CHEMISTRY 5570

Advanced Analytical Chemistry

Lecture 21

Mass Spectrometry

Combining MS with Chromatography Techniques

GC-MS

Analytes from GC column are fed into the MS ion source where the molecules are ionized.

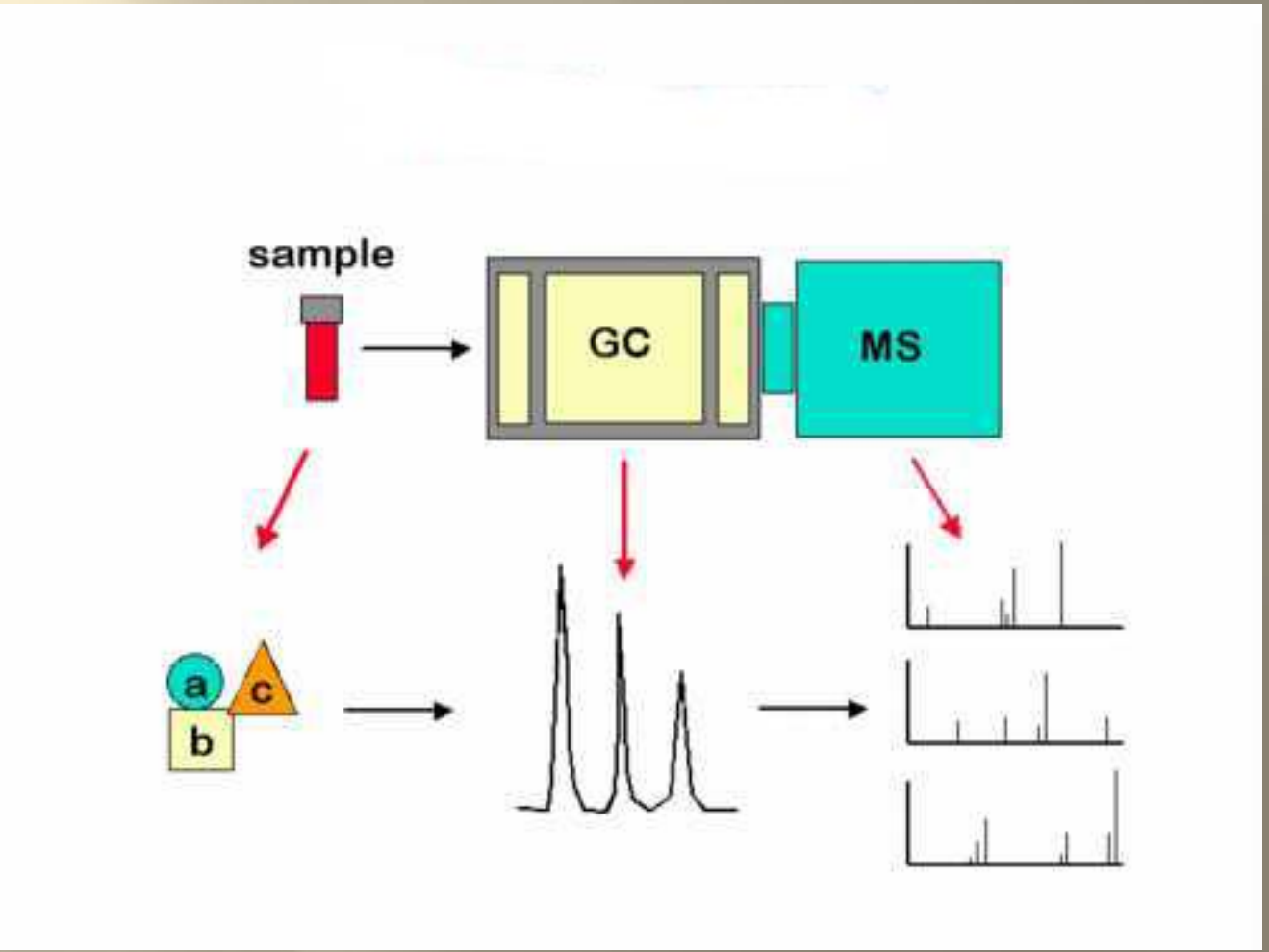
The molecular ions break apart or "fragment".

These fragments are separated according to their mass-to-charge ratio (m/z) and the intensity (ion current) of each type of ion is recorded.

Plot total ion current versus m/z – mass spectrum.

Mass Spectrometry

GC-MS



Mass Spectrometry (MS)

Liquid Chromatography / Mass spectrometry

Much more difficult, since the analyte is in a liquid mobile phase not a gas mobile phase.

Most analytes separated by HPLC are thermally stable and non-volatile (liquids) (unlike in GC) – so not ionized easily by EI or CI techniques.

MS must be at 10^{-6} torr

Mass Spectrometry (MS)

Liquid Chromatography / Mass spectrometry

Much more difficult, since the analyte is in a liquid mobile phase not a gas mobile phase.

However there are advantages:

Advantages:

More definitive identifications

Wide range of analytes can be studied

Sensitivity (pg)

Mass Spectrometry

LC-MS

Ideal Interface:

- **Has no reduction in chromatographic performance**
- **No chemical modifications**
- **High sample transfer**
- **Reliable and reproducible**

Mass Spectrometry

LC-MS

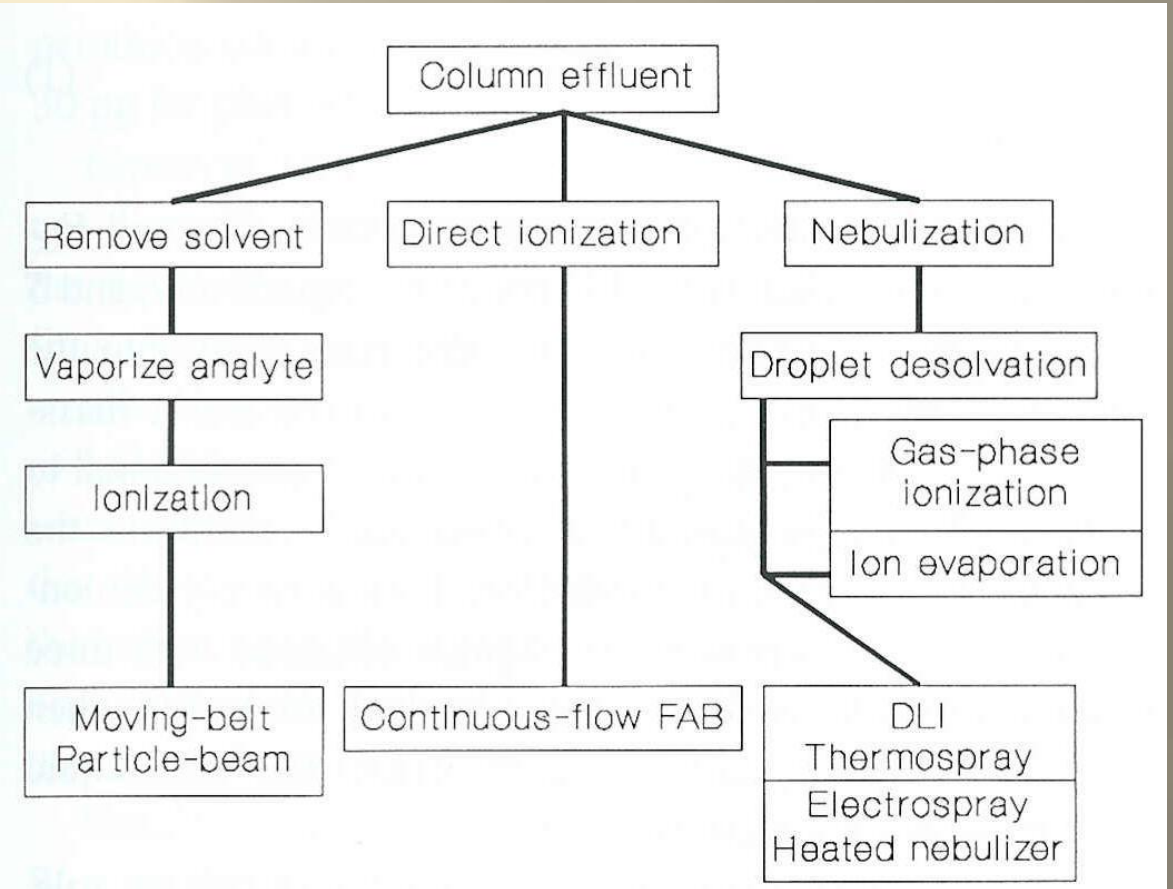


Fig. 1.5. Three general strategies to LC-MS interfacing.

Mass Spectrometry

Ion source

- Moving-belt 1977
- Direct-liquid-introduction 1980
- Thermospray 1983
- Frit FAB/continuous-flow FAB 1985/1986
- Atmospheric-pressure chemical ionization 1986
- Particle-beam 1988
- Electrospray 1988

Mass Spectrometry

Thermospray 1983

The thermospray setup overcame many of the problems encountered with the moving-belt and direct-liquid-introduction interfaces and with the advent of this, LC–MS became a routine analytical tool in a large number of laboratories.

It was also the first interface in which ionization is effected directly from solution within the interface itself, i.e. the mass spectrometer was not used to produce ions from the analyte simply to separate them according to their m/z ratios.

