Exploiting High Resolution Accurate Mass (HRAMS) QToF MS/MS for the Identification of Unknown Analytes in Biomedical Devices Leachates and Extracts

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Outline

- Biomedical Device
  - Industry Overview
  - Regulatory Aspects
  - Leachables and Extractables Analysis
- High Resolution Mass Spectrometry
  - Instrumentation
    - Time of Flight (TOF) Mass Spectrometers
  - MS Data Overview
  - Resolution and Accuracy
- Case Studies
  - L&E Contaminant Analysis
  - Surfactant Analysis
  - Study of Polyurethane Acrylic Adhesive Hydrolytic Stability

Medical Devices

- Medical Device - any healthcare product that does not achieve its principal intended purpose by chemical action or by being metabolized.

Biomedical Device- Regulatory Background

- Regulations, like drugs, defined in CFR Title 21
- Biomedical Device regulated by CDRH (Center for Device and Radiological Health) Division of FDA
- Three Biomedical Device Classes – Class I, Class II and Class III
- Class I and II – 510K regulatory route (no clinical trial based on substantial equivalence to predicate device)
- Class III – IDE/PMA (involves clinical trial ; requires testing to demonstrate safety and efficacy of new product)
- Chemical Testing of Biomedical Devices required to demonstrate safety or equivalence, i.e. “Leachables and Extractables” (L&E)
- Objective of L&E Testing
  - Identify and Quantitate “leachates” - chemical compounds that might leach from the material under simulated physiological conditions
  - Identify and Quantitate “extractables” chemical compounds that could potentially migrate from the material under more extreme conditions of temperature and solvent.

Leachable and Extractable Testing Aspects

- Leachables and Extractables (L&E) Test Guidance
  - ISO 10993-12:2002 “Sample Preparation and Reference Materials”
  - ISO 10993-18:2005 “Chemical Characterization of Materials”
  - ISO 10993-13:2010 “Identification and Quantification of Degradation Products from Polymeric Medical Devices

- Extraction Conditions
  - Solvent
  - Solvent:Mass ratio
  - Time and Temperature
  - Mode of Extraction
    - Soxhlet Extraction in swellable solvent (for crosslinked
    - (elastomeric) materials such as butyl rubber, silicones,polyurethane)
    - Repeated soak with heat and shaking
    - Polymer Dissolution (for non-cross linked polymers)

ISO 10993 Guided Chemical Analysis –Identification and Quantification

- Select analytical method justifying sensitivity and selectivity
- GC/MS and LC/UV and LC/MS required
  - Typically require analytical method with ppm sensitivity for plastics monomers and plastics additives.
- Semi-targeted screen – many possible analytes known (plastics monomers) but many unknown (degradants and contaminants, polymerization byproducts)
- If a polar analyte detected by UV (many chemicals are detectable at 1-100ppm concentrations by UV), it must be chemically identified, preferably source reported and amount quantified. The specificity of UV spectra is not sufficient enough for chemically id.
- If a volatile analyte detected by GC/FID or GC/MS, it must be identified. NIST library is a great aid to id but often analyte is not in the library.

Significant Challenges of Leachable and Extractable Testing of Biomedicals Device

- Device is a multiplicity of diverse materials
  - Different plastics
  - Adhesives
  - Metals
  - Lubricants
  - Manufacturing aids (surfactants)
  - Coatings
- Unknown Degradant Profile
  - Process effects on diverse materials yielding unknown degradants
    - Sterilization, molding, laser etching
  - Device Aging Effects
    - Shelf Life, hydrolytic aging, photoaging
- Raw material variability
  - Each raw material source has potentially different impurity composition
- All plastics contain a significant proportion of processing additives
  - E.g. antioxidants, heat stabilizers, UV absorbers, mold release agents and dispersants
  - Same plastic, e.g. polypropylene, but different additives package.
    - Not all polypropylenes are equivalent

UPLC Coupled to
G2-S (Waters) Q-Tof High Resolution Mass Spectrometer

ANALYTICAL FUNCTION: Chemical and Structural Identification of unknown component analytes comprising a complex mixture.

UPLC Function : Separation of a complex liquid mixture into pure components
Mass Spectrometer Function: Determine the molecular mass of each pure component
Molecular Mass , if accurate enough, can be a powerful tool for chemical identification

Definitions - Mass

Carbon $_{6}C^{12}$ isotope = 12 Da = 12 g/mole

1 atomic mass unit (amu) = 1 Dalton = $1.660538921(73) \times 10^{-27}$ kg

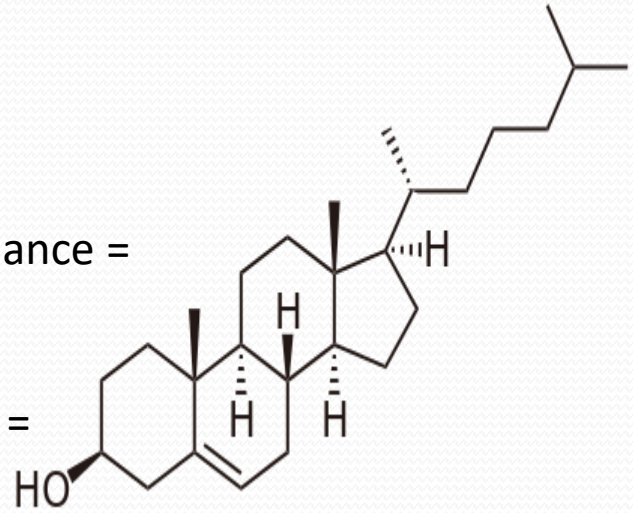
Cholesterol

Elemental composition: $C_{27}H_{46}O$

Molecular weight (based on natural isotopic abundance = 386.65 g/mole

Theoretical exact monoisotopic mass (C^{12}, H^{1}, O^{16}) = 386.35486 Da

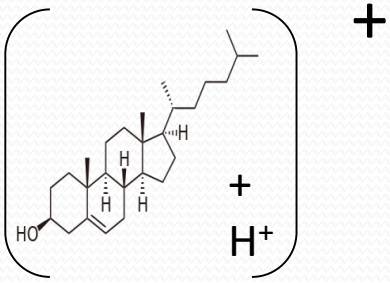
Nominal mass = 386 Da



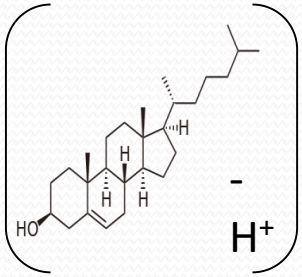
Definitions – Mass to Charge (M/Z)

- Mass spectrometer does not detect mass but mass to charge (m/z) at various levels of accuracy

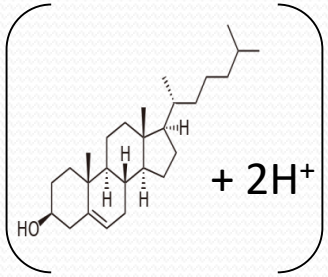
Cholesterol Singly Positively Charged Ion



Singly Negatively Charged Ion



Doubly Positively Charged Ion



$$m/z = \frac{386 + 1}{1} = 387$$

$$m/z = \frac{386 - 1}{1} = 385$$

$$m/z = \frac{386 + 2}{2} = 194$$

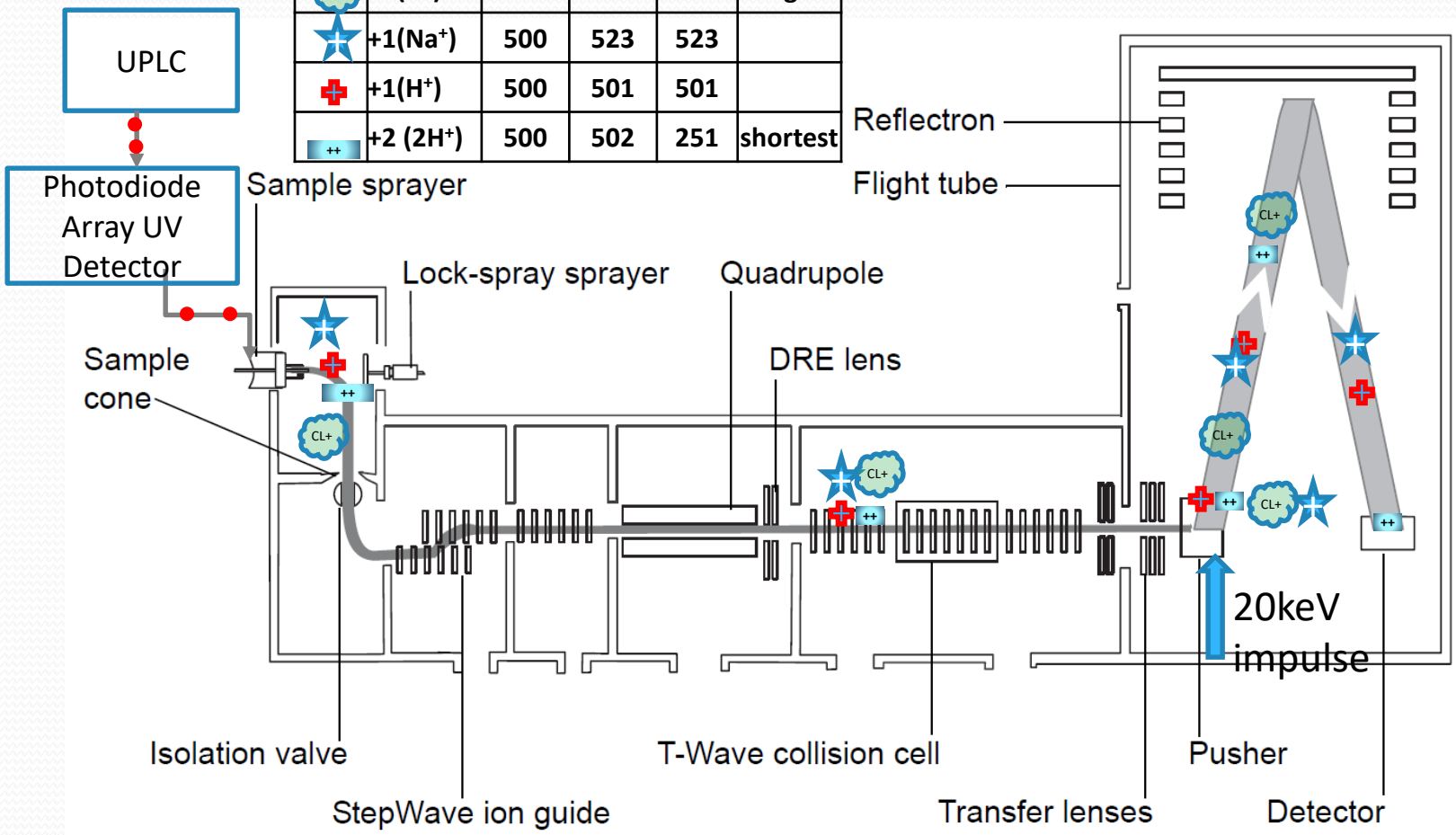
$$m/z = \frac{386.3549 + 1.0072}{1} = 387.3549$$

$$m/z = \frac{386.3549 - 1.0072}{1} = 385.3477$$

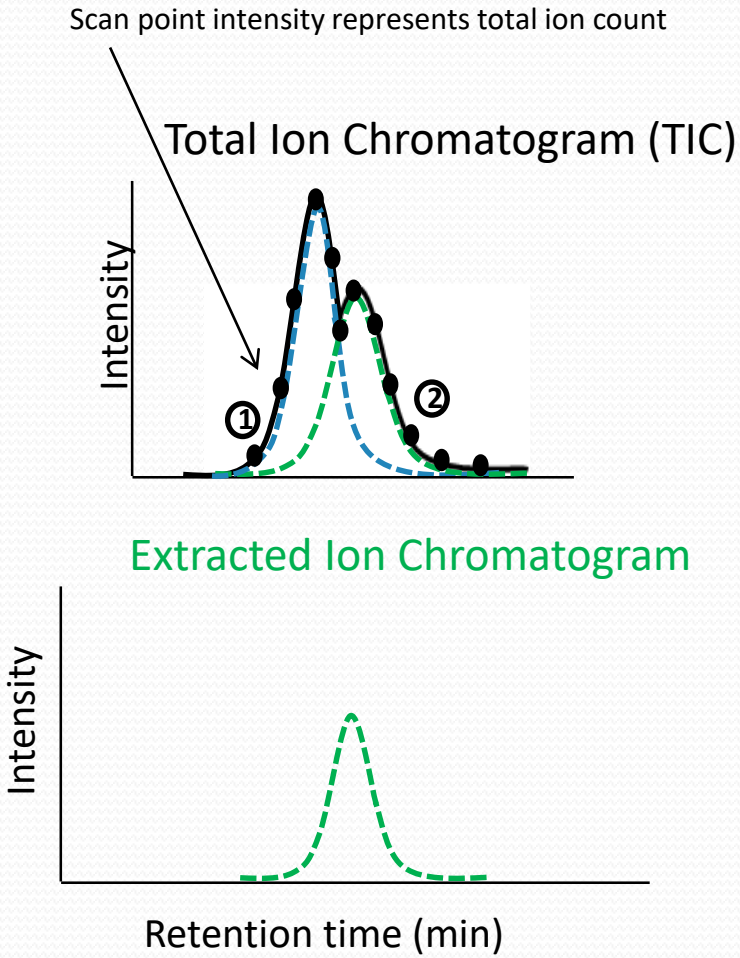
$$m/z = \frac{386.3549 + 2.0144}{2} = 194.1847$$

Full Scan (Positive Mode) High Resolution MS Operation

Ion	Charge	Ana Mass	Ion Mass	m/z	TOF
CL+	+1(H^+)	500	1001	1001	longest
	+1(Na^+)	500	523	523	
	+1(H^+)	500	501	501	
++	+2 ($2H^+$)	500	502	251	shortest



Total Ion Chromatogram and Mass Spectra Output of Detected Ion Data



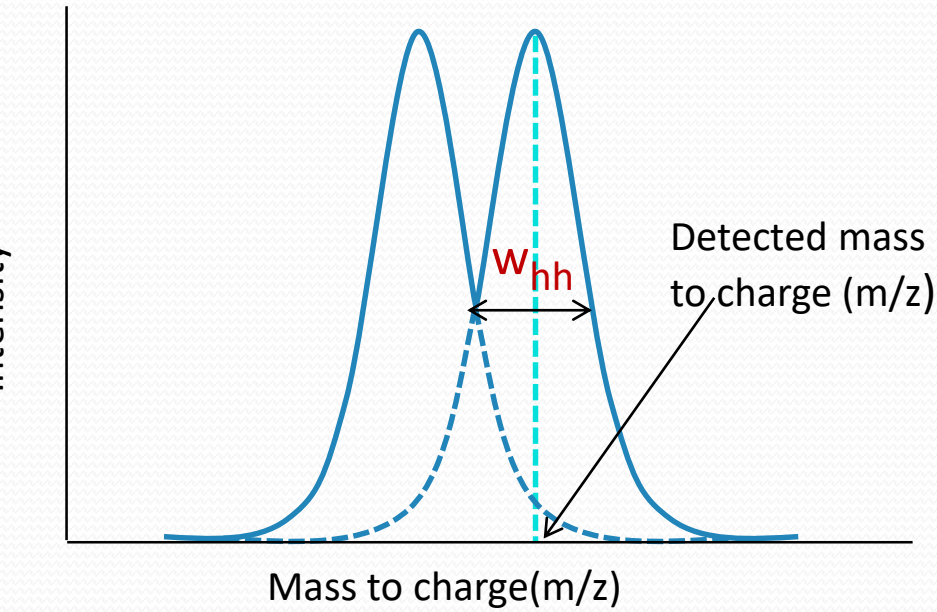
Relationship between TOF and m/z

$$m/z = k(TOF)^2$$

- Time of flight (TOF) data yields the mass spectrum, a plot of ion Intensity as a function of TOF which is proportional to m/z.
- Each push yields a mass spectrum
- Many (20,000) mass spectra are added to yield a **SCAN POINT**

