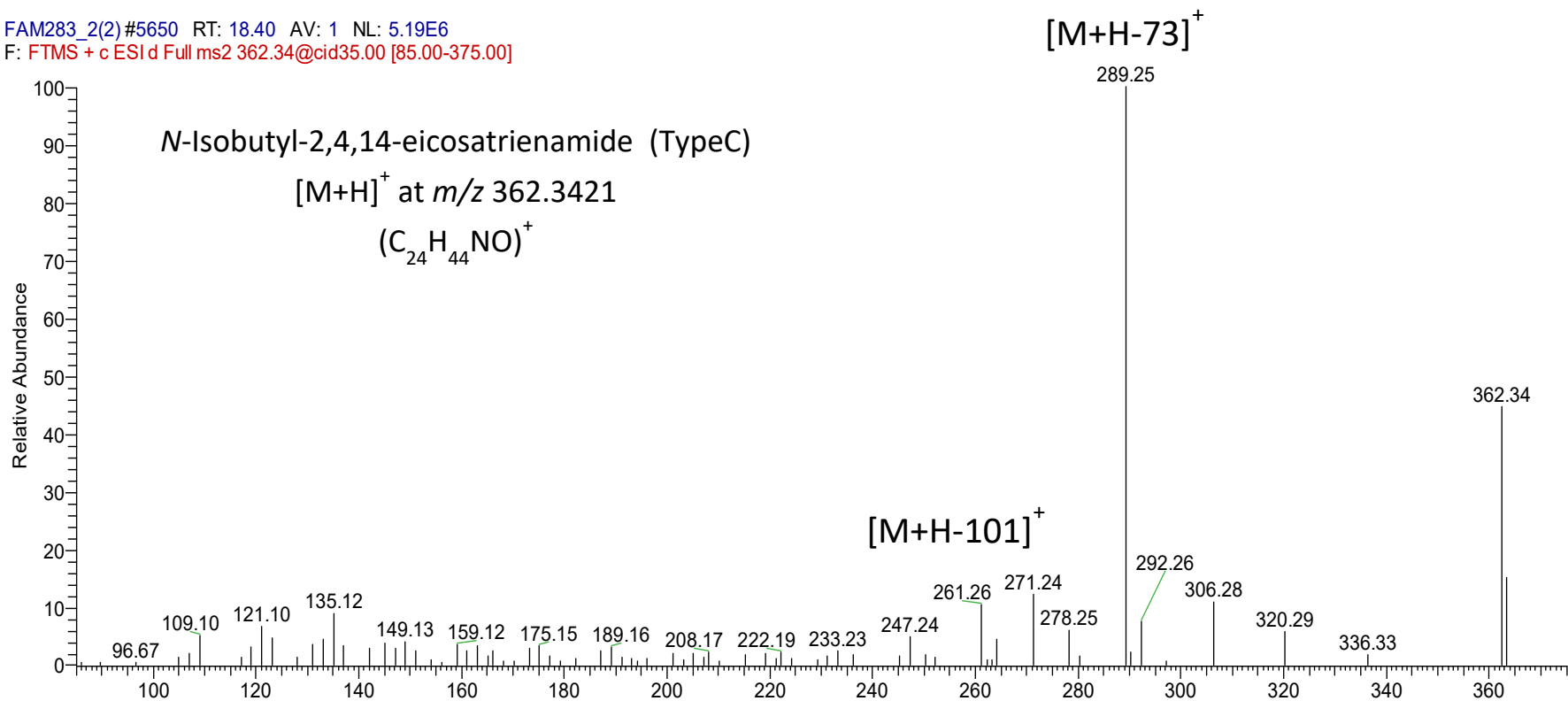


Figure S3. MS₂ spectrum of *N*-isobutyl-2,4,14-eicosatrienamide (Type C piperamide).

FAM283_2(2) #5854 RT: 19.04 AV: 1 NL: 8.90E5
F: FTMS + c ESI d Full ms2 350.34@cid35.00 [85.00-365.00]

