Best Practices for OINDP Pharmaceutical Development Programs
Leachables and Extractables

IV. Analysis of Leachables and Extractables

PQRI Leachables & Extractables Working Group

PQRI Training Course
12-13 April 2007
Chicago, IL

Key Points to Consider

- Identification of leachables and extractables is a problem in Trace Organic Analysis.
- "Trace Organic Analysis" can be defined as the qualitative and quantitative analysis of a complex mixture of trace level organic compounds contained in a complex matrix.
- Other similar problems include:
  - Analysis of pollutants in environmental matrices
  - Organic geochemical analysis
  - Metabolite profiling in biological matrices

Key Points to Consider (continued)

- Trace Organic Analysis problems require (in general):
  - Some knowledge of the chemical nature of the analyte mixture and matrix (*Supplier Information*).
  - Removal/extraction of the complex mixture of organic compounds from the matrix.
  - Separation of the complex mixture of organic compounds into individual chemical entities.
  - Compound specific detection of the individual chemical entities within the complex mixture.

Compound Specific Detectors
(from the ridiculous to the sublime)

- Single-crystal X-ray Spectrometer
- FTIR (Fourier Transform Infrared Spectrophotometer)
- NMR (Nuclear Magnetic Resonance Spectrometer)
- Mass Spectrometer
  - GC/MS
  - LC/MS

What is a Mass Spectrometer???????

- Sample inlet system
- Ion Source
- Mass analyzer
- Detector

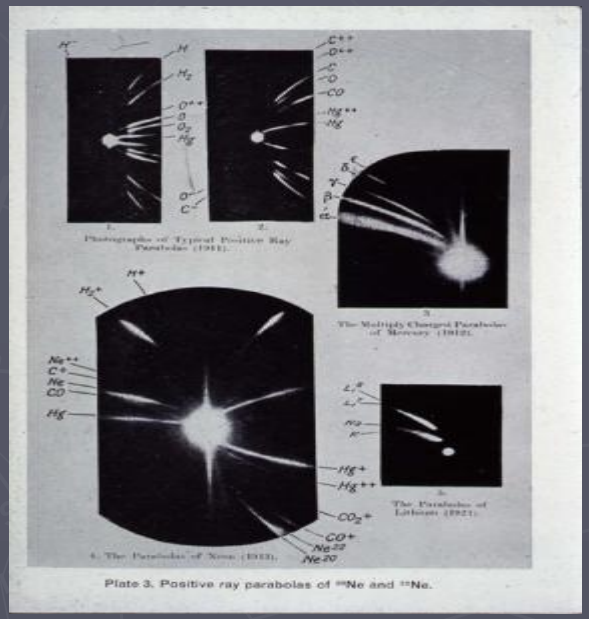


Plate 3. Positive ray parabolas of ^{20}Ne and ^{22}Ne.

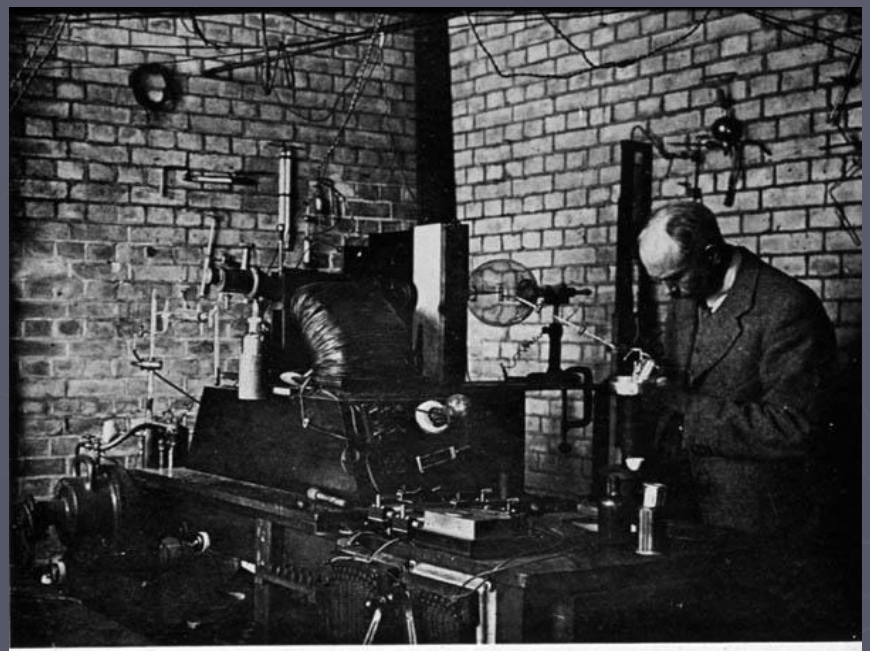


Plate 1. F. W. Aston with second mass spectrograph.

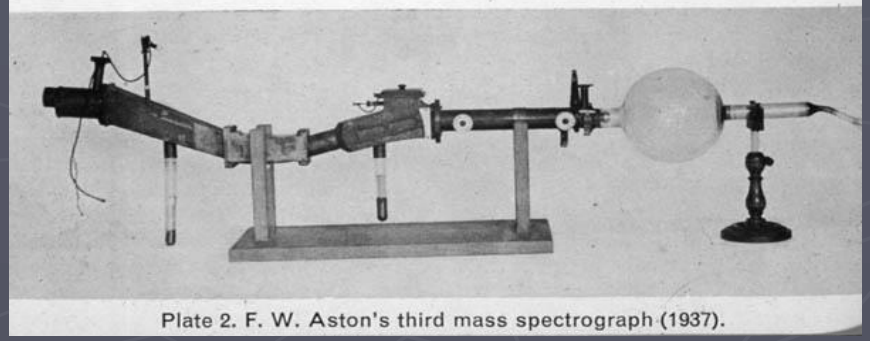
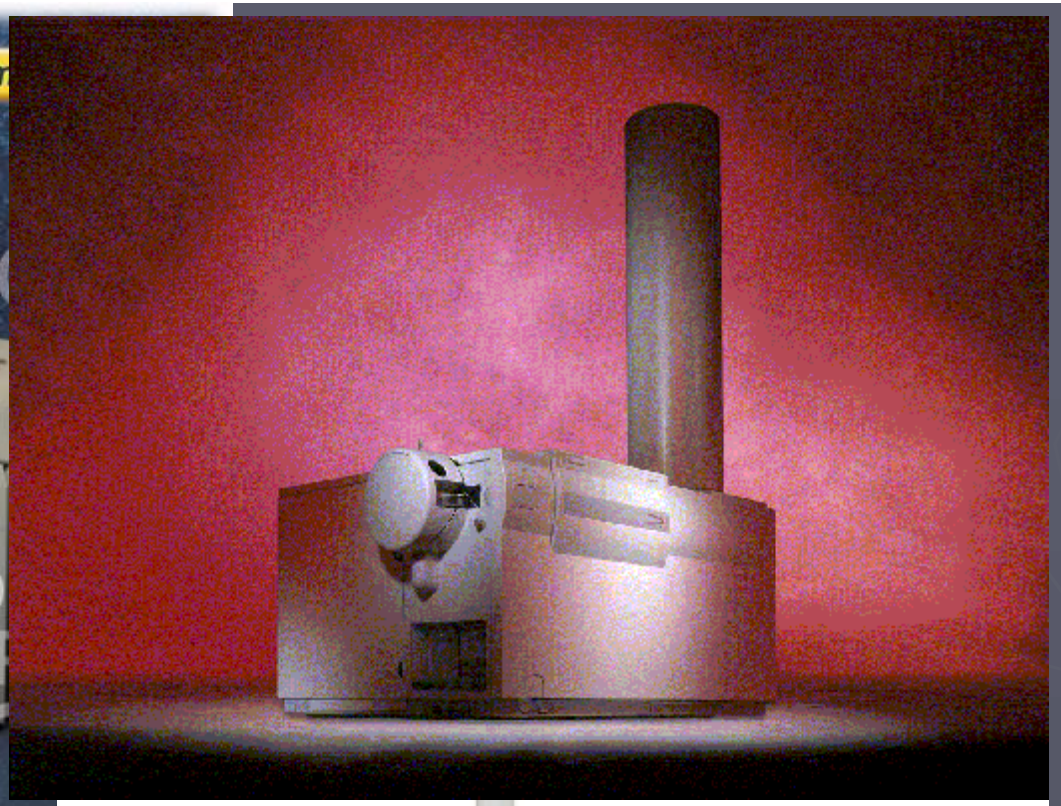


Plate 2. F. W. Aston's third mass spectrograph (1937).


AutoSpec Ultim
Pesticides
PCBs
PHARMACEUTICAL
CLINICAL
ENVIRONMENTAL
ANALYTICAL
Dioxin




IonSpec

Sample Inlet Systems

- Direct Insertion Probes (EI/CI/FAB)
- Batch Inlet Systems (AGIS/heated septum inlets/"targets")
- Gas Chromatographs
- Liquid Chromatographs (with or without separation in an HPLC column)

Ionization Processes

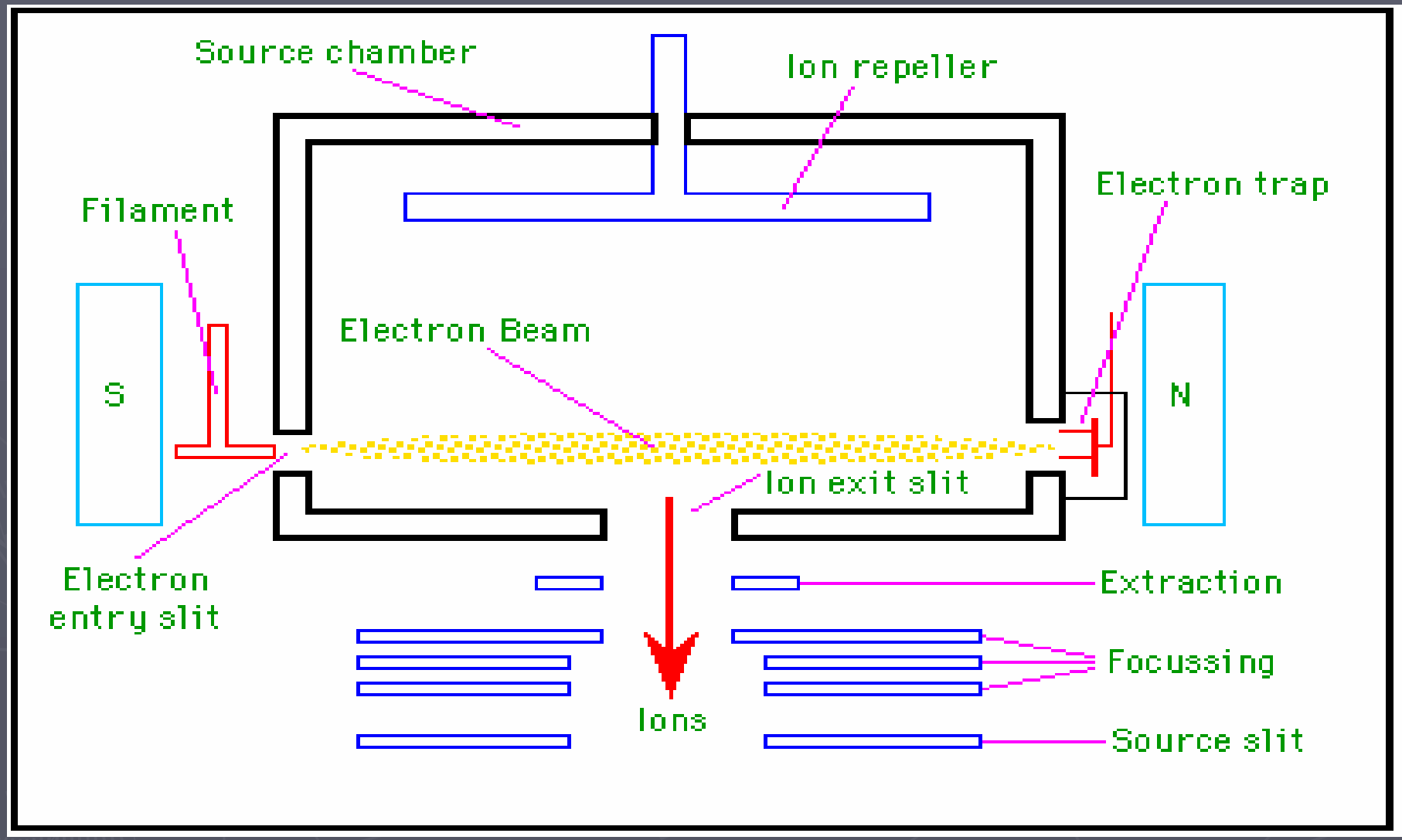
- EI - electron ionization
- CI - chemical ionization
- FI - field ionization
- FAB - fast atom bombardment
- LSIMS - liquid secondary ion
- TSP - thermospray
- ESI - electrospray
- APCI - atmospheric pressure CI
- MALDI - matrix assisted laser desorption

Mass Analyzers

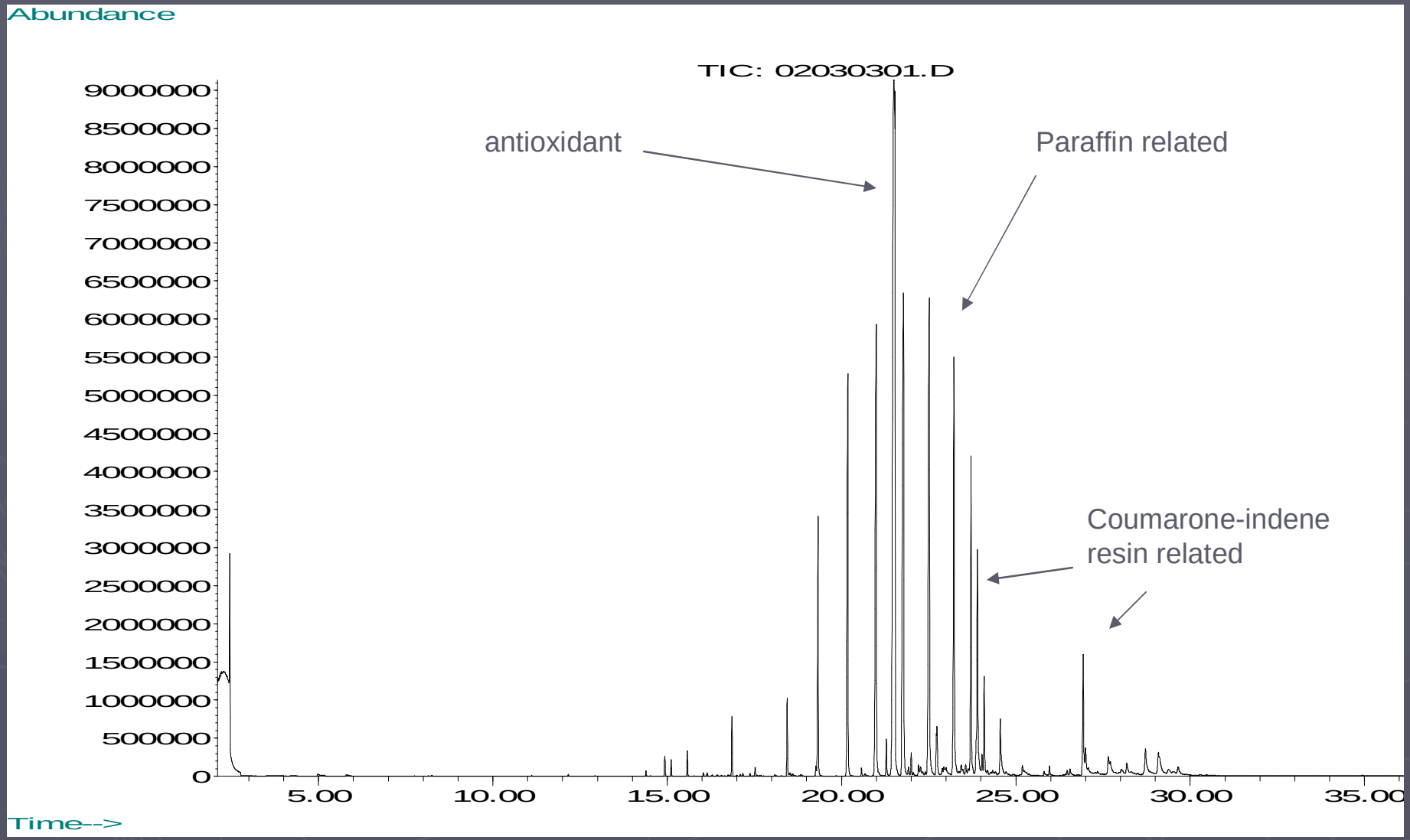
- Magnetic sector (high resolution)
- Quadrupole filter ("benchtop"/Triple Quad.)
- Time-of-flight (scan rate/mass range/accurate mass measurements)
- Quadrupole ion trap (MS^n)
- Ion cyclotron resonance (FTMS/ultra-high resolution/mass range/accurate mass measurements)