Definition - Mass Resolution

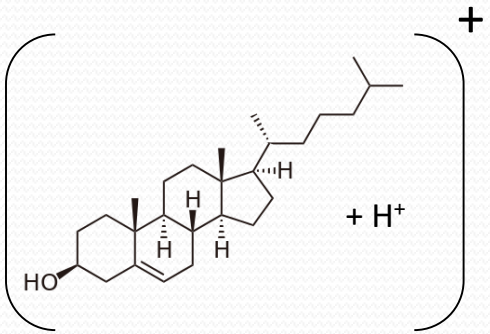
$$\text{Resolution} = \frac{m/z}{w_{hh}} = \left(\frac{TOF}{\Delta TOF} \right)^2$$

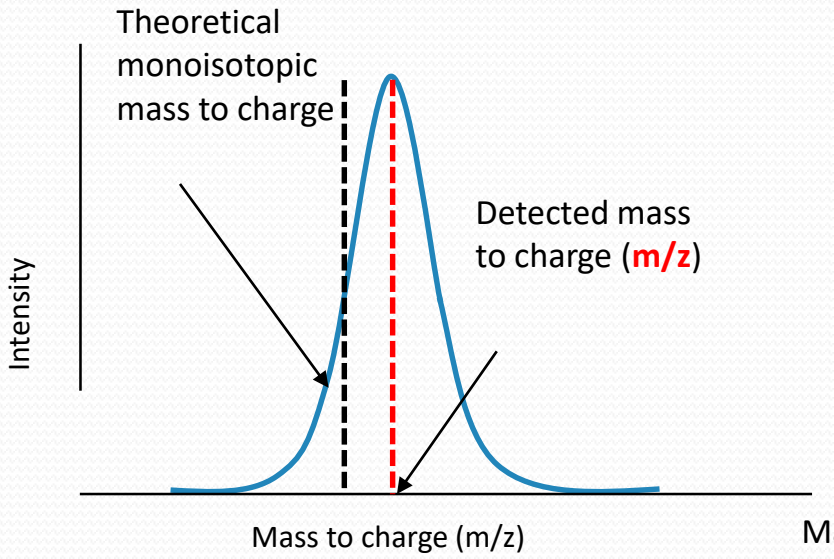


MS Instrument		Res	W_{hh} @ m/z= 500
Tof	High Res	30,000	0.0167
Quadrupole	Low Res	500-1000	0.5-1.0

Definition - Mass Accuracy

$$\text{Mass Accuracy} = \frac{(\mathbf{m/z} - \text{theoretical m/z})}{\text{theoretical m/z}}$$



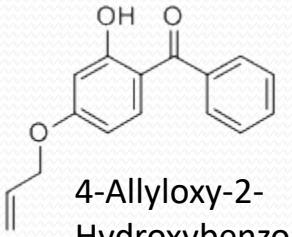


Theoretical m/z = 386.3549
Example detected **m/z = 386.3599**

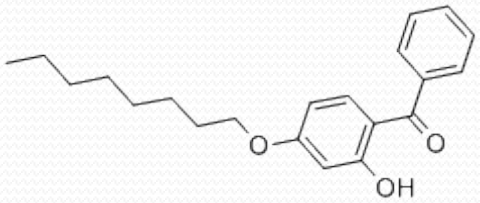
$$\text{Mass Accuracy} = \frac{(386.3599 - 386.3549)}{386.3549} = 1.29 \times 10^{-5}$$

Mass Accuracy = $1.29 \times 10^{-5} \times 1{,}000{,}000 = 12.9$ parts per million (ppm)

Plastics Additive UV Data

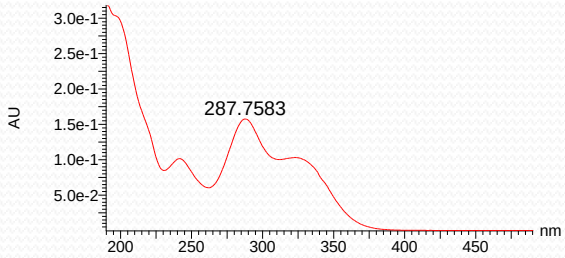


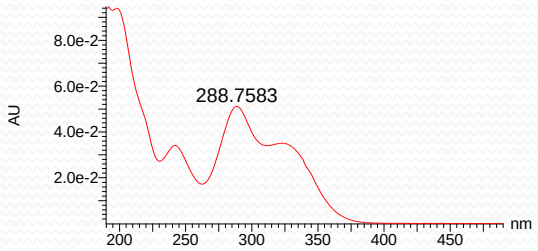
4-Allyloxy-2-Hydroxybenzophenone
CAS 2549-87-3



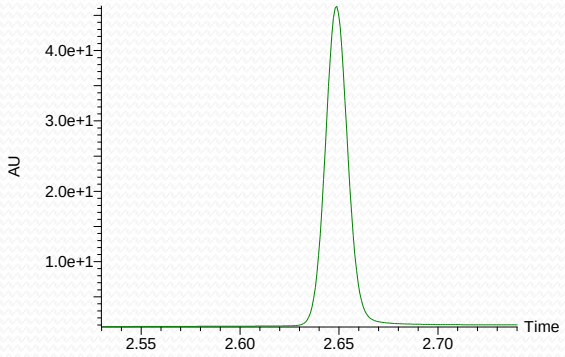
Octabenzone
CAS 1843-05-6

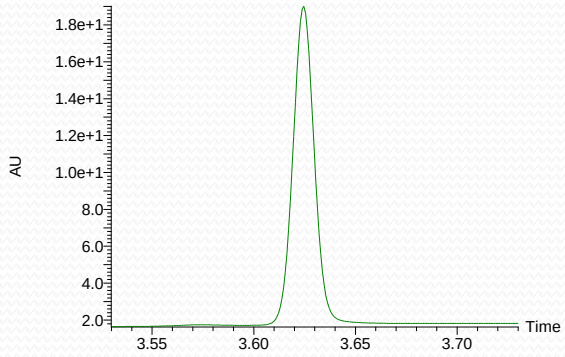
UV Spectrum





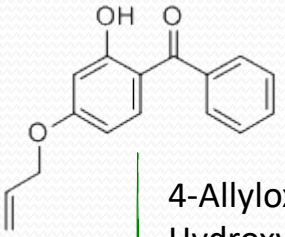
Total photodiode array UV chromatogram



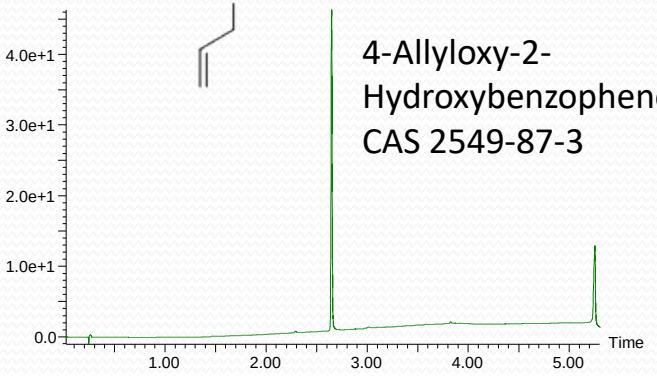


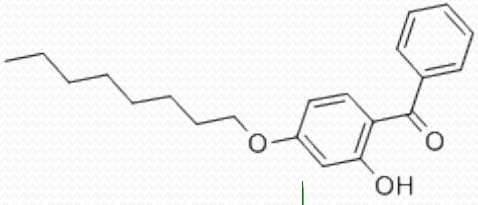
Plastics Additives UV and MS TIC Data

Total λ photodiode array UV chromatogram

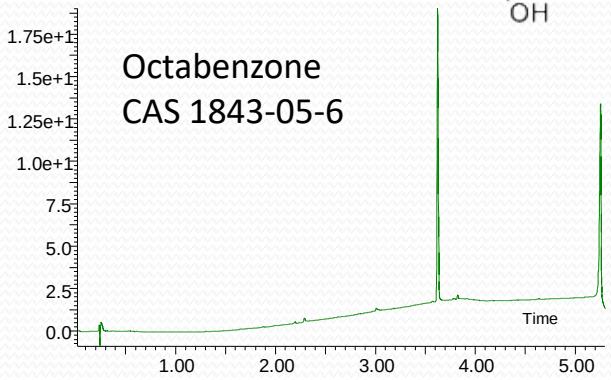


4-Allyloxy-2-Hydroxybenzophenone
CAS 2549-87-3

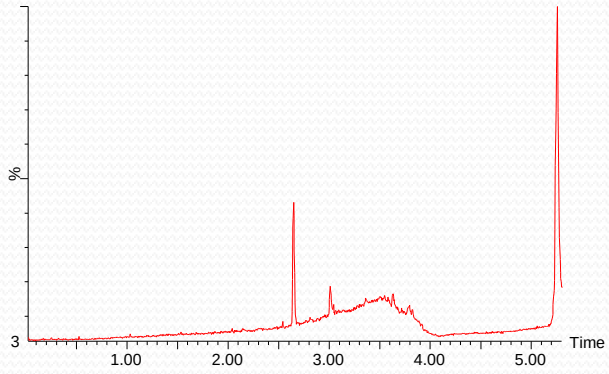


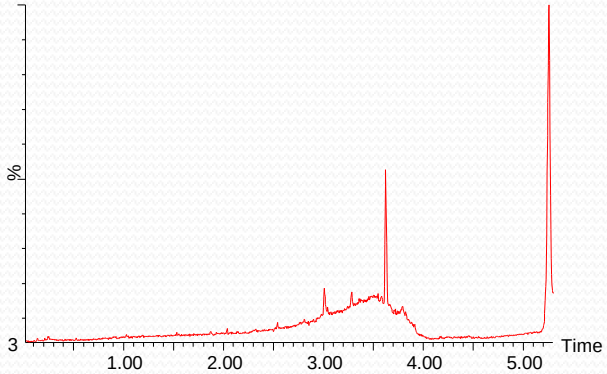


Octabenzone
CAS 1843-05-6

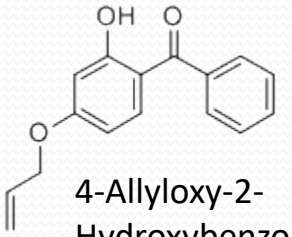


Total ion chromatogram (TIC)

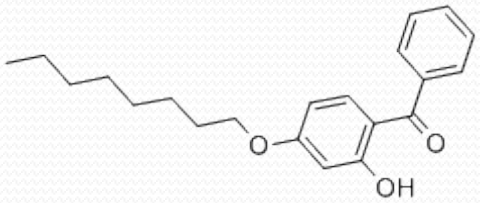




Plastics Additive MS Data

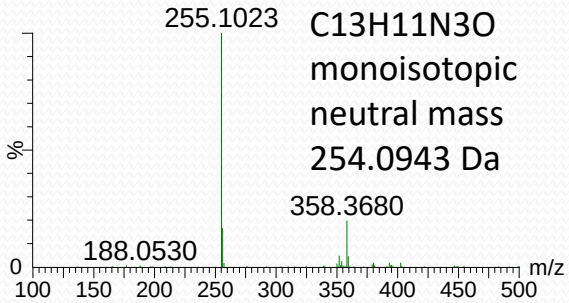


4-Allyloxy-2-Hydroxybenzophenone
CAS 2549-87-3

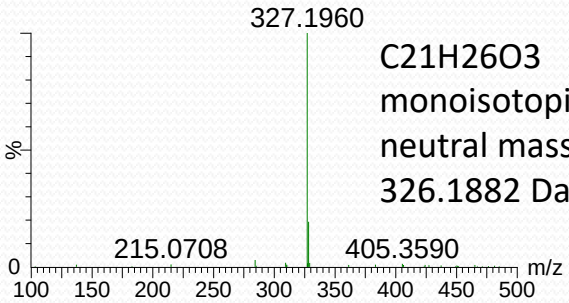


Octabenzone
CAS 1843-05-6

Mass spectrum

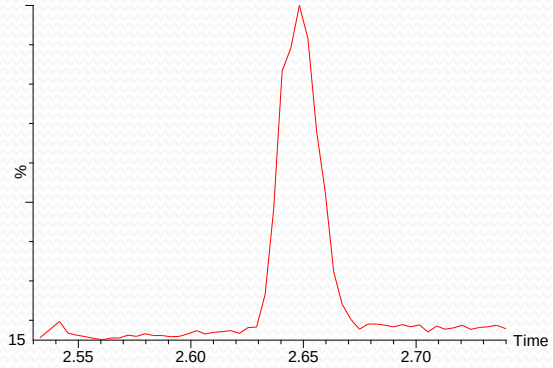


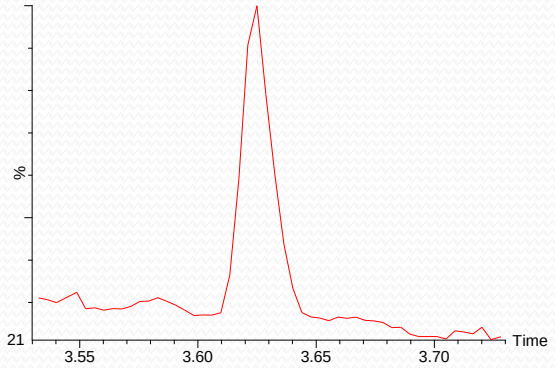
C13H11N3O
monoisotopic
neutral mass
254.0943 Da



