



UNIVERSITAS
GADJAH MADA

Aplikasi LC-MS/MS untuk Analisis Komponen Halal

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 NEWS EKONOMI BOLA TEKNO SAINS ENTERTAINMENT OTOMOTIF LIFESTYLE PROPERTI TRAVEL EDUKASI KOLOM IMAGES
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Dalam Sehari, 1.200 Anjing Dikonsumsi di Kota Solo
 Kompas.com - 21/02/2018, 13:22 WIB

The Daily Telegraph
 Thursday, January 17, 2013
Horse meat in burgers for years
 telegraph.co.uk
 No 99011 £1.20

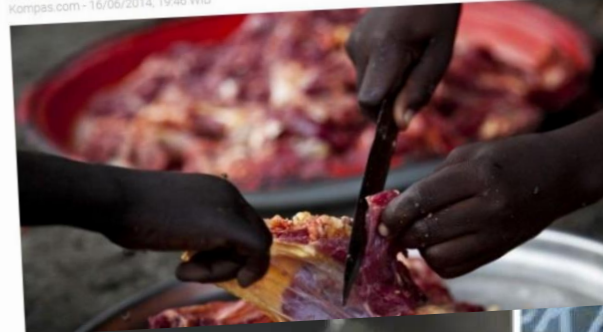


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Kurang Pengawasan, Sebagian Daging di DIY Tercampur Daging Babi
 KONTRIBUTOR YOGYAKARTA, GANDANG SAJARWO
 Kompas.com - 16/06/2014, 19:45 WIB



Tribunnews UPDATE

KRONOLOGI DAGING SAPI TERNYATA BABI



BANDUNG, JABAR



TRIK LICIK JUAL DAGING BABI

CNN Indonesia
 Home Nasional Internasional Ekonomi Olahraga Teknologi Hiburan Gaya Hidup

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BPOM Perintahkan Produsen Tarik Viostin DS dan Enzyplex

Rahman Indra, CNN Indonesia | Rabu, 31/01/2018 16:37 WIB

Bagikan :



Halal vs Haram

1,9 milyar populasi muslim dunia

Janganlah kamu mengikuti langkah-langkah setan, sesungguhnya setan itu musuh yang nyata bagimu."

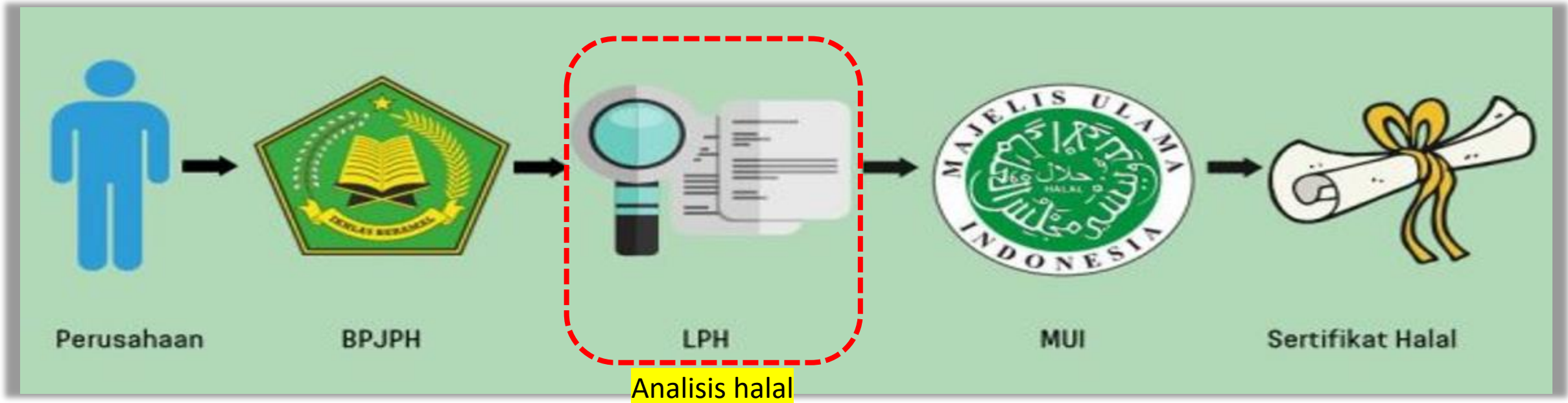
210 juta muslim di Indonesia

➤ Q

"Diharamkan bagimu (memakan) bangkai, darah, daging babi, (daging hewan) yang disembelih atas nama selain Allah, yang tercekik, yang terpukul, yang jatuh, yang ditanduk, dan diterkam binatang buas,...."



Alur Sertifikasi Halal (UU JPH No. 33 Tahun 2014)





Outline

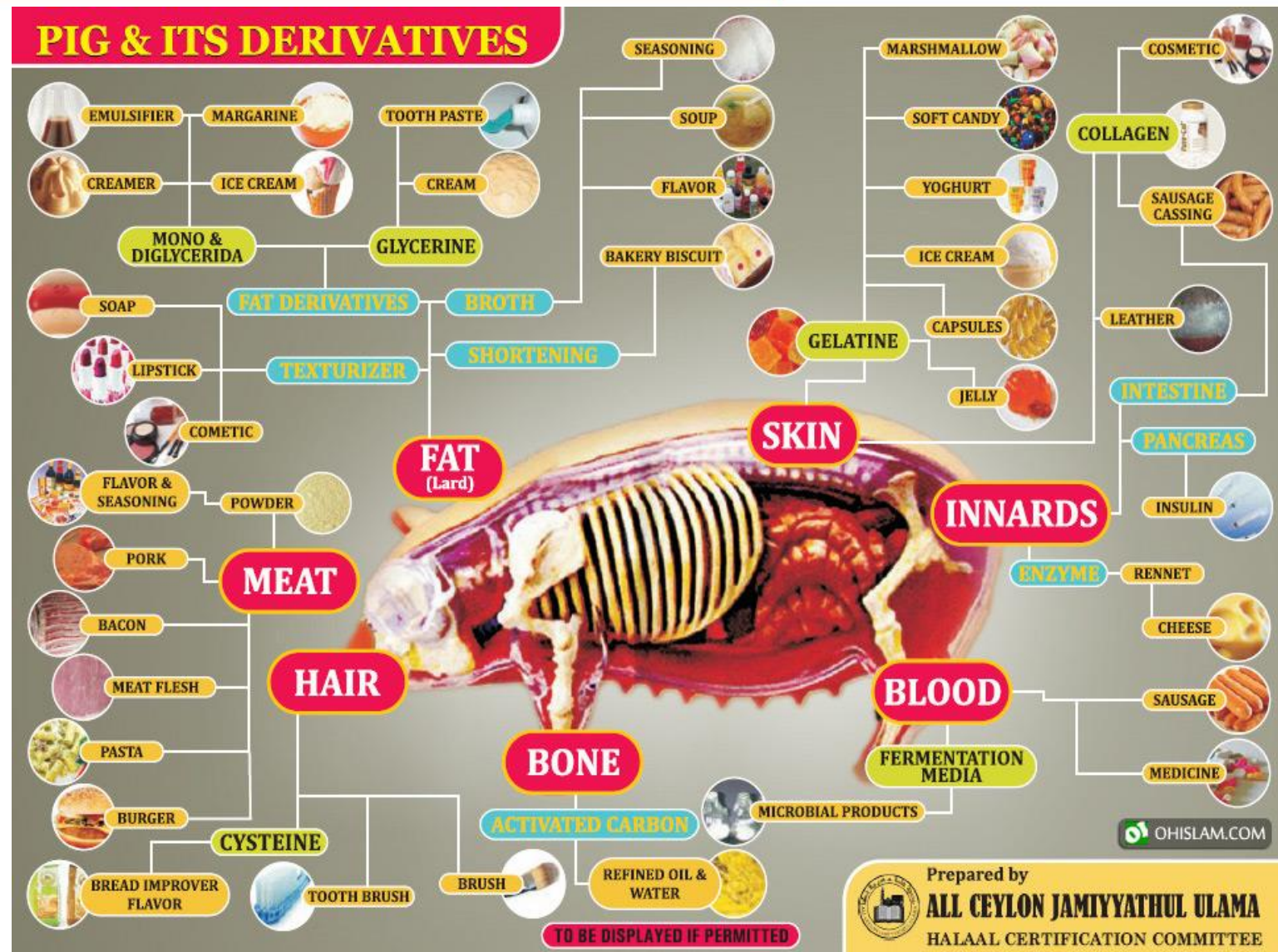
Kenapa LC-MS/MS diperlukan untuk Analisis Halal?

Marker Peptida untuk Analisis Halal dengan HRMS

Contoh Aplikasi LC-MS/MS untuk Analisis Halal



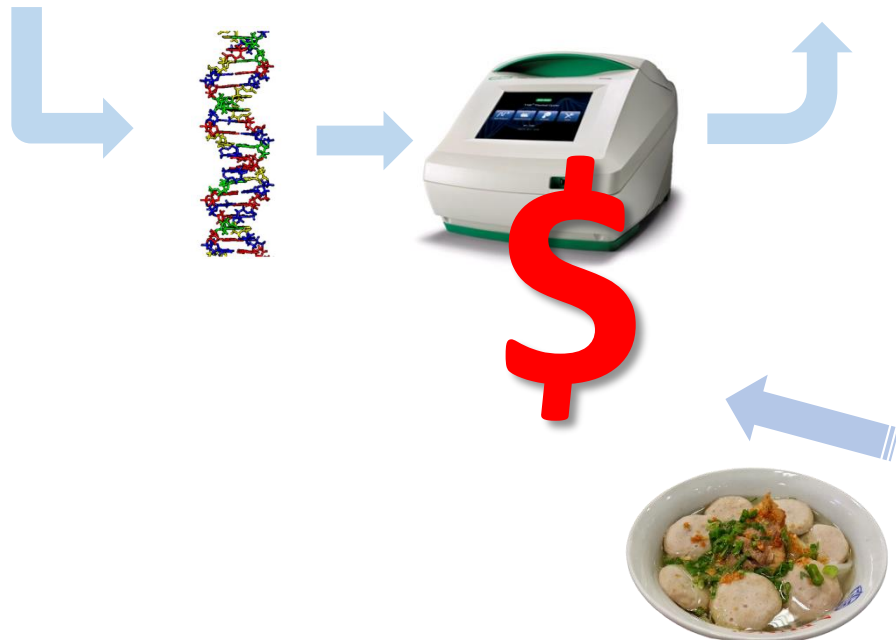
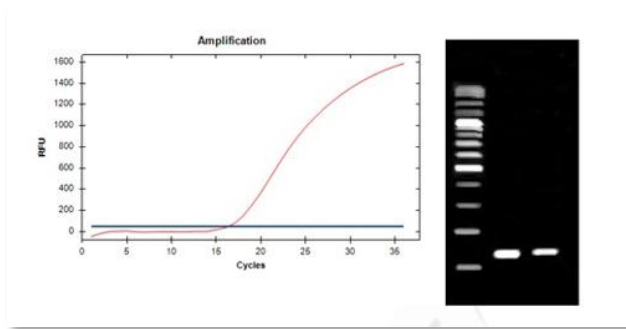
Kenapa LC-MS/MS diperlukan untuk Analisis Halal?




MADA

PCR dan realtime PCR sebagai *gold standard* analisis halal

Daging dan produk olahan :
(babi, tikus anjing?)



 = Rp...Rp..Rp



Low DNA or no DNA sample

False negative





Analisis halal = identifikasi spesies

Variasi genetik

sapi

-GGAGCCATATTAGATAGA-

babi

-GGAGCAATTTTGATAGA-

Desain primer spesifik spesies



PCR & rPCR

DNA

Based

Analysis

Protein Based Analysis

sapi

Gly Ala Ile Leu asp Arg

babi

Gly Ala Ile Phe asp Arg

Variasi urutan asam amino protein

Biomarker protein

2D-elektroforesis



Spektrometri masa

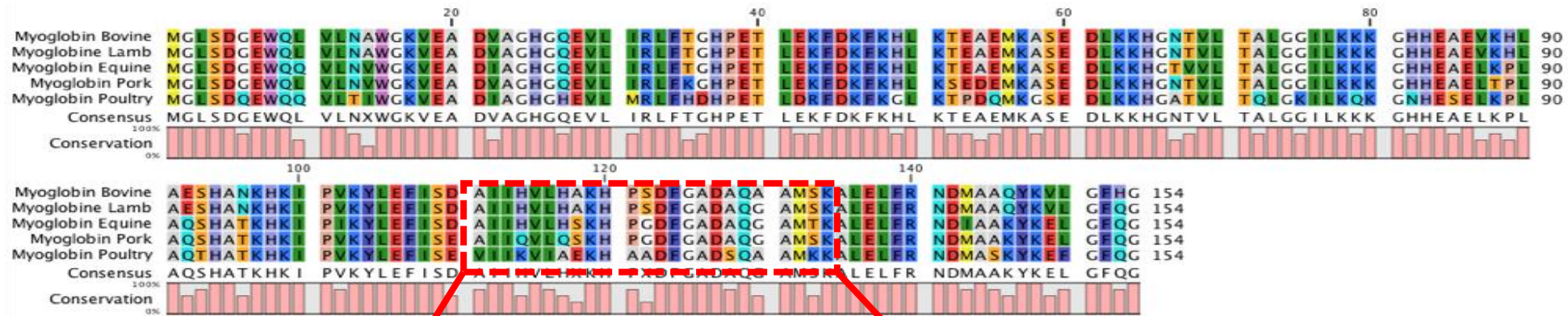


Marker Peptida

Peptida untuk Analisis Halal dengan LC-MS/MS



Mencari marker peptida



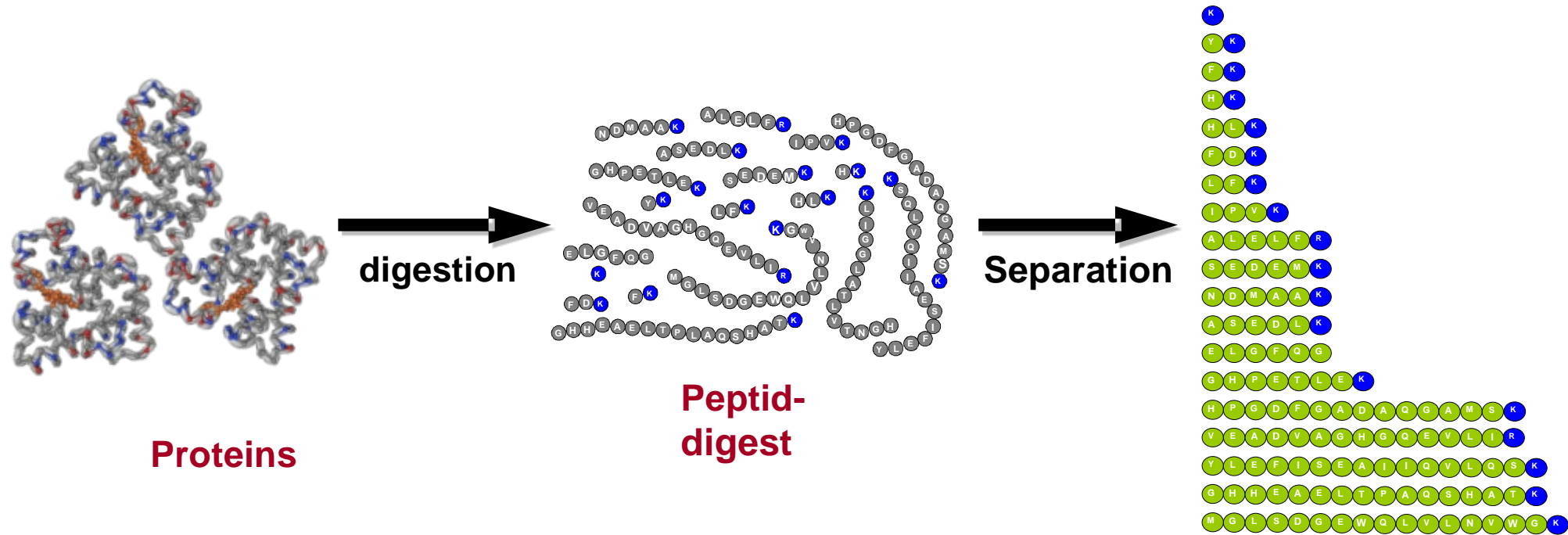
Bagian protein

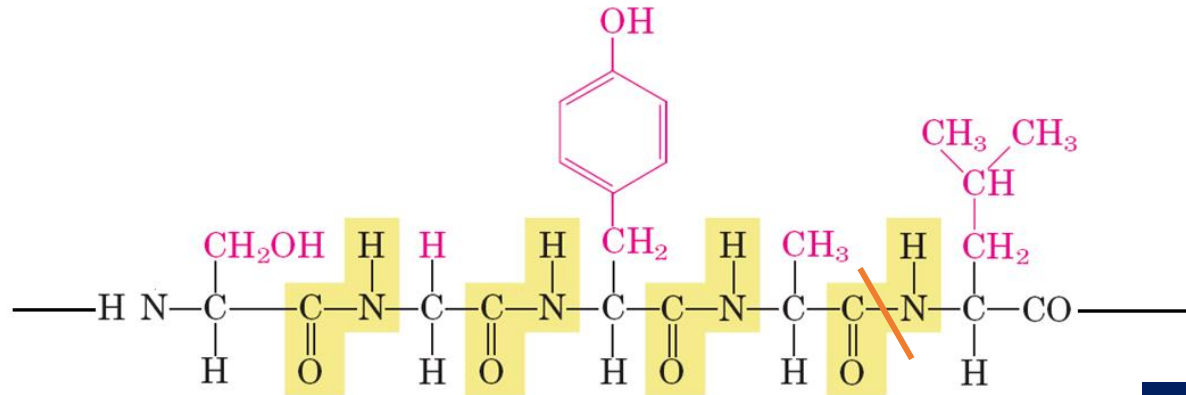
Massa dan urutan asam amino spesifik

Beef	YLEFISDA I I H V L HAK H P SDF G A DA QA A MSK A LE LFR	m1
Horse	YLEFISDA I I H V L H S K H P GDF G A DA QA G A MTK A LE LFR	m2
Pork	YLEFISEA I I Q V L Q S K H P GDF G A DA QA G A MSK A LE LFR	m3
Lamb	YLEFISDA I I H V L HAK H P SDF G A DA QA G A MSK A LE LFR	m4

biomarker

Protein → peptida

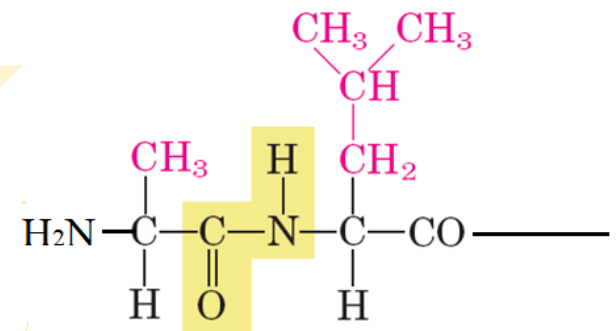
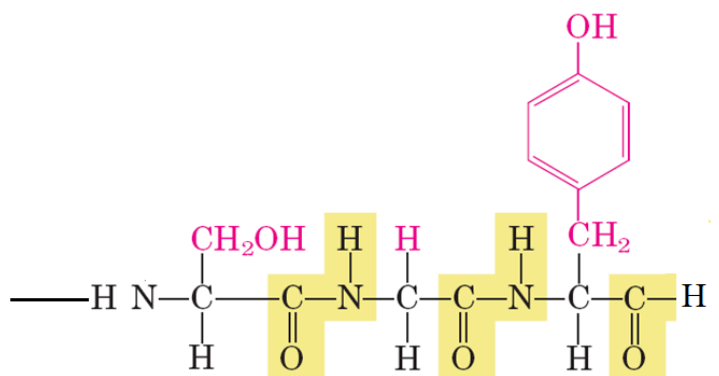