Mass Spectrometry

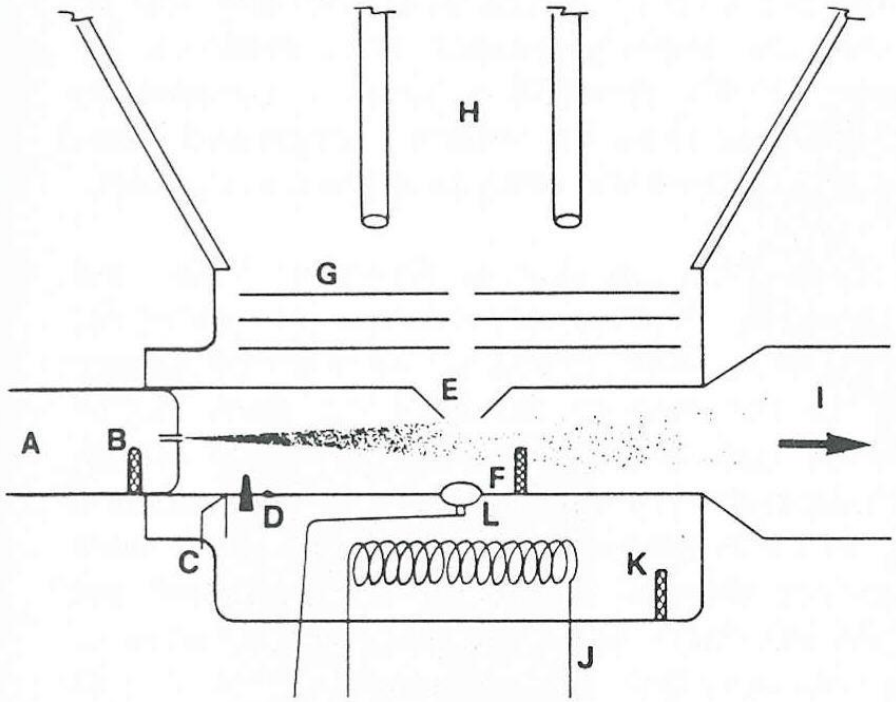
Thermospray interface

Ionization involving this interface comprises the following four stages:

- **the formation of droplets from the HPLC eluate**
- **charging of these droplets**
- **desolvation of the droplets**
- **the formation of ions from the analyte**

Mass Spectrometry

Thermospray interface



Schematic of Thermospray Interface

A Direct heated vaporizer
B Vaporizer thermocouple
C Filament*
D Discharge electrode*
E Ion exit cone
F Aerosol thermocouple
G Lenses
H Quadrupole assembly
I Liquid nitrogen trap and forepump
J Source block heater
K Source thermocouple
L Repeller

*Dependent on model and manufacture

Figure 4. Schematic of a thermospray LC/MS interface. [From reference 11]

Mass Spectrometry

Thermospray interface

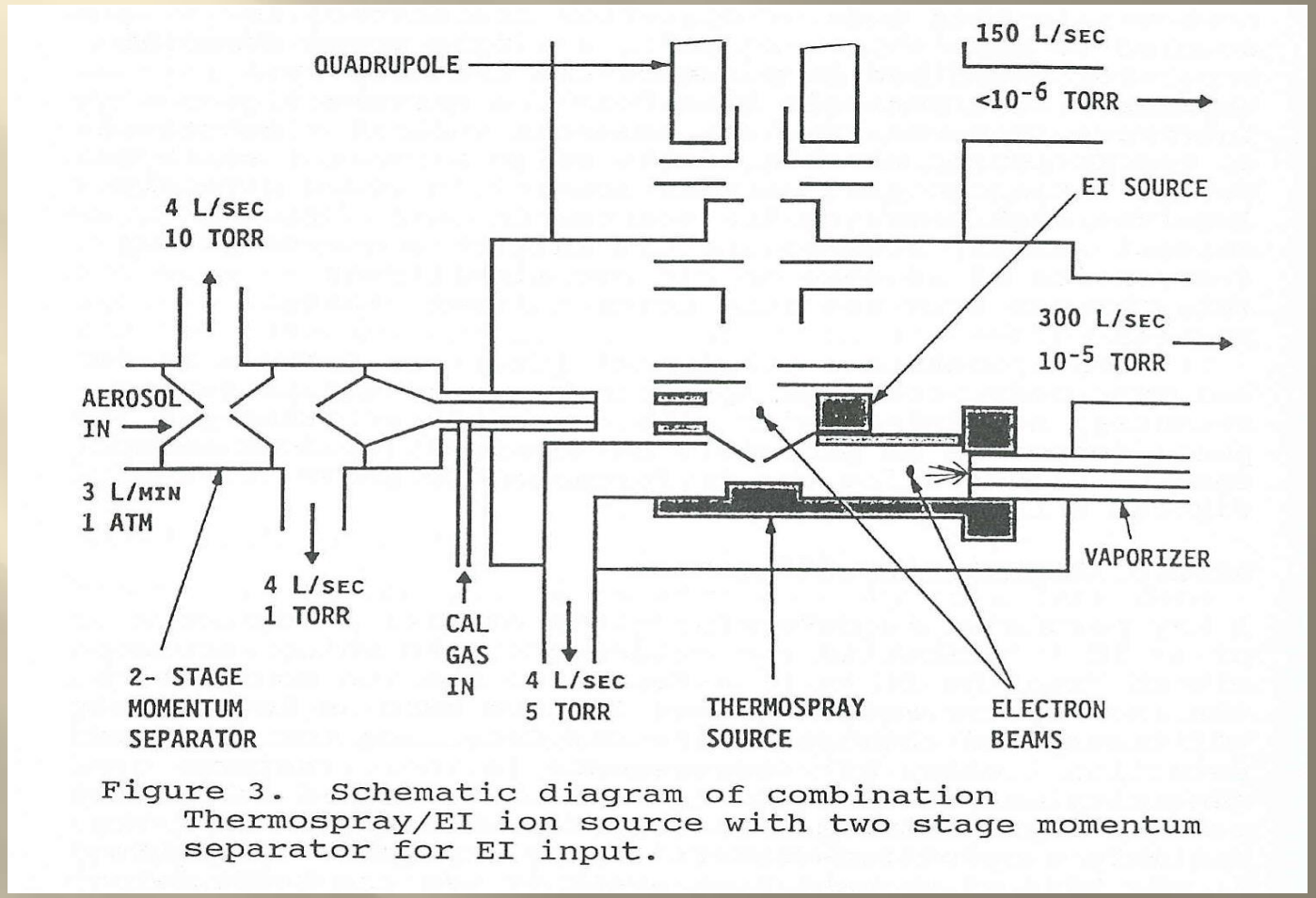


Figure 3. Schematic diagram of combination Thermospray/EI ion source with two stage momentum separator for EI input.

Mass Spectrometry

Thermospray interface

A thermospray system consists of

- a heated capillary through which the LC elutant flows, the temperature of this capillary is controlled to bring about 95% vaporization of the liquid.

-the vapour produced acts as a nebulizing 'gas' and aids the break-up of the liquid stream into droplets.

-the droplets undergo desolvation as they traverse a heated region of the interface and ions are formed from analytes contained in the liquid stream by means of ion–molecule reactions.

-the ions formed are then directed though a sampling cone at 90° to the direction of vapour flow.

-downstream of the end of the capillary are usually a filament and/or a discharge electrode, which provids secondary methods of ionization, while opposite or slightly downstream of the sampling cone is a repeller or retarding electrode.

Mass Spectrometry

Thermospray – Disadvantages

- The presence of a buffer is usually needed so must be present in the HPLC mobile phase.
- Decomposition of some thermally labile analytes occurs.
- Thermospray is not suitable for high-molecular-weight (>1000 Da) analytes.
- The reproducibility of analytical results is affected by a number of experimental parameters and is sometimes difficult to control.
- The formation of adducts may confuse the assignment of molecular weight.
- Usually little structural information is immediately available and repeller induced fragmentation or MS–MS is required.
- Thermospray generates a significant amount of solvent-associated chemical noise at low mass which makes this region unusable for analytical purposes. Since the ions generated from the analyte are molecular species, this is not usually a problem but must be considered when spectra are being interpreted.

Mass Spectrometry

Thermospray – Advantages

- Is easier to use than other available alternatives.
- Can operate under a wide range of HPLC conditions, regular HPLC flow rates and mobile phases containing high percentages of water.
- Can allow unequivocal determination of molecular weight as thermospray spectra usually contain ions simply from molecular species with little fragmentation being observed.
- The addition of a discharge electrode and a filament to the thermospray source widens the range of compounds that may be studied and HPLC solvents that may be accommodated.
- Since the sample is ionized directly from solution it is protected from heat and many thermally labile analytes may be studied with little or no degradation.
- The sensitivity is compound-dependent but generally high sensitivity is possible by using one of the ionization methods available (thermospray, filament and discharge).

Mass Spectrometry

Ion source

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- Direct-liquid-introduction 1980
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- Atmospheric-pressure chemical ionization 1986
- Particle-beam 1988
- Electrospray 1988

Mass Spectrometry

Atmospheric-pressure chemical ionization 1986

Although work demonstrating APCI was published in parallel with that demonstrating ESI, APCI was not widely adopted until ESI was commercialized, which occurred in the wake of Fenn's work.

Atmospheric-pressure chemical ionization (APCI) is another of the techniques in which the stream of liquid is dispersed into small droplets, in this case by the combination of heat and a nebulizing gas.

As such, APCI shares many common features with thermospray.

The differences between the techniques are the methods used for droplet generation and the mechanism of subsequent ion formation.

