Marine Organic Geochemistry

Analytical Methods – II.

Methods For Characterization of Macromolecular Organic Matter.

Mass Spectrometry

Reading list

Biopolymer/macromolecular organic matter analysis

- Hedges J.I. (1991) Lignin, Cutin, Amino Acid and Carbohydrate Analyses of Marine Particulate Organic Matter. In *Marine Particles: Analysis and Characterization* (Eds. D.C. Hurd and D.W. Spencer) Geophysical Monograph 63, pp. 129-137
- Mopper K. and Furton K.G. (1991) Extraction and Analysis of Polysaccharides, Chiral Amino Acids and SFE Extractable Lipids from Marine POM. In *Marine Particles: Analysis and Characterization* (Eds. D.C. Hurd and D.W. Spencer) Geophysical Monograph 63, pp. 151-161.
- Chappe B., Michaelis W. and Albrecht P. (1980) Molecular fossils of Archaebacteria as selective degradation products of kerogen. In *Advances in Organic Geochemistry, 1979* (Eds. A.G. Douglas and J.R. Maxwell). pp. 265-274. Pergamon Press
- Mycke B. an Michaelis W. (1986) Molecular fossils from chemical degradation of macromolecular organic matter. *Org. Geochem*. **10**, 847-858.
- Meuzelaar H.L.C, Haverkamp J. and Hileman F.D. (1982) *Pyrolysis-Mass Spectrometry of Recent and Fossil Biomaterials. Compendium and Atlas.* Elsevier
- Hedges J.I. and Ertel J.R. (1982) Characterization of lignin by capillary gas chromatography of cupric oxide oxidation products. *Anal. Chem*. 54, 174-178.
- Stock L.M. and Wang S.H. (1986) Ruthenium tetroxide catalyzed oxidation of coals. *Fuel* **65**, 1552.

Reading list

Mass Spectrometry

General reference

- McLafferty F.W. (1980) *Interpretation of Mass Spectra, 3rd Edition*, University Science.
- Meuzelaar H.L.C, Haverkamp J. and Hileman F.D. (1982) *Pyrolysis-Mass Spectrometry of Recent and Fossil Biomaterials. Compendium and Atlas.* Elsevier.
- Peters K.E. and Moldowan J.M (1993) The Biomarker guide. Prentice-Hall. 363 pp.

Specific topics

- Kujawinski E.B., Freitas M. A., Zang X., Hatcher P.G., Green-Church K.B., and Jones R.B. (2002) The application of electrospray ionization mass spectrometry (ESI-MS) to the structural characterization of natural organic matter. *Org. Geochem.* **33**, 171-180.
- Sturt H.F., Summons R.E., Smith K., Elvert M. and Hinrichs K.-U. (2004) Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry – new biomarkers for biogeochemistry and microbial ecology. *Rapid Comm. Mass Spectrom.* **18**, 617-628.

Characterization of Macromolecular Organic Matter: Analytical Approaches

1. Direct Spectroscopy (e.g. FTIR, NMR)

Advantages:

- quantitative
- non-destructive (IR & NMR)
- rapid

Disadvantages

- Lower resolution information

Characterization of Macromolecular Organic Matter: Analytical Approaches

2. Chemical degradation ("Chemolysis")

Advantages

- Very selective
- Carbon skeletons can be preserved - more biochemical information (molecular-level)

Disadvantages

- Time-consuming - low sample throughput
- Solubility limitations of many reagents
- Non-quantitative or semi-quantitative

Characterization of Macromolecular Organic Matter: Analytical Approaches

3. Thermal degradation ("pyrolysis")

Advantages

- relatively rapid
- semi-quantitative
- can analyze samples irrespective of solubility

Disadvantages

- Complex distributions of products
- Secondary reactions ?

Best approach: A combination of these techniques

Organic matter concentration (demineralization) procedures

- Removal of carbonates (HCl)
- Removal of silicates (HF) 40-50% HF, <40°C overnight
- Removal of pyrite ($LiAlH_4$, density separation, $CrCl_2$)*

- **Prone to sample fractionation/alteration*

Chemical degradation (chemolysis) methods

Reagent(s)	*Deg. Type*	*Site(s) of attack*	*Biochemical(s)*
H_2SO_4	acid hydrolysis	glycoside link	polysaccharides
HCl	acid hydrolysis	peptide link	proteins
CuO	basic oxidative hydrolysis	ether link ester link	lignin cutin
BBr_3	ether cleavage	ether link	ether lipids
Ru/C	hydrogenolysis	ether link	lignin ether lipids
KOH	basic hydrolysis (saponification)	ester link	cutin ester-bound lipids
Raney Ni	desulfurization	sulfur link	S-macromol.
MeLi/MeI		di/polysulfides	S-macromol
RuO_4	oxidation	double bonds	aromatic systems functional groups

Molecular-level Characterization of Polysaccharides

Method:

- Acid hydrolysis of polymer to monomers

Hydrolysis

- If crystalline cellulose (vascular plants) present - must pre-treat sample with 72 wt% H_2SO_4 to soften fibers prior to hydrolysis.
- Hydrolysis usually performed by reflux in 1M H_2SO_4 for 3hr

Limitations

- Simple sugars are unstable under hydrolysis conditions so must balance competing reactions of production vs destruction of monosaccharides.
- Efficiency of hydrolysis dependent upon polysaccharide composition.
- Not all polysaccharides in environmental samples are hydrolyzable.

Molecular-level Characterization of Polysaccharides

Analysis

1. GC of equilibrated anomeric mixtures as volatile trimethylsilyl derivatives without pre-treatment to remove troublesome carbonyl. (Cowie and Hedges)

2. GC of alditol acetate derivatives (formed via reduction followed by ester formation, (Klok)

- Advantages:
- only one peak per sugar and high resolution via capillary GC columns
- can use GC-MS for identification
- suitable for isotopic analysis by GC-irMS?
- Disadvantages:
- Procedurally complex
- loss of information (one alditol can be formed from more than one aldose or ketose)

3. Direct analysis by HPLC using fluorescent derivatives (Mopper)

- Advantages:
- Can perform in aqueous system
- High sensitivity
- Disadvantages
- low resolution of HPLC vs GC
- some derivatives are unstable.

Molecular-level Characterization of Protein Amino Acids

Method

- Acid hydrolysis (basic hydrolysis causes extensive racemization and loss of some amino acids).
- Typical reaction conditions: 6N HCl at 100°C for 24 hr
- Chromatographic separation: 3 different approaches
  - 1. Ion exchange chromatography
  - 2. HPLC
  - 3. GC

Molecular-level Characterization of Lignin

Method:

- CuO alkaline oxidative hydrolysis
- CuO procedure breaks apart lignin polymers (β-O-4 linked phenolic macromolecule) into simple phenols that can be separated and quantified by HPLC or by GC (after derivatization).

General Procedure:

- 0.5 g sediment + CuO + NaOH
- Bomb 170°C, 3 hr
- filter products
- extract with diethylether
- dry (anhydrous Na_2SO_4)
- derivatize (in pyridine) with BSTFA
- GC(/MS) or HPLC-UV/Vis

Efficiency of phenol yield from CuO oxidation

- Vanillyl 30%
- Syringyl 90%
- Others ?

Thermal degradation (pyrolysis) methods

Mechanism of pyrolytic cleavage:

- Primarily free radical process involving chain scission followed by propagation and termination steps.
- Functional groups and heteroatomic linkages particularly susceptible to cleavage

Mode of Use

- On-line
- Pyrolysis-Gas Chromatography (Py-GC)
- Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS)
- Pyrolysis-Mass Spectrometry (Py-MS)
- Off-line
- Py-trap

Flash Pyrolysis

- Rapid heating (< 5s) to high temperatures (>500°C) to promote dissociation of macromolecule with minimal opportunity for secondary reactions.
- Used for structural characerization.

Static pyrolysis

- Slow or isothermal heating in a closed system in the presence of absence of water.
- Designed to mimic geological heating (hydrothermal systems, petroleum generation in the subsurface).

Mass Spectrometry

What is Mass Spectrometry?

- The separation of matter according to atomic and molecular mass.
- Used in analysis of organic compounds of molecular mass up to 200,000 Daltons.
- Most versatile, sensitive and widely used analytical method available today.

Principle:

- Mass spectrometers use the difference in the mass-to-charge ratio (m/e or m/z) of ionized atoms or molecules to separate them from each other.
- MS is useful for the quantification of atoms or molecules, and also for determining chemical, structural and isotopic information about molecules.
- Molecules have distinct fragmentation patterns that provide chemical information (structural elucidation).

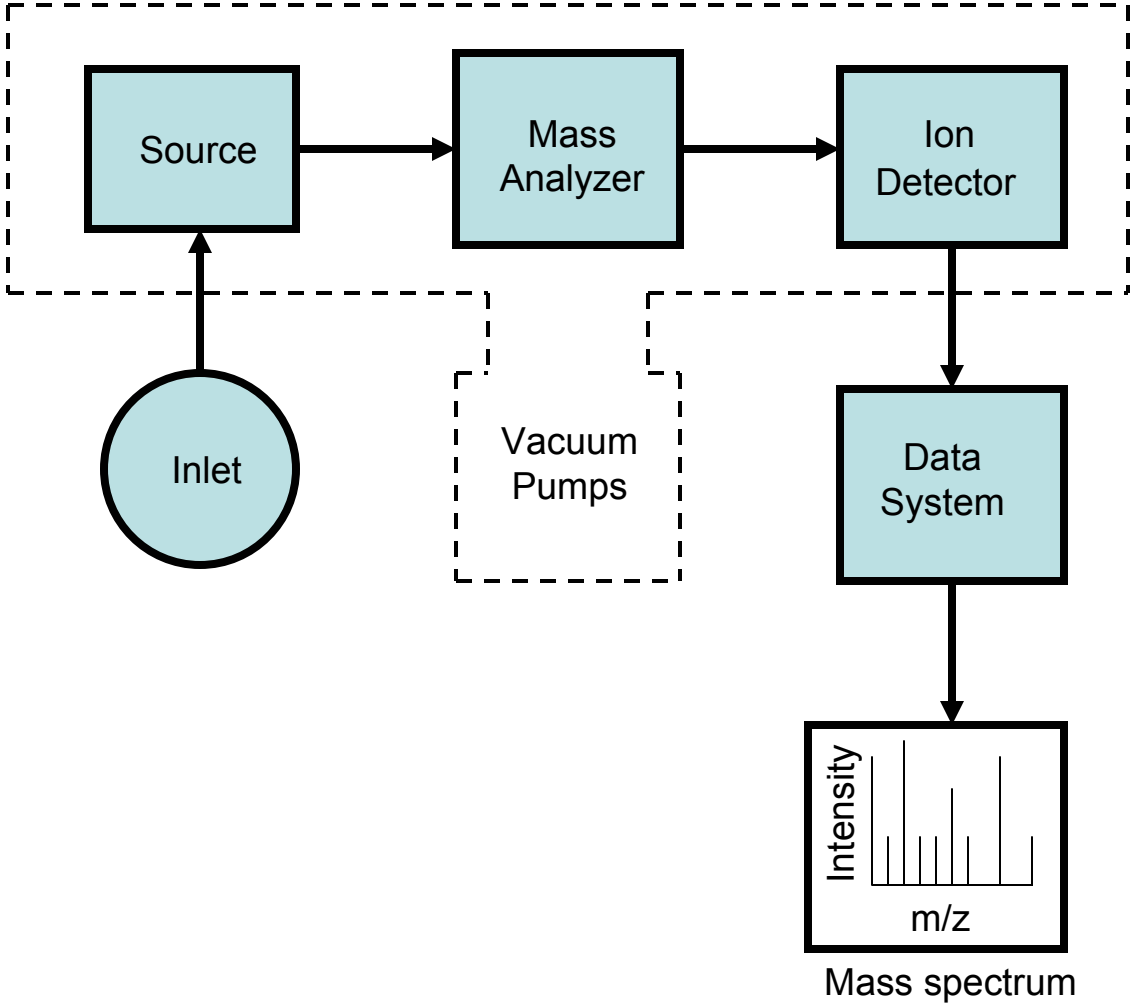
Basic Components of a Mass Spectrometer

General operation:

1. Create gas-phase ions
2. Separate the ions in space or time based on their mass-charge ratio.
3. Measure the quantity of ions of each mass/charge ratio.

Since MS systems create and manipulate gas-phase ions, they operate under high vacuum.

Magnetic-sector, quadrupole and time-of-flight mass analyzers also require extraction and acceleration ion optics to transfer ions from the source region to the mass analyzer.

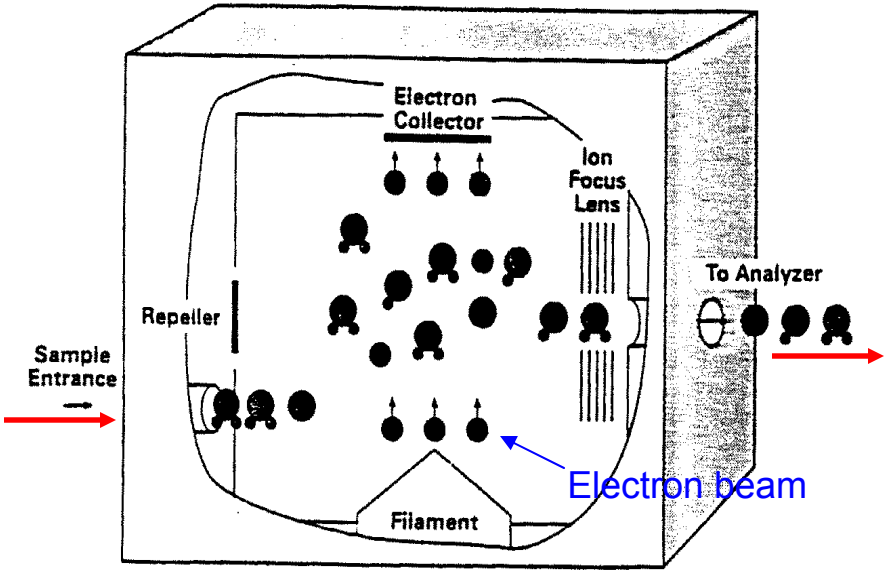


Ionization Methods

- **Electron Impact (EI) ionization**
- An EI source uses an electron beam, usually generated from a tungsten filament, to ionize gas-phase atoms or molecules.
- An electron from the beam knocks an electron off the analyte to create ions.

- *EI is the most common ionization method for routine GC/MS analysis*
- *EI is a relatively harsh ionization technique and can lead to extensive fragmentation of the molecule (good and bad).*

- *Typical ionization conditions 35-70 eV*
- *12-20 eV = low eV (less fragmentation).*



Tungsten filament

Ionization Methods

Chemical Ionization (CI)

- CI uses a reagent ion to react with the analyte molecules to form ions by either proton or hydride transfer:
- $MH + C_2H_5^+ \rightarrow MH_2^+ + C_2H_4$
- $MH + C_2H_5^+ \rightarrow M^+ + C_2H_6$
- The reagent ions are produced by introducing a large excess of methane or another gas (e.g. ammonia) relative to the analyte into an EI source. Electron collisions produce CH_4^+ and CH_3^+ which react further with methane to form $C_2H_5^+$.