Protease : tripsin, khimotripsin



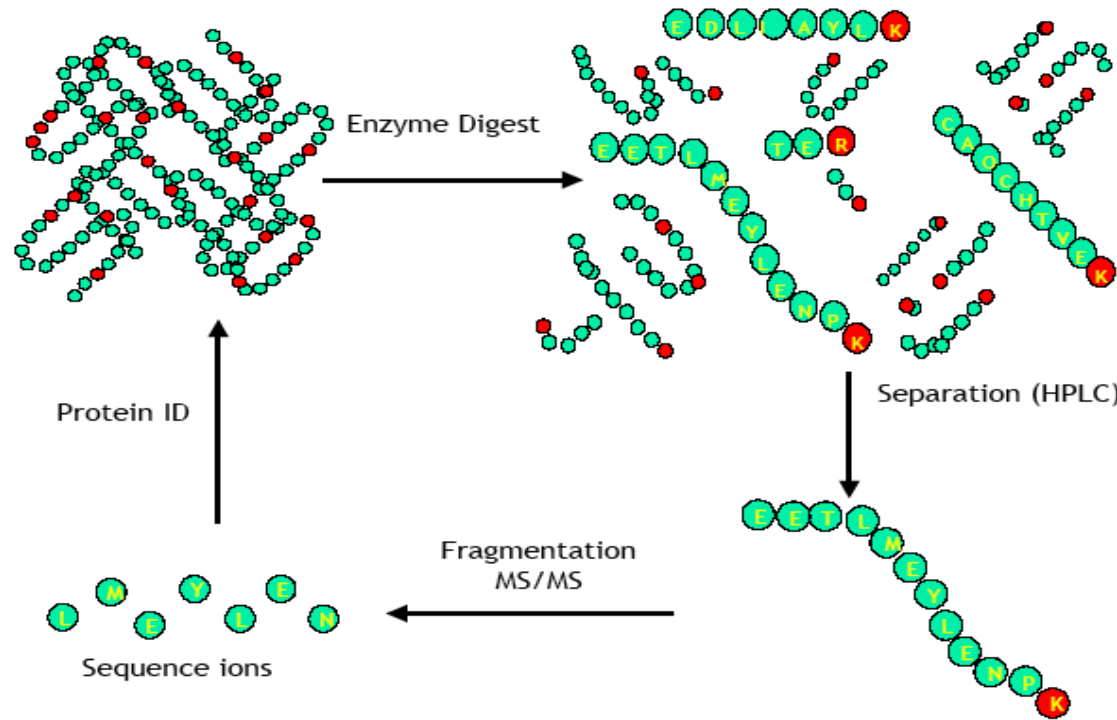
H₂O



Protease menghidrolisis ikatan peptida secara spesifik



Analisis Peptida dengan Spektrometri Massa

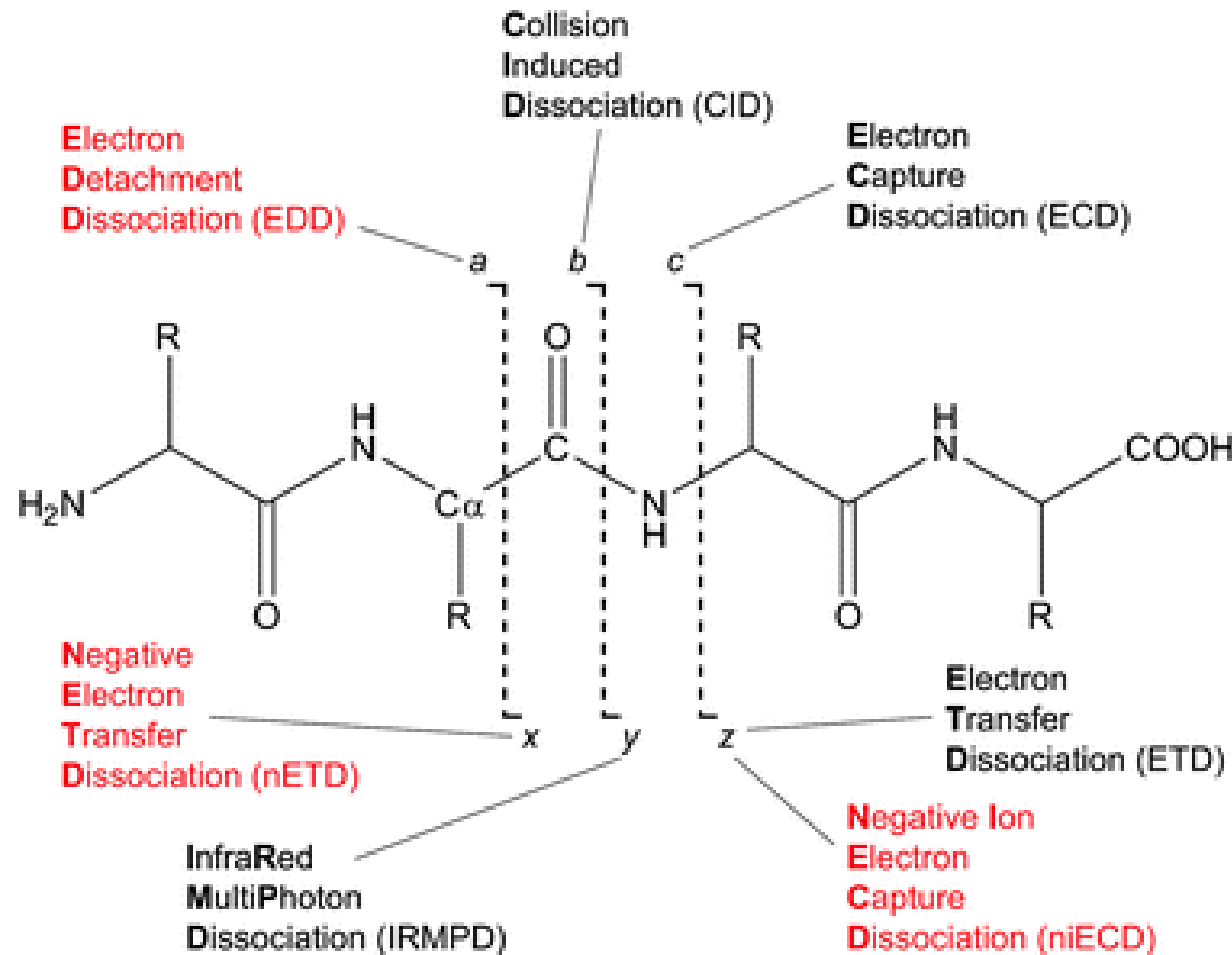


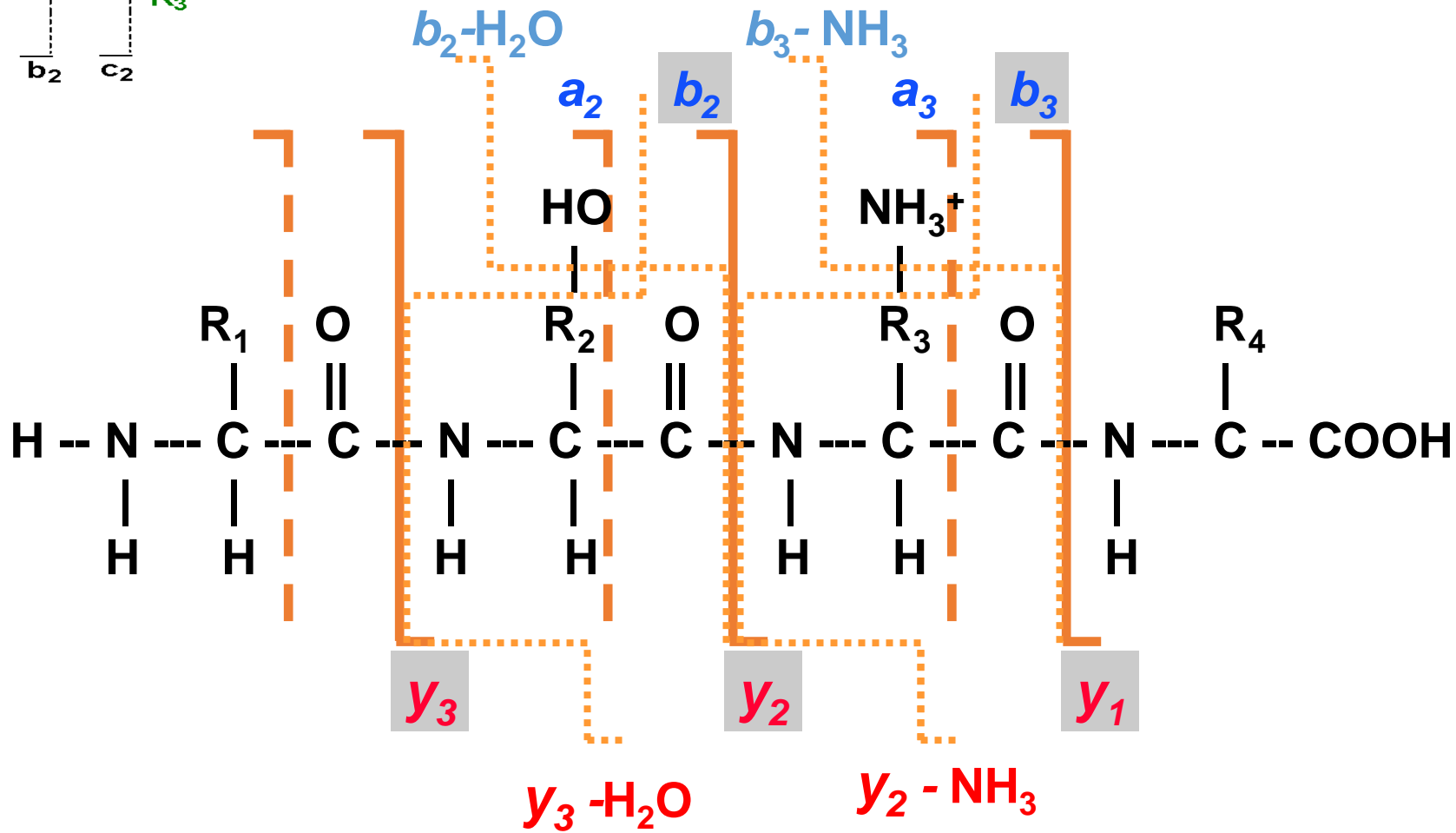
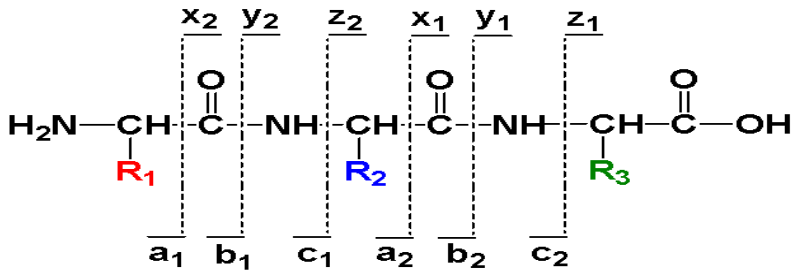
MS¹ → m/z peptida

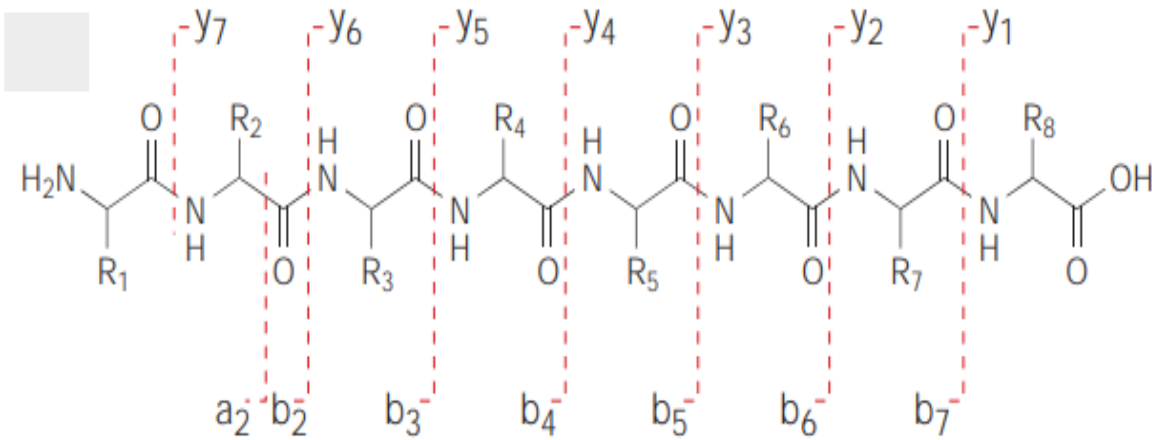
MS² → urutan asam amino peptida
(fragmentasi)

Tandem MS

Fragmentasi peptida (MS²)







P E T L E K

b fragment ions

P E T L E

P E T L

P E T

P E

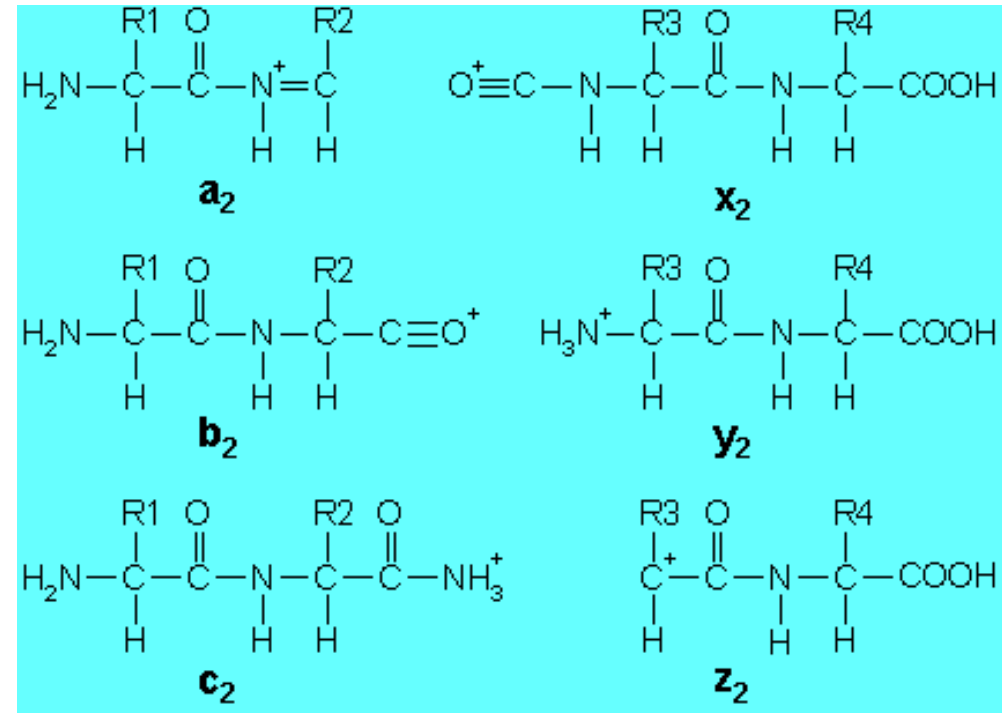
y fragment ions

E T L E K

T L E K

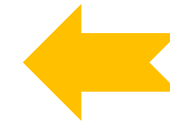
L E K

E K

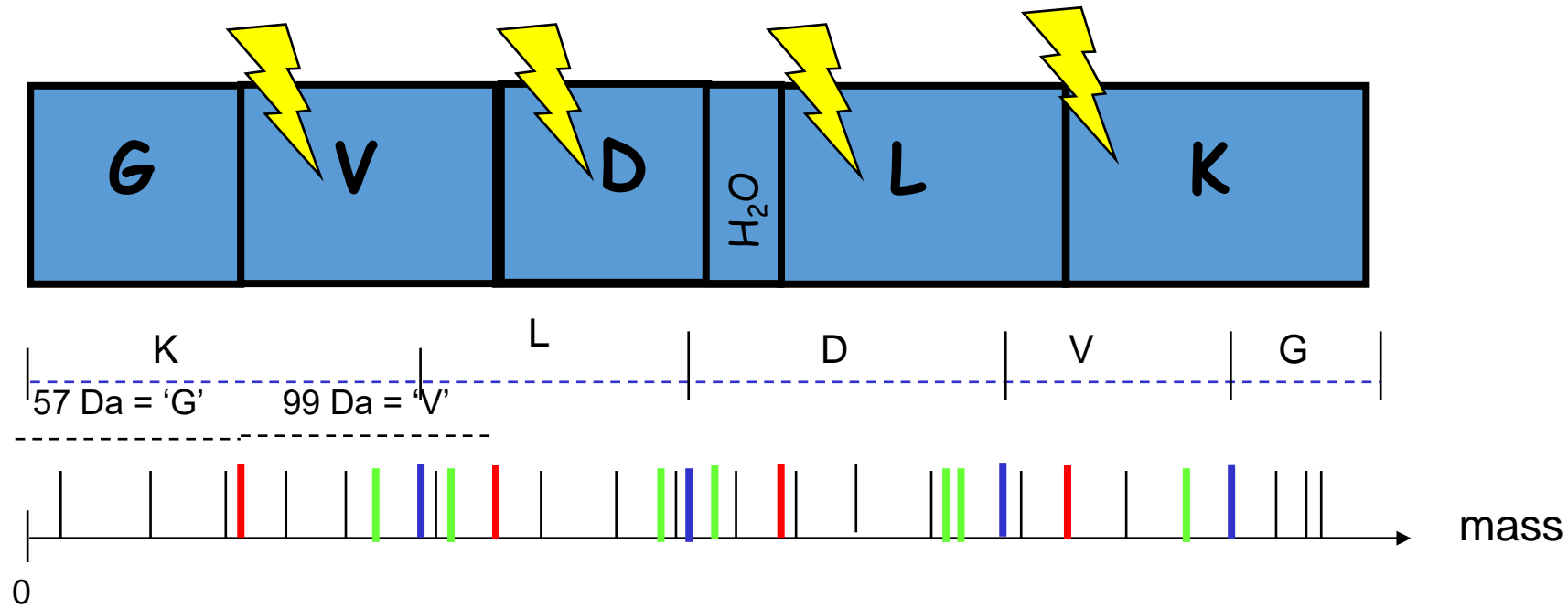


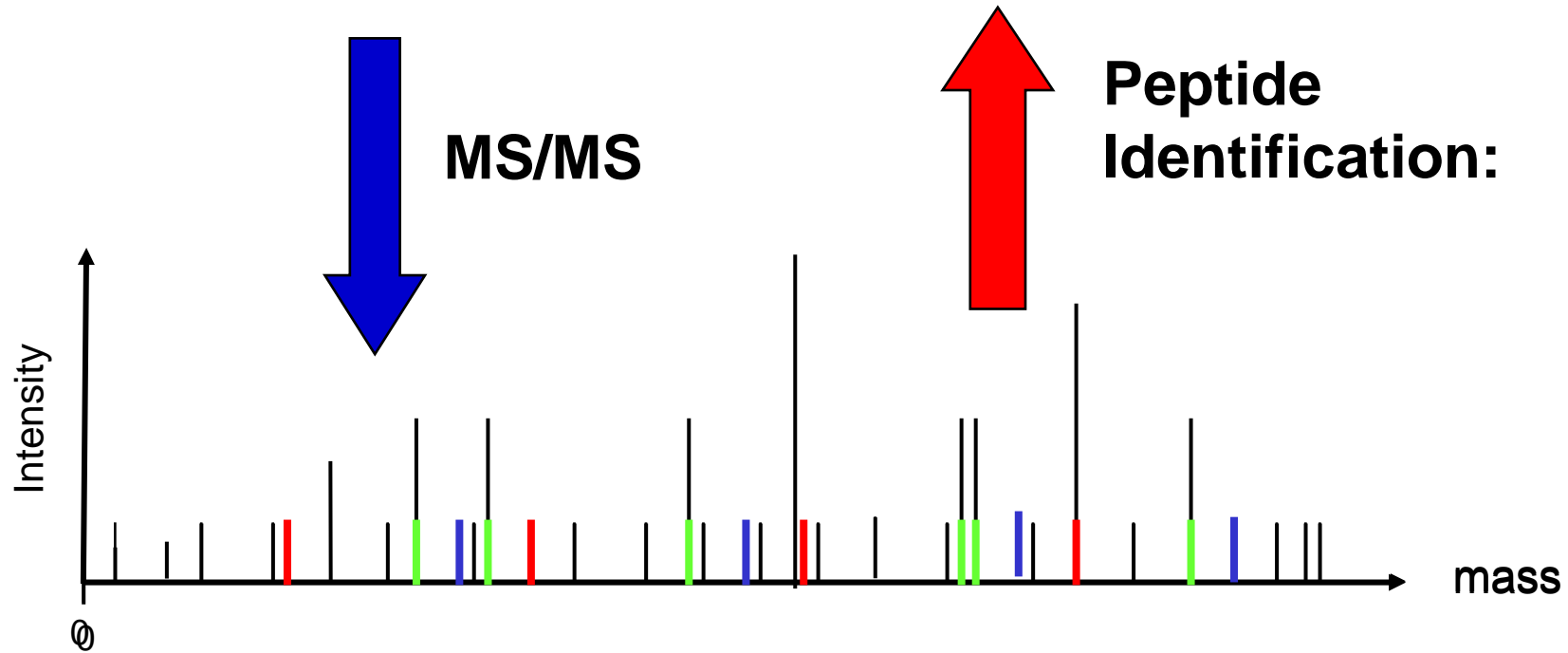
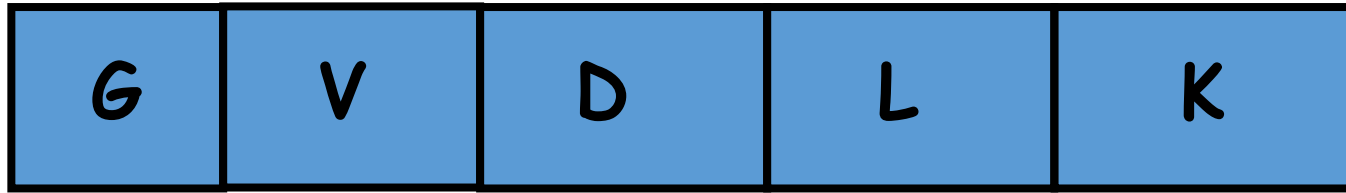


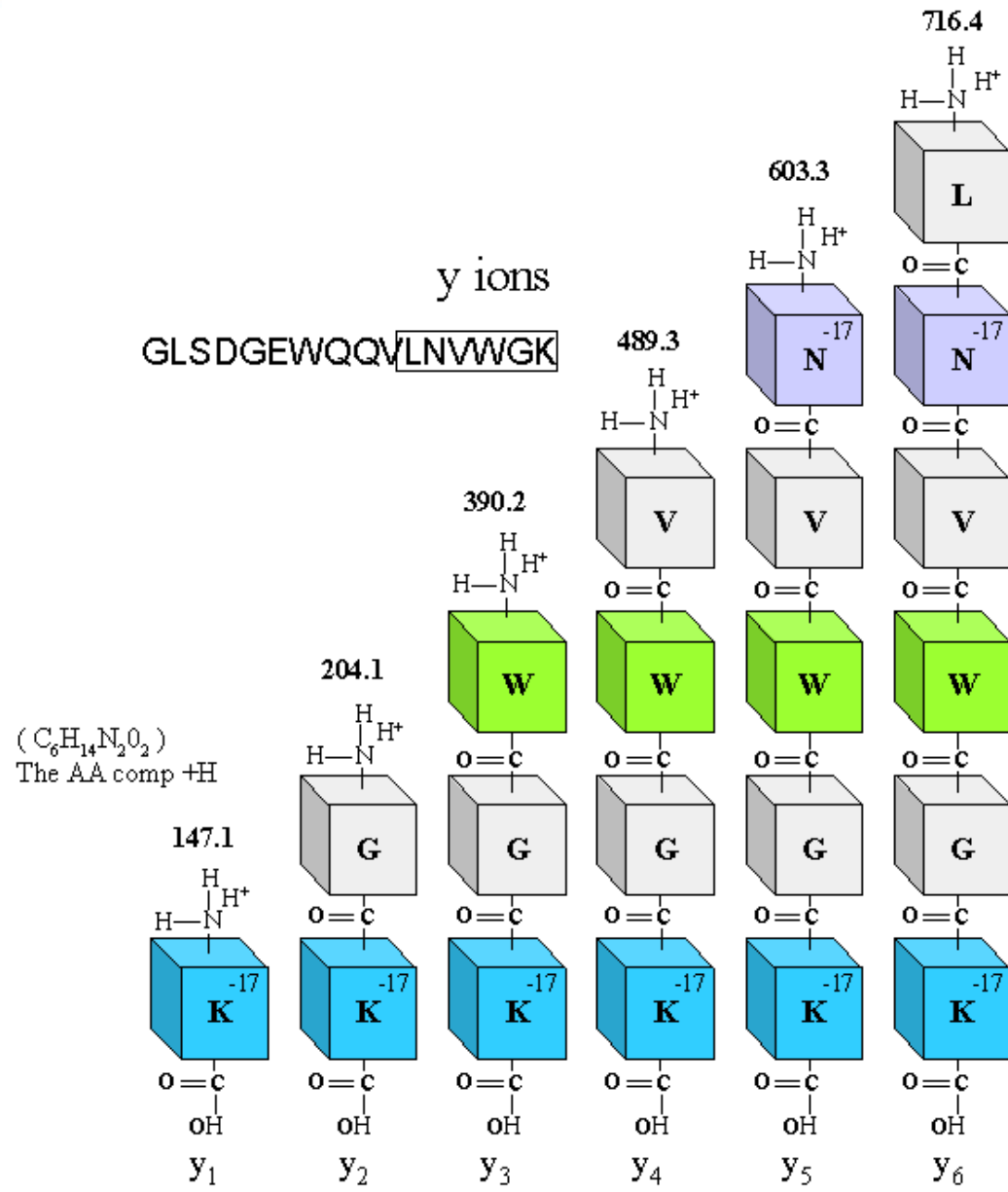
code	name	monoisotopic mass (Da)
G	glycine	57.021464
A	alanine	71.037114
S	serine	87.032028
P	proline	97.052764
V	valine	99.068414
T	threonine	101.047678
C	cysteine	103.009184
I	isoleucine	113.084064
L	leucine	113.084064
N	asparagine	114.042927
D	aspartic acid	115.026943
Q	glutamine	128.058578
K	lysine	128.094963
E	glutamic acid	129.042593
M	methionine	131.040485
H	histidine	137.058912
F	phenylalanine	147.068414
R	arginine	156.101111
Y	tyrosine	163.063329
W	tryptophan	186.079313
	carboxymethylated cysteine	161.051049
	carbamidated cysteine	160.030648
	oxidised methionine	147.035399