Mass Spectrometry

Atmospheric-pressure chemical ionization (APCI)

1st generation

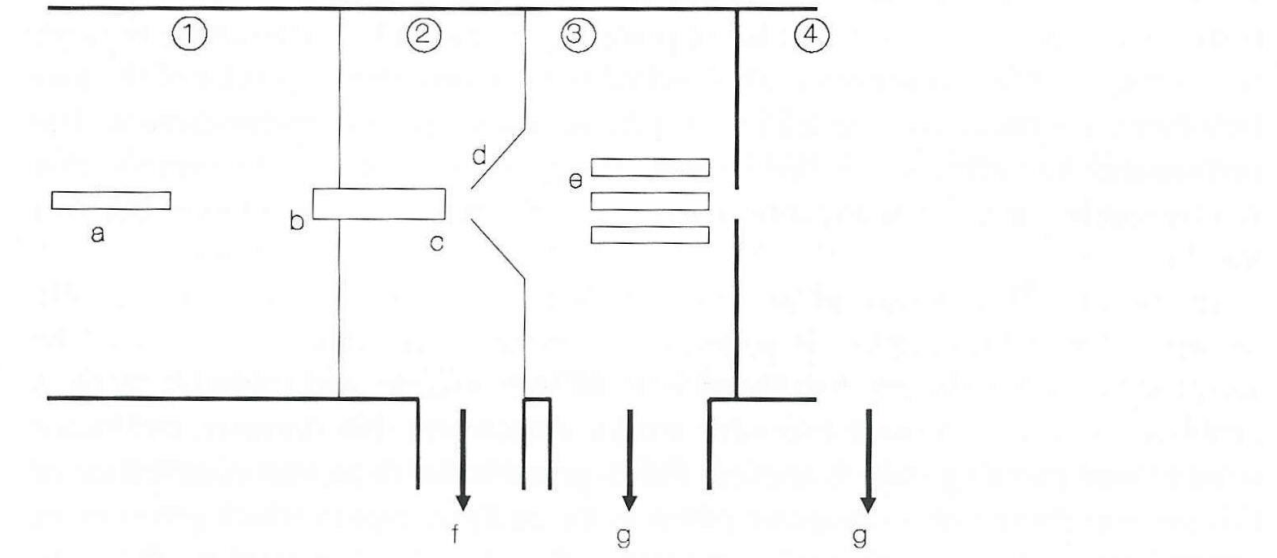


Fig. 1.15. Schematic drawing of the atmospheric-pressure ion source as a molecular beam apparatus. (1) Atmospheric-pressure ionization source region, (2) first pumping stage, (3) second pumping region, and (4) mass analyzer region. (a) Transfer tube from LC (either electrospray needle assembly or APCI heated nebulizer), (b) sampling orifice, (c) nozzle, (d) skimmer, (e) quadrupole, hexapole, or octapole ion collection and focusing device, (f) high through-put mechanical pump, and (g) turbomolecular pumps.

Mass Spectrometry

APCI

- The liquid is sprayed through a heated (250-400 ^{o}C) vaporizer at atmospheric pressure.
- The heat vaporizes the liquid, and the solvent molecules are ionized by electrons discharged from a corona needle.
- The solvent ions transfer charge to the analyte molecules through chemical reactions (chemical ionization).
- The analyte ions then pass through a capillary sampling orifice into the mass analyzer.

Mass Spectrometry

APCI

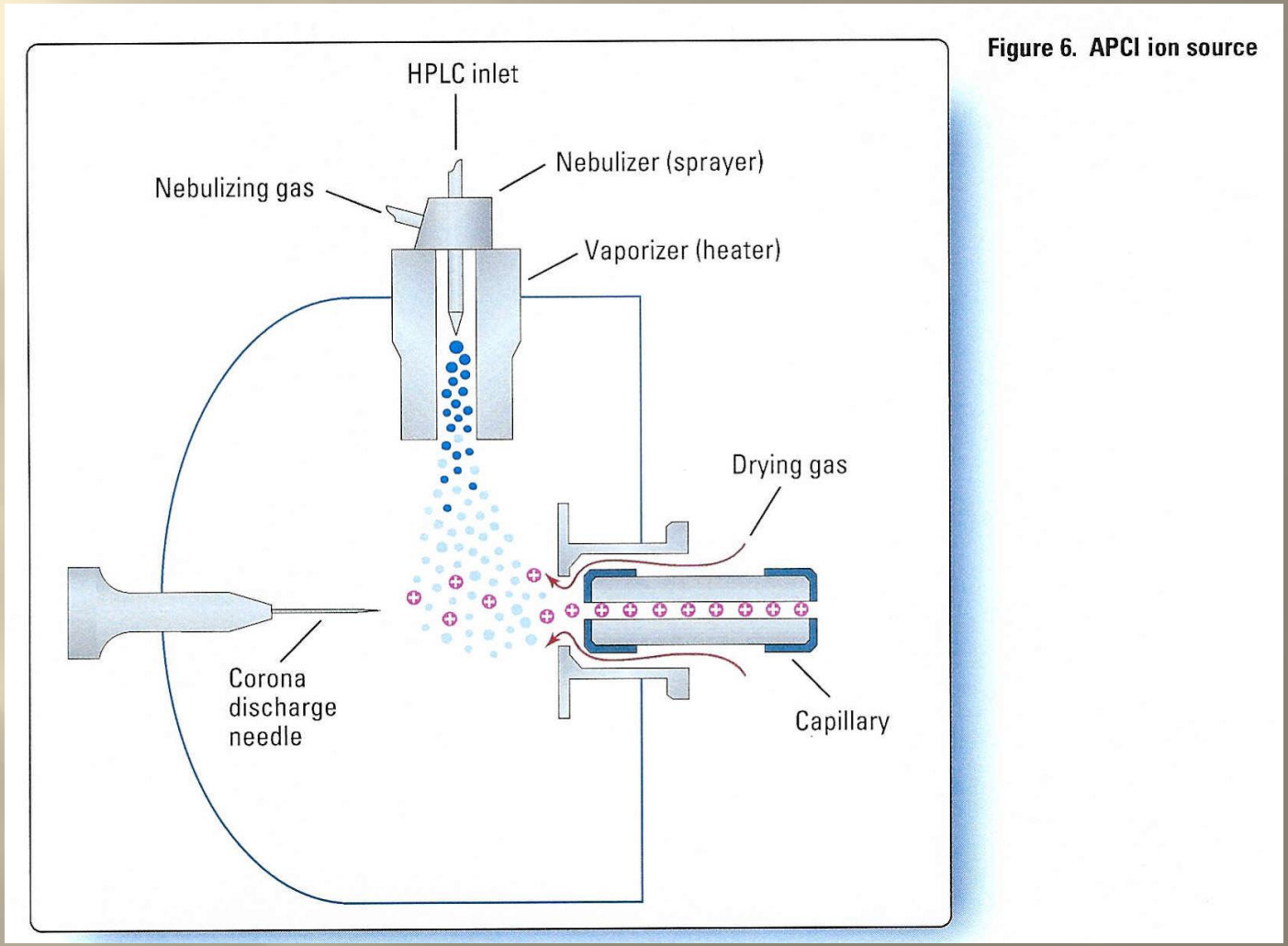


Figure 6. APCI ion source

Mass Spectrometry

APCI - Disadvantages

- APCI spectra can contain ions from adducts of the analyte with the HPLC mobile phase or organic modifiers, such as ammonium acetate, that may be present. The presence of ions such as (M + NH4)+ and (M + CH3COO)− may hinder interpretation of the spectra obtained.
- Structural information is not usually available unless cone-voltage fragmentation or MS–MS is used.
- APCI is not able to function effectively at very low flow rates.
- APCI is not suitable for analytes that are charged in solution.

Mass Spectrometry

APCI – Advantages

- **APCI produces ions from solution and compounds with a degree of thermal instability may be studied without their decomposition.**
- **APCI is best applied to compounds with low to moderately high polarities.**
- **APCI is a soft ionization technique which usually enables the molecular weight of the analyte under study to be determined.**
- **APCI is able to deal with flow rates up to 2 $mlmin^{-1}$ and is, consequently, directly compatible with 4.6 mm HPLC columns.**
- **APCI is more tolerant to the presence of buffers in the mobile phase stream than is ESI.**
- **APCI is more tolerant to changes in experimental conditions than ESI, including gradient elution.**

Mass Spectrometry

Atmospheric-pressure Photoionization Interface (APPI)

Atmospheric-pressure photoionization (APPI) is a relatively new technique. Just as in APCI, a vaporizer converts the LC eluent to the gas phase.

