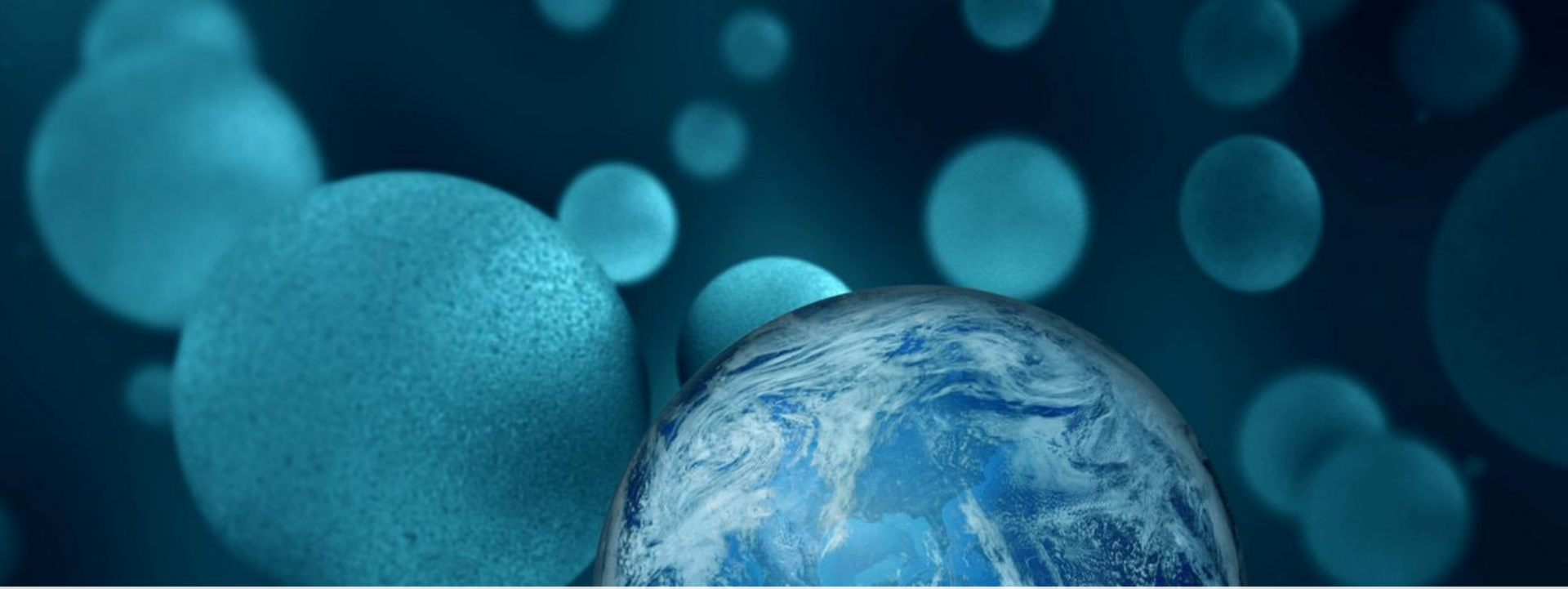

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Využití cílené proteomiky pro kontrolu falšování potravin: identifikace peptidových markerů v mase pomocí LC- Q Exactive MS/MS

Michal Godula Ph.D.
Thermo Fisher Scientific

Meat Substitution

Přihlášení Předplatné Dnes

29. květen 2017 31°C, 14°C

HOSPODÁŘSKÉ NOVINY

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Kurzy $23,63

Pokuta za koňské maso v kuličkách IKEA? Půl milionu korun

ČTK, mil 25. 4. 2013 17:41 Zpravodajství 0

- **Veterinární správa udělila pokutu půl milionu za koňské maso v kuličkách IKEA. Švédský nábytkářský řetězec se kvůli tomu odvolal. Naopak Tesco, které za koninu dostalo za koninu pokutu 650 tisíc korun, se odvolávat nebude.**

reklama

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Meat Substitution

Motivation : $$$

- Addition of meat from undeclared species to a specific meat product in order to lower production cost and increase profitability

  Cost per kg: Horse meat << Beef meat

An international issue

- It is economic fraud
- It represents health issues due to specific dietary restrictions
- It is an ethical problem
- It is also an important cultural and religious issues

How testing is done?

- Two-dimensional polyacrylamide gel electrophoresis and western-blot analysis
- Qualitative Real-Time PCR
- Enzyme-linked immunosorbent assay (ELISA)

Challenges

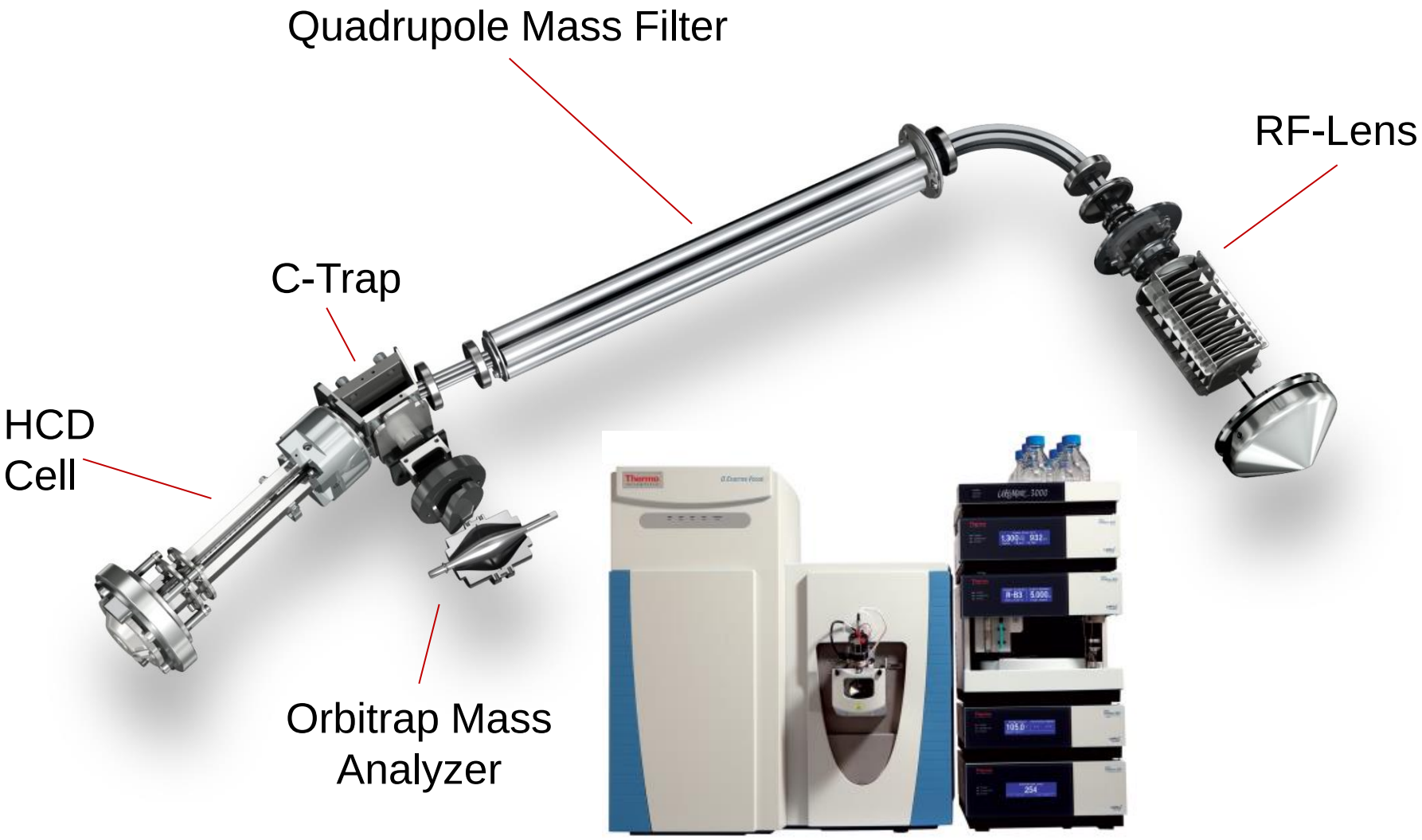
- These methods are mostly qualitative
- Molecular information obtained is limited
- Data can't be revisited post-acquisition for data mining
- They are not generic approaches and need to be heavily customized

