

MALDI-ToF and ESI-Ion Trap Mass Spectrometry

Schedule-UNH Tutorial; Glycomics Center, Durham, NH

9:30-9:40 Greeting and introduction

- a.) Schedule for the day
- b.) PEG Support: Resources & Aspirations
- c.) Overview of principle instruments

9:40-10:00 Tour of laboratory and mass spectrometry facility

10:00-10:30 Tutorial: MS considerations

- a) Tracking fragments, energetics, comprehensive, library support

10:30-10:50 Coffee break

10:50-11:20 Chromatographic methods: HPLC

11:20-11:50 MS of oligosaccharides: General principles

12:00-2:30 Lunch Flag Hill Vineyards' (16)

2:30-3:00 GSLs, N-linked, O-linked glycans: Sample handling, processing, and analysis

3:00-3:30 Bioinformatics: Library matching of MSⁿ data

3:30-5:00 Lab

5:00-5:30 Ice cream on the Boston & Maine

Laboratory exercises:

Overview of AXIMA-CFR with target preparation and analysis

Overview of LTQ-Nanomate with sample analysis

Wet Lab demo: GSL extraction, SPE, permethylation

Mass Spectrometry

Basics

- Generate ions from sample compounds
- Separate the ions based on mass-to-charge ratio
- Determine the mass of the compounds of interest

Mass Spectrometry

General Principles

- Measure mass-to-charge ratio (m/z)
 - not “mass”
 - z isn't always 1
- Compounds must be ionized/ionizable

Components of Mass Spectrometer

Basics for UNH in-house equipment

- Source: generate gas-phase ions
 - MALDI: Matrix-Assisted Laser Desorption/Ionization
 - ESI: Electrospray Ionization
 - other types (mainly GC apps): electron impact, chemical ionization
- Mass Analyzer: separation of ions according to mass-to-charge ratio (m/z)
 - Tof: Time of Flight
 - Ion Trap: 3D quadrupole IT and linear quadrupole IT
 - other types: quadrupole, triple quadrupole, Q-Tof, Orbitrap, etc.
- Detector: convert gas-phase ion signal into electric current

MALDI

- Matrix-Assisted Laser Desorption/Ionization
- Solid sample
- Co-crystallized with “matrix”
 - usually aromatic compound
 - we use 2,5-dihydroxy benzoic acid (DHB)
- Sample bombarded with UV laser (N₂ 337 nm)
- Mechanism still not completely understood
 - Laser beam excites matrix
 - ablation of matrix produces plume of molecules
 - ionization takes place in the plume
- Ions ready for analysis

Time of Flight

- Ions are accelerated by an applied electric field
- Potential energy related to charge and voltage
 - $E_p = qU$
- When accelerated, potential energy becomes kinetic energy
 - $E_k = 0.5mv^2$
- Distance of flight tube is known, time is measured
 - $v = d/t$
- Substitute and rearrange
 - $t = (d/(2U)^{0.5}) \times (m/q)^{0.5}$

MALDI

Special Features

- Charge state (z) generally always 1, making mass spectral interpretation simple
- Matrix produces considerable background, particularly less than 800-900 m/z
 - problematic for smaller molecules
- While considered “soft” (relative to EI), harsher than ESI
 - non-methylated sialic acids are labile

ESI

- Electrospray ionization
- Liquid sample (in solution)
- Applied electric field at the emitter (counter electrode is the MS inlet) causes the flowing solution to be aerosolized
- At higher flow rates (standard ESI), nebulizing gas is used to remove solvent
- At low flow rates (nanoelectrospray), nebulizing/sheath gas is typically not required
- Ions enter the mass spectrometer through an ion transfer capillary

ESI

- Charge polarity
 - positive mode
 - negative mode
- Charge states
 - multiple charging common for larger masses
- Charge adducts
 - in positive mode, $[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[M+K]^+$, etc.
 - in negative mode, $[M-H]^-$, $[M+H_2PO_4]^-$, etc.