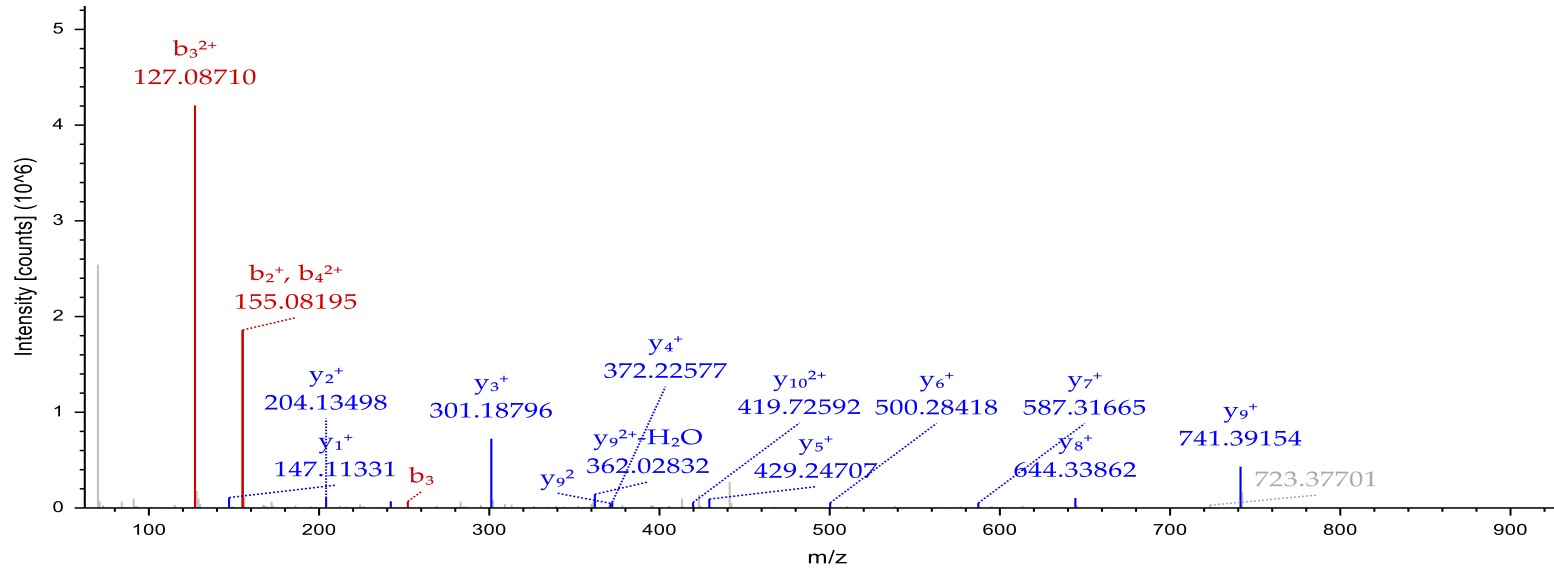
High resolution MS





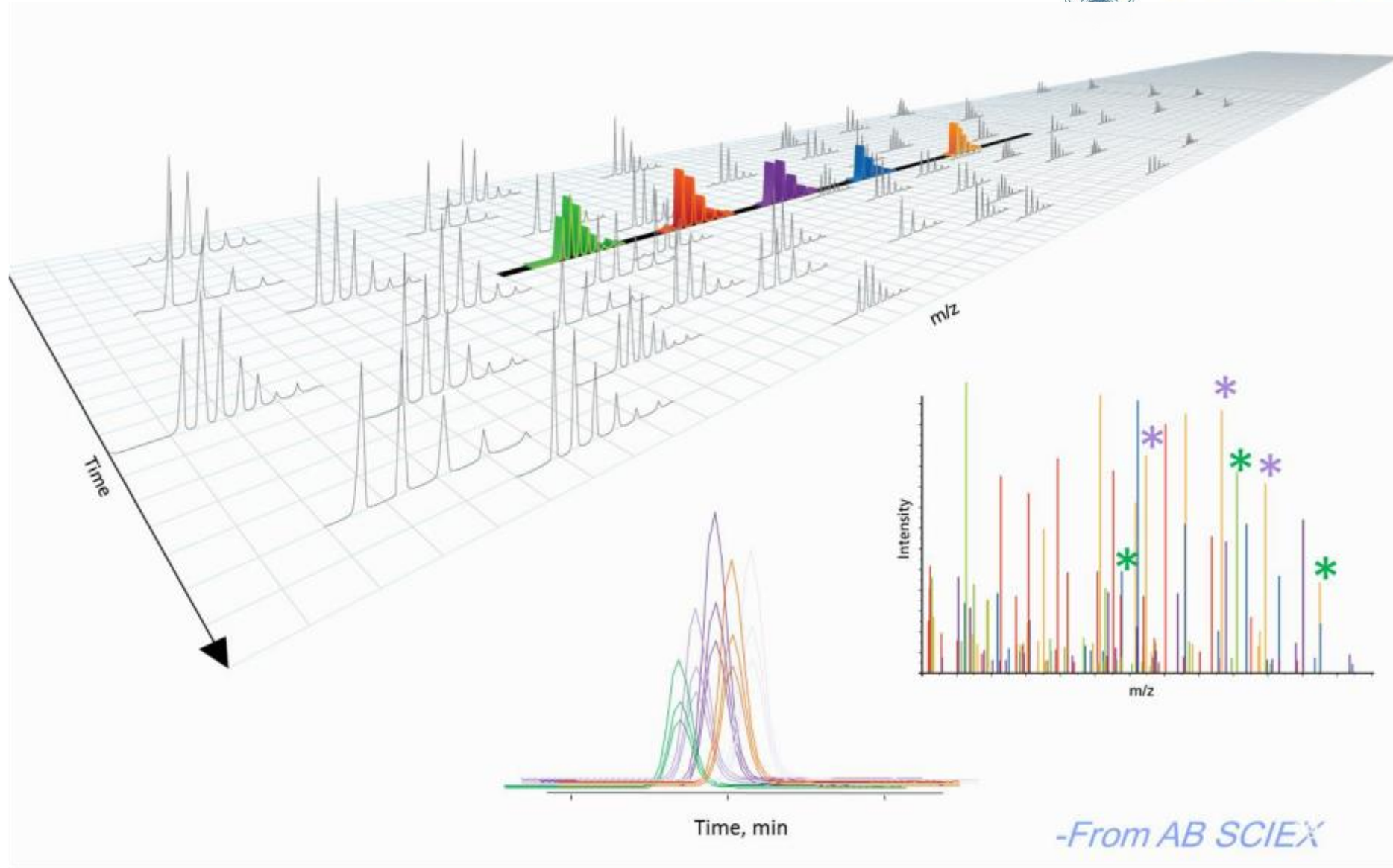


por100msms.raw #1309 RT: 12.2834 min
 FTMS, 448.2353@hcd27.00, z=+2, Mono m/z=448.23534 Da, MH+=895.46340 Da, Match Tol.=0.6 Da



#1	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	#2
1	58,02874	29,51801	G			11
2	155,0815	78,04439	P	838,44174	419,72451	10
3	252,13427	126,57077	P	741,38898	371,19813	9
4	309,15573	155,0815	G	644,33621	322,67175	8
5	396,18776	198,59752	S	587,31475	294,16101	7
6	467,22487	234,11608	A	500,28272	250,645	6
7	524,24634	262,62681	G	429,24561	215,12644	5
8	595,28345	298,14536	A	372,22415	186,61571	4
9	692,33621	346,67175	P	301,18703	151,09715	3
10	749,35768	375,18248	G	204,13427	102,57077	2
11			K	147,1128	74,06004	1

GPPGSAGAPGK



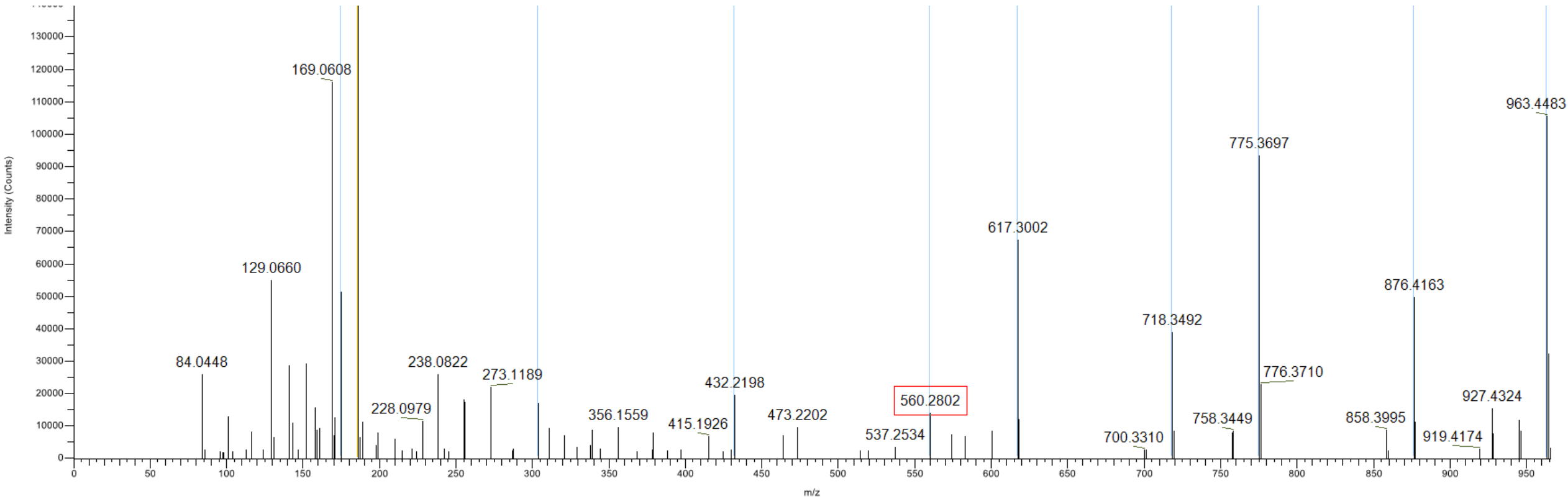


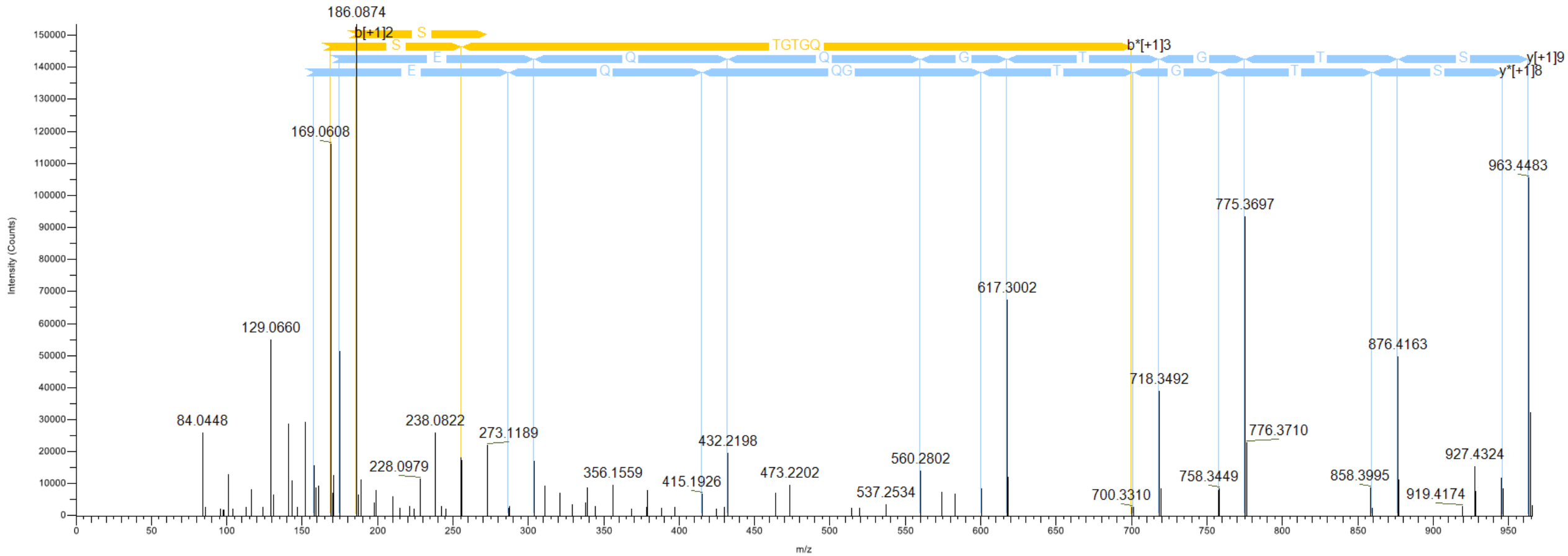
Identifikasi peptida



Proteins	Peptides	Search Input	Result Filters	Peptide Confidence	Search Summary		
	A2	Sequence	Protein Group Accessions	Modifications	pRS Probability	pRS Site Probabilities	
+	<input type="checkbox"/>	DLWtAPEHLR	P25092	T4(Phospho)	100.0 %	T(4): 100.0	
+	<input type="checkbox"/>	sVADELK	Q9C0D7	S1(Phospho)	100.0 %	S(1): 100.0	
+	<input type="checkbox"/>	NVPFsELNLK	Q14966	S5(Phospho)	100.0 %	S(5): 100.0	
+	<input type="checkbox"/>	AEEDEILNRsPR	P27824	S10(Phospho)	100.0 %	S(10): 100.0	
+	<input type="checkbox"/>	EVVWNVDDDGVIIsLNFEcDK	P52179	S13(Phospho); C18(Carba...	100.0 %	S(13): 100.0	
+	<input type="checkbox"/>	KQsLGELIGTLNAAK	P60174	S3(Phospho)	100.0 %	S(3): 100.0; T(10): 0.0	
+	<input type="checkbox"/>	LPLsLDLPsPRPFALPLGsPR	Q3MIN7	S4(Phospho); S9(Phospho)...	100.0 %	S(4): 100.0; S(9): 100.0; S(19): 100.0	
+	<input type="checkbox"/>	AQtPPGPSLSGSK	Q9UQ35	T3(Phospho)	99.9 %	T(3): 99.9; S(8): 0.0; S(10): 0.0; S(12): 0.0	
+	<input type="checkbox"/>	QLsSGVSEIR	P04792	S3(Phospho)	99.3 %	S(3): 99.3; S(4): 0.7; S(7): 0.0	
+	<input type="checkbox"/>	NATVGQsVLNIK	P28328	S7(Phospho)	98.9 %	T(3): 1.1; S(7): 98.9	
+	<input type="checkbox"/>	EsEVAHVdQLFDDASR	Q15047	S2(Phospho)	98.3 %	S(2): 98.3; S(15): 1.7	
+	<input type="checkbox"/>	LGDVsPTQIDVsQFGSFK	P46821	S5(Phospho)	96.6 %	S(5): 96.6; T(7): 3.4; S(12): 0.0; S(16): 0.0	
+	<input type="checkbox"/>	ESVPEFPLsPPK	P16949	S9(Phospho)	94.4 %	S(2): 5.6; S(9): 94.4	
+	<input type="checkbox"/>	LTPVsPESSSTEEK	Q13501	S5(Phospho)	94.2 %	T(2): 5.8; S(5): 94.2; S(8): 0.0; S(9): 0.0; S(10): 0.0; T(11): 0.0	

MS/MS







b	58.02875	186.08733	273.11936	374.16704	431.18851	532.23619	589.25766	717.31624	845.37482	974.41742	1130.51854
b*(+·	41.00220	169.06078	256.09281	357.14049	414.16196	515.20964	572.23111	700.28969	828.34827	957.39087	1113.49199
b°(+·	40.01818	168.07676	255.10879	356.15647	413.17794	514.22562	571.24709	699.30567	827.36425	956.40685	1112.50797
y	1091.50763	963.44905	876.41702	775.36934	718.34787	617.30019	560.27872	432.22014	304.16156	175.11896	1148.52910
y*(+·	1074.48108	946.42250	859.39047	758.34279	701.32132	600.27364	543.25217	415.19359	287.13501	158.09241	1131.50255
v°(+·	1073.49707	945.43849	858.40646	757.35878	700.33731	599.28963	542.26816	414.20958	286.15100	157.10840	1130.51854



GQSTGTGQQER

Peptida Biomarker Daging Babi

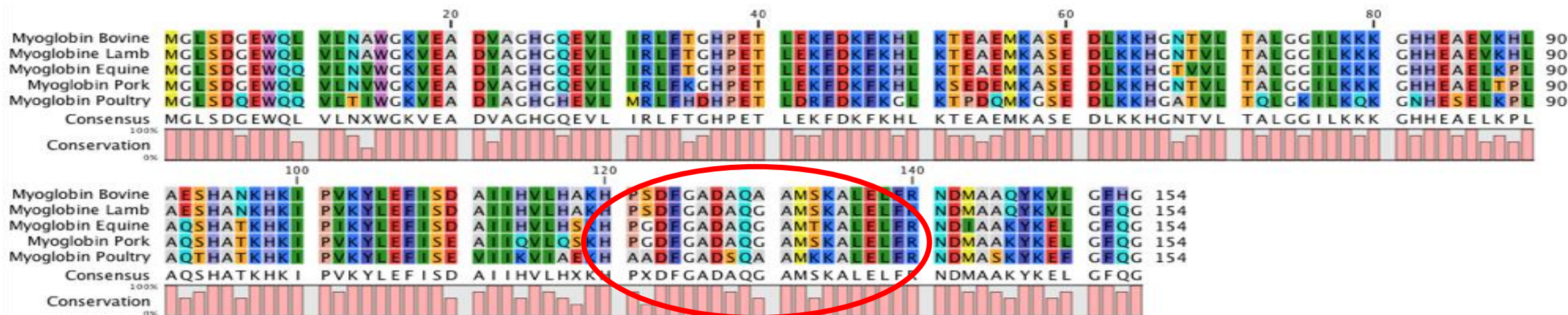
Protein target di daging



Myoglobin

Myosin

β -Hemoglobin



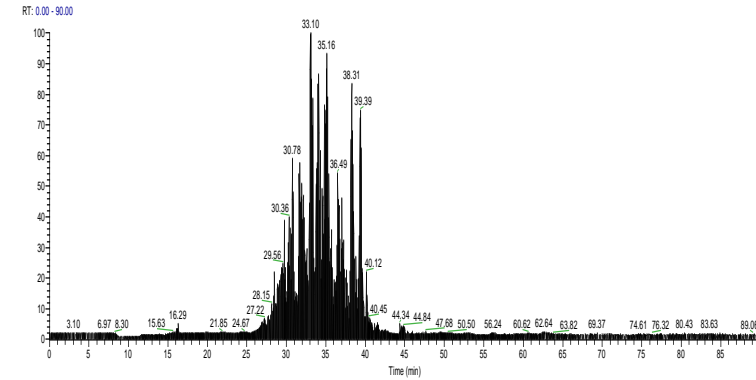
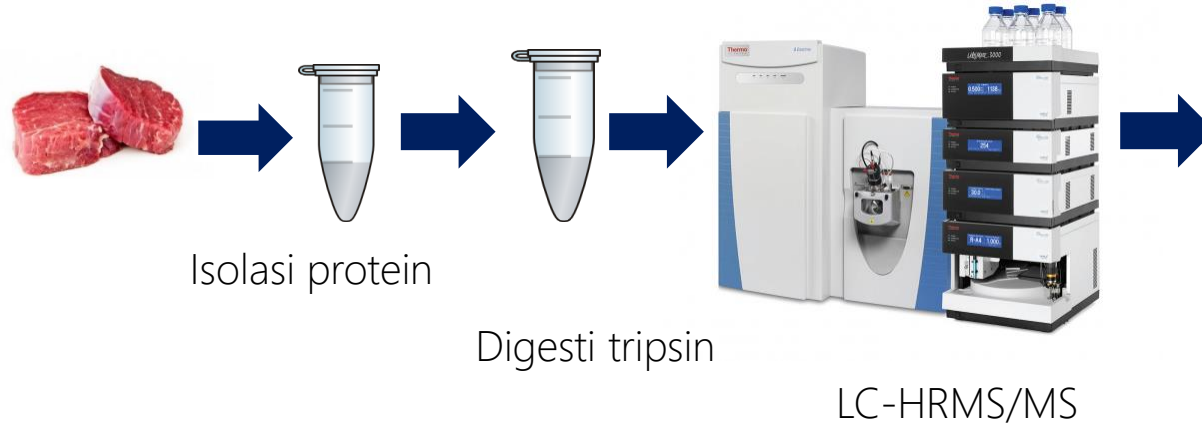
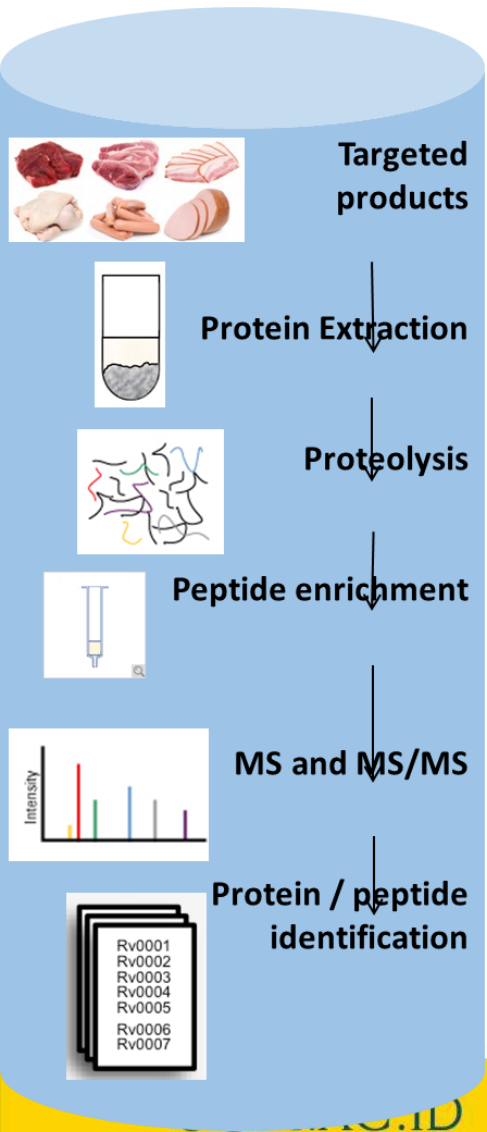
Myoglobin