What About Mass Spectrometry Options

High Resolution Accurate Mass

- Can perform the same level of quantitation as MS/MS
- Selectivity obtained by accurate mass measurement (only m/z needed)
- Less false positives and negatives
- No need to setup instrument (SRM) before analysis
- Unlimited number of compounds in a run – perfect for screening
- Automated data processing

Q Exactive™ MS - a 3D View



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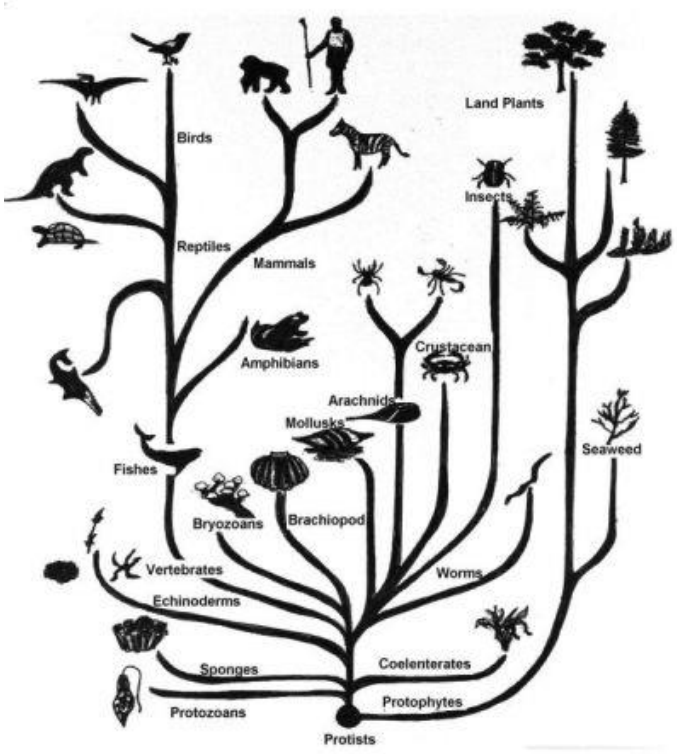
Why Bottom-up Proteomics Workflow Is An Interesting Option To Develop An MS Based Assay?



All life forms are related by common ancestry and descent. The construction of phylogenies provides explanations of the diversity seen in the natural world.

Today, phylogenies are usually constructed using **DNA sequence data.**

Relationship between genes and species is central for meat speciation

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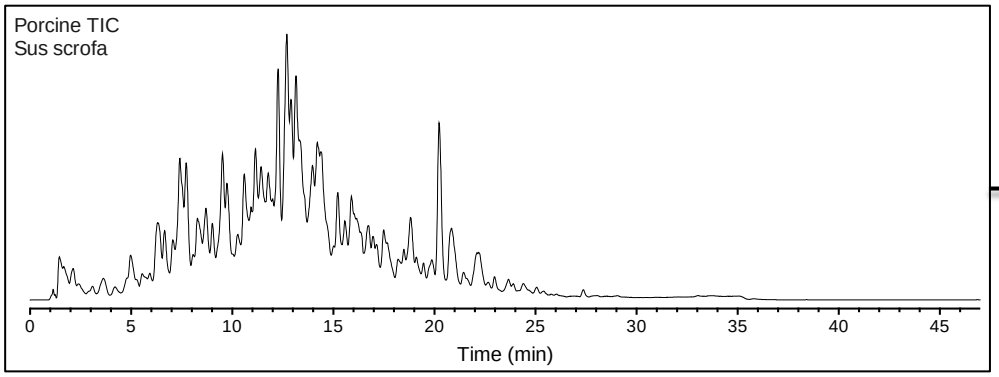
Traditional Peptide Fingerprinting Approach Using MS



Generate a mass list (MS and MS/MS data)

Database search (MASCOT/SEQUEST)

MS and MS/MS matching

Peptide fingerprinting

Beef

17 specific peptides

Horse

23 specific peptides

7 429 common unique peptides identified

Pork

14 specific peptides

Lamb

9 specific peptides

Using a relatively high abundance threshold

…

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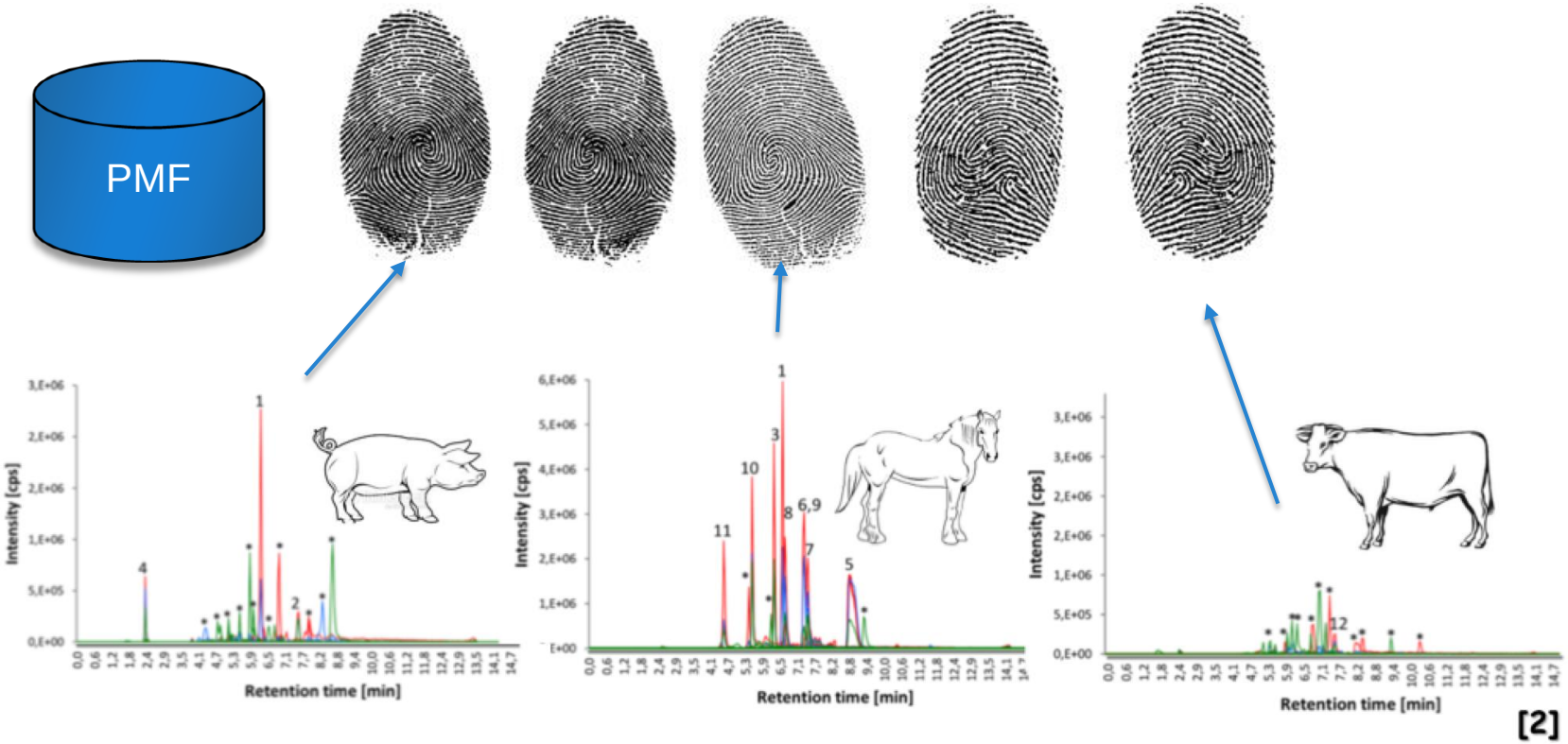
Peptide Mass Fingerprinting