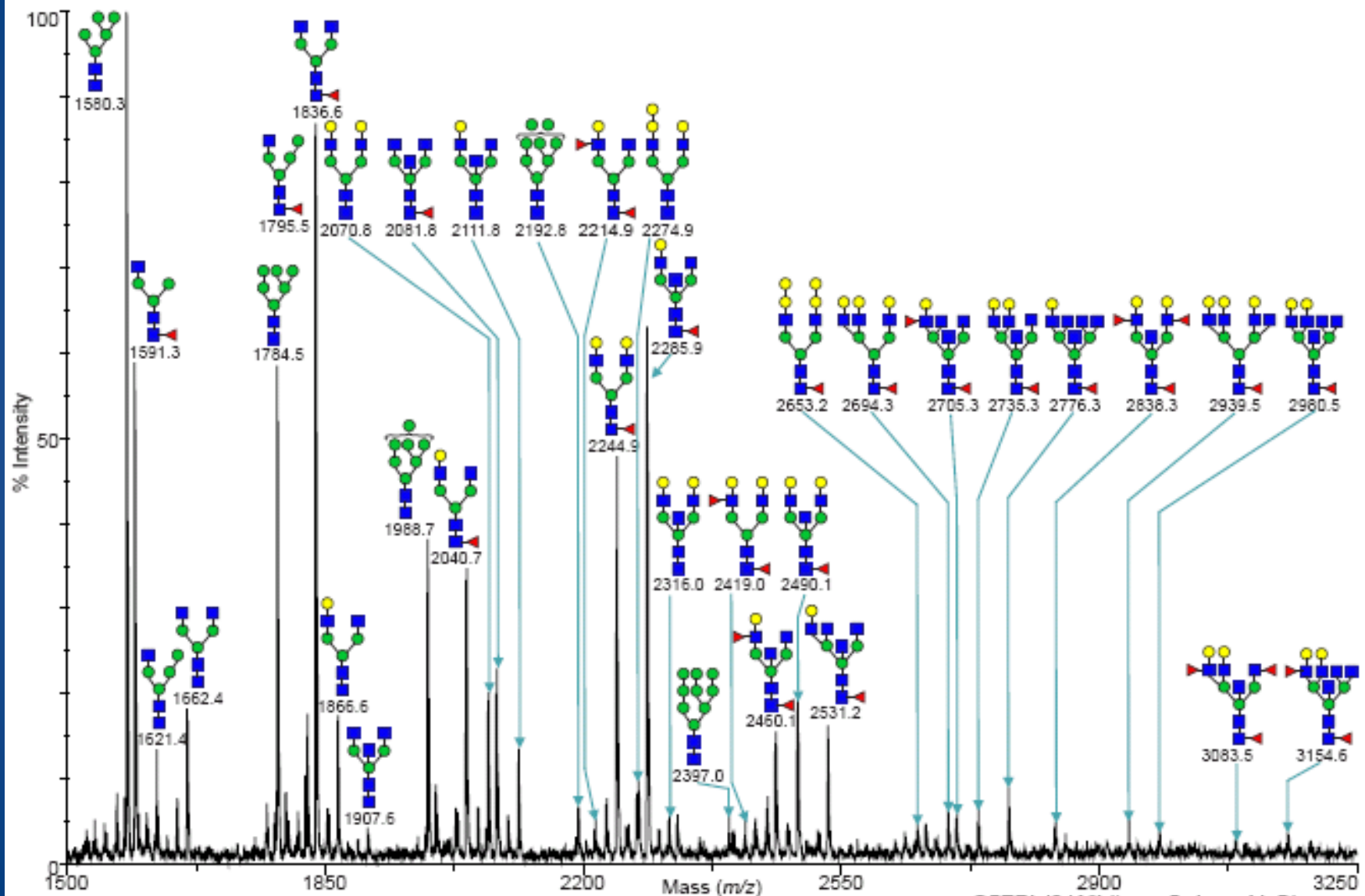
Ion Trap

- Two basic types
 - 3D Quadrupole Ion Trap (Paul trap)
 - Linear Ion Trap
 - larger capacity than Paul trap
- Physics (instrumental parameters identical)
- Ions can be stored, selected, and dissociated

