MS technologies

2023 AEIC Fall Meeting

Jeff Shippar – Senior Technical Manager, Eurofins Food Testing, US

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Mass Spectrometry technology

Many different types

- **MS**
- **MSMS**
- **qTOF**
- **Triple quadrupole (QQQ)**
- **Orbitrap**
- MALDI
- Magnetc Sector

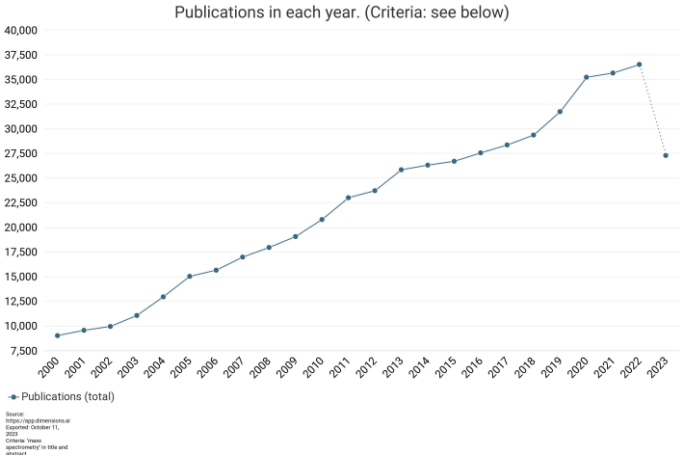
…and many more

…and many different front ends

- **Direct infusion**
- **HPLC/UHPLC (LCMS)**
- **GC (GCMS)**
- **Supercritical fluid (SFE)**
- IC
- nanoLC
- microLC
- Drift tube
- …and many more

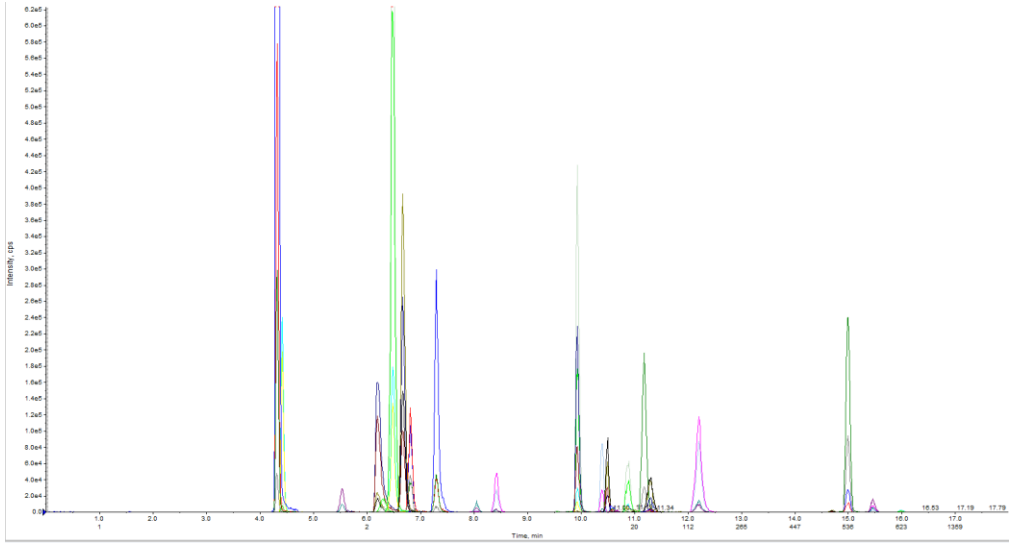
Outline

- Mass Spectrometry (MS) technology
  - Triple Quadrupole LCMS
  - Challenges for Protein analysis
- High Resolution Mass Spectrometry
- “Emerging” LCMS approaches for protein quant and ID
  - Parallel Reaction Monitoring (PRM)
  - Ion mobility



Mass Spectrometry

- **Target mass (m) / Target charge (z) = m/z**
- **Advantages**
  - Specificity
  - Sensitivity
  - Repeatability
  - Curve range
  - Turnaround time
  - Flexibility



MS technology – Food Analysis

Food and Chemical Toxicology 126 (2019) 313–321

Quantitation of seven transmembrane proteins from the DHA biosynthesis pathway in genetically engineered canola by targeted mass spectrometry

Michelle L. Colgrave[a,*], Keren Byrne[a], Sapna Vibhakaran Pillai[b], Bei Dong[b], Antonio Leonforte[c], Joanne Caine[d], Lukasz Kowalczyk[d], Judith A. Scoble[a], James R. Petrie[b], Surinder Singh[b], Xue-Rong Zhou[b]

[a] CSIRO Agriculture and Food, 306 Carmody Rd, St Lucia, QLD 4067, Australia
[b] CSIRO Agriculture and Food, GPO Box 1600, Canberra, ACT 2601, Australia
[c] Nuseed, 5 Ballinger St, Horsham, VIC, 3400, Australia
[d] CSIRO Manufacturing, 343 Royal Parade, Parkville, VIC, 3052, Australia

Food Chemistry: X 5 (2020) 100080

An improved LC–MS method to profile molecular diversity and quantify the six main bovine milk proteins, including genetic and splicing variants as well as post-translationally modified isoforms

Guy Miranda[a,*], Leonardo Bianchi[a], Zuzana Krupova[a,1], Philippe Trossat[b], Patrice Martin[a,*]

[a] UMR GABI, INRAE, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[b] ACTALIA, pôle expertise analytique, 39801 Poligny, France

J AOAC Int. 2020 Nov 30;qsaa162. doi: 10.1093/jaoacint/qsaa162. Online ahead of print.