[2]

marker	species	protein	biomarker peptide sequence
1	pig/horse	troponin T/unknown	YDIINLR
2	pig	myosin-4	TLAFLFAER
3	horse	myosin-2	EFEIGNLQSK
4	pig	myosin-1 and myosin-4	SALAHAVQSSR
5	horse	myoglobin	YLEFISDAIIHVLHSK
6	horse	myosin-1 and myosin-2	VVETMQTMLDAEIR
*	Unspecific signals		

marker	species	protein	biomarker peptide sequence
7	horse	pyruvate kinase	IYVDDGLISLQVK
8	horse	hemoglobin	FLSSVSTVLTSK
9	horse	myoglobin	HGTVVLTALGGILK
10	horse	myoglobin	VEADIAGHGQEVLIR
11	horse	myosin-1	LVNDLTGQR
12	cattle	myosin-1	TLALLFSGPASGEAEGGPK

Bargen *et al.* (2013), Journal of Agricultural and Food Chemistry, 61:11986-11994

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What Are The Main Limitations Of This Analytical Approach?

- It relies heavily on the quality of the MS and MS/MS data
- It strongly relies on bioinformatics and parameterizations
- It requires highly skilled scientists to obtain comprehensive results

Is this really appropriate for implementation in a routine food analysis laboratory ?

Can we propose alternative strategies ?

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Targeted Bioinformatics Analysis Example: Myoglobin

Extraction of gene information

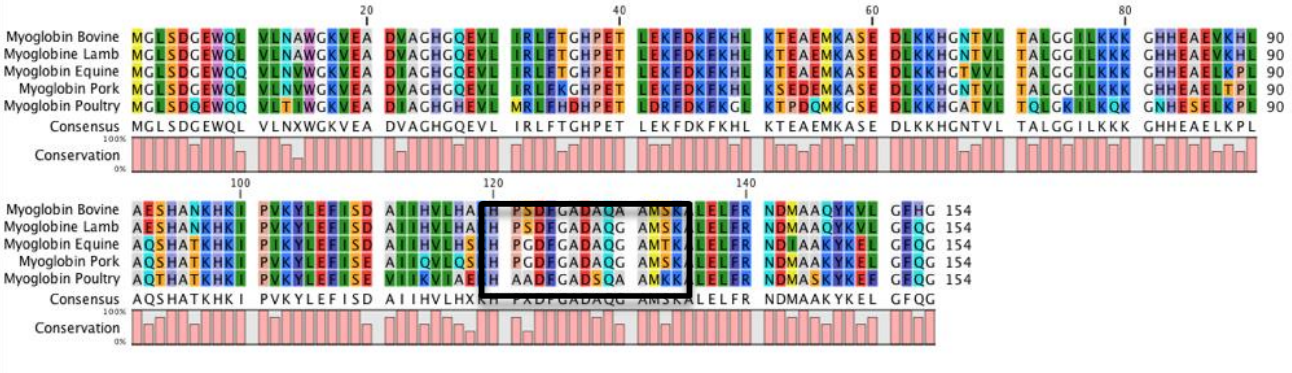
In silico sequence alignment

In silico protein translation and alignment

Sequence analysis and *In silico* tryptic digestion

Generate proteotypic mass lists

Myoglobin is the primary oxygen-carrying protein of muscle tissues
It is a highly abundant protein



Proteotypic peptides can be identified (120-134)

120 134

Beef YLEFISDA IIHVLHAKHP SDFGADAQAA MSKALELFR
Horse YLEFISDA IIHVLHSKHP GDFGADAQGA MTKALELFR
Pork YLEFISEA IIQVLQSKHP GDFGADAQGA MSKALELFR
Lamb YLEFISDA IIHVLHAKHP SDFGADAQGA MSKALELFR

	Proteotypic peptides	Mass (m/z)
Beef	HPSDFGADAQAAMSK	x_1
Horse	HPGDFGADAQGAMTK	x_2
Pork	HPGDFGADAQGAMSK	x_3
Lamb	HPSDFGADAQGAMSK	x_4