In silico tryptic digestion

	120	134	<i>m/z</i> teoritis (z=2)
Beef	↓	↓	766,8435
Horse	HP SDFGADAQAA MSK		751,8383
Pork	HP GDFGADAQGA MTK		744,8304
Lamb	HP SDFGADAQGA MSK		759,8357



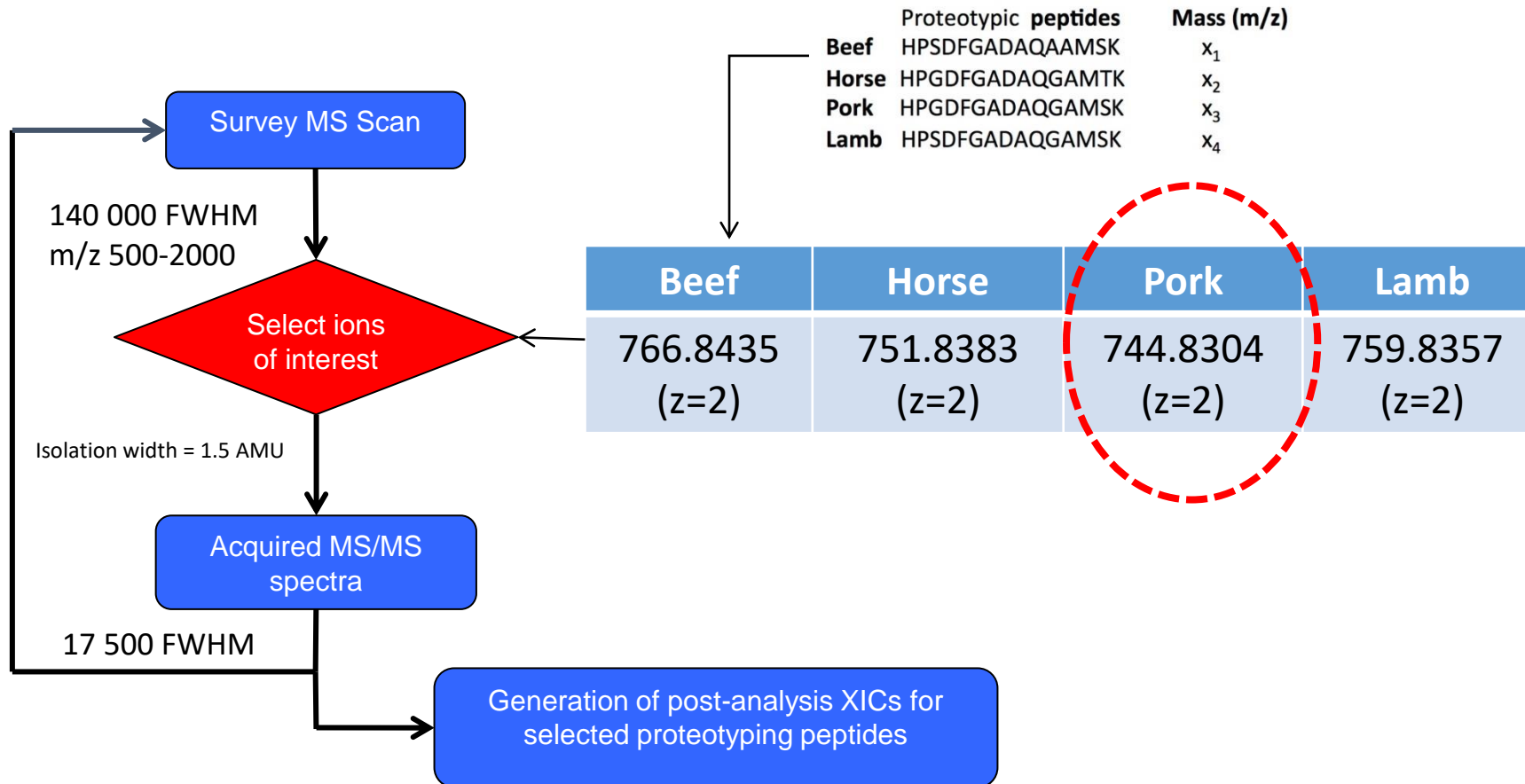
Experimental



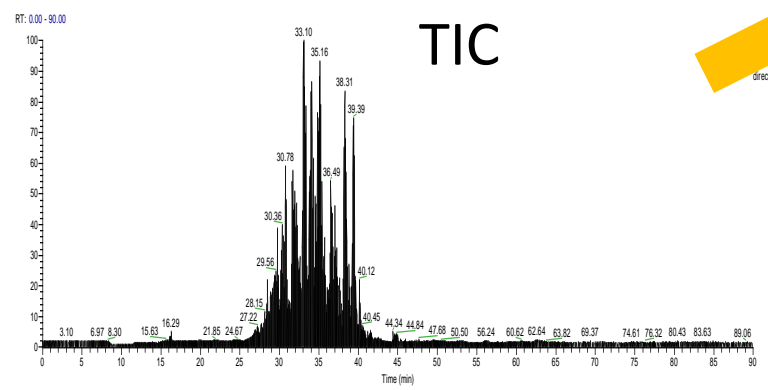
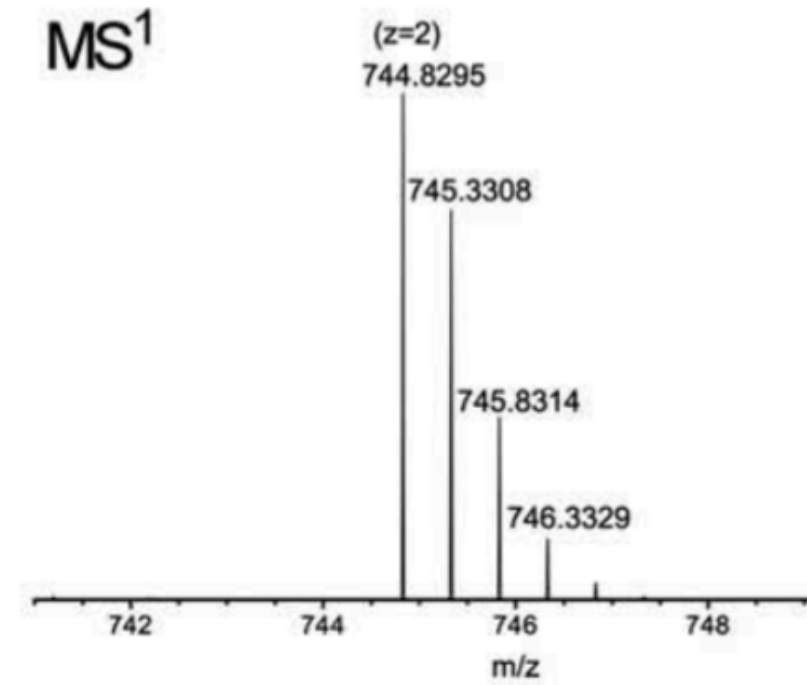
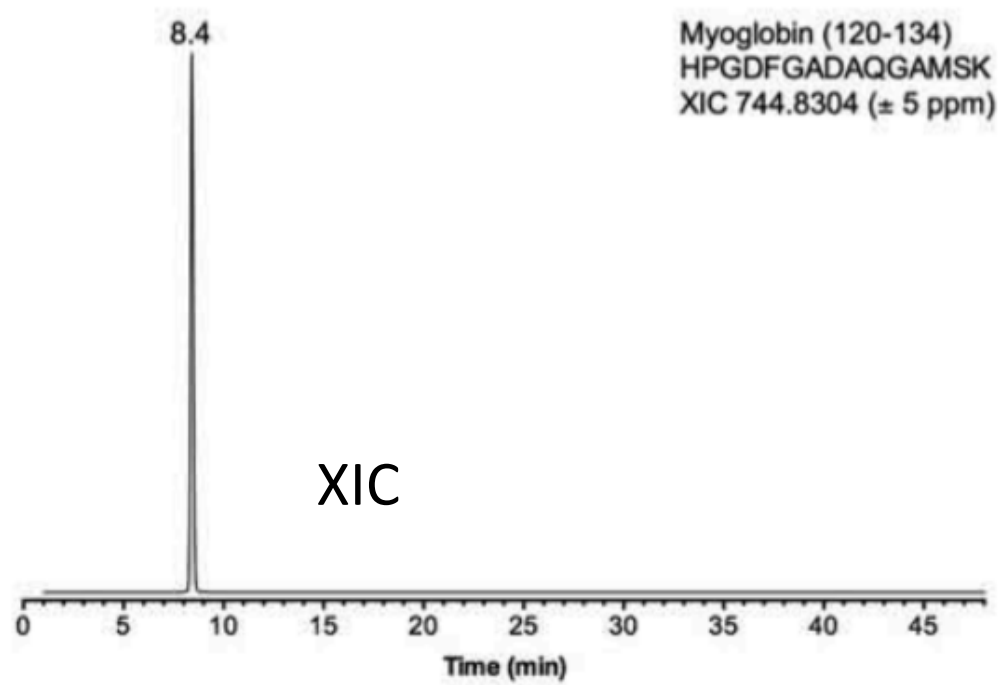
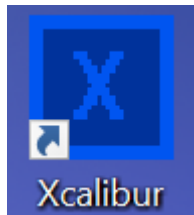
TIC (raw data)



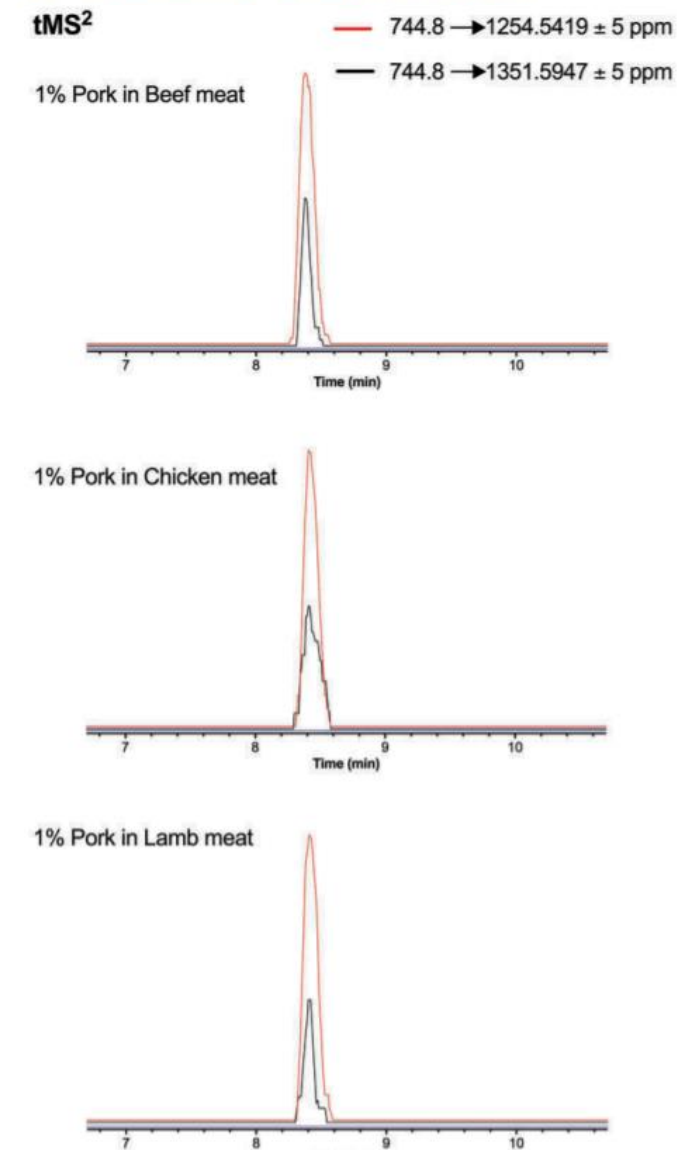
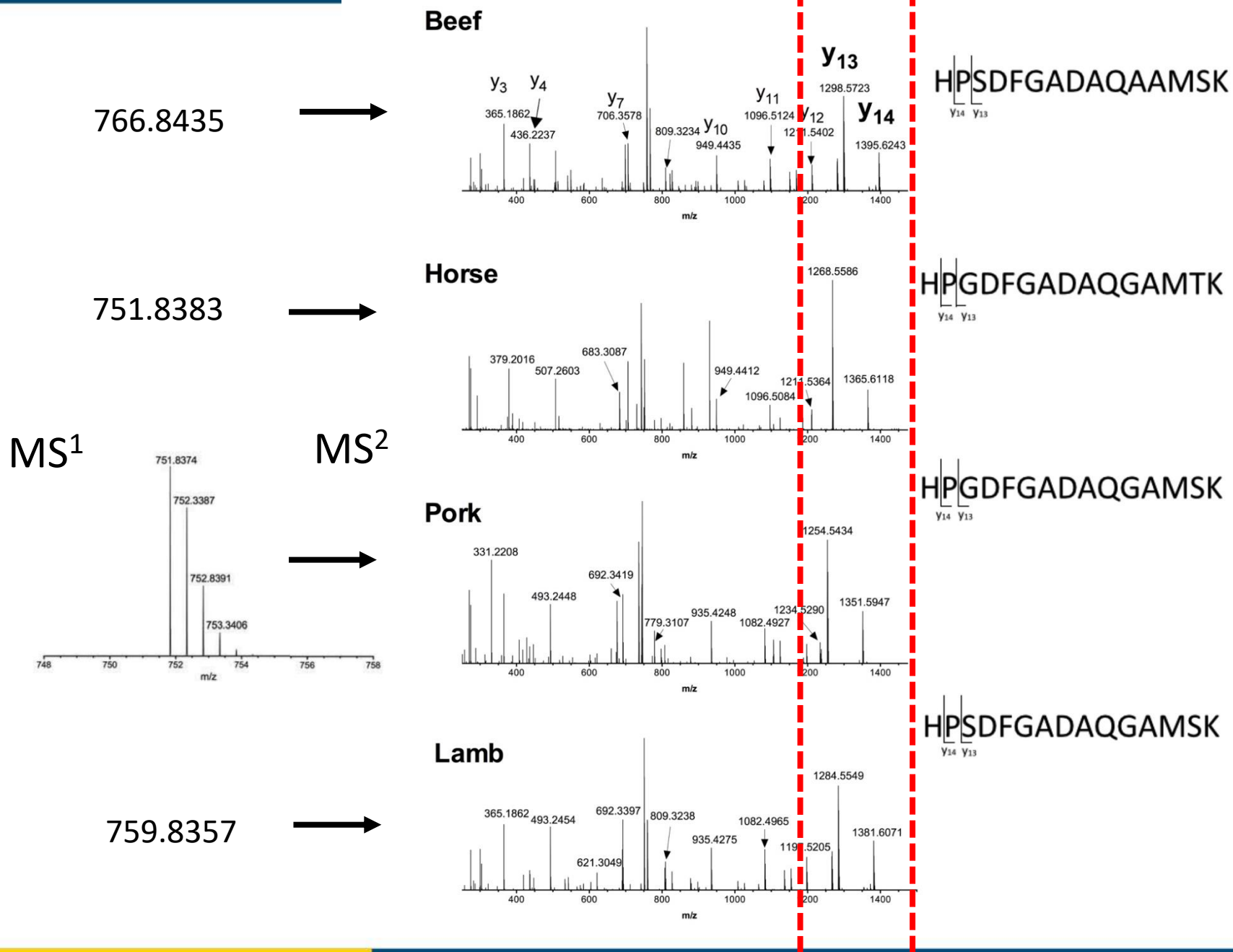
Ekstraksi ion dari TIC (XIC)



Extracted ion chromatograms with $m_n \pm 5$ ppm



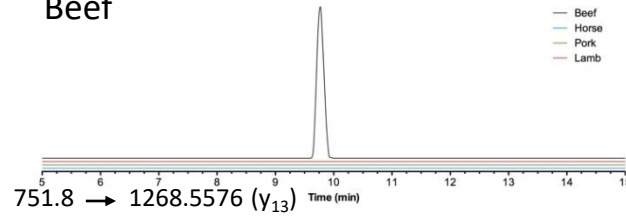
Orduna, et al, 2017 <https://doi.org/10.1080/19440049.2017.1329951>



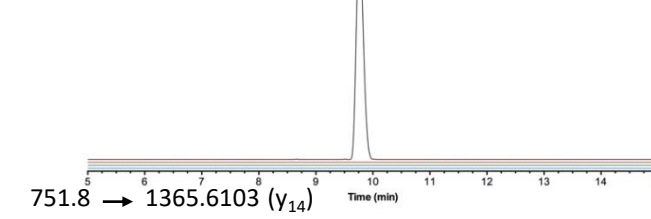


766.8 → 1298.5681 (y_{13})

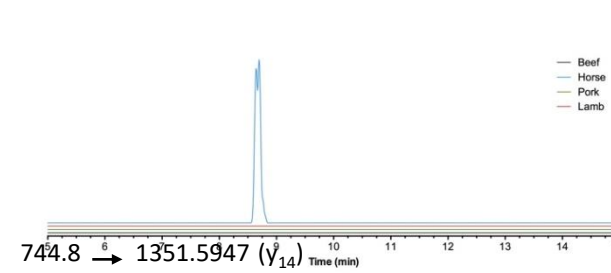
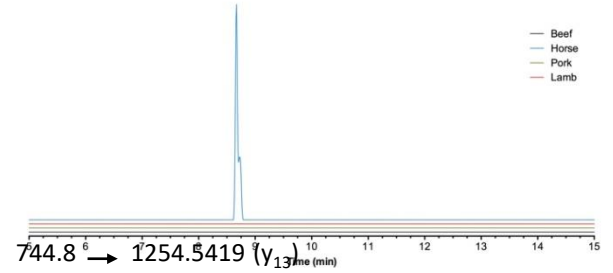
Beef



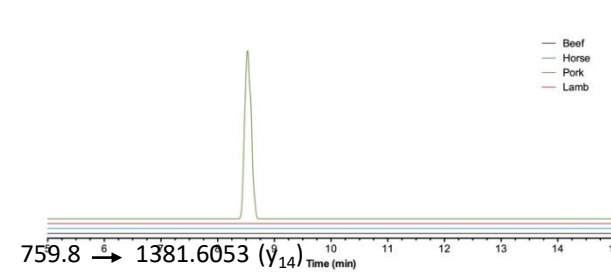
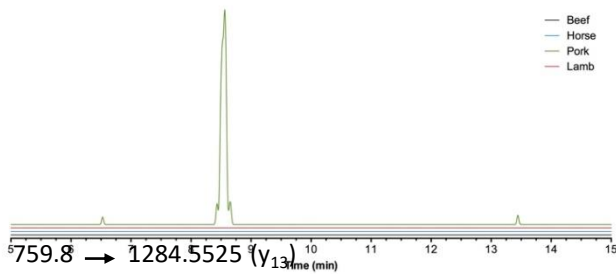
766.8 → 1395.6209 (y_{14})



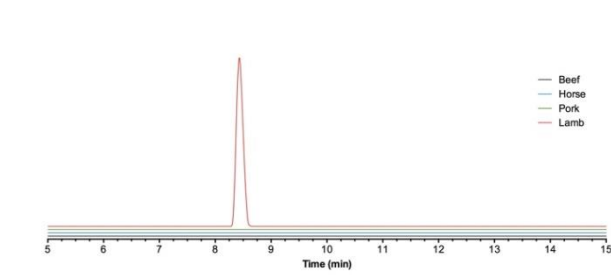
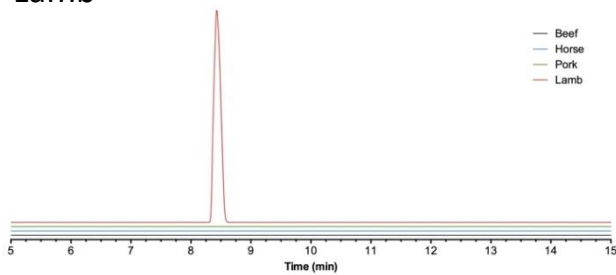
Horse



