

# Myth Busters: The Truth About Metabolomics and High Resolution Gas Chromatography Time-of-Flight Mass Spectrometry

David E. Alonso, Joe Binkley, and Lorne Fell | LECO Corporation, St. Joseph, MI USA

## Introduction

### Background

- The best approach for comprehensive profiling of metabolites is through a combination of instrumental techniques (e.g., GC-MS, LC-MS, FT-NMR, etc.).
- GC-MS offers analytical advantages that complement other analytical techniques.
- The intent of this poster is to dispel the myths about the shortcomings of GC-MS as a technique for metabolomic studies.

### The Myths

- GC-MS is only applicable for the analysis of non-polar compounds.
- Derivatization is required, is a waste of time, and provides inconclusive results.
- Only compounds with m/z values <400 can be analyzed by GC-MS.
- GC-EI MS fragmentation is problematic and hinders identification of metabolites.
- MS/MS experiments are necessary for confident characterization of metabolites (See 4 above).
- High resolution mass spectrometry is not required for metabolomic studies.

### Metabolomics: General Analytical Challenges

- Chemical Diversity of Compounds
- Complexity of Biological Matrices
- Confident Compound Identification and Quantification (bottleneck)

### The Solution: High Resolution GC-TOFMS

- High Quality Spectral Data
  - Comprehensive
  - Search Against Well-Established Databases (NIST, Wiley)
- Excellent Mass Accuracy Values (<1 ppm) = Robust Formulas for Fragment, Molecular, and Adduct Ions
- High Resolution Deconvolution™ (HRD™)
- High Resolving Power (up to 50,000) = Increased Selectivity for:
  - Discovery
  - Confirmation
  - Comprehensive Profiling of Complex Samples

## Experimental

### GC-MS Analysis Capabilities

Alcohols	Ionic Compounds	Organophosphates
Alkaloids	Lipids	Phenolics
Amino Acids	Nucleosides	Polar Organics
Carotenoids	Nucleotides	Prostaglandins
Catecholamines	Organic Acids/Diacids	Sterols
Eicosanoids	Organic Amines	Terpenes
Essential Oils	Organic Bases	Waxes

