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ANALYTICAL TECHNIQUES FOR TRACE ORGANIC COMPOUNDS—III

# MASS SPECTROMETRY IN TRACE ORGANIC ANALYSIS

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# Analytical techniques for trace organic compounds—III: Mass spectrometry in trace organic analysis

Abstract - Mass spectrometry is a very sensitive and structure specific method for qualitative and quantitative analysis. The commercially available instruments achieve mass separation by various principles, such as magnetic deflection, quadrupole fields, ion cyclotron resonance or time-of-flight. Direct combination of a mass spectrometer with chromatographic separation represents a powerful analytical tool. Depending on the analytical problem at hand, various ionization techniques can be used.

## INTRODUCTION

For organic trace analysis, the mass spectrometer (MS) is unique in the combination of limit of detection, broad applicability and specificity. Any compound that can be converted to a positively or negatively charged particle in the gas phase or ejected by some means into the gas phase can be analyzed by mass spectrometers qualitatively (identification or structure determination) and quantitatively (amount present in a given sample). Such an analysis can be accomplished for components present as a simple mixture directly, because most compounds exhibit mass spectra that are sufficiently different from each other that they can be detected and measured in the presence of one another. For more complex mixtures, the mass spectrometer is usually coupled directly to a separation technique such as gas chromatography (GC) or liquid chromatography (LC), *i.e.*, GC/MS or LC/MS.

## TYPES OF MASS SPECTROMETERS

There are a number of mass spectrometer systems commercially available and they differ in the physical principle used for the separation of the charged particles (ions). Chronologically, they fall in the following categories:

(a) single focussing magnetic deflection mass spectrometer
(b) double focussing magnetic deflection mass spectrometer
(c) time-of-flight (TOF) mass spectrometer
(d) quadrupole mass spectrometer
(e) ion cyclotron mass spectrometer
(f) Fourier transform mass spectrometer (FTMS)
(g) ion trap mass spectrometer (ITD)
(it should be noted that e and f, and to certain extent g, operate in part on the same principle, ion cyclotron resonance).

These different operating principles lead to different performance characteristics in terms of mass resolution, mass range, limit of detection, scan speed, as well as size, cost and ease of operation (see Table I). More detailed information on the topics discussed below can be found in the various reference books, such as that by Watson (ref. 1).

One important characteristic of all mass spectrometers is the need for very low pressure ($10^{-3}$ to $10^{-7}$ Pa, depending on the type of instrument), from the point where the sample is introduced to the measurement of the final signal by the electron multiplier used as the detector for all types of scanning mass spectrometers (except the FTMS). Since the sample must be placed into the instrument itself (rather than into a separate sample cell or holder as with most other analytical instruments), contamination of the mass spectrometer, particularly the ion source, is one of the major experimental problems and causes of downtime. Fortunately, because mass spectrometers are very sensitive instruments, only a very small amount of material is necessary to obtain a good signal. Commercial instruments are designed such that their vacuum systems (consisting of mechanical forepumps and either diffusion pumps or turbomolecular pumps) efficiently remove the unionized sample that has entered the vapor phase.

For electron or chemical ionization (see below), the relatively volatile compounds can be introduced into the ion source of the mass spectrometer, either from a gas reservoir (*via* a molecular leak) or directly from a gas chromatograph. Less volatile samples are introduced on a sample probe inserted into the ion source through a vacuum lock. The compound is then carefully vaporized by heating the probe to a temperature (up to 300 °C at the most to avoid pyrolysis) at which it reaches a vapor pressure sufficient to produce a good ion current upon ionization. Some ionization techniques do not require vaporization of the sample (see below).

In a *magnetic deflection mass spectrometer*, the ions produced in the ion source are accelerated by a potential gradient of a few kV, which injects them through a narrow slit into a magnetic field that deflects the ions according to their mass-to-charge ratio, $m/z$ (most ions formed are singly charged, *i.e.*, z=1). By scanning the magnetic field from low

to high values, ions of increasing mass are focussed onto the collector slit and pass through to be recorded by an electron multiplier. In the case of a double focussing mass spectrometer, an electric field of a few hundred volts is placed before (forward Nier-Johnson geometry) or after (reverse Nier-Johnson geometry) the magnetic field to eliminate the effect of the small spread in energy of the ions originally produced. This configuration, therefore, permits higher resolution and a smaller physical size of the spectrometer and, for these reasons, practically all commercially available magnetic mass spectrometers are of this type. Because of the geometry and focussing principles involved, many components of a magnetic deflection mass spectrometer require high mechanical precision and alignment but, if properly installed and operated, maintain performance very well and reproducibly.