CI is a softer ionization technique.

Ionization Methods

Fast-atom Bombardment (FAB)

- In FAB a high-energy beam of neutral atoms, typically Xe or Ar, strikes a solid sample causing both desorption and ionization.
- The atomic beam is produced by accelerating ions from an ion source through a charge-exchange cell. The ions pick up an electron in collisions with neutral atoms to form a beam of high energy atoms.

FAB causes little fragmentation and usually gives a large peak corresponding to the molecular weight (molecular ion).

Electrospray ionization (ESI)

- The ESI source consists of a very fine needle and a series of skimmers.
- A sample solution is sprayed into the source chamber to form droplets. The droplets carry charge when they exit the capillary and, as the solvent evaporates (desolvation), the droplets disappear leaving highly (multiply) charged analyte molecules.

ESI is particularly useful for large biological molecules (e.g. proteins, peptides) that are difficult to vaporize or ionize, or beyond the mass range of the analyzer.

Ionization Methods

Field ionization (FI) and Field Desorption (FD)

- Molecules can lose an electron when placed in a very high electric field.
- High fields can be created in an ion source by applying a high voltage between a cathode and an anode - called a “field emitter”. A field emitter consists of a wire covered with microscopic carbon dendrites, which greatly amplify the effective field.

FI causes little fragmentation. Used extensively in characterization of humic and fulvic acids (soil science).

Laser Ionization (LIMS)

- A laser pulse ablates the material from the surface of the sample, and creates a microplasma that ionizes some of the sample constituents.
- The laser pulse accomplishes both vaporization and ionization of the sample.

Matrix-assisted laser desorption ionization (MALDI)

- Macromolecules are dispersed in a solid matrix such as nicotinic acid or glycerol.
- A UV laser pulse ablates the matrix which carries some of the large molecules into the gas phase in an ionized form.

MALDI is a LIMS method for vaporizing and ionizing large biological molecules (e.g., proteins, DNA fragments). See MALDI-TOF-MS

Ionization Methods

Resonance Ionization (RIMS)

- One or more laser beams are tuned in resonance to transitions of a gas phase atom or molecule to promote it above its ionization potential and create an ion.
- Solid samples must be vaporized by heating, sputtering or laser ablation.

Secondary Ionization (SIMS)

- A primary ion beam - such as $^{3}He^{+}$, $^{16}O^{+}$, or $^{40}Ar^{+}$ - is accelerated and focused onto the surface of a sample and sputters material into the gas phase. Approximately 1% of the sputtered material comes off as ions, which can then be analyzed by the MS.

SIMS has the advantage that material can be continually sputtered from a surface to determine analyte concentrations as a function of distance (spatial and depth profiling).

SIMS basis of Accelerator Mass Spectrometry and Ion Microprobe MS

Thermal Ionization (TIMS)

- A sample is deposited on a metal ribbon, such as Pt or Re, and an electric current heats the metal to a high temperature.
- The ribbon is often coated with graphite to provide a reducing effect.

TIMS is used for elemental or refractory materials.

Mass Analyzers

- **Magnetic-Sector MS**
- The ion optics in the ion-source chamber extract and accelerate ions to a kinetic energy (K.E.) given by:

$$K.E. = 0.5\ mv^2 = eV$$

- where:
  - m = mass of the ion
  - v = velocity of the ion
  - e = the charge
  - V = applied voltage of the ion optics.

- The ion enters the flight tube between the poles of a magnet and are deflected by the magnetic field, H. Only ions of m/e ratio that have equal centrifugal and centripetal forces pass through the flight tube:

$$mv^2/r = Hev;\ \text{centrifugal} = \text{centripetal forces}$$

- Where:
  - r = radius of curvature of the ion path:

$$r = mv/eH$$

- Thus:

$$m/e = H^2r^2/2V$$

- This equation shows that m/e of the ions that reach the detector can be varied by:
- - Changing H (magnetic field) "*magnet scan*"
- - Changing V (accelerating voltage) "*voltage scan*".

Mass Analyzers

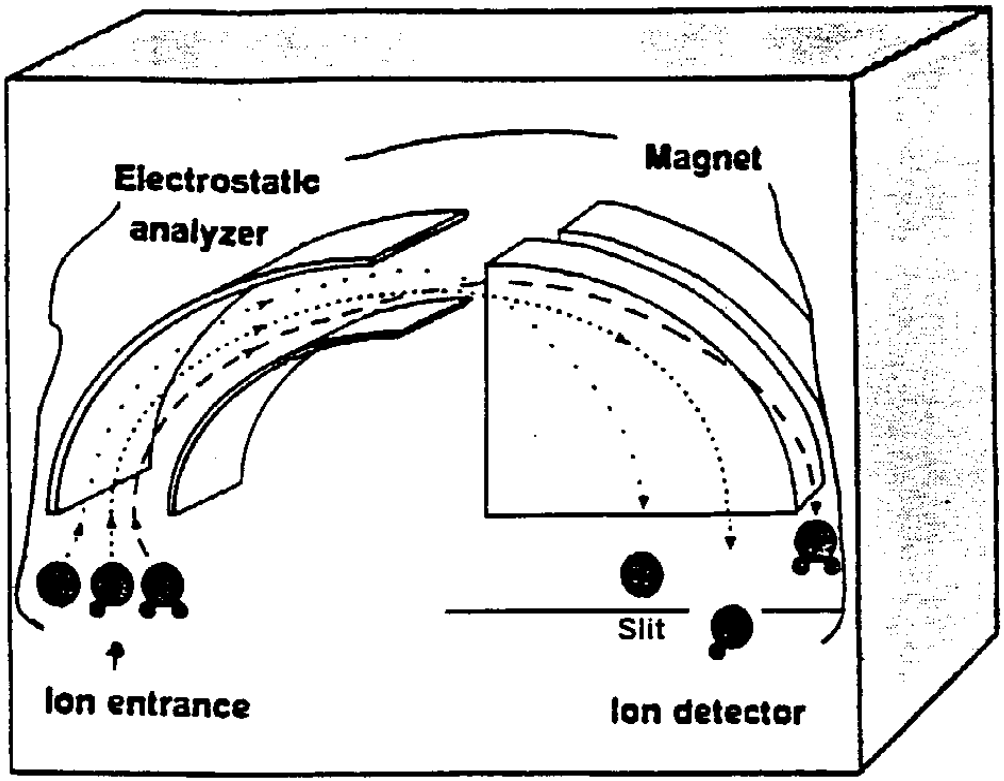
Magnetic-Sector MS

- *Instrumentation:*
- Single focus analyzers: A circular beam path of 180, 90 or 60 degrees can be used. The various forces influencing the particle separate ions with different m/e ratios.
- Double focussing analyzers: An electrostatic field is added to separate particles with different kinetic energies.

- *Magnetic sector MS provides nominal to high mass resolution.*

- *Most common mass analyzer for determination of isotope ratios.*

Magnetic-Sector MS



Mass Analyzers

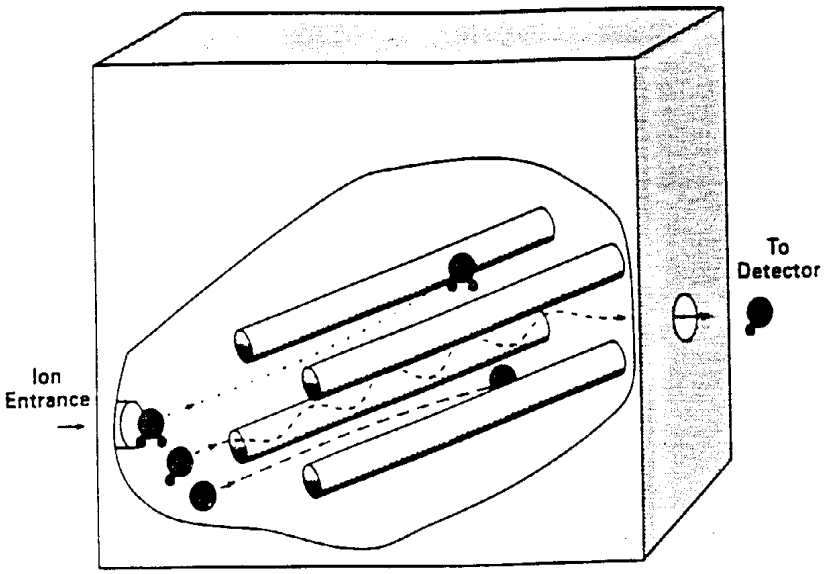
Quadrupole MS

- A quadrupole mass filter consists of four parallel metal rods.
- Two opposite rods have an applied potential of (U+Vcos(wt)), and the other two rods have a potential of –(U+Vcos(wt)) where:
  - U is a dc voltage
  - Vcos(wt) is an ac voltage.
- The applied voltage affects the trajectory of ions travelling down the flight path centered between the four rods. For given ac and dc voltages only ions of certain m/e ratio pass through the quadrupole filter, others are thrown out.
- A mass spectrum is obtained by monitoring the ions passing through the quadrupole filter as voltages on the rods are varied.

Quadrupole MS provides nominal mass resolution.

Most common mass analyzer for routine GC/MS applications ("Bench-top" GC/MS).

Quadrupole MS



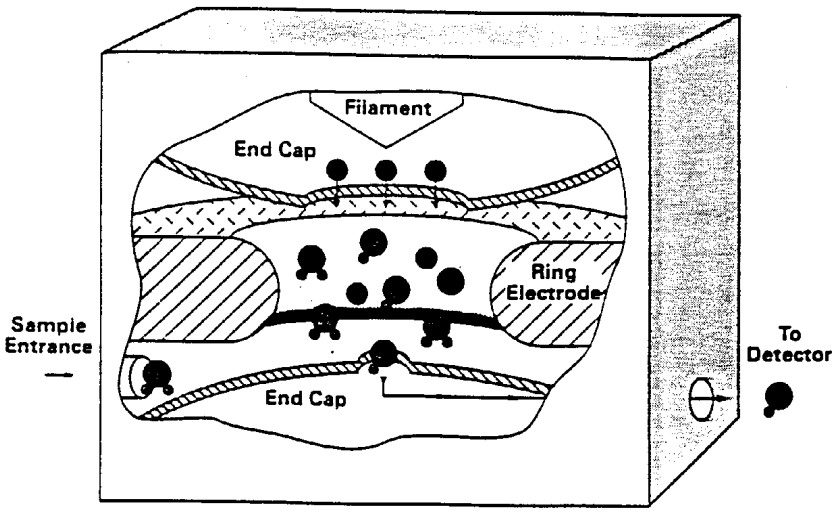
Mass Analyzers

Ion-Trap MS

- The ion trap MS uses three electrodes to trap ions in a small volume. The mass analyzer consists of a large ring electrode separating two hemispherical electrodes.
- A mass spectrum is obtained by changing the electrode voltages to eject the ions from the trap.

- *The advantages of Ion Trap MS include compact size, the ability to trap and accumulate ions to increase signal-to-noise, and the ability to perform MS-MS, or MSn experiments.*
- *Common benchtop MS for GC or LC.*
- *Ion Trap MS provides nominal mass resolution*

Ion-Trap MS

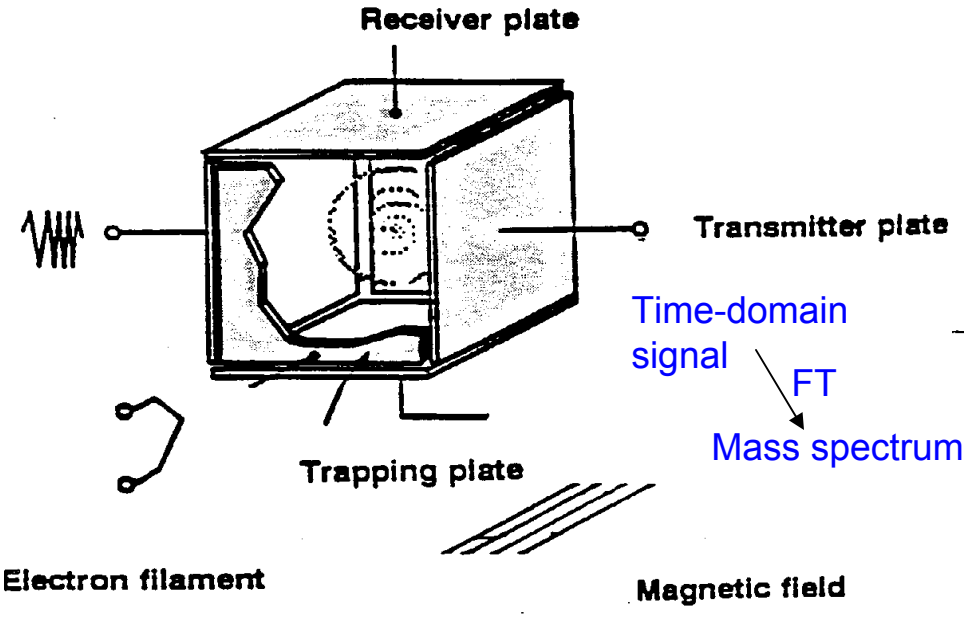


Mass Analyzers

Fourier-Transform Ion Cyclotron Resonance MS (FT-ICR)

- FT-ICR MS takes advantage of ion cyclotron resonance to select and detect ions.
- Ions are trapped within a cubic cell under the influence of small trapping potentials and a constant magnetic field. The frequency of the signal measured at the receiver plate is proportional to ion mass.

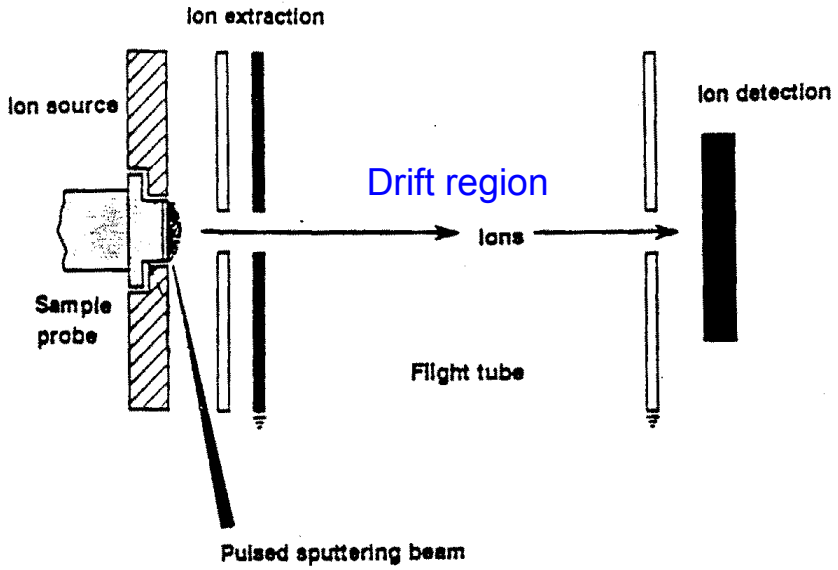
FT-ICR MS provides extremely high-resolution (accurate) mass measurement.