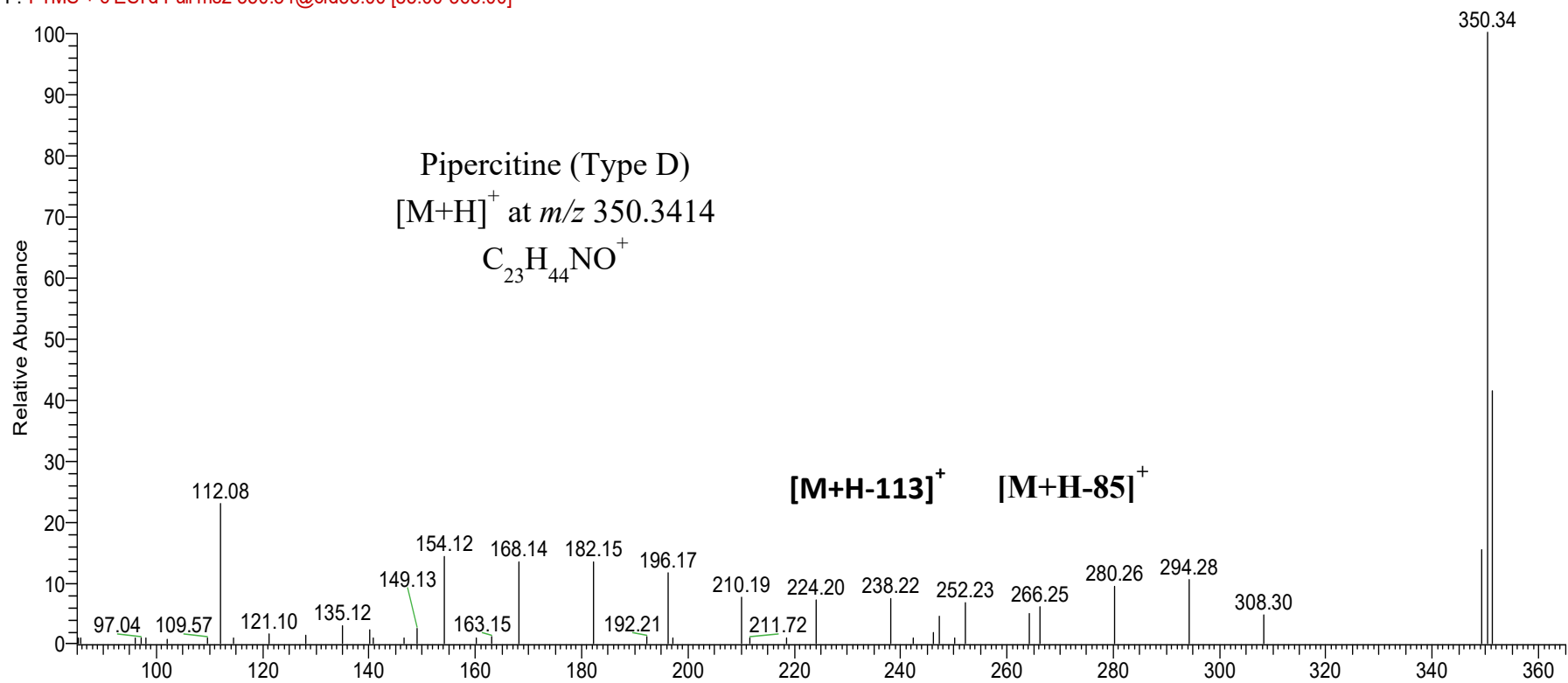
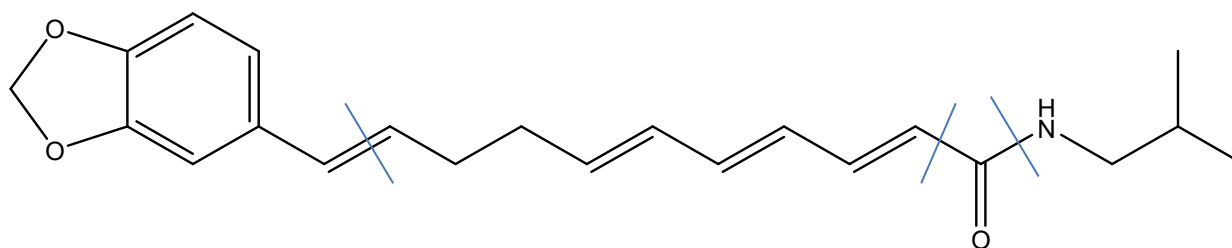


Figure S4. MS₂ spectrum of pipercitine (Type D piperamide).



(2E,4E,6E,10E)-11-(1,3-benzodioxol-5-yl)-N-(2-methylpropyl)undeca-2,4,6,10-tetraenamide

FAM283_2(2) #4622 RT: 15.09 AV: 1 NL: 3.74E6

F: FTMS + c ESI d Full ms2 356.22@cid35.00 [85.00-370.00]

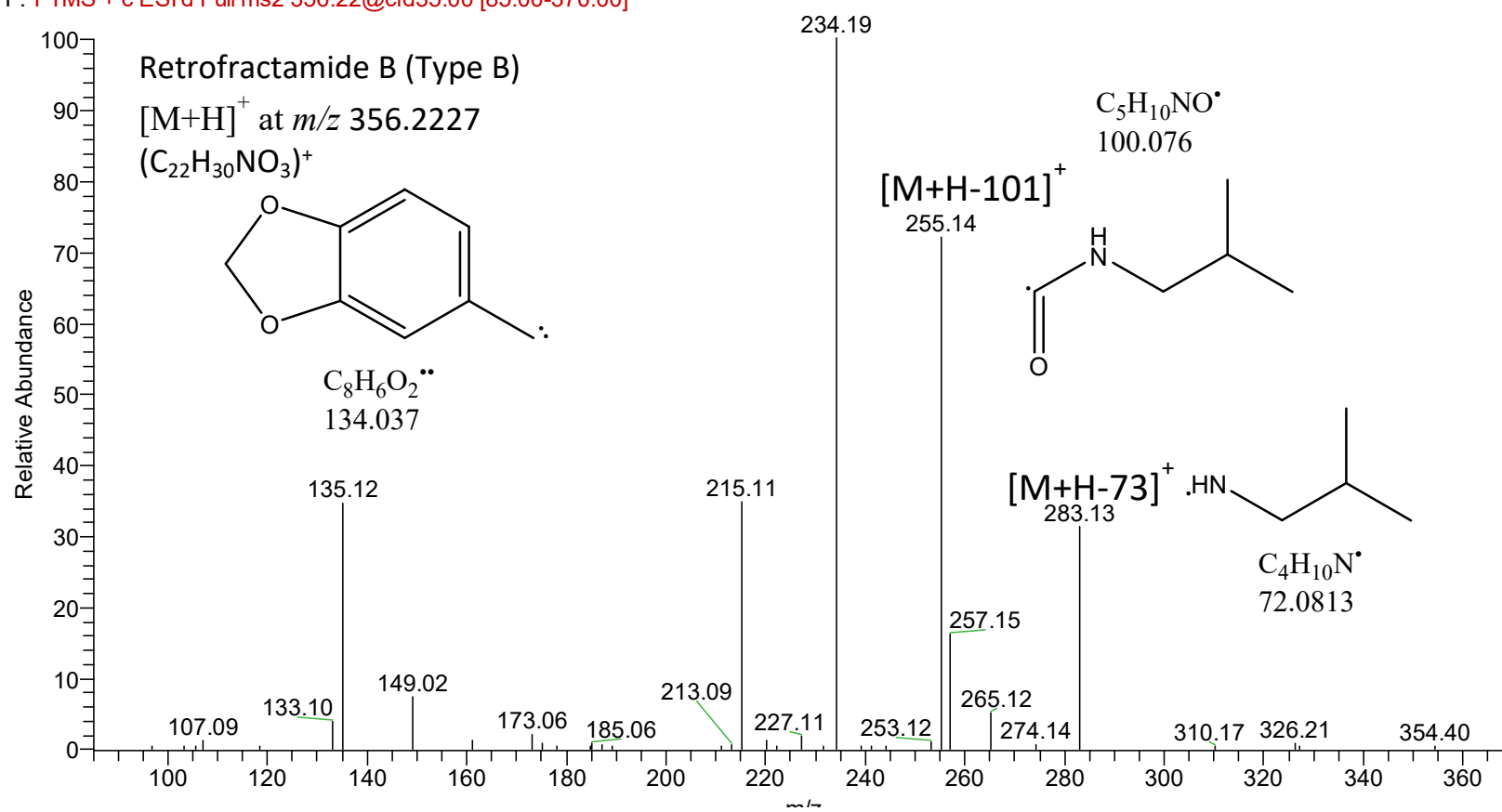


Figure S5. MS₂ spectrum of retrofractamide B (Type B piperamide).