In contrast, *quadrupole* mass spectrometers separate ions by passing only those of a certain *m/z* value (or range) through the center of a set of four cylindrical or hyperbolic rods to which both dc and rf fields are applied. Changing of these fields at a fixed rf frequency allows scanning the mass spectrum, which is recorded by an electron multiplier placed behind the rod system. There are no defining slits involved and the ion source can therefore be very simple, also because the accelerating voltage is very low (5-30 V). Thus, the instrument is quite simple; only the rods have to be aligned with good precision. However, the low kinetic energy of the ions produced makes them very susceptible to changes in the electric field, particularly in the ion source and in the space before the entrance to the rod system, and the low velocity of ions of higher mass discriminates against their efficient transmission.

The *time-of-flight* mass spectrometer generates very short pulses of ions that are then allowed to drift along a tube 20-100 cm in length, at the end of which is placed the electron multiplier. Since all ions are accelerated by the same electric field, their velocity depends on their mass-to-charge ratio, which can be determined by very accurately measuring their arrival time. The TOF mass spectrometer is very sensitivie, chiefly because all ions of the pulse are detected, in contrast to the magnetic and quadrupole spectrometers that record only a very small fraction (the inverse of the resolution setting) at a time during the scan. Presently, the TOF principle is used practically only in plasma or laser desorption mass spectrometry (see below). Again, no slits are involved and precision alignment is necessary only if one wishes to obtain relatively high resolution through energy focussing or reflectors that generate a V-shaped flight path.

The *Fourier transform* mass spectrometer is also slitless and operates in a pulsed mode. Ions are generated (generally by electron ionization or laser ionization) in a box in the center of a high magnetic field (usually a superconducting magnet) and maintained at high vacuum. A short pulse excites the ions that follow a circular path when an rf field is applied to two opposite sides of the box. The FTMS does not use an electron multiplier to collect and measure the ions but measures the field induced in another pair of plates by the motion of the ions within the box. Again, the mechanical design is extremely simple, since the separation of the ions is purely a matter of the electric and magnetic fields causing the ion cyclotron motion. The main drawback is the need for observing the motion of the ions for a relatively long time (generally $10^{-2}$ to 10 sec), particularly when high resolution is desired. This is the reason why the process requires very low pressure to avoid losing the ions through collision with residual gas molecules. Another limitation is the relatively low dynamic range caused by the distorting effects of the space charge when more than a total of $10^6$ ions are created in the box. Finally, the geometry of superconducting magnets makes the ionizing region rather inaccessible, because it must be very close to, if not within, the box. For all these reasons, FTMS has not yet become a routine instrument in the analytical laboratory, although it is potentially very promising.

As mentioned above, the *ion trap* also utilizes ion cyclotron resonance as the mass resolving principle but uses only electric fields applied to a ring-electrode and two cap electrodes, placed above and below the ring, to confine the ions in motion. By varying the potential of the ring electrode, ions of increasing *m/z* value are ejected and recorded by an electron multiplier. In contrast to FTMS, much higher pressures can be tolerated in the cell and the ion trap is therefore an ideal detector for a capillary gas chromatograph, an application where the relatively low resolution and mass range (compared to FTMS) do not matter. It is produced commercially for this application by Finnigan-MAT and called ITD (ion trap detector). This and other mass spectrometer systems suitable as detectors in gas chromatography are discussed elsewhere (ref. 2).