Mass Analyzers

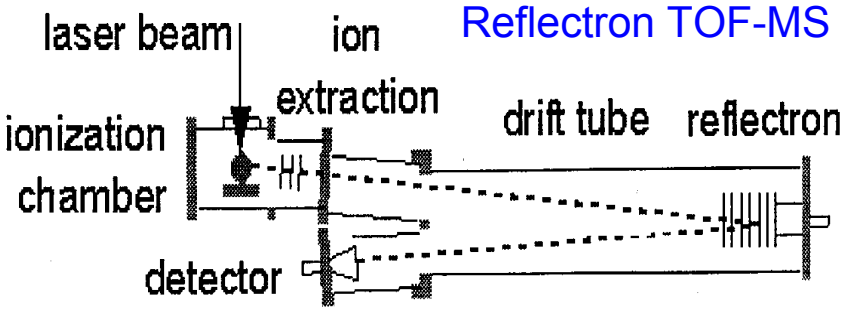
Time-of-Flight (TOF) MS

- A TOF MS system uses the differences in transit time through a drift region to separate ions of different masses.
- It operates in pulsed mode so ions must be produced or extracted in pulses.
- An electric field accelerates all ions into a field-free drift region with a kinetic energy of qV, where q is the ion charge and V is the applied voltage.
- Since the ion kinetic energy = 0.5 mv^2 lighter (smaller) ions have a higher velocity than heavier ions, and reach the detector at the end of the drift region sooner.



The advantages of TOF-MS are the ability to measure very large masses, and fast MS acquisition rate.

TOF-MS provides nominal to medium resolution.



Ion Detectors

- Channeltron
- Daly detector
- Electron multiplier tube (EMT)
- Faraday cup (used in isotope ratio mass MS)
- Microchannel plate (used in TOF-MS)

Interpretation of Mass Spectra

Important Features of Mass Spectra

Molecular Ion ($M^{+\cdot}$)

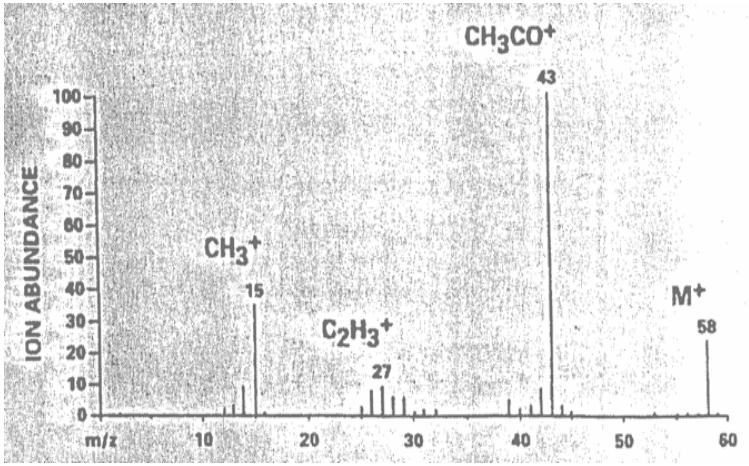
- Intensity will depend on stability of molecular structure and ease of fragmentation

Base Peak (B^{+})

- May be molecular ion or favored fragment ion, depending on structure

Fragment Ions

- May be formed by cleavage, loss of neutral fragments or by structural rearrangement
- May be many or few

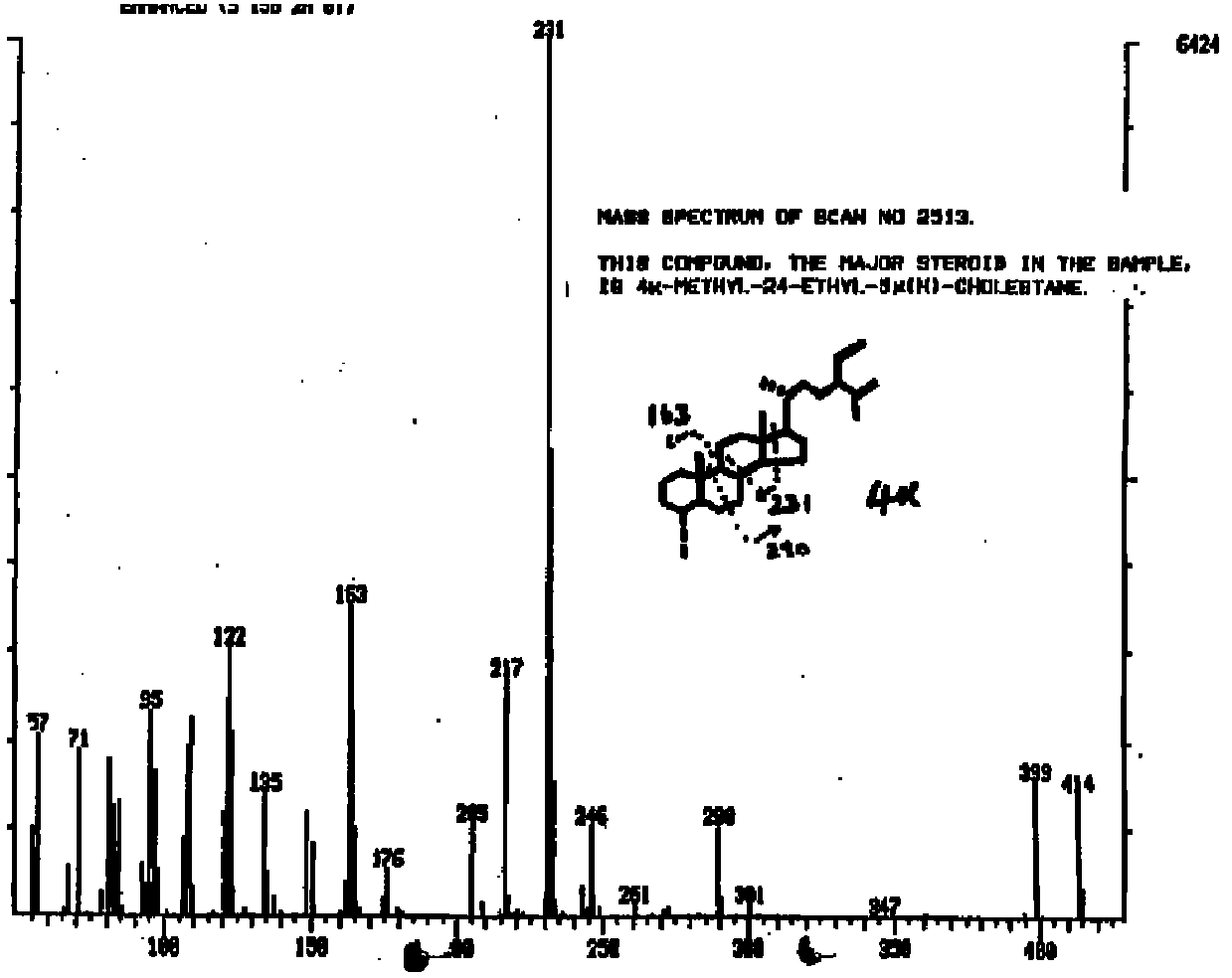


The mass spectrum (EI) of acetone, CH_2COCH_3, contains many fragment ions as well as the molecular ion at m/z 58

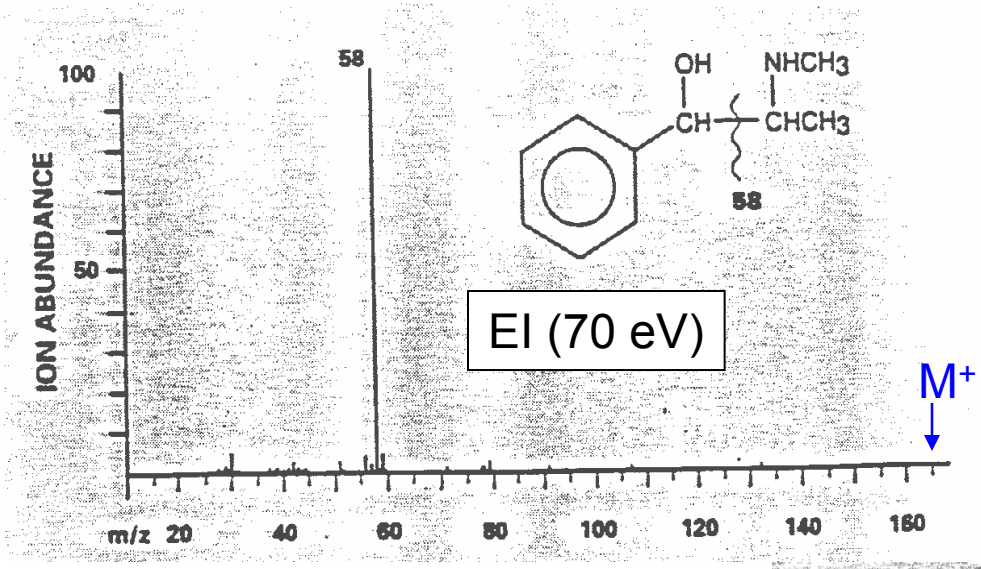
Interpretation of Mass Spectra

Major Influences on Mass Spectral Fragmentations of Organic Compounds

- 1. Ring Structures
- 2. Branching points
- 3. Double bonds
- 4. Aromaticity
- 5. Stereochemistry
- 6. Functionality

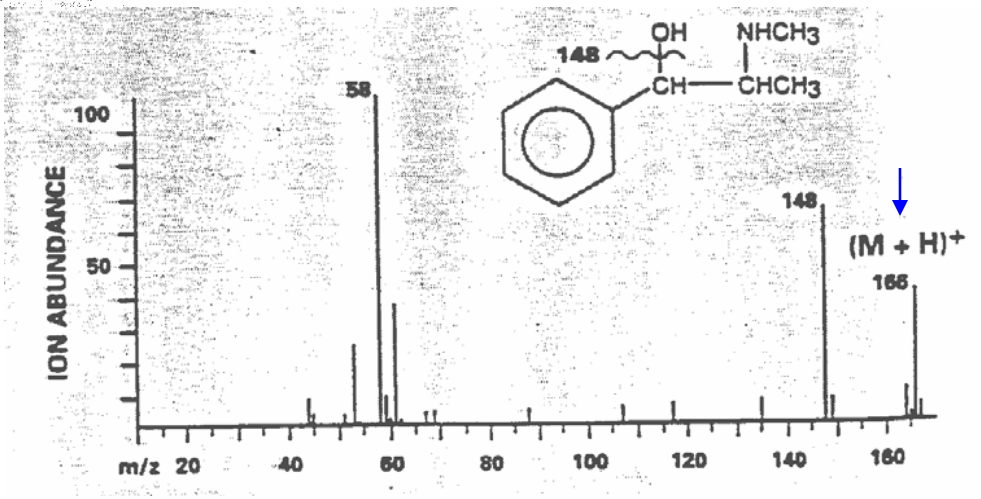


Interpretation of Mass Spectra



Ephedrine (mw 165)

M^+ (absent)



Interpretation of mass spectra

Mass Resolution

- R = resolution required to baseline separate a pair of ions having the same nominal mass:

$$R = M/\Delta m$$

- Where:

  M = nominal mass of ions to be separated

  Δm = difference in mass

- e.g. CO^+ (27.995) and N_2^+ (28.006), nominal mass = 28

- $\Delta m = 0.011$, R = 2,500

Interpretation of mass spectra

Isotopic abundances and precise masses of selected elements

Element	Symbol	Nominal Mass	Precise Mass	Abundance
Hydrogen	H	1	1.0078	99.99
	D	2	2.014	0.01
Carbon	^{12}C	12	12.0000	98.91
	^{13}C	13	13.0034	1.09
Nitrogen	^{14}N	14	14.0031	99.6
	^{15}N	15	15.0001	0.04
Oxygen	^{16}O	16	15.9949	99.76
	^{17}O	17	16.9991	0.04
	^{18}O	18	17.9992	0.20
Sulfur	^{32}S	32	31.9721	95.02
	^{33}S	33	32.9715	0.76
	^{34}S	34	33.9679	4.22
Chlorine	^{35}Cl	35	34.9689	75.77
	^{37}Cl	37	36.9659	24.32
Bromine	^{79}Br	79	78.9183	50.5
	^{81}Br	81	80.9163	49.5
Fluorine	F	19	18.9984	monoisotopic
Iodine	I	127	126.9045	monoisotopic
Phosphorus	P	31	30.9738	monoisotopic

Mass Defect: the difference between the nominal and exact mass. The mass defect can assume both positive and negative values.