GC/MS – Circa Late 1980s

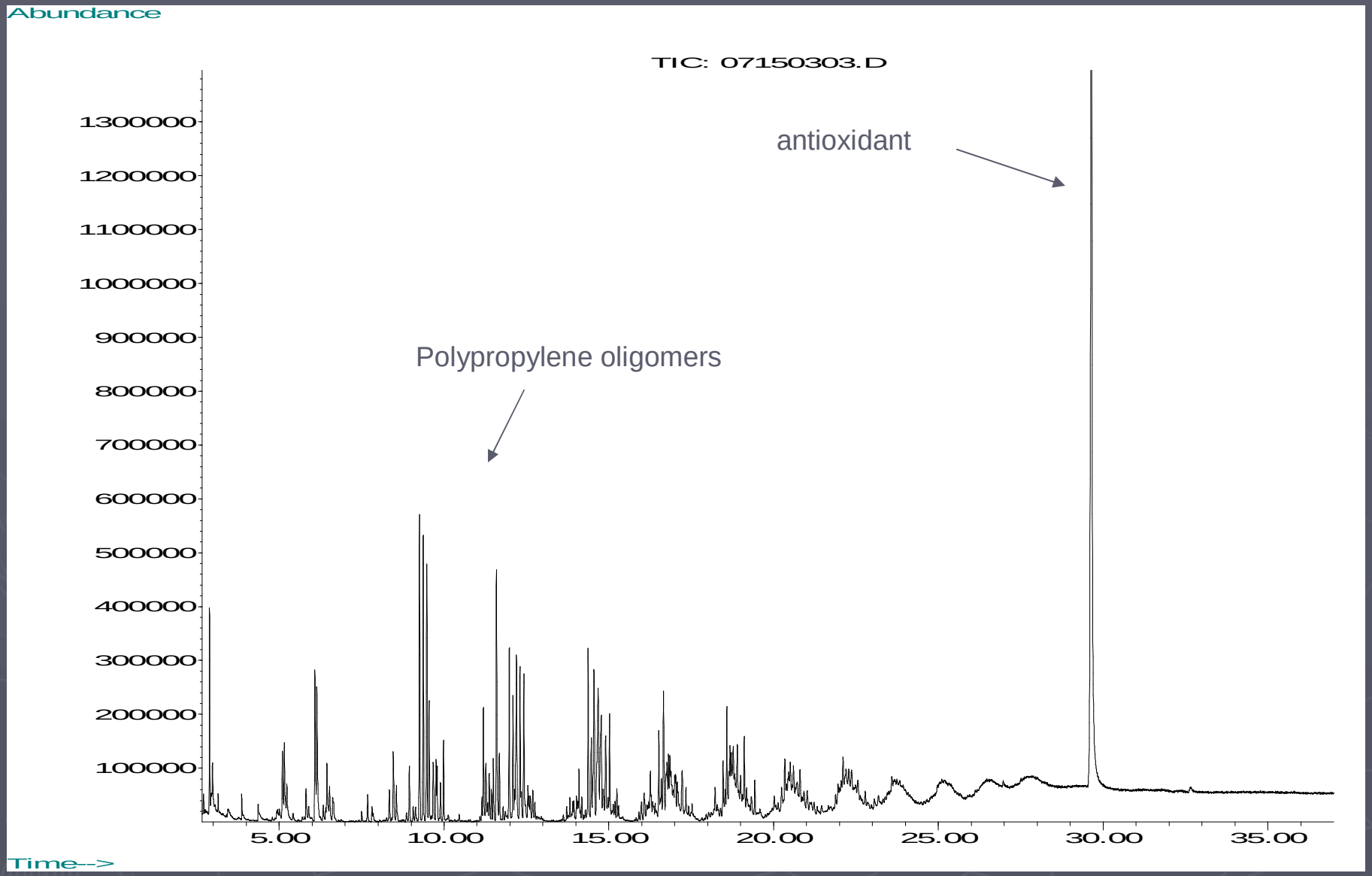
GC/MS – Electron Ionization



GC/MS Extractables Profile of an Elastomer



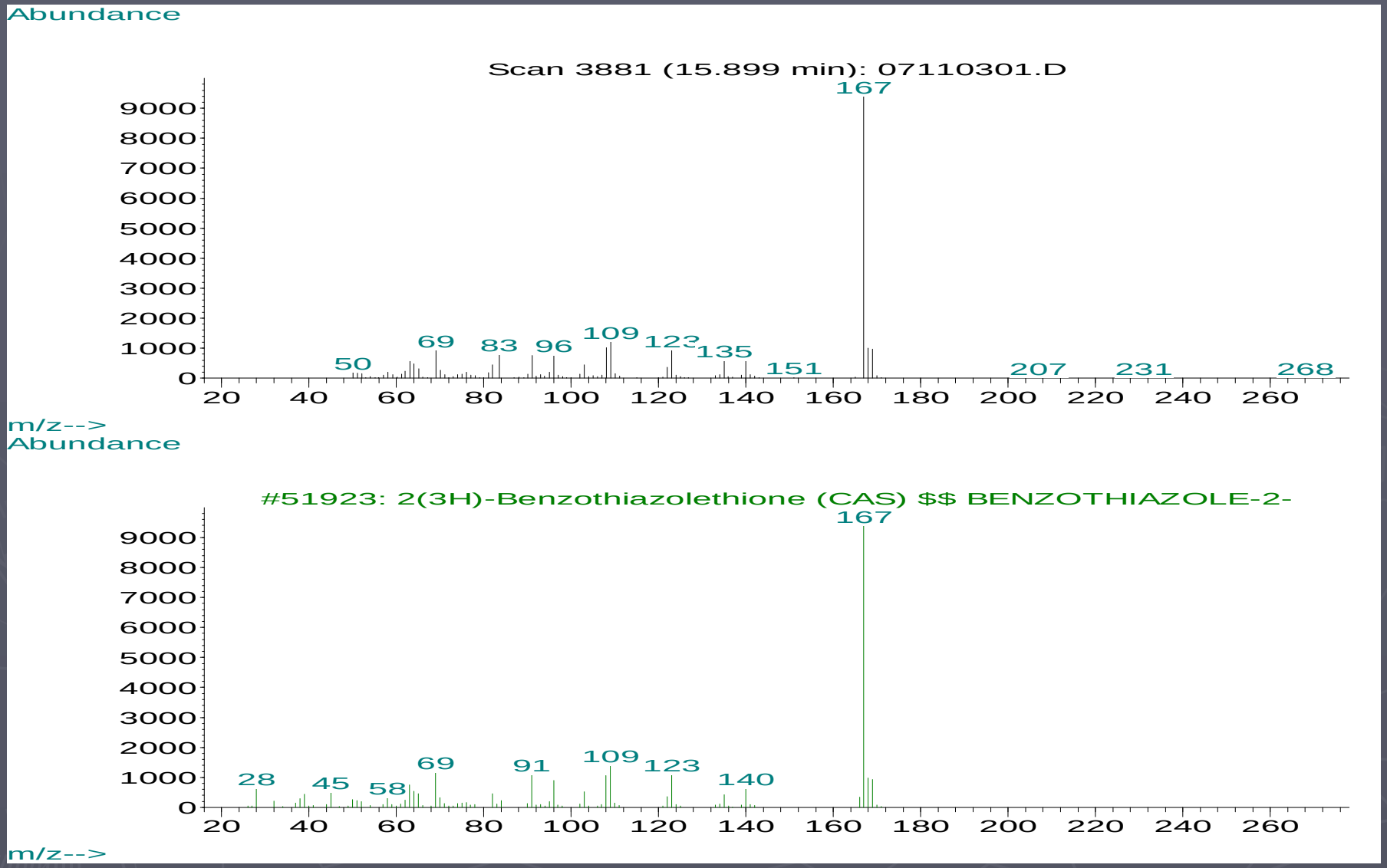
GC/MS Extractables Profile of Polypropylene



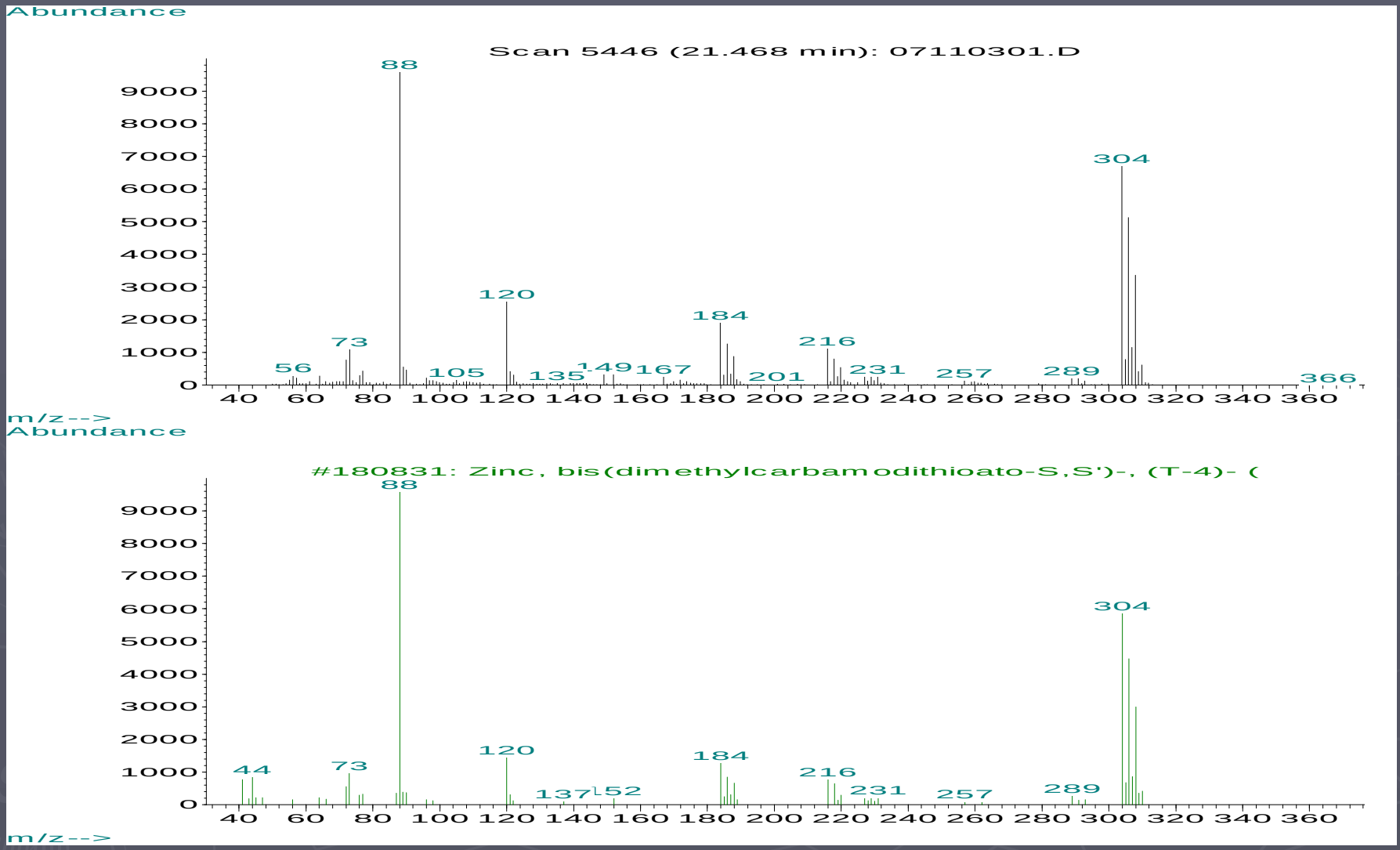
Interpreting a Mass Spectrum

- Confirm molecular weight!!!!!!!!!!
  - Adduct ions/multiply charged ions (LC/MS)
  - Alternate ionization techniques (EI/CI and APCI/ESI)
  - Note features of the molecular ion (obvious heteroatoms/nitrogen rule)
- Rationalize significant fragmentation processes:
  - Structures of fragment ions
  - Mechanisms for fragment ion formation