Determination of total and A1–Type β–casein in milk and milk–derivedingredients by liquid chromatography – mass spectrometry using characteristic tryptic peptides

Stefan Ehling [1], Meibo Wang [2], Luke Weber [1]

AOAC Official Method 2017.11
Identification of Pea, Rice, and Soy Protein in Raw Materials and Finished Goods
ESI HPLC-MS/MS
First Action 2017

AOAC Official Method 2017.12
Identification of Milk Proteins in Raw Materials and Finished Goods
ESI HPLC-MS/MS
First Action 2017

CODEX ALIMENTARIUS
International Food Standards
World Health Organization
Food and Agriculture Organization of the United Nations

AOAC INTERNATIONAL

ISO

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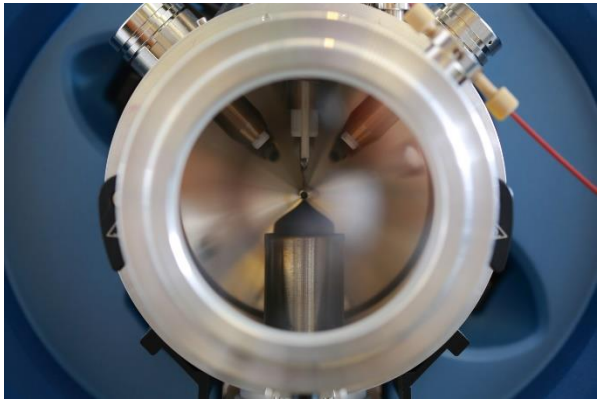
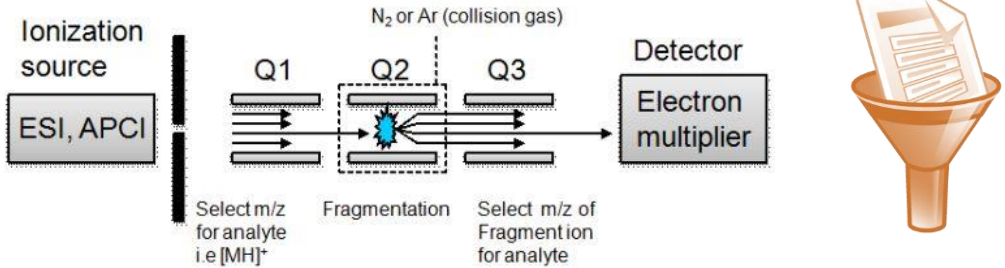
LCMS Triple quadrupole (QQQ)





TY - CHAPJO - Topics on Drug MetabolismAU - Jinsong NiAU - Josh RoweTI - Microdosing Assessment to Evaluate Pharmacokinetics and Drug Metabolism Using Liquid Chromatography-Tandem Mass Spectrometry TechnologyY1 - 2012-02-22 N1 29249 UR http://www.intechopen.com/books/export/citation/ReferenceManager/topics-on-drug-metabolism/microdosing-assessment-to-evaluate-pharmacokinetics-and-drug-metabolism-using-lc-mc-ms-technology ER -

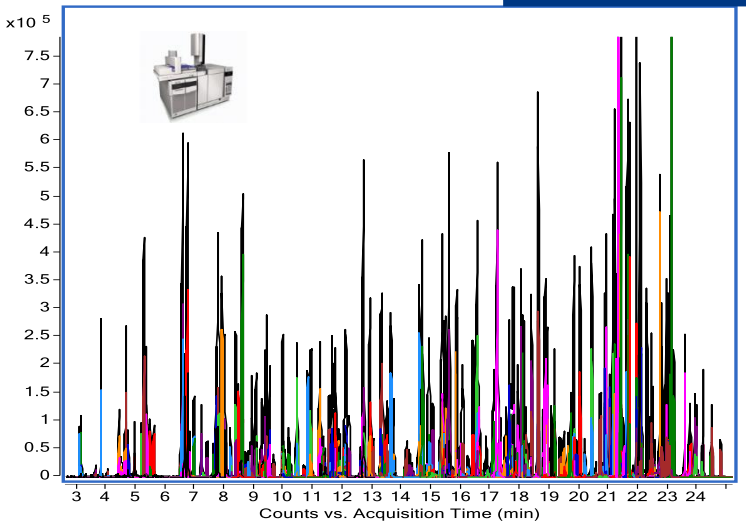
LCMS is fast, sensitive

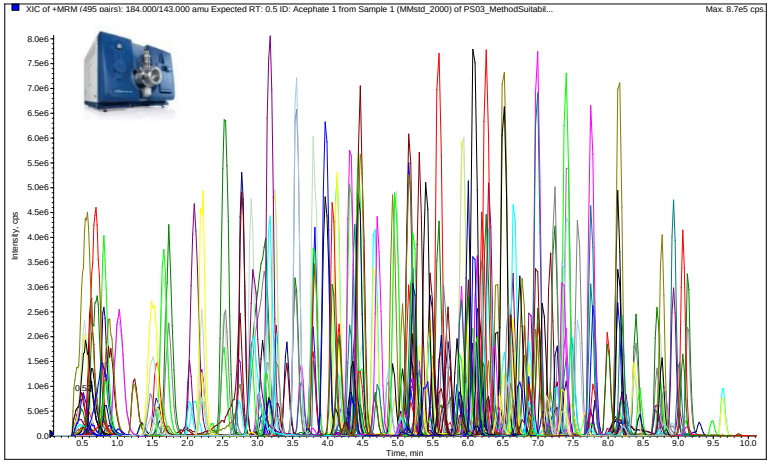
Pesticide analysis

Sample Preparation

QuEChERS

GC-MS/MS | **LC-MS/MS**





GC-MS/MS
(~290 analytes)

BOTH
(~235 analytes)

LC-MS/MS
(~460 analytes)