## RESOLUTION

Useful resolution falls into three ranges: (1) So-called unit resolution is adequate to resolve species differing by one mass unit (instruments of even lower resolving power find practically no use, except beyond mass 5000). (2) High resolution instruments generate very narrow ion beams so that ions having the same integral mass, but differing in elemental composition and therefore in their fractional mass (*e.g.*, propylbenzene, $C_9H_{12}$ of mass 120.0939 and acetophenone, $C_8H_8O$ of mass 120.0575) can be separated; these instruments also permit the measurement of the mass of these ions with an accuracy of $1:10^5$ or $1:10^6$; this allows the determination of the elemental composition of the ion, based on the exact mass

TABLE 1. Comparison of various mass analyzers

| Type | Mass Range | Mass Resolution | Cost[a] | Complexity | Comments |
|---|---|---|---|---|---|
| I. Magnetic Deflection | | | | | |
| 1) Single focussing | 1-1000[b] | 1:3000 | medium | simple | Used for routine qualitative and quantitative analysis, very stable and reliable. |
| 2) Double focussing (small to medium size) | 1-2000[b] | 1:30,000 | medium | medium | Used for quantitative analysis where high resolving power is required; allows determination of elemental composition by exact mass measurement. |
| 3) Double focussing (large size) | 1-15,000[b] | 1:150,000 | high | high | Research instrument for structure determination. |
| II. Quadrupole | | | | | |
| 1) Mass selective detector (for GC) | 1-800 | 1:800 | low | very simple | Produced only by Hewlett Packard; small quadrupole completely integrated with gas chromatograph and optimized for EI only; very useful for routine qualitative and quantitative analysis by GCMS. |
| 2) Medium mass range | 1-1500 | 1:1000 | medium | simple | All quadrupole mass spectrometers require frequent calibration and tuning to maintain reproducible performance, because operational parameters have a large effect on ion transmission. |
| 3) High mass range | 1-4000 | 1:2000 | medium | medium | |
| III. Ion Cyclotron Resonance | | | | | |
| 1) Ion trap detector (for GC) | 20-650 | 1:500 | low | simple | Produced only by Finnigan-MAT; useful for routine qualitative and quantitive analysis but even more sensitive to operating conditions than quadrupoles. |
| 2) Fourier transform | 1-20,000[c] | variable[d] | high | medium to high | Ions must be in a very low pressure environment for mass analysis, therefore, interfacing with GC and LC difficult; also difficult to operate with FAB ionization; research instrument only; may eventually become more routine. |
| IV. Time-of-Flight | | | | | |
| 1) $^{252}$Cf Plasma Desorption | 1-35,000 | <1000[e] | medium | simple | The only commercially available instrument is the BIN-10K of BIO-ION. |
| 2) Laser Desorption | 1000-300,000[f] | 500-10,000 | medium | simple | The only commercially available instrument is the Model 2000 of VESTEC. |

| | | | | | |
|---|---|---|---|---|---|
| V. Tandem Instrument | | | | | |
| 1) Triple quadrupole | 1-2000 | 1:2000 | medium | medium | Useful for detection and quantitative analysis of "target compounds" in complex mixtures with or without preseparation. |
| 2) Magnetic deflection | | | | | |
| a) double focussing MS-1 + additional electric field as MS-2 | 1-3000 | unit mass[g] 1:100 | high | medium | |
| b) double focussing MS-1 + additional magnetic field as MS-2 | 1-3000 | unit mass[g] 1:500 | high | medium | |
| c) double focussing MS-1 double focussing MS-2 | 1-15,000[h] 1-15,000[h] | unit mass[g] unit mass[i] | very high | high | Highly skilled operators required; research instrument for the determination of the structure of large complex molcules, especially in mixtures. |
| 3) Fourier Transform (see III. 2) | | | | | |

[a] Ranges from low (about US$50,000) to very high (US$1,500,000).

[b] At full accelerating voltage [usually 3 kV for 1), 3-8 kV for 2), and 8-10 kV for 3)]; proportionally higher range at reduced accelerating voltage at expense of resolution and limit of detection.

[c] Theoretically up to mass 900,000.

[d] Ranging from unit resolution when operating in wide band (frequency) mode to very high in narrow band mode. In the latter case, resolution in excess of 1:1,000,000 at *m/z* 78 has been obtained but resolution is inversely proportional to mass.

[e] While the peaks are broad, their center of mass can be measured with an accuracy of 1:5,000 or better.

[f] By matrix assisted laser desorption [M. Karas and F. Hillenkamp, Anal. Chem. 60, 2299 (1988)].

[g] MS-1 capable of same high resolution as in conventional mode (up to 1:150,000) but usually operated at unit mass resolution to provide the high intensity ion beam necessary to obtain good signal at MS-2.