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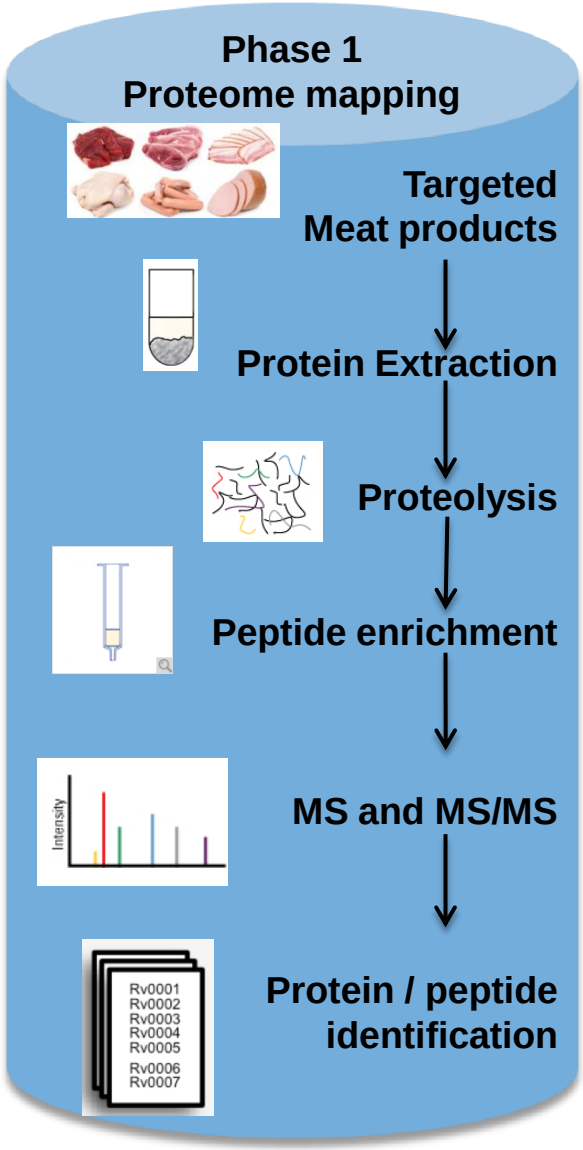
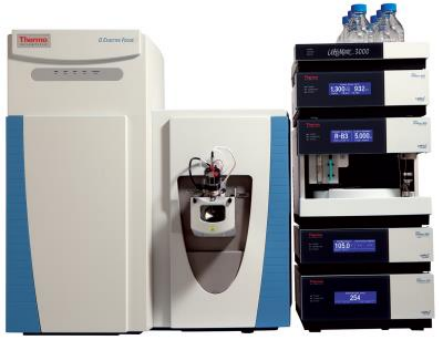
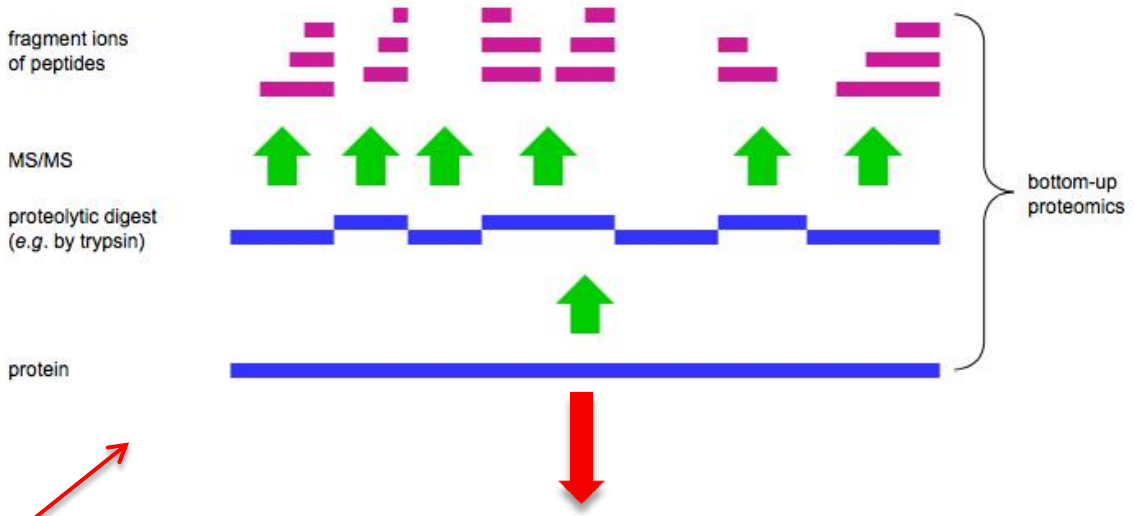
How Bottom-up Proteomics Can Be Used For Meat Speciation







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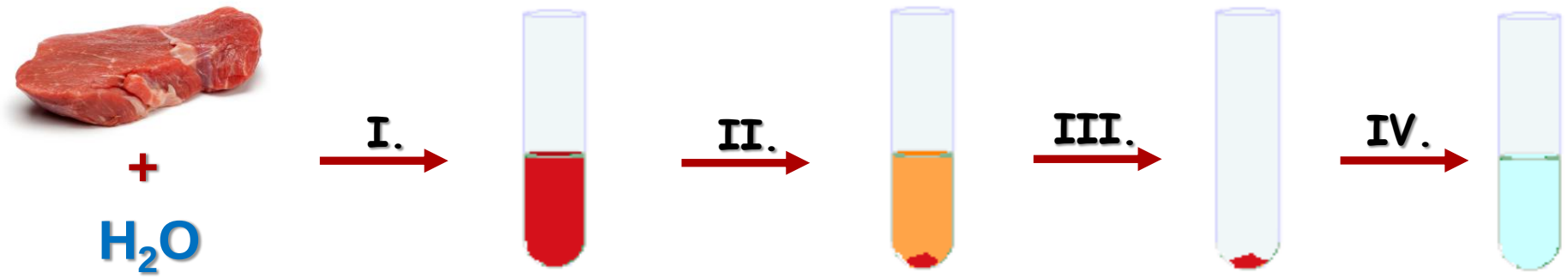
Bottom-up Proteomics Sample Preparation

1. Meat sample mixed with water (1:5) is homogenized and the mixture is sonicated
2. Proteins in the suspension are precipitated with acetone (1:1)
3. Acetone is discarded and the generated protein pellet is dryed to remove all traces of acetone.
4. Protein pellets are dissolved in ammonium bicarbonate (pH 8.5).
5. Proteins are denatured by heating at 120˚C
6. Reduced with Dithiothreitol (DTT) and alkylated with Iodoacetamide IAA
7. Proteomic grade **trypsin** is added and the reaction is performed at 40ºC for 24h. Trypsin cleavage occurs after basic amino acids : **Lys (K) & Arg (R)**



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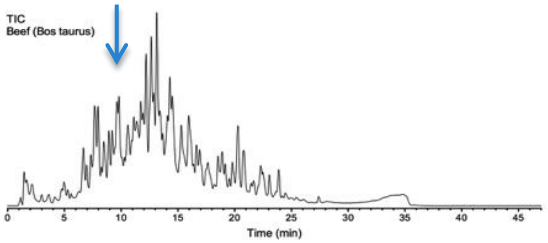
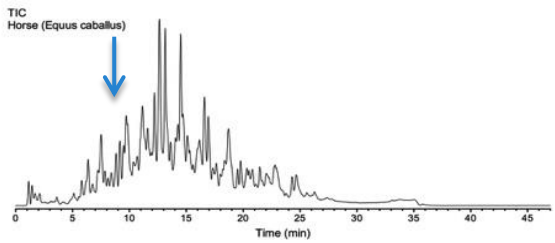
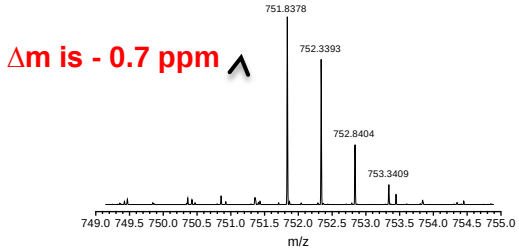
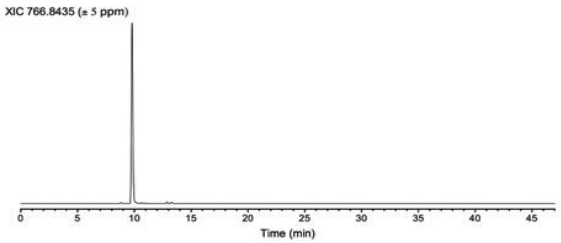
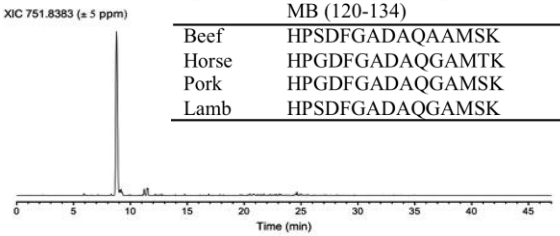
Each Targeted Peptides Can Be Detected And Extracted From Tics