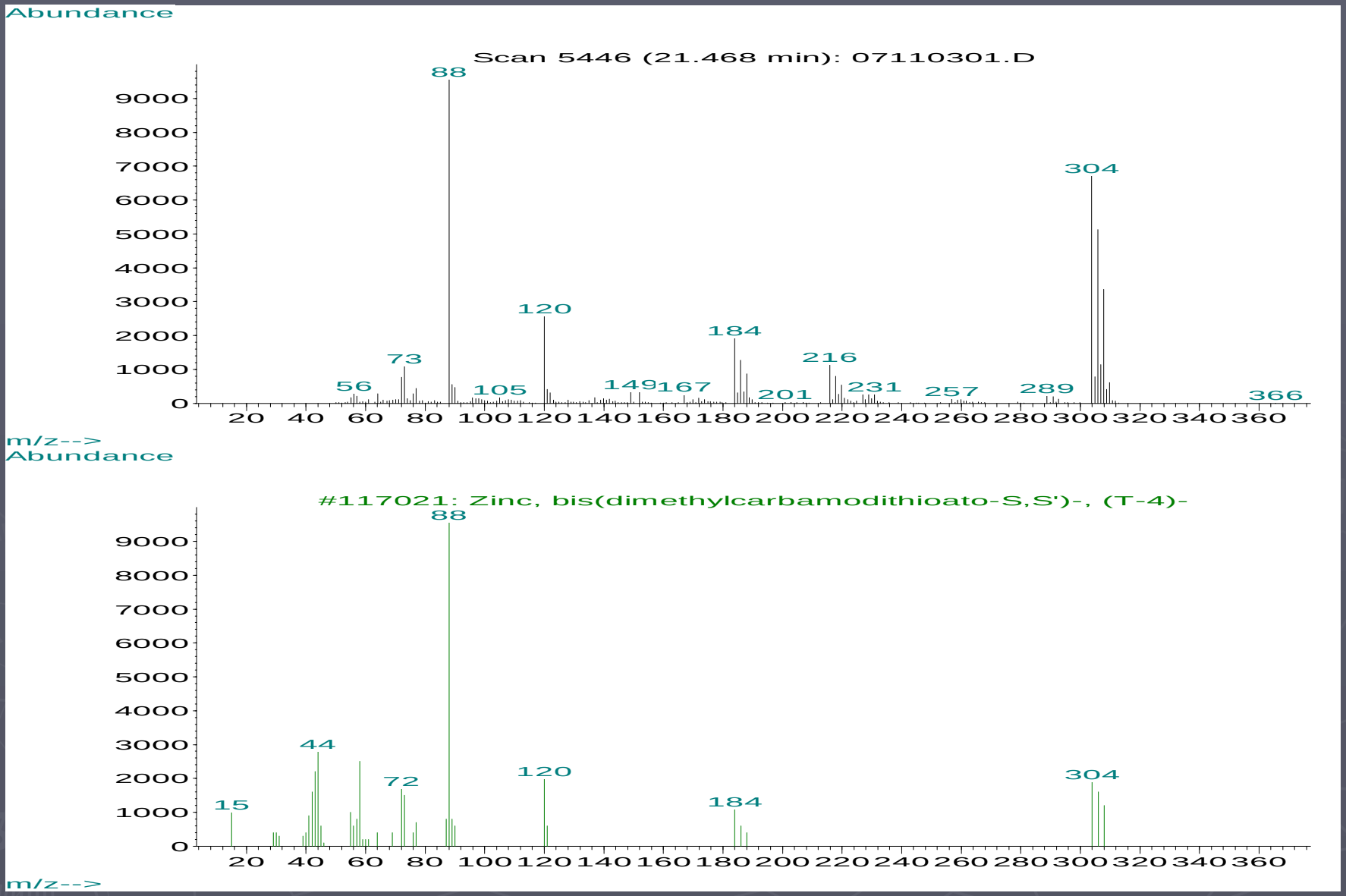
“Good” Library Search Result



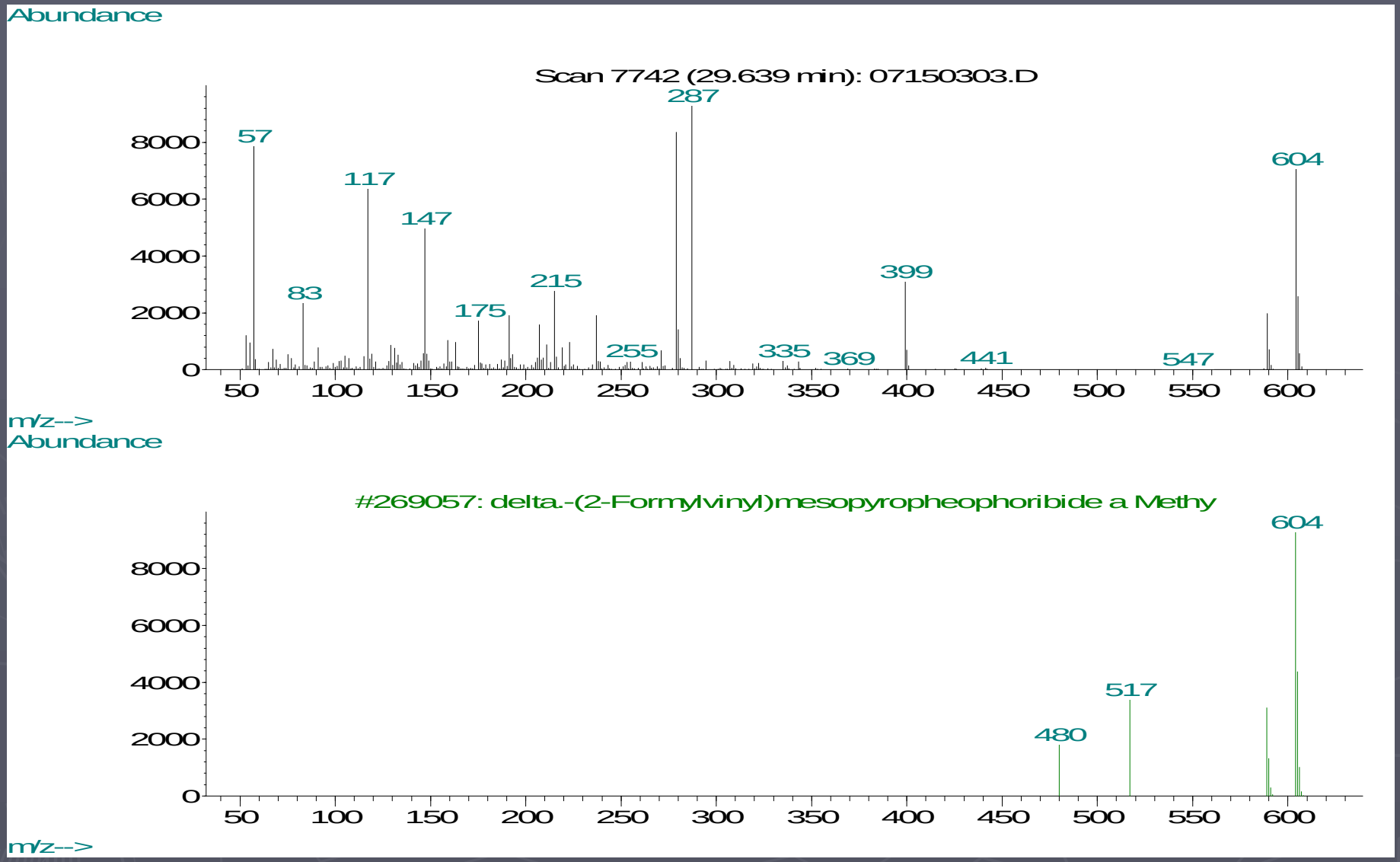
“Good” Library Search Result



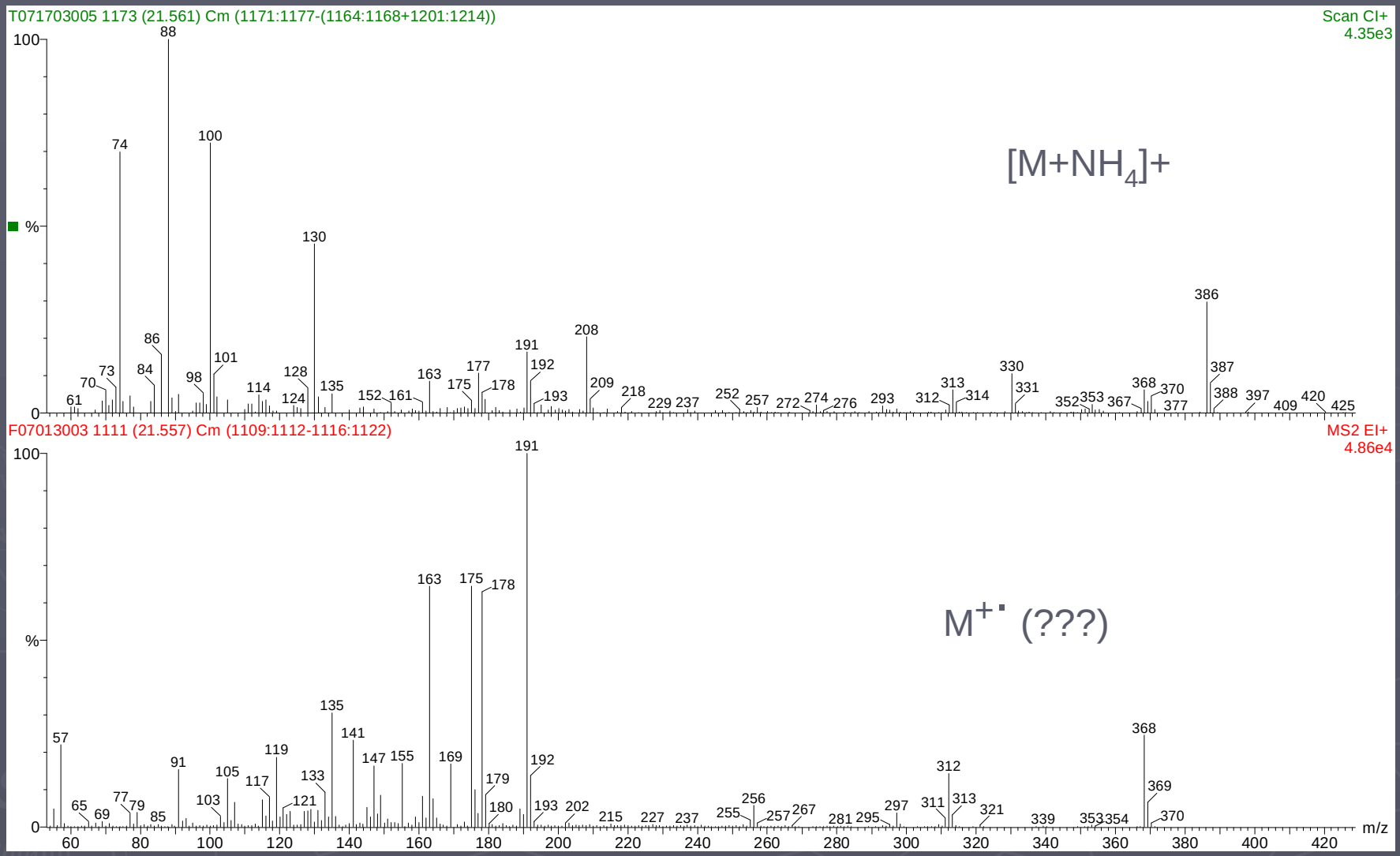
“Questionable” Library Search Result



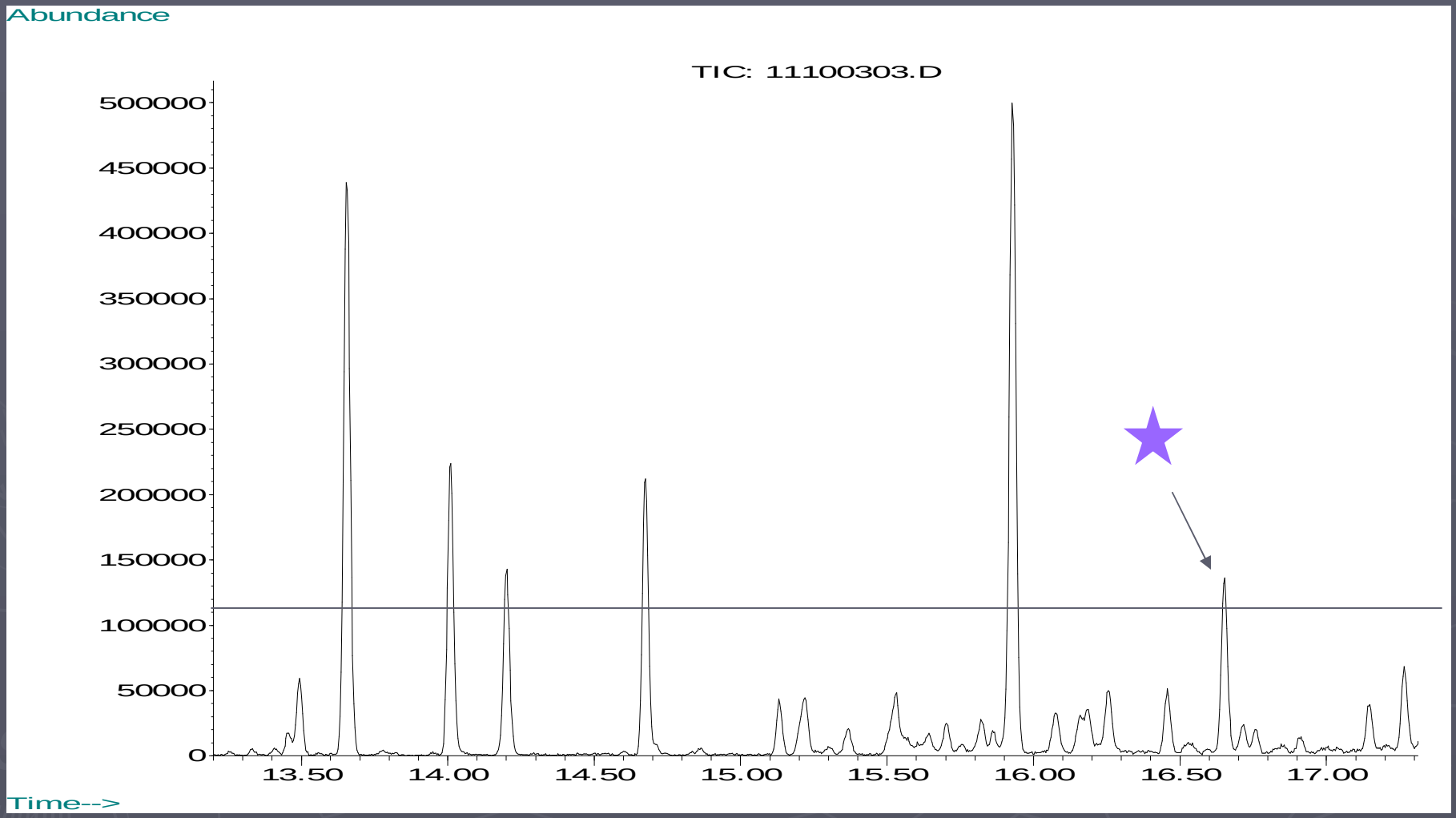
“Questionable” Library Search Result



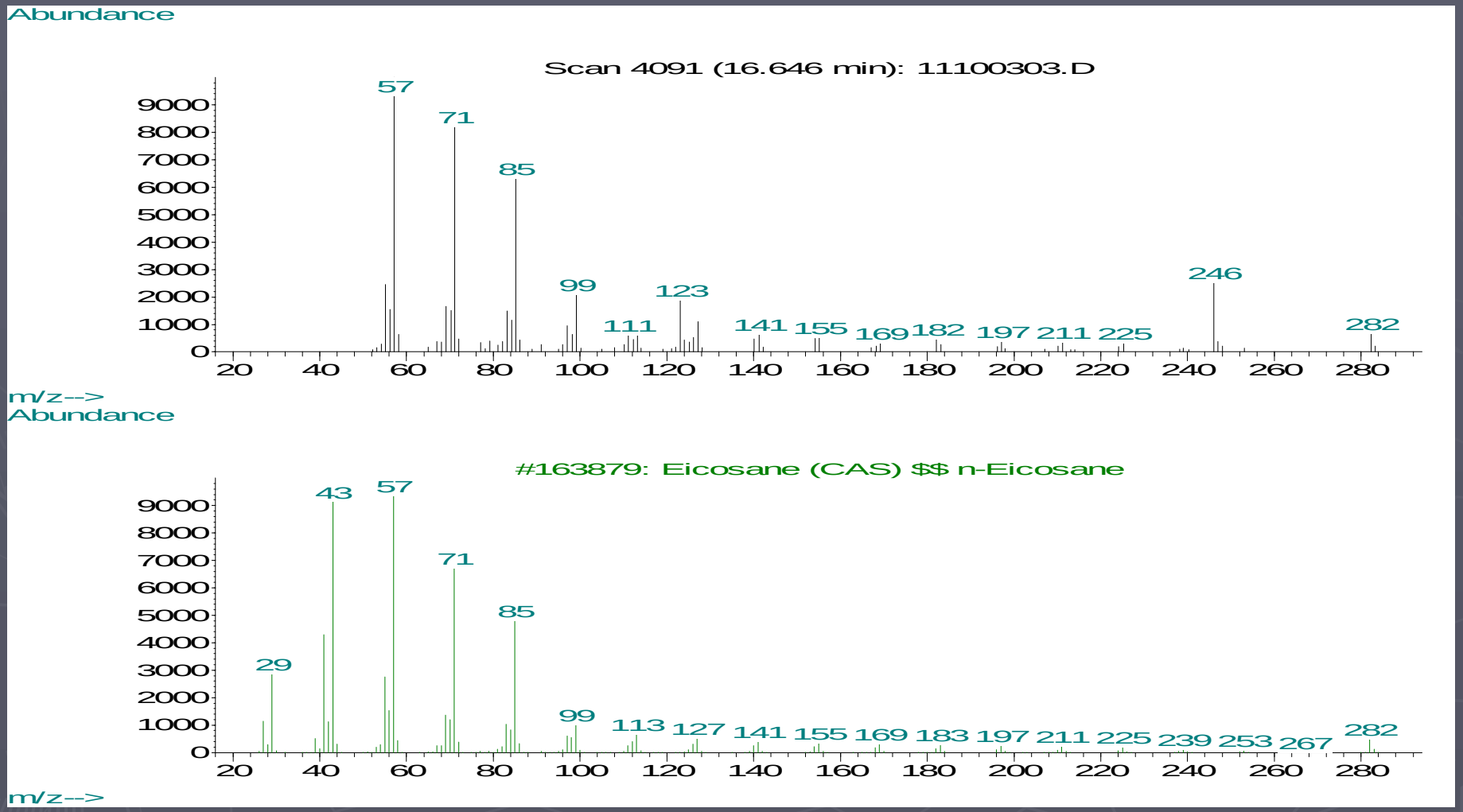
Example of Chemical Ionization (ammonia reagent gas)