[h] This full mass range cannot be exploited because precursor ions of do not fragment sufficiently upon collision.

[i] In principle up to 1:10,000 but the signal is in general very low; more than unit resolution is usually not required for the interpretation of the data.

of the elements and their isotopes making up the particular ion. Clearly, a high resolution mass spectrometer provides more specificity and more selectivity, but the higher resolution is achieved at the expense of detection limits (FTMS is an exception to this statement, at least theoretically). (3) Accurate mass measurement for the determination of elemental composition becomes more and more useless beyond mass 1000, because it is more and more difficult to achieve the resolution necessary to separate ions differing by a few millimass units. Furthermore, a mass measurement accuracy of $1:10^5$ and $1:10^6$ would still encompass a large number of different combinations of elements, thus eliminating the possibility to determine the elemental composition of an ion from its accurate mass. However, meaningful "unit mass" assignment requires an accuracy within a few tenths of a mass unit in the region beyond mass 1000. At mass 5000, a mass assignment to ±0.3 u corresponds already to an accuracy of ~1:17,000!

## MASS RANGE

The mass ranges also fall into roughly three categories: (1) The range from mass 12-500 is very useful because it covers the vast majority of synthetic organic compounds, as well as a large fraction of naturally occurring substances that need to be identified and quantitated: volatile or soluble aliphatic and aromatic hydrocarbons, solvents, pesticides, plasticizers, drugs, essential oils, fatty acids, steroids, by-products of chemical productions, drug metabolites, etc. (2) The range from mass 12-1000 is mainly the domain of low molecular weight compounds that are polar due to their polyfunctionality, such as mono- and oligosaccharides or polyhydroxysteroids. These have to be rendered less polar and more volatile by derivatization with relatively heavy chemical entities, such as the trimethylsilyl group [$(CH_3)_3Si$-] that adds 72 mass units for each hydroxyl group. (3) The mass range from 1000-10,000 and beyond (up to a few hundred thousand), which is accessible with special ionization techniques applicable to polymers of biological subunits such as amino acids and pentoses or hexoses, *i.e.*, polypeptides and polysaccharides or a combination thereof. These compound types are rarely involved in "trace analysis", although they often have to be characterized in minute amounts.

## LIMIT OF DETECTION

The limit of detection is, of course, of utmost importance in trace analysis. In mass spectrometry, this parameter is dependent on a number of factors. Among these are the mode of introduction of the sample into the mass spectrometer; the efficiency of ionization; the efficiency of transmission of the resulting ions, from their point of origin (the ion source) through the mass separating system to the detector; the efficiency of ion detection; the "noise" in the detector and associated electronics; the scan speed, etc. In general, the detectability ranges from $10^{-9}$ to $10^{-12}$ g but can be pushed far below that for certain compounds or by certain experimental procedures. For the analysis of traces of organic compounds present in a large bulk sample, pre-separation, extraction and enrichment play, of course, additionally important roles.

## DATA ACQUISITION AND PROCESSING

Today there is hardly a commercially available mass spectrometer that is not integrated with a computer for data acquisition, data processing and display. More recently, some instruments use this computer for the control and operation of the mass spectrometer itself. For the acquisition of the data, a computer (mini or micro) is required because of the high scan speed (~1 sec/scan) of today's mass spectrometers and the large number of consecutive scans acquired when operating the spectrometer in conjunction with a chromatographic system, where one wishes to record many complete spectra as each fraction elutes. The vast amount of data generated in this mode necessitates the use of a reasonably powerful computer (with respect to speed, core memory and secondary storage devices, such as high capacity disks). The computer-aided operation of the mass spectrometer alluded to above may turn out to be a mixed blessing because it renders the spectrometer inoperative in the case of a computer failure, may complicate trouble-shooting and could make it impossible to carry out experiments not anticipated by the designers of the hardware/software configurations. Computer control of the mass spectrometer is easier and more necessary (and therefore more prevalent) with quadrupoles than with magnetic deflection instruments.