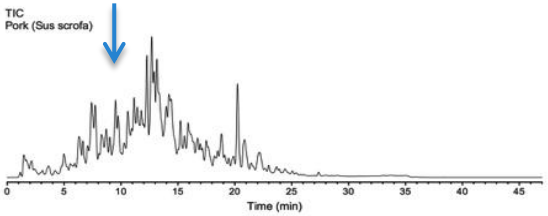


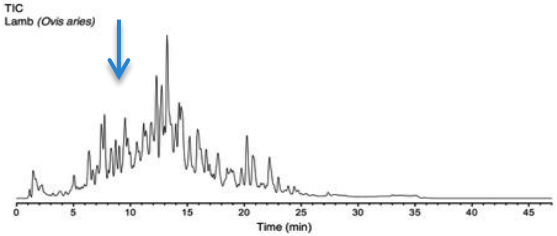




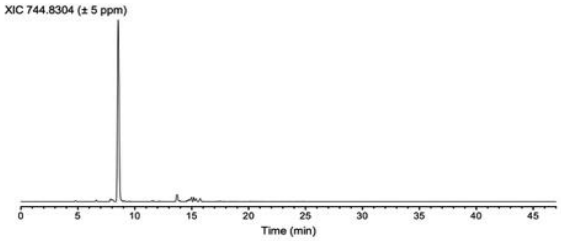
Species	Tryptic peptide sequence MB (120-134)	Theoretical mass (z=2)	Observed mass (z=2)	Mass accuracy (ppm)
Beef	HPSDFGADAQAAMSK	766.8435	766.8436	0.13
Horse	HPGDFGADAQGAMTK	751.8383	751.8378	-0.67
Pork	HPGDFGADAQGAMSK	744.8304	744.8314	1.34
Lamb	HPSDFGADAQGAMSK	759.8357	759.8363	0.79

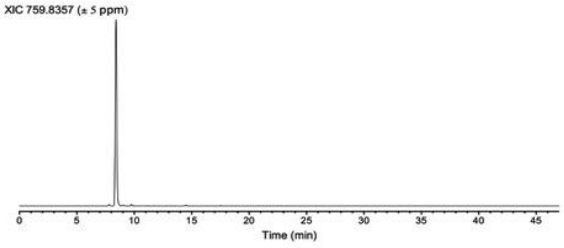
Very complex TIC's
Over 7000s of unique peptides can be found.





Need for very high resolution data (>100 000).





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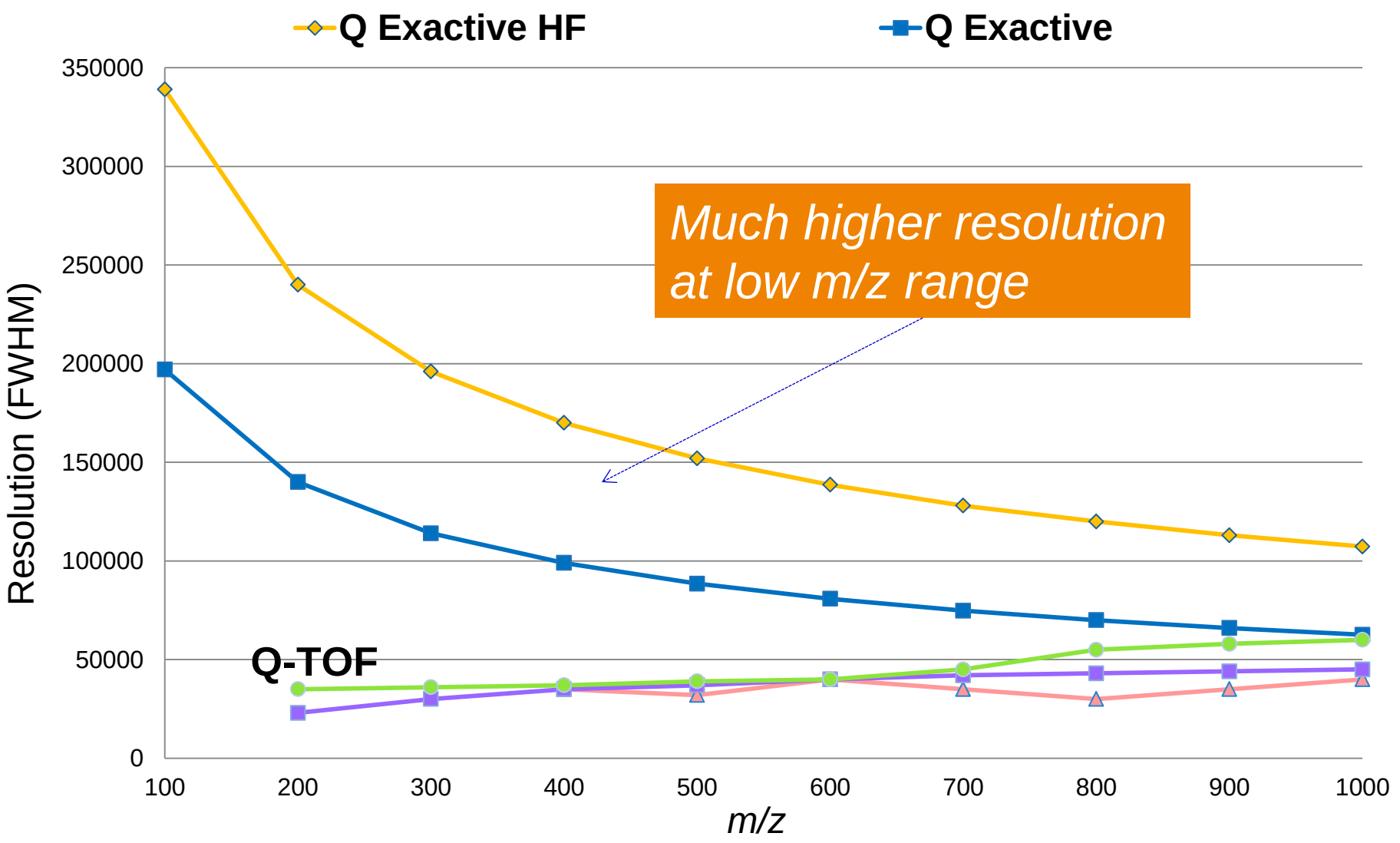
Resolution vs. m/z