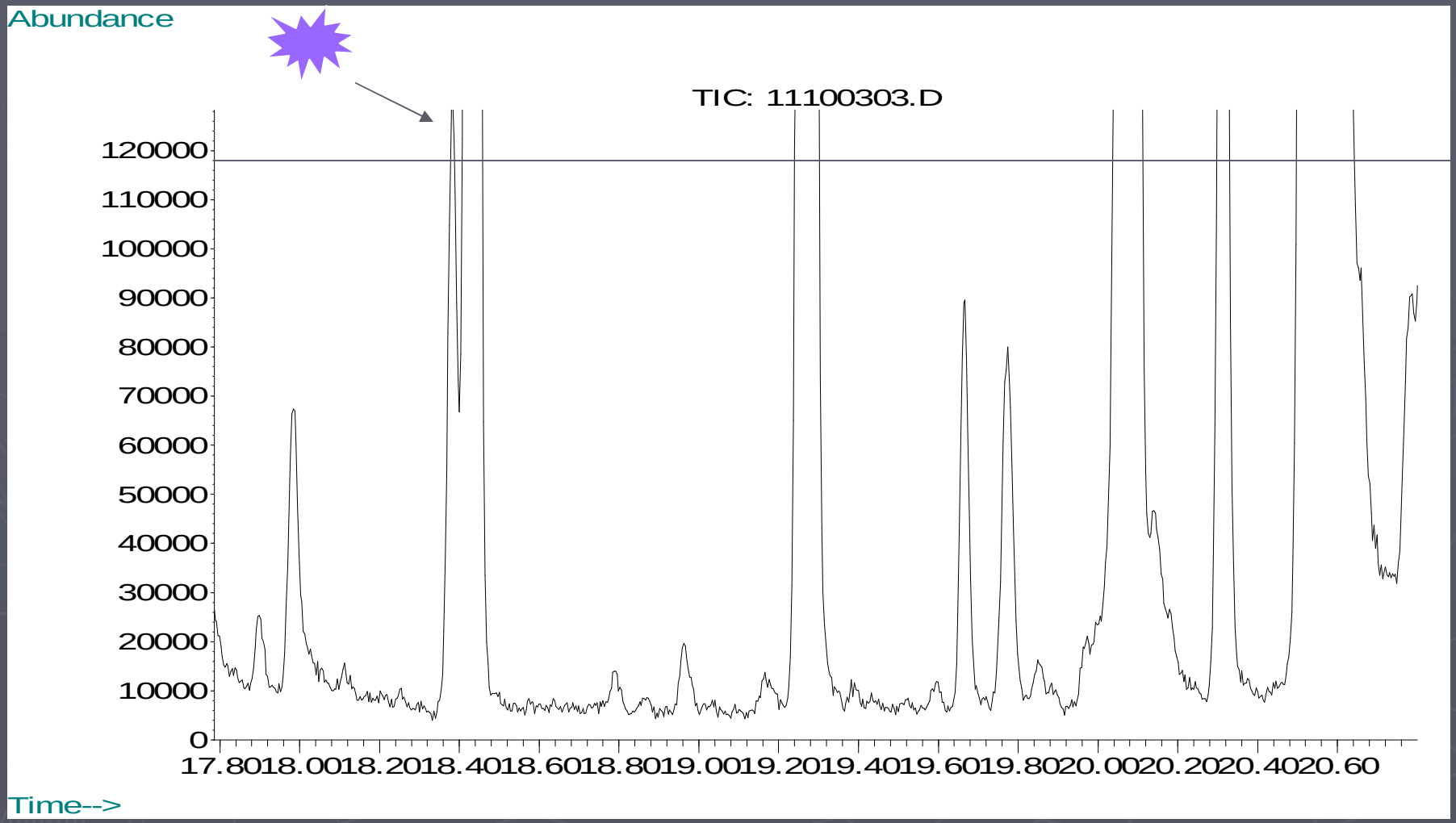
Leachables Profile – 1 Week Timepoint Expanded Section



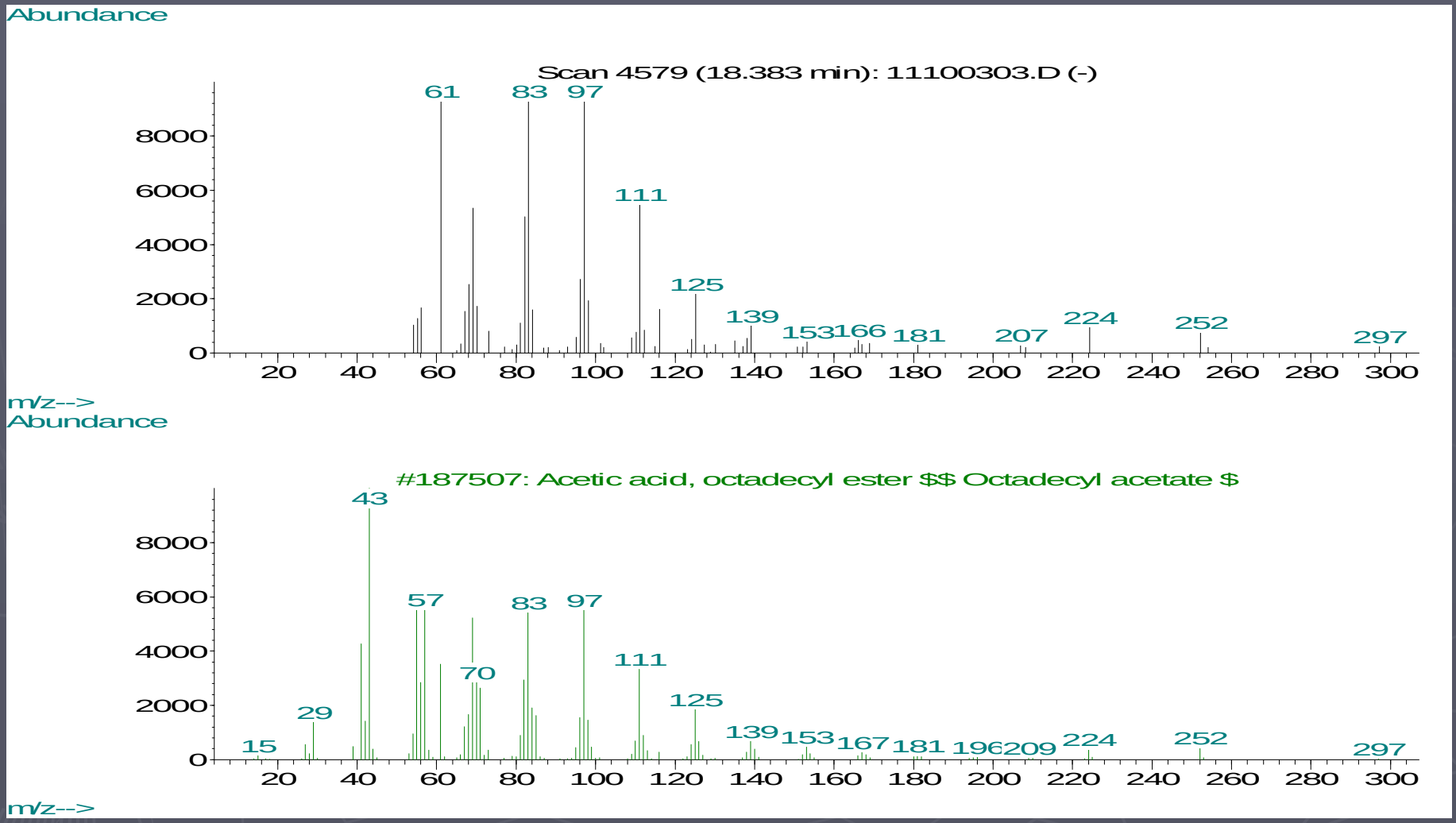
Library Search Identification of



Leachables Profile – 1 Week Timepoint Expanded Section (2)



Library Search Identification of



Key Points to Consider

- EI is under “kinetic control”. Therefore, EI spectra are reproducible and spectral libraries can be employed (remember limitations of libraries!!!!!!).
- CI is under “thermodynamic control”. It is useful for molecular weight confirmation but no libraries.
- GC/MS provides both a chromatographic retention time and mass spectrum (spectra) which can be compared with an authentic reference material (Good luck finding it).

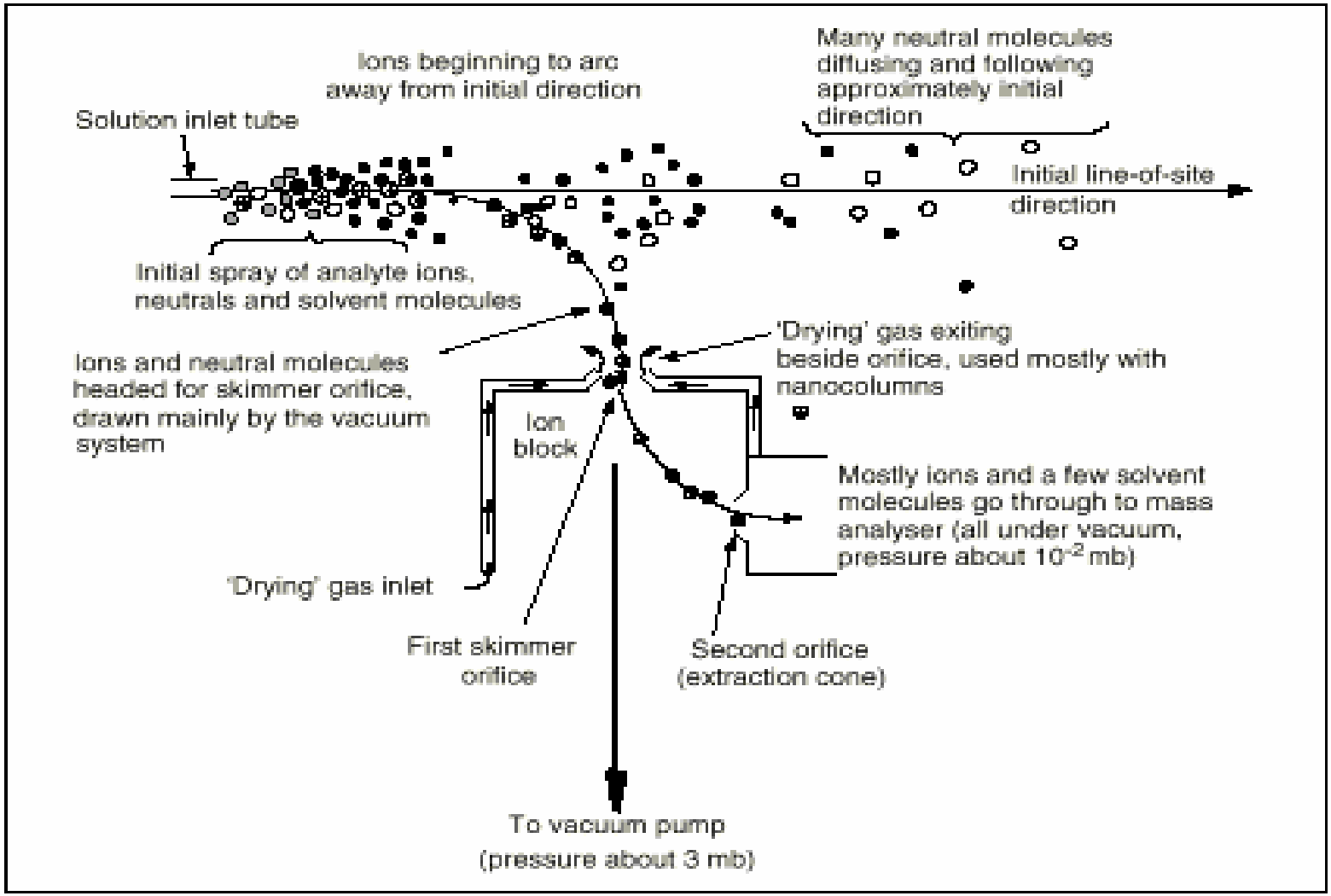
LC/MS Interfaces/Ionization Processes

- Moving belt/wire (transport device)
- Thermospray (unique ionization process)
- Continuous flow FAB (transport device)
- Particle-beam (transport device)
- Electrospray (unique ionization process)
- APCI (transport device/ionization process)

A “Modern” LC/MS System

“Bench-top” LC/MS Systems

Schematic of an Orthogonal API Source



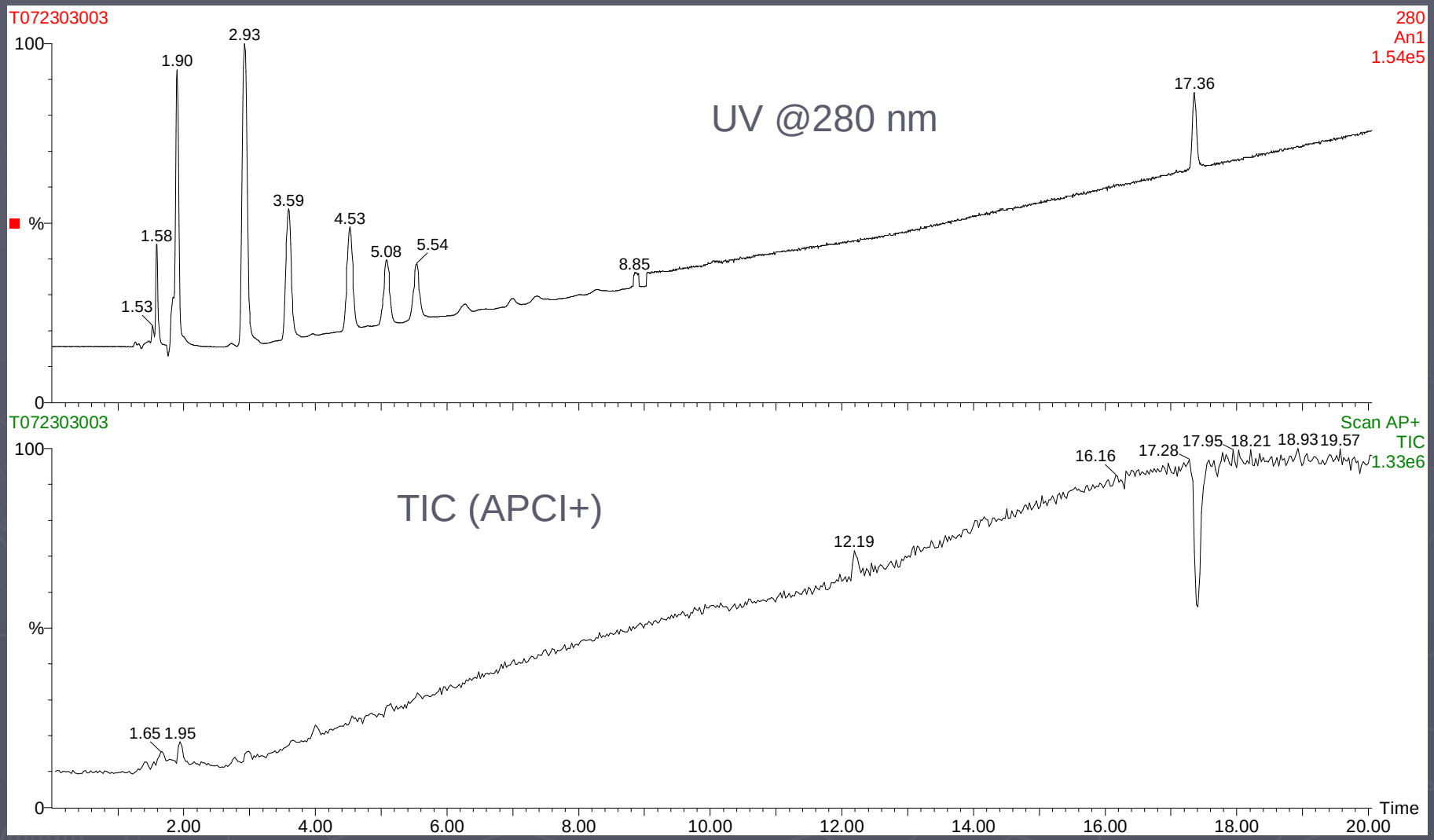
Features of Electrospray

- Often reflects solution chemistry (multiply charged ions observed)
- Operates at atmospheric pressure
- Uses a strong electric field
- Flow-rate (optimum performance at uL/min)
- Soft-ionization process
- Extremely rugged