FAM283_2(2) #4864 RT: 15.86 AV: 1 NL: 2.33E6
F: FTMS + c ESI d Full ms2 382.24@cid35.00 [95.00-395.00]

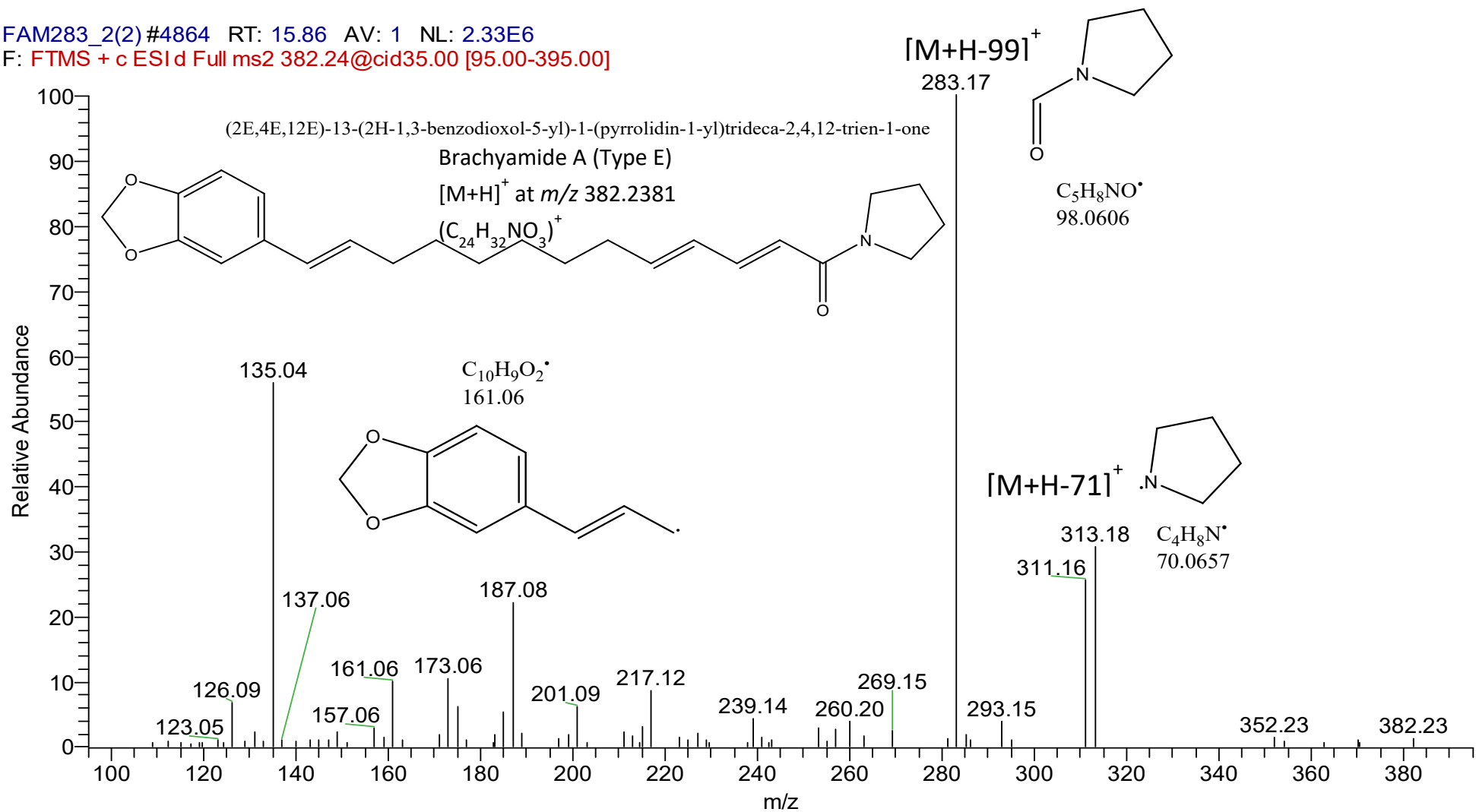


Figure S6. MS₂ spectrum of brachyamide A (Type E piperamide).

FAM283_2(2) #3718 RT: 12.17 AV: 1 NL: 1.52E6
F: FTMS + c ESI d Full ms2 258.15@cid35.00 [60.00-270.00]

