Analytical Chemistry Capabilities Supporting Bioscience Research

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1. Introduction

Sandia's Materials Characterization Department employs a variety of tools to support bioscience research for both internal and external customers. The capital investment and expertise accessible internally enables cutting-edge analytical support. Additional methods and instrumentation not discussed here are also available. Analytical chemistry consists of three categories of techniques: Collection, Separation, and Detection. Examples of Separation and Detection are presented.

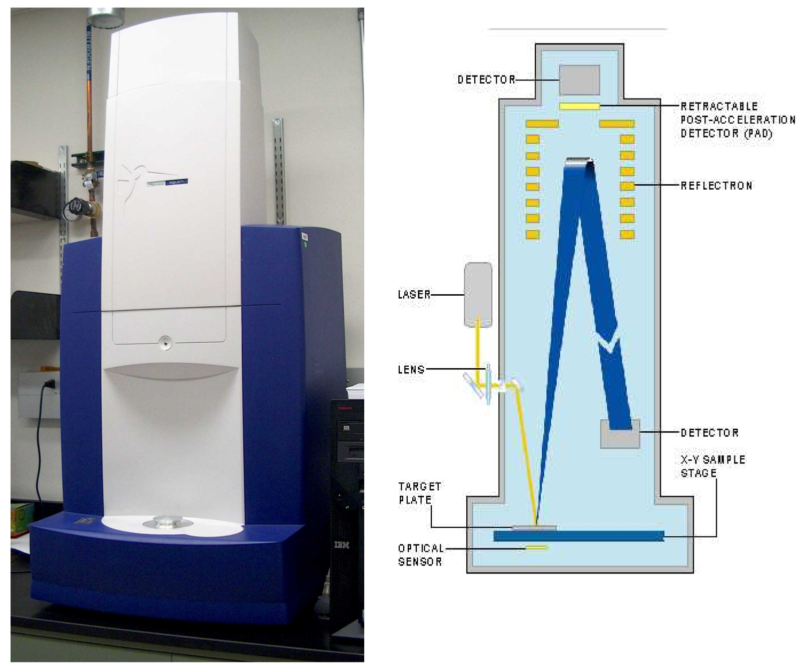

Figure 1. Photo and ion optic schematic of high resolution reflectron mode time-of-flight (TOF) mass spectrometer.

2. Challenging Small Molecules

We use "traditional" gas chromatography / mass spectrometry (GC/MS) tools for small molecule identification and quantification; however some species are not amenable to these tools. Figure 2 shows TOF-MS analysis (using instrument in Fig. 1) of a thermally labile synthetic boron compound[1]. The high resolution mass measurement and isotope distribution confirms composition. BENEFIT: rapid high accuracy mass measurement.

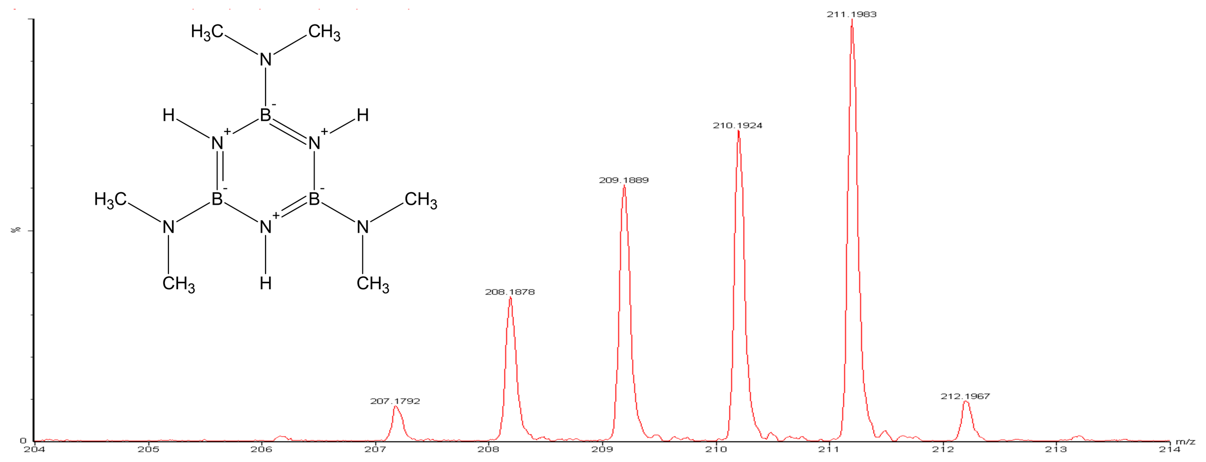

Figure 2. High resolution TOF mass spectrum of synthetic boron.