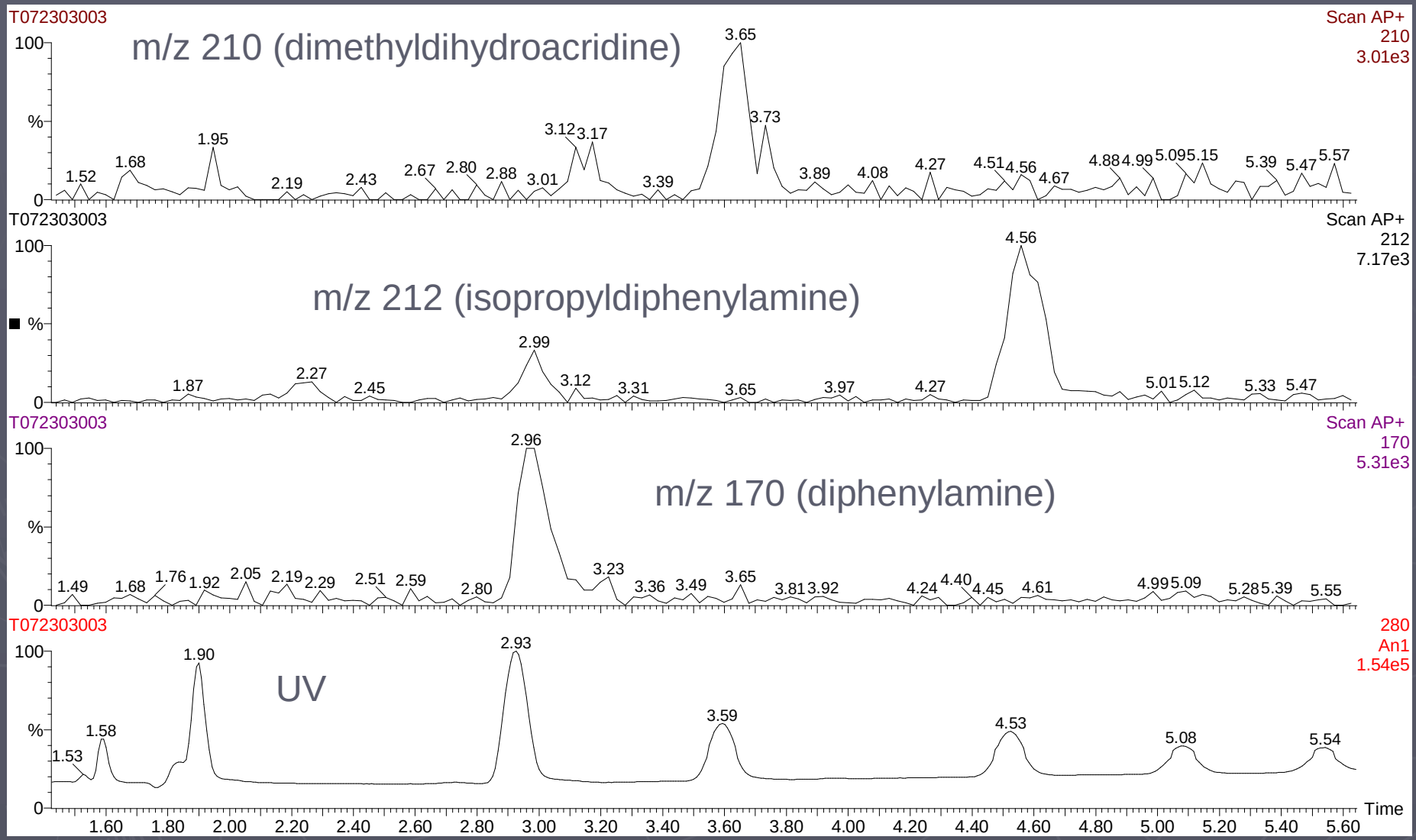
Features of APCI

- Gas phase ionization process
- Operates at atmospheric pressure
- Flow-rate (optimum performance at mL/min)
- Uses a corona discharge
- Soft-ionization process
- Extremely rugged

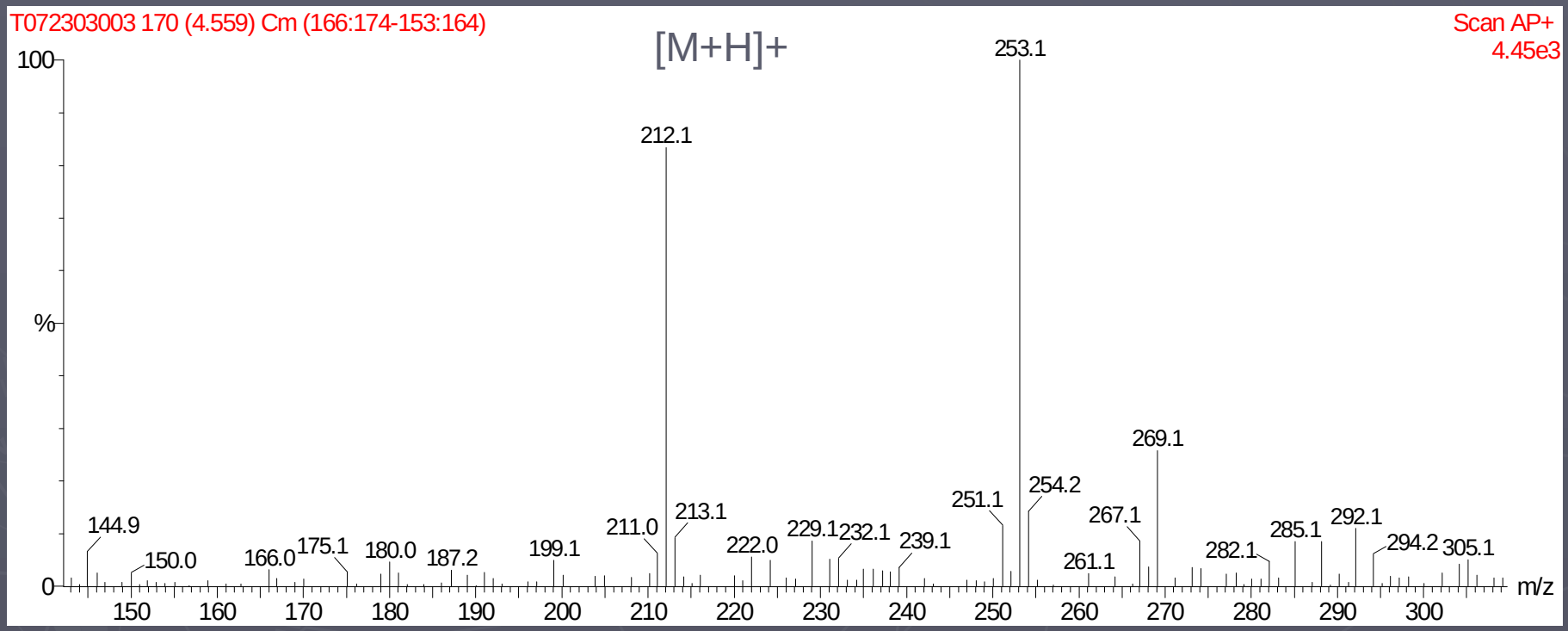
APCI LC/MS Extractables Profile(s)



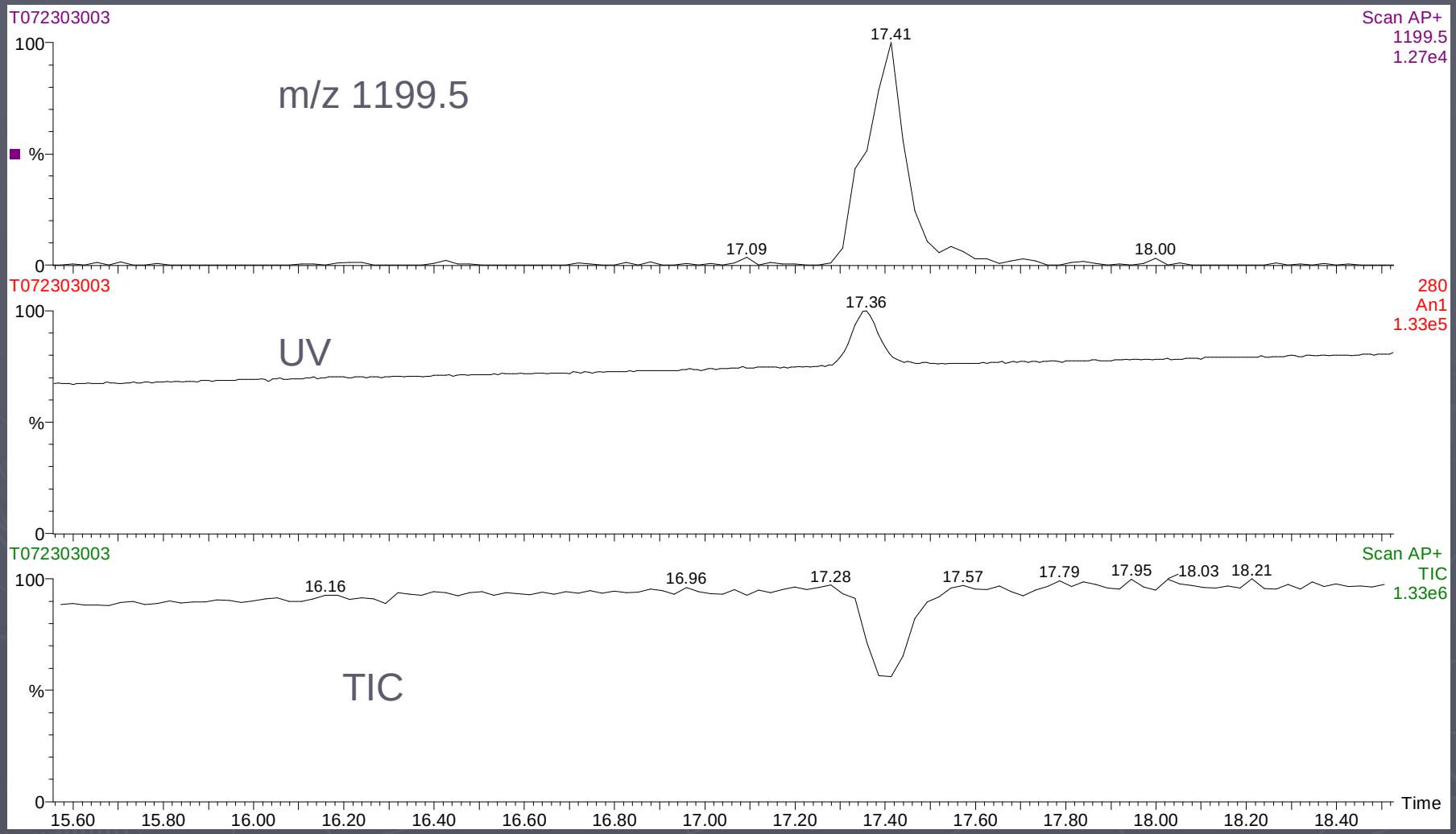
Mass Chromatograms (Diphenylamine Acetone Condensate)



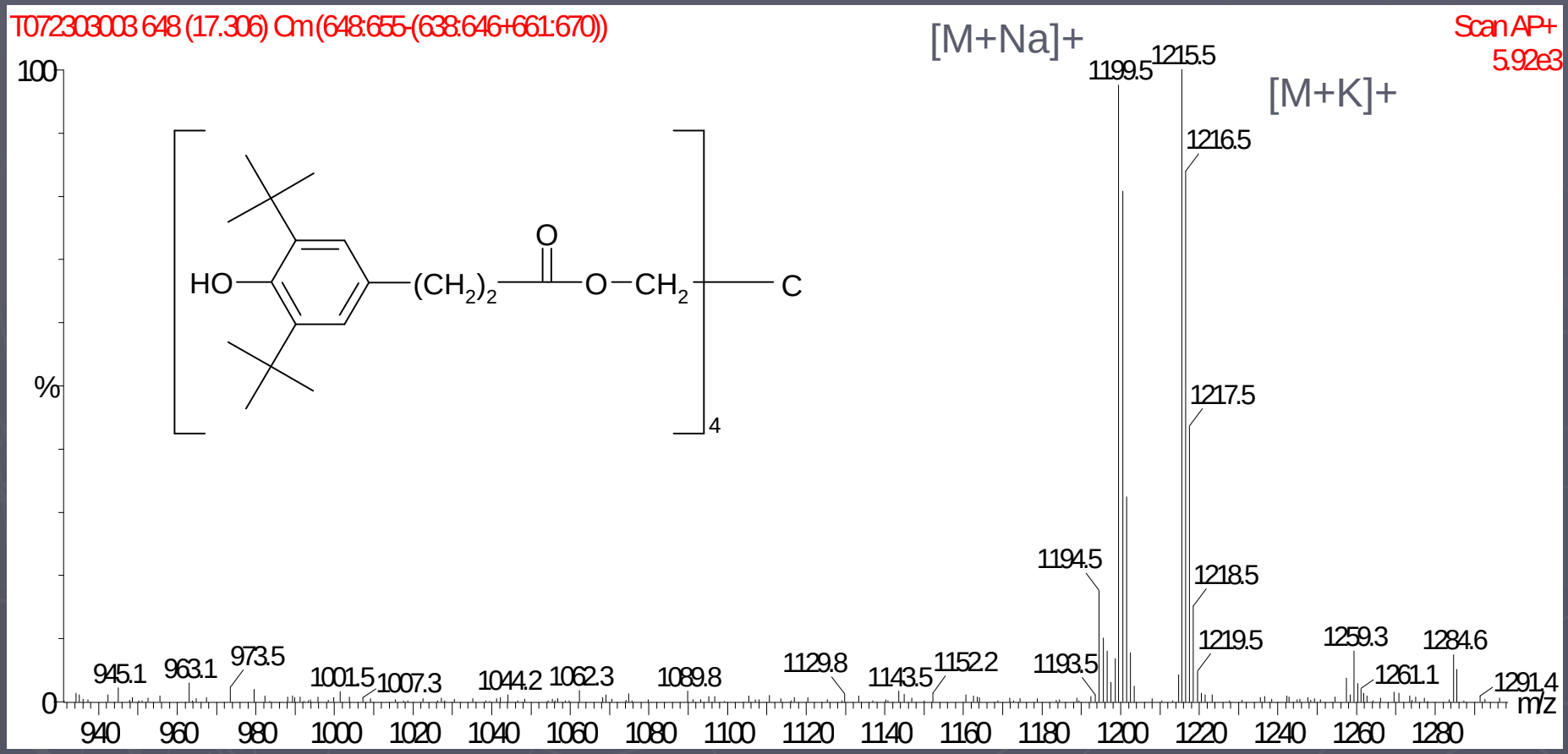
APCI+ Mass Spectrum of Isopropyldiphenylamine