C21H26O3
monoisotopic
neutral mass
326.1882 Da

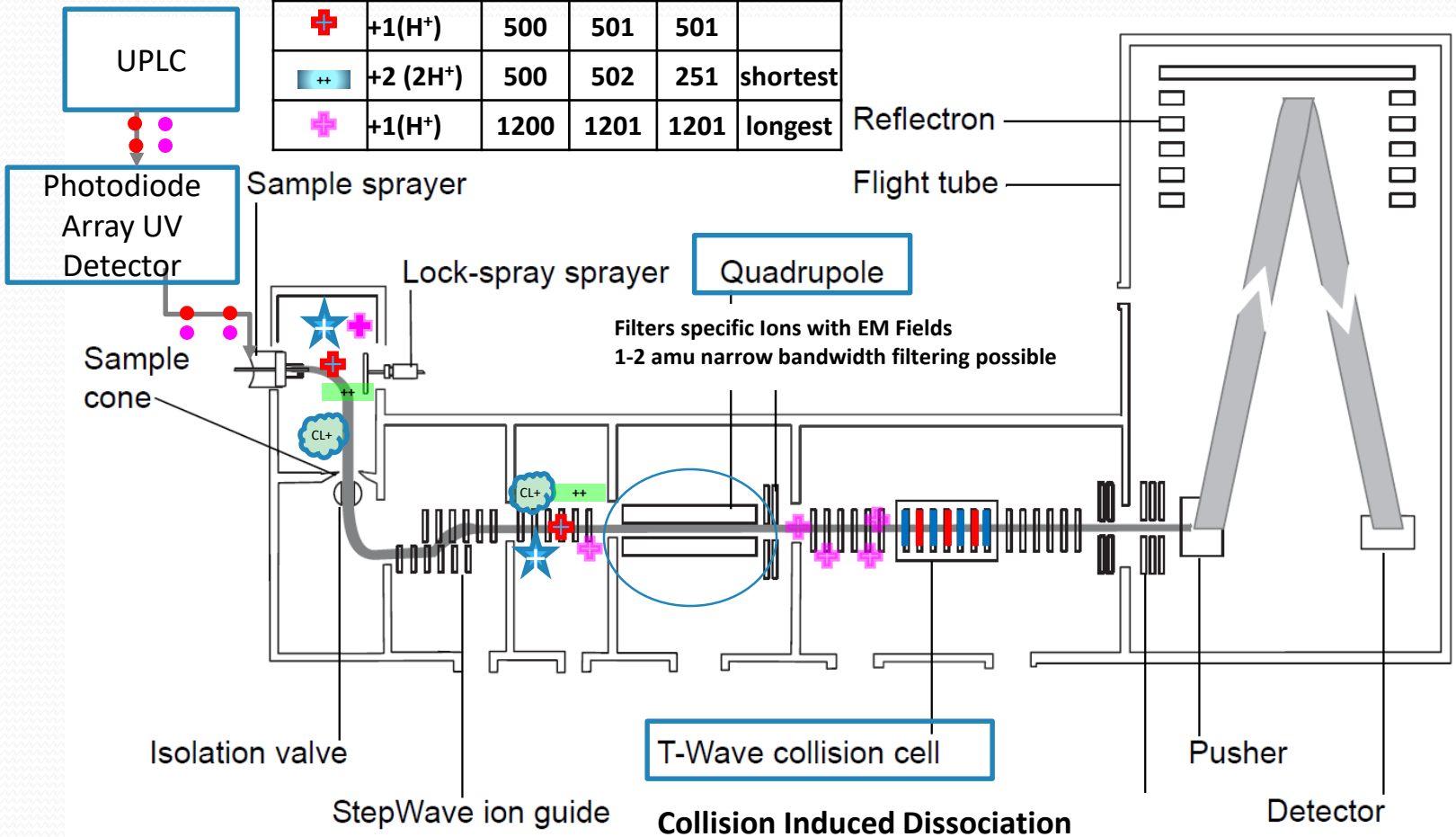
Total ion chromatogram





MSMS High Resolution MS Operation

Ion	Charge	Ana Mass	Ion Mass	m/z	TOF
CL+	+1(H^+)	500	1001	1001	
	+1(Na^+)	500	523	523	
	+1(H^+)	500	501	501	
++	+2 ($2H^+$)	500	502	251	shortest
	+1(H^+)	1200	1201	1201	longest



Collision Induced Dissociation (CID) – "Fragmentation"
Low Pressure Argon Gas Filled Chamber

Ion Fragmentation In Collision Cell

$Ar + M^{+} \xrightarrow{eV} F^{+} + N + Ar$

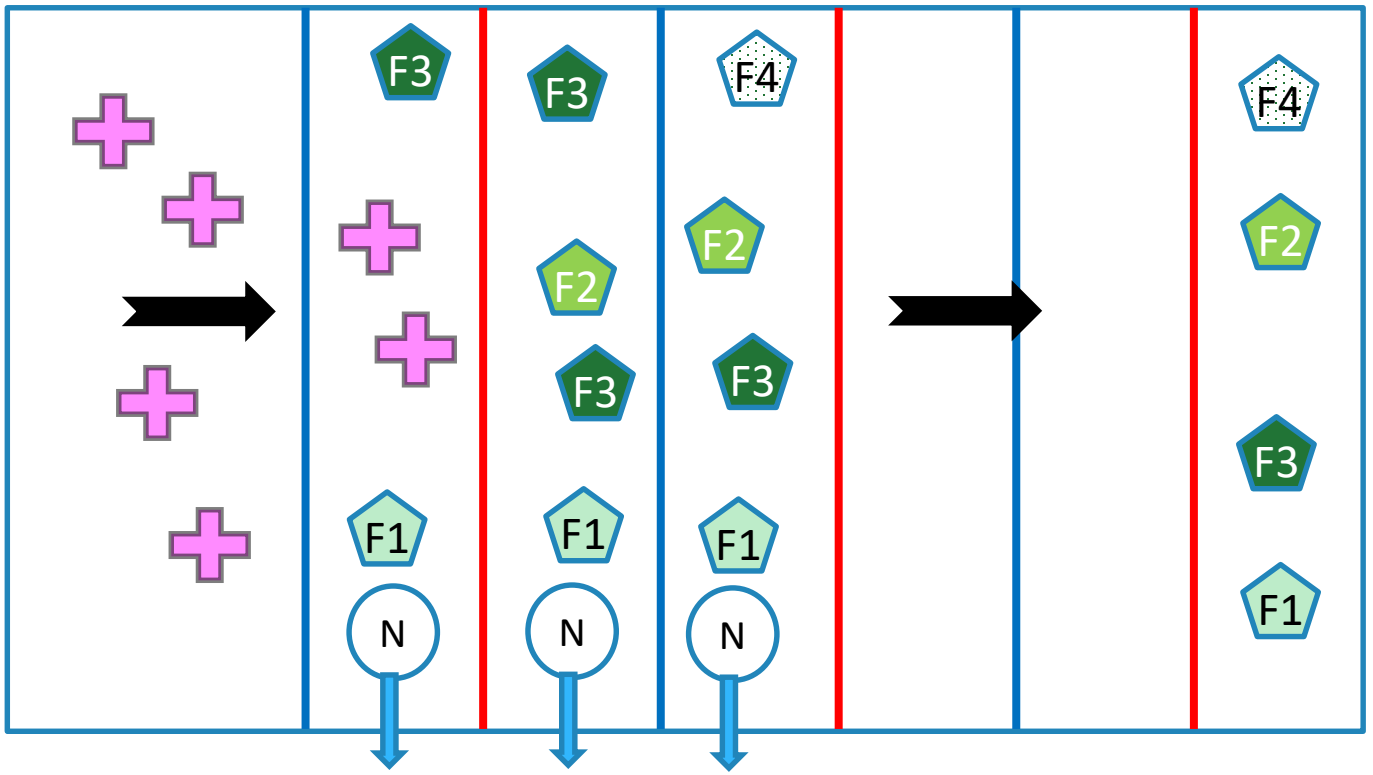
M^{+}= Molecular Ion N= neutral fragment F^{+}= ion fragment M>F1>F2>F3>F4

Fragmentation – Collision Induced Dissociation (CID)

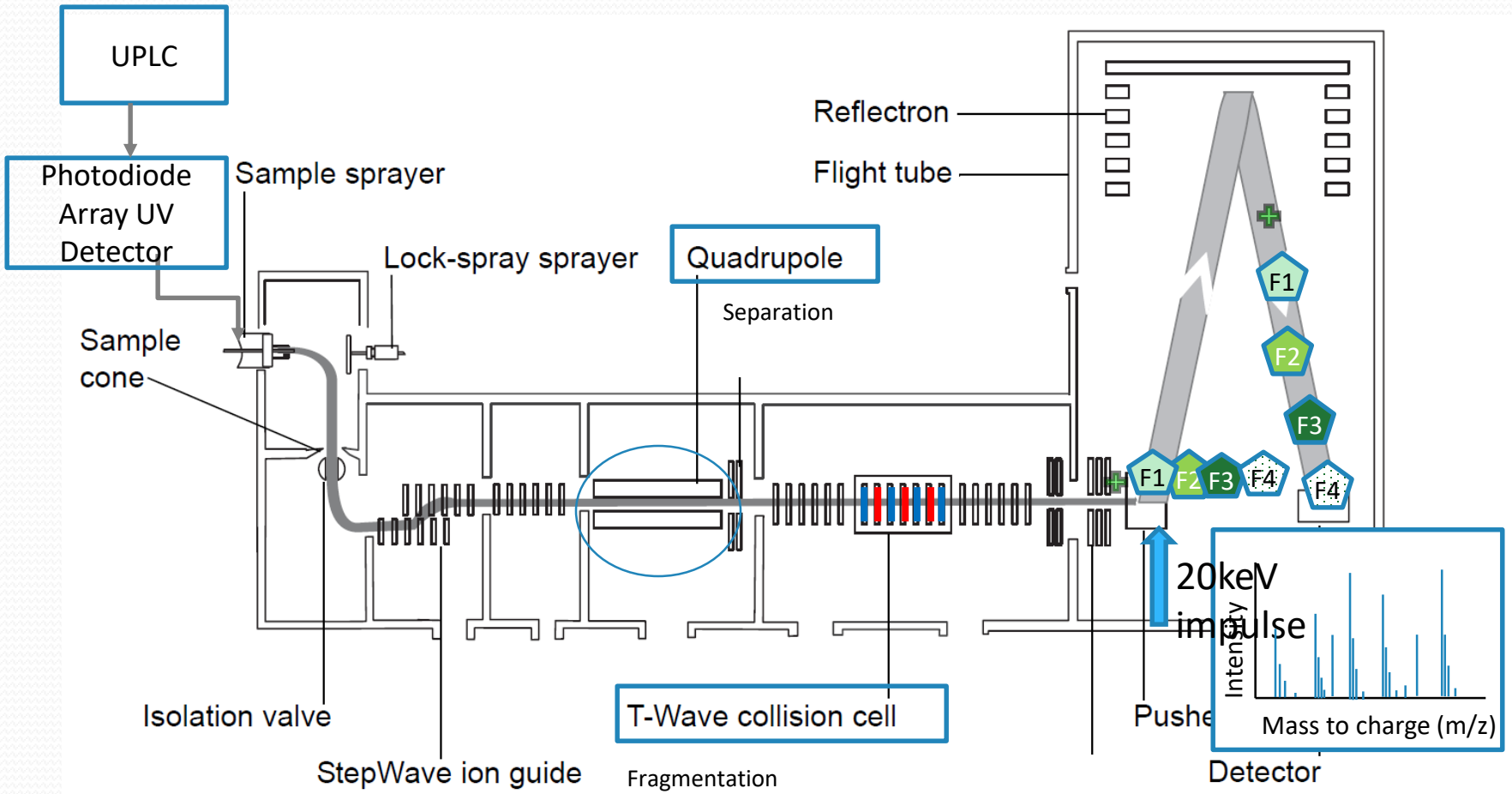
Site Specificity -Preferential fragmentation at polar bonds, clusters

Structural Information Encoded in Fragments

Searchable MSMS Fragmentation Libraries (Similar to EI GC/MS libraries, e.g. NIST) generated with characteristic spectra. (Platform dependent) chemically identify fragments

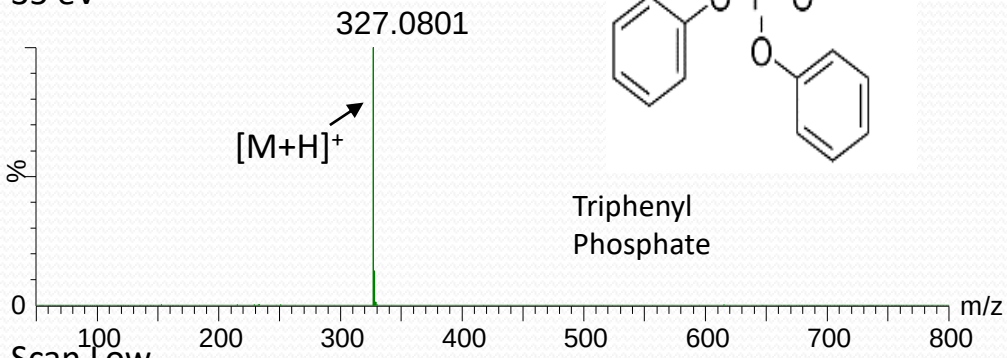


MSMS High Resolution MS Operation



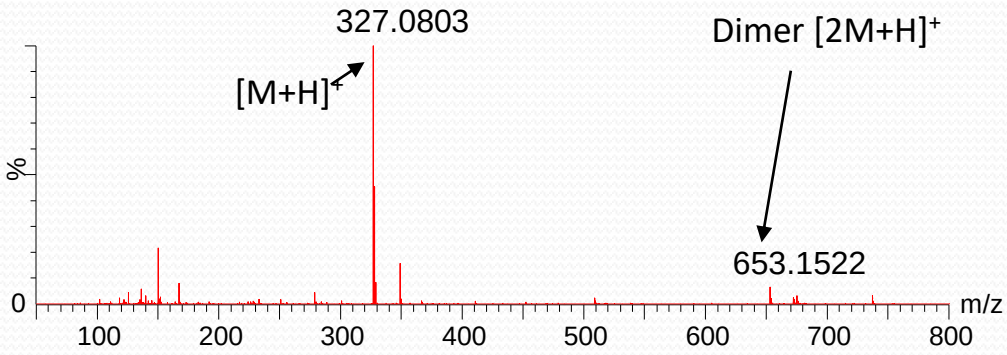
Triphenyl Phosphate High E MSMS of Dimer