MS Complications

- Connectivity & Energy Distribution

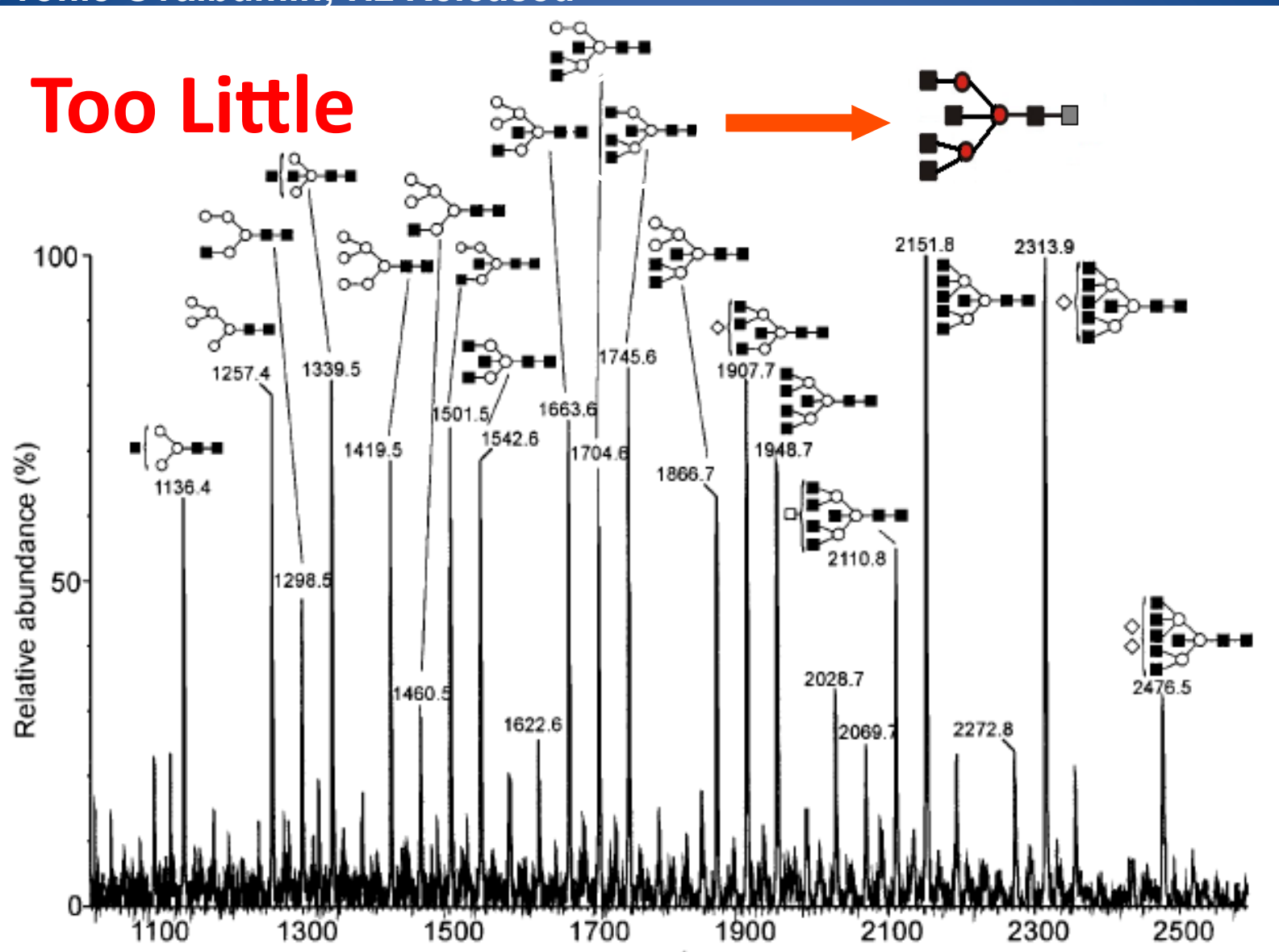
Too much, loose connectivity; Too little, no detail