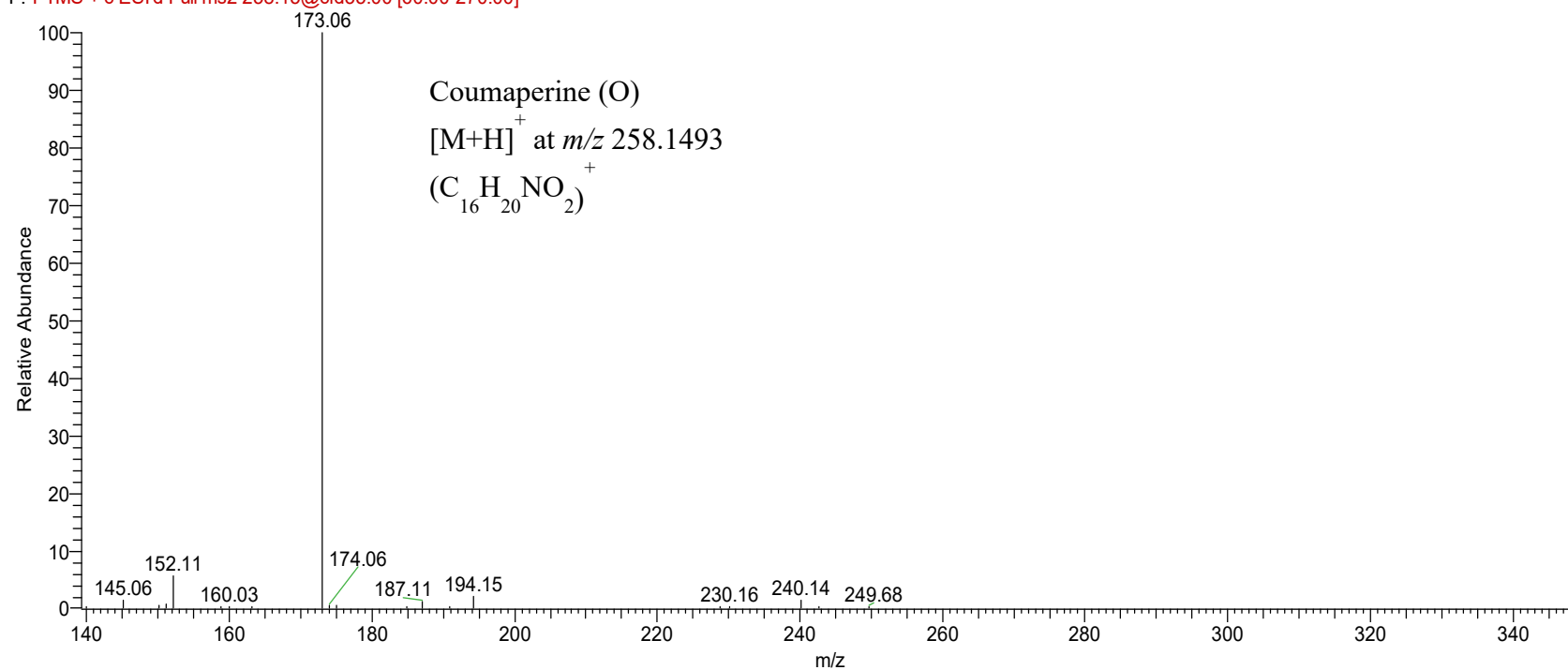


Figure S7. MS₂ spectrum of coumaperine (Type O piperamide).

FAM283_1#482 RT: 10.14 AV: 1 NL: 1.58E5
T: FTMS - c ESI d Full ms2 563.14@cid45.00 [145.00-575.00]

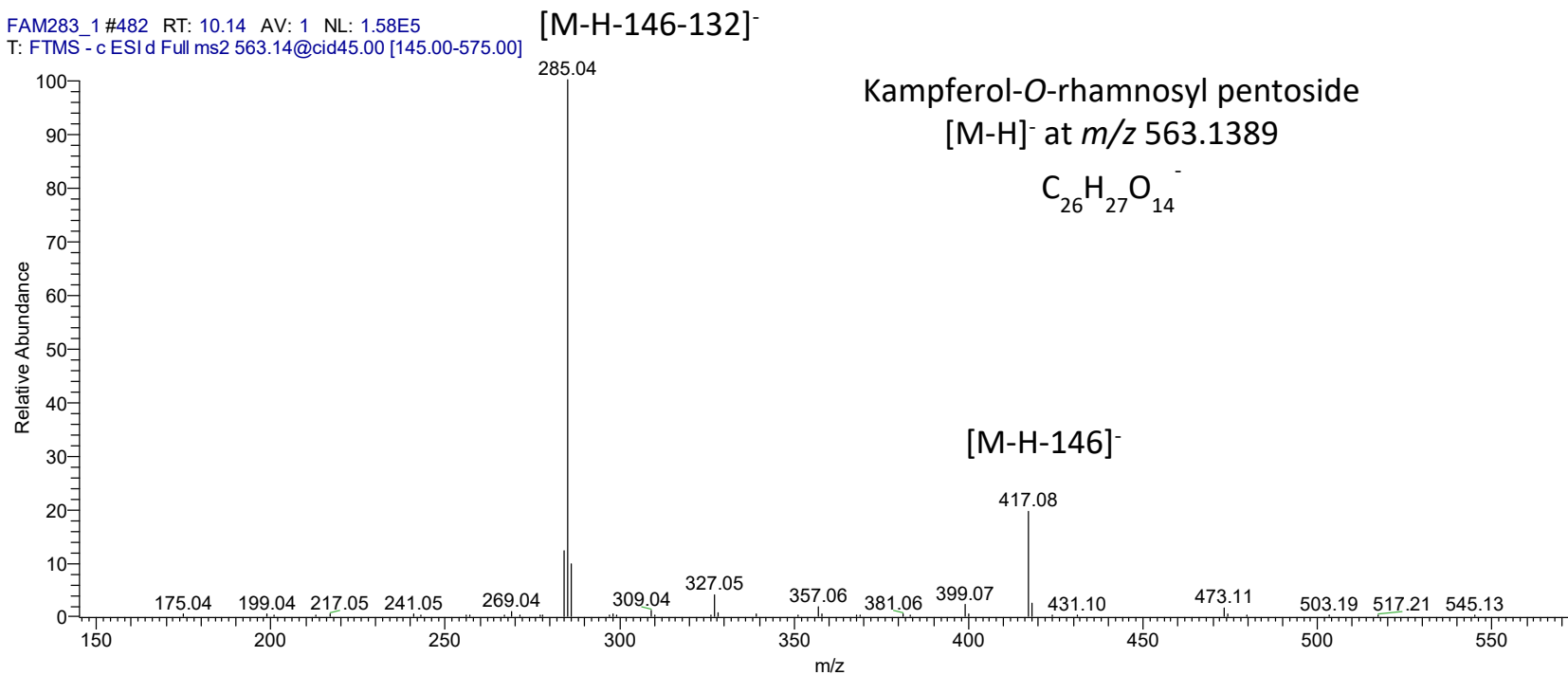


Figure S8. MS₂ spectrum of kampferol-*O*-rhamnosyl pentoside (flavonoid)