MS/MS of 653 m/z
20 - 35 eV



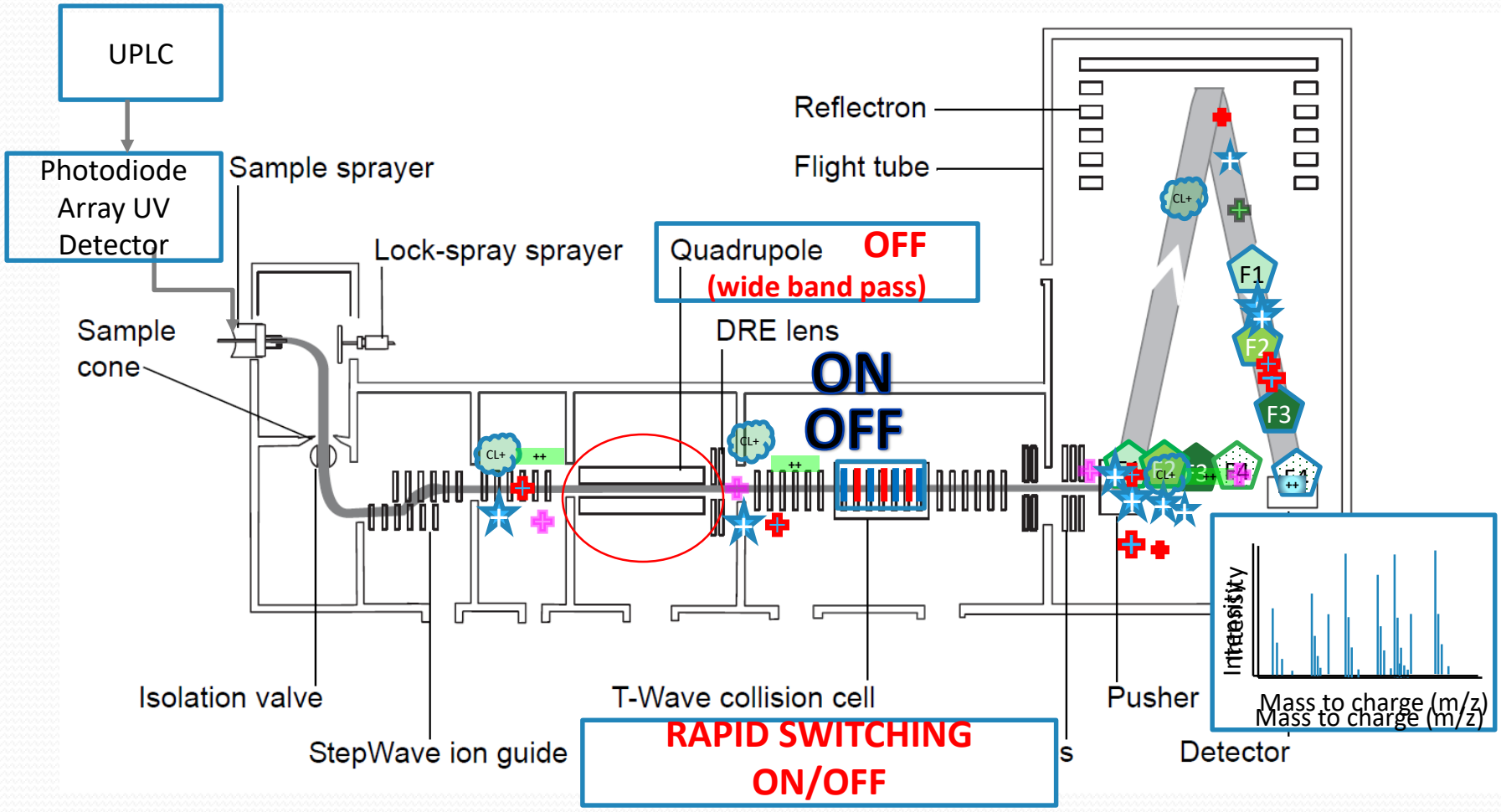
Triphenyl
Phosphate

MS Full Scan Low
Energy

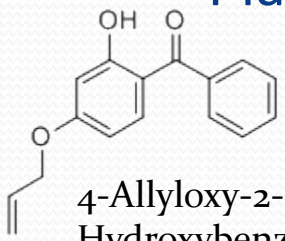


MS^E High Resolution MS Operation

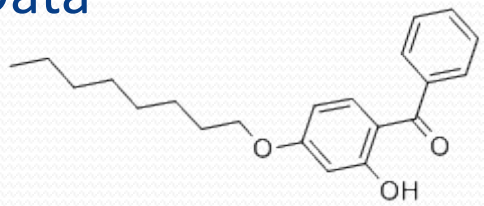
Fragmentation without Quad Selection



Plastics Additive MS^E Data



4-Allyloxy-2-Hydroxybenzophenone
CAS 2549-87-3



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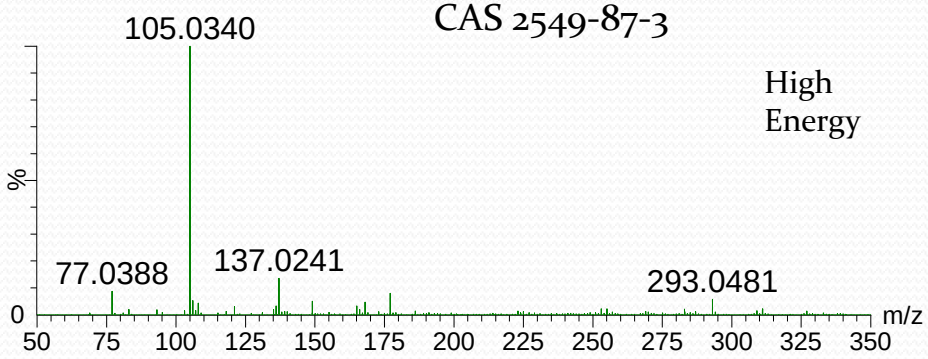
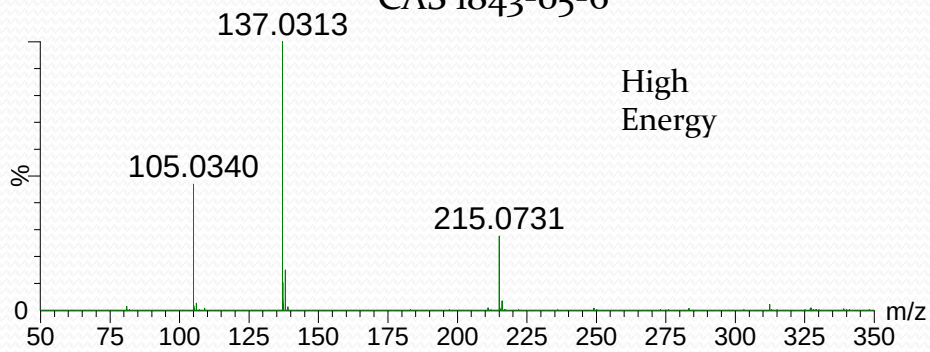
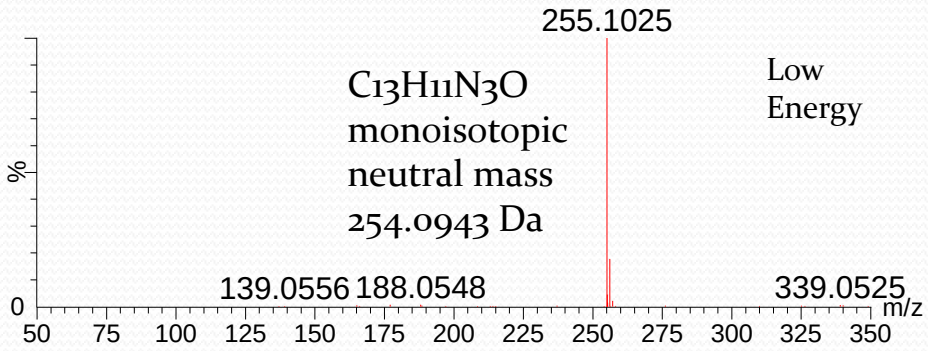
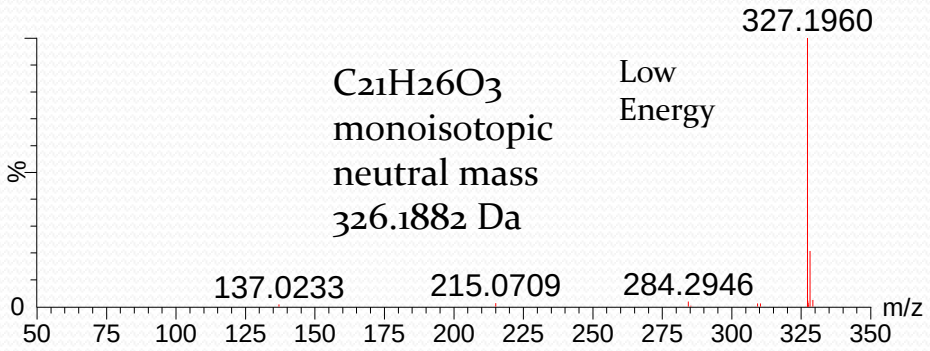


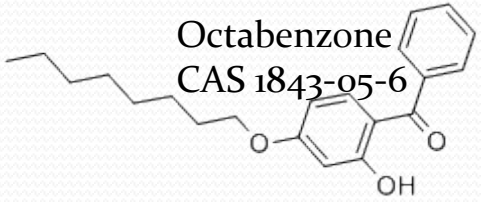




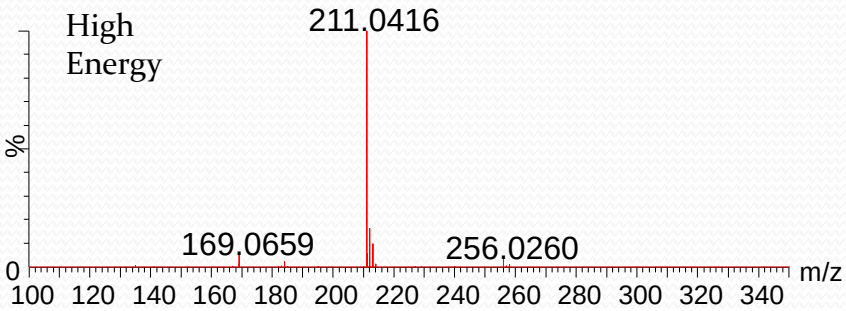


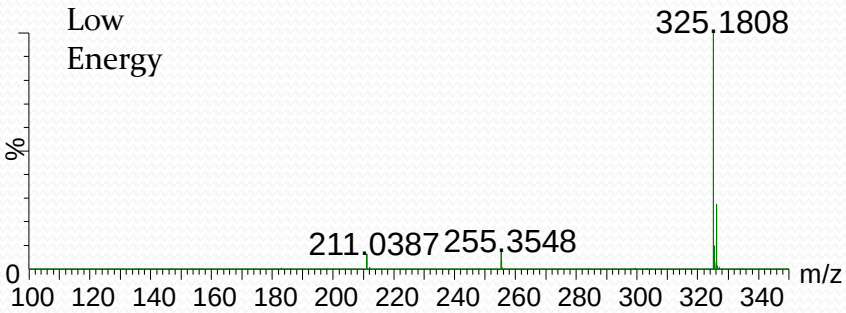
Plastics Additive MS^E Data

$C_{21}H_{26}O_3$
monoisotopic
neutral mass
326.1882 Da

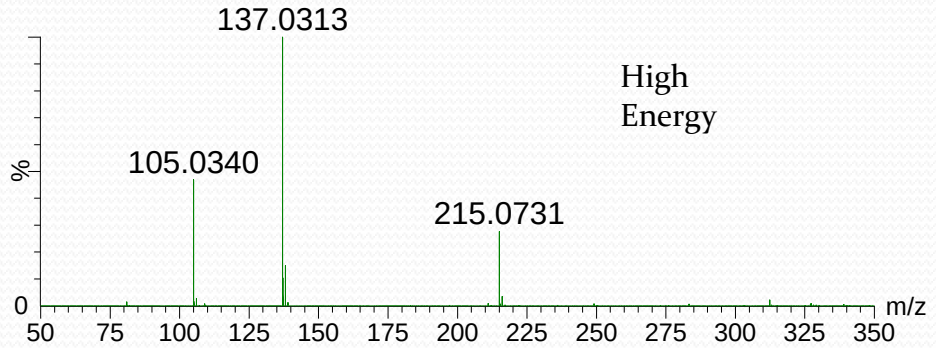


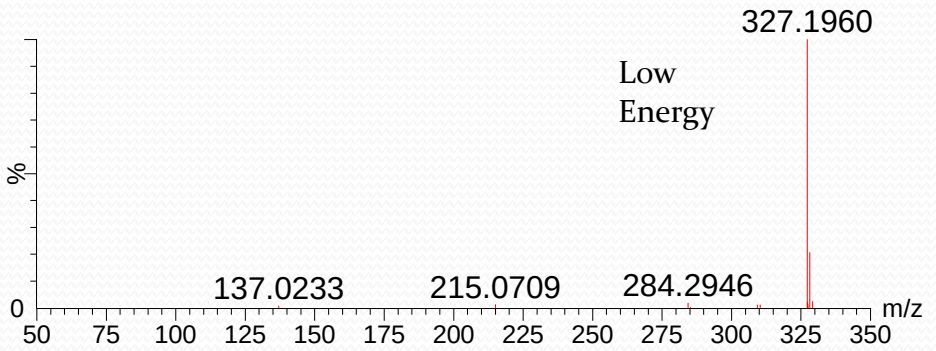
Negative Mode MS



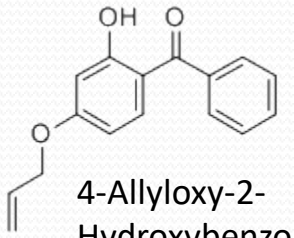


Positive Mode MS

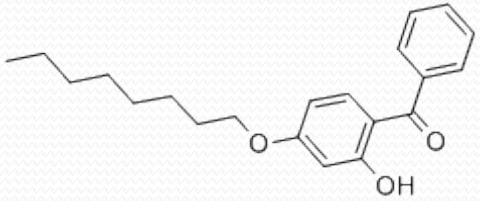




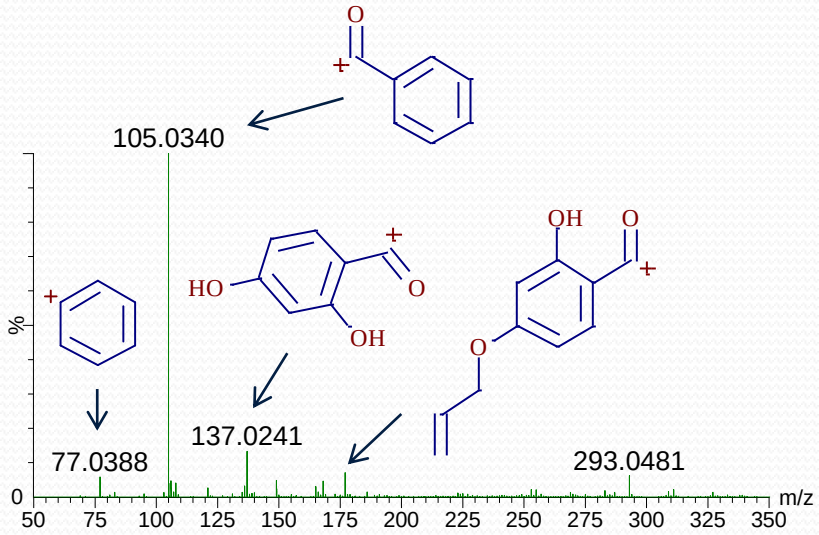
Theoretical Prediction of Plastic Additive Fragment Ions in MassFrontier (Thermo) Software