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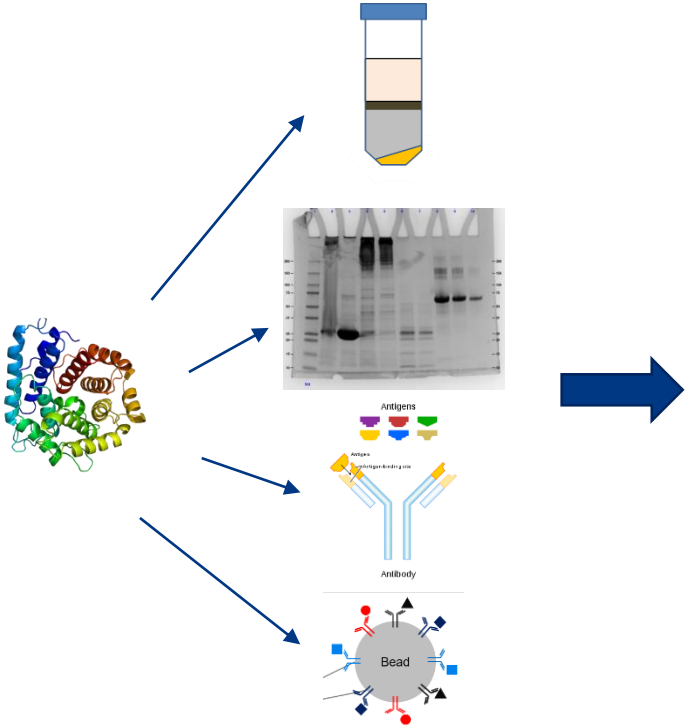
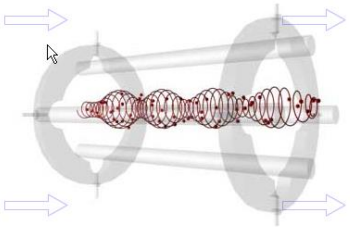
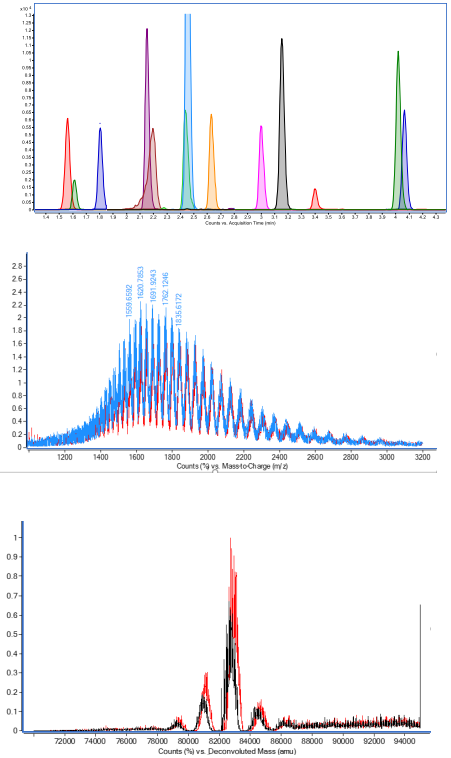
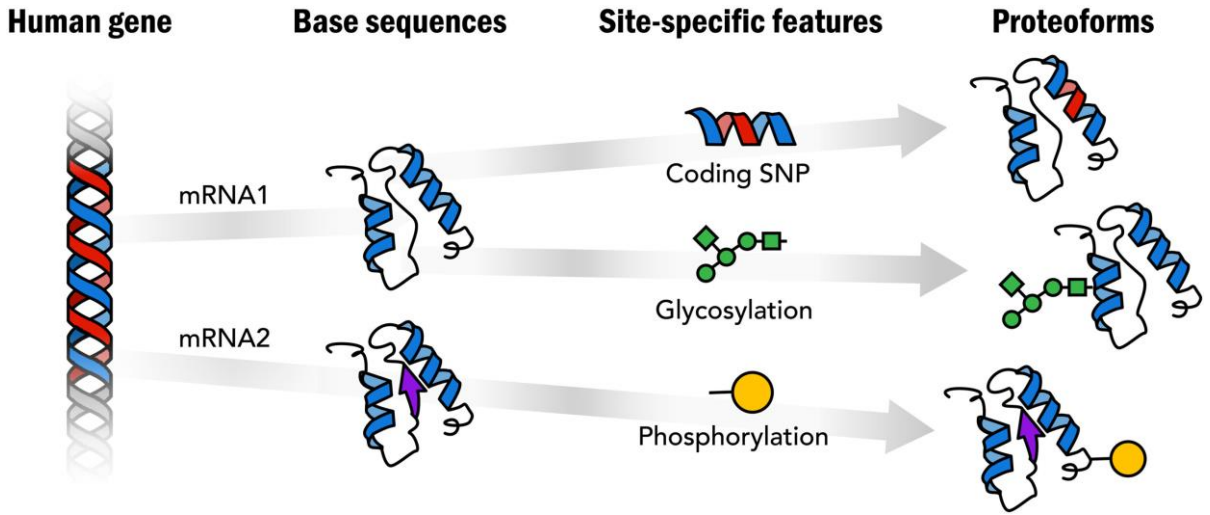
LCMS is versatile







Challenges - Proteoforms



•Lloyd M. Smith et al. The Human Proteoform Project: Defining the human proteome.*Sci. Adv.***7**,eabk0734(2021). DOI:10.1126/sciadv.abk0734

Proteoform
- Not one form or state
- Multiple, variable modifications
- Standard material vs sample material

Post Translational Modifications (PTMs) affect
- Chromatography
- Observed mass (range)
- Observed peptides

(Also Food Processing effects)