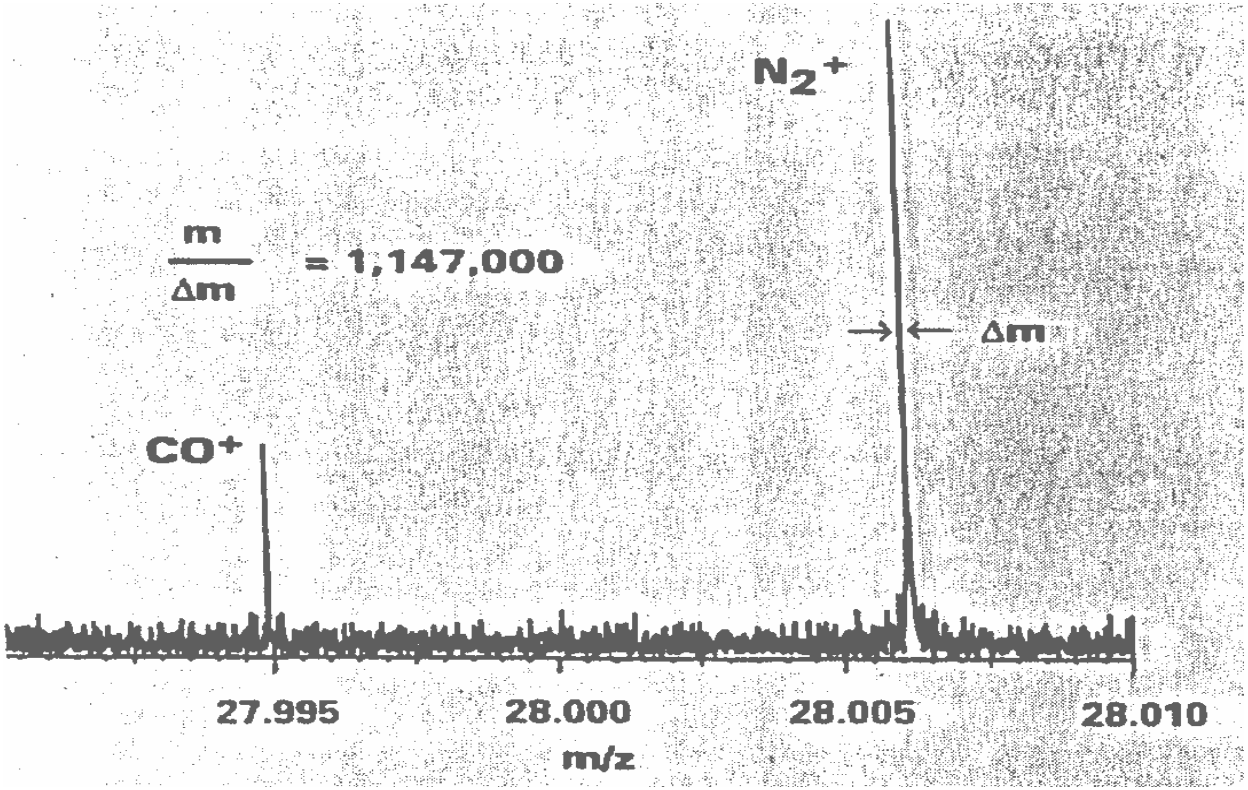
Interpretation of mass spectra

Highest base-line resolved mass for selected doublets at a resolution of 1 part in 25000

Doublet	Δ Mass	Highest Resolved Mass (25000 x ΔMass)
$C - H_{12}$	0.0939	2347
$C_2H_8 - {}^{32}S$	0.0905	2263
$CH_4 - O$	0.0364	910
${}^{32}S - O_2$	0.0277	692
${}^{13}CH - N$	0.0081	203
$C_3 - {}^{32}SH_4$	0.0034	85

Interpretation of mass spectra

High resolution mass spectrum (FT-ICR-MS) of carbon monoxide and nitrogen



Gas Chromatography-Mass Spectrometry (GC/MS) and Liquid Chromatography-Mass Spectrometry (LC/MS)

Objective:

- Identification and Quantification of components in complex mixtures.
- GC/LC: Separates components of complex mixture according to molecular size, shape, polarity.
- MS: Permits recognition of individual components as they sequentially elute from GC.

Approach

Compound Identification

- Mass Spectra
- Mass Chromatography

Compound Quantification

- Total (Reconstructed) Ion Current (TIC/RIC)
- Mass Chromatography

- MS scans across a given mass range (e.g. 50 - 500 amu) at a set rate (e.g. 1 scan/sec).
- Spectra are collected ("acquired") for each scan over a time (usually corresponding to the length of the GC run).

Mass Chromatography/Mass Fragmentography

- Use: single ion monitoring
- multiple ion monitoring
- Can select ions characteristic of
- compound type
- carbon number
- stereochemistry

Example 1

- Mass Spectra are collected for unrelated compounds A, B and C separated from a mixture by GC

- Mass x,y and z are found to be uniquely characteristic for compounds A,B and C respectively.

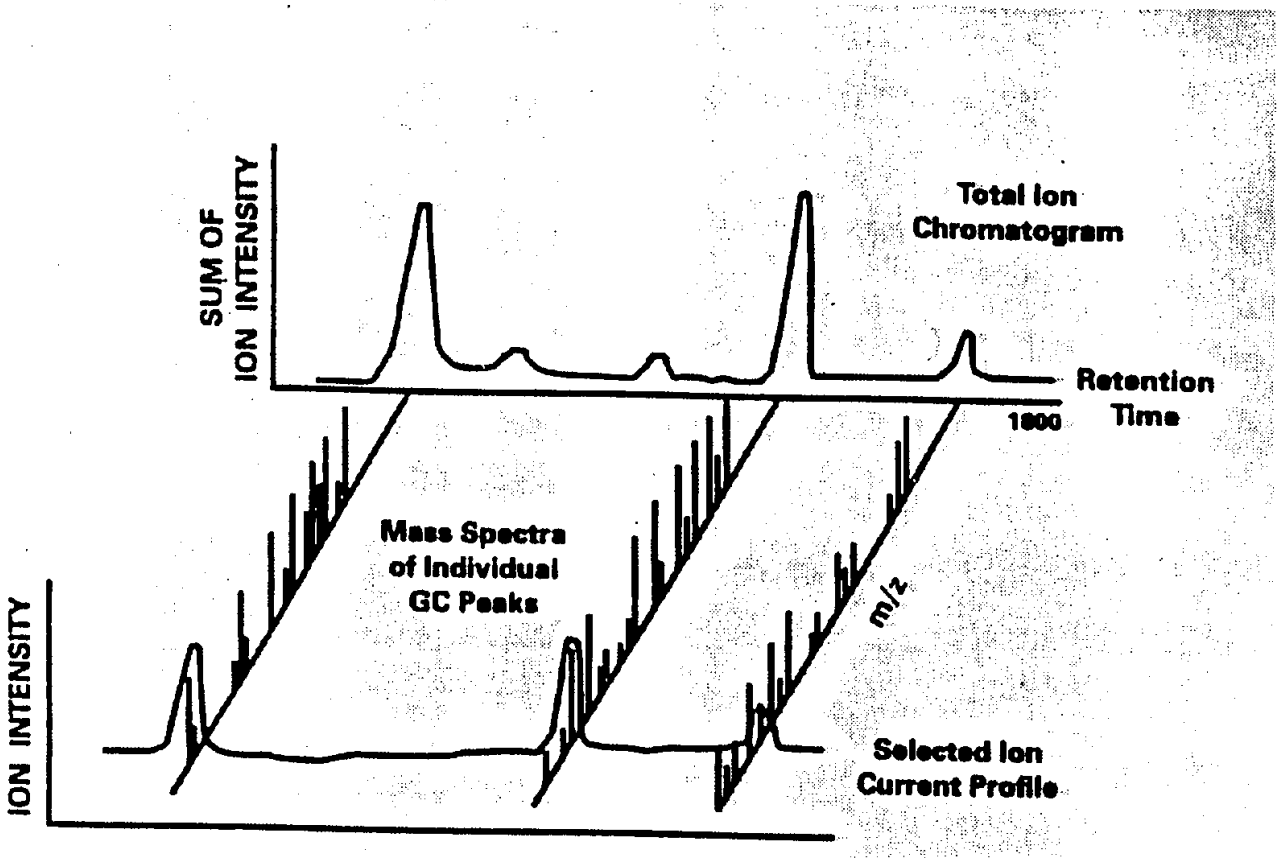
- Can perform mass chromatography using diagnostic ions

Mass Chromatography/Mass Fragmentography

Example 2.

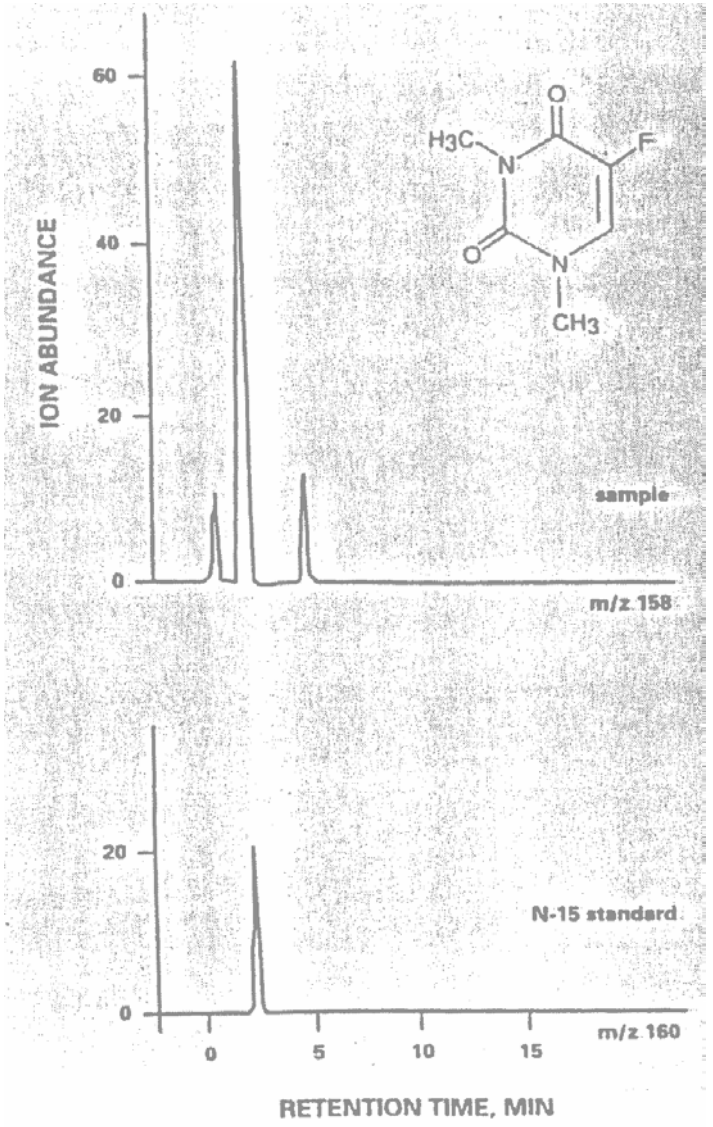
- For related compounds A and A' can select a common ion to study their distributions in complex mixtures
- This is a very good method for recognition, characterization and "fingerprinting" of homologous series.

Mass Chromatography/Mass Fragmentography



Quantitative mass spectral analysis of the dimethyl derivative of 5-flourouracil using isotopically labeled standard

Advantages
Use of standards with similar retention times and ionization efficiencies to analytes.
(= more accurate quantification).



Mass Chromatography/Mass Fragmentography

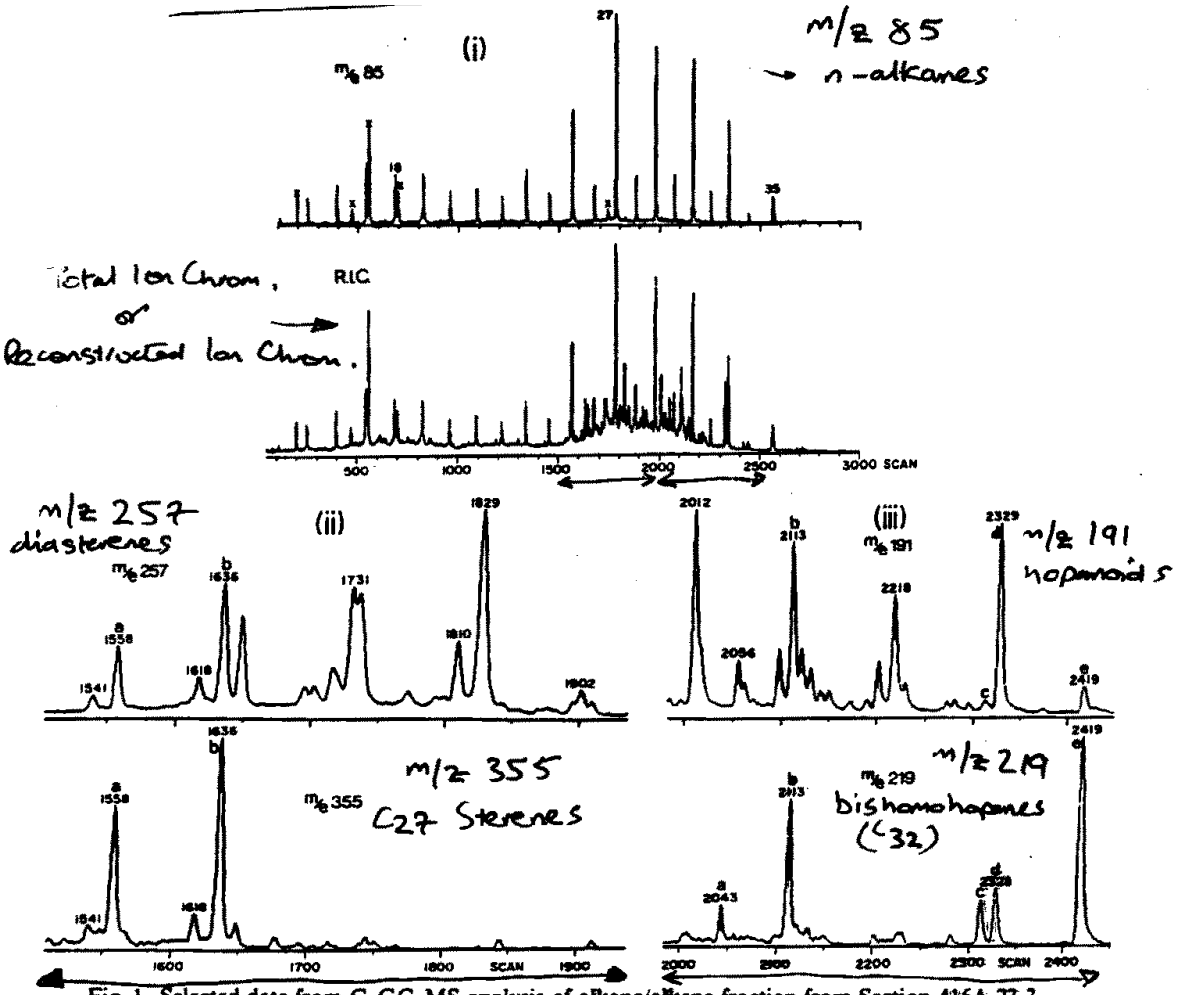
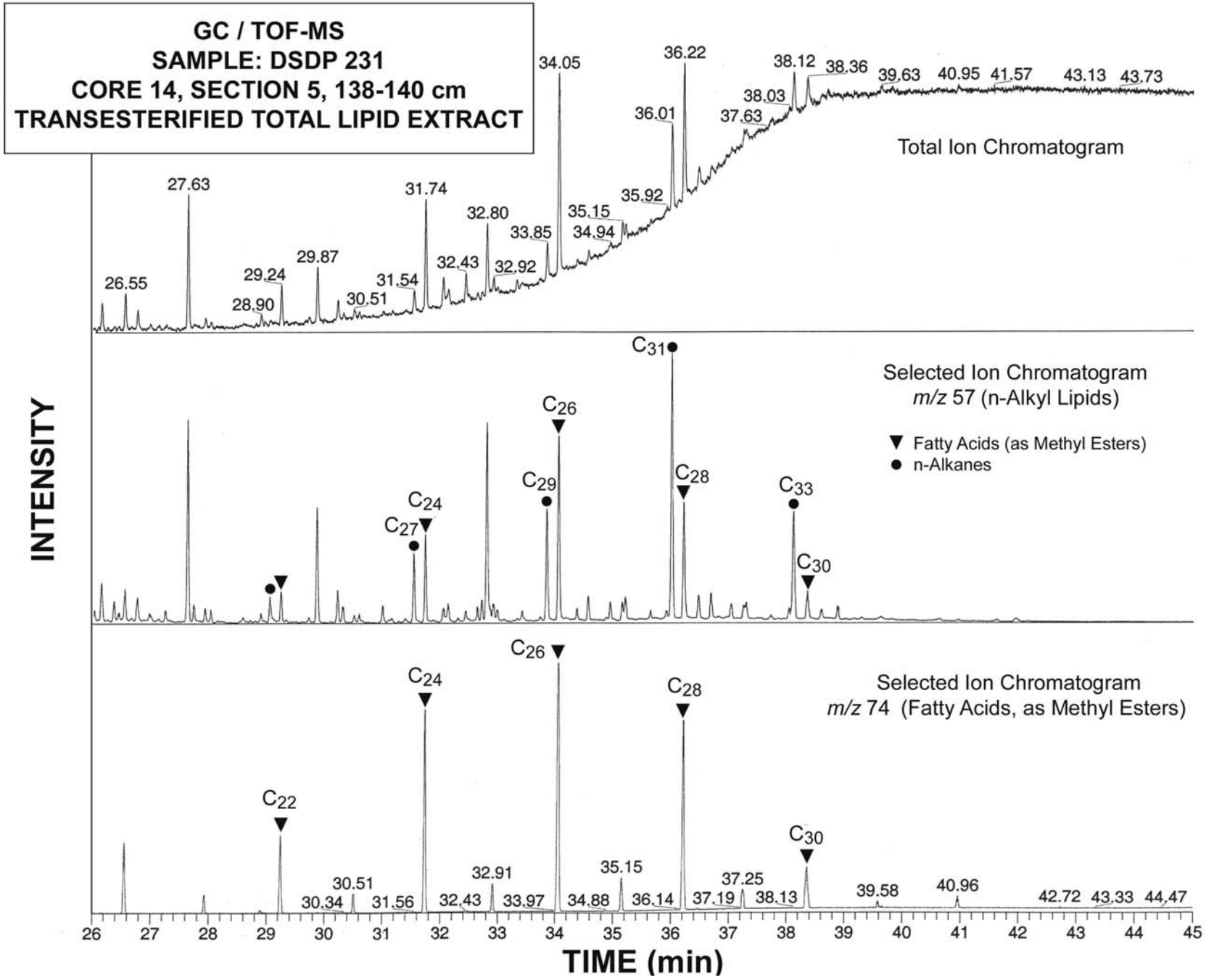