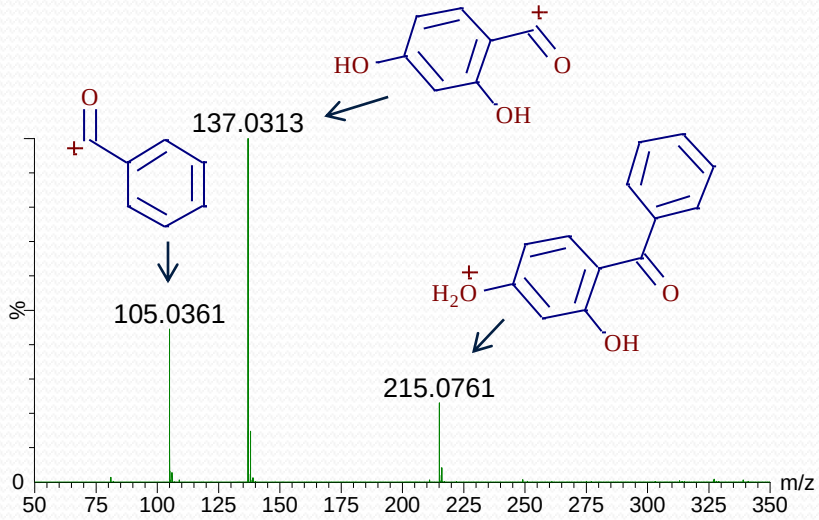


4-Allyloxy-2-Hydroxybenzophenone
CAS 2549-87-3



Octabenzone
CAS 1843-05-6



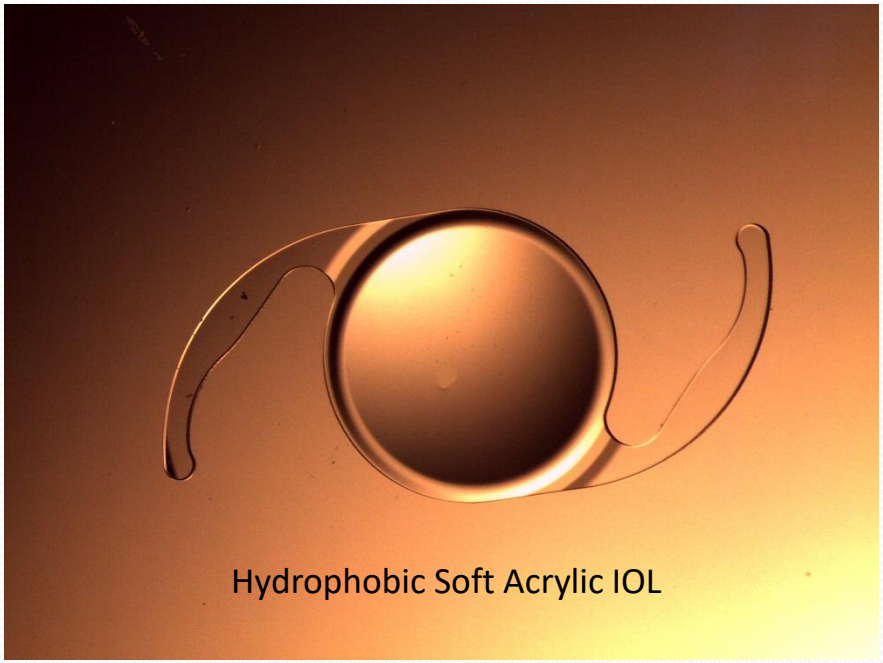


Contaminant Structure Verification Using Mass Frontier Software (MFS)

- Mass Frontier (Thermo Scientific) Small Molecule Structural Elucidation Software
- MassFrontier is a predictive software that generates structures of expected fragments from a given precursor ion
- Mass Frontier generates fragments using predictive algorithms based on
  - rules of ionization, fragmentation and rearrangement
  - MSMS Collision Induced Dissociation (CID) Fragmentation library based on 120,000 published small molecule fragmentation mechanisms
- The software predicts fragments as well as the pathways of their formation and so yields precursor/fragment pairs.

Case Study I

E&L Study of Acrylic Intraocular Lens (IOL) Device
Chemical Identification of Low Level UV absorber Contaminant in Hydrophobic Acrylic Intraocular Lens (IOL) Extract



Hydrophobic Soft Acrylic IOL

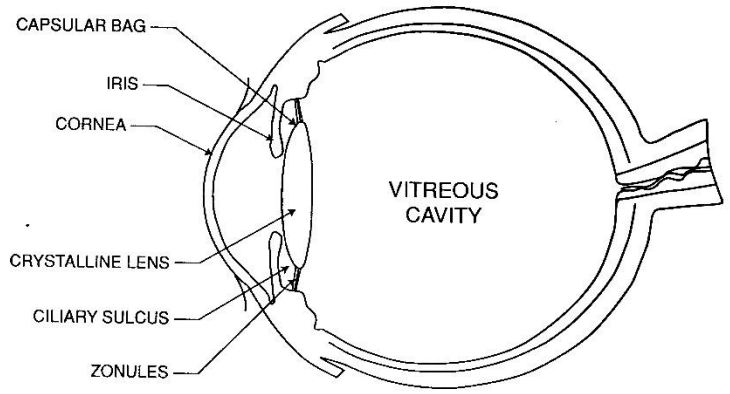
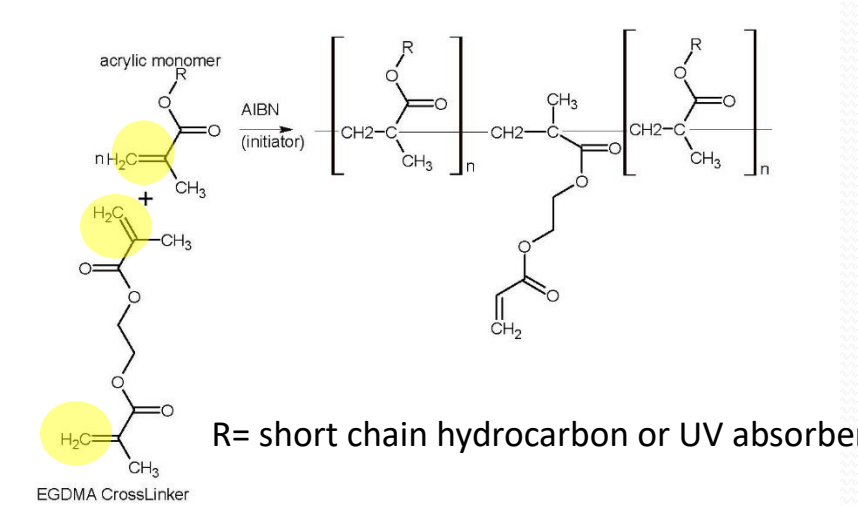


Figure 1 Sagittal view of the human eye showing the location of the natural crystalline lens. Cataract is the opacification of the natural lens. In modern surgery, the cataract is removed through an incision peripheral to the cornea.

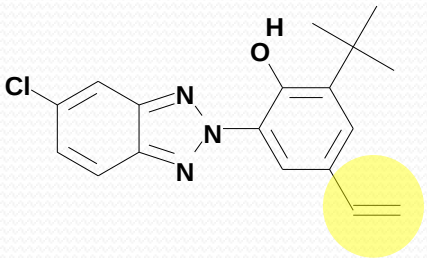
Acrylic Chemistry

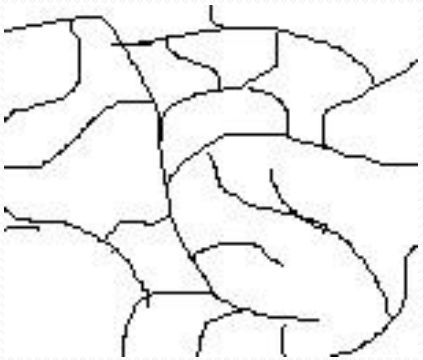
Lens Acrylic Components

Acrylic Monomers
Initiator (Azoisobutyronitrile)
Acrylic Crosslinker
Benzotriazole (UV absorber) monomer



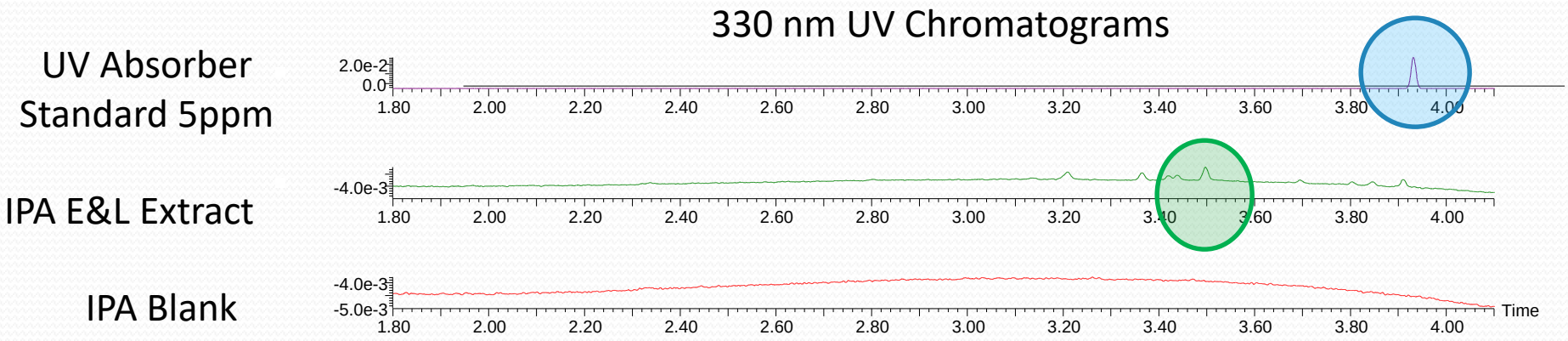
R= short chain hydrocarbon or UV absorber





Elastomeric Polymer (acrylics, polyurethane, EPDM rubber, silicone, PVC)
Elastomer swells in a chemically compatible solvent; it does not dissolve
Acrylics swell in alcohols, e.g. isopropanol (IPA)

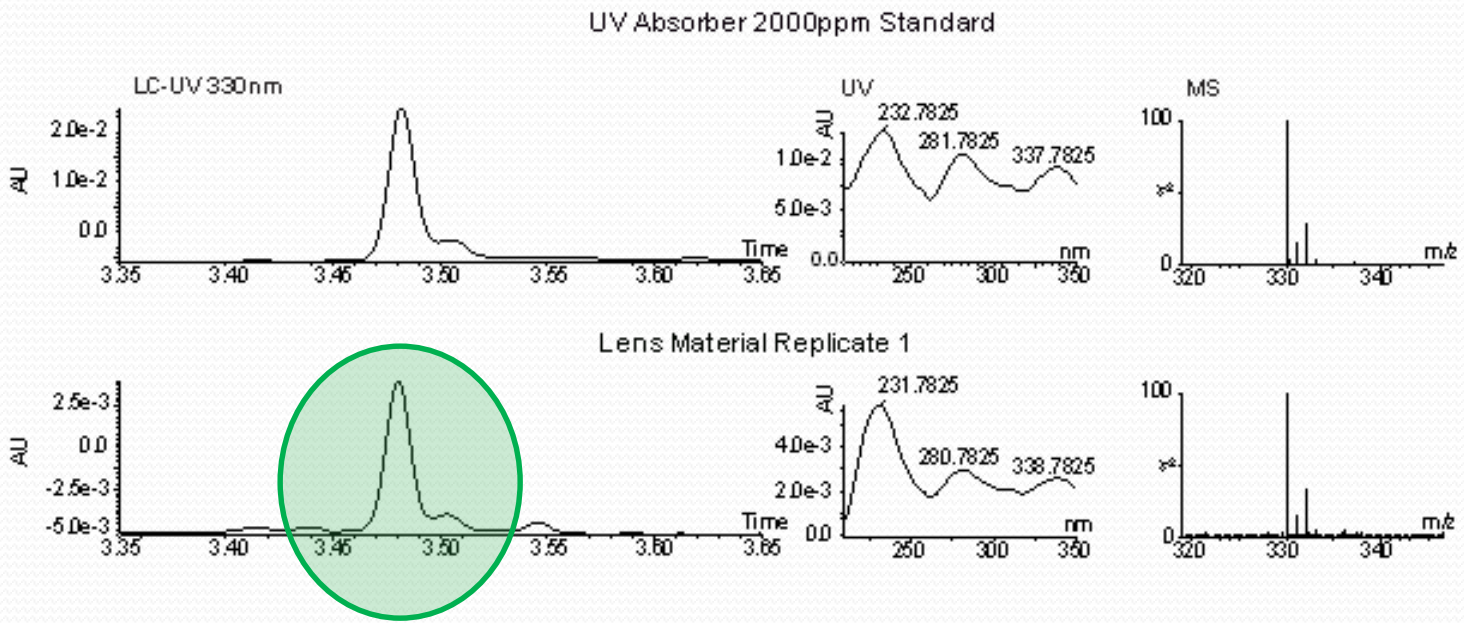
UV Detection



- UV absorber monomer elutes at 3.68 minutes
- Unknown residual analyte detected at RT 3.45 minutes
- 330nm absorbance characteristic of UV absorber, but not UV absorber monomer (no coelution).
- Possible sources of ipa extractable UV absorber related analyte
  - A) generated from the monomer as a degradant.
  - B) Introduced with UV absorber monomer (as a contaminant)

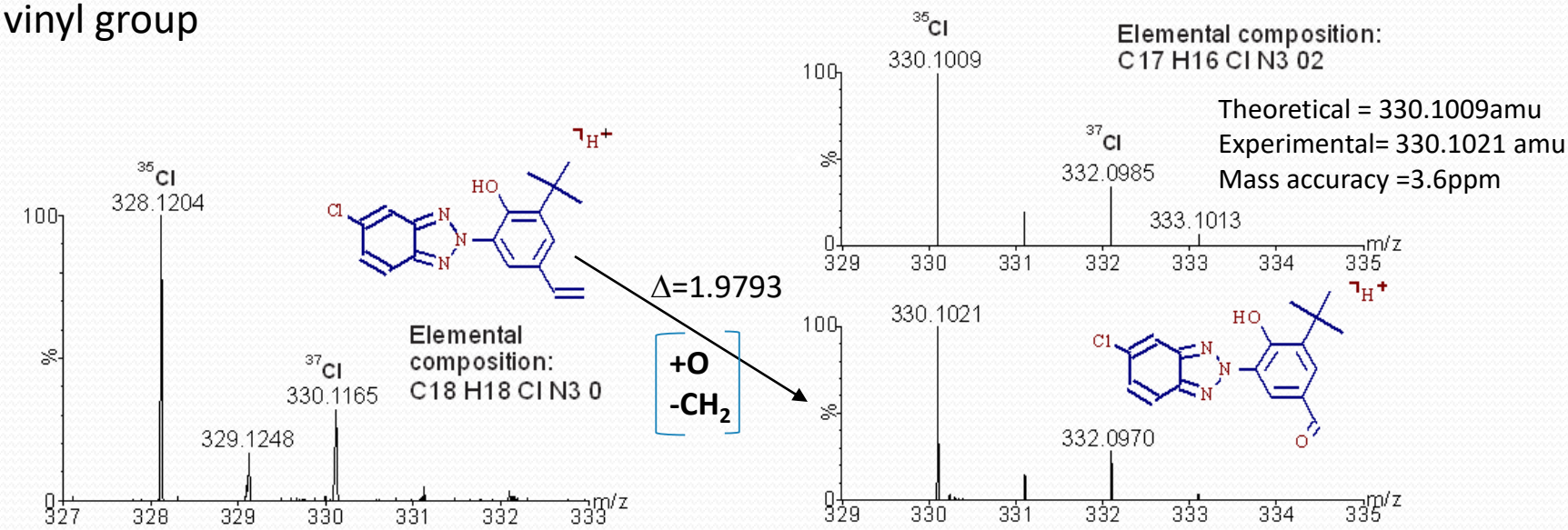
Contaminant Investigation – Analyte Source

- We identified an impurity in a high concentration UV absorber standard that matched the device impurity in UPLC retention time, UV response, and mass spectrum