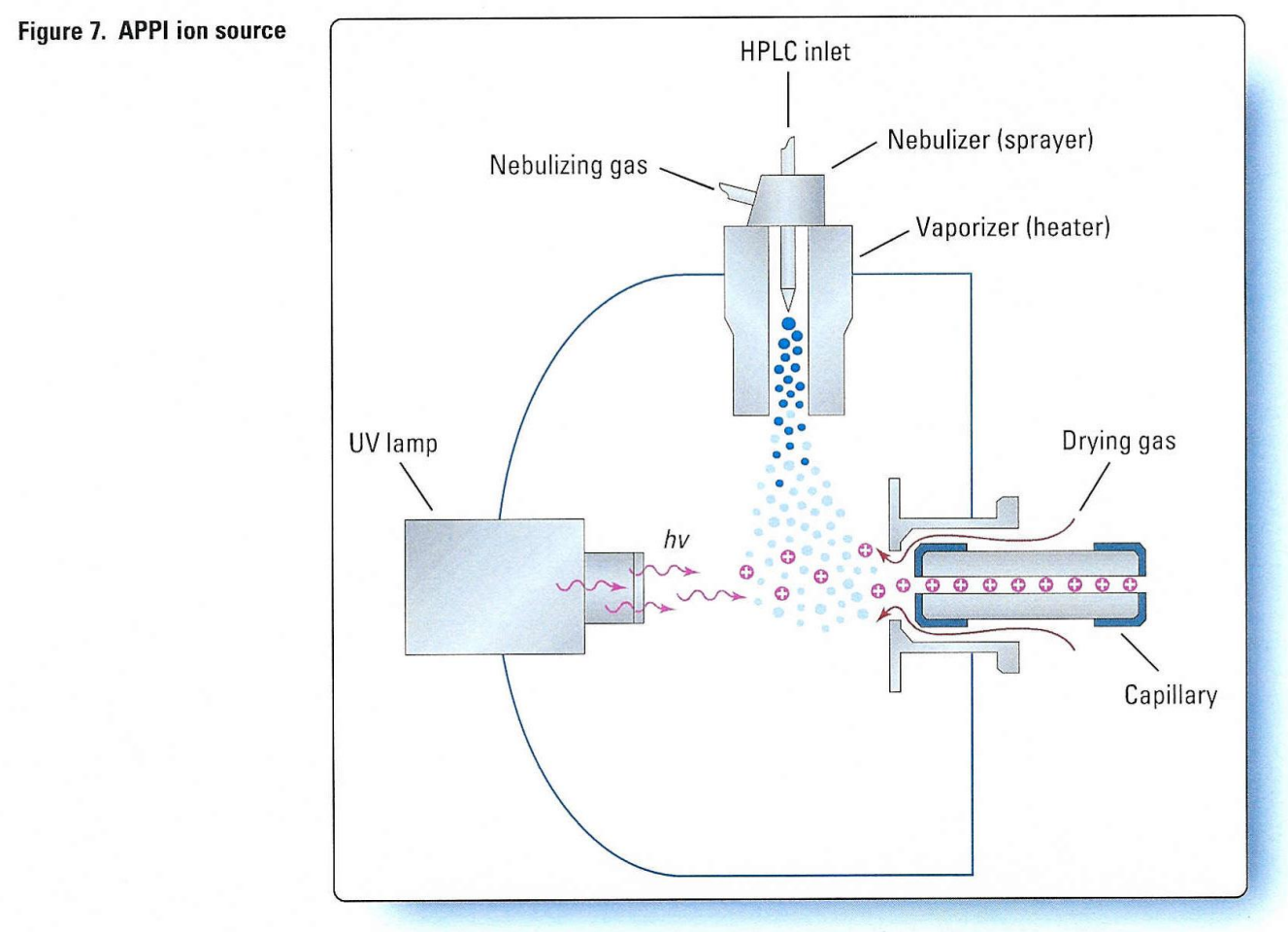
A discharge lamp generates photons in a narrow range of ionization energies. The range of energies is chosen to ionize analyte molecules while minimizing ionization of solvent molecules.

The resulting ions pass through a capillary sampling orifice into the mass analyzer.

Atmospheric-pressure photoionization is applicable to the same compounds as APCI, however it responds better to highly nonpolar compounds and low flow rates (<100 uL/min).

Mass Spectrometry

Atmospheric-pressure Photoionization Interface (APPI)



Mass Spectrometry

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- Electrospray 1988

Mass Spectrometry

Electrospray 1988

High performance liquid chromatography is an effective technique for the separation of compounds of high molecular weight. However, two major problems for the study of this type of molecule have severely limited the application of LC–MS. Specifically,:

The inability to ionize, in an intact state, many of the labile and/or involatile molecules involved.

Should ionization be possible, the lack of appropriate hardware to allow the mass analysis and efficient detection of the ions of high m/z ratio involved.

Electrospray is an ionization method that overcomes these problems.

Mass Spectrometry

Electrospray 1988

A liquid, in which the analyte(s) of interest have been dissolved, is passed through a capillary (typically stainless steel), at atmospheric pressure, maintained at high voltage (3 to 4 kV).

The liquid stream breaks up with the formation of highly charged droplets which are desolvated as they pass through the atmospheric-pressure region of the source towards a counter electrode.

Mass Spectrometry

Electrospray interface – 1st generation

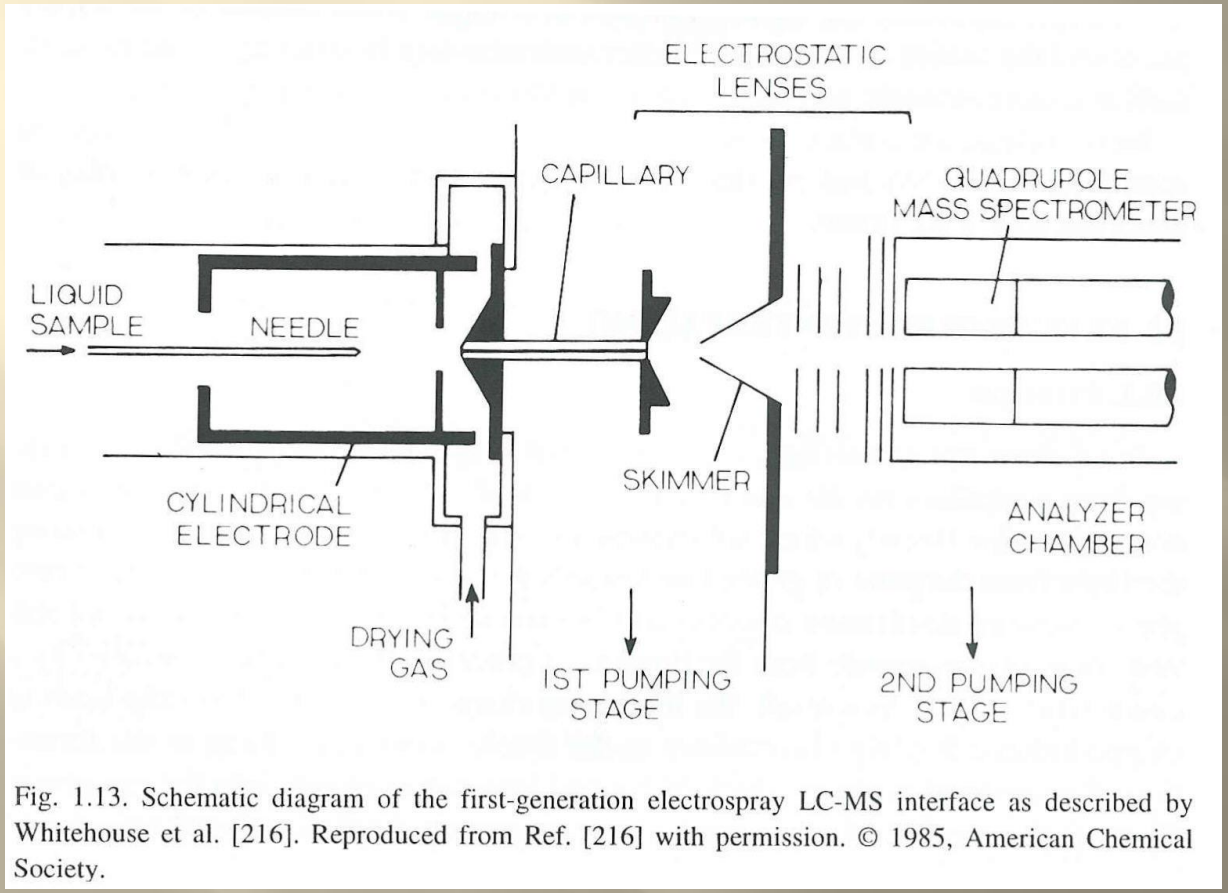
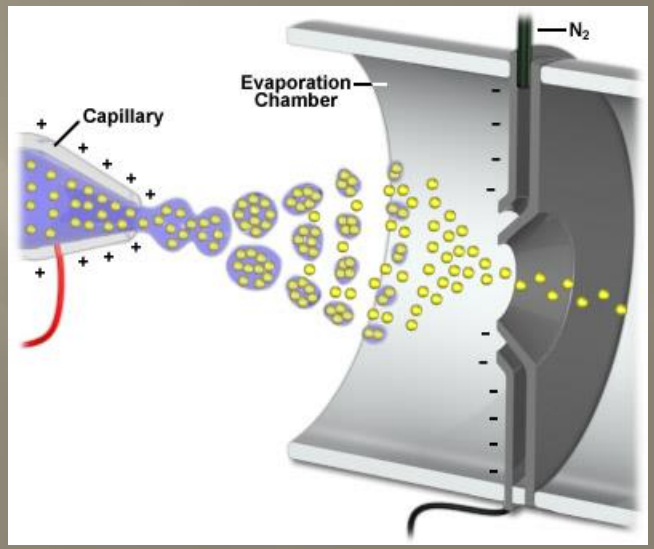
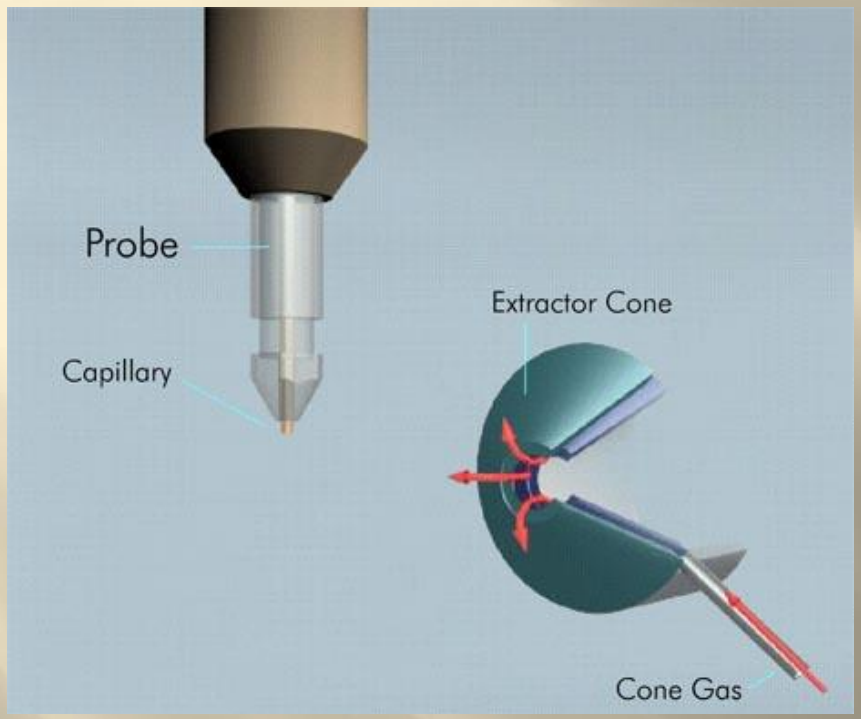


Fig. 1.13. Schematic diagram of the first-generation electrospray LC-MS interface as described by Whitehouse et al. [216]. Reproduced from Ref. [216] with permission. © 1985, American Chemical Society.