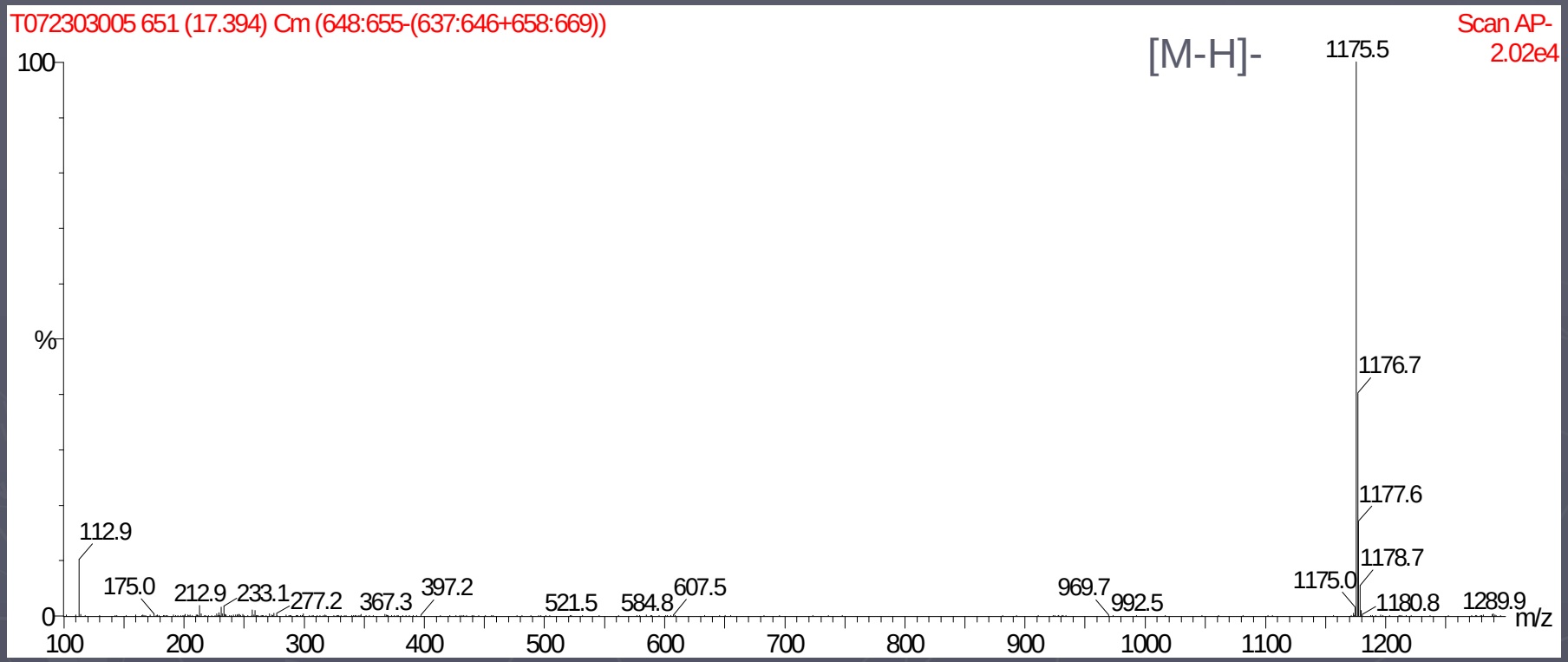
Irganox 1010



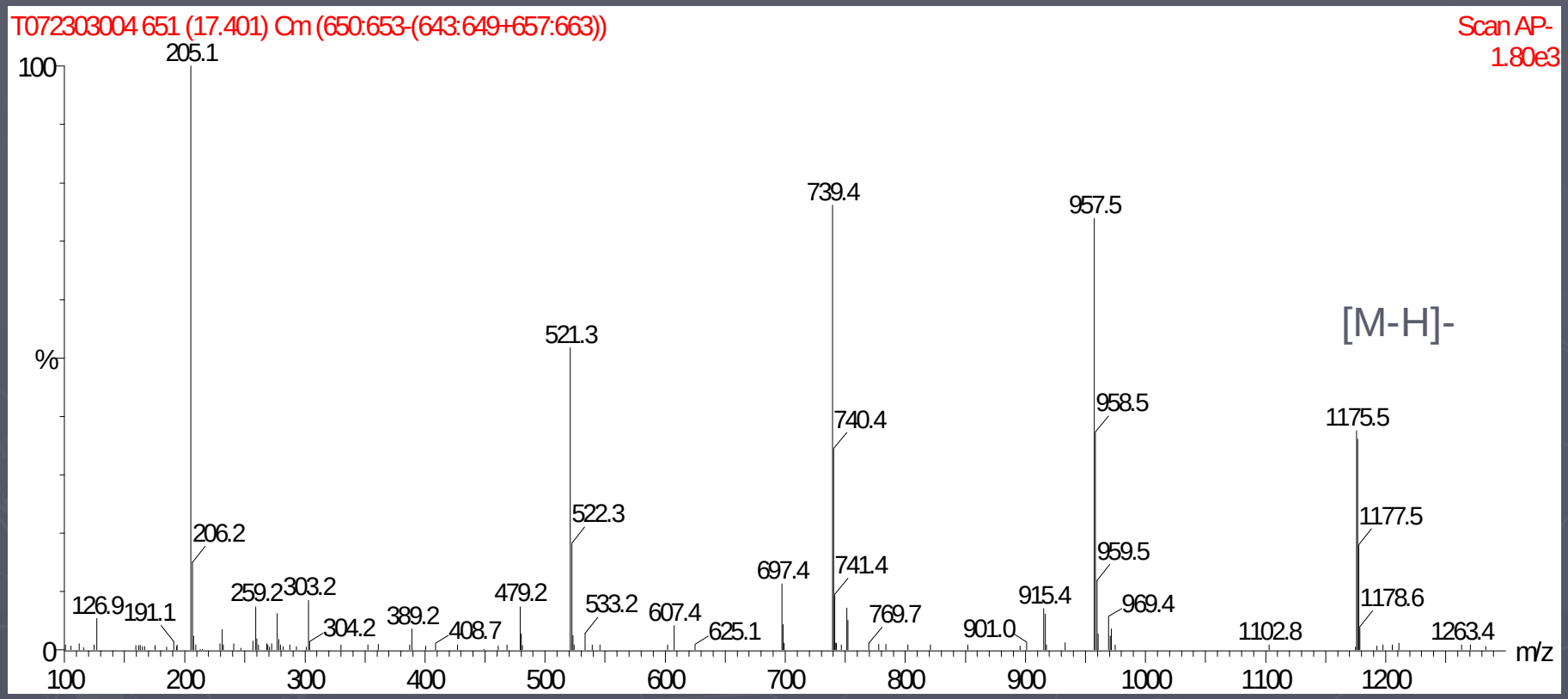
APCI+ Mass Spectrum Irganox 1010



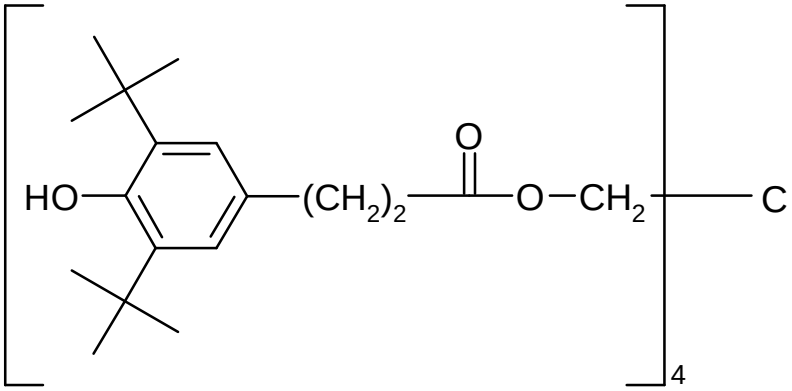
APCI- Mass Spectrum Irganox 1010
(no induced fragmentation)

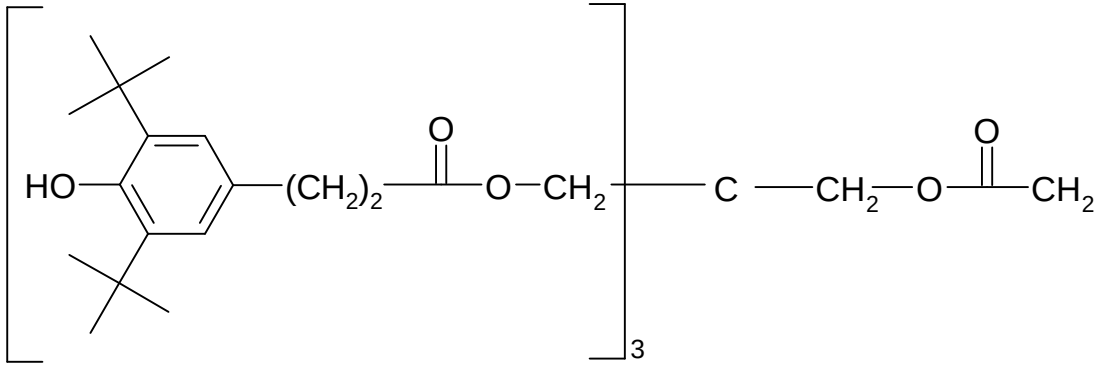


APCI- Mass Spectrum Irganox 1010
(induced fragmentation in the ion source)



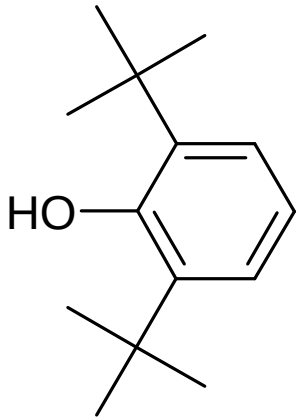
Structure and Fragmentation of Irganox 1010 in Negative Ion APCI