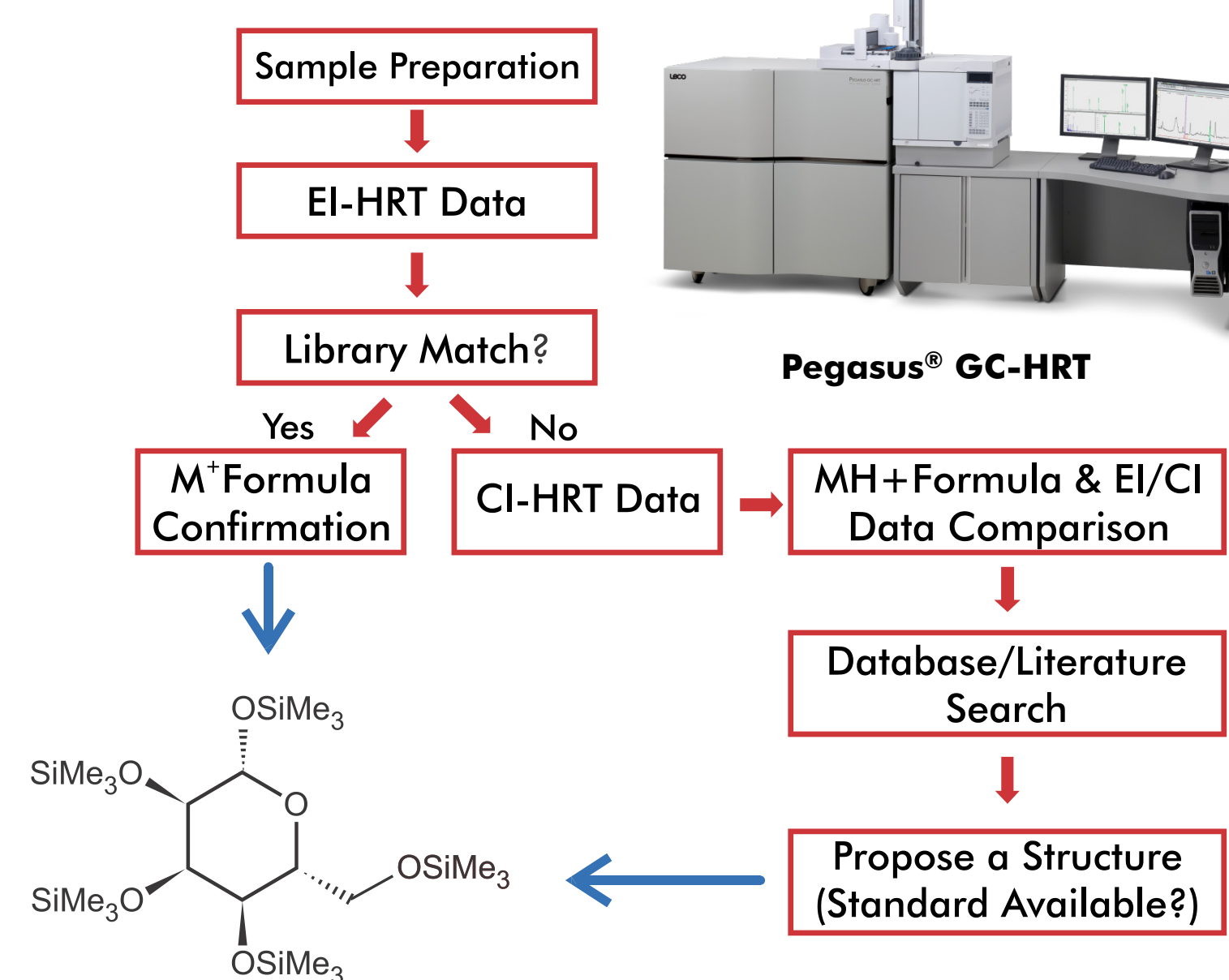
### General Sample Preparation Methods

Samples (100 µL) were dried for 2 hours using a Speed Vac and then for 4 hours in a lyophilizer (3 mbar, -50°C). The samples were derivatized with MEOX (25 µL, 60°C, 1 hr) and MSTFA (75 µL, 60°C, 1hr) and transferred to autosampler vials for analysis.