C57BL/6 Wildtype Colon - N-Glycans

Profile Ovalbumin; Hz Released

Too Little



Quasi-equilibrium theory (QET)

- 1) Molecular ions in various electronic (and vibrational) states are produced by EI (or PI).
- 2) Ions in excited electronic states undergo rapid internal conversion to the ground state. → Rapid conversion of electronic energy to vibrational energy.
- 3) Intramolecular vibrational redistribution (IVR) occurs rapidly also. → Transition state theory, or, Rice-Ramsperger-Kassel-Marcus (RRKM) theory. ⇒ QET or RRKM – QET

$$k_i(E) = \frac{\nu}{(1 - E_0/E)^s}$$

The diagram shows the equation $k_i(E) = \frac{\nu}{(1 - E_0/E)^s}$ with three red boxes and arrows pointing to specific parts: 'frequency factor' points to ν , 'number of oscillators' points to s , and 'critical energy' points to E_0 .

The Energetics

MS Complications

- Connectivity & Energy Distribution

Too much, loose connectivity; Too little, no detail

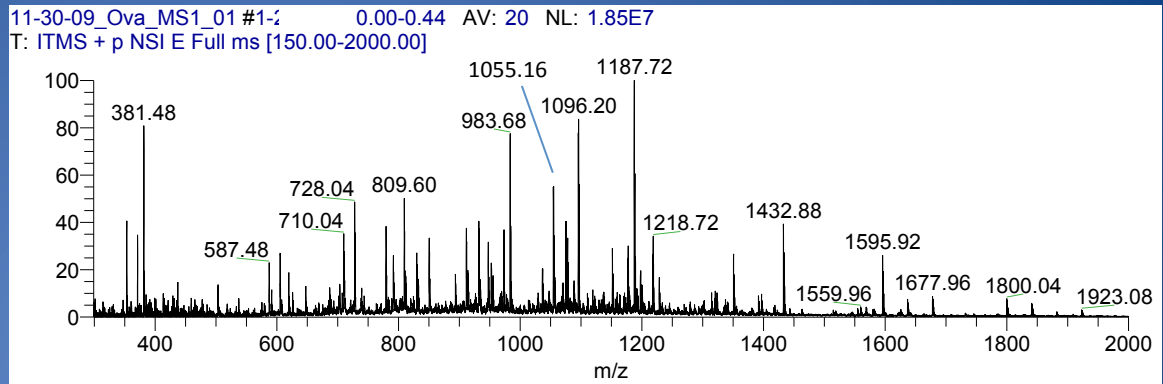
Solution

MSⁿ & Data Dependent Solutions,
e.g., Library

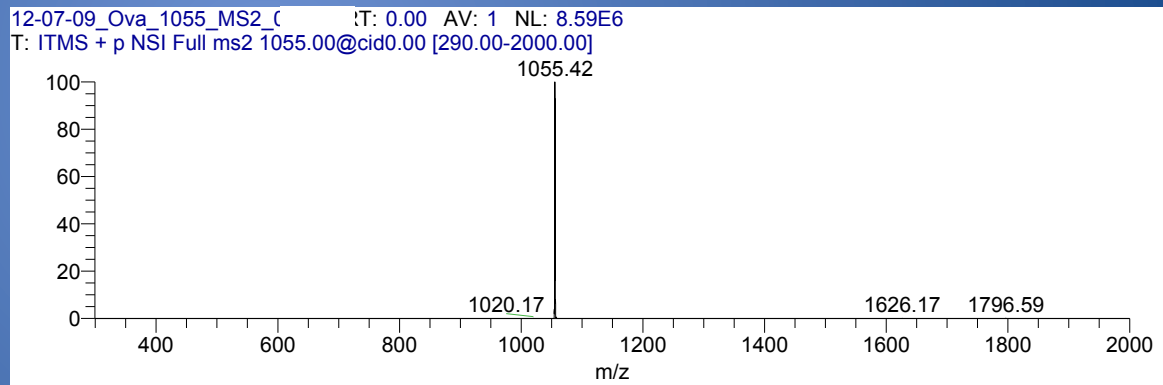
Ion Trap

Isolation and fragmentation

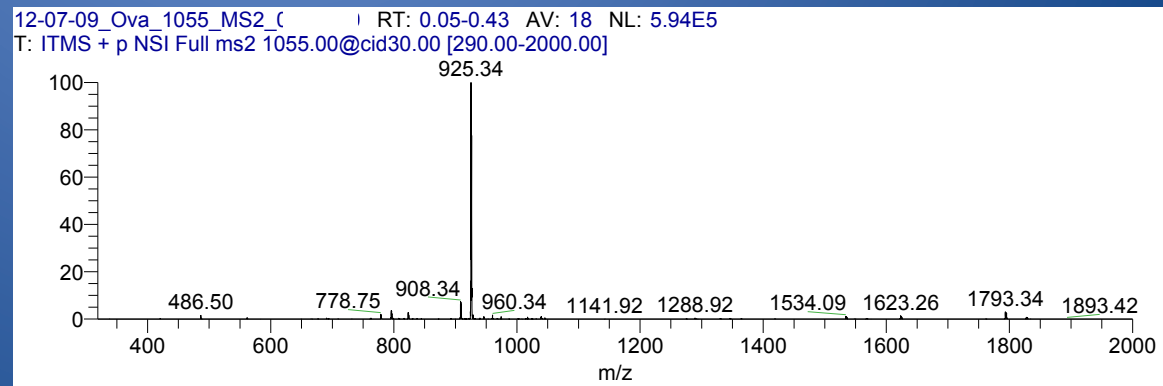
ESI-IT Mass spectrum of reduced and permethylated ovalbumin N-glycans



Isolation of m/z 1055 glycan from ovalbumin glycan mixture
All other ions are ejected
Essentially, MS^2 without applied collision energy

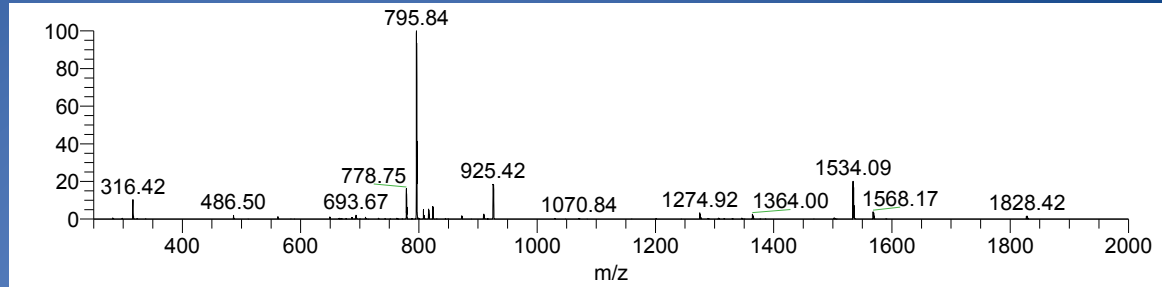


Isolation and fragmentation of m/z 1055 glycan
Labile cleavage dominates spectrum
Other mass analyzers (QqQ, Qtof) can do MS^2 but not MS^n