m/z 957

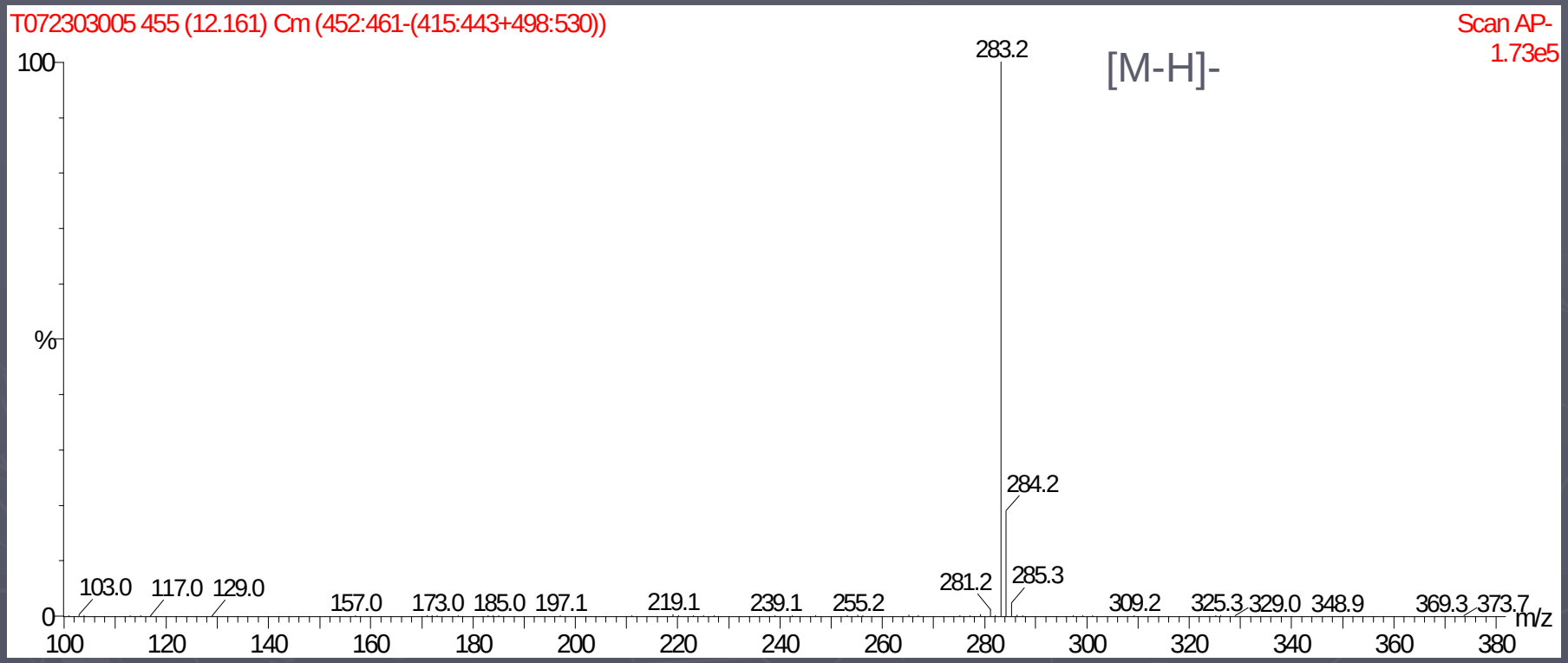
-



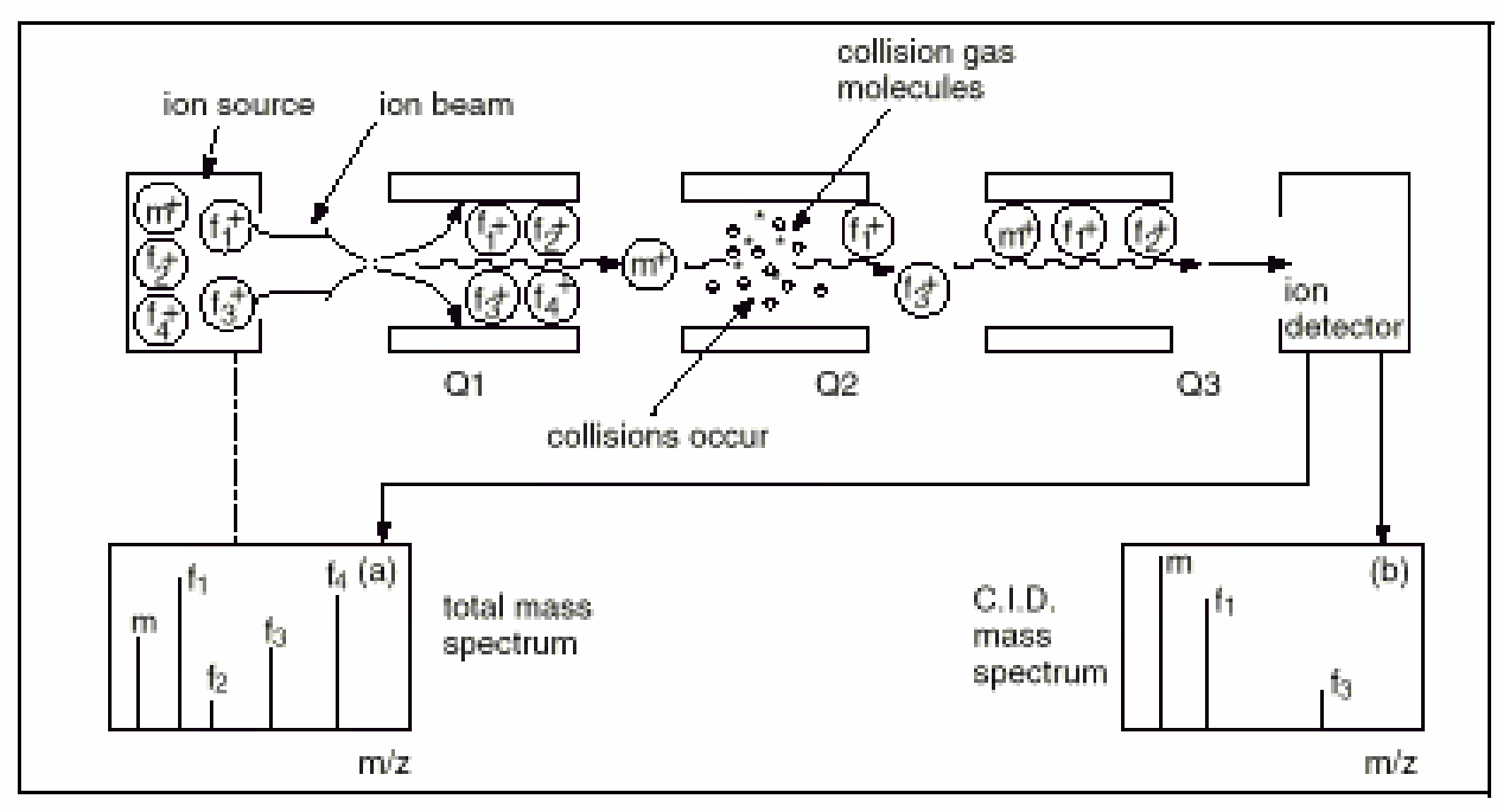
-

m/z 205

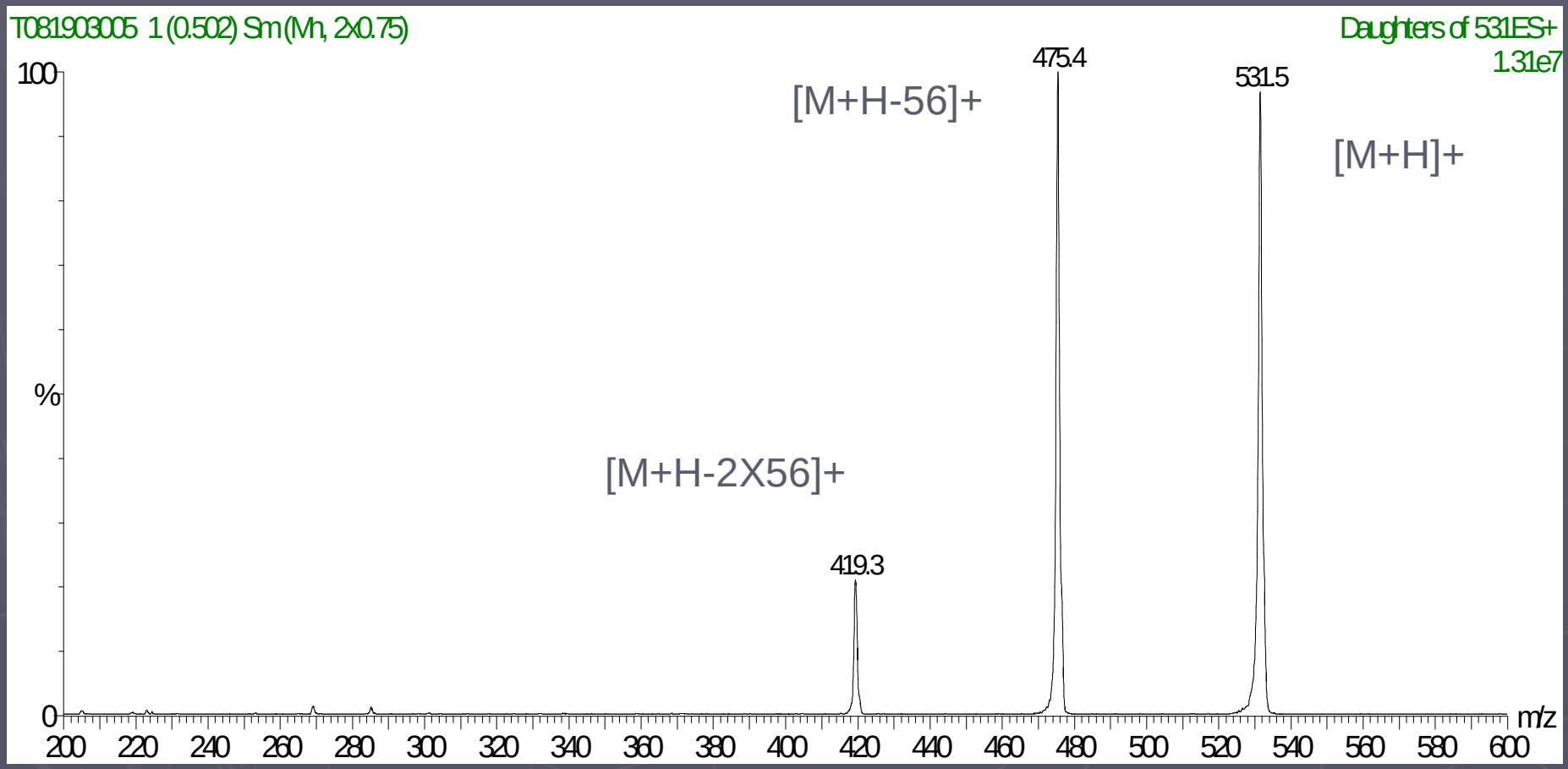
APCI- Mass Spectrum of Stearic Acid



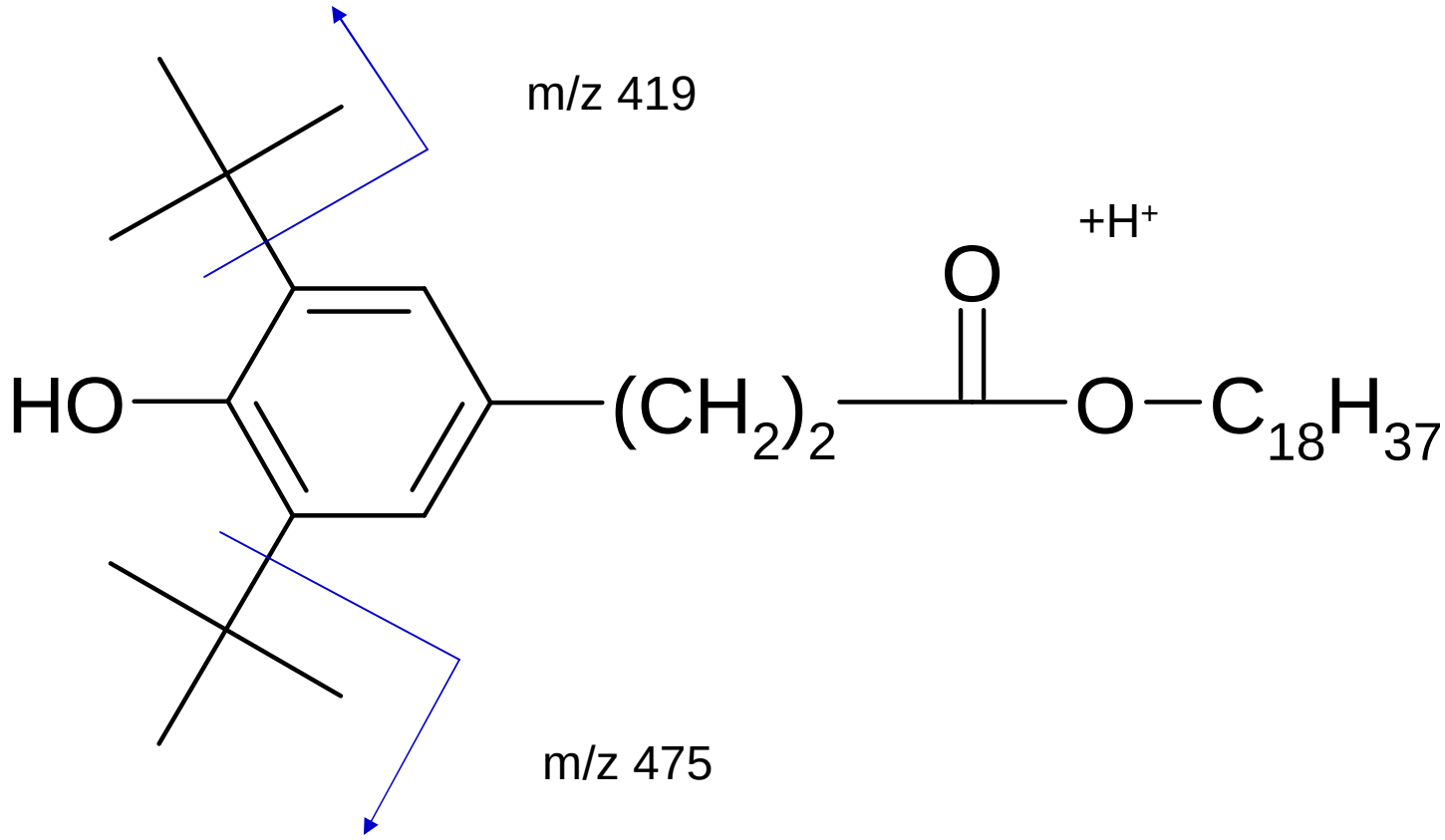
Triple Quadrupole Mass Spectrometer



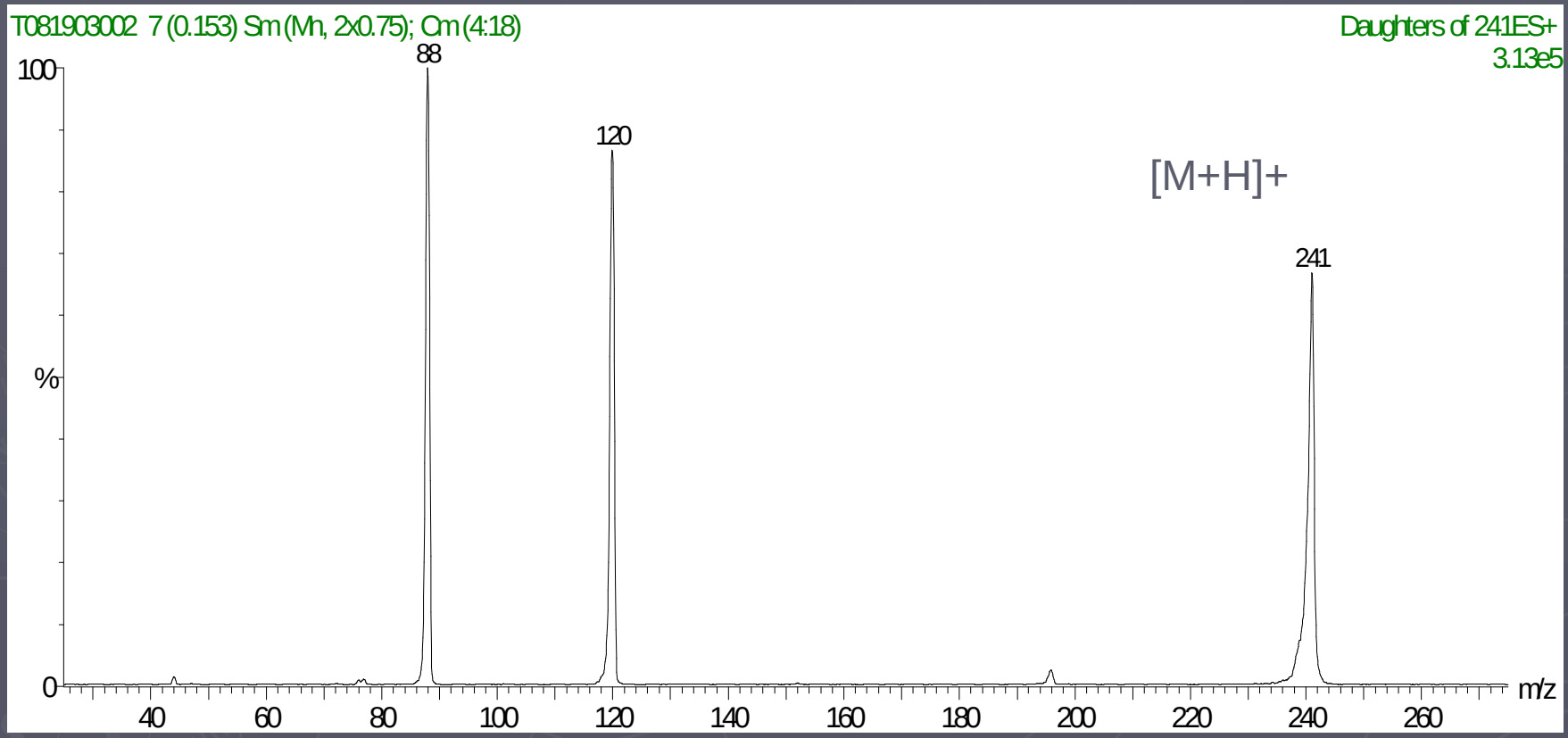
MS/MS Spectrum of Irganox 1076
(ESI+; products of m/z 531)



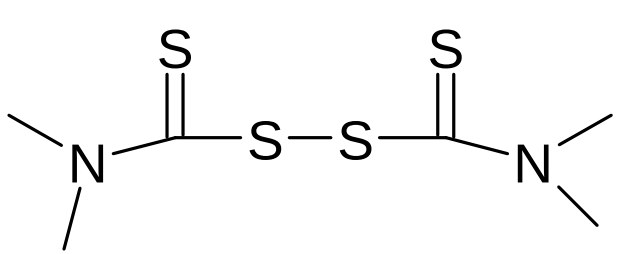
Structure and Fragmentation of Irganox 1076 in Positive Ion APCI (MS/MS)



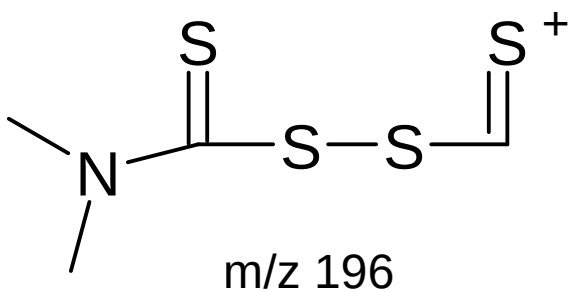
MS/MS Spectrum of Tetramethylthiuram Disulfide (ESI+; products of m/z 241)



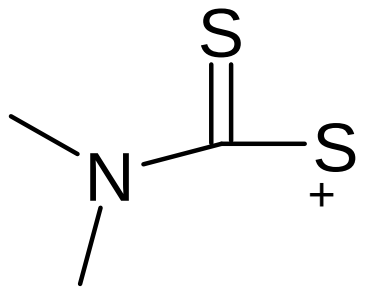
Structure and Fragmentation of Tetramethylthiuram Disulfide in Positive Ion APCI (MS/MS)