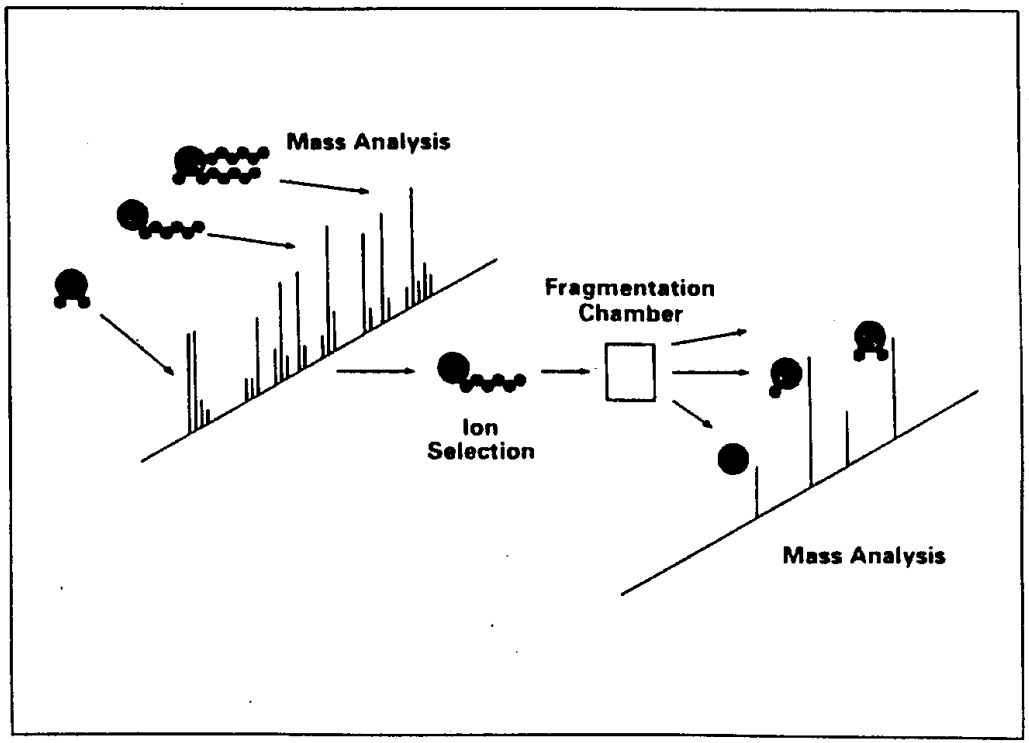
Fig. 1. Selected data from C–GC–MS analysis of alkane/alkene fraction from Section 416A-23-3.


GC / TOF-MS
SAMPLE: DSDP 231
CORE 14, SECTION 5, 138-140 cm
TRANSESTERIFIED TOTAL LIPID EXTRACT
Total Ion Chromatogram
Selected Ion Chromatogram
m/z 57 (n-Alkyl Lipids)
Fatty Acids (as Methyl Esters)
n-Alkanes
Selected Ion Chromatogram
m/z 74 (Fatty Acids, as Methyl Esters)
INTENSITY
TIME (min)

Tandem Mass Spectrometry (MS/MS)

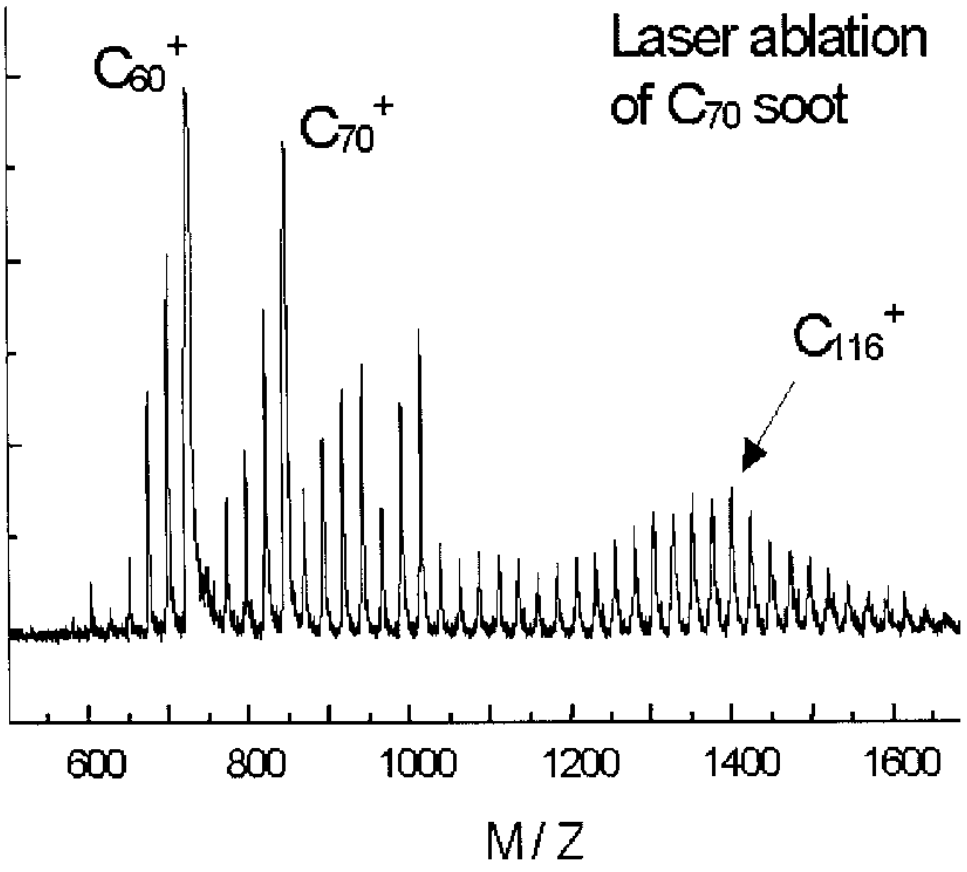
In MS/MS, the first mass analyzer selects one m/z value for fragmentation; the second mass analyzer produces the mass spectrum of the fragments.

In ion trap and FT-ICR systems, this process can be repeated multiple times (MS^n)



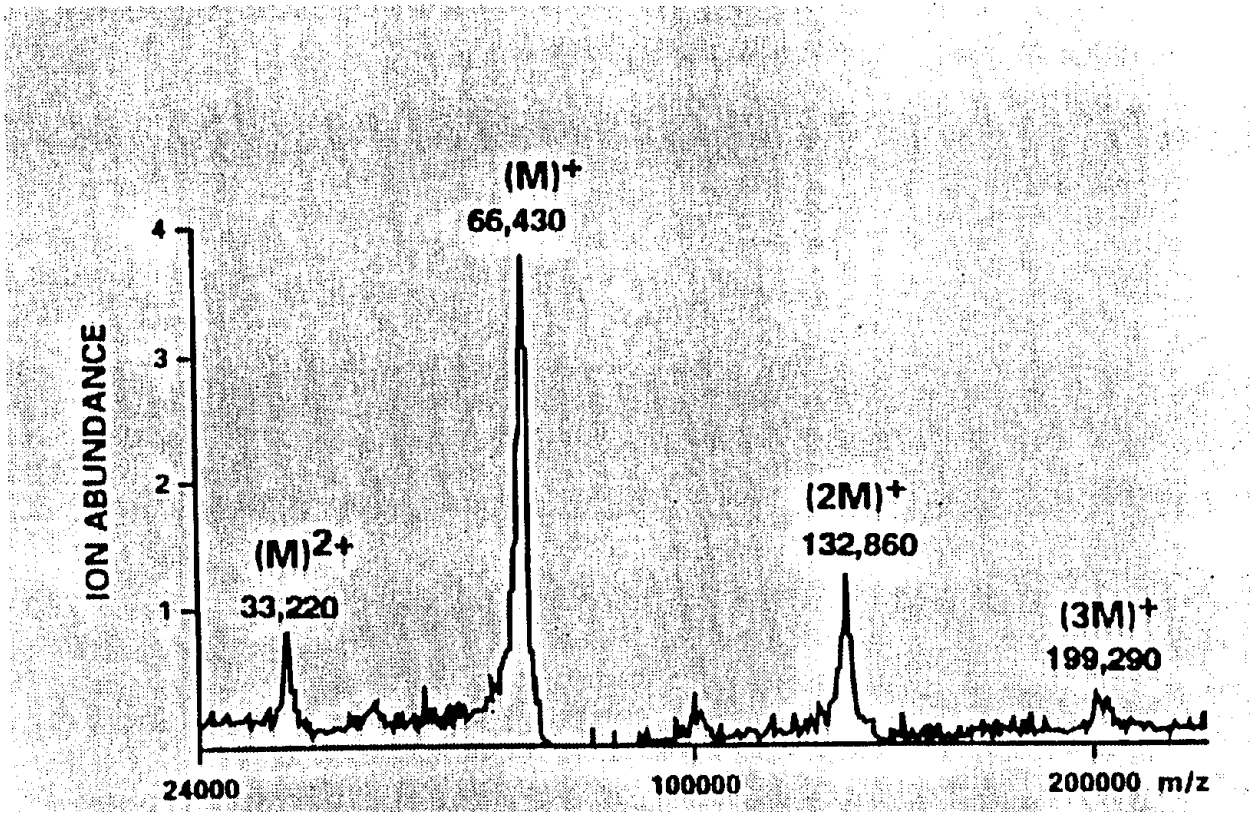
Mass Spectrometry of high mass organic compounds

MALDI-TOF/MS



Mass Spectrometry of high mass organic compounds

MALDI-TOF/MS of bovine serum albumin (protein)