Pork



Lamb

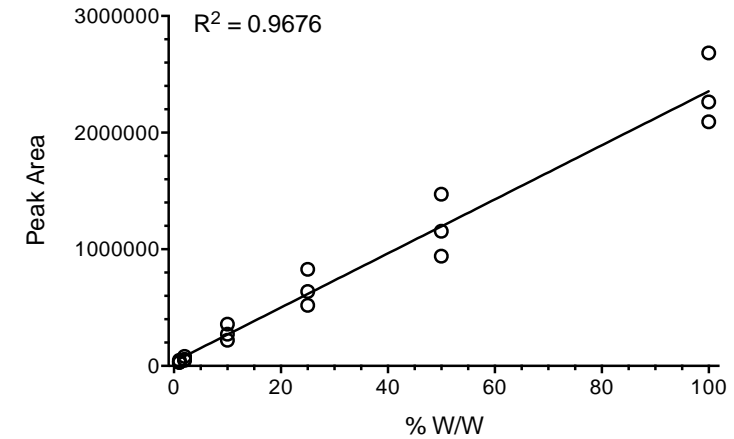
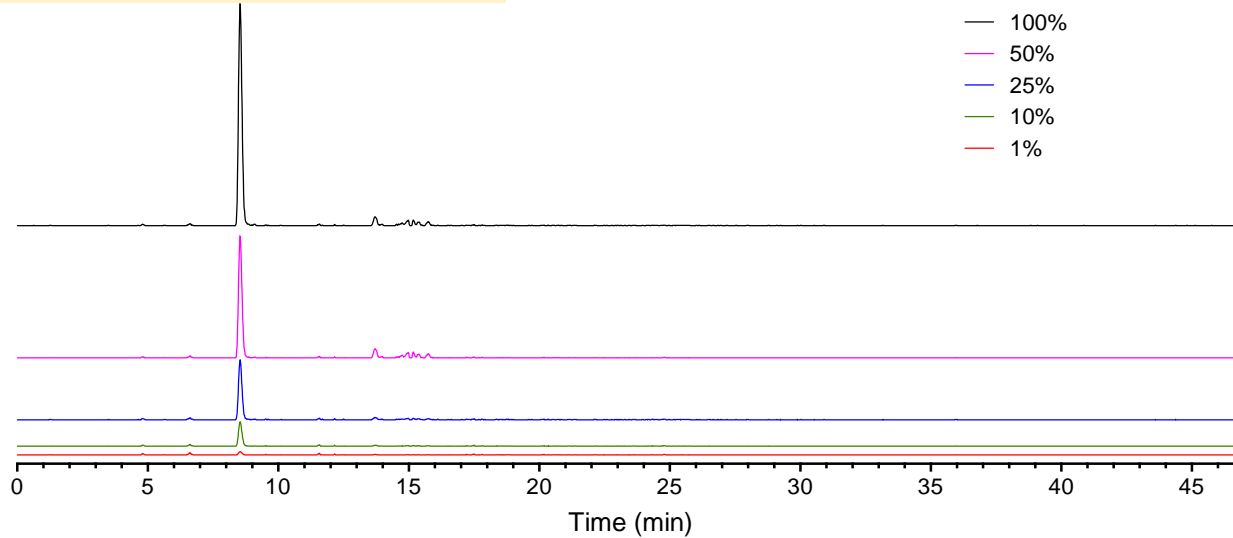


XIC spesifik dapat digunakan untuk membedakan antar spesies



Kuantifikasi dengan XIC m/z 744.8304 \pm 5 ppm ($z=2$)

Peptida myoglobin babi



Peak area XIC vs konsentrasi



Species	Protein	Peptide sequence	AA position	Theoretical mass (z = 2)	Observed mass (z = 2)
Pork	Myoglobin	HPGDFGADAQGAMSK	120–140	744.8304	744.8295
Horse	Myoglobin	HPGDFGADAQGAMTK	120–140	751.8383	751.8374
Beef	Myoglobin	HPSDFGADAQAAMSK	120–140	766.8435	766.8447
Lamb	Myoglobin	HPSDFGADAQGAMSK	120–140	759.8357	759.8368
Chicken	Myoglobin	HAADFGADSQAAMK	120–139	710.3197	710.3199
Pork	Myosin-1	TLAFLFTGAAGADAEAGGGK	619–638	912.9600	912.9593
Horse	Myosin-1	TLALLFSGPASADAEAGGK	619–637	888.4623	888.4612
Beef	Myosin-1	TLALLFSGPASGEAEGGPK	619–637	901.4702	901.4718
Lamb	Myosin-1	TLAFLFSGAASAEAEAGGGAK	619–638	927.9652	927.9648
Chicken	Myosin-1	TLALLFASAGGEPEASGGGGK	618–639	945.4838	945.4831
Pork	Myosin-2	TLAFLFSGAQTGEAEAGGTK	619–638	978.4891	978.4884
Horse	Myosin-2	TLALLFSGAQTADAEAGGVK	617–636	960.5073	960.5061
Beef	Myosin-2	TLAFLFSGTPTGDSEASGGTK	619–639	1022.4971	1022.4968
Lamb	Myosin-2	TLALLFSGTPTAESESGGTK	617–636	984.0020	984.0026
Chicken	Myosin-2	TLALLFANYGGAEAEASGGGGGGK	618–642	1084.5346	1084.5359
Pork	β -Haemoglobin	FFESFGDLSNADAVMGNPK	42–60	1023.4673	1023.4665
Horse	β -Haemoglobin	FFDSFGDLSNPGAVMGNPK	42–60	1000.4646	1000.4635
Beef	β -Haemoglobin	FFESFGDLSTADAVMNNPK	40–58	1045.4804	1045.4794
Lamb	β -Haemoglobin	FFEHFGDLSNADAVMNNPK	40–58	1076.9915	1076.9905
Chicken	β -Haemoglobin	FFASFGNLSSPTAILGNPMVR	42–62	1113.5724	1113.5729

Peptida Biomarker Gelatin



Protein target gelatin

α 1- Kolagen

α 2- Kolagen



- $\alpha 1$

BOVINE
Porcine

```
MFSFVDLRLLLLLAATALLTHGQEEGQEEG---QEEDIIPVTCVQNGRLRYHDRVWKPVP
MFSFVDLRLLLLLAATALLTHGQEEGQEEGQQGQEEEDIIPVTCVQNGRLRYHDRVWKPVP
*****
```

BOVINE
Porcine

```
CQICVCDNGNVLCDDVICDELKDCPNAKVPTDECCPVCPEGQESPTDQETTIGVEGPKGDT
CQICVCDNGNVLCDDVICDEIKNCPSARVPAGECCPVCPEGEVSPIDQETTIGVEGPKGDT
*****
```

BOVINE
Porcine

```
GPRGPRGPAGPPGRDGI PGQPGLPGPPGPPGPPGPPGLGGNFAPQLSYGYDEKSTGISVP
GPRGPRGPSGPPGRDGI PGQPGLPGPPGPPGPPGPPGLGGNFAPQLSYGYDEKSAGISVP
*****
```

- $\alpha 2$

PORCINE
BOVINE

```
MLSFVDTRILLLLAVTSCLATCQSLQEATARKGPTGDRGPRGERGPPGPPGRDGDGIPG
MLSFVDTRILLLLAVTSCLATCQSLQEATARKGPSGDRGPRGERGPPGPPGRDGDGIPG
*****
```

PORCINE
BOVINE

```
PPGPPGPPGPPGLGGNFAAQYDGKGVGAGPGPMGLMGRGPPGAVGAPGPQGFQGPAGEP
PPGPPGPPGPPGLGGNFAAQFDAKG--GGPGPMGLMGRGPPGASGAPGPQGFQGPPEP
*****
```

PORCINE
BOVINE

```
GEPGQTGPAGARGPPGPPGKAGEDGHPGKPRPGERGVVGPQGARGFFGTPLPGFKGIR
GEPGQTGPAGARGPPGPPGKAGEDGHPGKPRPGERGVVGPQGARGFFGTPLPGFKGIR
*****
```

Digesti trypsin *in silico*



α 1

bovine

porcine

mass	position	#MC	peptide sequence	mass	position	#MC	peptide sequence
1.987,9831	448-468	1	GEPGPTGIQGPPGPA GEEGK R	1.973,9675	451-471	1	GEPGPTGVQGPPGPA GEEGK R
1.975,9943	1062-1083	1	SGDRGETGPA GPA GPIGPV G AR	1.961,9787	1065-1086	1	SGDRGETGPA GPA GPV GPV G AR
1.834,8790	781-801	1	GEA GPSGPA GPTGARGA PGD R	1.864,8896	784-804	1	GETGPSGPA GPTGARGA PGD R
1.831,8820	448-467	0	GEPGPTGIQGPPGPA GEEGK	1.817,8664	451-470	0	GEPGPTGVQGPPGPA GEEGK
1.721,8313	352-369	1	GEGGPQGPRGSEGPQGV R	1.709,8313	355-372	1	GEA GPQGARGSEGPQGV R
1.696,7884	1014-1032	1	EGAPGA EGSPGRDGSPGAK	1.706,8092	1017-1035	1	EGAPGA EGSPGRDGAPGPK
1.672,8989	304-321	1	GLPGERGRPGA GPAGAR	1.698,9146	307-324	1	GLPGERGRPGPPGPA GAR
1.601,8254	115-131	2	GDTGPRGPRGPA GPPGR	1.617,8204	118-134	2	GDTGPRGPRGSPGPPGR
1.560,8128	1066-1083	0	GETGPA GPA GPIGPV GAR	1.546,7972	1069-1086	0	GETGPA GPA GPV GPV GAR
1.496,7525	171-186	0	STGISV PGPMGSPGPR	1.466,7420	174-189	0	SA GISV PGPMGSPGPR
1.406,7426	1310-1320	2	NWYISKNPKEK	1.392,7270	1313-1323	2	NWYISKNPDK
1.318,6862	505-519	1	GPA GERGAPGPA GPK	1.334,6811	508-522	1	GPA GERGSPGPA GPK
1.281,6181	781-795	0	GEA GPSGPA GPTGAR	1.311,6287	784-798	0	GETGPSGPA GPTGAR
1.063,5755	310-321	0	GRPGA GPAGAR	1.089,5911	313-324	0	GRPGPPGPA GAR
1.018,5540	121-131	1	GPRGPA GPPGR	1.034,5490	124-134	1	GPRGPSGPPGR

 α -2

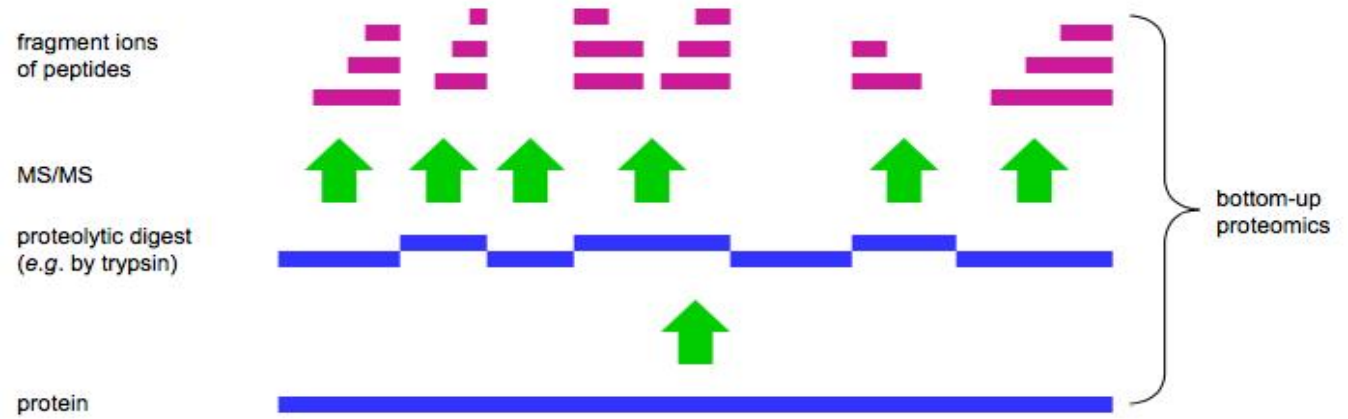
bovine

porcine

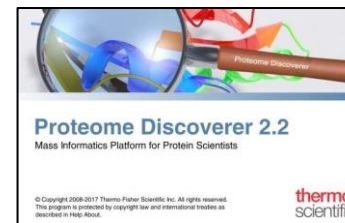
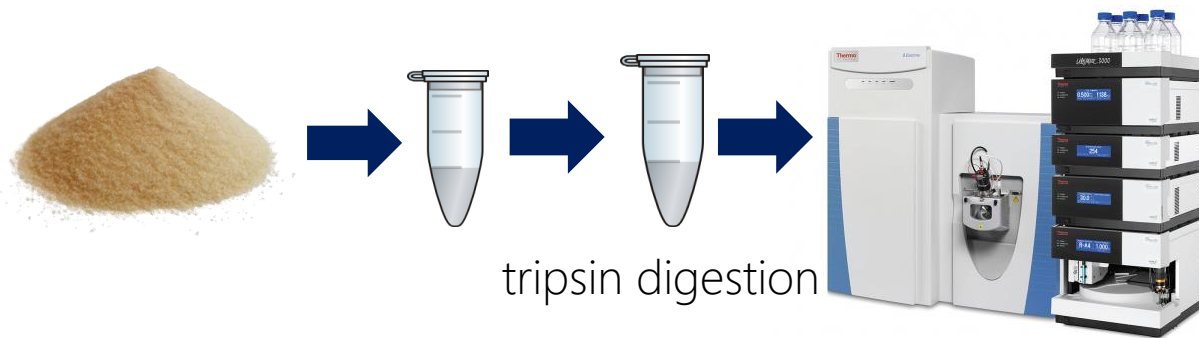
mass	position	#MC	peptide sequence	mass	position	#MC	peptide sequence
1.889,9576	497-517	2	GPSGDPGKA GEKGHAGLAGA R	1.946,9790	499-519	2	GPTGDPGKNGEKGHAGLAGA R
1.959,9518	359-379	1	GEPGAVGQPGPPGPSGEEGK R	1.931,9205	361-381	1	GEPGAA GPQPPGPSGEEGK R
1.920,0072	1122-1138	1	SPTSLRPKDYEVDA TLK	1.916,0123	1124-1140	1	SPPSLRPKDYEVDA TLK
1.944,9926	1204-1219	2	AQPEDIPVKNWY RNSK	1.915,9773	1206-1221	2	AQPENIPAKNWY RNSK
1.904,9321	670-691	1	DGARGAPGAI GAPGAPANG DR	1.890,9164	672-693	1	DGARGAPGAVGAPGAPANG DR
1.864,9187	485-504	1	GEPGNIGFPGPKGPSGDPGK	1.878,9344	487-506	1	GEPGNIGFPGPKGPTGDPGK
1.906,9365	587-607	1	GERGPPGESGAA GPTGPIGS R	1.876,9259	589-609	1	GERGPPGESGAA GPAGPIGS R
1.871,8379	925-943	1	DGNPGNDGPPGRDQQPGHK	1.818,8113	927-945	1	DGNPGSDGPPGRDQQAGHK
1.781,9180	793-813	0	TGPPGPSGISGPPGPPGPA G K	1.793,9544	795-815	0	IGPPGPSGISGPPGPPGPA G K
1.803,8507	359-378	0	GEPGAVGQPGPPGPSGEEGK	1.775,8194	361-380	0	GEPGAA GPQPPGPSGEEGK
1.772,9401	379-397	1	RGSTGEIGPAGPPGPPGLR	1.771,9197	381-399	1	RGPNGEVGSAGPPGPPGLR
1.751,8670	995-1012	2	GPSGPQGIRGDKGEPGDK	1.765,8827	997-1014	2	GPSGPQGIRGEKGEPGDK
1.737,9030	572-589	1	GIPGEFGLPGPAGARGER	1.763,9187	574-591	1	GIPGEFGLPGPAGPRGER
1.770,8841	656-673	2	GETGLRGGDIGSPGRDGAR	1.756,8684	658-675	2	GETGLRGDVGSPGRDGAR



Eksperimental



Genome annotation



- Protein spesifik spesies
- Peptida spesifik spesies

HRMS analysis
Thermo Exactive
Orbitrap

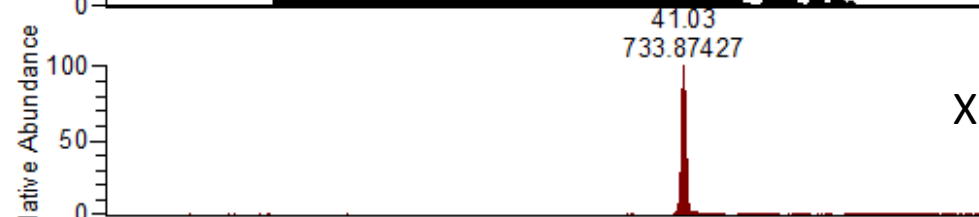


Sekuence	MH ⁺ (z=1) teori	MH ⁺ (z=1) terukur	z=2	z=3
SAGISVPGPMGPSGPR	1466,74202	1466,74190; $\Delta = - 0,08$	733.87459	489.58575

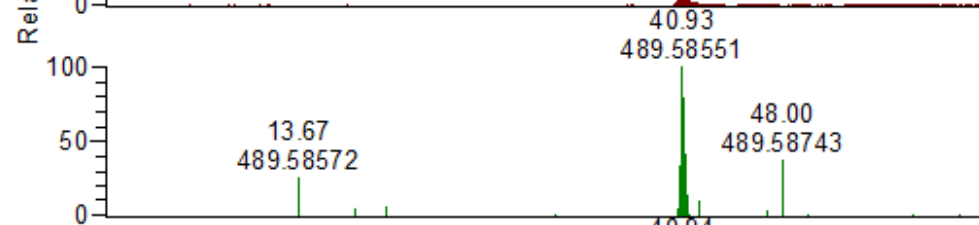
RT: 0.00 - 90.01 SM: 3G



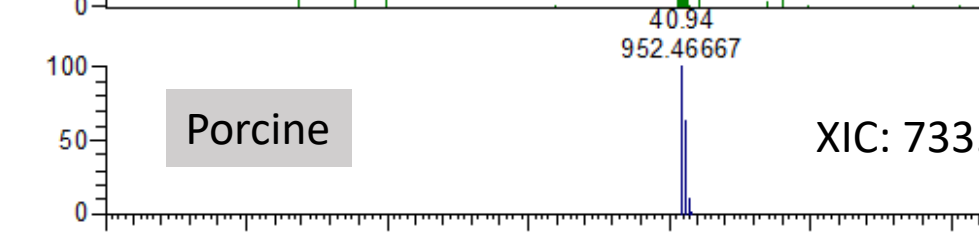
TIC



XIC: 733.87459

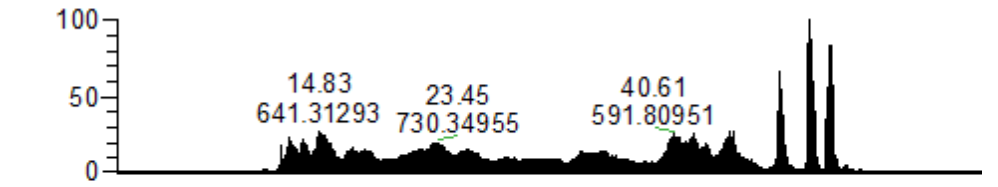


XIC: 489.58575



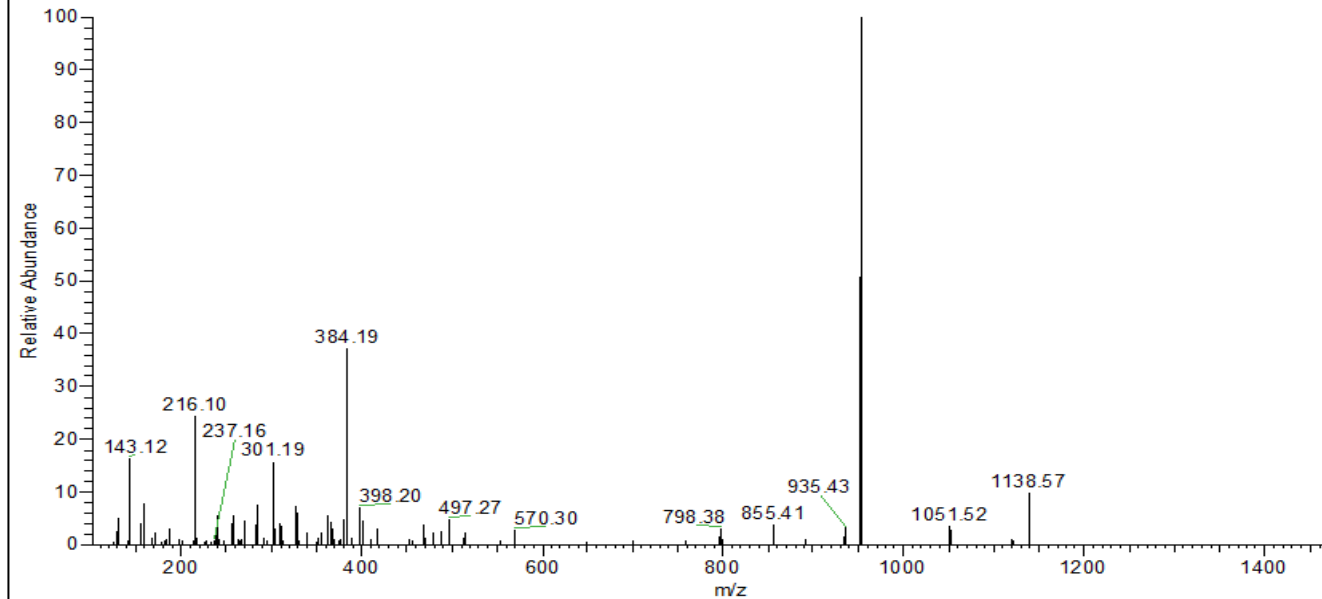
XIC: 733.87459 → 952.46667

RT: 0.00 - 90.00 SM: 3G

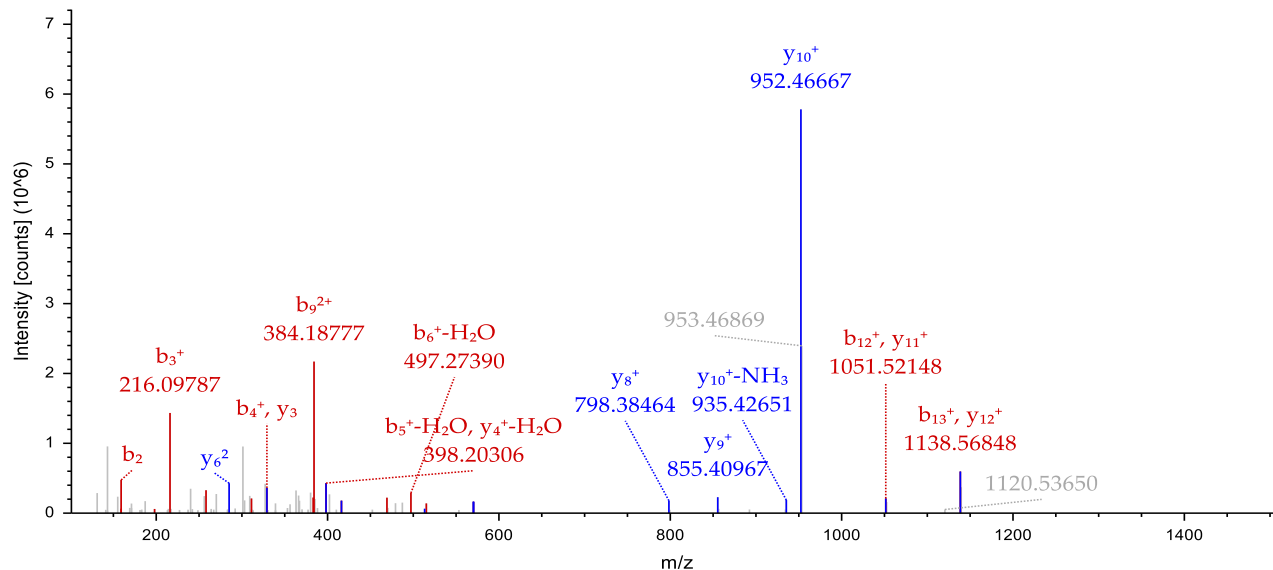


Bovine

por100msms (2) #12848 RT: 40.94 AV: 1 NL: 5.77E6
 F: FTMS +p NSI d Full ms2 733.8748@hcd27.00 [101.0000-1515.0000]



por100msms.raw #12848 RT: 40.9354 min
 FTMS, 733.8745@hcd27.00, z=+2, Mono m/z=733.87447 Da, MH+=1466.74166 Da, Match Tol.=0.6 Da