## IONIZATION METHODS

An important factor that influences the applicability of mass spectrometry to various aspects of organic trace analysis is the method by which the sample molecules are ionized. Historically, *electron ionization* (EI), which was almost exclusively used until 1970, involves bombardment of the molecules (present at a pressure of ~$10^{-4}$ Pa in the ion source of the mass spectrometer) by an electron beam of 50-70 eV kinetic energy. Upon interaction of the electron with the molecule, an electron is ejected from the latter, thus generating a positive molecular radical ion, $M^{+\cdot}$. This species may survive long enough to be accelerated and transmitted through the mass analyzer, a process which takes about $10^{-4}$ sec in a magnetic deflection mass spectrometer and somewhat longer in quadrupoles that employ a much lower accelerating voltage (~10 eV, as contrasted to 3-10 keV). Alternatively, the molecular ion may dissociate first into fragment ions (and neutral particles). A recording

of the abundance of all these ions separated by their mass (or actually their mass-to-charge ratio, *m/z*) represents the mass spectrum of the compound subjected to electron ionization. The interpretation of mass spectra is discussed elsewhere (refs. 1,3). The EI process requires the analyte to be present in the gas phase at ~$10^{-4}$ Pa. This method is limited to compounds that can be vaporized under these conditions without thermal decomposition. Gases or low boiling liquids are introduced into the ion source from a reservoir. Compounds of proper volatility can be efficiently introduced *via* a gas chromatograph coupled to the mass spectrometer. Less volatile samples are introduced on a heatable probe inserted into the ion source *via* a vacuum lock. These volatility requirements limit EI to compounds of low polarity and molecular weight (generally below 500).

Another mode of ionization particularly useful for the detection of a compound at the trace level (alone or in complex mixtures) rather than for the identification of an unknown is so-called "*chemical ionization*" (CI) (ref. 4), which was developed in the late 1960s. In the positive ion mode, it makes use of the addition of a positively charged species (generally a proton) or the abstraction of a negatively charged species (most often an hydride ion) to generate $(M+H)^+$ or $(M-H)^+$, giving rise to ions that have an *m/z* value one mass unit higher or lower than the molecular weight of the compound being analyzed. The former is the more frequently encountered case, while the latter is only observed if the loss of an hydride ion forms a more stable positively charged species (*e.g.*, in the case of primary or secondary aliphatic alcohols, where the oxonium ion generated by protonation of the hydroxyl group is less favored than the protonated carbonyl group formed upon abstraction of an hydride ion from the α-carbon atom).

Negative chemical ionization involves either the capture of an electron, abstraction of a proton, or addition of a negatively charged species ($Cl^-$ or $O^-$, etc.). The resulting negative ion of the compound of interest is mass analyzed in the same way as positive ions except that the acceleration potential has to be reversed and, in the case of a magnetic deflection mass spectrometer, the magnetic field (and the polarity of the electrostatic field in double focussing instruments) also has to be reversed.

Chemical ionization is carried out in a plasma in which ionizing chemical species abound. The simplest such reactant ion is $CH_5^+$, generated by electron ionization of methane (the reagent gas) present in the ion source at a pressure high enough (a few Pa) to cause multiple collisions of the primary ions with neutral $CH_4$. Collision of $CH_5^+$ with the analyte introduced into the ion source leads to transfer of a proton to the analyte molecule, or sometimes (see above) abstraction of an hydride ion. The high abundance of thermal electrons (*i.e.*, ~0 eV kinetic energy) leads to efficient capture by analyte molecules of reasonably high electron affinity, thus forming abundant negative radical anions ($M^{-\cdot}$).

As in EI, the analyte is generally introduced into the chemical ionization source in the vapor form, either through a gas chromatograph or vaporized from a conventional solids introduction probe. It is, however, also possible to obtain CI mass spectra from compounds that are not volatile enough to be vaporized into the vacuum of the ion source without decomposition. Such materials can produce useful CI spectra by depositing them onto a heatable metal tip (preferably covered with a film of heat stable polymer). The tip is then inserted into the ion source to protrude into the ionization chamber, where it comes in direct contact with the reactant ions of the chemical ionization plasma. This process is generally referred to as "direct chemical ionization".