Contaminant Chemical Characterization Using Accurate Mass

- Experimental accurate monoisotopic mass of UV absorber = 328.1204amu
  of UV absorber impurity = 330.1021amu
- Software aided chemical formula determination
  - Assumed element content C, O, H, N, Cl
  - one chlorine containing chemical formula, C17H16ClN3O2 (monoisotopic mass 330.1009amu) matched the accurate experimental mass within 5 ppm
- Chemical deduction suggested the impurity structure with an aldehyde group replacing the vinyl group

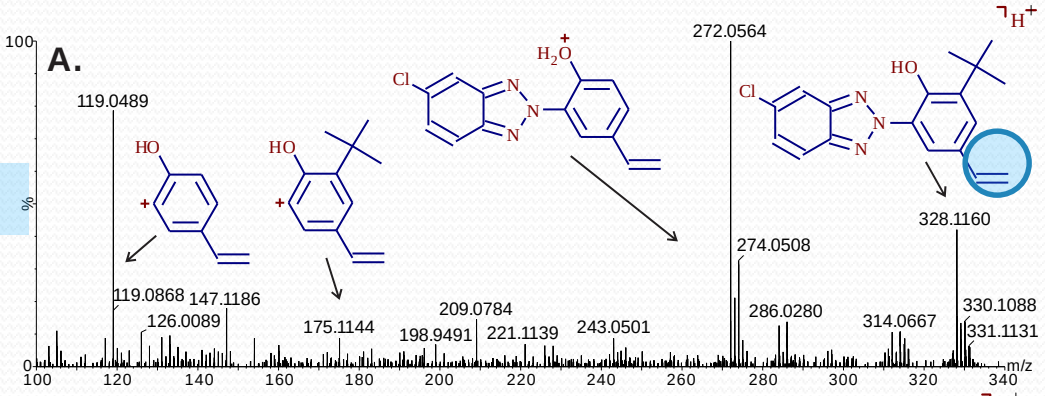


UV Absorber Monomer

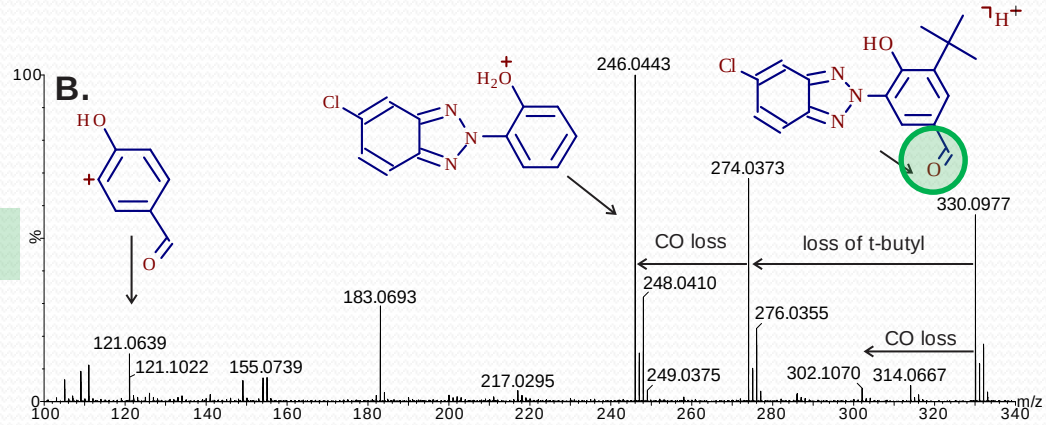
Contaminant

Comparison of Experimental MS^E High Energy Spectral Fragments and MFS Theoretical Predicted Fragments

UV Absorber



Contaminant



Application of High Resolution MS to Surfactant Fingerprinting

- Surfactant Uses in Biomedical and Pharmaceutical
  - Formulation Solubilization or Compatibilization
    - OTC drugs, cosmetics, pharmaceutical formulations)
  - Foam Control
  - Drug Delivery Application
  - Wipe down of clean room surfaces
  - Final package (e.g. cap and closure) critical cleaning
  - Process equipment cleaning
  - Final cleaning of biomedical devices prior to final packaging

Surfactant Chemistry

- Non-ionic
  - General Architecture

Linear Polymeric Surfactant Molecule

Hydrophobic Tail

R=cetyl, undecyl

Hydrophilic Head

PEG (polyethylene glycol)

$+CH_2CH_2O+_n$

PPG (polypropylene oxide repeat unit)

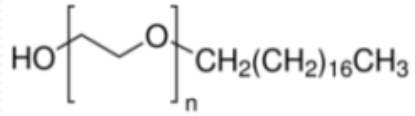
PBO (polybutylene oxide)

  - High mass
  - Liquids, paste, waxes

TICs of Several Non-ionic Surfactants

- Surfactants Often Coelute in LC due to chemical similarity
- Polymeric chains are not chromatographically resolved. Broad chromatographic peaks encompass surfactant molecules covering a range of molecular weights (polydisperse)
- All surfactants shown not UV detectable at 20-200 ppm concentration

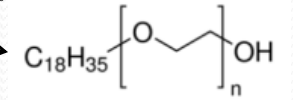
average M_n ~4,670



Brij S100

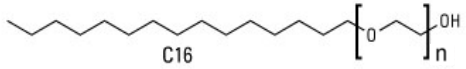
One Unsat bond

avg. M_n ~1,150



Brij O20

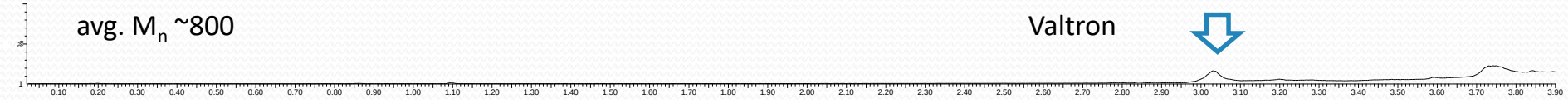
avg. M_n ~1,120



Brij 58

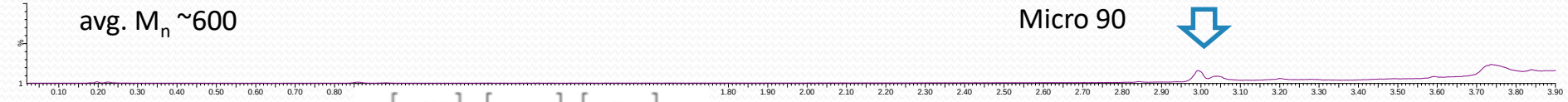
avg. M_n ~800

Valtron

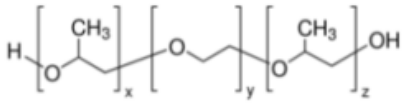


avg. M_n ~600

Micro 90



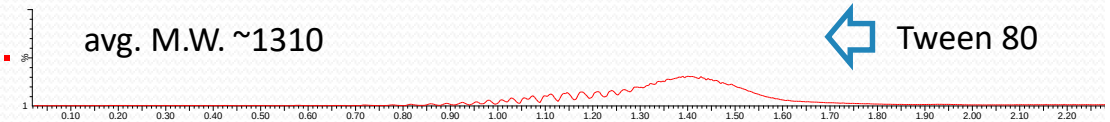
avg. M_n ~2000

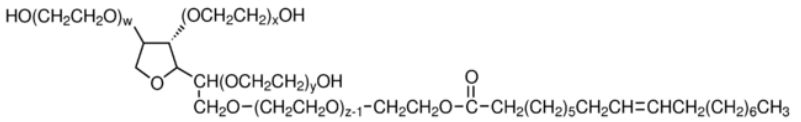


PPG-PEG-PPG (Poloxamer)

avg. M.W. ~1310

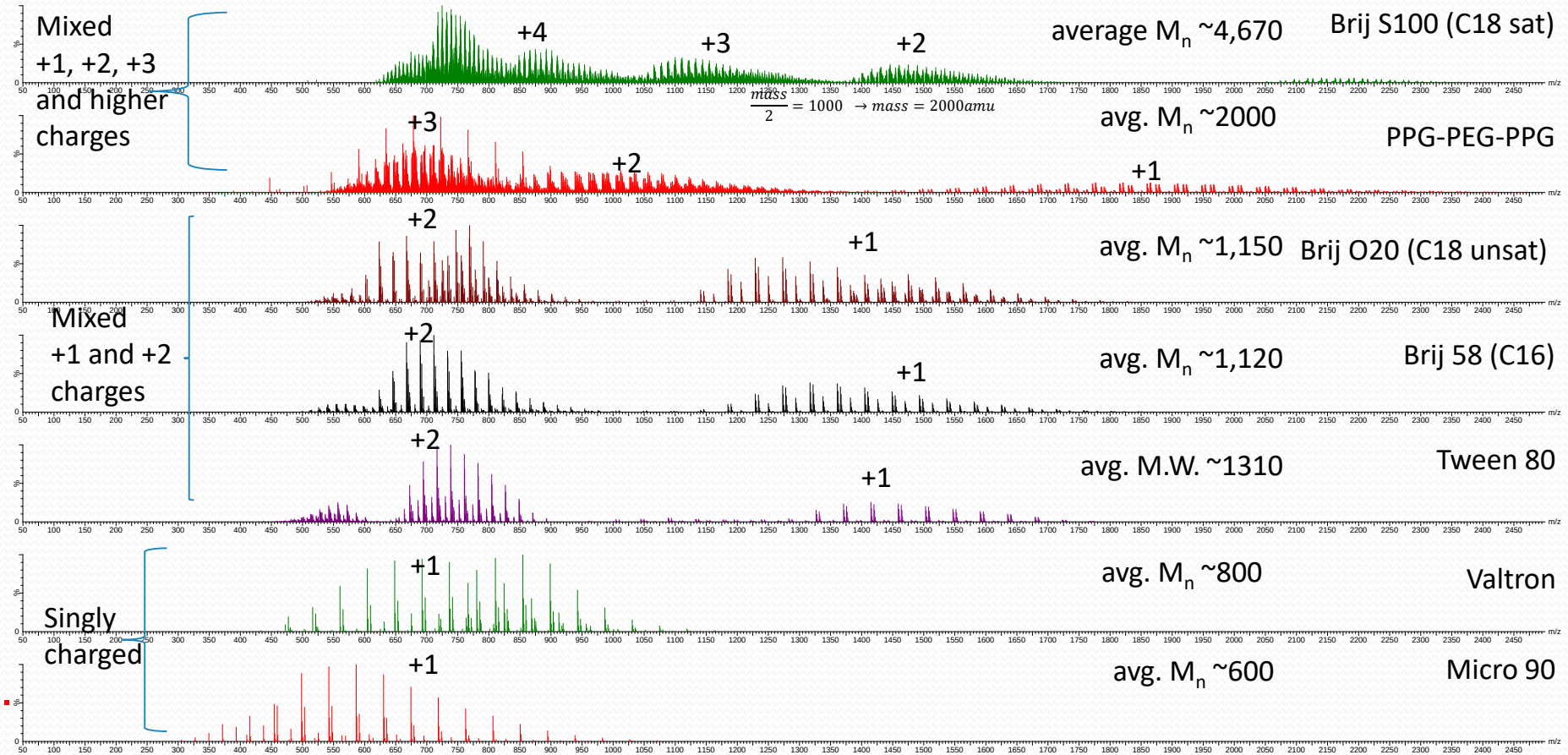
Tween 80





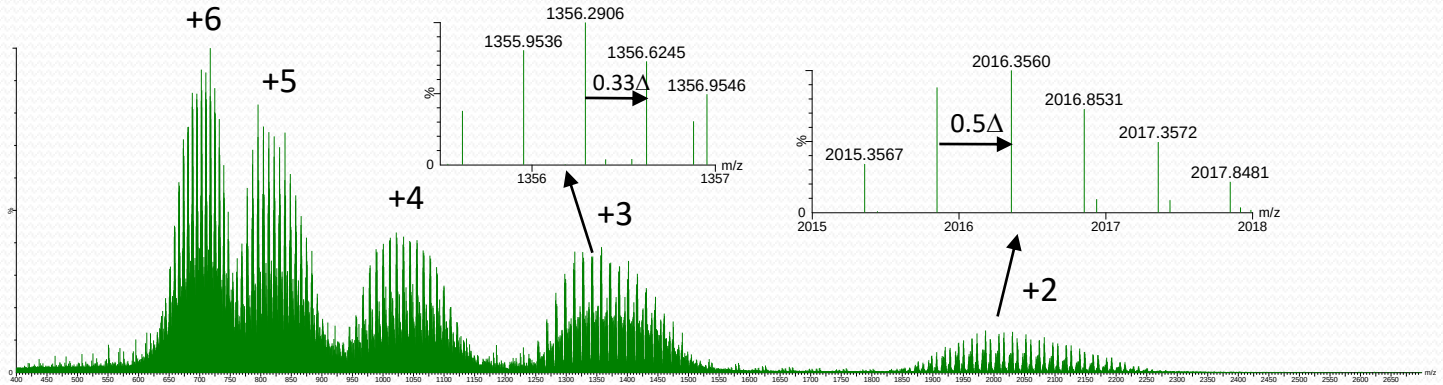
Combined Mass Spectra of Surfactants

- Higher Molecular Weight Surfactants Tend to Multiply Charge in ESI
- Molecular weight distribution can be experimentally estimated from mass spectral data
- Each surfactant displays a unique mass spectral fingerprint despite similarity in structure