FAM283_1 #570 RT: 11.37 AV: 1 NL: 4.42E4
F: FTMS - c ESI d Full ms2 312.12@cid45.00 [75.00-325.00]

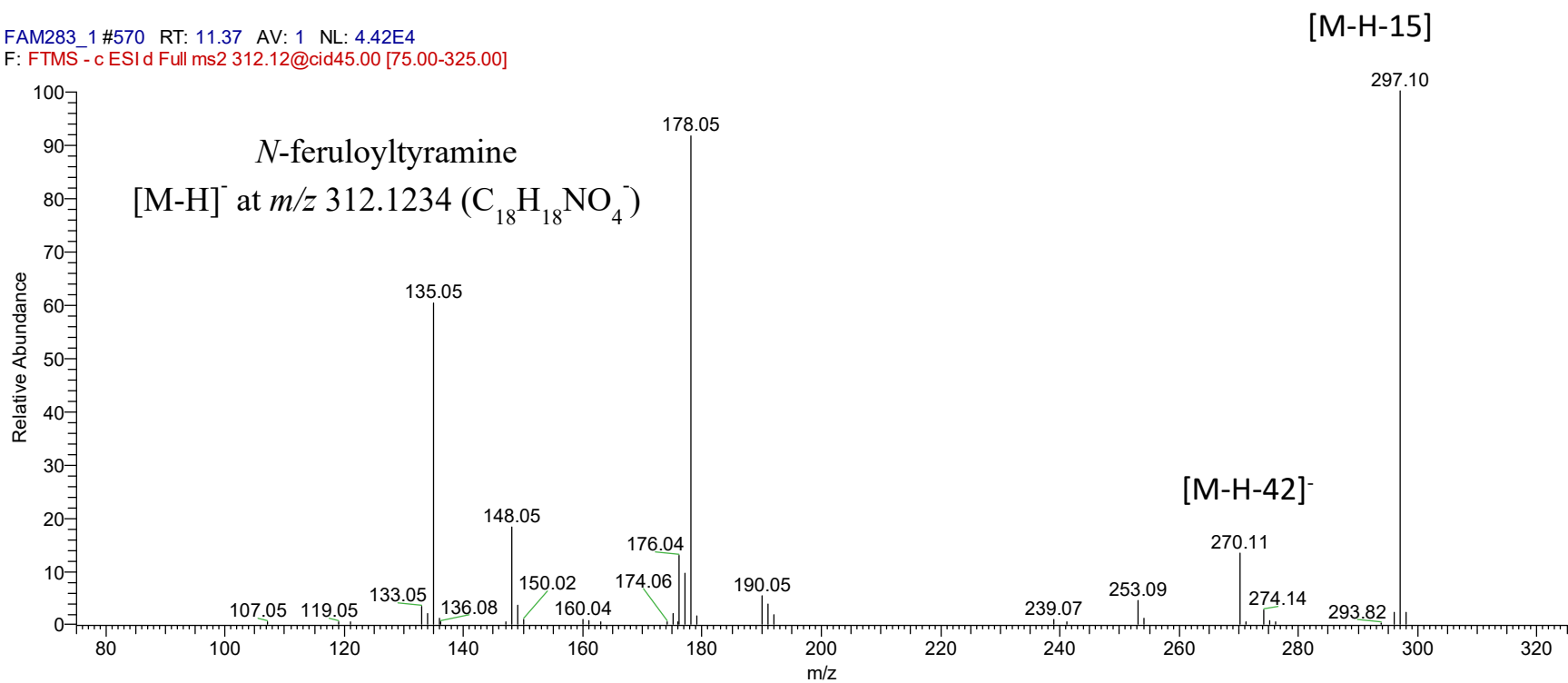


Figure S9. MS₂ spectrum of 3,4-Bis[(4-hydroxyphenyl) methyl]oxolan-2-one *N*-feruloyltyramine (phenylamide)

FAM283_1 #604 RT: 11.84 AV: 1 NL: 1.11E4
F: FTMS - c ESI d Full ms2 327.29@cid45.00 [80.00-340.00]