Since in all these processes relatively little kinetic energy is transferred to the resulting ion, little or no fragmentation takes place. Chemical ionization, therefore, generally produces few, if any, structure specific fragment ions but the spectra exhibit strong signals due to the molecular species, particularly if isobutane rather than methane is the reagent gas. These spectra are, therefore, especially suitable to indicate the presence or absence of a particular known molecule of interest ("target compound") or for the quantitative analysis of that compound with a low limit of detection. This is because (1) the resulting peak in the mass spectrum is found at higher mass than a fragment would be and is, therefore, in a region of the spectrum where there is less background and other interferences; (2) all the ions initially formed appear as the molecular species (M+H, M-H or related adducts) and none are lost by fragmentation; and (3) other compounds present do not interfere (unless they are isomers or isobars). In the negative ion mode, the limit of detection can be further decreased by conversion of the analyte to a derivative of much increased electron capture efficiency, such as the conversion to a pentafluorobenzoyl ester or amide. In this respect, negative CI resembles electron capture GC (ref. 2).

Another form of CI is *atmospheric pressure ionization* (API). As the term implies, ionization takes place in a gas (air or nitrogen) kept at atmospheric pressure. The analyte is introduced into this gas and ionized by a plasma formed upon corona discharge (ref. 5). Ionization is very efficient (near 100%) but only a small fraction of the ions can be transferred into the mass spectrometer analyzer, which involves a pressure drop of 6-8 orders of magnitude. Still, API is a very sensitive method, probably $10^3$-$10^4$ times more than EI. For this reason, it is mainly used for trace analysis, particularly for the detection of trace organic compounds in air. Recently, API sources have been used for the coupling of liquid chromatography with mass spectrometers. In this case, the high

(~atmospheric) pressure is generated by the evaporating solvent and a "drying gas" (such as nitrogen) that helps desolvating the resulting microdroplets (ref. 6).

Both electron ionization and chemical ionization require vaporization of the compound to be analyzed prior to ionization. This precludes the analysis of substances having a vapor pressure of less than $10^{-4}$ Pa at 250 °C, the maximum temperature at which the ion source is generally operated. Ionization techniques that eliminate this requirement were sought ever since the power of mass spectrometry for the determination of the structure of complex organic molecules was realized. The first of these techniques, *field desorption* (FD) (ref. 7), causes compounds to ionize, which are deposited on a sharp point (such as a needle or an edge) and subjected to a high potential (10-20 keV). The potential gradient of a few MeV/cm that develops at these sharp points causes the ejection of an electron from the molecule, generating a molecular radical ion ($M^{+\cdot}$) which is then injected into the mass analyzer. Multipoint emitters produced by growing carbon or silicon dendrites on a thin tungsten wire by vacuum deposition are now most widely used. The sample is deposited on the emitter by evaporating a drop of a solution of the compound of interest placed on the emitter. Because of the high kinetic energy of the resulting ions, FD ionization is generally limited to magnetic deflection mass spectrometers. The method is particularly useful for the analysis of non-volatile, non-polar compounds. For more polar substances, fast atom or ion bombardment (see below) is more convenient and simpler to use.

A more recent development, which permits the ionization of non-volatile, polar and thermally sensitive compounds, involves the bombardment of a solution of the compound in a liquid matrix of low vapor pressure (such as glycerol) with neutral atoms (ref. 8)) or ions of 5-30 keV kinetic energy. This is referred to as *fast atom bombardment* (FAB) and *matrix-assisted secondary ion mass spectrometry* (liquid SIMS or LSIMS). These methods allow the ionization of polar molecules of molecular weights in excess of 10,000, and have been a boon to research in polypeptide and polysaccharide chemistry in the past few years. However, these aspects are of limited utility to trace analysis, although they have made it possible to carry out structural analyses at the microgram level and below.

All of these methods, FD, FAB and LSIMS, are "soft" ionization techniques, which mainly generate molecular radical ions ($M^{+\cdot}$) in the case of FD and protonated molecular ions, $(M+H)^+$, in the case of FAB and LSIMS. The term "soft ionization" implies that little excess energy is imparted onto the ionized species, which, therefore, remains mainly intact and does not form fragments that would be characteristic of the structure of the molecule in question. Such fragmentation has to be induced by another process, such as collision with a neutral gas molecule (most commonly helium) followed by mass analysis of the fragment ions in a second mass spectrometer, hence the term "tandem mass spectrometry" (see below).