3. Biodiesel Applications

There are significant investments in biodiesel research today – the "fuel" being oils and triglycerides. Direct analysis by TOF-MS can be a screening tool and/or save analysis time over traditional GC/MS like in a biodiesel ASTM method[2]. Mixture analysis by TOF-MS (Fig. 3) provides high mass accuracy and resolution to confirm species WITHOUT separation. BENEFIT: simple qualitative composition assessment, high mass accuracy confirms bond saturation levels.

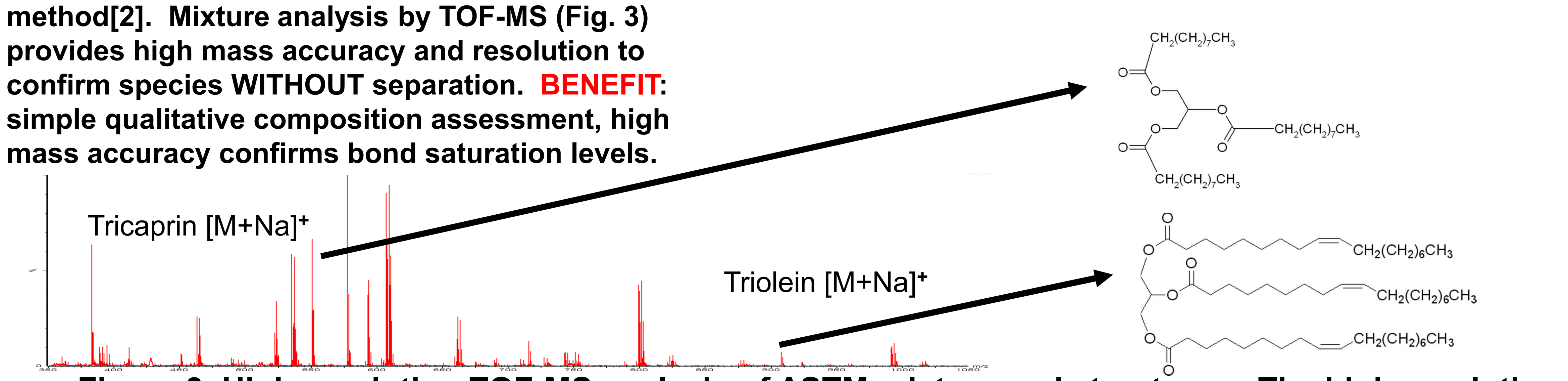

Figure 3. High resolution TOF-MS analysis of ASTM mixture and structures. The high resolution data allows confirmation of saturation level of triglycerides.

4. Very Large Biomolecules

Matrix-assisted laser desorption ionization (MALDI) using the instrument in Fig. 1 is the method of choice for confirmation of molecular weight of large (>1,000 dalton) and very large (>50,000 dalton) molecules such as proteins, peptides, and other biopolymers. Molecular weight measurement is useful for bioscience research related to identification, differentiation, expression, modification, purification, and a variety of other applications. BENEFIT: accurate high mass measurement of intact biopolymers.

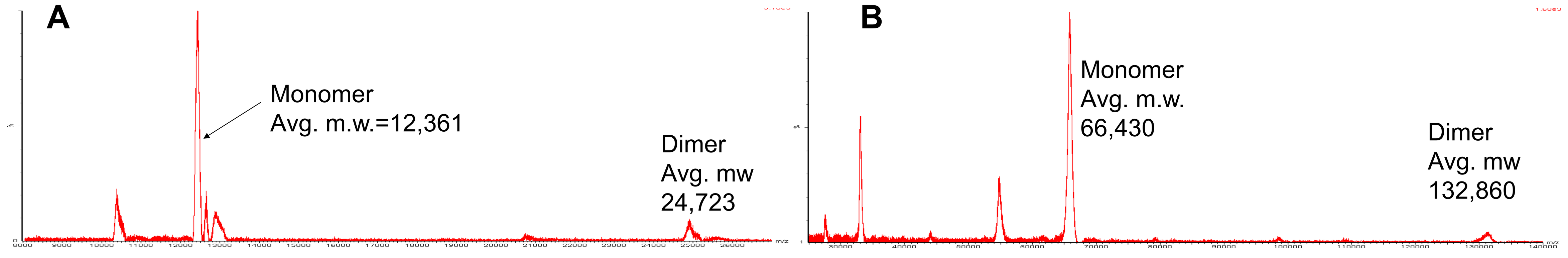

Figure 4. MALDI TOF-MS spectra of A) cytochrome C (equine); and B) bovine serum albumin.