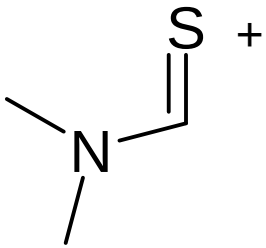


m/z 241

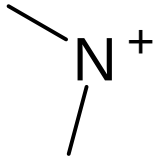


m/z 196

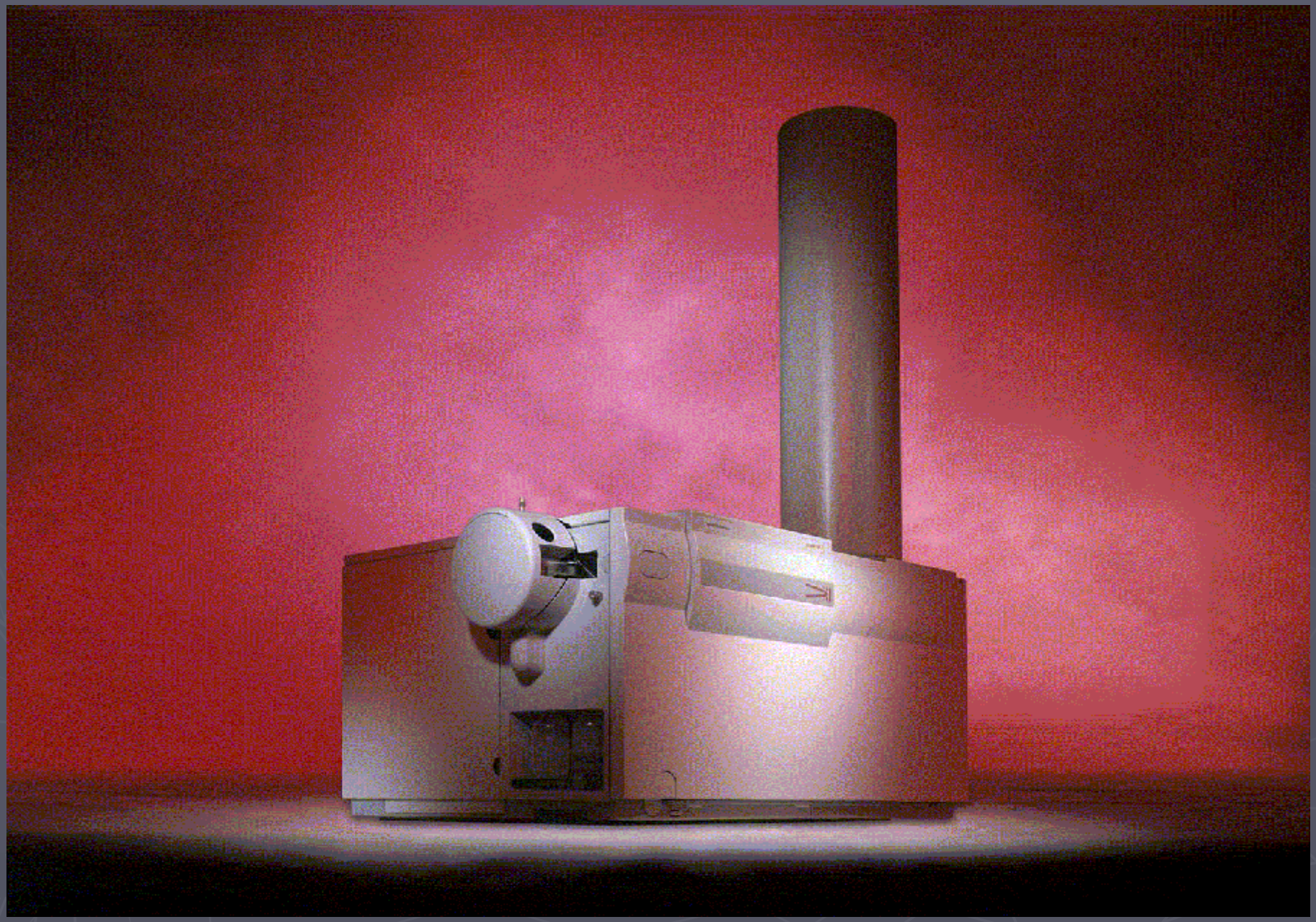




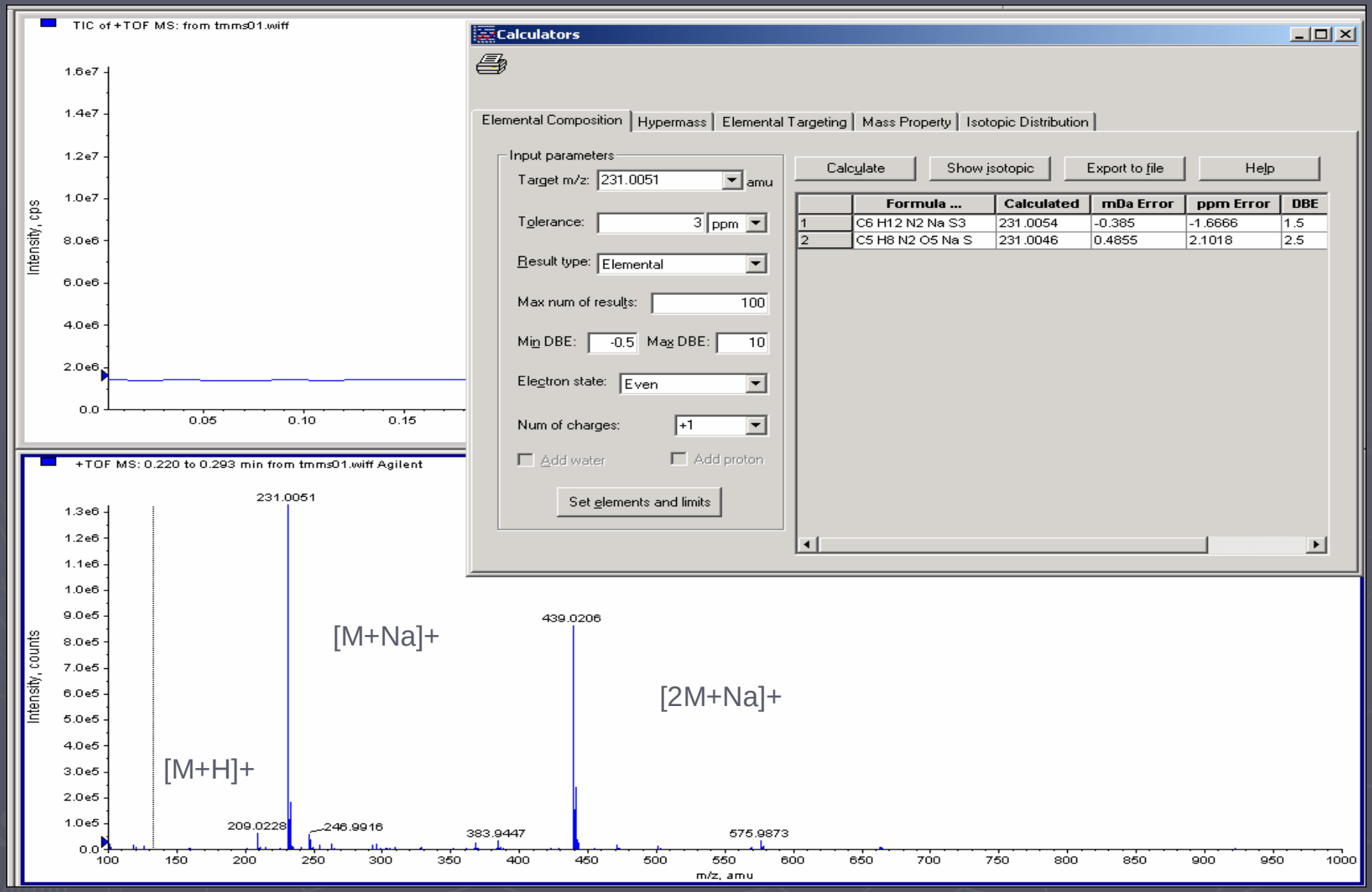
m/z 88



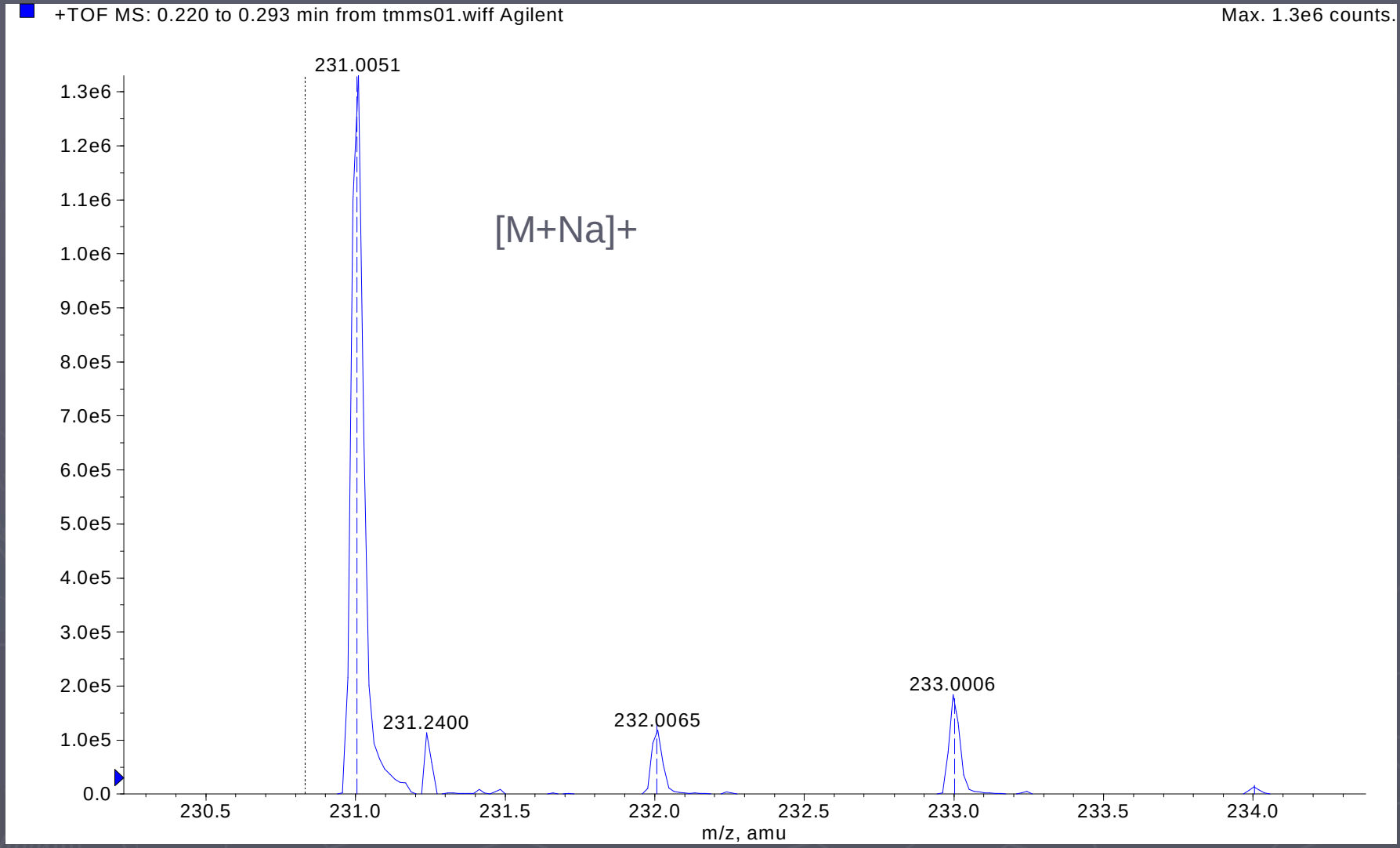
m/z 44



ESI+ TOF Mass Spectrum of TMTMS



Expanded ESI+ TOF Mass Spectrum of TMTMS



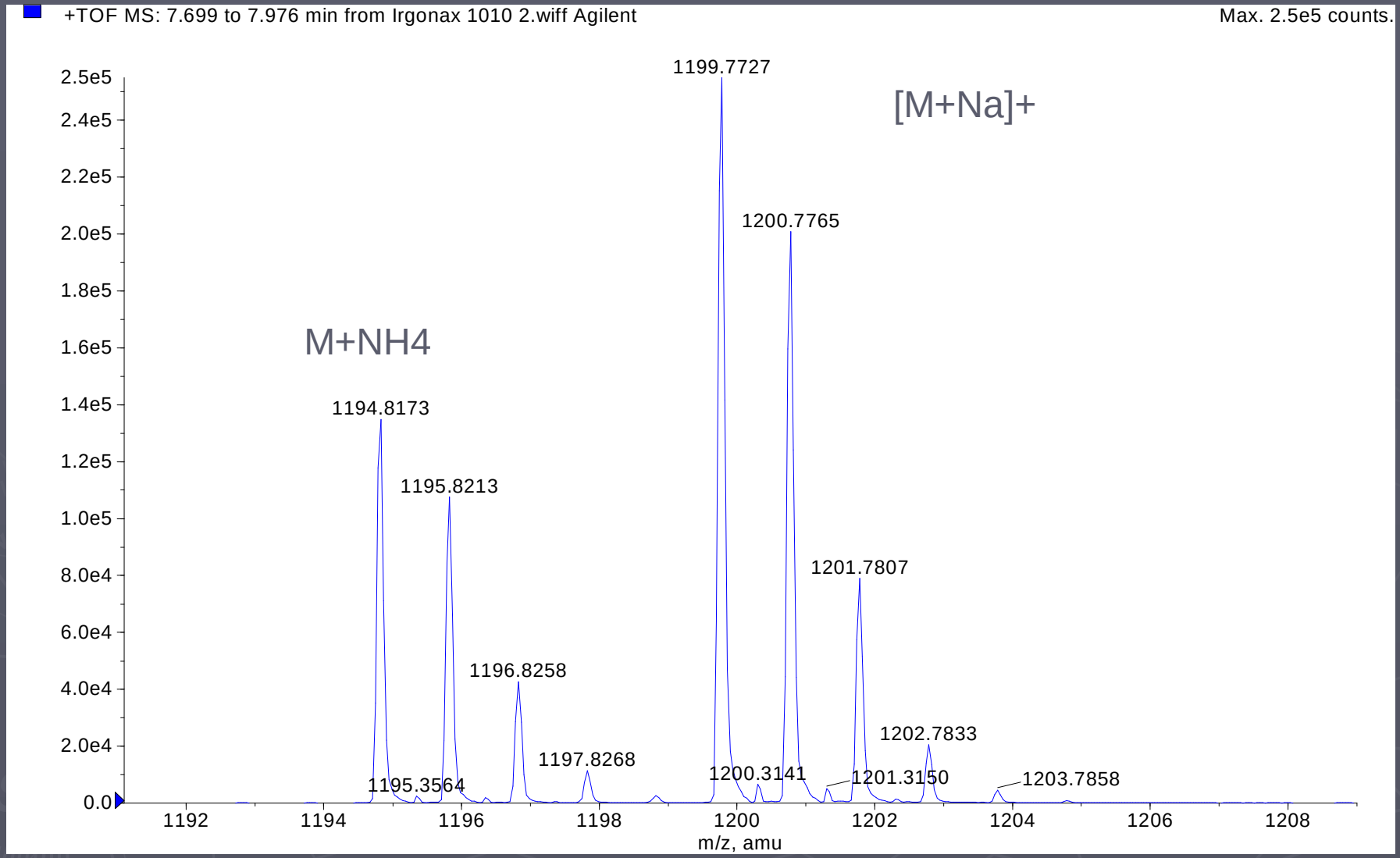
Elemental Composition Report for TMTMS from ESI+ TOF-MS

Single Mass Analysis - displaying only valid results
Tolerance = 10.0 PPM / DBE: min = -0.5, max = 20.0

Monoisotopic Mass, Odd and Even Electron Ions
43463 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)

Minimum:					-0.5
Maximum:			2.0	10.0	20.0
Mass	Calc. Mass	mDa	PPM	DBE	Formula
231.0052	231.0052	0.0	0.2	8.0	12C4 1H2 14N9 23Na 32S
	231.0051	0.1	0.6	9.5	12C11 1H7 14N2 32S2
	231.0060	-0.8	-3.6	1.5	12C6 1H12 14N2 23Na 32S3

Expanded ESI+ TOF Mass Spectrum of Irganox 1010



Elemental Composition Report for Irganox 1010 from ESI+ TOF-MS

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -0.5, max = 40.0

Monoisotopic Mass, Odd and Even Electron Ions
289500 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)

Minimum:				-0.5	
Maximum:		2.0	5.0	40.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
1199.7727	1199.7738	-1.1	-1.0	19.5	12C73 1H108 16O12 23Na
	1199.7763	-3.6	-3.0	22.5	12C75 1H107 16O12
	1199.7680	4.7	3.9	28.5	12C80 1H104 16O7 23Na

My Philosophy with LC/MS

- For CMC impurity profiling studies use APCI as a primary technique.
- Reproduce the UV chromatogram.
- Use every trick in the book to make APCI work (mobile phase additives, alternate acquisition techniques, etc.).
- Use electrospray to complement and confirm APCI.

IPAC-RS Proposed Identification Categories

Table 2. Identification Categories for Structure Elucidation of Extractables and Leachables by GC/MS and LC/MS	
Category	**Identification Data**
A	Mass spectrometric fragmentation behavior
B	Confirmation of molecular weight
C	Confirmation of elemental composition
D	Mass spectrum matches automated library or literature spectrum
E	Mass spectrum and chromatographic retention index match authentic specimen

Identification Categories

- A *Confirmed* identification means that identification categories A, B (or C), and D (or E) have been fulfilled.
- A *Confident* identification means that sufficient data to preclude all but the most closely related structures have been obtained.
- A *Tentative* identification means that data have been obtained that are consistent with a class of molecule only.