### Instrument Parameters

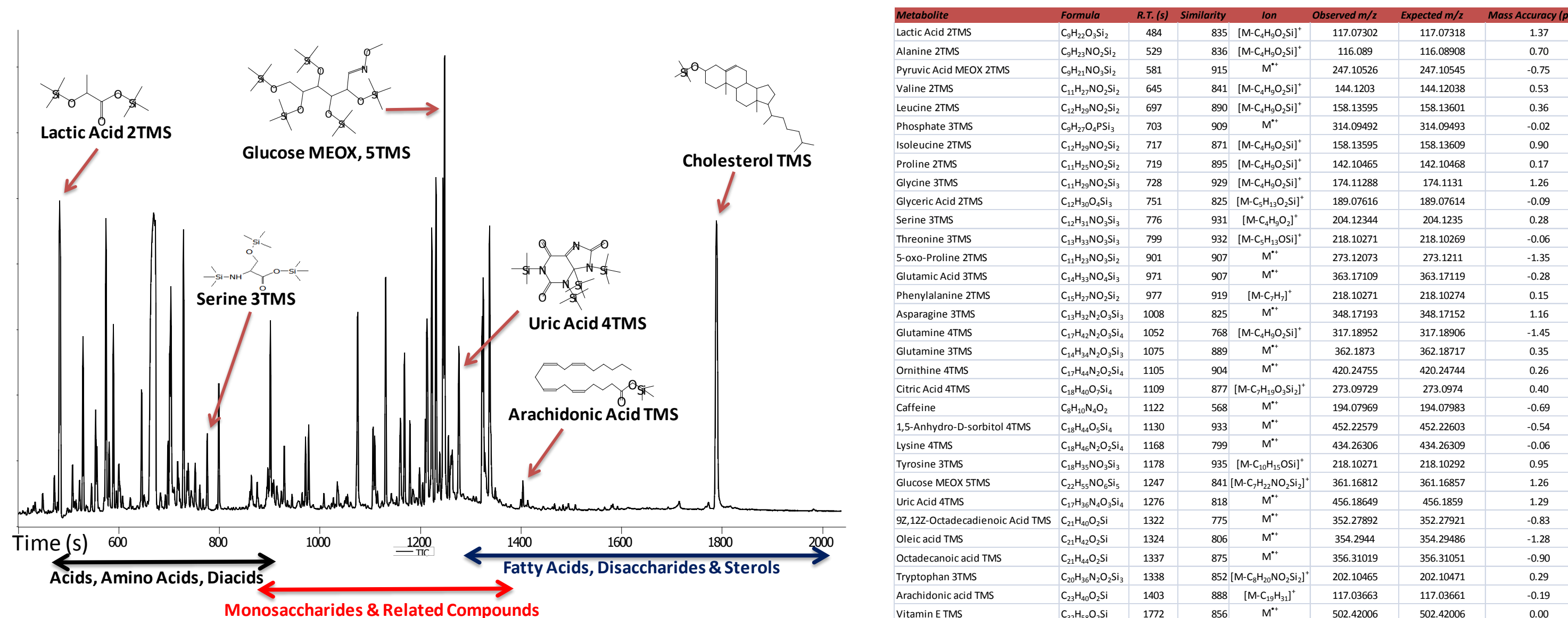
GC		Agilent 7890 with Gerstel MPS Auto Sampler	
Column	Restek Rxi-5 MS (30 m x 0.25 mm x 0.25 µm)	Carrier Gas, Flow	He, 1.0 mL/min Constant Flow
Injection Volume & Split	1 µL, Split 10:1 (CI, Splitless)	Inlet Temperature	270°C
Temp. Program	70°C (2 min) to 280°C at 20°C/min (3 min)		
MS		LECO Pegasus® GC-HRT	
Transfer Line Temp.	300°C	Ion Source Temp.	EI 250°C; CI 200°C
Ionization	EI (70 eV); CI (140 eV)	Mass Range	EI 35–510; CI 60–1000
Acquisition Rate	12 spectra/second	Calibration (Internal)	PFTBA
Reagent Gas	5% Ammonia in Methane		