UNIVERSITAS GADJAH MADA

Konfirmasi MS²

SAGISVPGPMGPGPR

No	b ⁺	b ²⁺	Seq.	y ⁺	y ²⁺	No
1	88,0393	44,52329	S			16
2	159,07642	80,04185	A	1379,71	690,3586	15
3	216,09788	108,5526	G	1308,6729	654,8401	14
4	329,18195	165,0946	I	1251,6514	626,3294	13
5	416,21397	208,6106	S	1138,5674	569,7873	12
6	515,28239	258,1448	V	1051,5353	526,2713	11
7	612,33515	306,6712	P	952,46691	476,7371	10
8	669,35662	335,182	G	855,41415	428,2107	9
9	766,40938	383,7083	P	798,39268	399,7	8
10	897,44986	449,2286	M	701,33992	351,1736	7
11	954,47133	477,7393	G	570,29944	285,6534	6
12	1051,5241	526,2657	P	513,27797	257,1426	5
13	1138,5561	569,7817	S	416,22521	208,6162	4
14	1195,5776	598,2924	G	329,19318	165,1002	3
15	1292,6304	646,8188	P	272,17172	136,5895	2
16			R	175,11895	88,06311	1

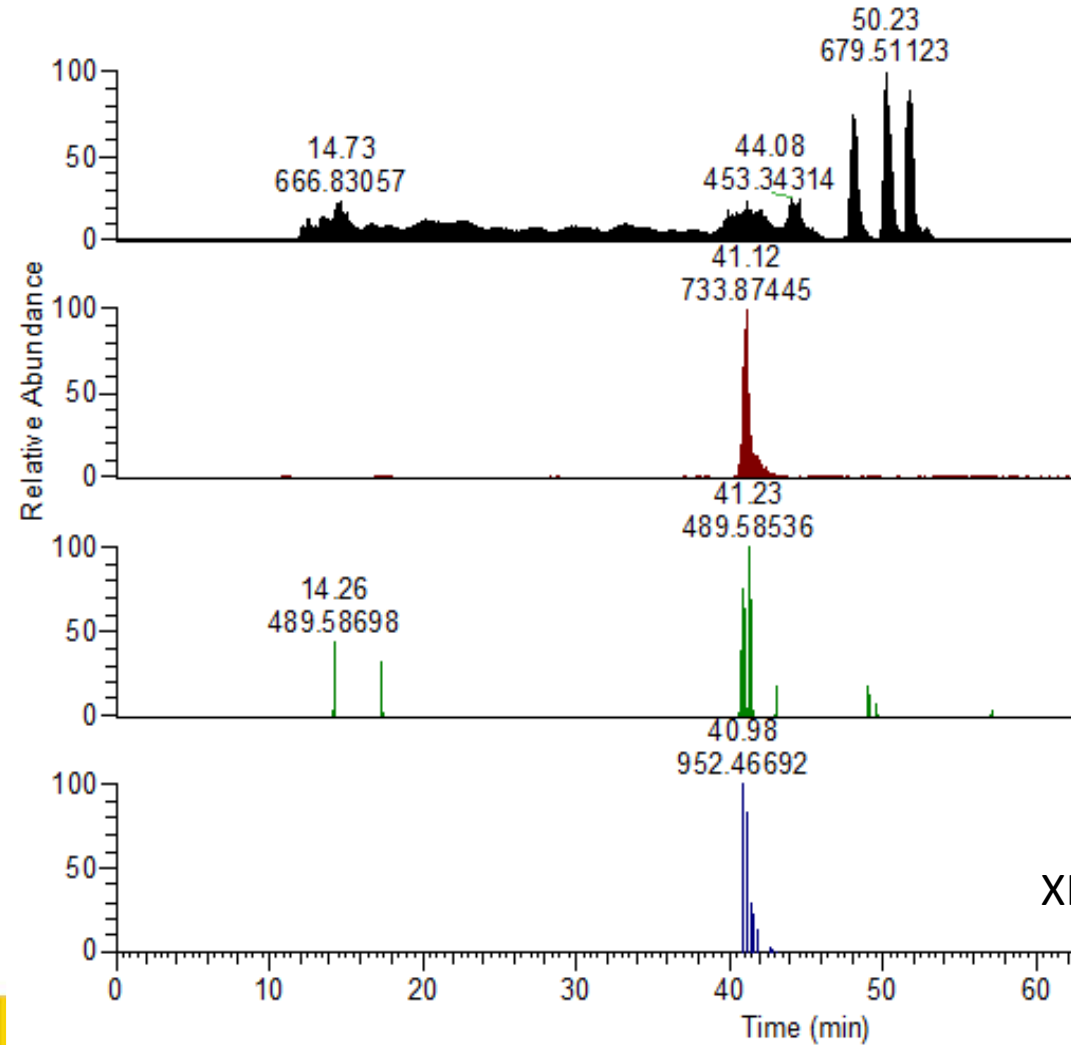


Porcine 50%

Porcine 25%

RT: 0.00 - 90.00 SM: 3G

RT: 0.00 - 90.01 SM: 3G

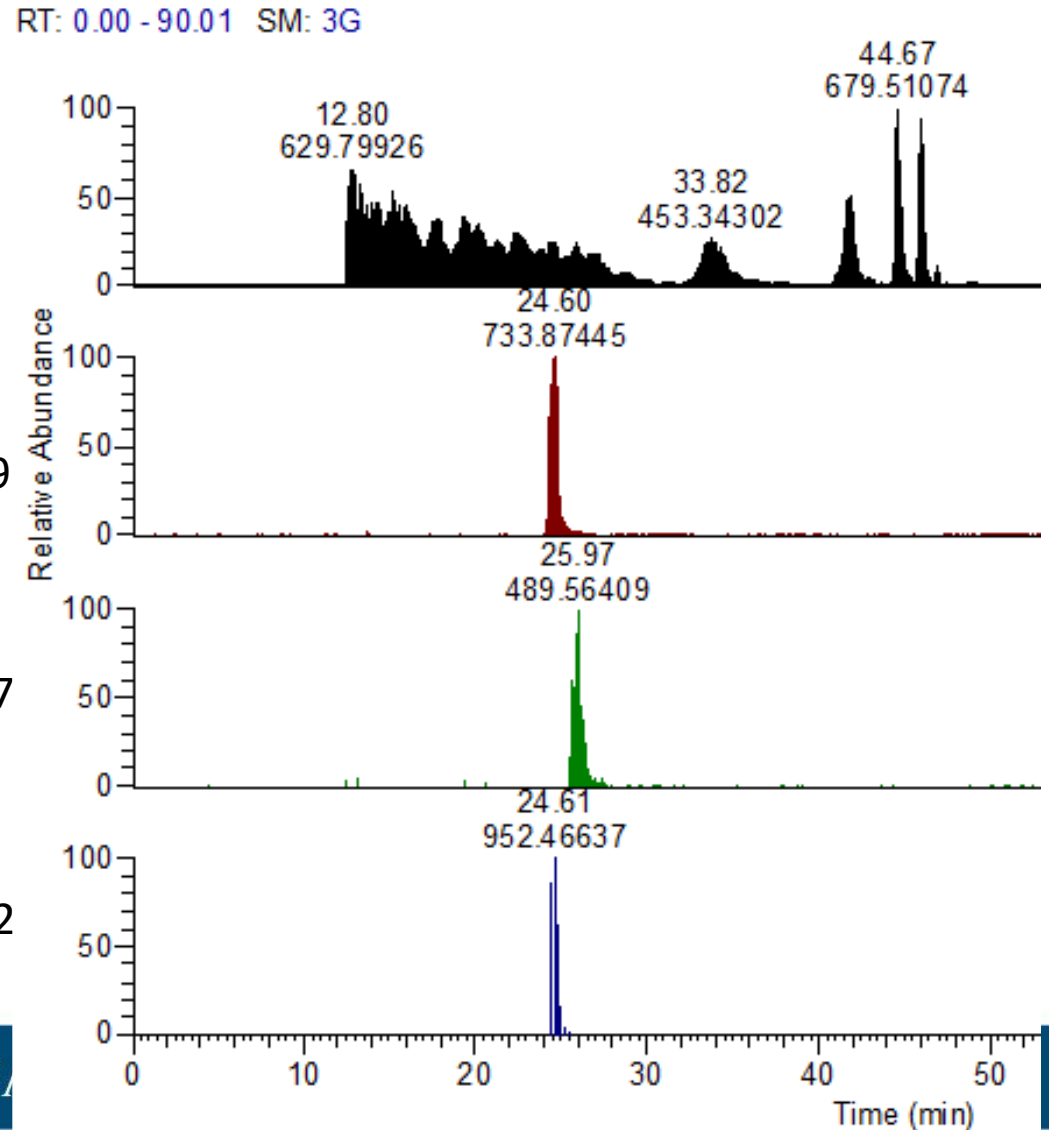


TIC

XIC: 733.87459

XIC: 489.5857

XIC: 733.87459 → 952



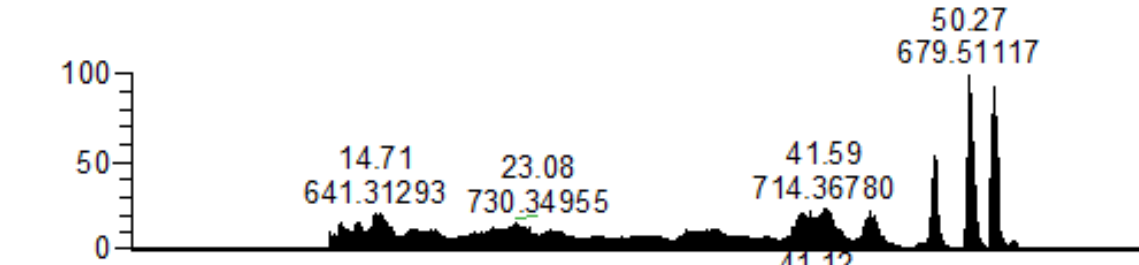


Porcine 10%

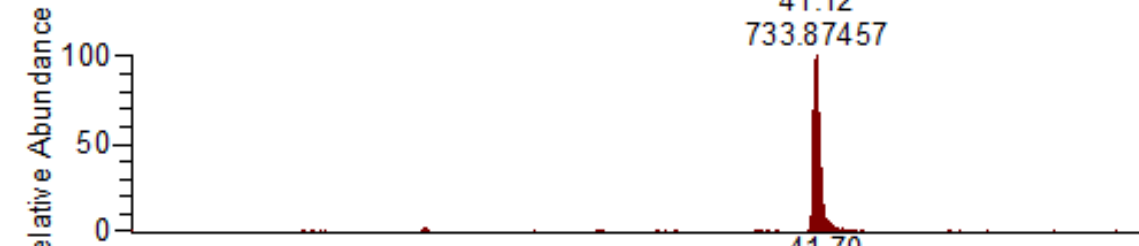
Porcine 5%

RT: 0.00 - 90.00 SM: 3G

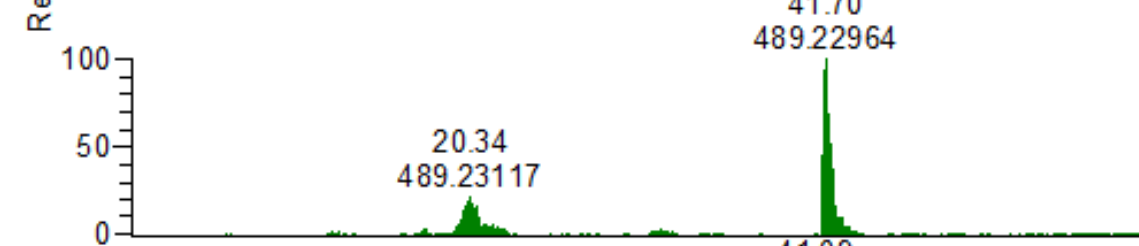
RT: 0.00 - 90.00 SM: 3G



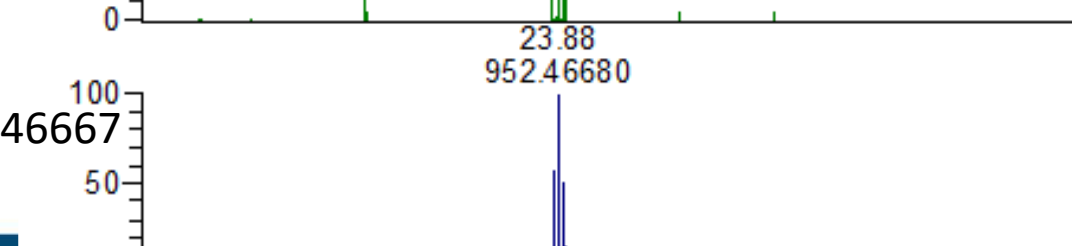
TIC



XIC: 733.87459



XIC: 489.58575



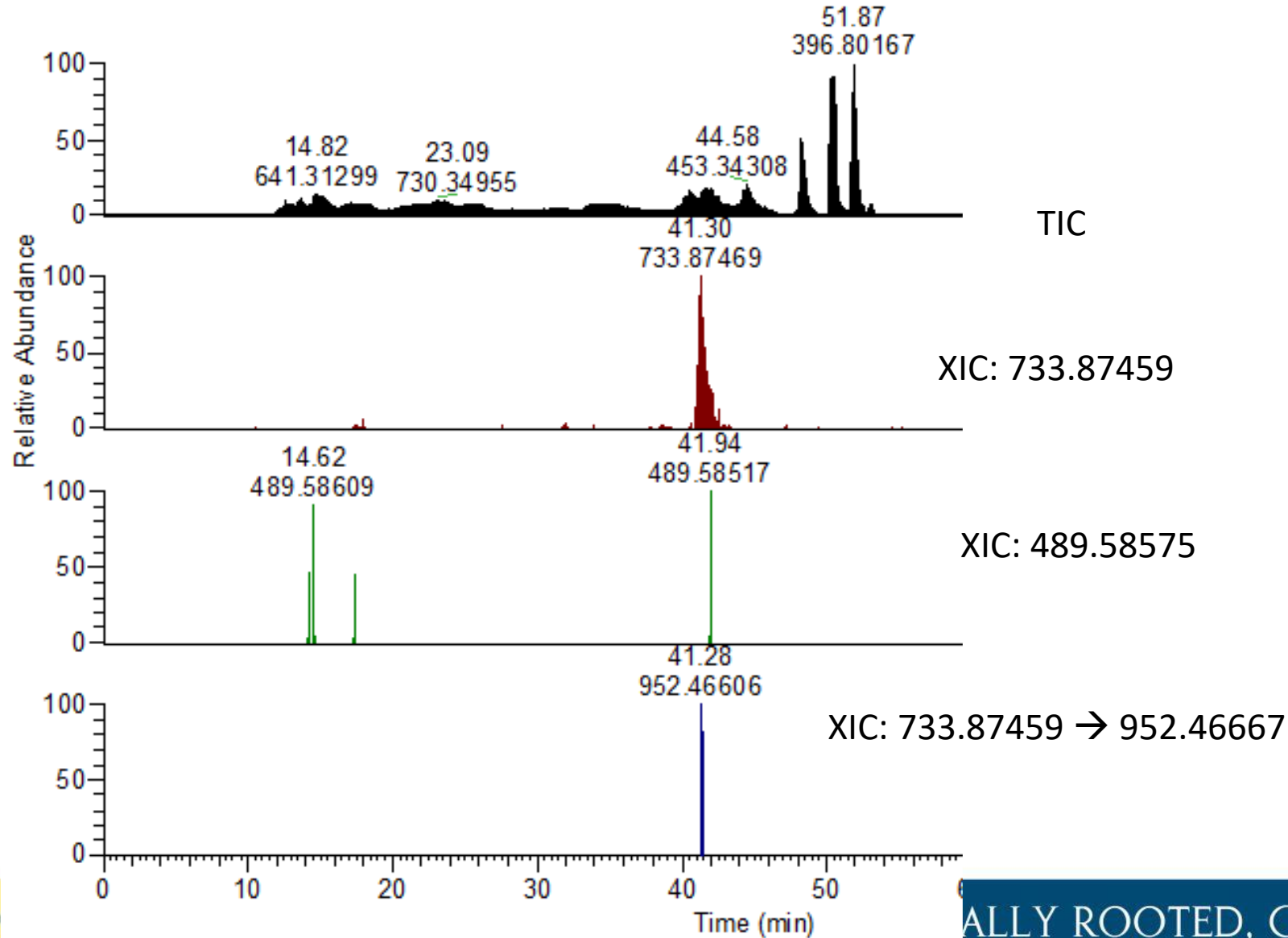
XIC: 733.87459 → 952.46667

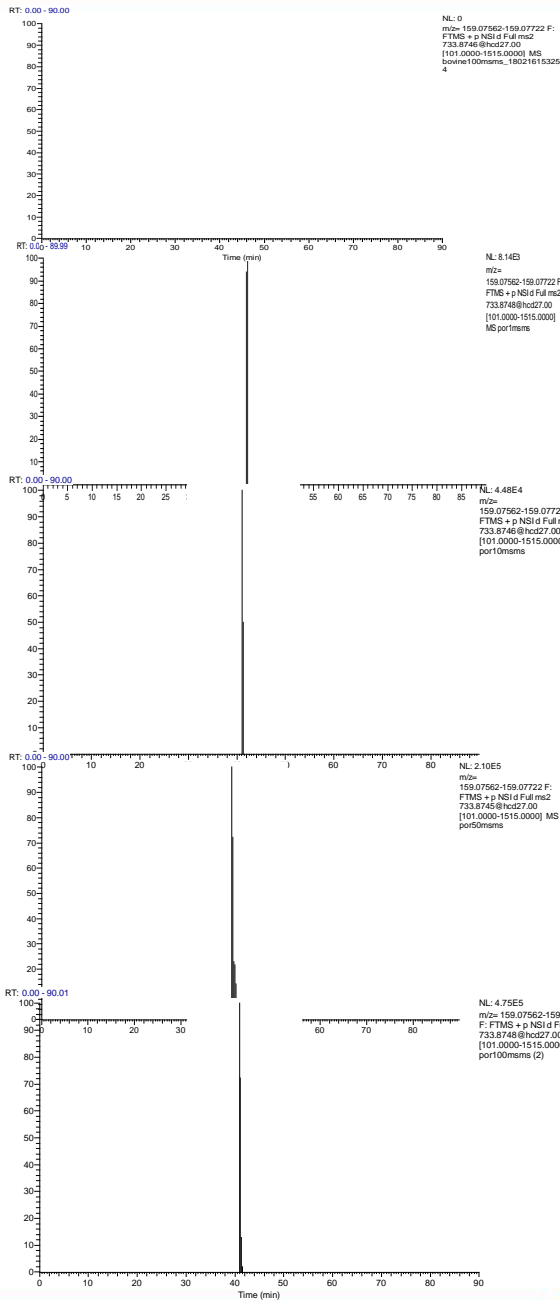




Porcine 1%

RT: 0.00 - 89.99 SM: 3G





Gelatin babi 0%

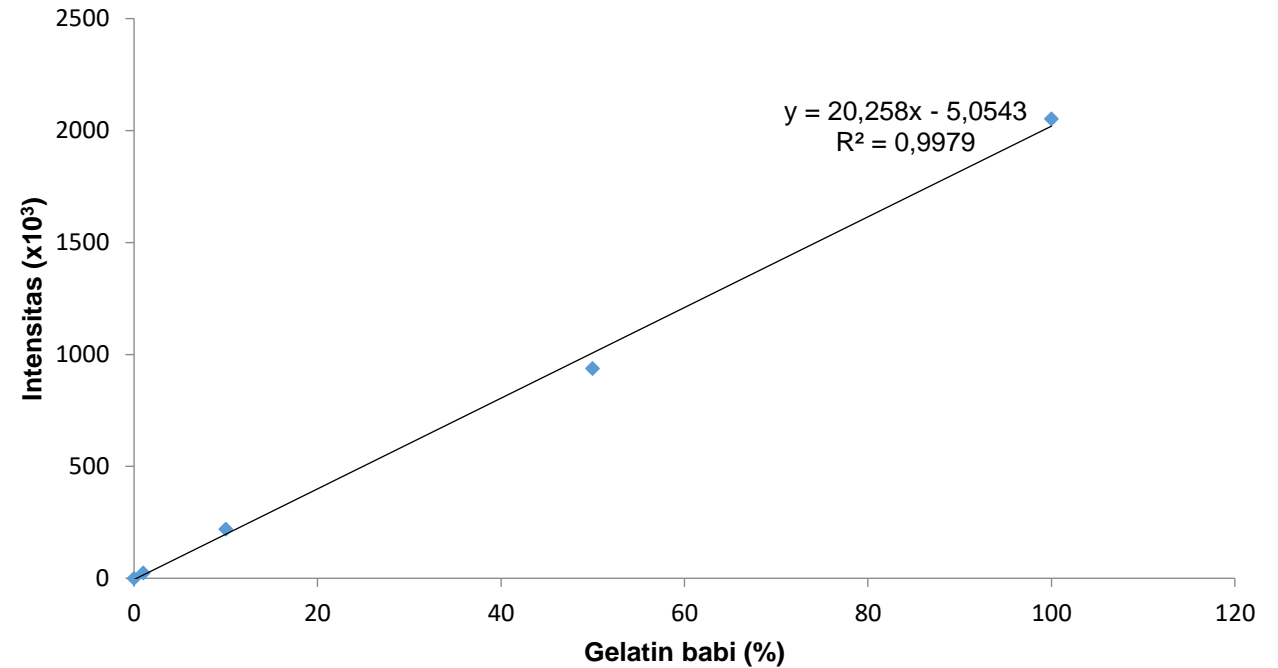
Gelatin babi 1%

Gelatin babi 10%

Gelatin babi 50%

Gelatin babi 100%

Gelatin babi vs Intensitas



High vs Low resolution MS



m/z
744.8304

MS² untuk
mendapatkan urutan
peptida

Mencari target biomarker,
kuatitasi dengan PRM



m/z
744

Memerlukan
daughter ion untuk
konfirmasi
qualitative (MS²)