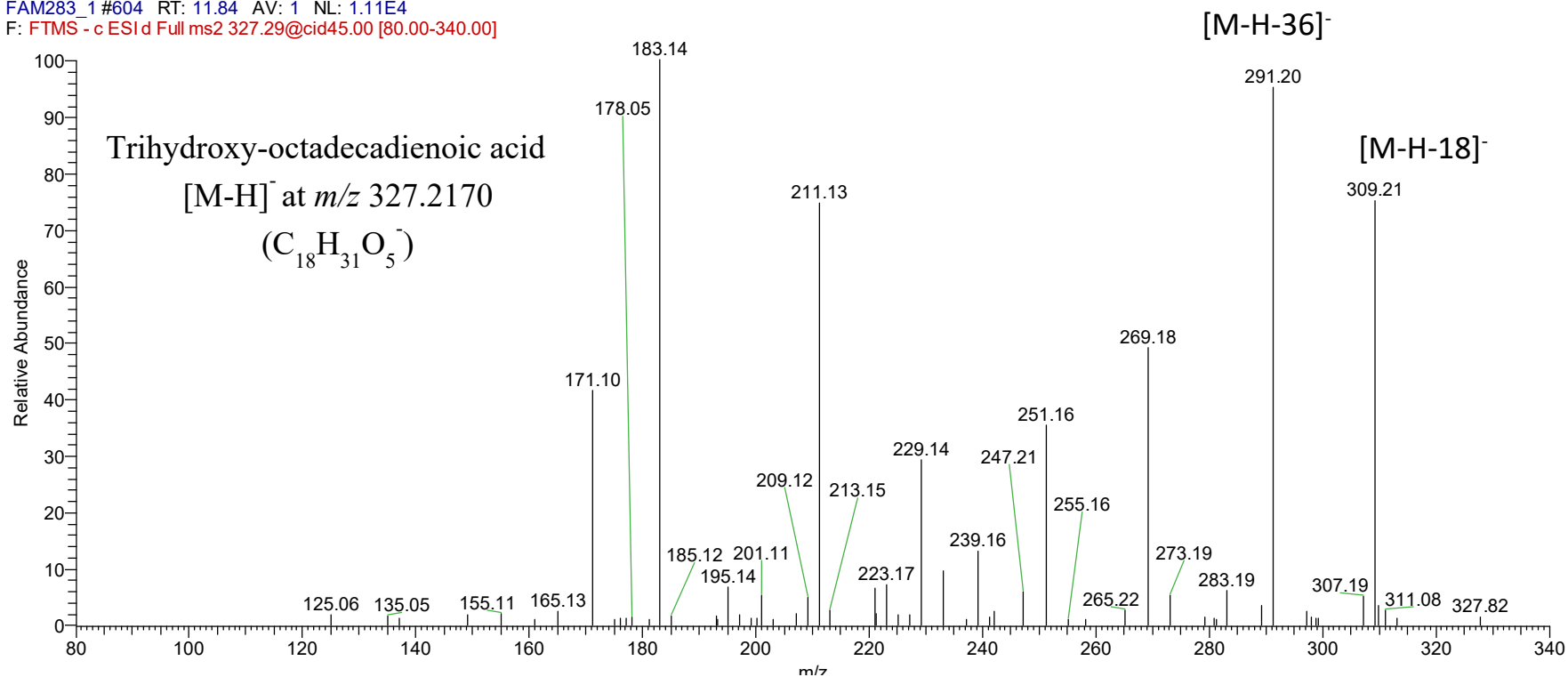


Figure S10. MS₂ spectrum of trihydroxy-octadecadienoic acid (fatty acid)

FAM283_2(2) #3672 RT: 12.02 AV: 1 NL: 2.73E5
F: FTMS + c ESI d Full ms2 290.18@cid35.00 [65.00-305.00]

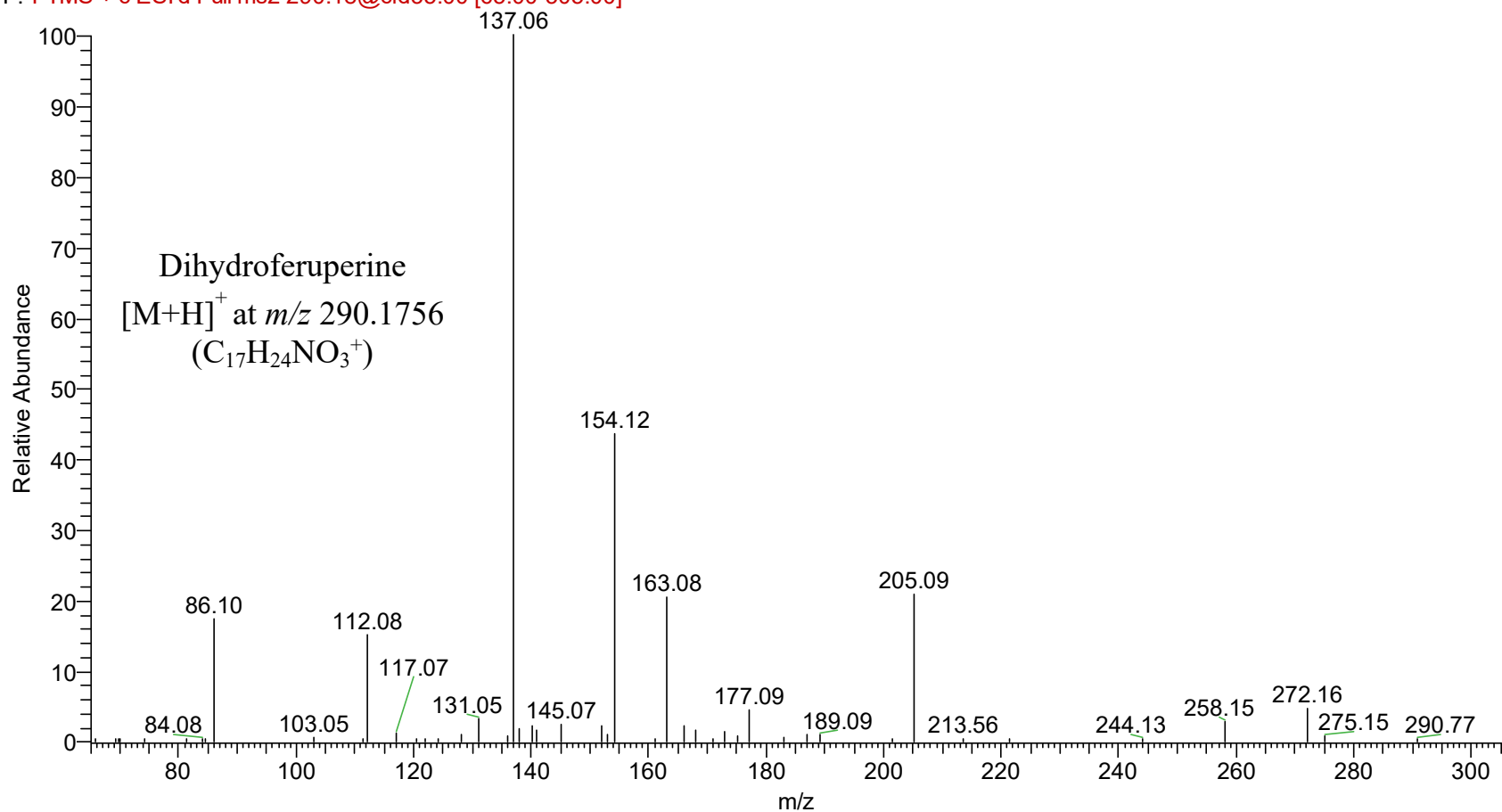


Figure S11. MS₂ spectrum of dihydroferuperine (Type O piperamide)

FAM283_2(2) #3716 RT: 12.17 AV: 1 NL: 1.88E6
F: FTMS + c ESI d Full ms2 258.15@cid35.00 [60.00-270.00]