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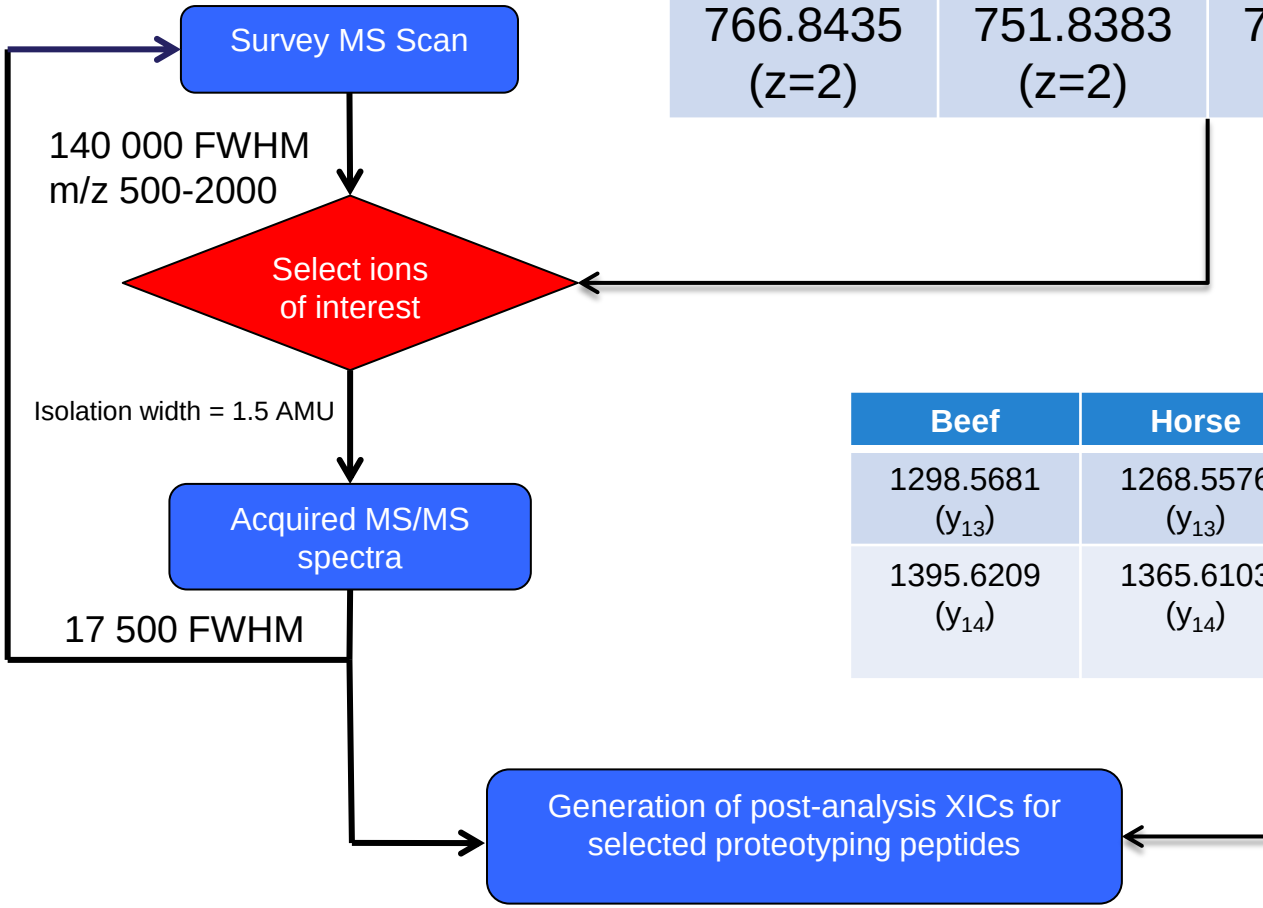
Data Dependent MS/MS Used For Targeted Work

Beef	Horse	Pork	Lamb
766.8435 (z=2)	751.8383 (z=2)	744.8304 (z=2)	759.8357 (z=2)



Beef	Horse	Pork	Lamb
1298.5681 (y_{13})	1268.5576 (y_{13})	1254.5419 (y_{13})	1284.5525 (y_{13})
1395.6209 (y_{14})	1365.6103 (y_{14})	1351.5947 (y_{14})	1381.6053 (y_{14})

Extracted ion chromatogram at y_{14} or y_{13} ± 5 ppm

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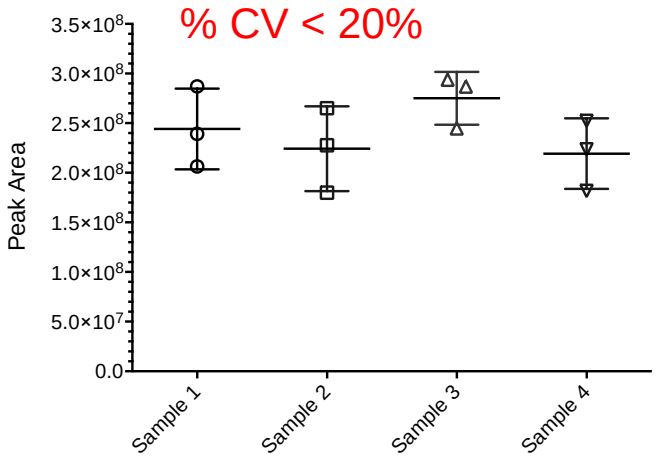
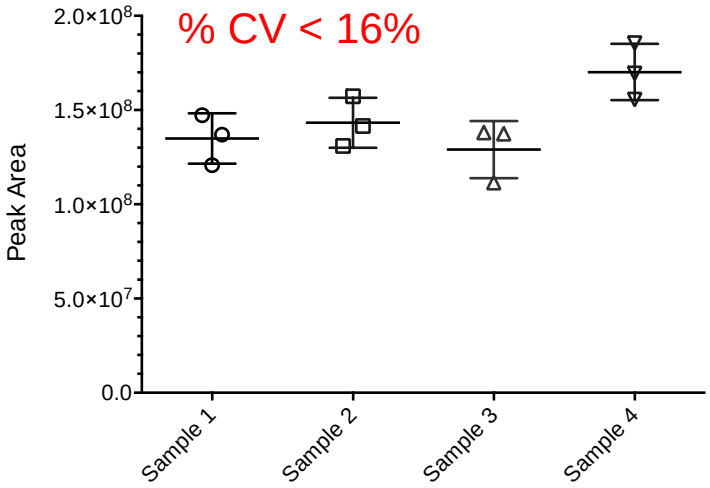
Assessment Of The Apparent Intra- And Inter Method Reproducibility

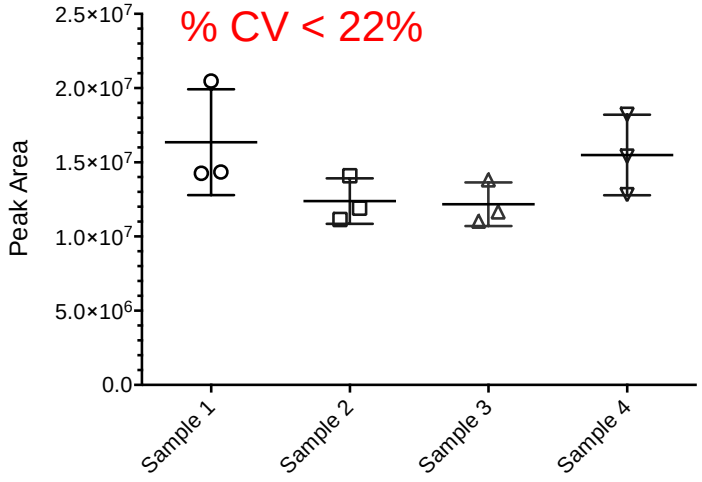
Beef (Myoglobin peptide 120-134)