Chronologically, the first method for the ionization of very large, polar molecules was $^{252}Cf$ ionization, also called *plasma desorption* (PDMS) (ref. 9). The sample is deposited on a thin metal foil and exposed in a vacuum to one of the two particles formed by the decay of $^{252}Cf$. The other particle triggers the timing signal of a TOF mass spectrometer. Only singly and multiply charged molecular species are obtained. Such signals have been recorded from compounds ranging in molecular weight up to ~35,000. While the method is very sensitive (a monolayer of molecules on an area less than 1 $cm^2$ suffices), it is not presently used in conventional trace organic analysis.

In another developing area, laser beams are used to desorb compounds of low volatility from a surface and ionize them (*laser desorption mass spectrometry*, LDMS). These two processes can occur either simultaneously by a using a single laser, or in two discrete consecutive steps involving two separate laser beams. Because at least the ionization step requires a pulsed laser of low wavelength, time-of-flight mass spectrometers or ion cyclotron resonance spectrometers (mainly of the FTMS type) are employed in such experiments. As with PDMS, the instrumentation for LDMS is not widely available and it is not frequently used for organic trace analysis. However, LDMS has great potential for the identification of trace organic contaminants on surfaces.

## TANDEM MASS SPECTROMETRY

Since some of the ionization methods described earlier (CI, FD, FAB, LSIMS) produce mainly molecular ions but little fragmentation, the latter can be induced by collision with a neutral gas (*collision induced decomposition*, CID). The resulting fragment ions can then be mass analyzed in a second mass spectrometer (or, in the case of FTMS, in a second pulsing sequence), hence the name *tandem mass spectrometry* (ref. 10). Thus, these techniques are useful to obtain more structural information when it is necessary for other reasons to use one of these soft ionization techniques. Furthermore, tandem mass spectrometry can be used to detect a particular compound in a complex mixture by selecting in the first mass spectrometer (MS-1) an ion known to be characteristic and abundant for this compound, fragmenting it further by CID, and recording with the second mass spectrometer (MS-2) either the entire product ("daughter") ion spectrum or only one (SIM-mode) or a few (MID-mode) of the product ions specific for this compound. This approach can also be used to obtain greater specificity. Although the overall limit of detection is higher, the signal-to-noise ratio is greatly improved because only product ions of the particular

precursor ions are sensed by MS-2. The price to be paid is in the complexity of the instrumentation and its higher cost.

Tandem mass spectrometry (another term is MS/MS) can be accomplished by either two magnetic deflection instruments with a short collision cell in between; by three consecutive quadrupoles where the first generates and mass separates the primary ("precursor") ions, the second is operated in the rf-only mode and filled with ~$10^{-2}$ Pa of gas (He or Ar) to serve as the collision region, and the third quadrupole is scanned to record the product ions; or by combinations of magnetic deflection and quadrupole mass spectrometers (so-called hybrid systems). Finally, FTMS allows tandem mass spectrometry separated in time rather than in space, by ejecting all but the precursor ion of interest, fragmenting it with a pulse of collision gas or a laser beam, and mass analyzing the product ions. For similar reasons, an ion trap can also be operated in an MS/MS-like mode.

The performance and complexity of tandem mass spectrometers vary widely and the proper choice of a system is governed by the field of application (see Table I). A more detailed discussion of this topic will appear in a subsequent report (ref. 11).

## MASS SPECTROMETRY IN COMBINATION WITH SEPARATION TECHNIQUES

The coupling of a mass spectrometer with a gas chromatograph (GC/MS) has been mentioned repeatedly above. It is, at the present time, the most common mode of mass spectrometry for the analysis of organic compounds. It combines a powerful separation technique with a fast and efficient method of introduction of the compound of interest into the ion source of the mass spectrometer. Both features make GC/MS the ultimate method in organic trace analysis, with negative chemical ionization of suitable or suitably derivatized compounds achieving the lowest limit of detection.

Because of this precedent, various methods for the coupling of a high pressure liquid chromatograph (HPLC) with a mass spectrometer have been developed but not with the same success. This is because, in contrast to GC that is very compatible with MS (both are gas phase methods and the mobile phase can be easily pumped out of the spectrometer by modern vacuum systems), the removal of a liquid is much more difficult. On the other hand, it is simple to collect fractions from a liquid chromatograph, evaporate the solvent and introduce the residue into the mass spectrometer manually *via* the vacuum lock. This is in contrast to the great difficulty in collecting a fraction from a gas chromatograph and transferring it separately into the mass spectrometer, the problem which gave the original impetus to the interfacing of GC with MS. The present state of LC/MS is discussed by Lamotte (ref. 12).