Mass Spectrometry

Electrospray interface - orthogonal



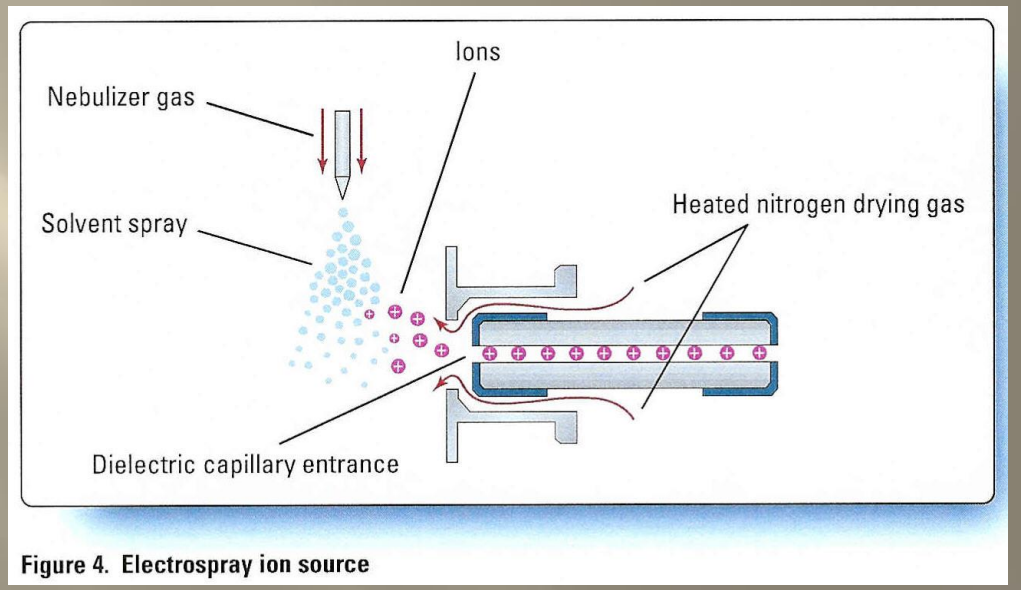


Figure 4. Electrospray ion source

Mass Spectrometry

Electrospray interface

Desolvation is assisted by a stream of a drying gas, usually nitrogen, being continually passed into the spraying region.

As the droplets shrink, the charge concentration in the droplets increases. The repulsive force between ions with like charges exceeds the cohesive forces and ions are ejected (desorbed) into the gas phase.

Analyte ions are obtained from these droplets which then pass through two differentially pumped regions into the source of the mass spectrometer.

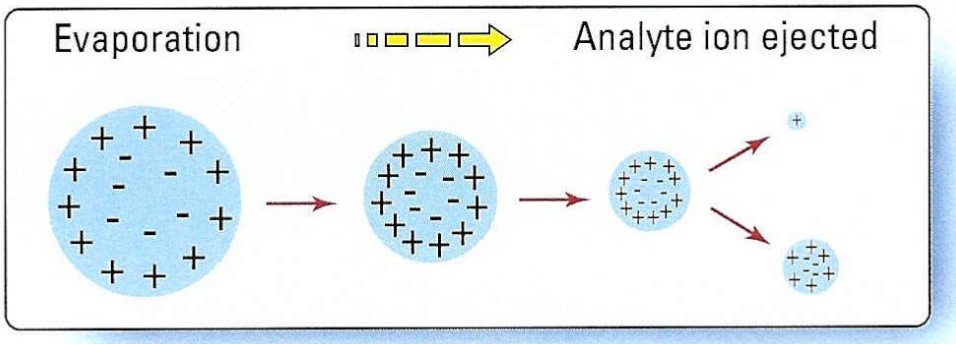


Figure 5. Desorption of ions from solution

Mass Spectrometry

Electrospray

The mechanism by which potential transfers from the liquid to the analyte, creating ions, remains a topic of controversy.

In 1968, Malcolm Dole first proposed the charge residue mechanism in which he hypothesized that as a droplet evaporates, its charge remains unchanged.

The droplet's surface tension, ultimately unable to oppose the repulsive forces from the imposed charge, explodes into many smaller droplets.

These Coulombic fissions occur until droplets containing a single analyte ion remain. When the solvent evaporates from the last droplet, a gas-phase ion forms.

Mass Spectrometry

Electrospray

In 1976, Iribarne and Thomson proposed a different model, the ion evaporation mechanism, in which small droplets form by Coulombic fission, similar to Dole's model.

However, according to ion evaporation theory, the electric field strength at the surface of the droplet is high enough to make leaving the droplet surface and transferring directly into the gas phase energetically favorable for solvated ions.

It is possible that the two mechanisms may actually work in concert: the charge residue mechanism dominant for masses higher than 3000 Da while ion evaporation dominant for lower masses.

Mass Spectrometry

Electrospray

Since ionization takes place directly from solution, thermally labile molecules may be ionized without degradation.

In contrast to most other ionization methods, the majority of ions produced by electrospray are multiply charged.

Electrospray is useful for large biomolecules such as proteins, peptides, etc… while still able to analyze smaller molecules.

Mass Spectrometry

Electrospray

In positive ionization mode:

a trace of formic acid is often added to aid protonation of the sample molecules.

In negative ionization mode:

a trace of ammonia solution or a volatile amine is added to aid deprotonation of the sample molecules.

Mass Spectrometry

Electrospray

Proteins and peptides are usually analyzed under positive ionization conditions.

Saccharides and oligonucleotides are usually analyzed under negative ionization conditions.

In all cases, the m/z scale must be calibrated by analyzing a standard sample of a similar type to the sample being analyzed (e.g. a protein calibrant for a protein sample), and then applying a mass correction.

Mass Spectrometry

Electrospray interface

In ESI, samples (M) with molecular masses up to ~1200 Da give rise to singly charged molecular-related ions.

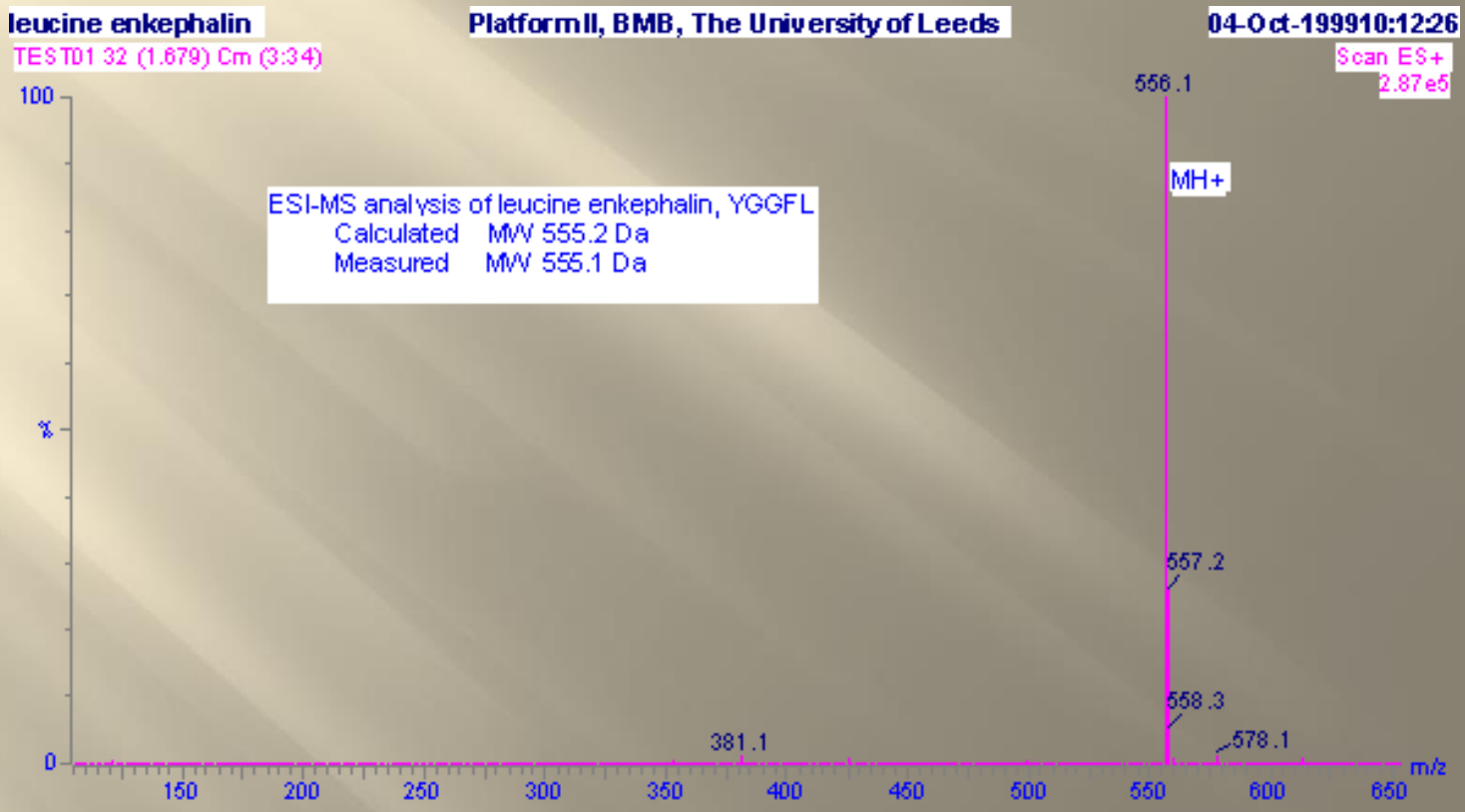
Protonated molecular ions of the formula (M+H)+ in positive ionization mode.