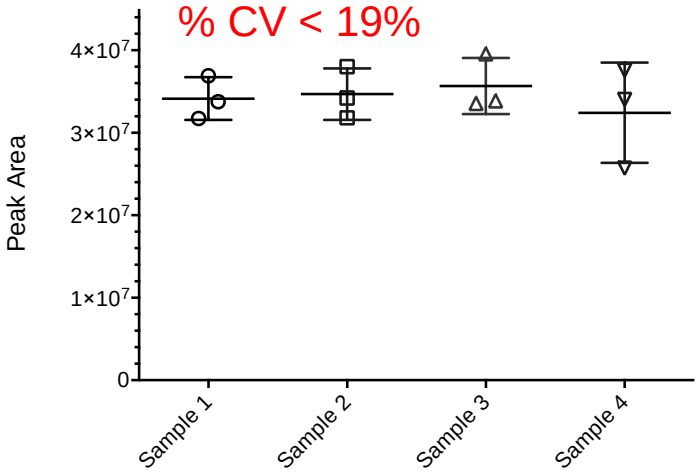
Horse (Myoglobin peptide 120-134)



Pork (Myoglobin peptide 120-134)



Lamb (Myoglobin peptide 120-134)



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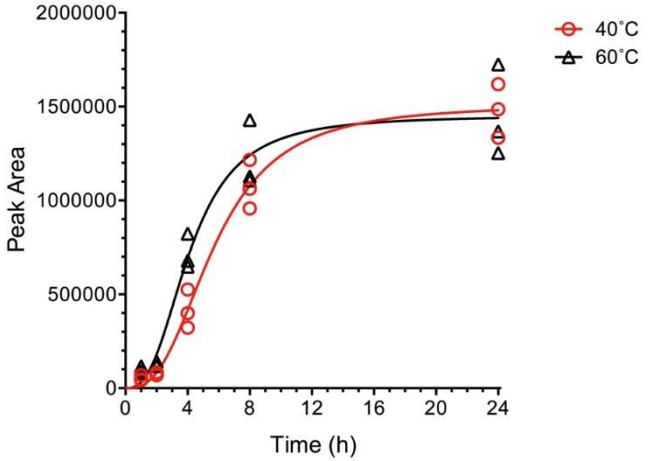
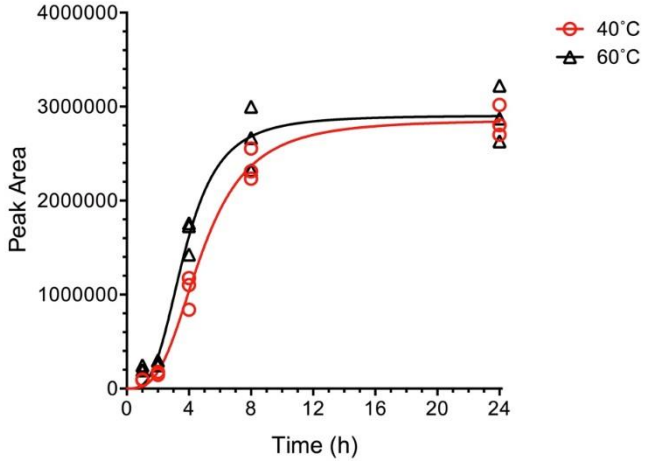
Tryptic Digestion Optimization

After 1h, peptides can be detected.
At 4h, we observed 30-40% of the maximum abundance.