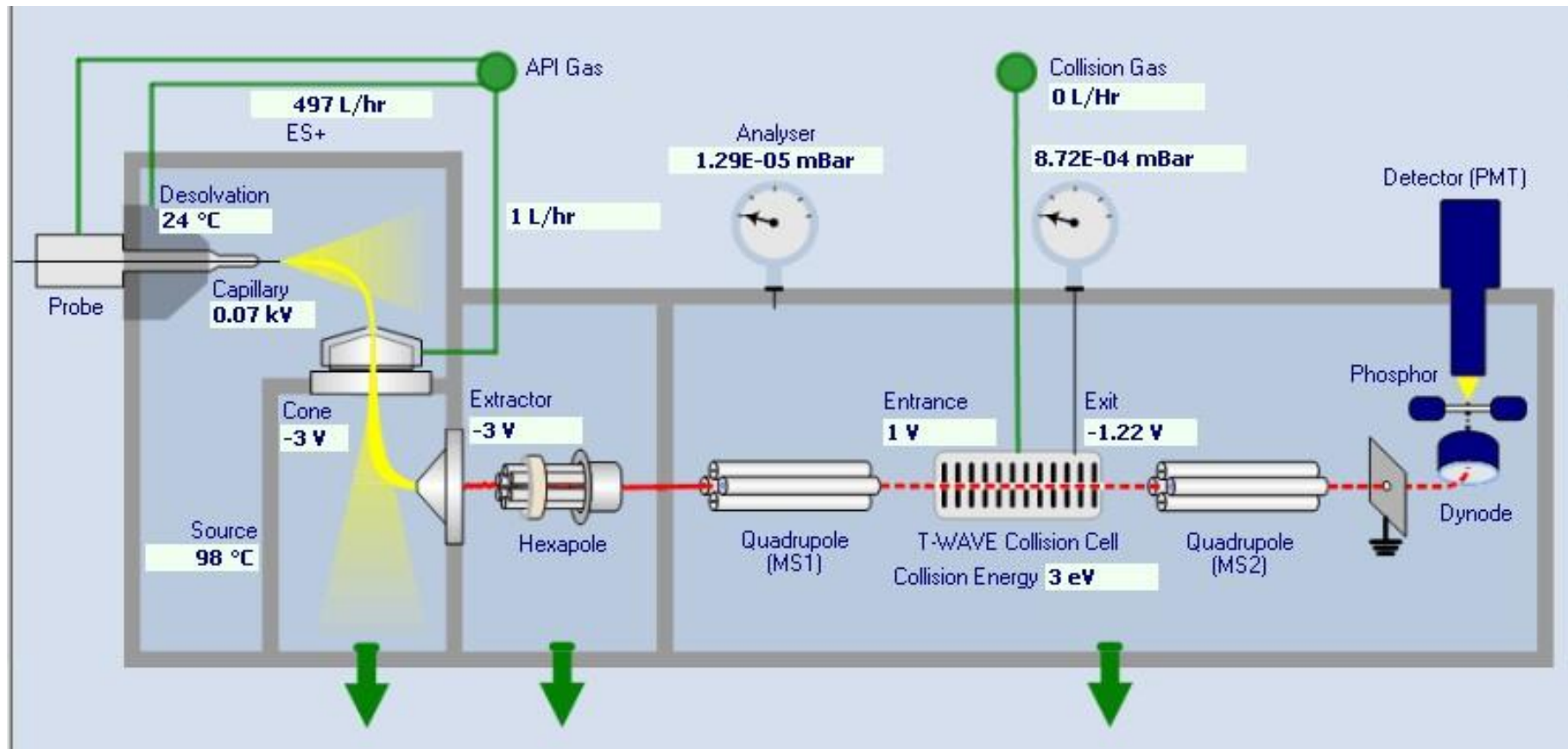
Analisis rutin dan kuantitatif
(MRM)



Contoh Aplikasi LC-MS/MS untuk Analisis Halal

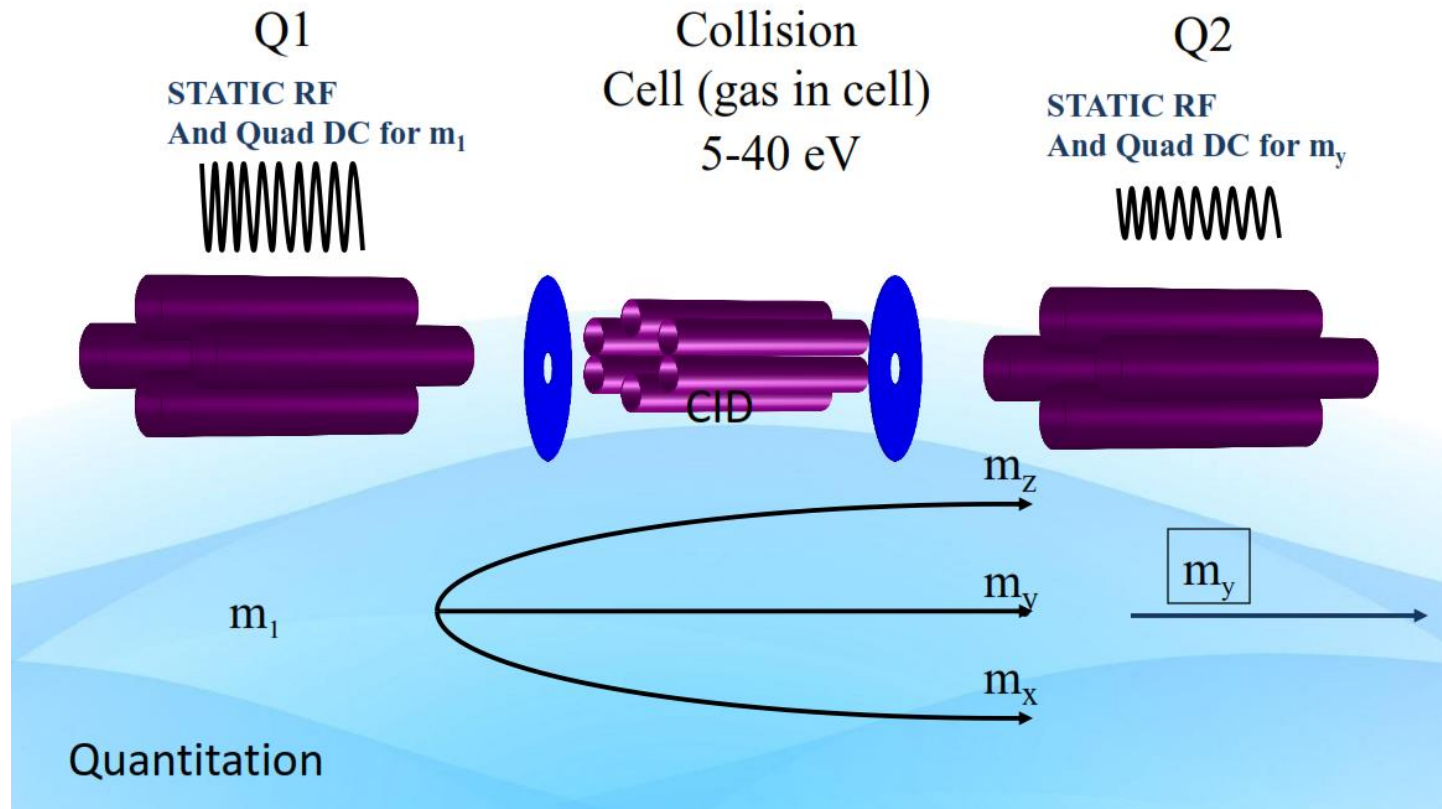


Inside TQD





MRM (Multiple Reaction Monitoring)





Food and Environmental

Are Pork Extracts Present in My Gummy Bears? Gelatin Speciation by LC-MS/MS

Chor Teck Tan¹ and Stephen Lock²
¹AB SCIEX Singapore, ²AB SCIEX Warrington (UK)

Overview

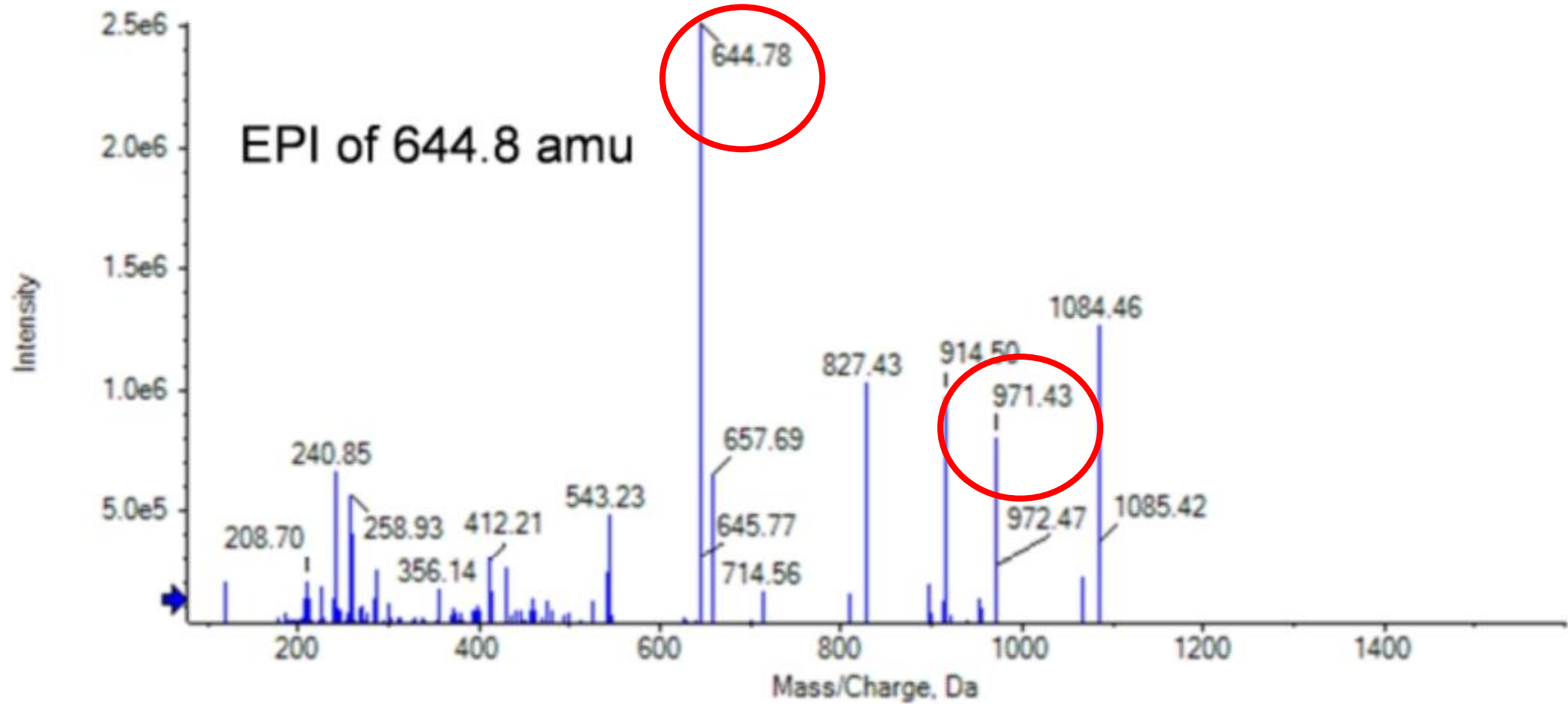
The accidental or fraudulent blending of meat and animal products from different species is highly relevant for consumers with ethical concerns against eating species such as horse or

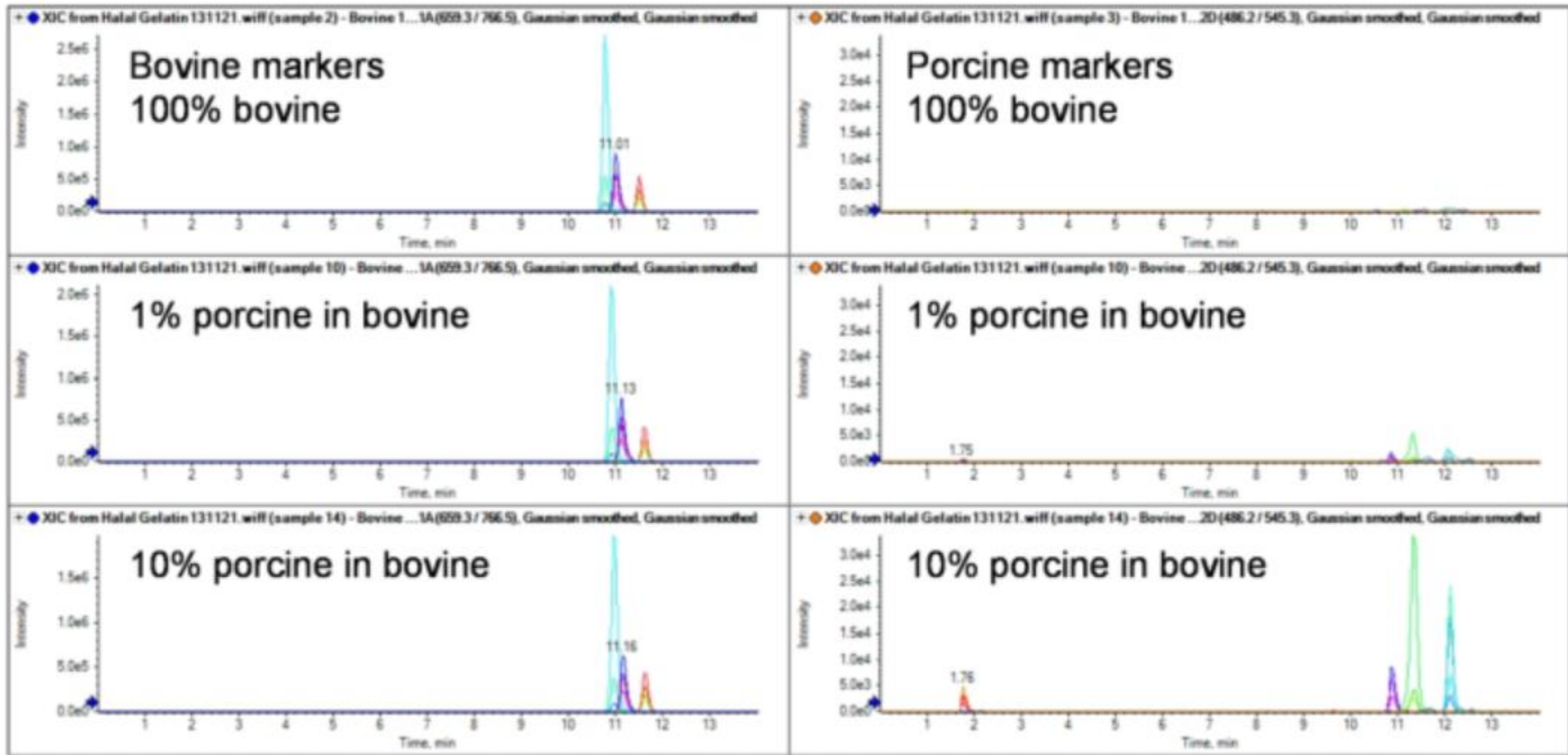


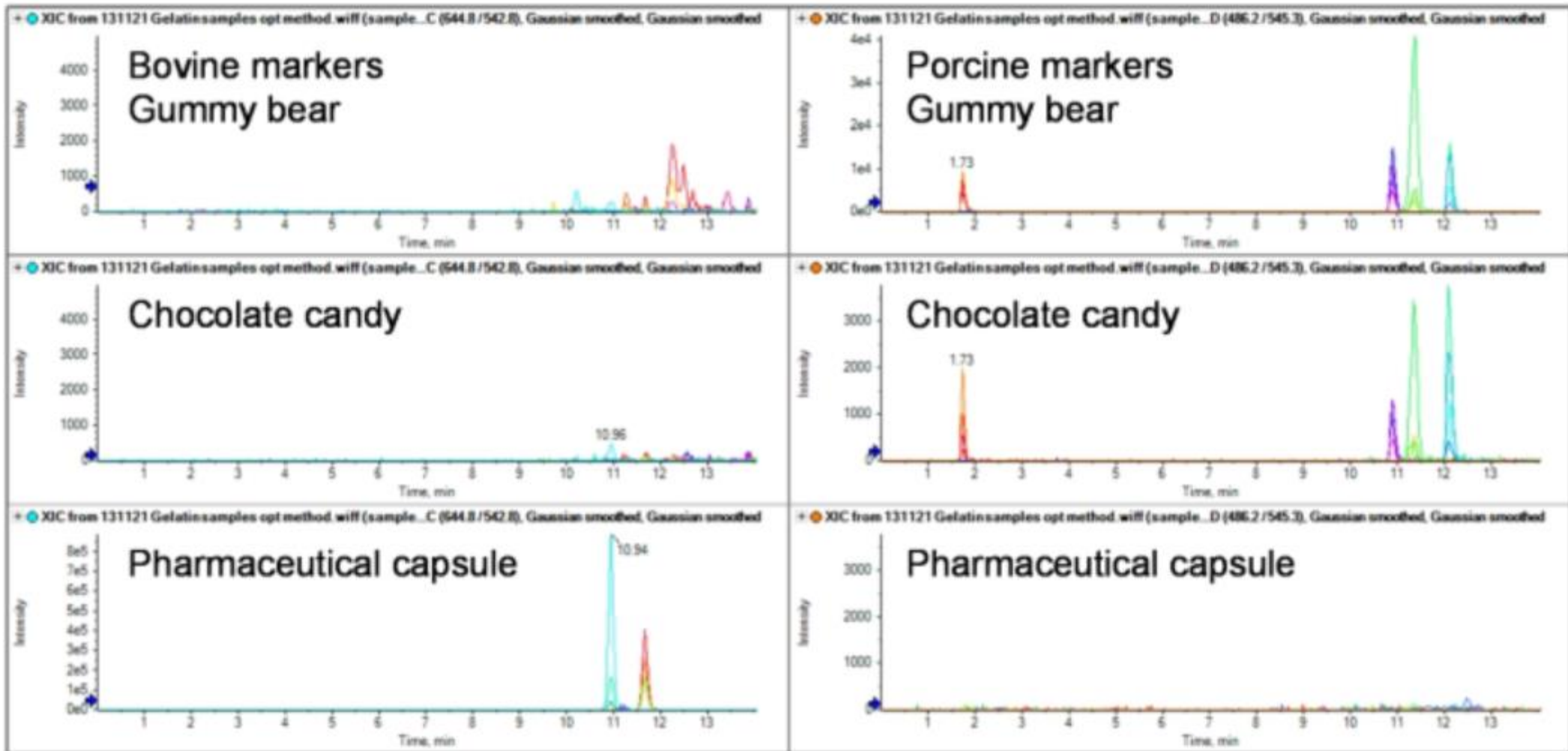


Contoh marker untuk analisis Rutin MRM

Gelatin Marker	Q1 (amu)	Q3 (amu)	CE (V)
<i>Pork gelatin 1</i>	1103	850.9	57.5
<i>Pork gelatin 2</i>	486.2	786.4	26.4
<i>Pork gelatin 3</i>	921.5	1050.6	49.4
<i>Pork gelatin 4</i>	620.8	618.3	28.3
<i>Beef gelatin 1</i>	659.3	766.5	34
<i>Beef gelatin 2</i>	781.4	991.6	42.3
<i>Beef gelatin 3</i>	644.8	971.5	37.3





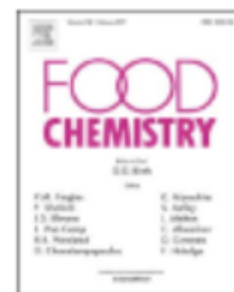




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Validation and theoretical justification of an LC-MS method for the animal species specific detection of gelatin



Anne J. Kleinnijenhuis^{a,*}, Frédérique L. van Holthoon^a, Griet Herregods^b

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^b Rousselot Expertise Center, Meulestedekaai 81, B-9000 Ghent, Belgium

ARTICLE INFO

Keywords:

Gelatin

LC-MS

Quantification

Collagen

Bovine

Porcine

TrustGel

ABSTRACT

Collagen is the most abundant protein family in mammals. Commercial edible gelatins are often produced from bovine and porcine skin and bone and consist mainly of partially hydrolyzed collagen type 1. The gelatin industry would benefit from a sensitive and reliable species detection method to unambiguously demonstrate species authenticity of their products. PCR and ELISA could in principle be used for this purpose. However, for gelatin, problems associated with false-positive and false-negative results, inconsistencies and low reactivity of commercially available kits have been observed with regard to ELISA and PCR methods. Therefore we developed a selective bottom-up LC-MS methodology for quantitative gelatin species determination with a lower limit of