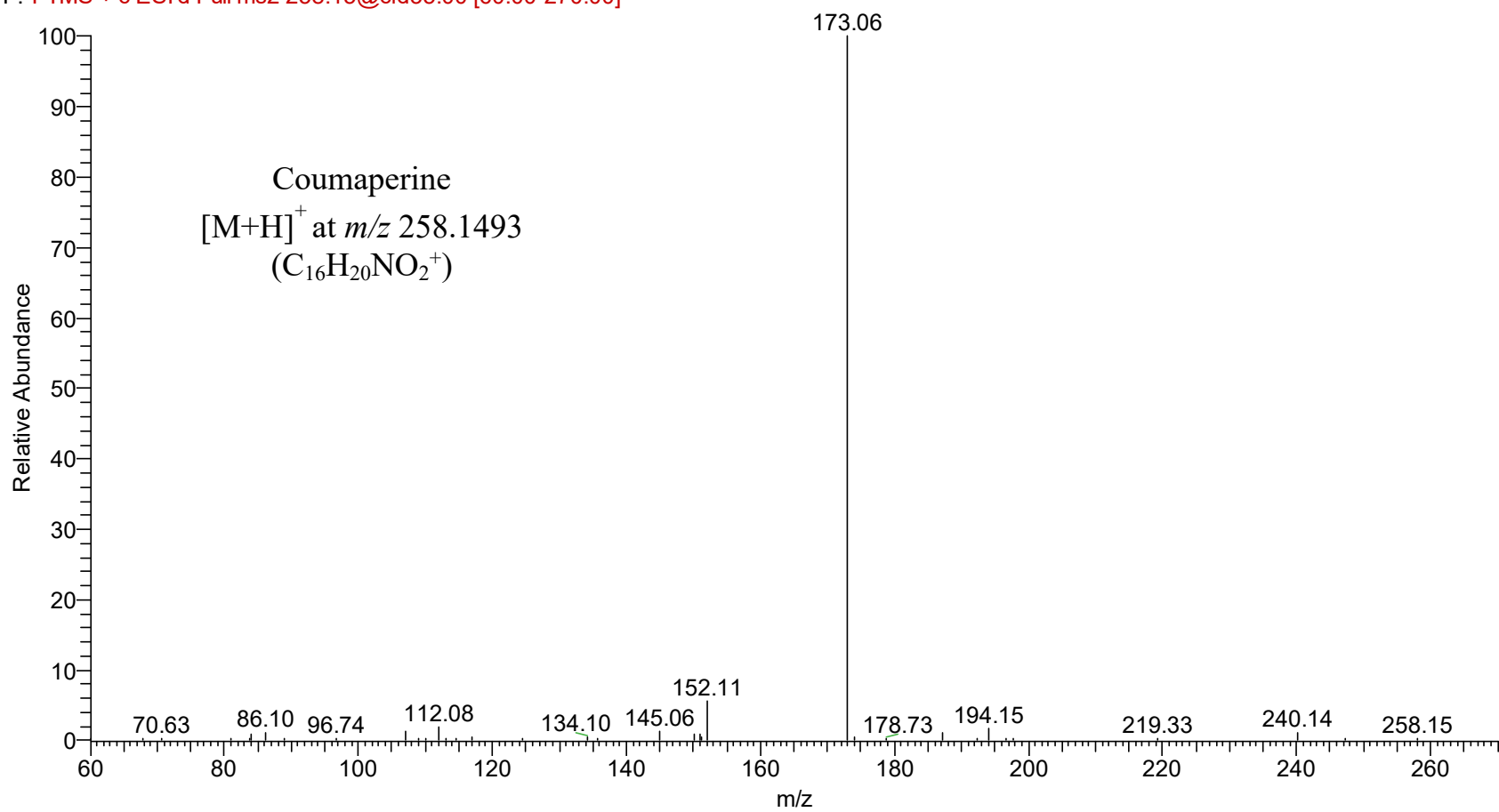


Figure S12. MS₂ spectrum of coumaperine (Type O piperamide)

FAM283_2(2) #3722 RT: 12.19 AV: 1 NL: 5.49E6
F: FTMS + c ESI d Full ms2 288.16@cid35.00 [65.00-300.00]