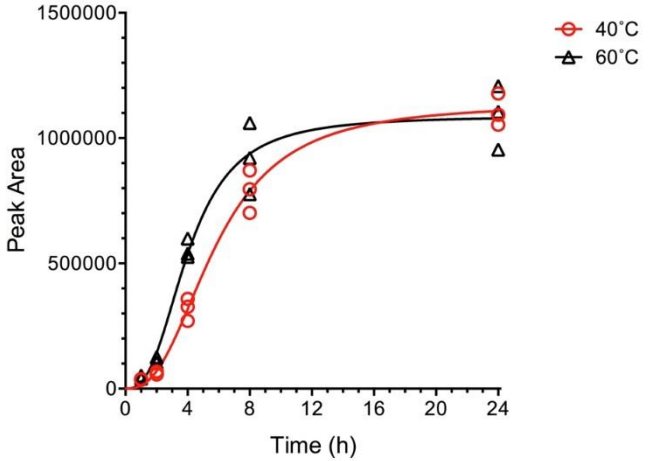
Beef



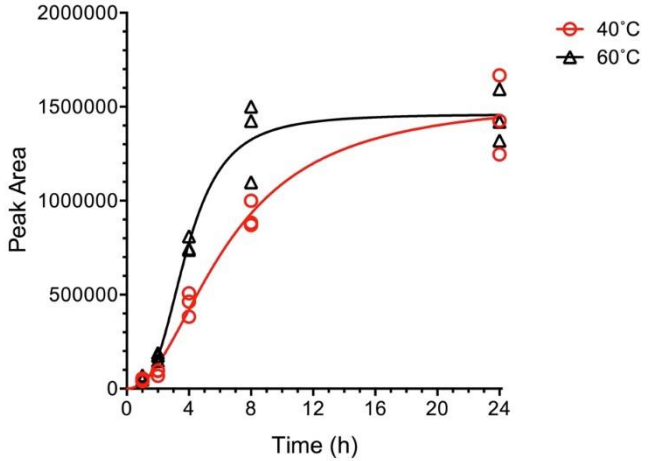
Horse



Pork



Lamb



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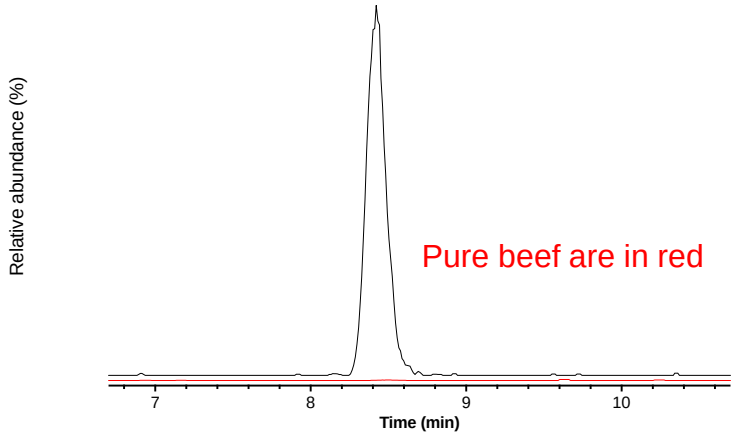
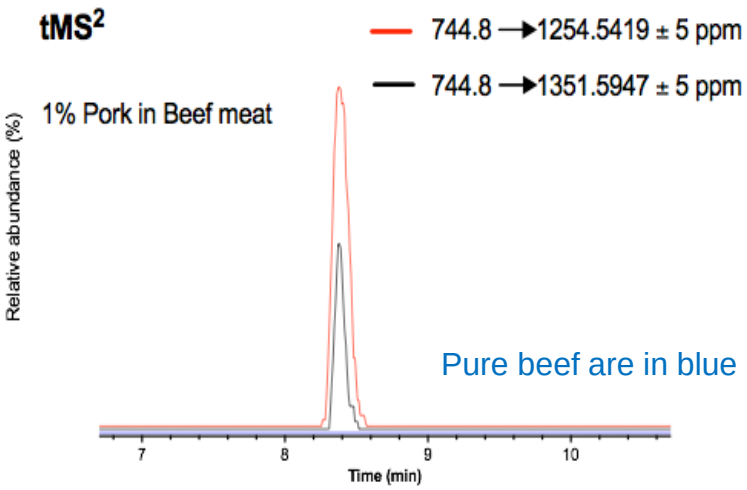
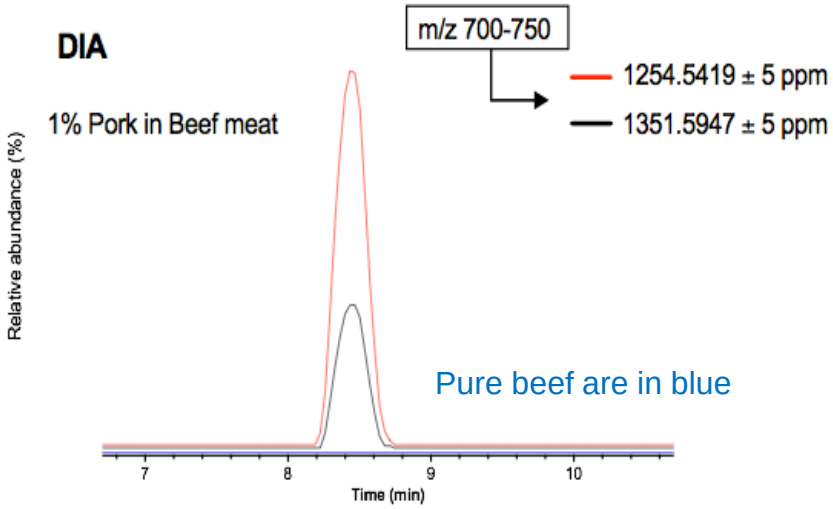
Chromatograms From Beef Meat Sample Spike With 1 % Pork Meat

MS^1
XIC 744.8304 ± 5 ppm







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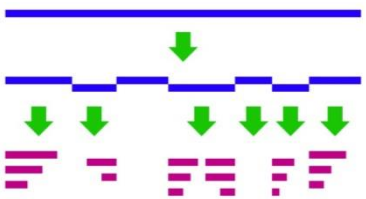
Routine Practive



Protein

Proteotypic peptides →

MS/MS spectra

We can use labeled peptides as internal standards !

Quality Control !

This is a must for methods to be used for routine analysis in multiple sites

LC-MS → **Targeted MS**

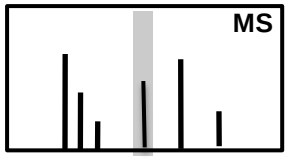
Introduction of internal standards
(^{13}C, ^{15}N)

Digestion

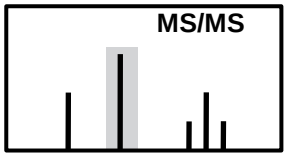
Fractionation

Homogenization/Lysis

1. Select precursor ion



2. Precursor fragmentation



3. Use Precursor-Fragment pairs for identification

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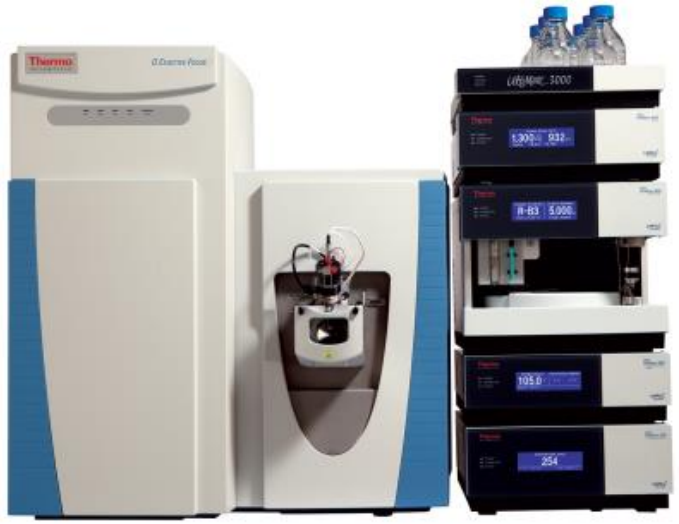
Conclusions

- Targeted bottom-up proteomics approach can be applied for meat species detection down to 0.1% w/w
- Quick and simple workflow for any laboratory
- High resolving power (140.000 FWHM) was needed to obtain sufficient selectivity
- Isotopically labelled peptides recommended for routine control
- Tested in routine – applying HPLC-Q Exactive



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Food Additives & Contaminants: Part A

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tfac20

Assessment of meat authenticity using bioinformatics, targeted peptide biomarkers and high-resolution mass spectrometry

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[b] Department of Environment & Food Safety, Thermo Fisher Scientific, San Jose, CA, USA

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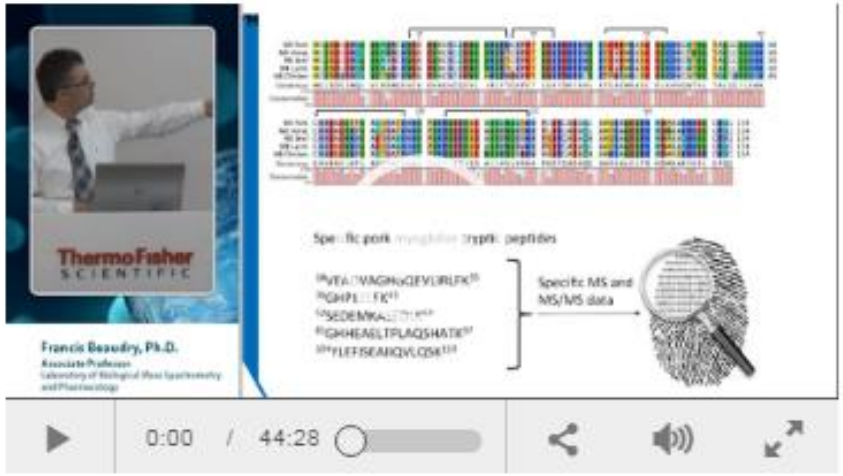
Webinar: Thermo Fisher Scientific Meat Adulteration Resource

Webinar



Meat Species Determination and Adulteration Detection using High-Resolution Mass Spectrometry and Proteogenomic Strategy

A new strategy is presented for identifying meat adulteration and authenticity by merging bioinformatics and a targeted bottom-up proteomic approach using LC coupled to Orbitrap HRAM mass spectrometry.

Video

Sample Preparation for Meat Species Determination and Adulteration using High-Resolution Mass Spectrometry

Learn how to carry out each of the sample preparation steps required for preparing meat samples prior to analysis by LC coupled to Orbitrap HRAM MS.

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