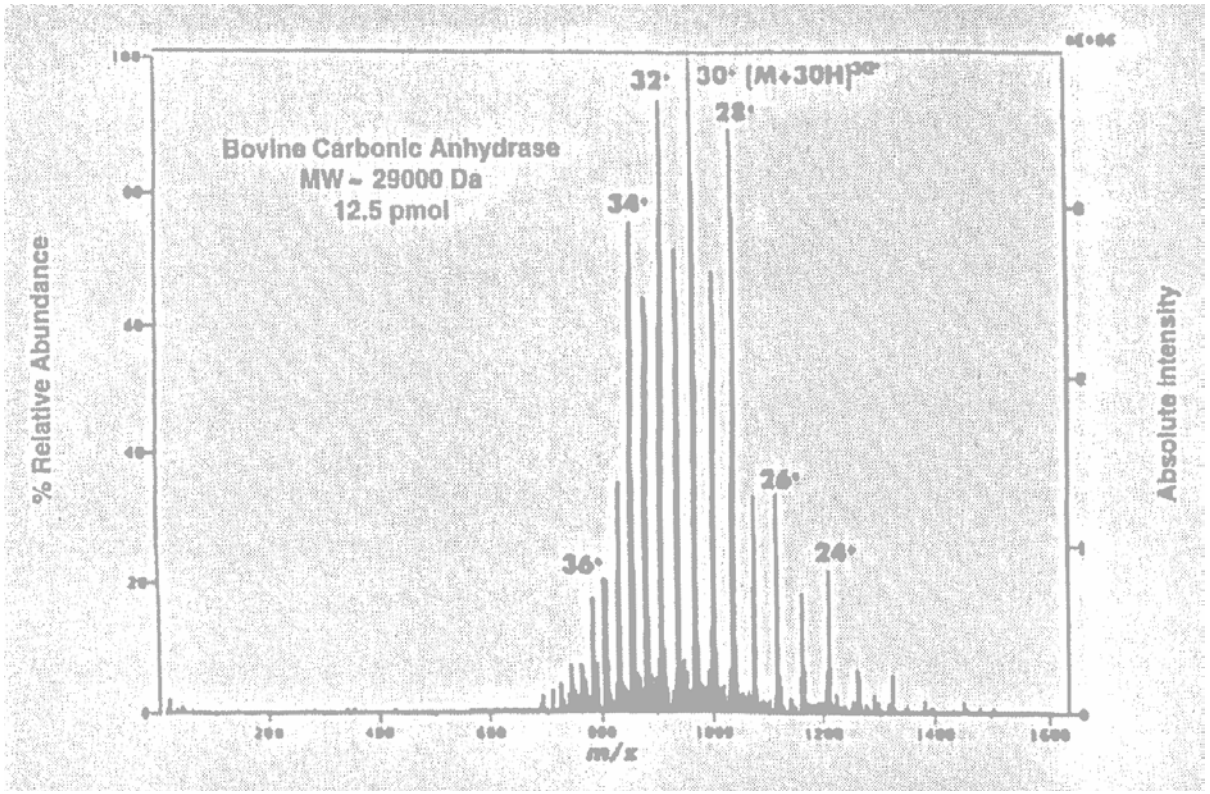


m/z

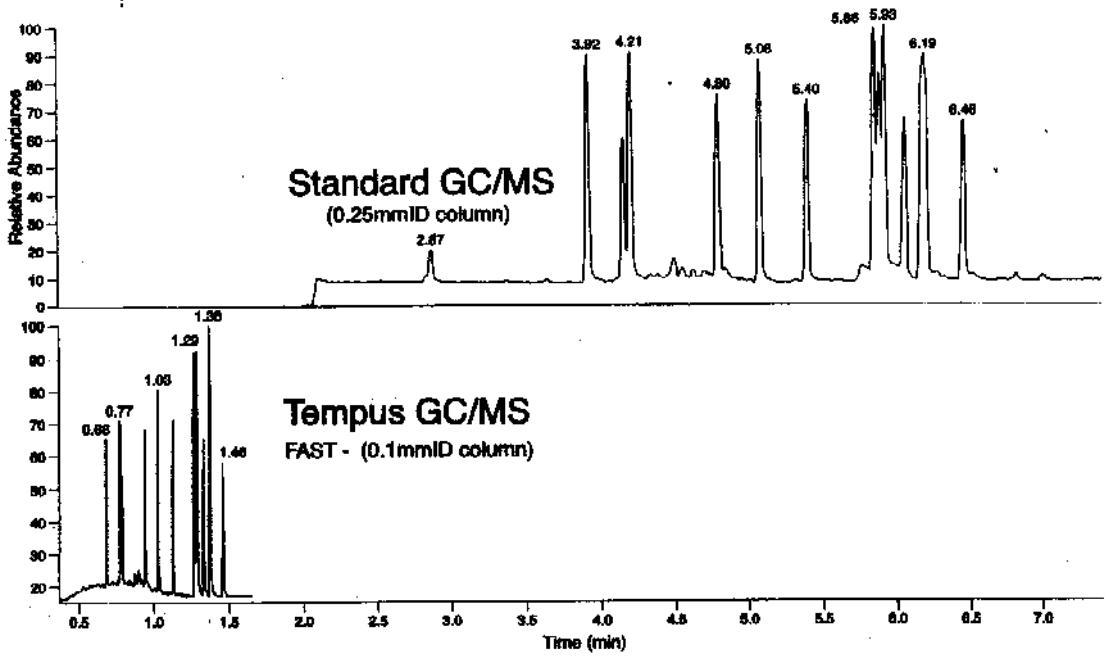
Mass Spectrometry of high mass organic compounds

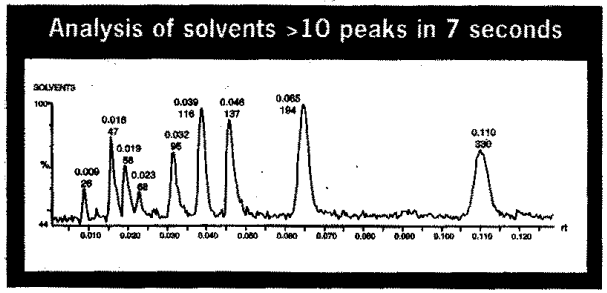
ESI/MS of bovine carbonic anhydrase (enzyme protein, mw 28,000 Da, 12.5 pmol)

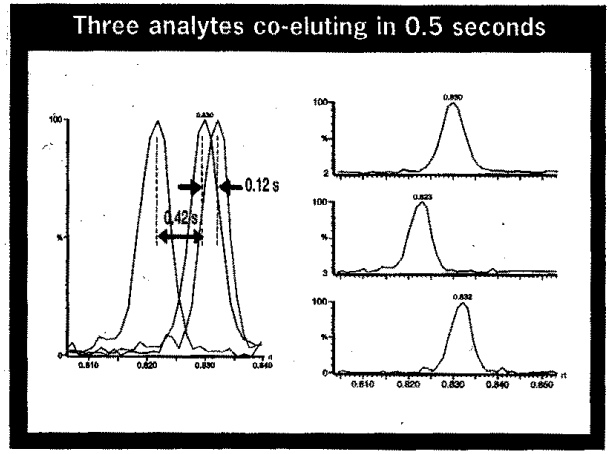
Electrospray ESI/MS of large biomolecules results in an array of multicharge ions with moderate m/z values.



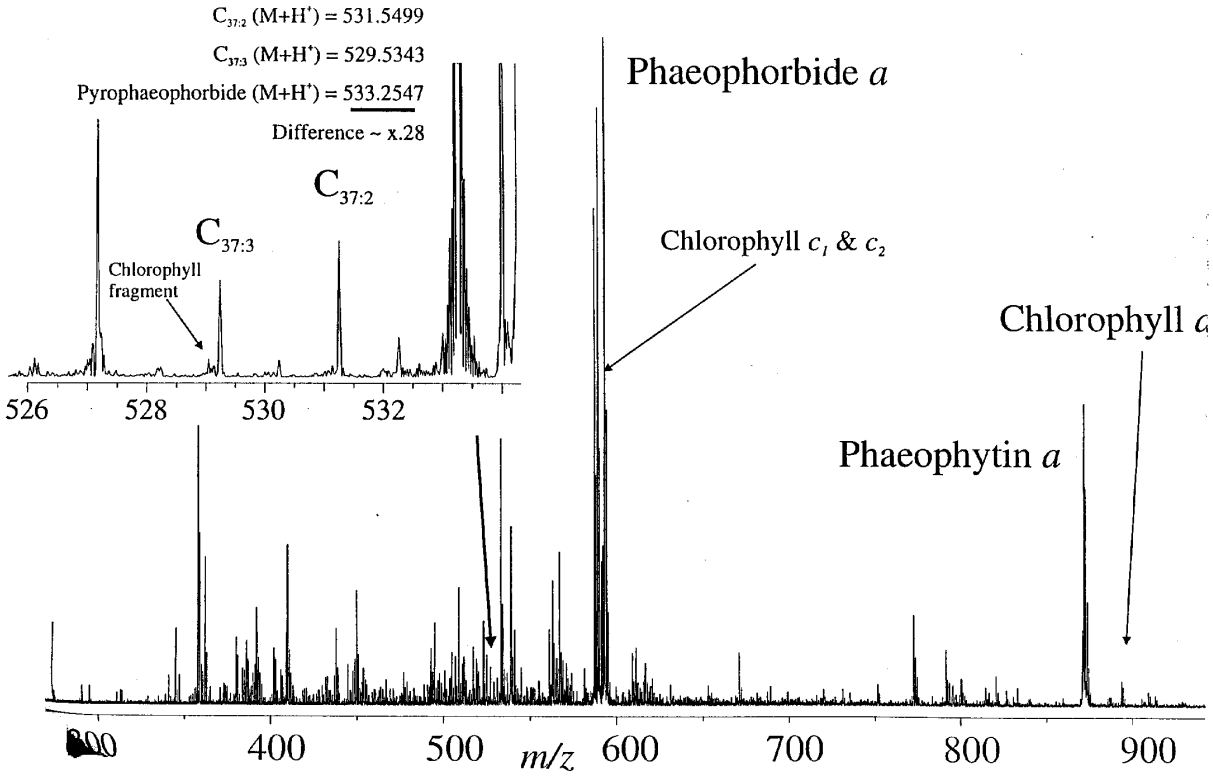
GC/TOF-MS


Standard GC/MS
(0.25mmID column)
2.67
3.92
4.21
4.90
5.08
5.40
5.86
5.93
6.19
6.46
Tempus GC/MS
FAST - (0.1mmID column)
0.66
0.77
1.03
1.22
1.36
1.46
Relative Abundance
Time (min)


Analysis of solvents >10 peaks in 7 seconds
SOLVENTS


Three analytes co-eluting in 0.5 seconds
0.12 s
0.42/s

MALDI-FTMS spectrum of DCM/ETOH extract of *Isochrysis Galbana*



ESI-FT-ICR-MS

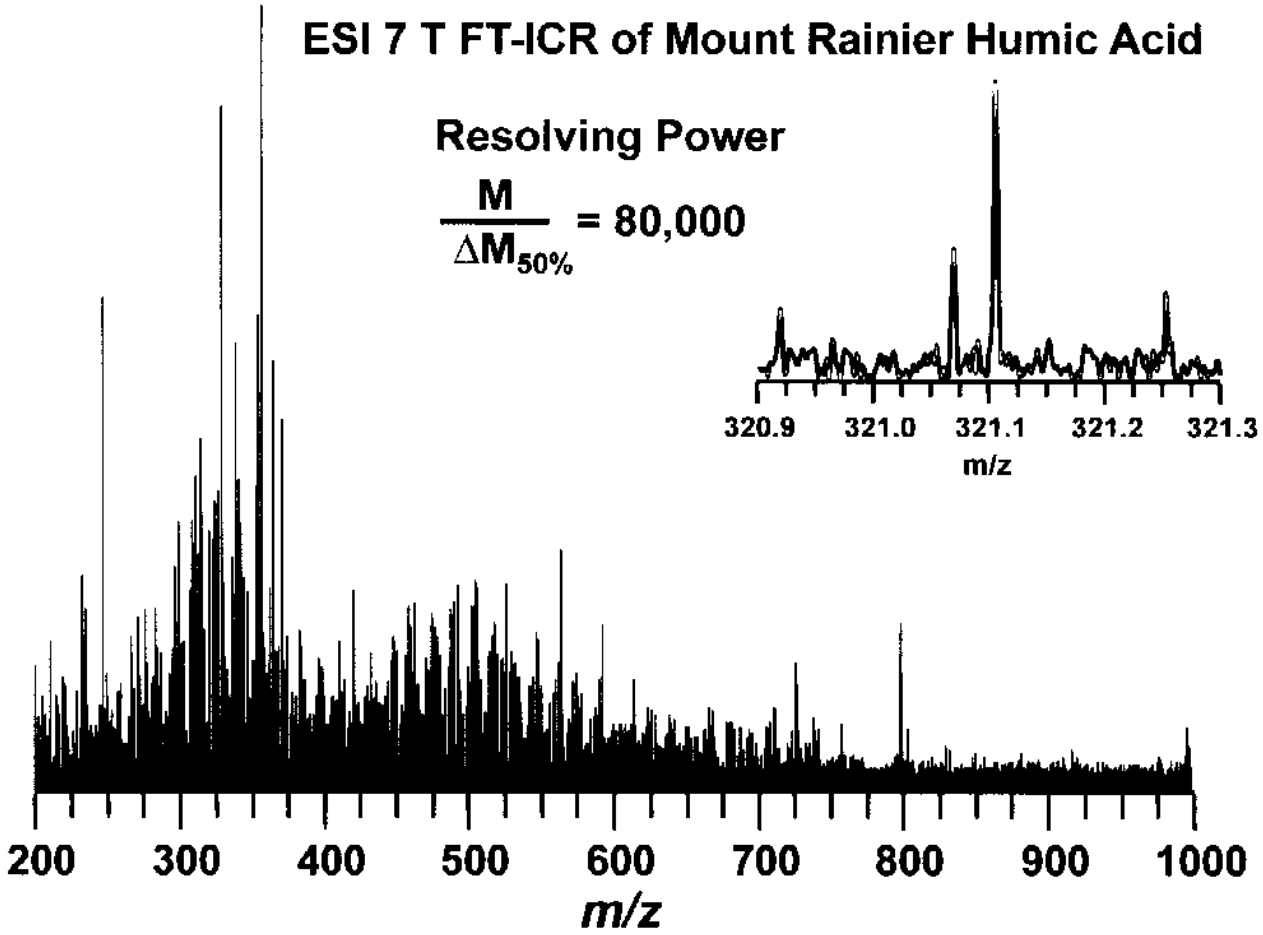


Fig. 2. ESI FT–ICR positive ion mass spectrum of the humic acid extract of a degraded wood sample from Mt. Rainier, WA, acquired on a 7 T FT ICR MS. The sample was prepared in 25:75 water: methanol at a concentration of 1.25 mg ml $^{-1}$. The spectrum represents the average of 18,000 scans. The inset is an expansion of the region around 321 *m/z* where the mass resolving power was approximately 80,000.

ESI-MS

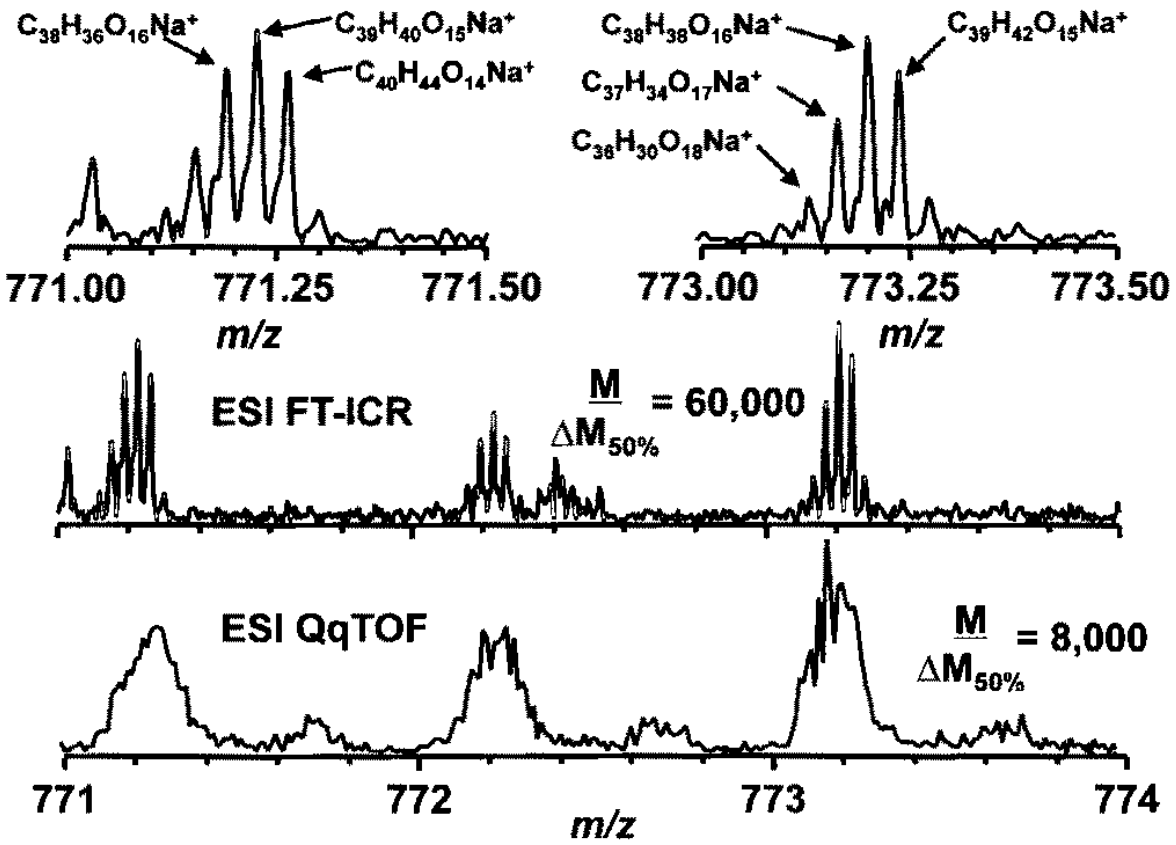


Fig. 3. Comparison of expanded spectra of Mt. Rainier humic acid. The ESI Qq-TOF spectrum (bottom) is an expansion of the 771–774 *m/z* range from Fig. 1. The ESI FT-ICR spectrum (middle) was acquired on a 9.4 T FT–ICR MS and the mass resolving power in this region was approximately 60,000. The top two mass spectra are further expansions of the FT ICR spectrum. The resolution of peaks in these regions was sufficient to allow the assignment of unique molecular formulas derived from proposed structures (values and errors in Table 1).