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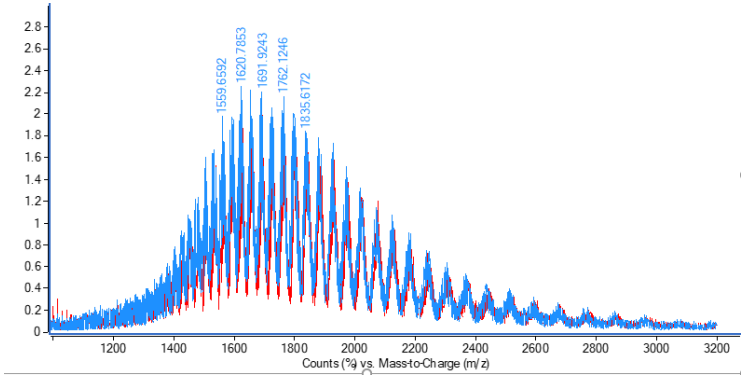
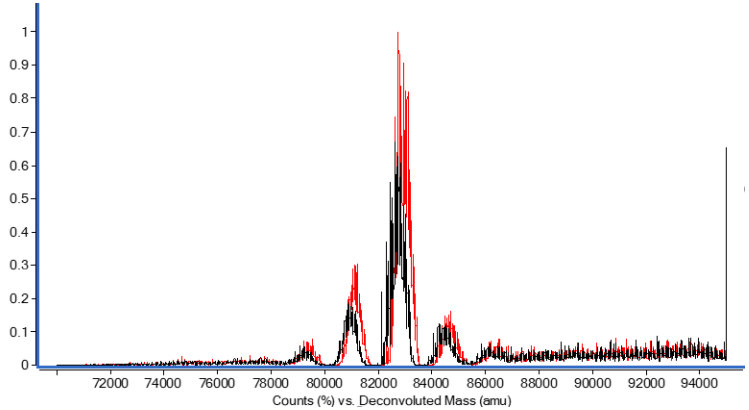
MS Challenges for targeted protein analysis (Top down)

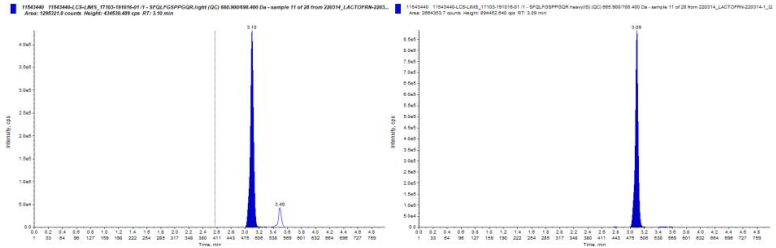
- Proteoforms – Multiple masses
- Multiple charge states – Decreased sensitivity
- Most samples are very complex. Many proteins.
- Need specific cleanups or multiple targets/measurements





Protein quantitation – Surrogate Peptide (Bottom up)

- Digestion to specific predicted fragments
  - Enzyme specificity
  - Complex samples
  - Quantitation using surrogate peptide
    - Sensitivity
    - Specificity
    - Stable Isotope Labeled (SIL) peptide
      - Internal Standard



APRKNVRWCTISQPEWFKCRRWQWRMKKLGAPSITCVRRAFALECIRAIAEKKADAVTLDGGMVFEAGRDPYKLRPV
AAEIYGTKESPQTHYYAVAVVKKGSNFQLDQLQGRKSCHTGLGRSAGWIIPMGILRPYLSWTESLEPLQGAVAKFFSAS
CVPCIDRQAYPNLCQLCKGEGENQCACSSREPYFGYSGAFKCLQDGAGDVAFVKETTVFENLPEKADRDQYELLCLNNS
RAPVDAFKECHLAQVPSHAVVARSVDGKEDLIWKLLSKAQEKFGKNKSRSFQLFGSPPGRDLLFKDSALGFLRIPSKVDS
ALYLGSRYLTTLKNLRETAEEVKARYTRVVWCAVGPEEQKKCQQWSQQSGQNVTCATASTTDDCIVLVLKGEADALNL
DGGYIYTAGKCGLVPVLAENRKSSKHSSLDCVLRPTEGYLAVAVVKKANEGLTWNSLKDKKSCHTAVDRTAGWNIPMG
LIVNQTGSCAFDEFFSQSCAPGADPKSRLCALCAGDDQGLDKCVPNSKEKYYGYTGAFRCLAEDVGDVAFVKNDTVWE
NTNGESTADWAKNLNREDFRLLCLDGTRKPVTEAQSCHLAVAPNHAVVSRSDRAAHVKQVLLHQQALFGKNGKNCP
DKFCLFKSETKNLLFNDNTECLAKLGGRPTYEEYLGTEYVTAIANLKKCSTSPLLEACAFLTR

Denature,
Digest

Measure
SFQLFGSPPGQR.light
SFQLFGSPPGQR.heavy,

GSNFQLDQLQGR.light
GSNFQLDQLQGR.heavy

https://www.uniprot.org/uniprot/P24627

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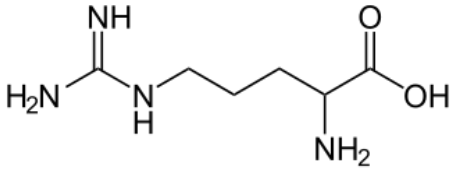
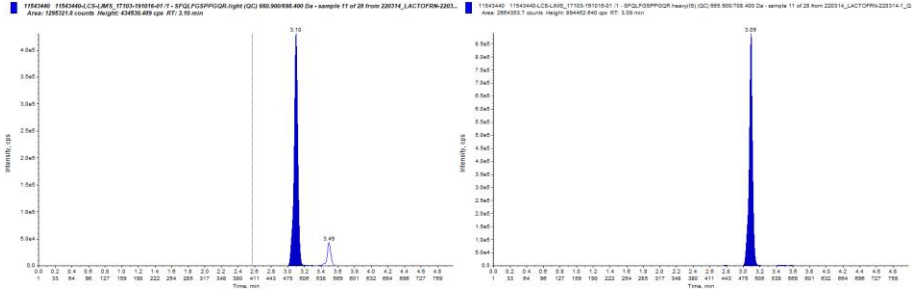
Custom Peptide Synthesis and Stable Isotope Labeled (SIL) Peptide