Deprotonated molecular ions of the formula (M-H)- in negative ionization mode.

Mass Spectrometry

Example of this type of sample analysis is shown in the m/z spectrum of the pentapeptide leucine enkephalin, YGGFL.

The molecular formula is $C_{28}H_{37}N_5O_7$ and the calculated molecular weight is 555.2692 Da.



The m/z spectrum shows dominant ions at m/z 556.1, which are consistent with the expected protonated molecular ions, (M+H+) under positive ionization conditions.

Mass Spectrometry

Electrospray interface

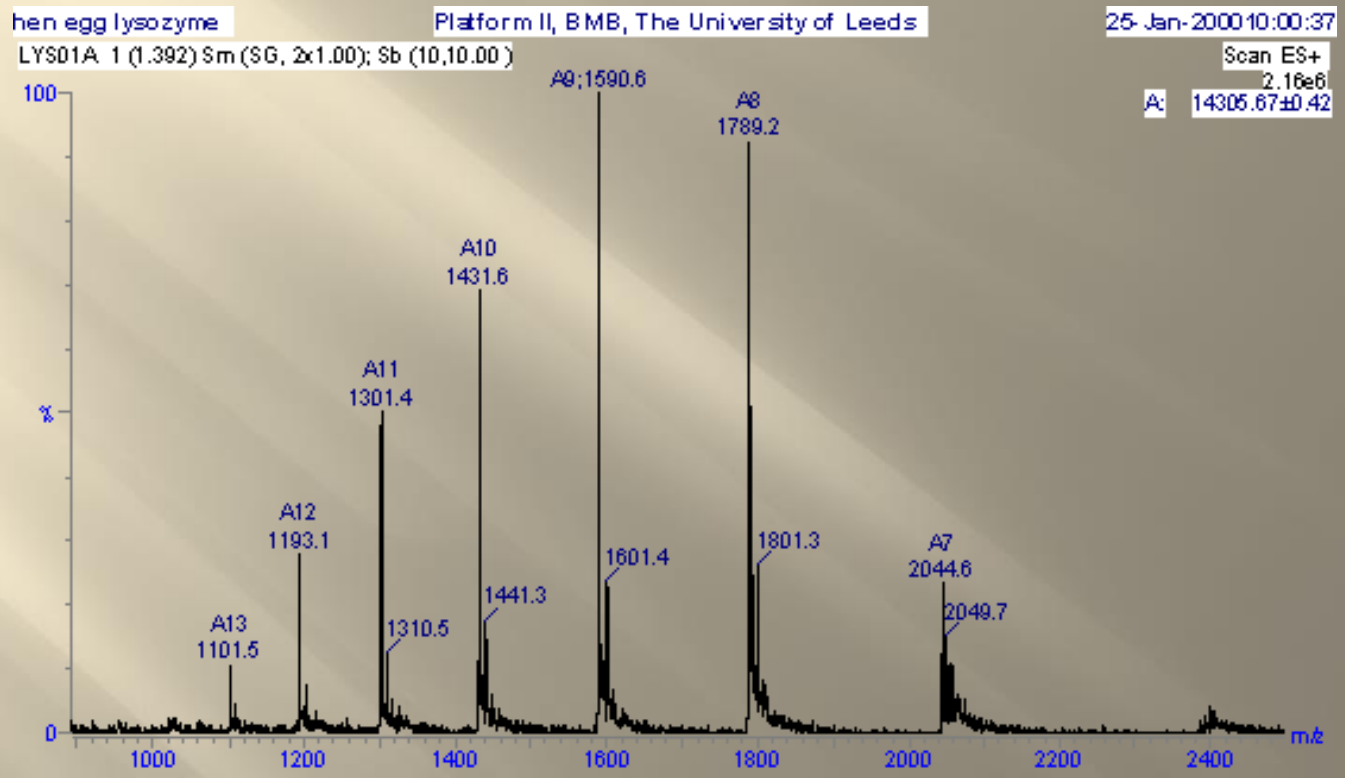
Samples (M) with molecular weights greater than ~1200 Da give rise to multiply charged molecular-related ions.

(M+nH)n+ in positive ionization mode

(M-nH)n- in negative ionization mode

Mass Spectrometry

Example is presented in the positive ionization m/z spectrum of the protein hen egg white lysozyme.



The m/z values can be expressed as follows: $m/z = (MW + nH+)/n$

where: m/z = the mass-to-charge ratio MW = the molecular mass of the sample

n = the integer number of charges on the ions H = the mass of a proton = 1.008 Da

Mass Spectrometry

Electrospray – Disadvantages

Electrospray is not applicable to non-polar or low-polarity compounds.

The mass spectrum produced from an analyte depends upon a number of factors and spectra obtained using different experimental conditions may therefore differ considerably in appearance.

Suppression effects may be observed and the direct analysis of mixtures is not always possible. This has potential implications for co-eluting analytes in LC–MS.

Electrospray is a soft-ionization method producing intact molecular species and structural information is not usually available.

Electrospray sources are capable of producing structural information from cone-voltage fragmentation but these spectra are not always easily interpretable. Experimentally, the best solution is to use a mass spectrometer capable of MS–MS operation but this has financial implications.

Mass Spectrometry

Electrospray – Advantages

Ionization occurs directly from solution and consequently allows ionic and thermally labile compounds to be studied.

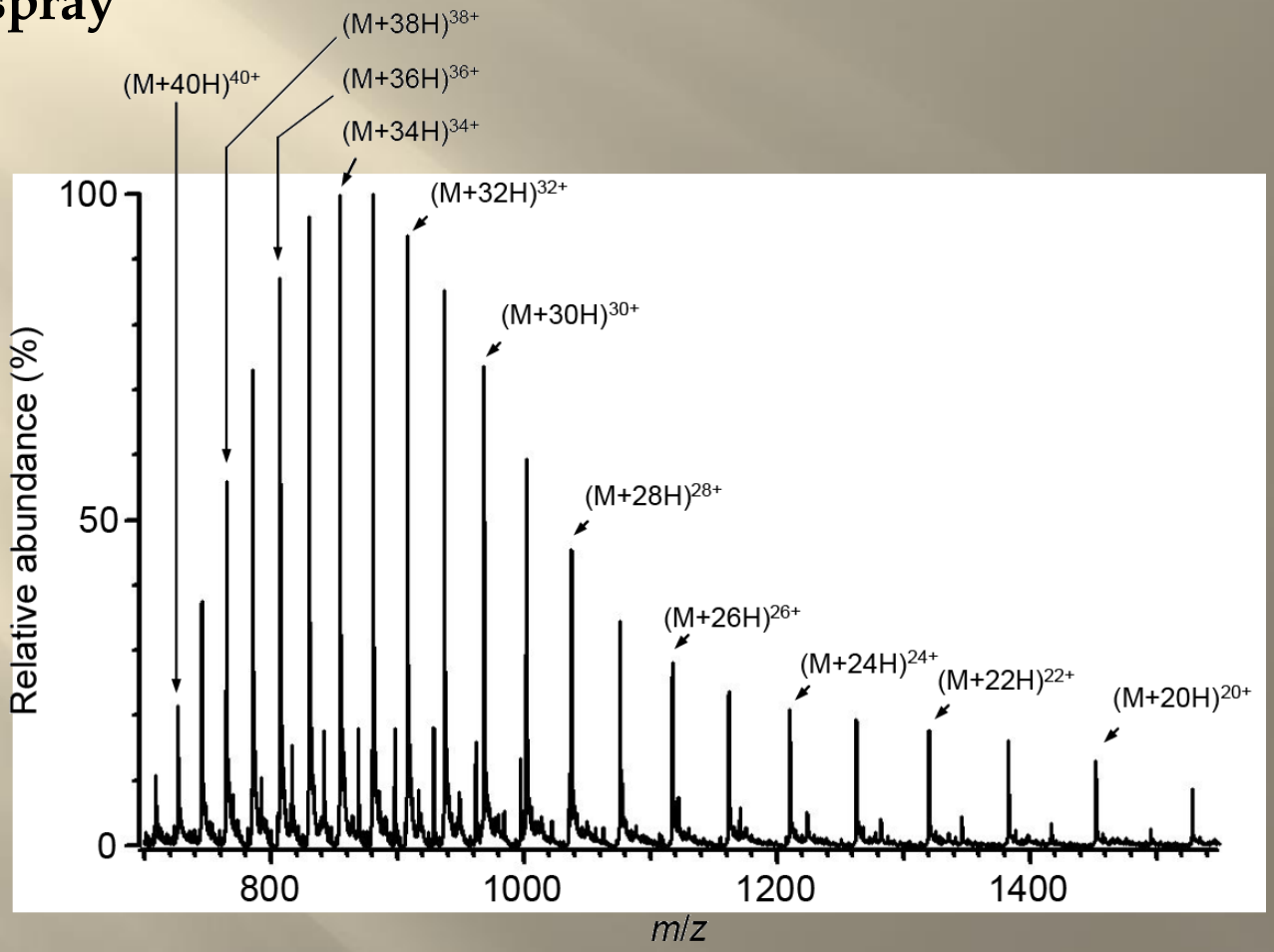
Mobile phase flow rates from nl min^{-1} to in excess of 1 ml min^{-1} can be used with appropriate hardware, thus allowing conventional and microbore columns to be employed.