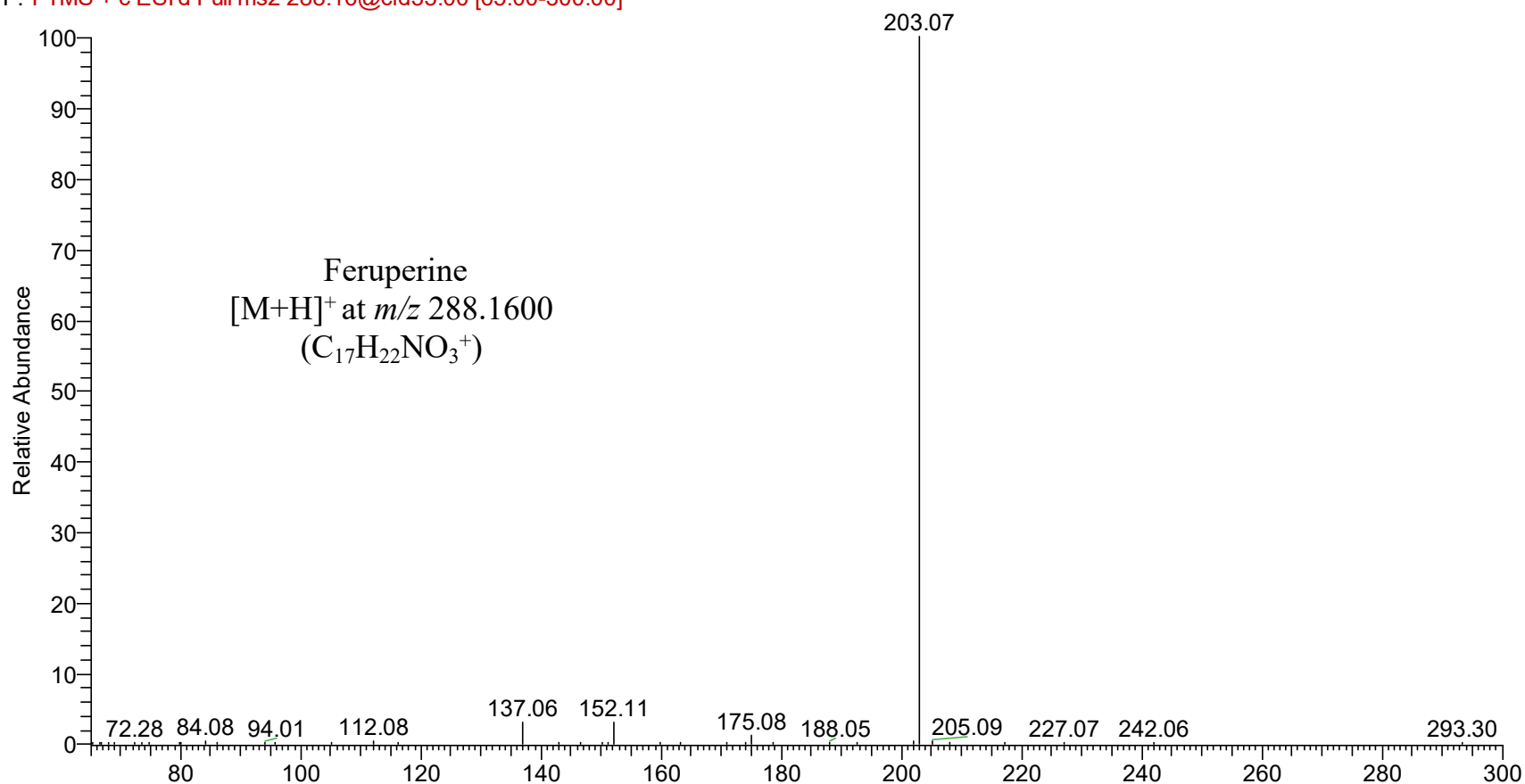


Figure S13. MS₂ spectrum of Feruperine (Type O piperamide)

FAM283_1 #628 RT: 12.18 AV: 1 NL: 1.95E5
F: FTMS - c ESI d Full ms2 298.14@cid45.00 [70.00-310.00]

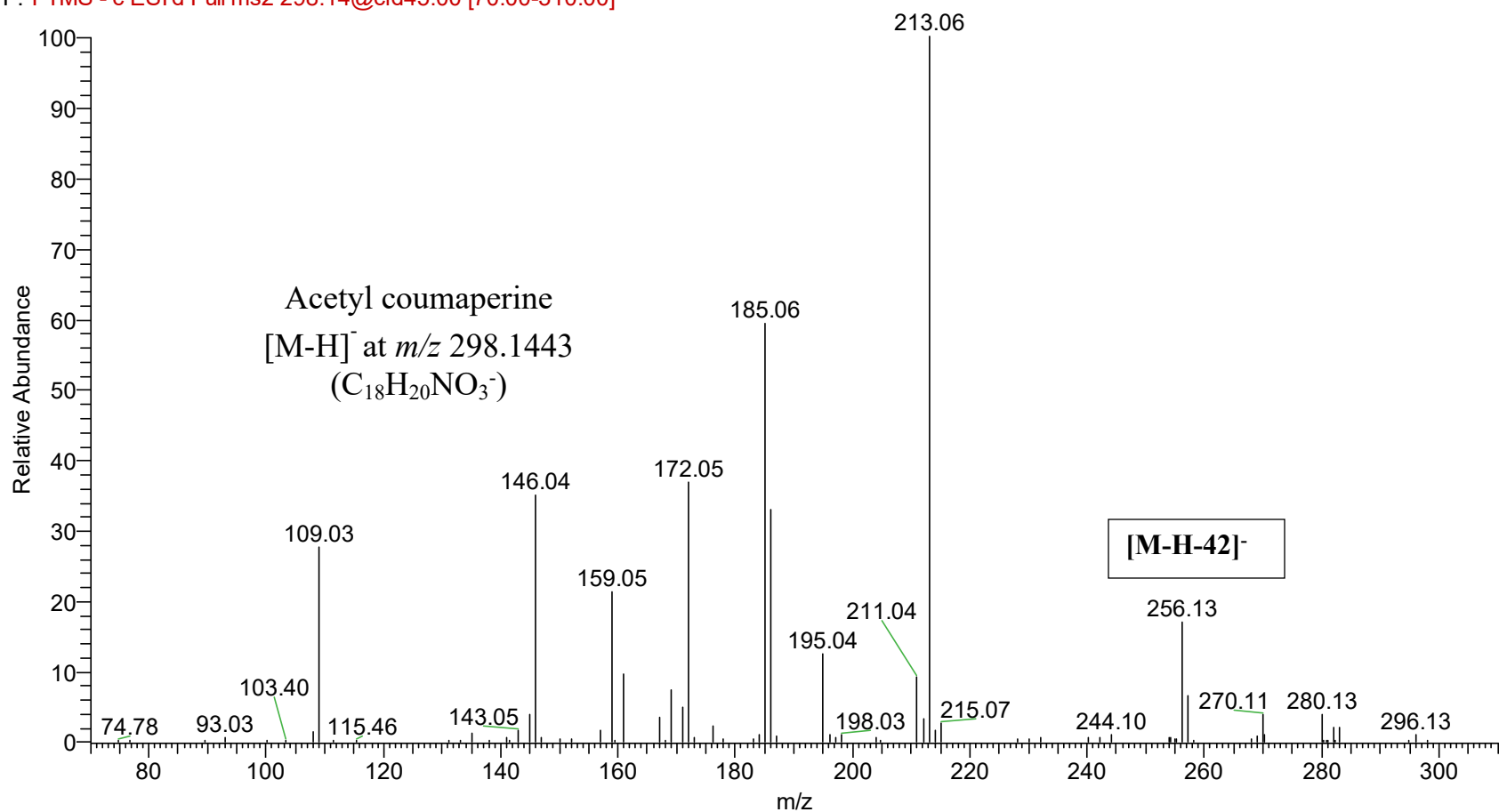


Figure S14. MS₂ spectrum of acetyl coumaperine (Type O piperamide)