Arginine

H2N-VLIVPQNFVVAAR^-OH
13 residues
>95% Purity
2500ug NET all material to be used in creating peptide mixture. See details
Amino Acid Analysis (AAA) - Concentration - Single

R^ = Arginine (13C6,15N4)



"Light" peptide = unmodified

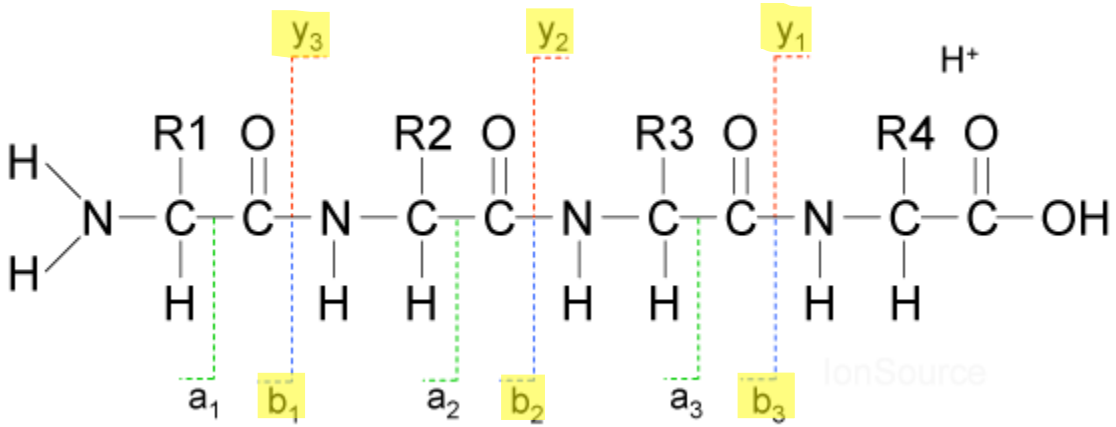
SIL peptide spiked in all samples and calibrators at the same concentration.
Will behave the same:

- Chromatography (e.g. LC retention time)
- MS Fragmentation

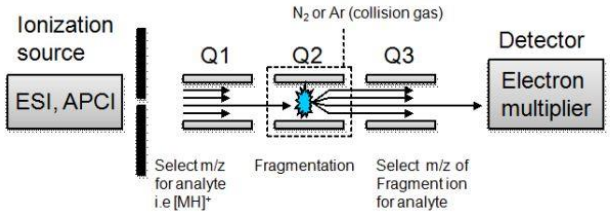
Observed Precursor Mass +10 amu

Observed Product Mass same or +10 (y/b ions)

Surrogate Peptide fragmentation



- “y” and “b” ions most commonly used
- Specificity from fragmentation patterns
- One for quant, one+ for confirmation



https://www.ionsource.com/tutorial/DeNovo/nomenclature.htm

Quantitation strategies

- Best option is protein of interest for calibration curve
  - Spiked at beginning of procedure
  - Experiences all sample treatment conditions (denaturation, modification, digestion)
  - Must be well characterized
- Peptides are very commonly used for standards
  - Light peptide neat curve with SIL
  - Matrix-matched preferred
  - SIL peptide only for endogenous compounds

$$Concentration\ light = \frac{Peak\ area\ light}{Peak\ area\ SIL} \times Concentration\ SIL$$

Emerging MS technologies

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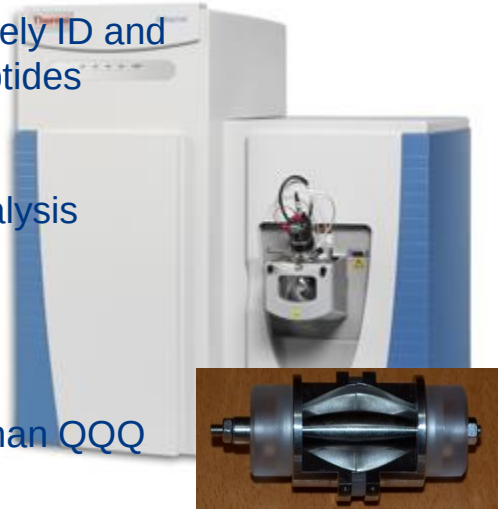
High Resolution Mass Spectrometry (HRMS)

- Time of Flight (TOF)
- Orbital Trap (FT)

Resolution is high enough to definitively ID and separate elemental isotopes and peptides