5. Liquid Chromatography MS/MS

Complex mixtures and biological samples are examples that require additional separation methods for enabling bioscience research. Figure 5 shows a high pressure UPLC instrument with a Q-TOF mass spectrometer capable of MS/MS mode allowing fragmentation and structure confirmation.

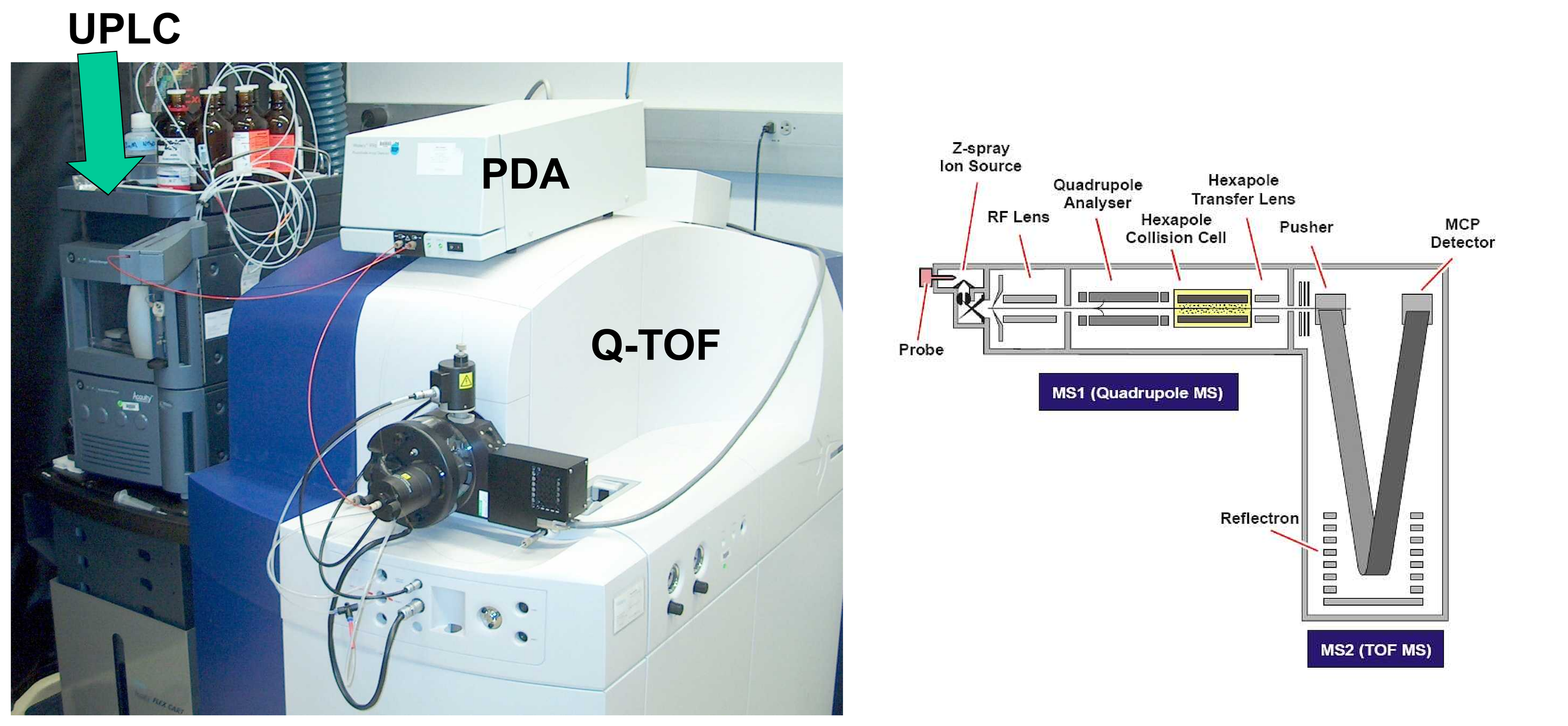

Figure 5: High pressure UPLC with Photodiode Array (PDA) Detector and Q-TOF mass spectrometer.

The Figure below illustrates a separation of 9 peptides of varying length. The experiment provides 4 dimensions of information for each species – (DIM-1) time axis representing separation or retention time, (DIM-2) optical absorbance with PDA detector, (DIM-3) total signal in ion counts, and (DIM-4) mass spectrum (mass to charge or m/z) of each peak. An additional data dimension (See Fig. 7, DIM-5) is possible by performing MS/MS which fragments a desired analyte. MS/MS data is most often used to determine additional chemical information such as peptide sequence. BENEFIT: high confidence multi-dimensional measurements and sequencing.