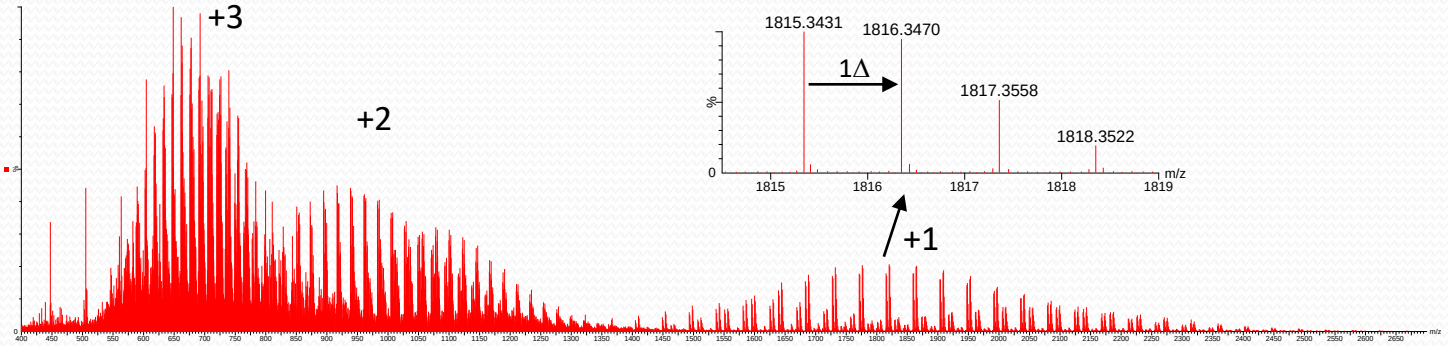


High Resolution Allows Identification of Multiply Charged Species

Brij S100



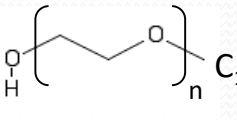
PPG-PEG-PPG



Brij 58 vs Brij O20 Surfactant MS Peak Separation

Brij O20

$HO(CH_2CH_2O)_{20}C_{18}H_{35}$

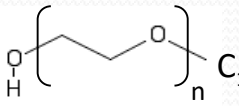


44.0262

$\Delta=C_2H_4O$

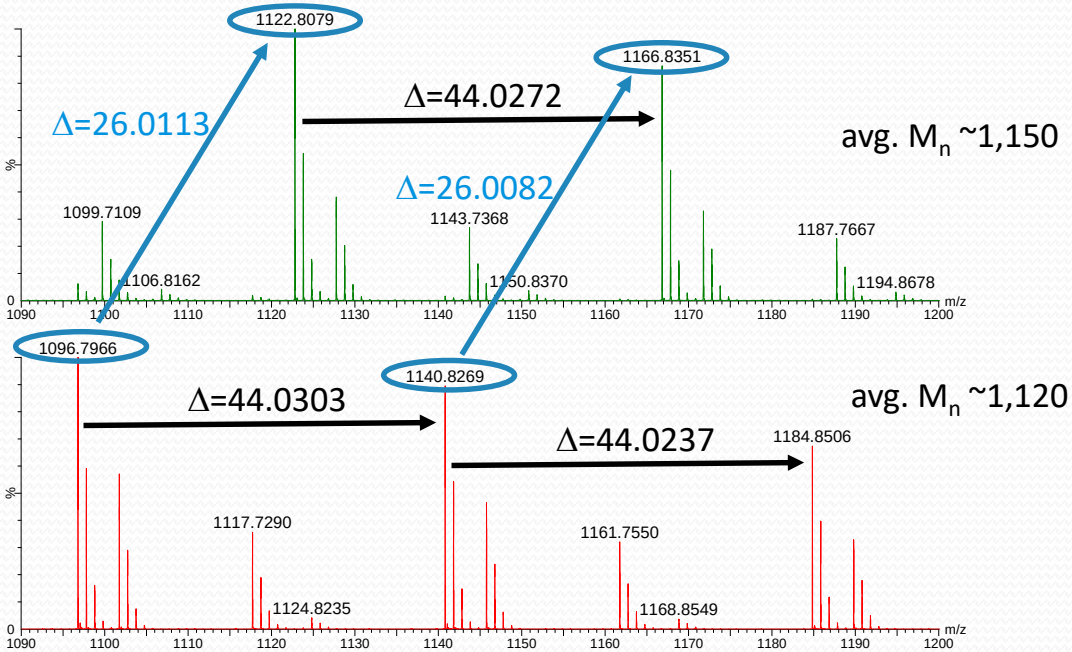
Brij 58

$HO(CH_2CH_2O)_{20}C_{16}H_{33}$



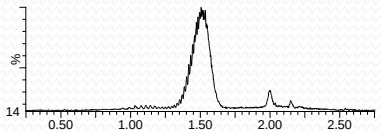
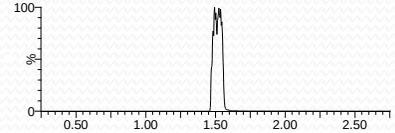
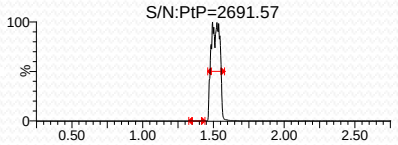
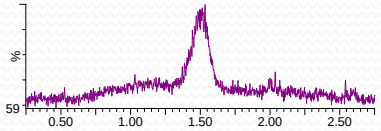
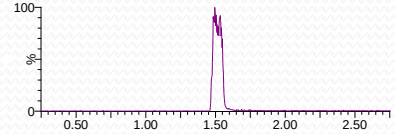
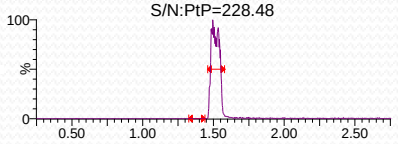
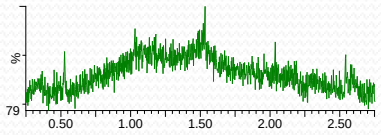
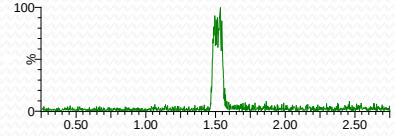
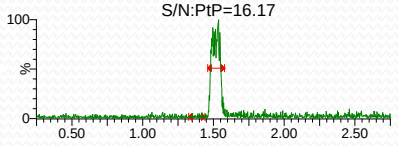
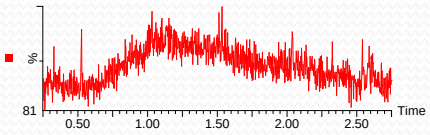
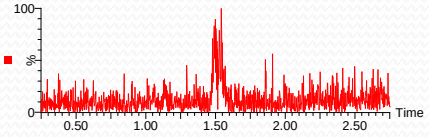
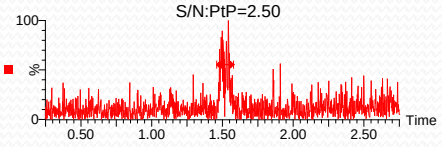
26.0157

$\Delta=C_2H_2$



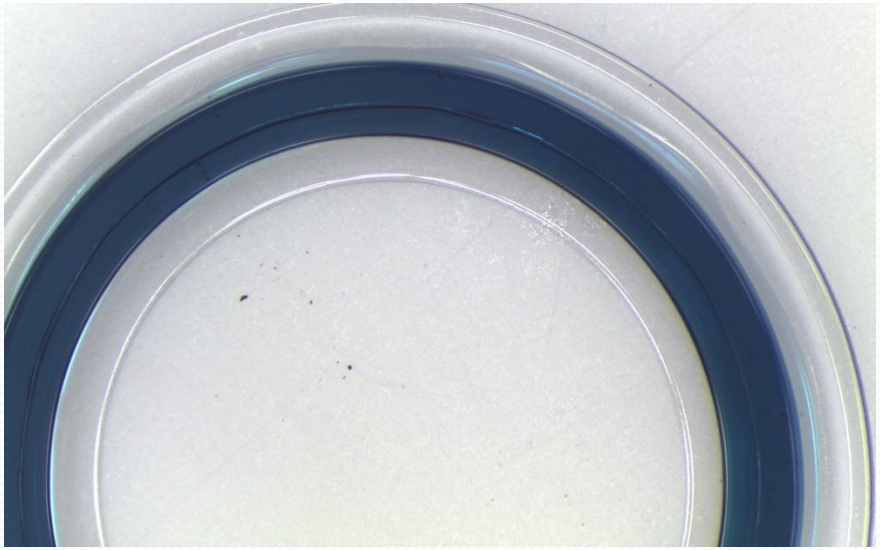
Water Soluble Wax Analysis

Water Soluble Wax Analysis - Accurate Mass Extracted Ion Chromatogram Improves Detection Limit

Wax Standard Concentration	Total Ion Chromatogram	Accurate Mass (+/- 0.01 Da) Extracted Ion Chromatogram Areas	Signal to Noise Calculation
10 ppm			
1 ppm			
100 ppb			
10 ppb			

Adhesive Hydrolytic Stability

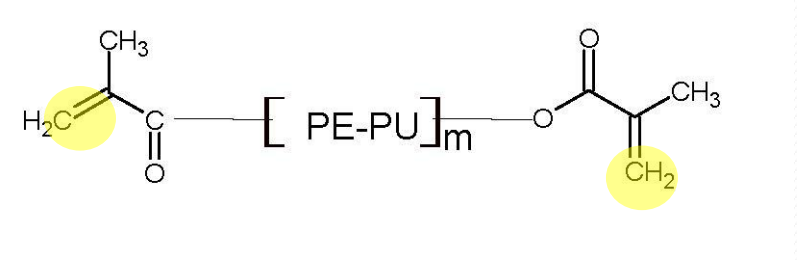
- Photo-cured acrylic-based adhesive for adhesion of acrylic parts of biomedical device
- Long term hydrolytic stability of the adhesive bond
  - Accelerated aging in water at 85°C for 2 months equivalent to five years real time at physiological conditions (35°C) with 10grams water/gram material.

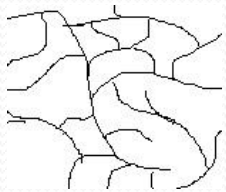


Adhesive Chemistry

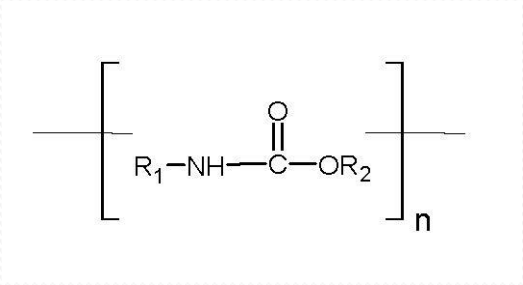
Macromer + Acrylic Crosslinker $\xrightarrow{\text{photoinitiator}}$ Adhesive Network

Polyetherurethane Acrylic Macromer Architecture

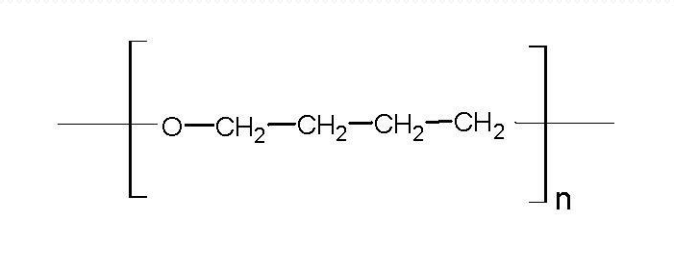




Crosslinked Adhesive



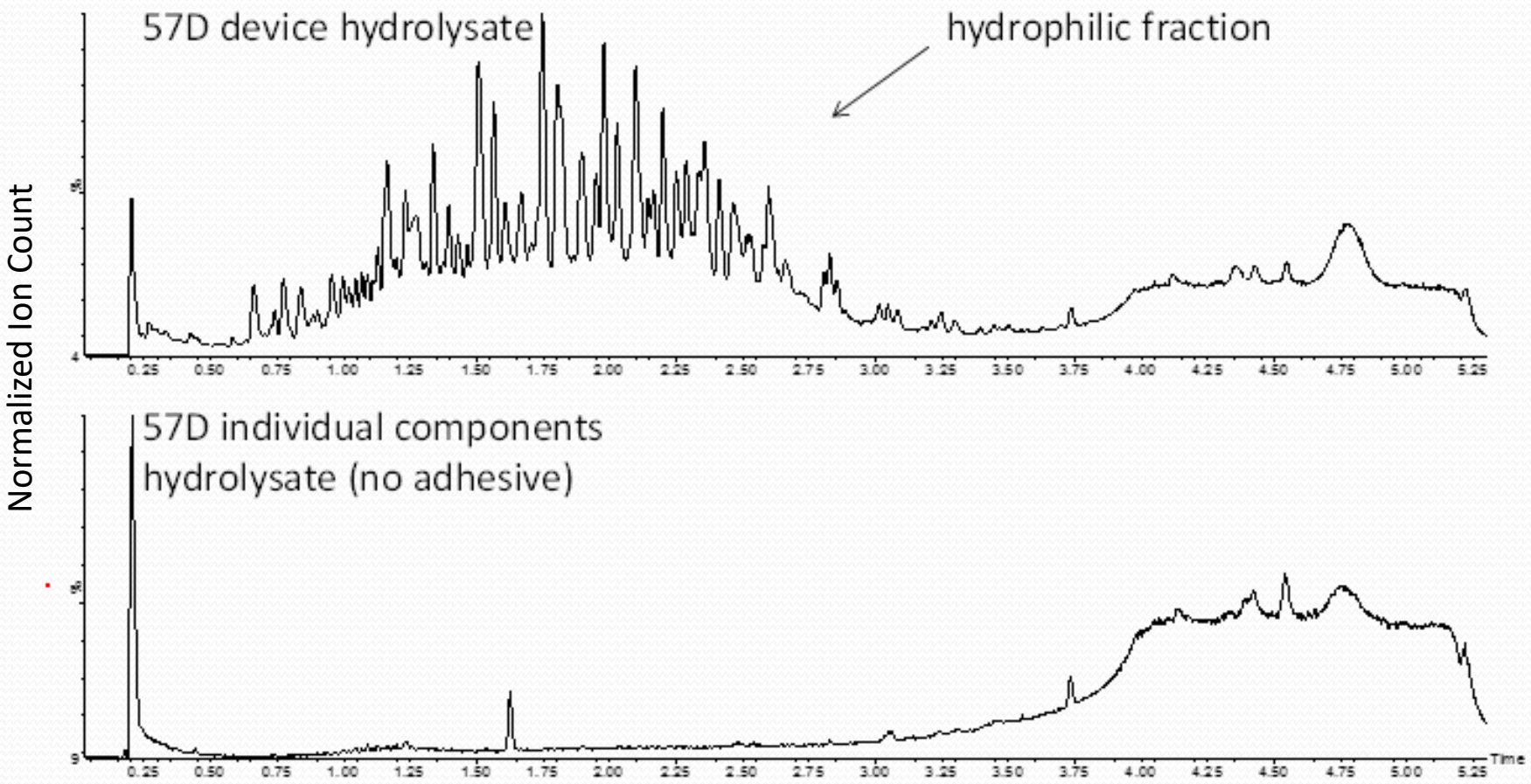
PU
Polyurethane



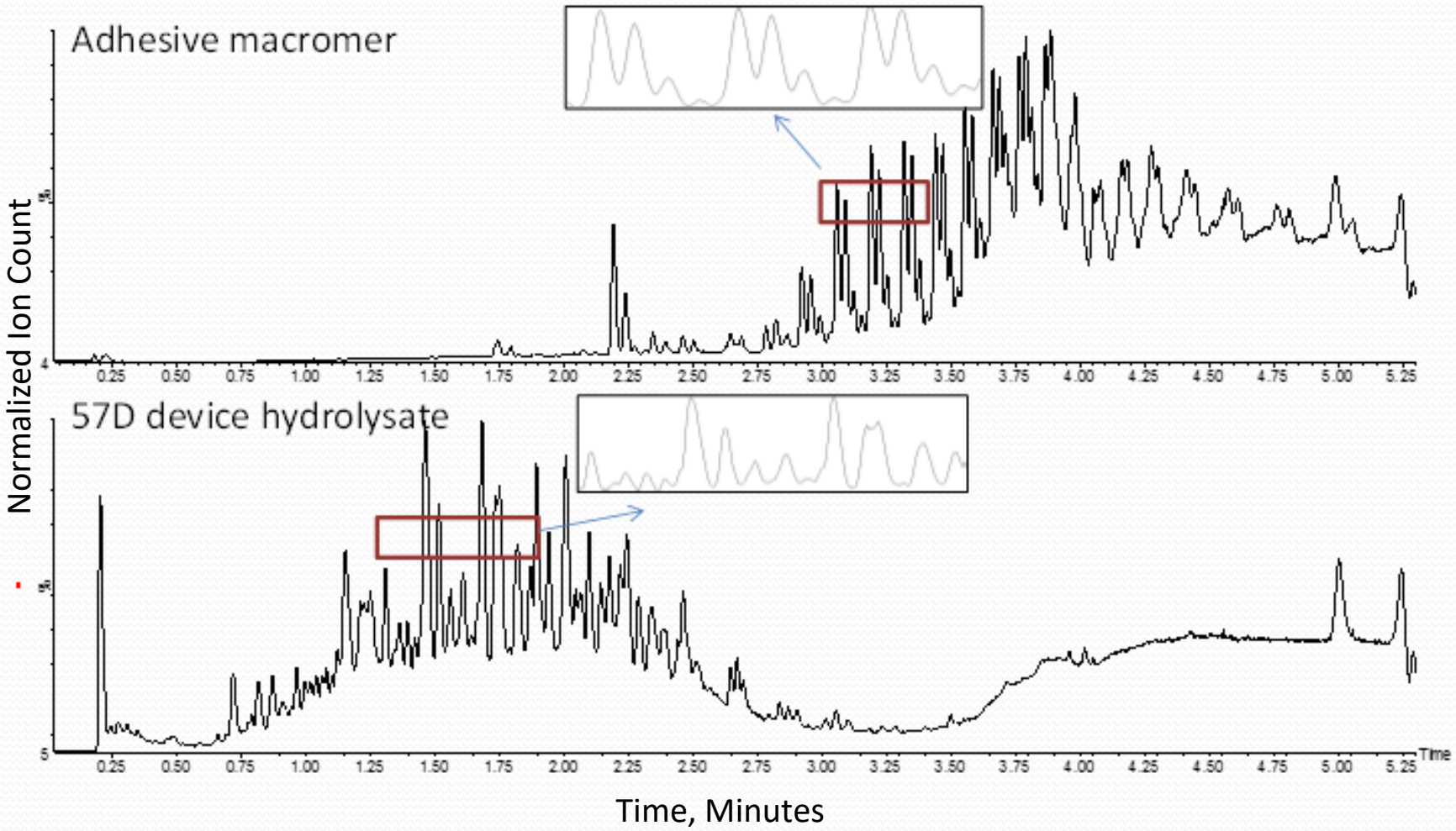
PE
polybutoxyether

Polyetherurethane macromer
MW≅ ?