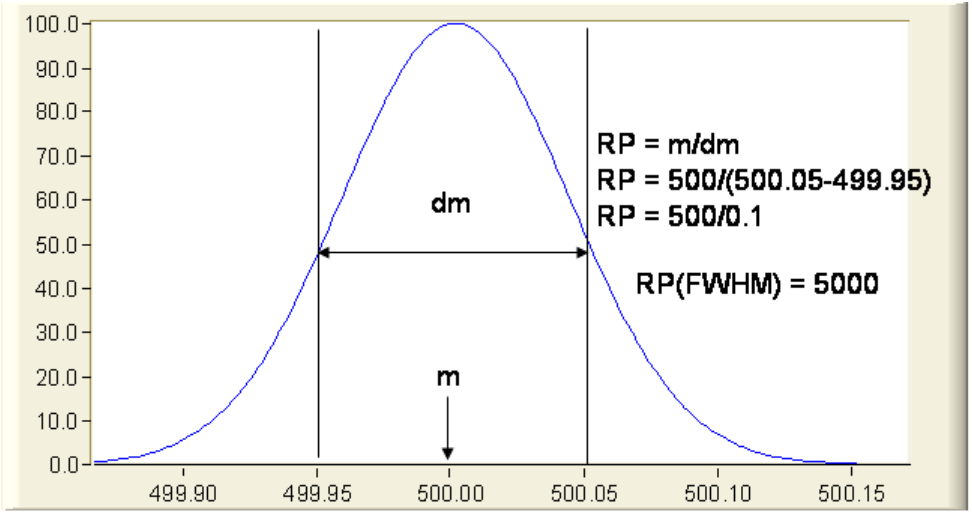
Peptide mapping, untargeted analysis



But…

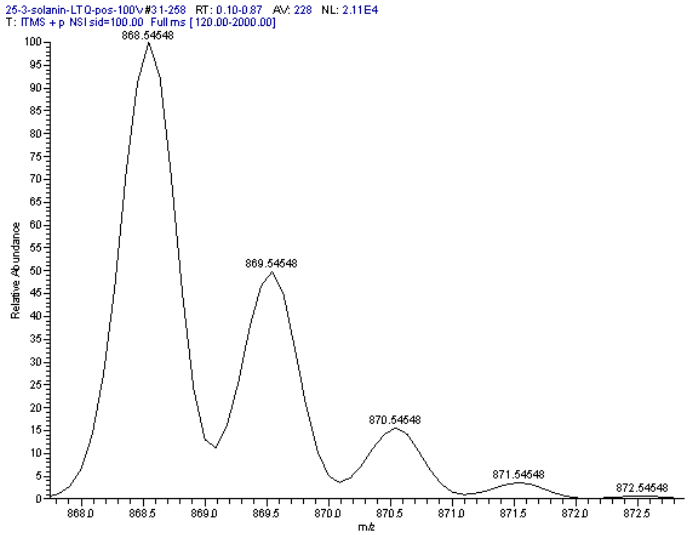
- Sensitivity typically much lower than QQQ
  - Microflow, nanoflow LC
  - Throughput

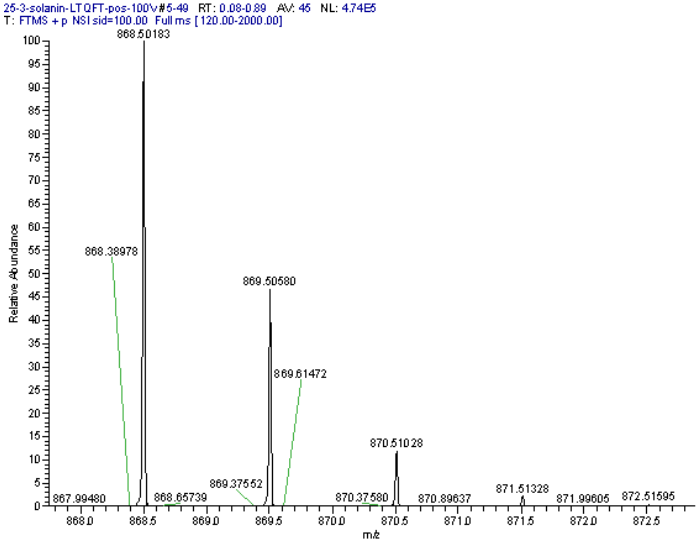
Mass Spec Resolution



- RP = Resolving Power
- FWHM = Full width at half maximum

Mass Spec Resolution





- Resolving power of ~2000 VS ~50,000
  - Need this level of detail to truly identify peptides
  - High resolution mass spectrometry typically greater than 20,000-30,000 (<5ppm mass error)

Targeted vs Non-Targeted Approach

TARGETED ANALYSIS

Question:

Is it in the sample?

MASS SPECTROMETRY and hyphenated techniques (especially LC and GC) are the tools most frequently used in non-targeted (food) analysis workflows

NON-TARGETED ANALYSIS

Question:

What is in the sample?

"Okay—who put my lunch through the mass spectrometer..?"

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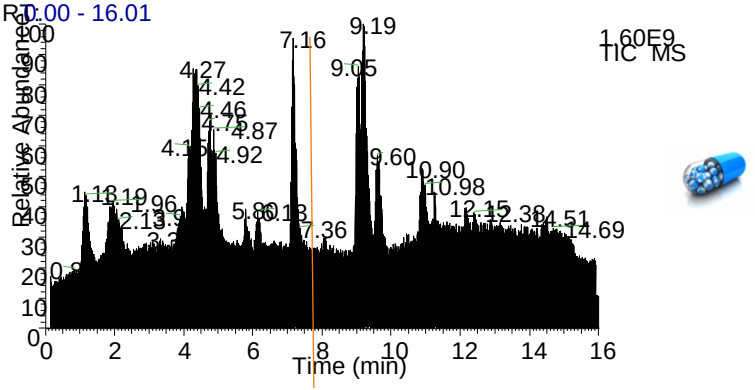
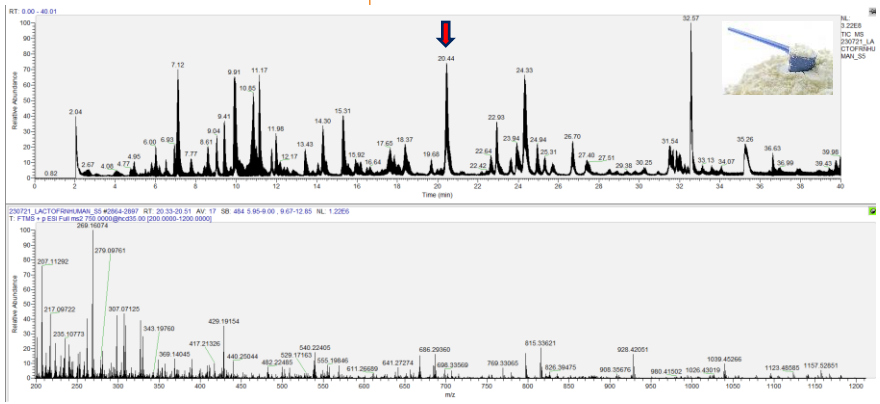
What does the data look like?