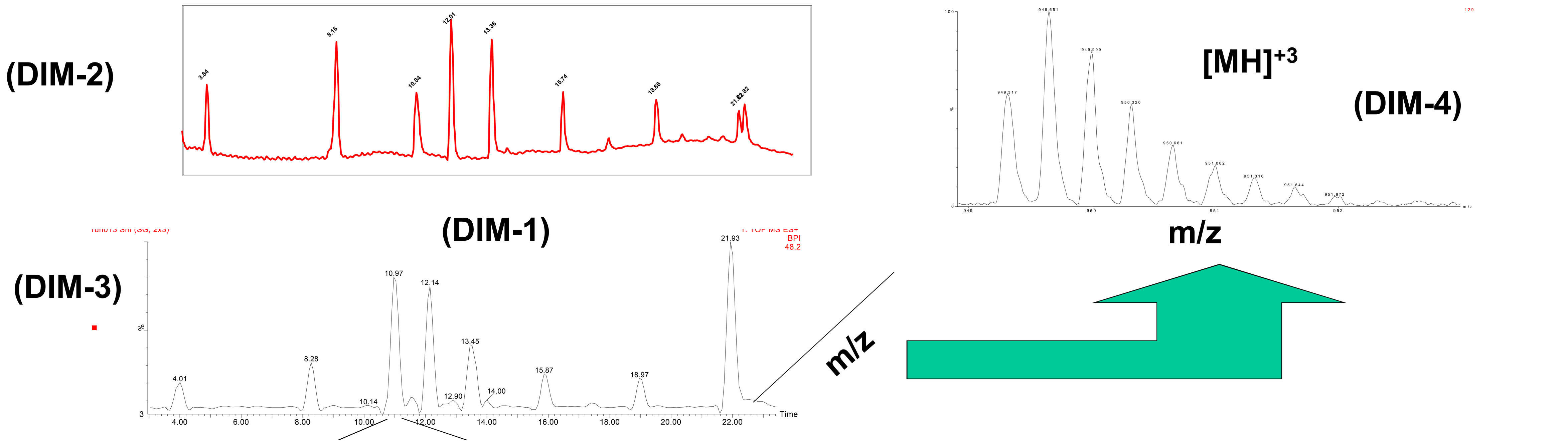

Figure 6. Separation and detection of peptide mixture providing 4 dimensional data.

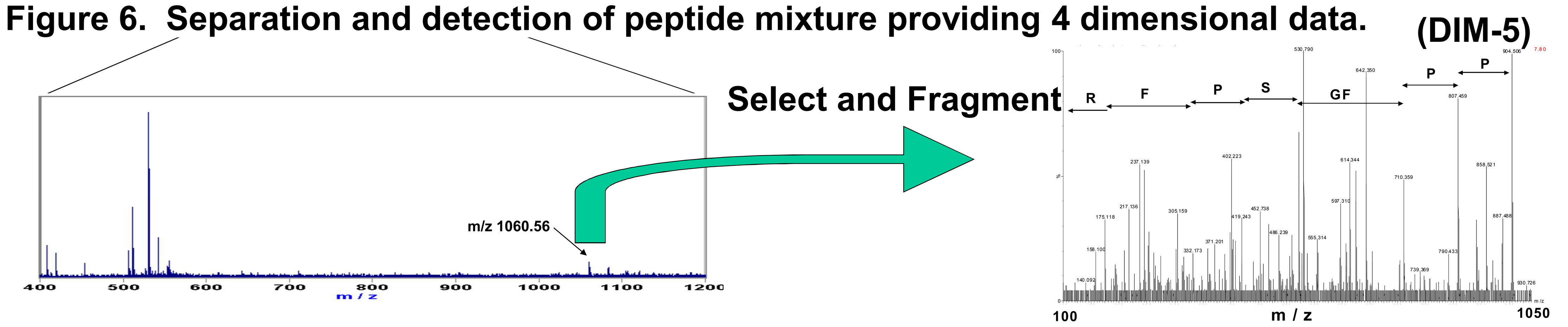

Figure 7. Example of MS/MS experiment – observed species is isolated on-line during separation (A) and subjected to fragmentation and measurement – generating MS/MS spectrum (B) of ions confirming sequence as RFPSFGPP. Provides an additional dimension of measurement capability.

3. Conclusions

Current capabilities are enabling measurements of molecular weight for challenging small molecules, biodiesel-related species, intact proteins and peptides, including sequence determination. Capabilities are in-house and a range of services from simple measurements to full collaboration are available.

References:

1. Niedenzu and Dawson, JACS, 1959, 81, 3561
2. ASTM 6584 Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters By Gas Chromatography.

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