Electrospray ionization, in contrast to the majority of other ionization methods, produces predominantly multiply charged ions of the intact solute molecule. This effectively extends the mass range of the mass spectrometer and allows the study of molecules with molecular weights well outside its normal range.

For high-molecular-weight materials, an electrospray spectrum provides a number of independent molecular weight determinations from a single spectrum and thus increased precision.

Mass Spectrometry

Electrospray



Mass Spectrometry

Ionization chemistry

For electrospray, formation of analyte ions in solution is essential to achieving good results.

Techniques to help ion formation include:

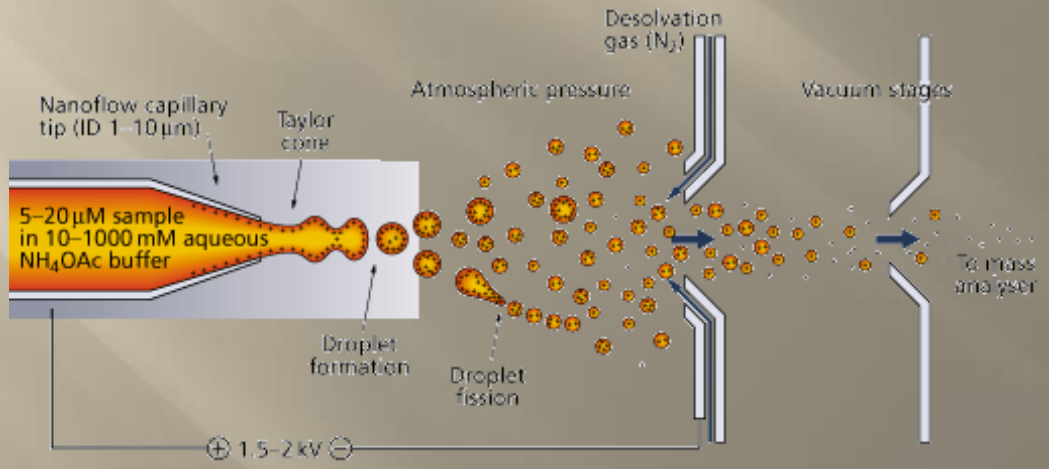
- **select more volatile buffers to reduce the buildup of salts in the ion source**
- **adjust solvent pH according to the polarity of ions desired and the pH of the sample**
- **use solvents that have low heats of vaporization and low surface tensions to enhance ion desorption**
- **make sure that gas-phase reactions do not neutralize ions through proton transfer or ion pair reactions**

Mass Spectrometry

Nanospray ionization - A low flow rate version of electrospray.

- **The flow rate of solute and solvent using this procedure is very low, 30 - 1000 nL/min, and so far less sample is consumed than with the standard electrospray ionization technique.**

- **A common application of this technique is for a protein digest mixture to be analyzed to generate a list of molecular masses for the components present, and then each component to be analyzed further by tandem mass spectrometric (MS-MS) amino acid sequencing techniques.**



Mass Spectrometry

LC-MS

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- **use solvents that have low heats of vaporization and low surface tensions to enhance ion desorption**
- **make sure that gas-phase reactions do not neutralize ions through proton transfer or ion pair reactions**

Mass Spectrometry

LC-MS

Solution chemistry is less critical for APCI because ionization occurs in the gas phase, not the liquid phase.

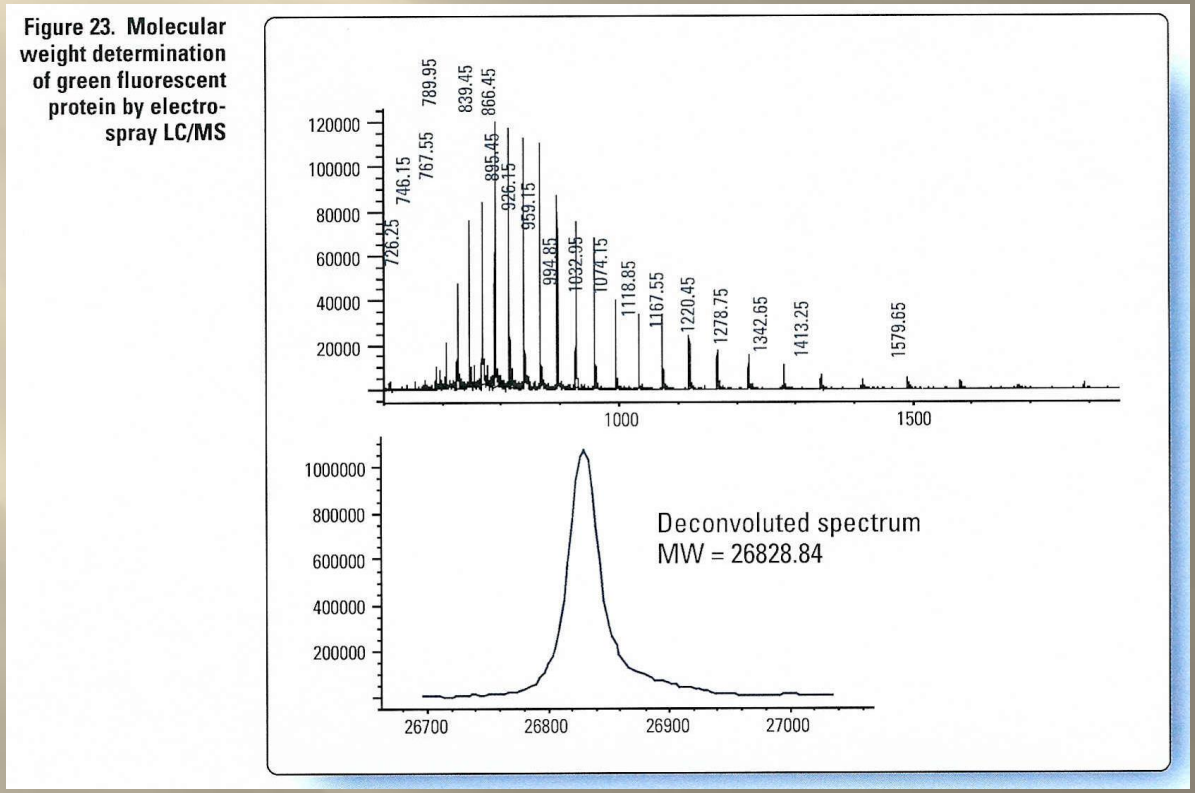
Solvent selection can still have an effect on signal response, so need to:

- ✓ **select more volatile solvents**
- ✓ **select solvents with a lower charge affinity than the analyte**
- ✓ **protic solvents generally work better than nonprotic solvents for positive ion mode**
- ✓ **for negative ionization, solvents that readily capture an electron must be used**

Mass Spectrometry

LC-MS Applications

Molecular Weight Determination
Example - Analysis of a 27,000-Dalton protein with 238 amino acids.



Mass Spectrometry

LC-MS Applications

Pharmaceutical Applications
Information from the MS can be used to determine even compounds that are not separated by LC.

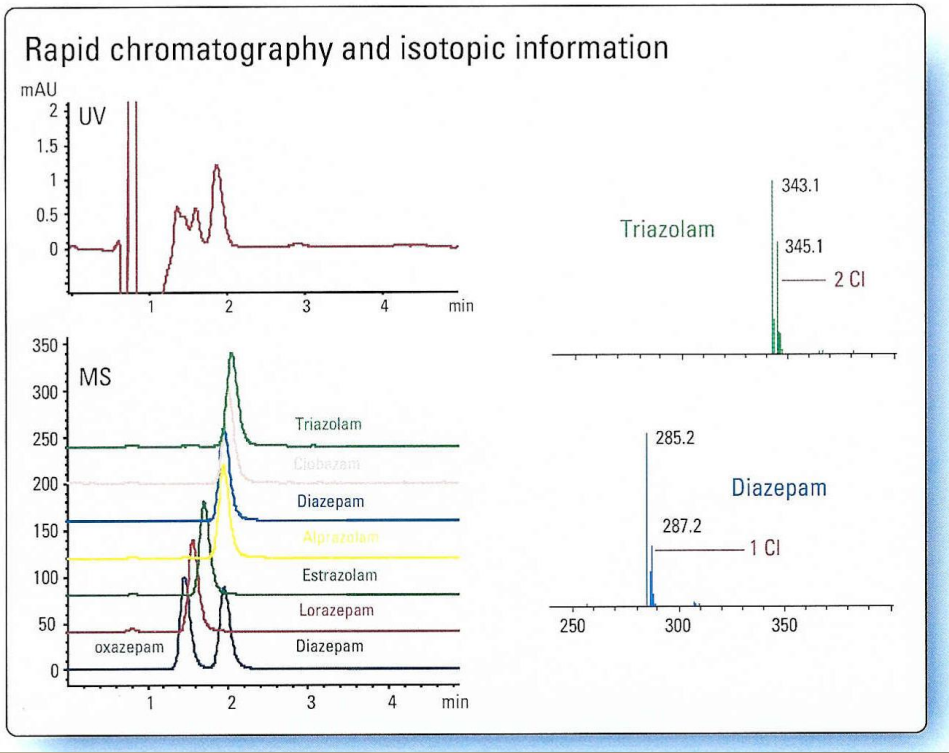


Figure 27. MS identification and quantification of individual benzodiazepines from an incompletely resolved mixture

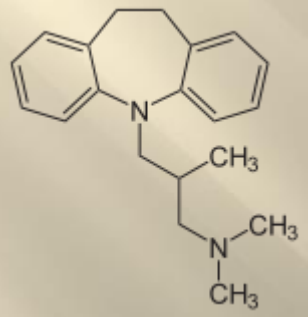
Mass Spectrometry

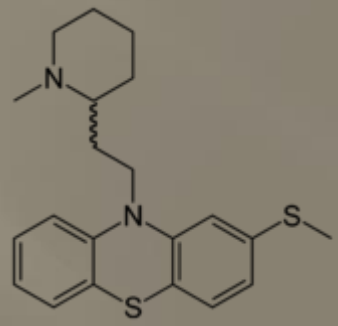
LC-MS Applications

Clinical Applications

Trimipramine (a tricyclic antidepressant) and Thioridazine (a tranquilizer) are at levels in urine that do not typically show up in the LC-UV.