Identification of Unknown Compounds

1. LC-HRMS(/MS) DATA OF THE "FEATURE"
2. ELEMENTAL FORMULA ESTIMATION
3. SEARCH IN MS LIBRARIES & (BIO)CHEMICAL DATABASES (SOFTWARE)
4. IDENTITY CONFIRMATION

Typically the most challenging and time-demanding step of the non-targeted analysis workflow…

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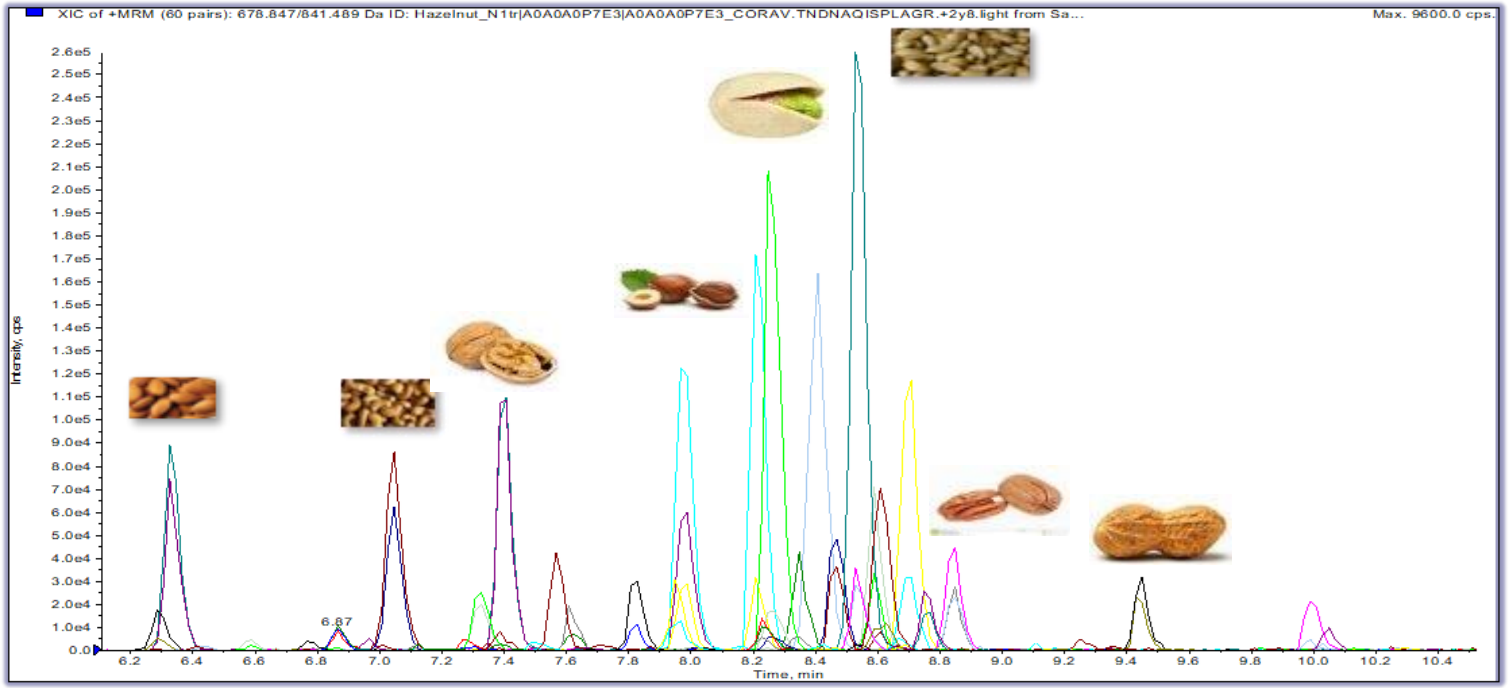
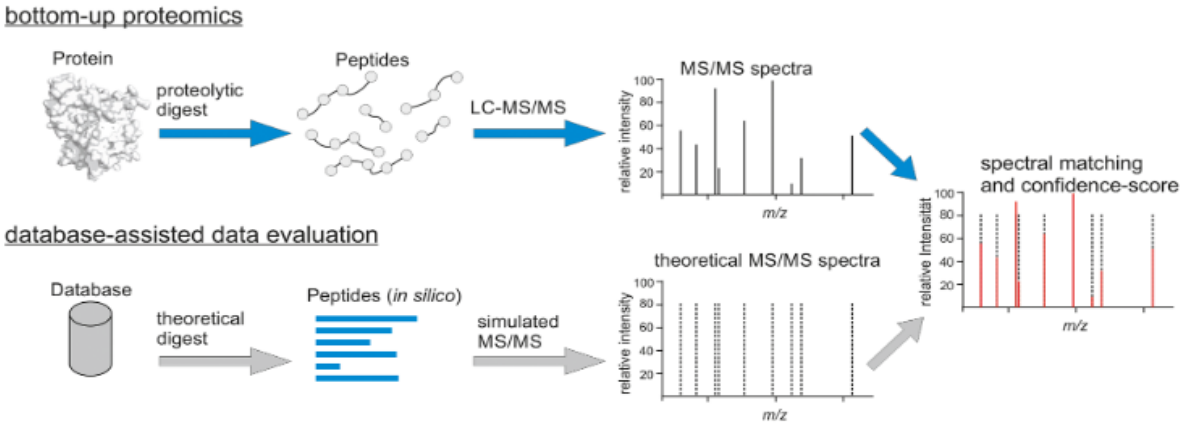
Food allergens LCMS