Marker

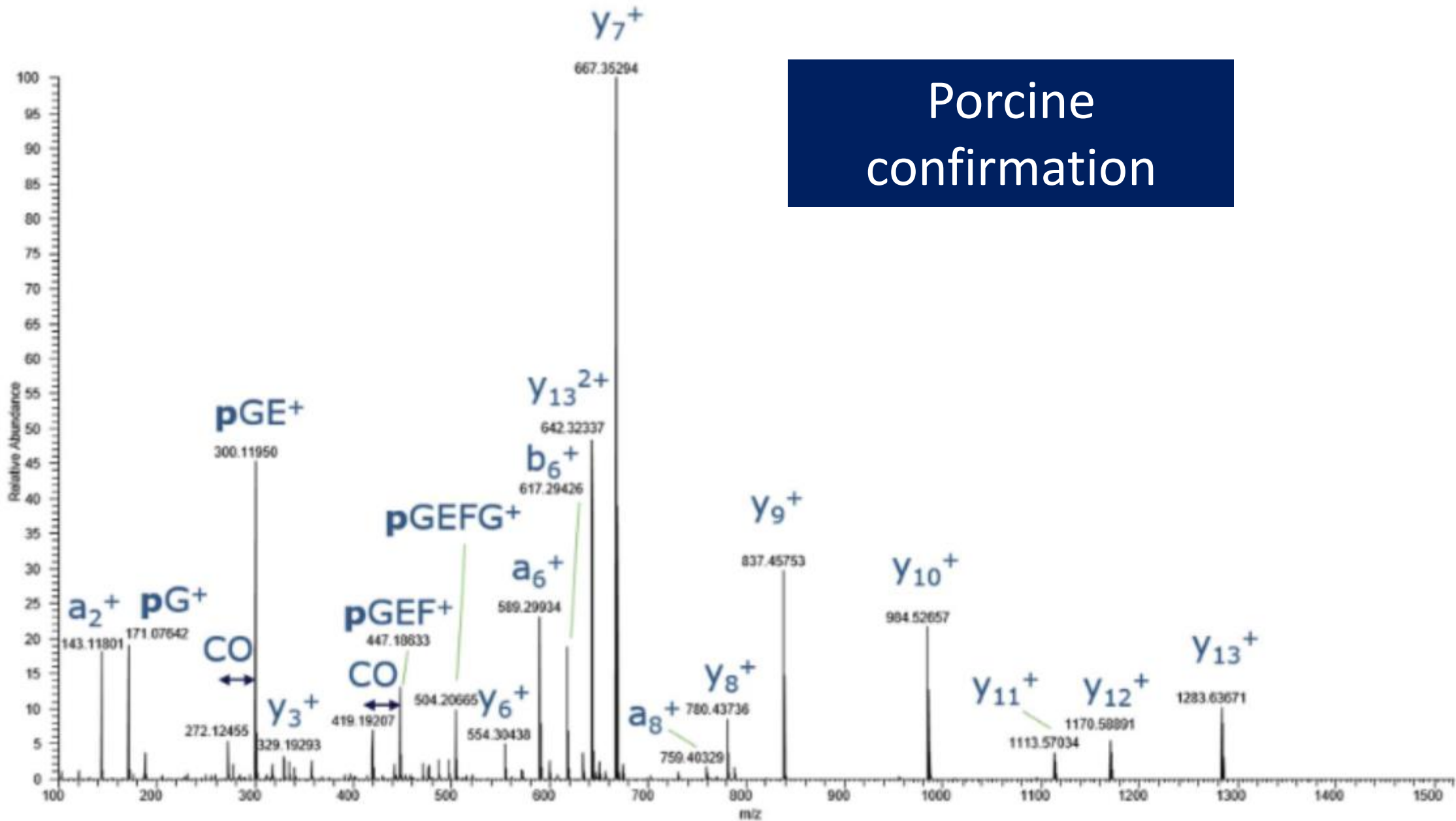
- Bovine (GETGPAGPAGPIGPVGAR) protein α -1 kolagen
- Porcine (GIPGEFGLPGPAGPR) protein α -2 kolagen

MRM

- capillary voltage 0.7 kV,
- The cone voltage (40–50 V) and collision energy (15–35 eV) were optimized for each peptide.
- Bovine 780.90 (2⁺) \rightarrow y_{11}^+ m/z 991.60 y_9^+ 823.50
- Porcine 727.40 (2⁺) \rightarrow y_7^+ m/z 667.35 and y_{13}^{2+} 642.32

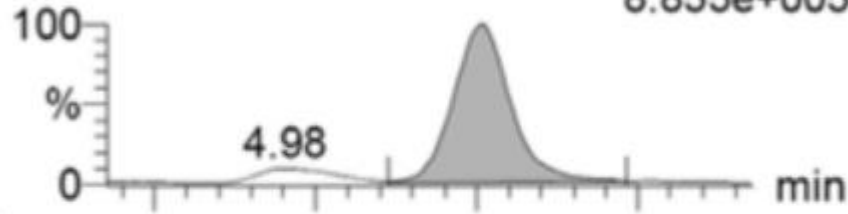


Porcine confirmation

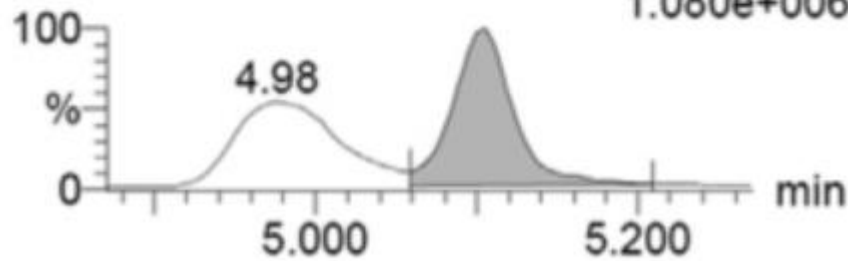


P_GlpGEFGLpGPAGPR

F3:MRM of 11 channels,ES+
727.4>667.35
8.855e+005

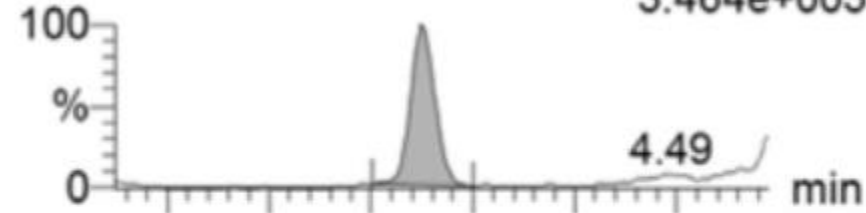


F3:MRM of 11 channels,ES+
727.4>642.3
1.080e+006

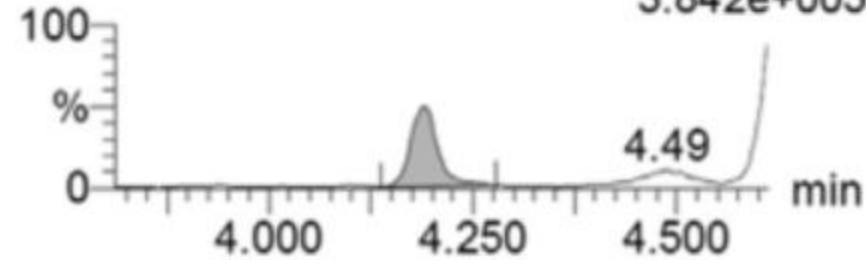


B_GETGPAGPAGPIGPVGAR

F5:MRM of 9 channels,ES+
780.9>991.6
3.464e+005



F5:MRM of 9 channels,ES+
780.9>823.5
3.842e+005



Validation

LLOQ 0.05%

Rec 111% (LLOQ), 124% (0.1%), 104% (2%)

Presisi, repetibilitas 3.1%, reproduc. 6%



Application News

No. AD-0164

Halal Authentication Analysis / LCMS-8060

**Detection and Differentiation of Bovine and Porcine Gelatins
in Food and Pharmaceutical Products By LC/MS/MS Method**

Udi Jumhawan, Jie Xing & Zhaoqi Zhan

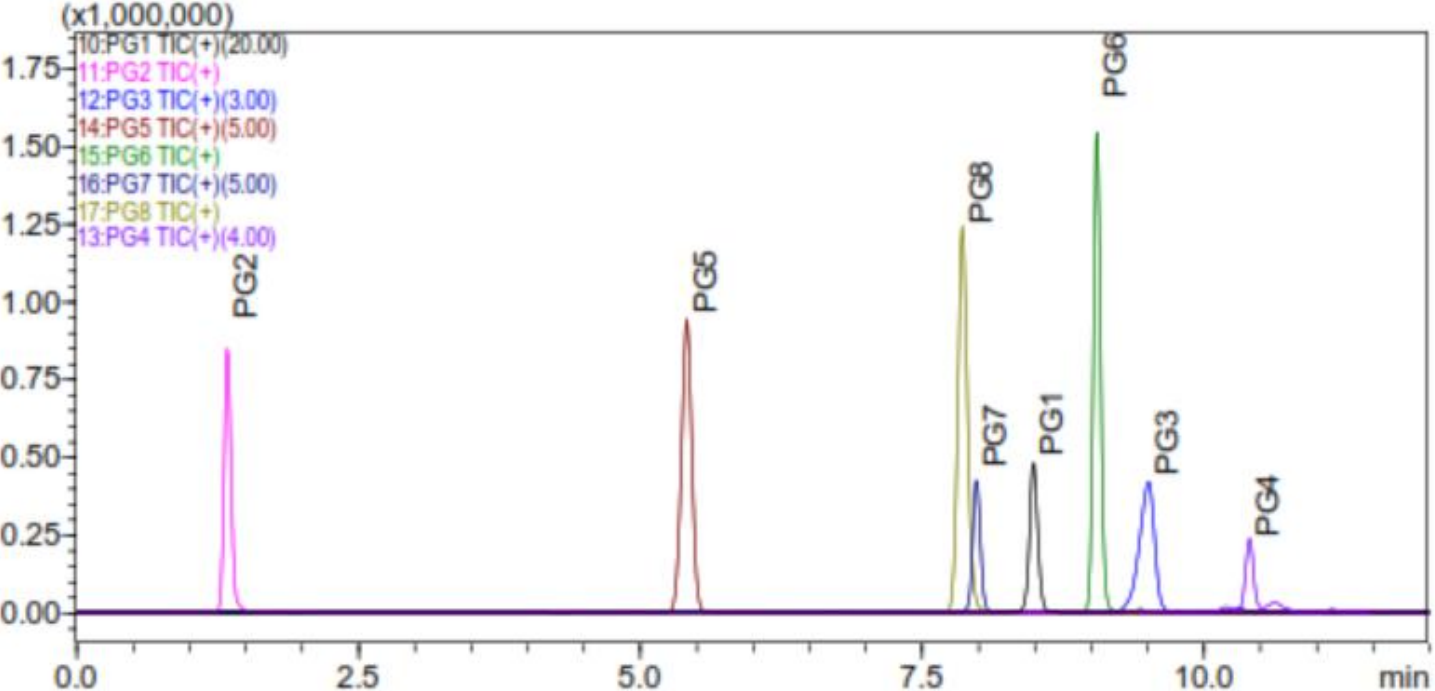
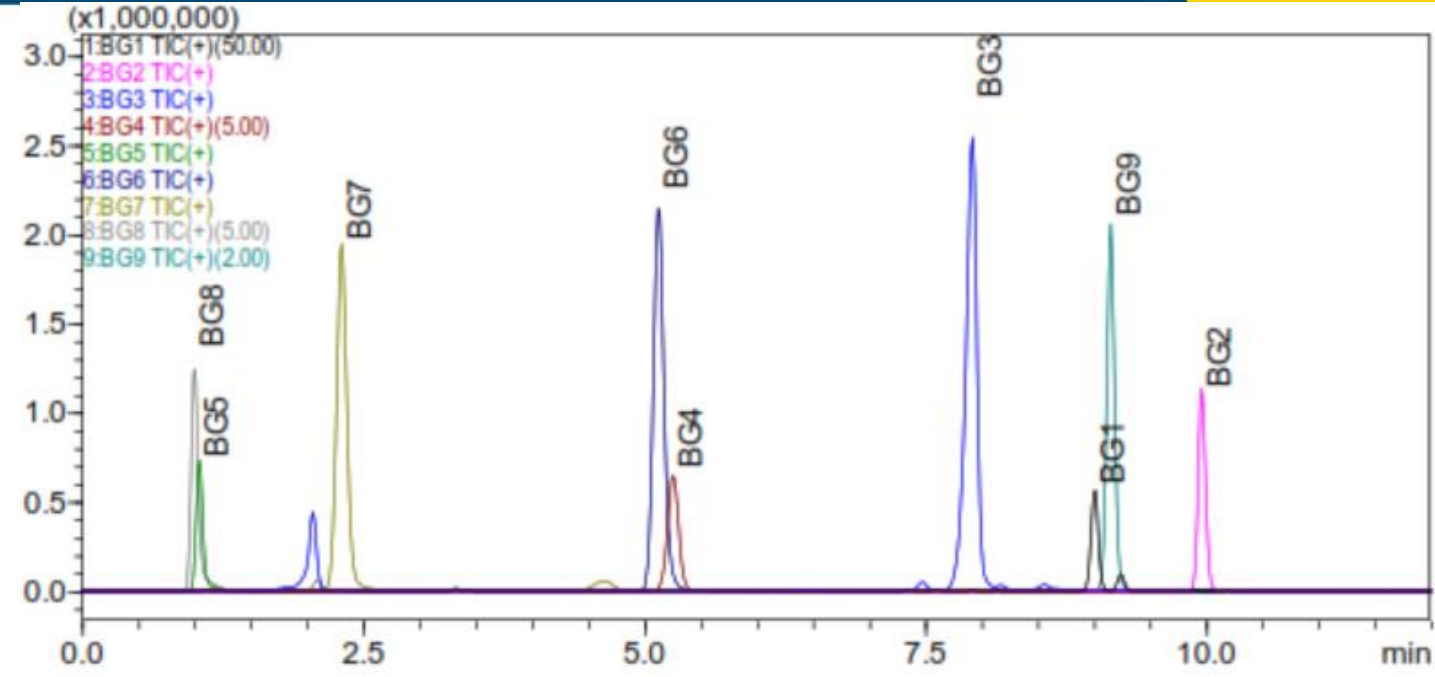
Application Development & Support Centre, Shimadzu (Asia Pacific) Pte Ltd, Singapore

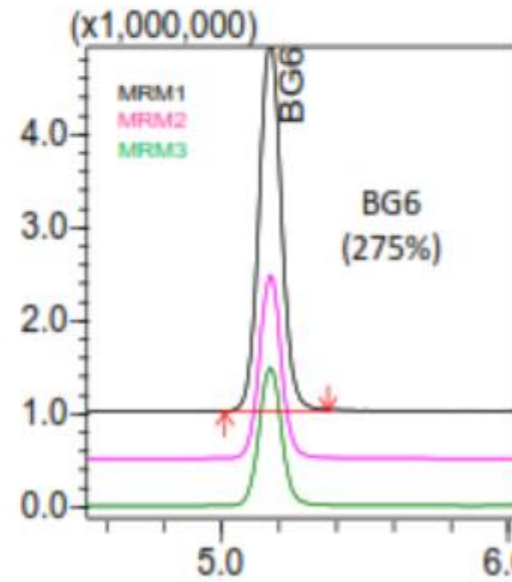
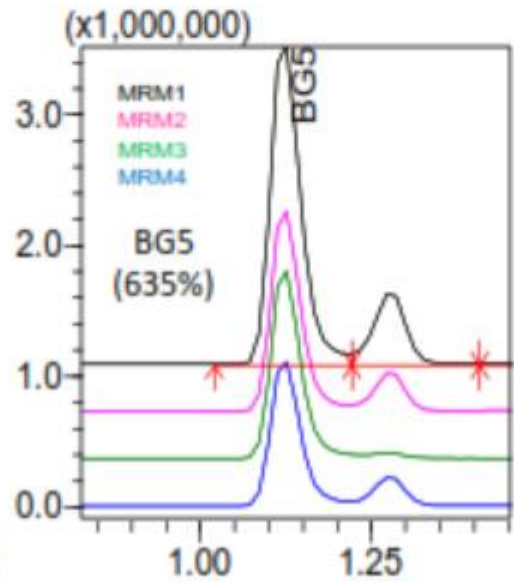
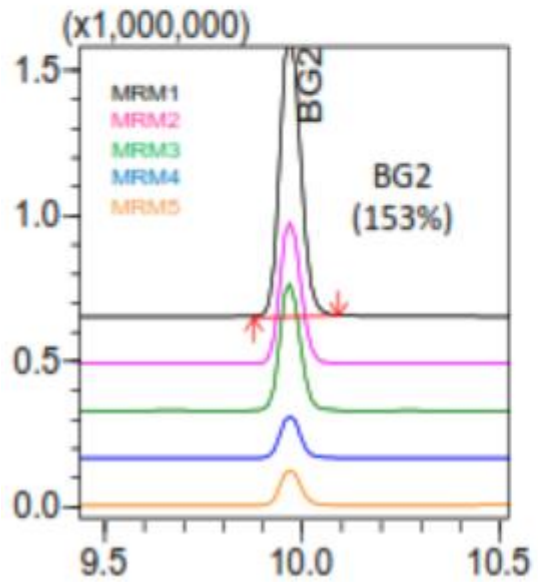
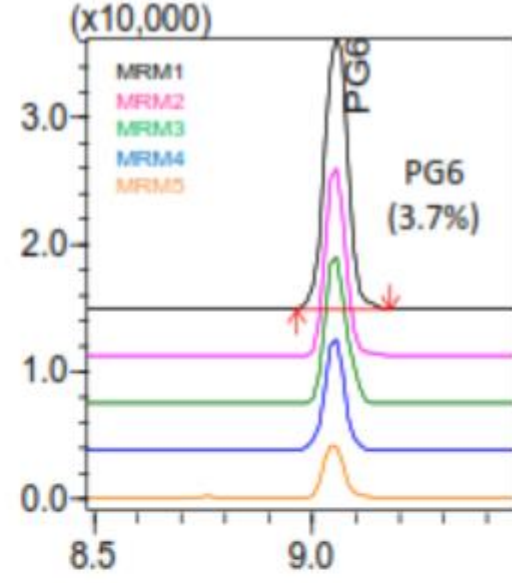
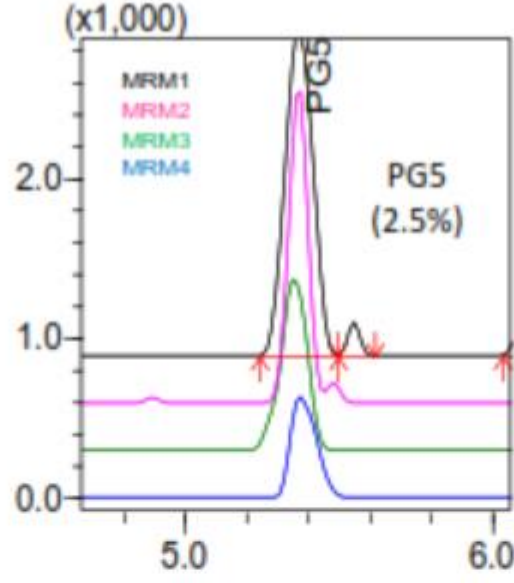
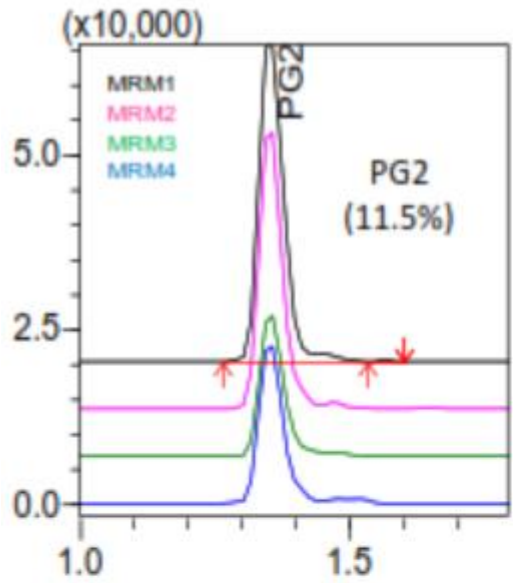


Protein	UniProt No.	Peptide marker (Abbr.)	Precursor ion & Charges	Number of MRM	RT (min)
Alpha-1 chain of type 1 bovine collagen	P02453	BG1	658.85++	4	9.0
		BG2	780.9++	5	9.9
		BG4	641.3++	5	5.3
		BG5	473.2++	4	1.0
Alpha-2 chain of type 1 bovine collagen	P02465	BG3	644.3++	3	7.9
		BG6	451.75++	3	5.1
		BG7	393.2++	4	2.3
		BG8	357.65++	3	0.9
		BG9	596.85++	5	9.2

Protein	UniProt No.	Peptide marker (Abbr.)	Precursor ion & charges	Number of MRM	RT (min)
Alpha-1 chain of type 1 porcine collagen	A0A1S7J210	PG2	486.25++	4	1.3
		PG5	656.3++	4	5.4
		PG6	773.9++	5	9.1
Alpha-2 chain of type 1 porcine collagen	A0A1S7J1Y9	PG1	1103.05++	3	8.5
		PG3	921.45++	5	9.5
		PG4	620.8++	5	10.4
		PG7	731.85++	5	8.0
		PG8	590.85++	5	7.9

TIC





No	Sample	Gelatin declaration & source	Number of detected peptide markers	
			Bovine	Porcine
1	Gummy bear I	Yes, beef	8	ND
2	Marshmallows I	Yes, pork	ND	5
3	Chewing gum	Yes, undeclared	ND	6
4	Marshmallows II	Yes, undeclared	6	ND
5	Gummy bear II	Yes, undeclared	8	ND
6	Pharma capsule I	Yes, undeclared	9	ND
7	Pharma capsule II	Yes, undeclared	9	4



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LOCALLY ROOTED, GLOBALLY RESPECTED