### Workflow

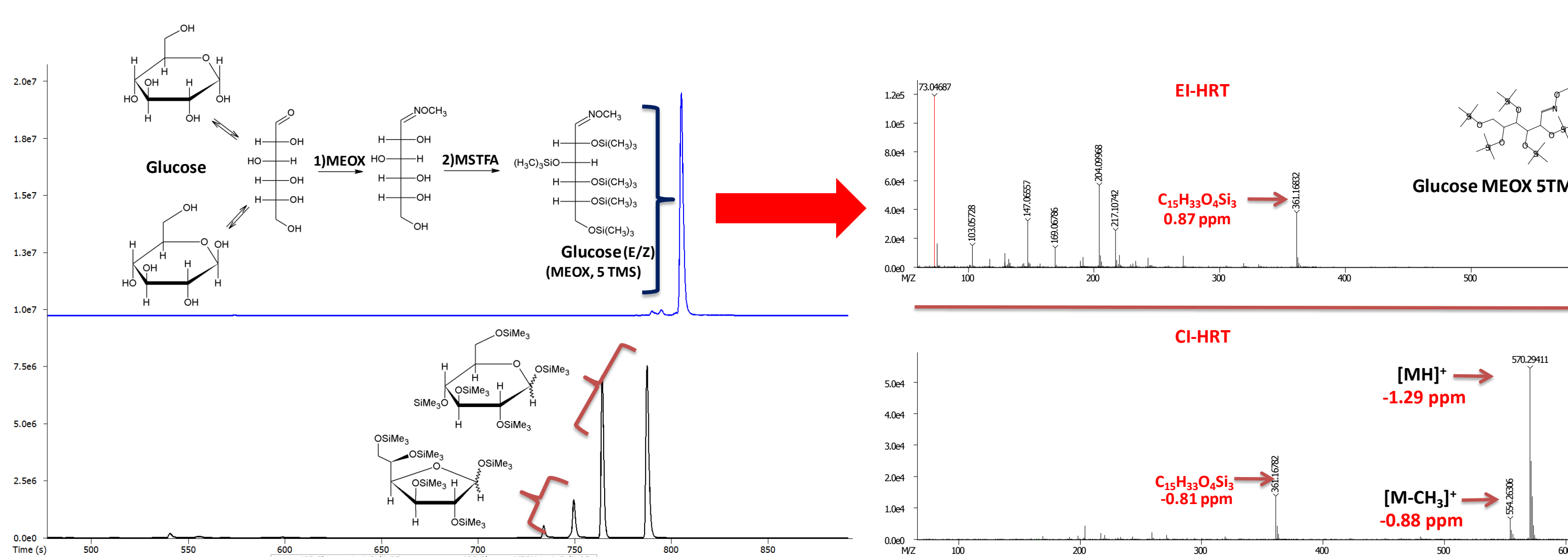


## Debunking The Myths (1–3)

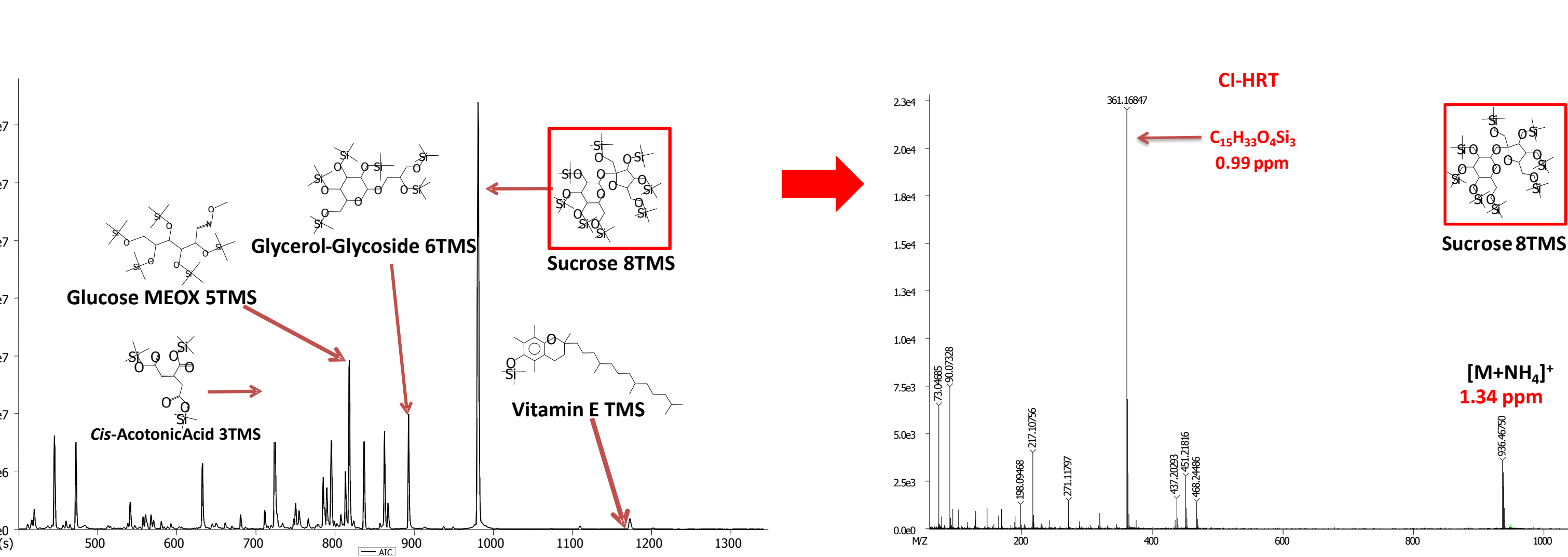
1) Analysis of a wide variety of metabolites (Human Plasma).



2) Derivatization is well-understood, routine, and can increase sensitivity while decreasing the complexity of samples (Human Serum).