Parallel Reaction Monitoring (PRM)

J. Agric. Food Chem. 2018, 66, 8935−8940



Journal of proteome research
pubs.acs.org/jpr
Article

Parallel Reaction Monitoring Mass Spectrometry Method for Detection of Both Casein and Whey Milk Allergens from a Baked Food Matrix

Bini Ramachandran, Charles T. Yang, and Melanie L. Downs*

Cite This: J. Proteome Res. 2020, 19, 2964−2976
Read Online

- Detect modified peptides
- Relative quant only
- TMI in some cases?

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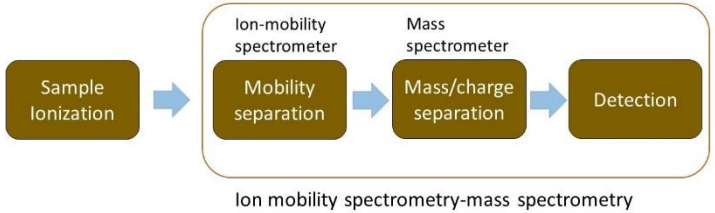
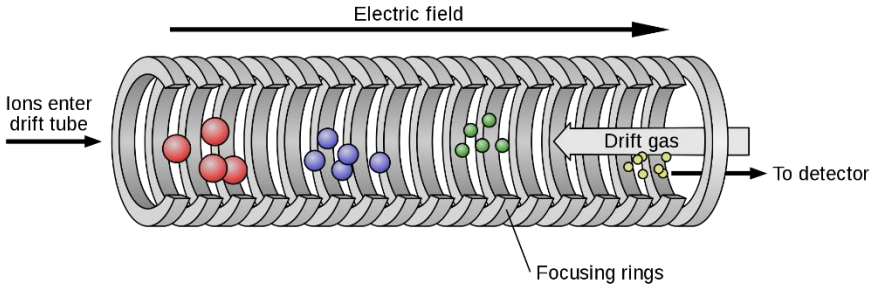
Ion mobility MS



Ion mobility spectrometry-mass spectrometry

- Isobaric (same m/z) analytes/interferences
- Chiral analysis
- Positional isomers



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Ion Mobility MS - Structures for lossless ion manipulations (SLIM)

Journal of the American Society for Mass Spectrometry

pubs.acs.org/jasms Research Article

High-Resolution Ion-Mobility-Enabled Peptide Mapping for High-Throughput Critical Quality Attribute Monitoring

James R. Arndt,* Kelly L. Wormwood Moser, Gregory Van Aken, Rory M. Doyle, Tatjana Talamantes, Daniel DeBord, Laura Maxon, George Stafford, John Fjeldsted, Bryan Miller, and Melissa Sherman

Cite This: *J. Am. Soc. Mass Spectrom.* 2021, 32, 2019−2032 Read Online

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From Moblion website (licensee):

SLIM separation technology is different. SLIM makes ions turn corners, not just travel straight. This enables a single-pass flight path up to 13 meters, providing resolving powers of >250 that can distinguish differences as small as 0.2% in collision cross section (CCS) values.

https://www.mobilionsystems.com/

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Ion Mobility MS - Structures for lossless ion manipulations (SLIM)

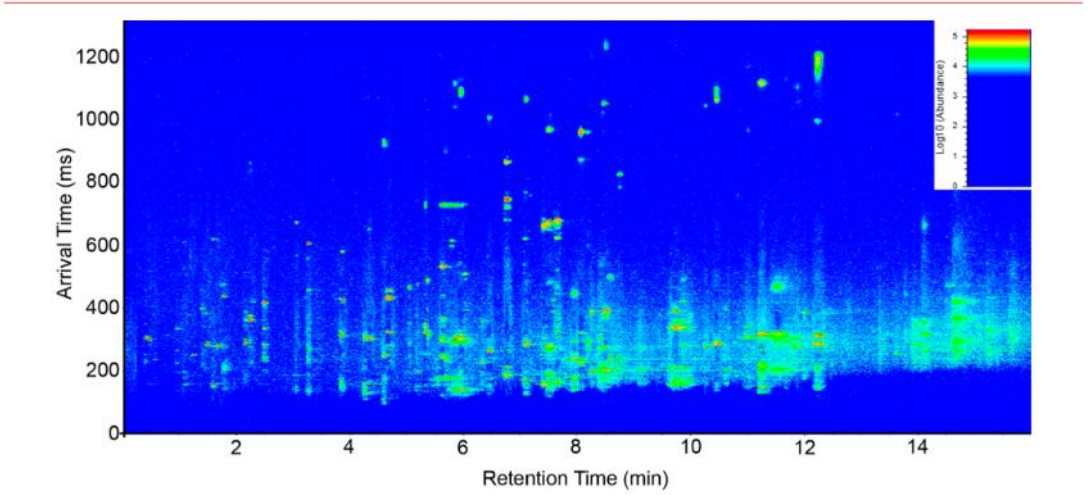

Figure 3. Two-dimensional arrival time vs retention time plot for the NISTmAb tryptic digest. Intensity scale is shown as the inset.

MOBILion SYSTEMS, INC.

BOKU

Simulations, experimental matching

https://www.mobilionsystems.com/

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Thank You!

Questions?

www.eurofinsUS.com/food

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