However if the analytes from the LC are transferred to the MS, a single quad has enough sensitivity to detect the compounds.





Mass Spectrometry

LC-MS Applications

Clinical Applications

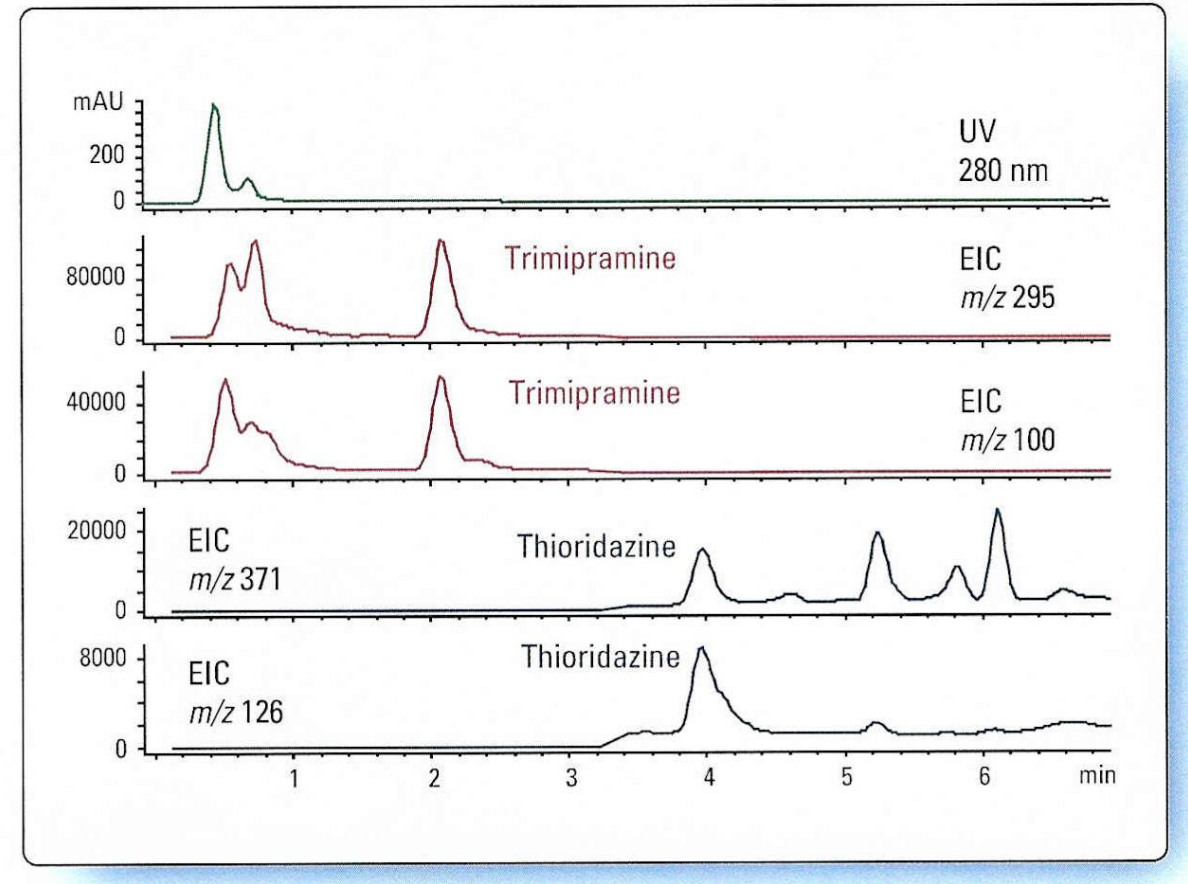


Figure 31. Trimipramine and thioridazine in a urine extract

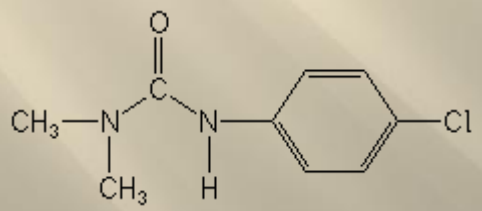
Mass Spectrometry

LC-MS Applications

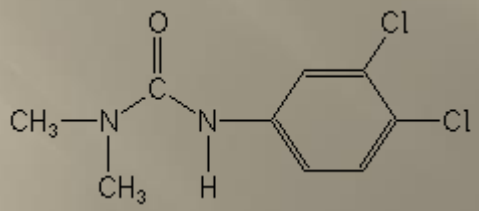
Environmental Applications

Phenylurea herbicides are used for pre- and post-emergence weed control in a wide variety of crops and are widely applied throughout the world. In general, these herbicides have long lifetimes in the environment.

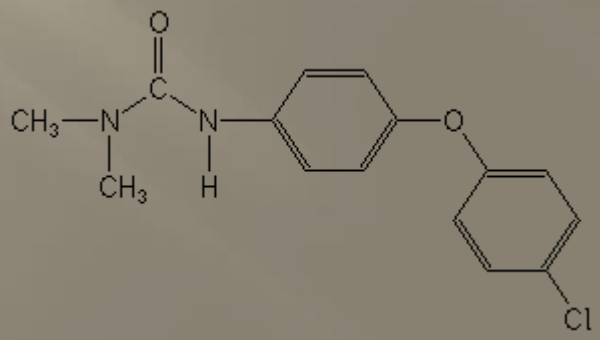
monuron



diuron



chloroxouron



Mass Spectrometry

LC-MS Applications

Environmental Applications

Phenylurea herbicides are very similar in structure and difficult to separate completely in LC, so UV detection is hard to interpret.

The monuron and diuron have one benzene ring and differ by a single chlorine giving very similar UV-vis spectra.

When analyzed using electrospray ionization on an LC-MS system, the compounds are easily identified.

Mass Spectrometry

LC-MS Applications
Environmental Applications

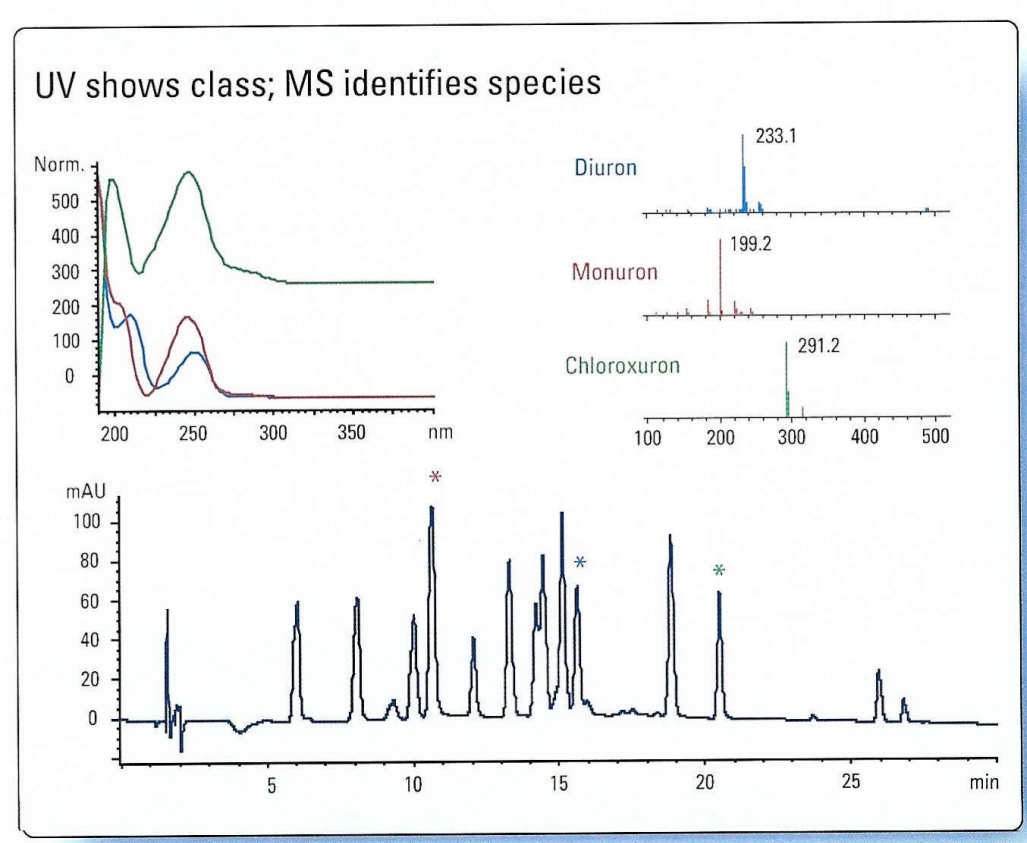


Figure 36. Chromatogram of phenylurea herbicide with UV and MS spectra

Assignment

Chem 5570