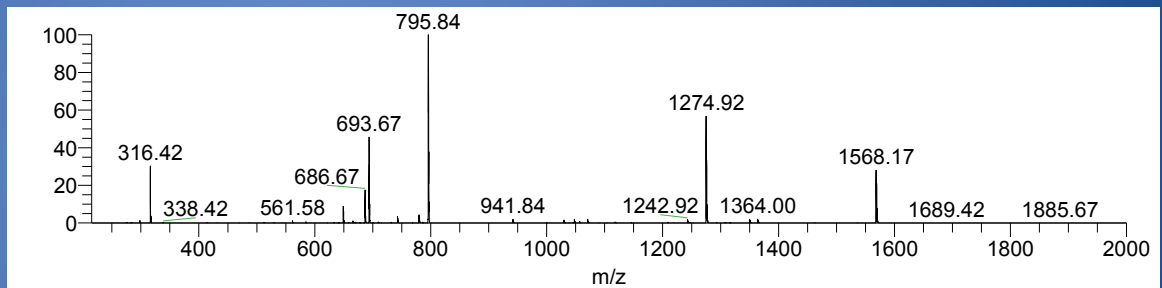


Ion Trap MSⁿ

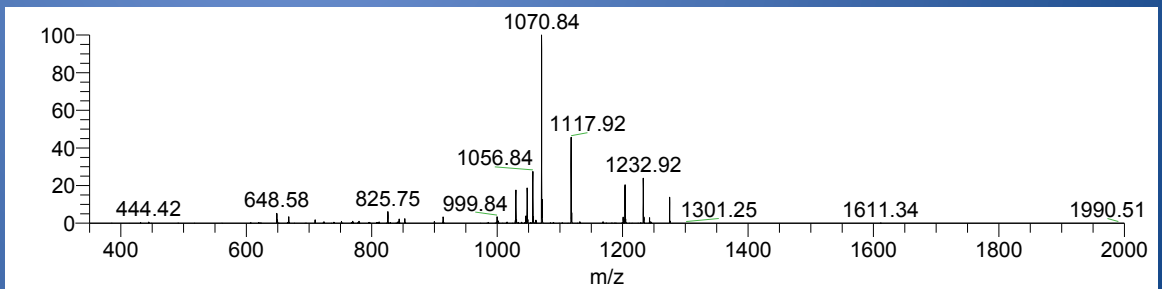
MS³ 1055 → 925



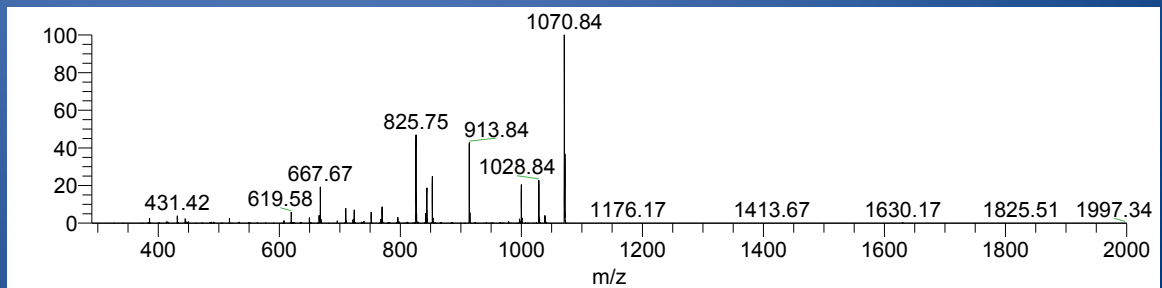
MS⁴ 1055 → 925 → 796



MS⁵ 1055 → 925 → 796
→ 1275



MS⁶ 1055 → 925 → 796
→ 1275 → 1071



Mass Spectrometry

Some Problems

- Ion suppression can occur in some samples
 - “matrix effect”
 - sample component(s) other than analyte affect measurement
 - can be endogenous (excessive salts) or exogenous (detergents, etc.)
 - can be difficult to track down, especially if endogenous
 - can be difficult to identify
 - diminished signal could be
 - limited sample?
 - ion suppression?
 - sample loss through processing?



Quasi-equilibrium theory (QET)

- 1) Molecular ions in various electronic (and vibrational) states are produced by EI (or PI).
- 2) Ions in excited electronic states undergo rapid internal conversion to the ground state. → Rapid conversion of electronic energy to vibrational energy.
- 3) Intramolecular vibrational redistribution (IVR) occurs rapidly also. → Transition state theory, or, Rice-Ramsperger-Kassel-Marcus (RRKM) theory. ⇒ QET or RRKM – QET

$$k_i(E) = \frac{\nu}{(1 - E_0/E)^s}$$

frequency factor

number of oscillators

The Energetics

critical energy