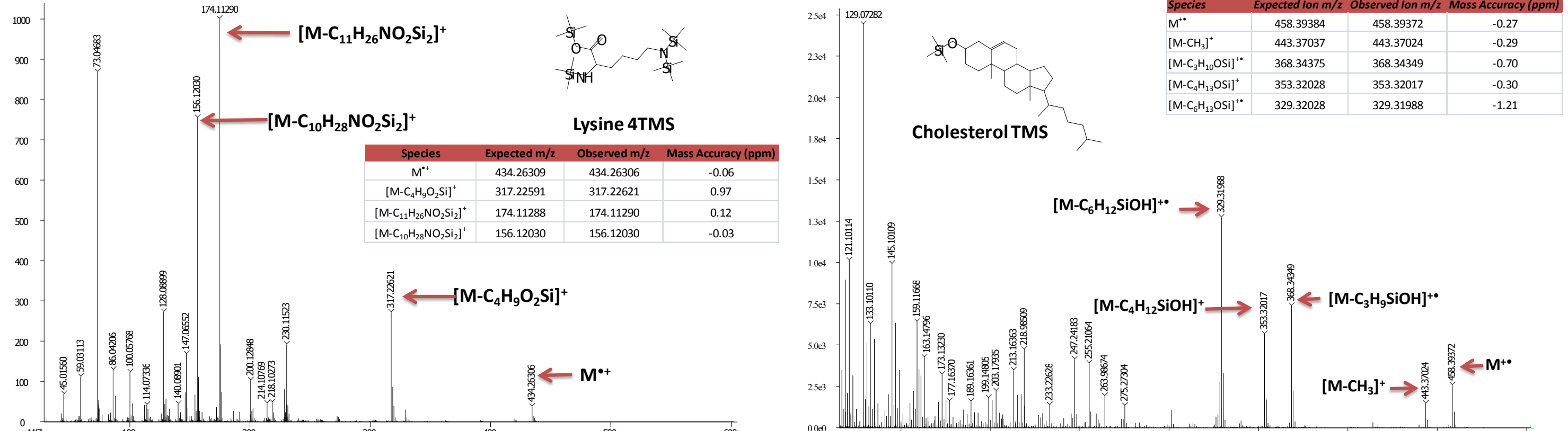


3) A workflow that includes CI-HRT data acquisition can provide valuable information for compounds with molecular weights >900 Da (Corn Extract).