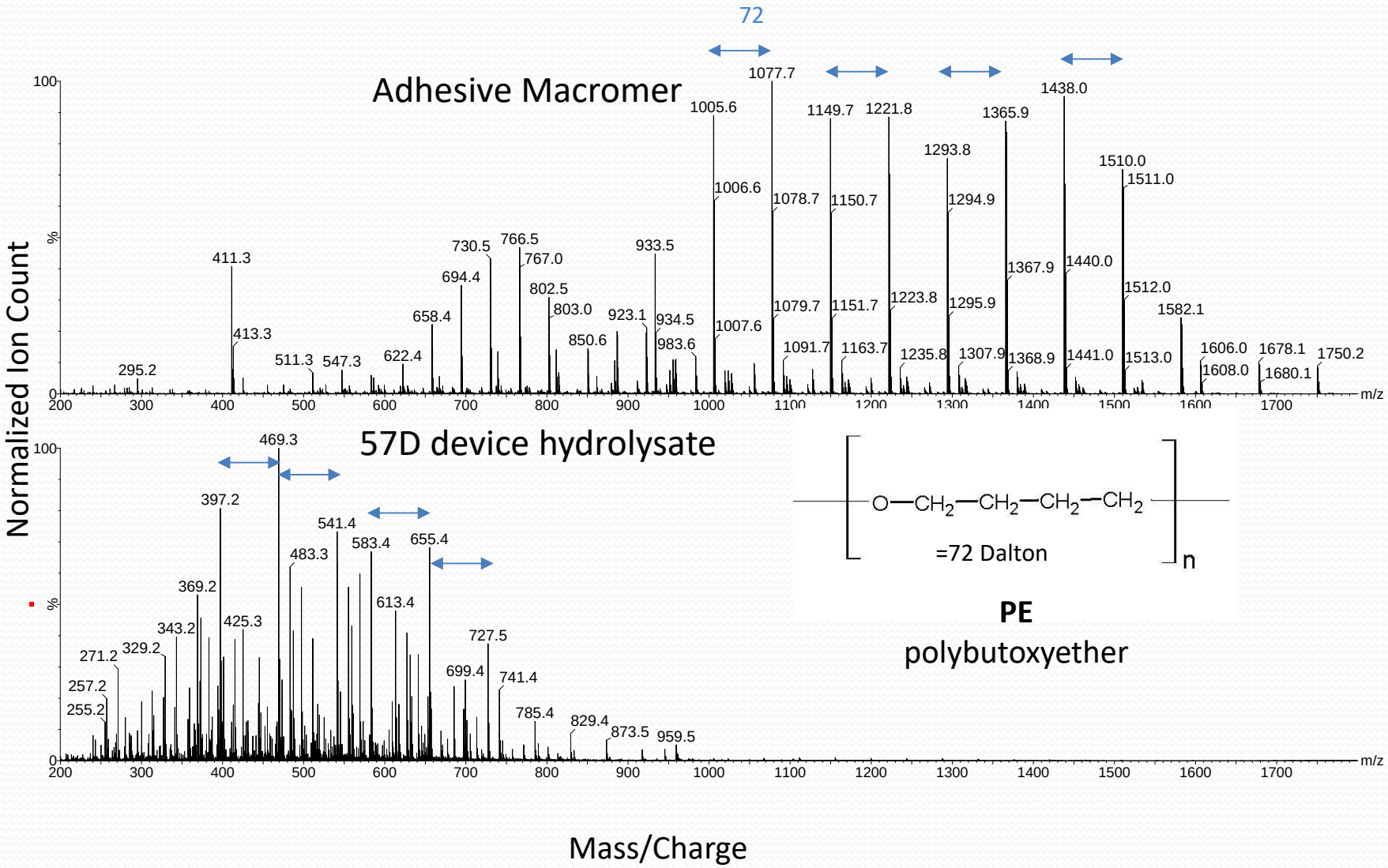
Total Ion Chromatograms of 5 Year Equivalent Aged Hydrolysates



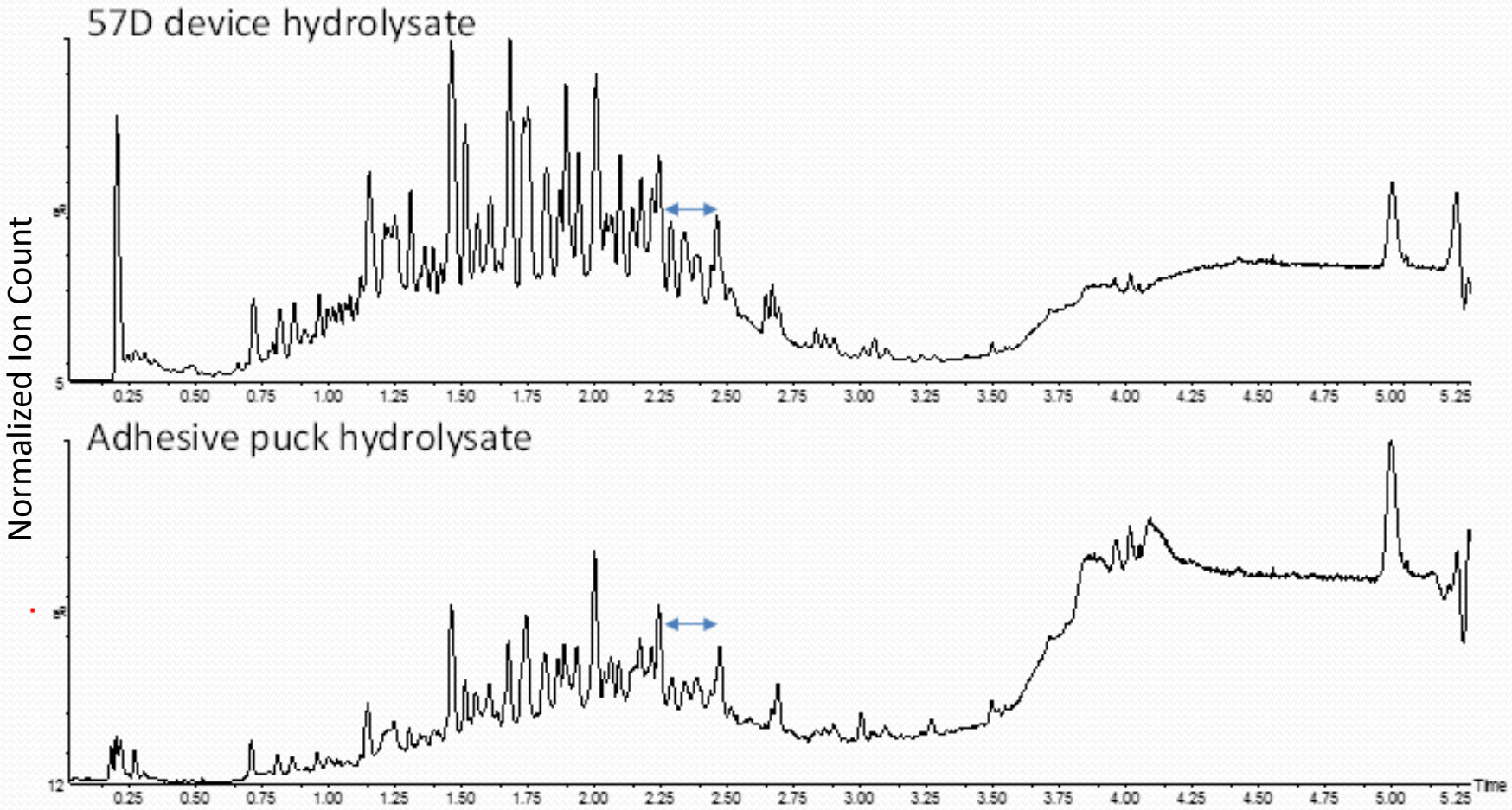
Total Ion Chromatograms – Macromer Std vs. Aged Hydrolysate



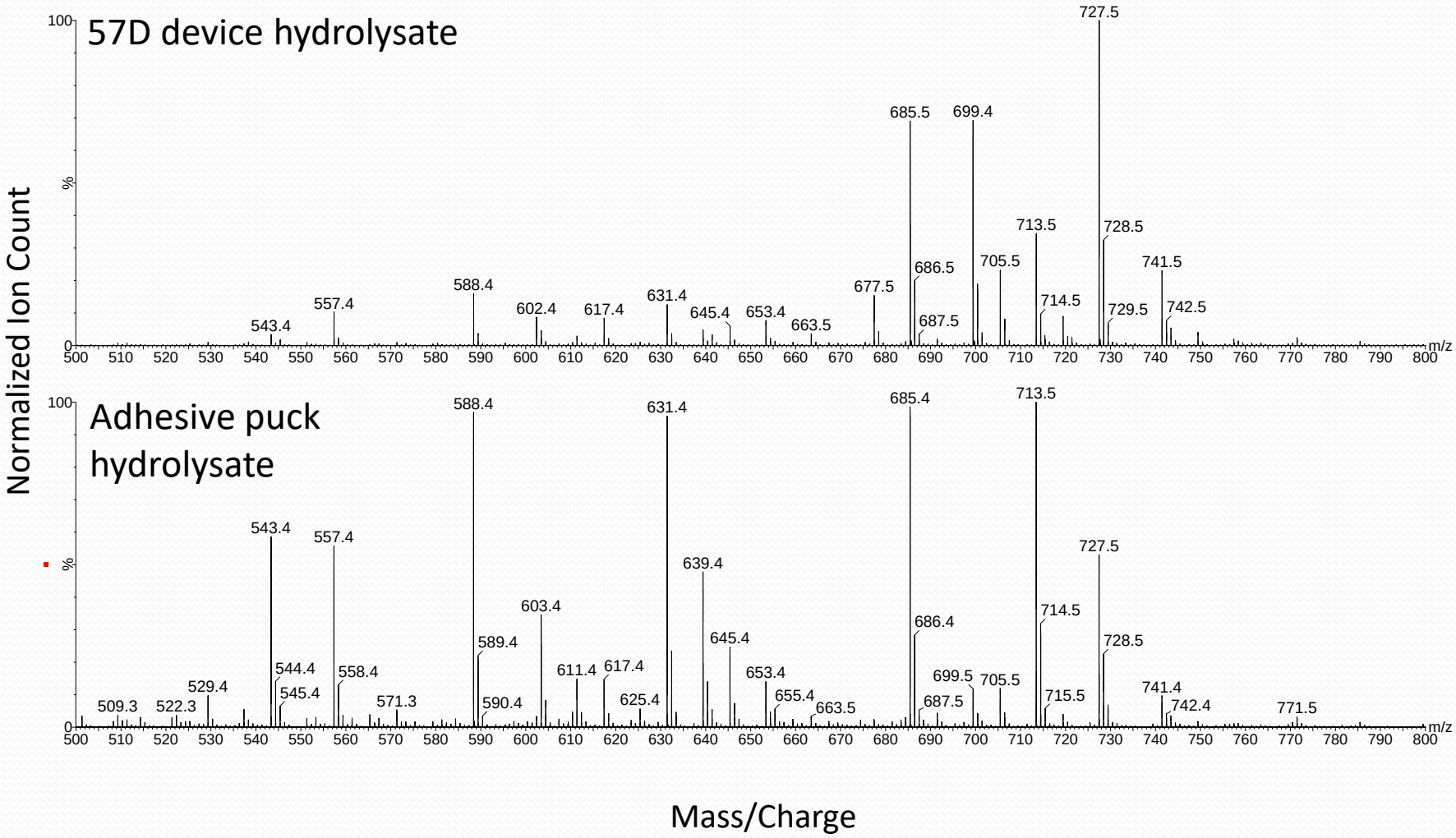
Combined Mass Spectra– Macromer Std vs. Aged Hydrolysate



Total Ion Chromatograms – Adhesive Puck vs. Aged Hydrolysate



Mass Spectra -Adhesive Puck vs. Aged Hydrolysate



Summary of Adhesive Case Study Results

- Macromer adhesive is not UV active not detectable by conventional analysis.
- Mass detection provided sensitive detection and mass spectral data rich in chemical structure information.
- The adhesive related analyte concentration in the device hydrolyate was estimated to be 45 ppm using the MS response of adhesive macromer standard solution for quantification. Low concentration equivalent to only 1% of the total macromer applied in the adhesive joint
- Prolonged hydrolysis of the adhesive puck at 100C beyond 170 hours did not result in an increase in the adhesive analyte solution concentration suggesting that hydrolysis was limited to a labile macromer fraction.
- We are currently working on ascertaining the source of this evidently limited but labile fraction.
- Evidence other than chemical did not indicate a hydrolysis problem
  - SEM analysis of the device adhesive joint after hydrolysis did not reveal any damage
  - Stress testing of hydrolytically aged devices did not detect any loss in the joint strength
  - Hydrolysate solutions were non-cytotoxic

Summary of Key Points

- Versatility of Waters G2-S QTOF Qual/Quan Instrument
- High Resolution Mass Spectrometry required for all three case studies. No other method has both sensitivity and selectivity needed.
- Fragment data acquired with QTOF MSMS is required for structural elucidation of small molecules.
- The performance of Mass Frontier software in fragment prediction was impressive. Its an important tool.
- Mass Spec Lab's fragment library built rapidly with MS^E technology is expected to be a superior tool for plastics and packaging additive identification in E&L studies performed for pharma and biomedical