ESI-FT-ICR-MS

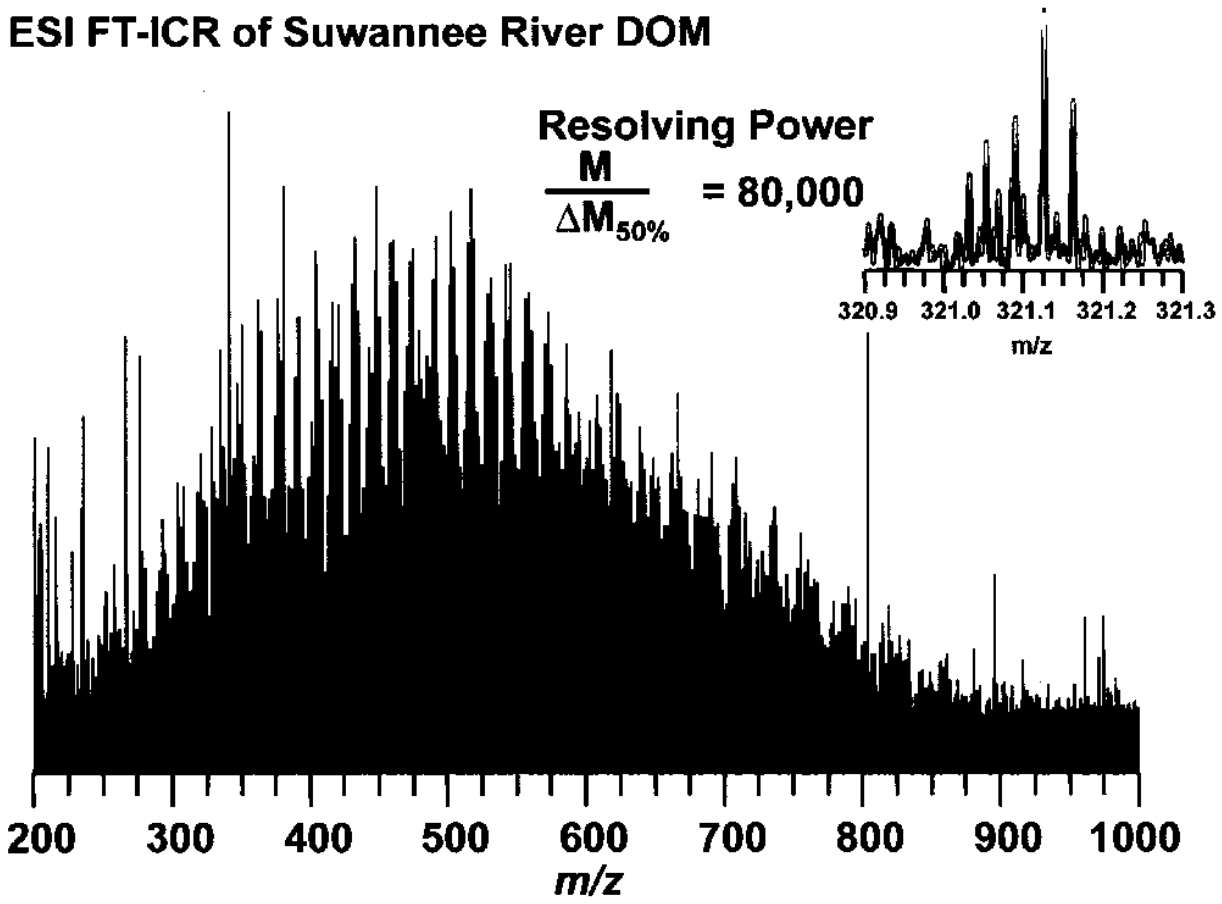


Fig. 4. ESI FT-ICR positive ion mass spectrum of dissolved organic matter (DOM) from Suwannee River. The spectrum was acquired in the same manner as that in Fig. 2.

High performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (HPLC/APCI-MS) of intact tetraether lipids

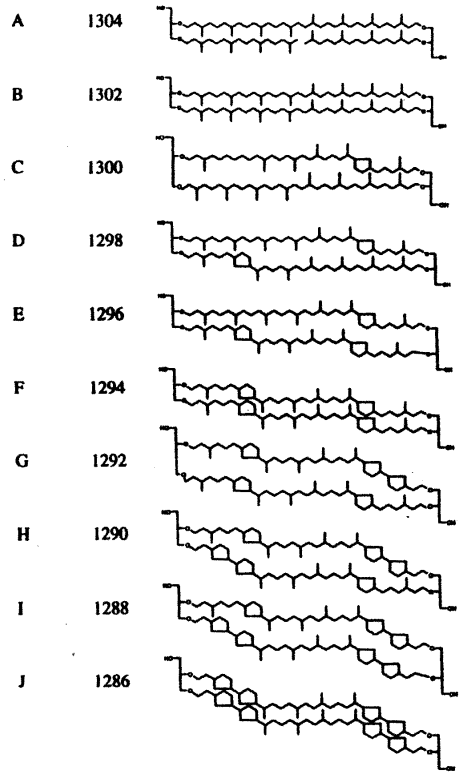


Figure 1. Structures and *m/z* values of protonated molecules of several archaeal tetraethers, previously identified.[7]

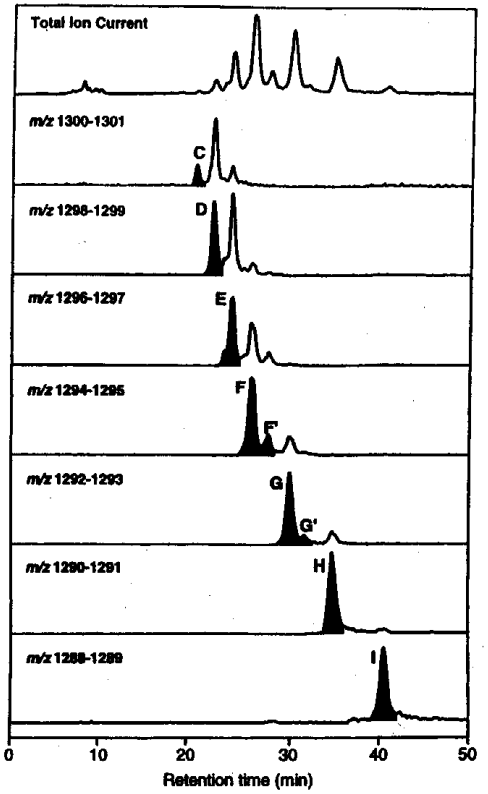


Figure 3. Total ion current and mass chromatograms of combined $[M+H]^+$ and $[M+H]^+ + 1$ ions of the GDGT fraction of *S. solfataricus*. Letters refer to structures shown in Fig. 1.

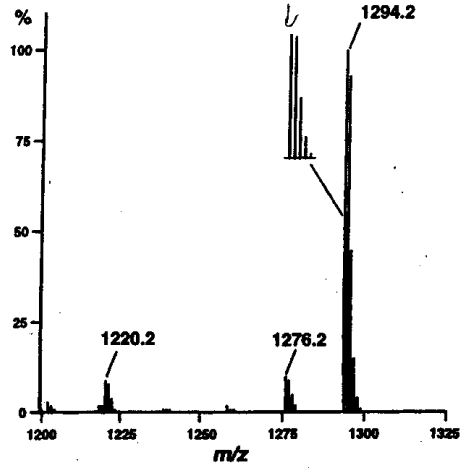
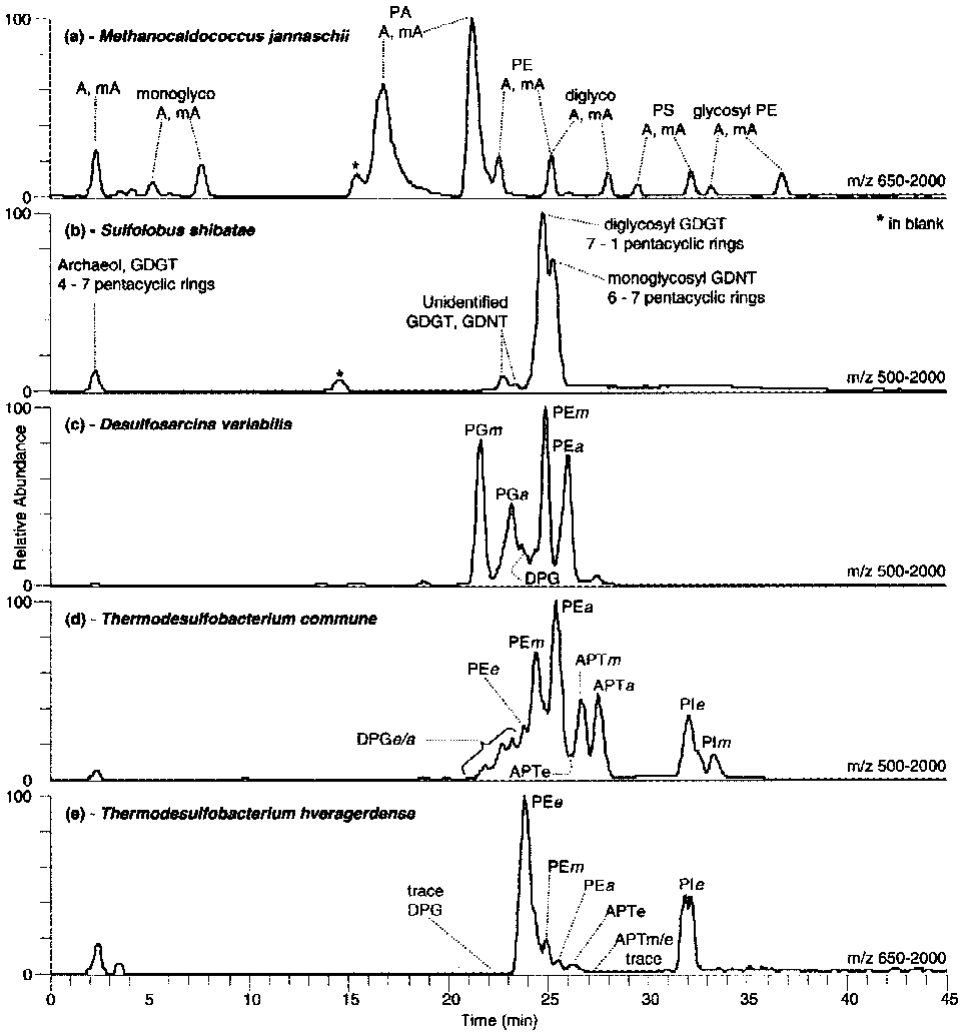


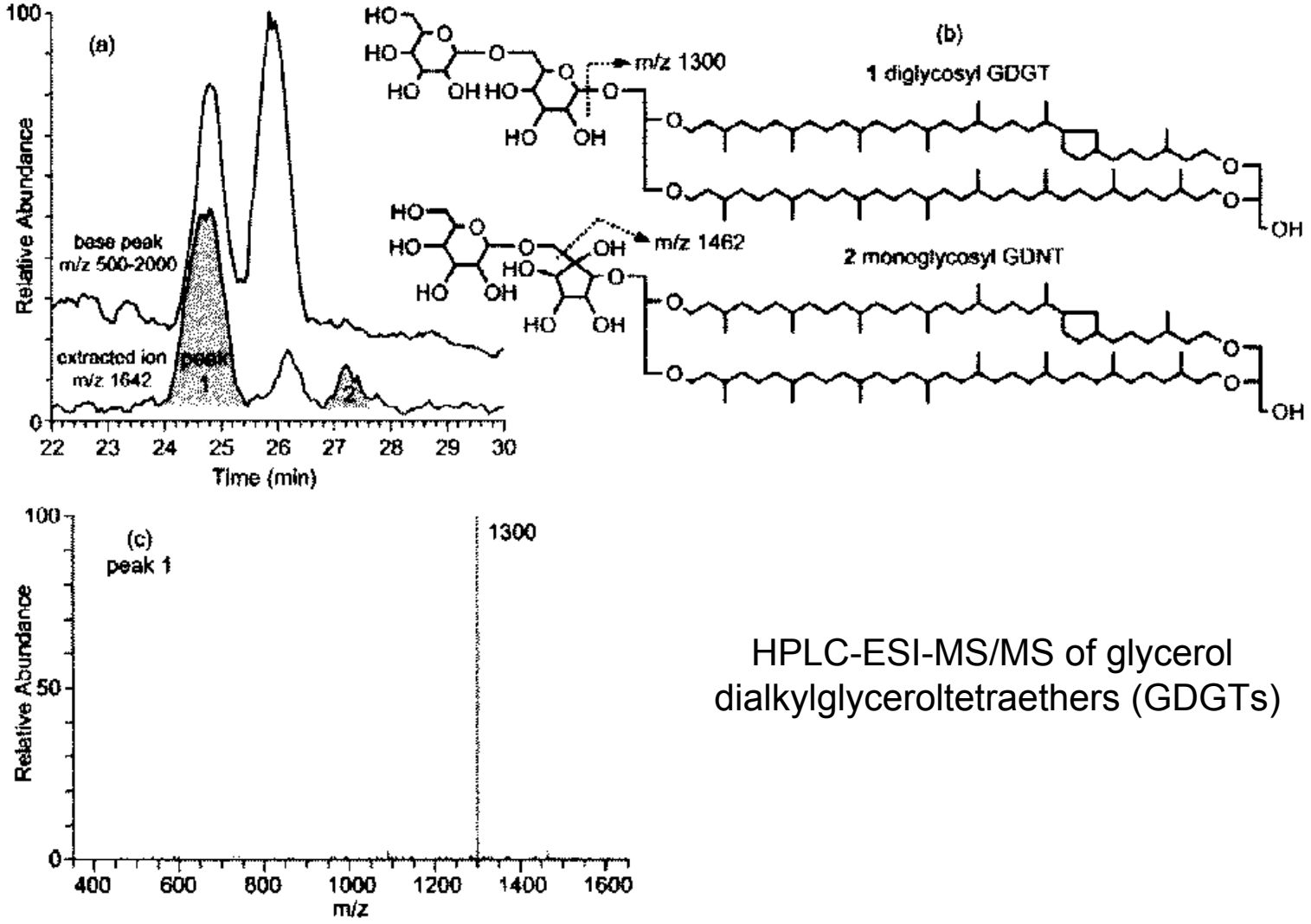
Figure 4. APCI mass spectrum of GDGT F with calculated ion distribution of protonated molecule (inset). Mass spectrum shown is corrected for background.