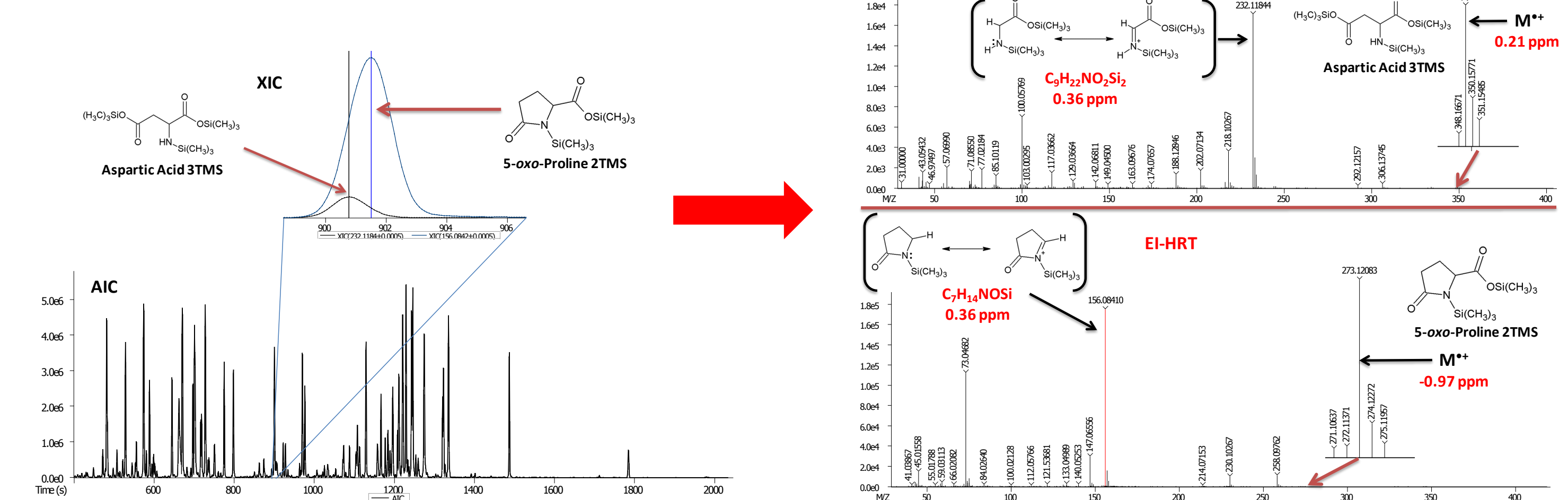


## Debunking The Myths (4-6)

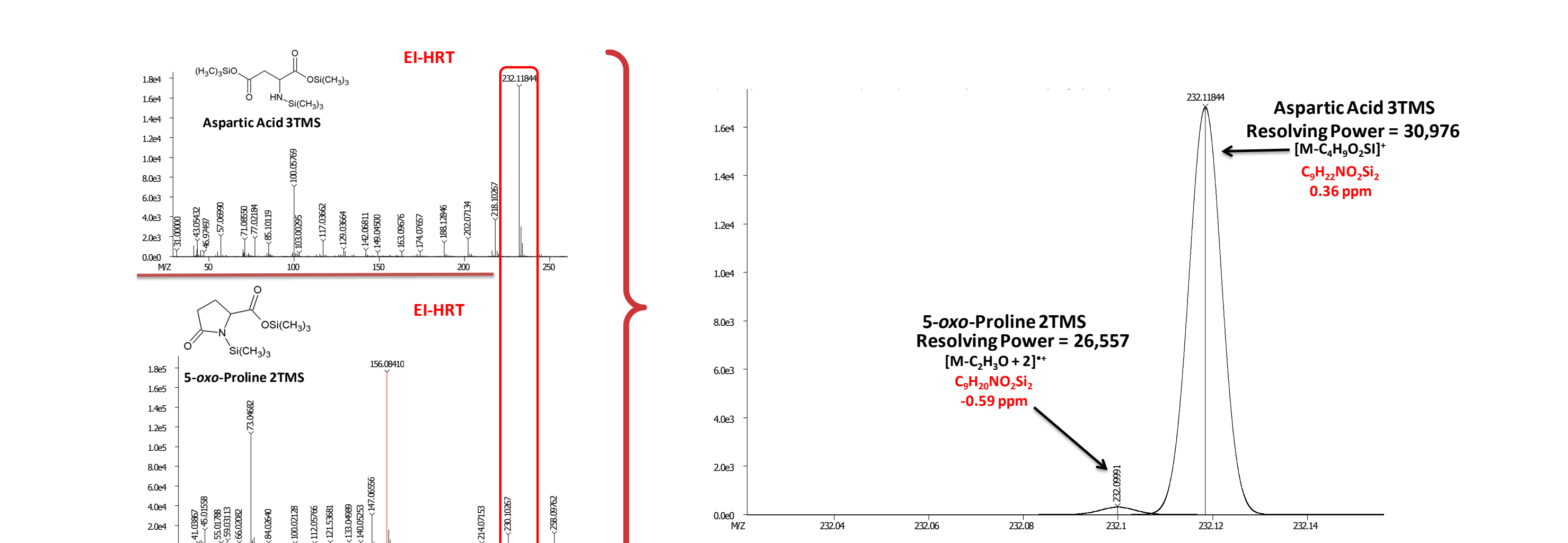
4) Fragmentation = structural characterization (Human Plasma).



5) Accurate mass ions provide confident structural characterization of metabolites (Human Serum). This enhanced selectivity, combined with HRD, facilitates the analysis of analytes in complex biological matrices.



6) High resolution minimizes isobaric interferences (Human Serum).



## Summary

- The data summarized in this presentation dispels myths associated with the utilization of GC-MS for analytes which are critical for metabolomic studies.
- GC-HRT provides high quality, accurate mass data for:
  - Spectral similarity searches (NIST, Wiley, etc.)
  - Formula determination (fragment, molecular & adduct ions)
- GC-HRT is an indispensable tool for the analysis of complex biological samples.