Hopmans et al 2000

High performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS) of intact polar lipids



Sturt et al. 2004



HPLC-ESI-MS/MS of glycerol dialkylglyceroltetraethers (GDGTs)

Sturt et al. 2004

Isotope Ratio Mass Spectrometry

Principle

- Isotope ratios can be precisely measured using a sector mass spectrometer (Faraday cup detectors).
- The MS precisely measures the ratio of currents from ion beams corresponding to different isotopes (e.g., for $^{13}C/^{12}C$, measure $^{13}CO_2^+$ (m/e = 45) and $^{12}CO_2^+$ (m/e = 44))
- Ratio is compared to a standard reference gas.

Conventional Method

- Introduction of gases via dual viscous inlet.

Continuous-flow mass spectrometry

- Elemental analyzer – irMS (EA-irMS).
- Isotope ratio monitoring-Gas Chromatography-Mass Spectrometry (irm-GC-MS, GC-irMS).

Element	Isotope	Measured as	Reference Std.
Carbon	$^{13}C/^{12}C$	CO_2	PDB
Nitrogen	$^{15}N/^{14}N$	N_2	Atm. N_2
Oxygen	$^{18}O/^{16}O$	CO_2	SMOW
Hydrogen	D/H	H_2	SMOW
Sulfur	$^{34}S/^{32}S$	SO_2	CDT

Isotope ratio Mass Spectrometry

HIGH-PRECISION CONTINUOUS-FLOW ISOTOPE RATIO MS ■

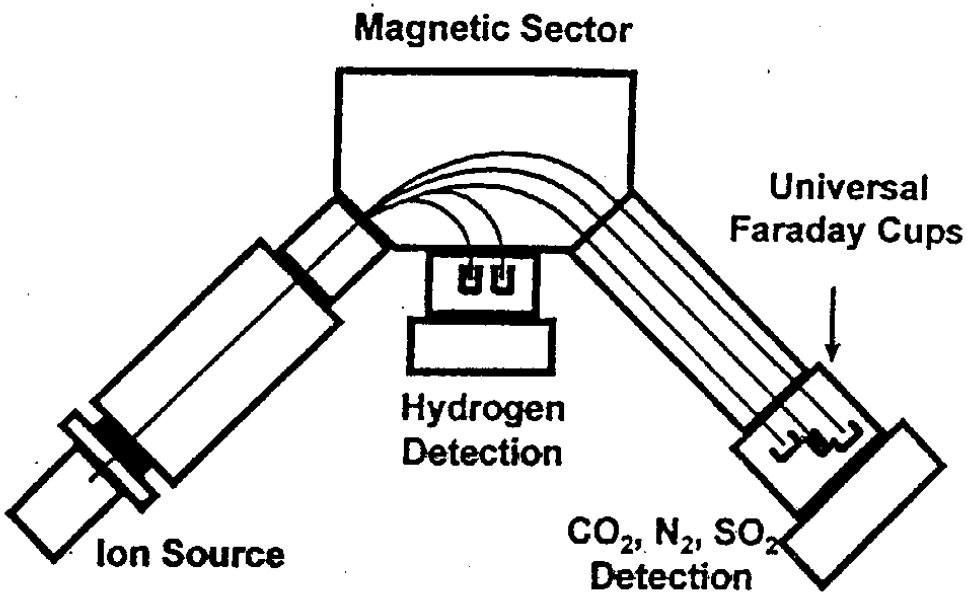
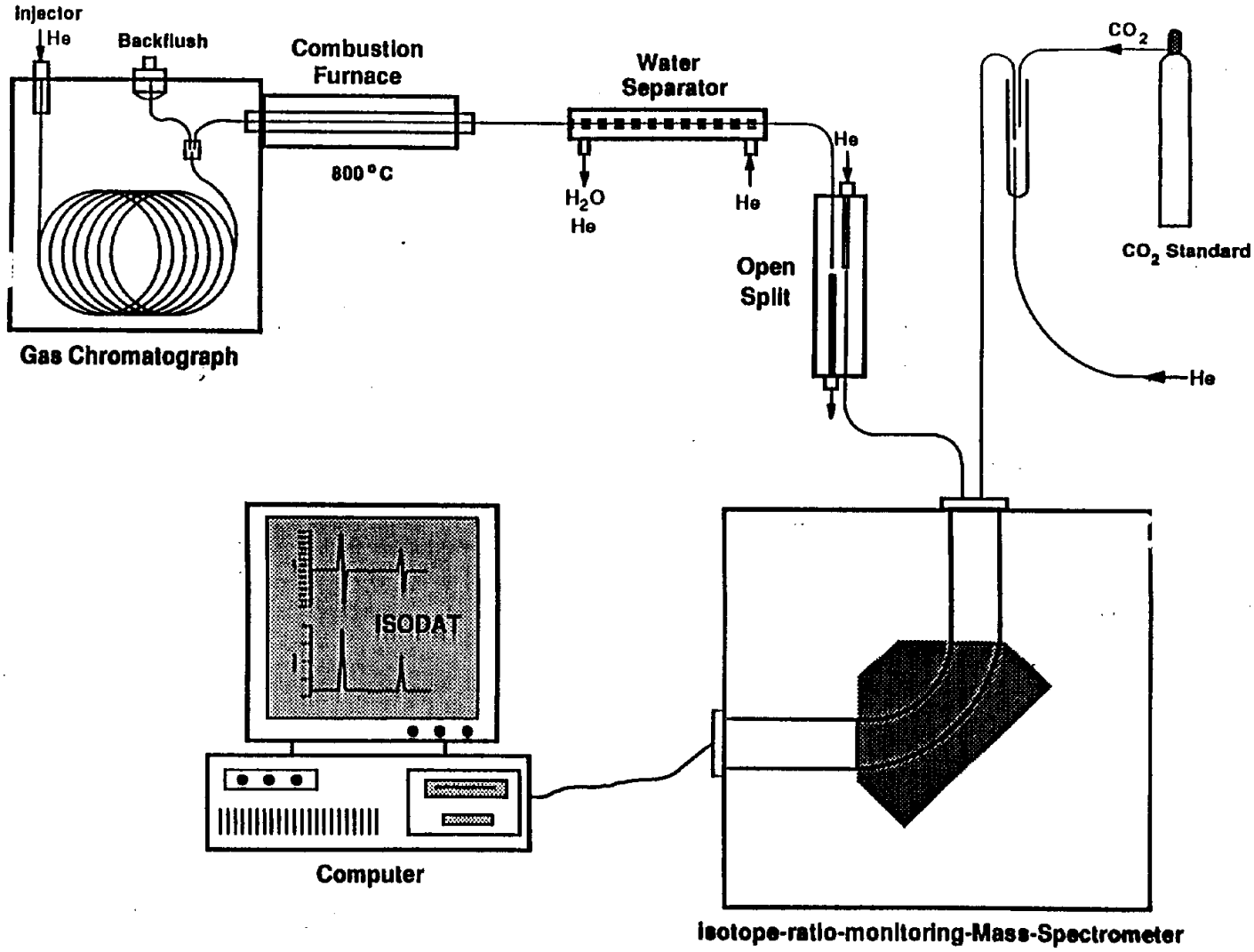
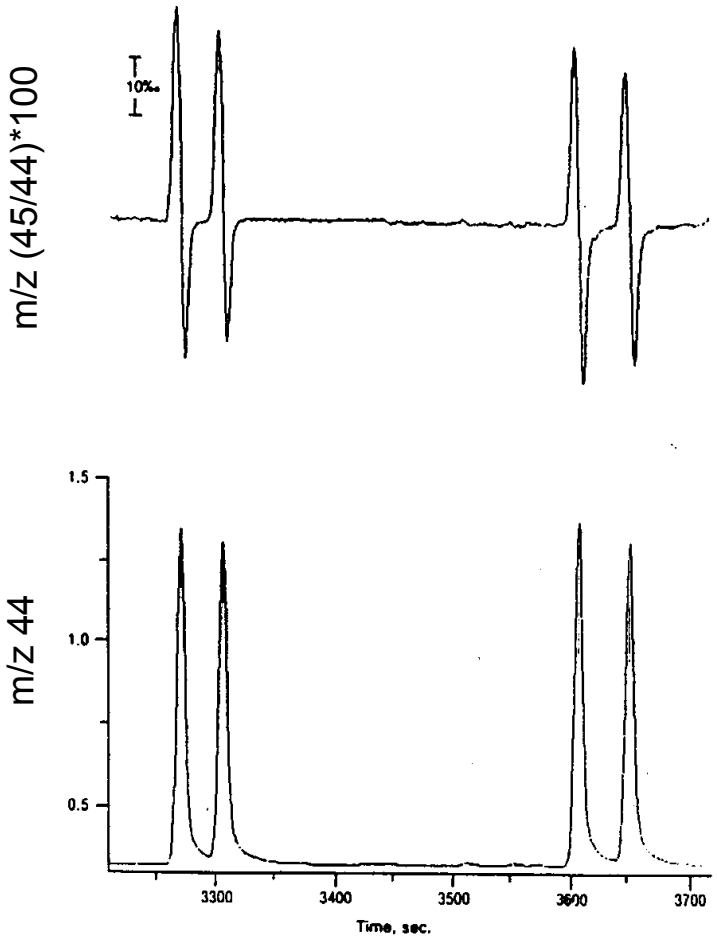


FIGURE 1. Diagram of a generic isotope ratio mass spectrometer, not including inlets. A tight electron impact ion source efficiently generates positive ions that are mass-analyzed by a single magnetic sector. Multiple Faraday cups permit simultaneous and continuous monitoring of the analyte major isotopomer masses in a split flight tube for hydrogen or for other gases. Universal collectors with a central narrow cup and two outer wider cups are used for capturing higher mass gases such as CO_2 and N_2.

GC-irMS or irmGC/MS

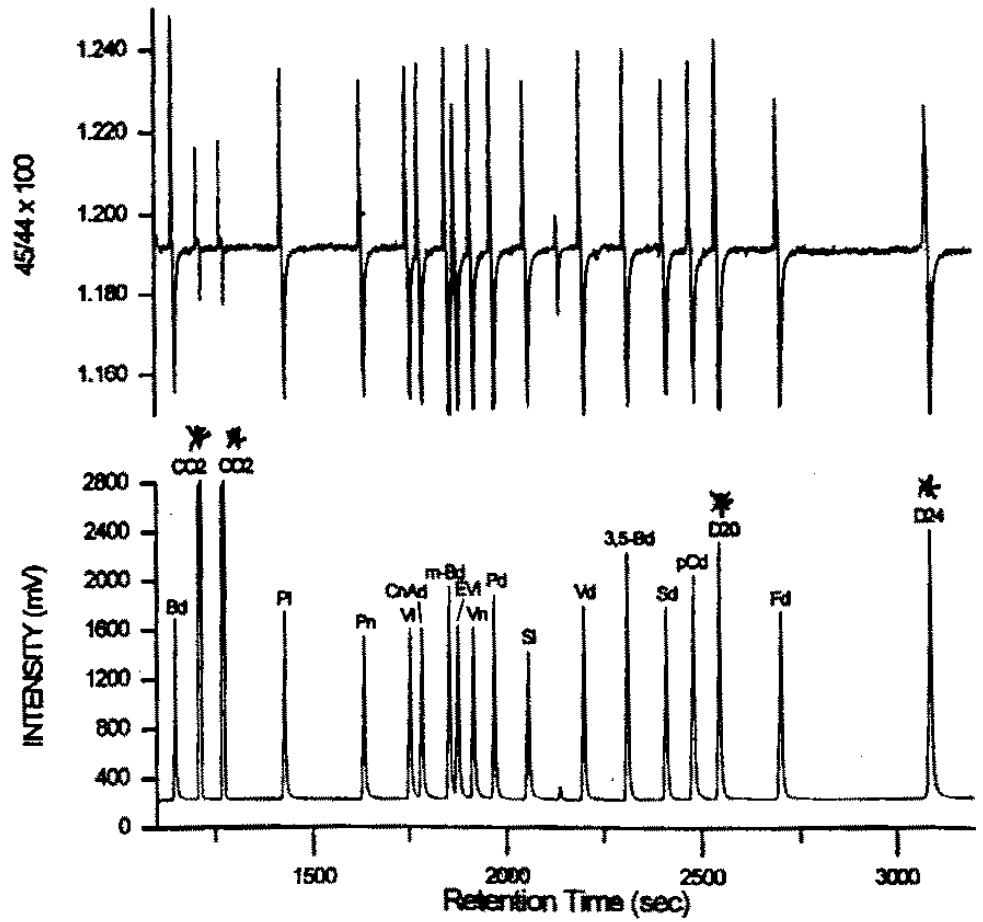


GC-irMS or irmGC/MS



ɔn of a chromatogram generated by irmGCMS: peaks represent CO_2 produced by the f *n*-heptadecane, pristane, *n*-octadecane and phytane. The lower trace shows a recording of ɔn current as a function of time and the upper trace depicts the instantaneous ratio of the mass-45 to mass-44 ion currents.

GC-irMS or irmGC/MS



* CO_2 standard pulses

* internal stds of known isotopic composition

Fig. 1. Partial *m/z* 44 and 45/44 chromatogram from irm–GC–MS of phenolic standards. Compound codes are as listed in Table 1. In addition, CnAd, *trans*-cinnamic acid (recovery standard); EVl, ethylvanillin (recovery standard); D20, perdeuterated C_{20} *n*-alkane (isotope standard); D24, perdeuterated C_{24} *n*-alkane (isotope standard); CO2, CO_2 standards.

Accelerator Mass Spectrometry

- Direct measurement of the proportion of ^{14}C atoms (relative to ^{13}C or ^{12}C) by accelerator mass spectrometry (AMS)
- Measurements are typically made on graphite (sometimes CO_2).
- Graphite formed by combustion of sample to CO_2 and reduction to graphite.
- Cs sputter source (SIMS) generates C^- ions (^{14}N does not make negative ions)
- Accelerator removes isobaric interferences (e.g. hydrides such as $^{13}CH^-$) by electron stripping.
- Sample size requirements: now as low as 25 μg C and measurement times as short as 20 min.