## CALIBRATION FOR QUANTITATIVE ANALYSIS

For quantitative analysis of components of a mixture without prior separation, calibration can, in principle, be carried out by measuring the signal for one or more ions specific for the analyte and comparing it to the signal obtained with a known amount of the pure compound. Since the mass spectrometer is very sensitive, it is difficult to introduce an exactly known but very small amount of sample into the ion source. Such measurements are, therefore, always made relative to an internal standard added before the analysis. When electron ionization is employed, ion currents are strictly additive because other components present have no effect on ionization efficiency or fragmentation of an individual compound. This is not necessarily so with chemical ionization, where large differences in proton affinity of the analytes may cause competition for the reactant ion. For these reasons, quantitation by mass spectrometry is usually carried out in combination with an on-line separation technique (GC or LC), except for gas analysis where the sample is continuously bled from a reservoir into the ion source.

For quantitative analyses of a specific compound, the limit of detection can be greatly decreased (generally by a factor of $10^2$) by setting the mass spectrometer to record only a particularly abundant and compound-specific ion rather than scanning the complete spectrum. This is called *single ion monitoring* (SIM) and is mainly used in conjunction with chromatographic separation (GC/MS or LC/MS). The resulting signal corresponds to the chromatographic peak but is free of any contribution of co-eluting compounds (or column bleed), which give no ion at that mass. With proper calibration, peak height or area can be used for quantitation. In order to increase specificity, more than one ion produced by the compound to be quantitated can be recorded by jumping from one $m/z$ to the next repetitively while the substance elutes from the chromatograph. The abundance ratio (which corresponds to a very abbreviated mass spectrum) is a check on the identity of the compound, and the constancy of the ratio indicates the homogeneity of the eluting fraction. This method, called *multiple ion detection* (MID), is also employed when using an isotopically labelled analog of the substance to be measured. Addition of a known amount of the labelled compound to the material to be analyzed greatly lessens the need for quantitative extraction, transfers, etc. during the analytical scheme, because the final measurement is the ratio of labelled vs. unlabelled (indigenous) material. It should be noted that a gas chromatograph is capable of at least partially separating labelled from unlabelled material and that the heavy isotopes cause slightly earlier elution. From a practical point of view, it is important to incorporate a sufficient number of isotopes so that the $m/z$ value

of the ion to be recorded is sufficiently separated from the natural isotope cluster of the compound to be analyzed. The incorporation of three or more deuterium atoms is the most common and economic approach. Details of quantitative mass spectrometry are summarized by Millard (ref. 13).

Competitive suppression of the signal from one component by another is frequently observed when using FAB or FD ionization. These methods are, therefore, rarely used for quantitative analysis, and then only with isotopically labelled internal standards which cancel out the suppresion effect on the quantitation.

## SUMMARY

Mass spectrometry is most widely used for three purposes: (1) Identification of organic compounds of otherwise known structure. For practical reasons (volatility, thermal stability), the vast majority of this work deals with compounds of molecular weight well below 1000 and most often employs electron ionization, which results in spectra of high informational content because it causes extensive fragmentation. (2) Elucidation of the hitherto unknown structure of organic compounds. For this task, high resolution mass spectrometry is of great advantage because it provides the elemental composition of the molecule and all the fragments. However, unless one deals with a member of a compound class for which the fragmentation is very well understood, additional data, such as ultraviolet, infrared and nuclear magnetic resonance spectra, are often needed to arrive at a unique structure. (3) Quantitative analysis of organic compounds of known structure and mass spectrum. Such analyses are carried out by measurement of the ion current generated by a molecular or fragment ion that is very abundant and sufficiently characteristic of the compound to be analyzed. Because of the unique properties of the mass spectrometer, isotopically labelled internal standards are frequently used that eliminate the need for quantitative sample manipulations (such as extractions, transfers, derivatizations, etc.). Electron ionization and chemical ionization are the most widely used methods for quantitative analysis. They are frequently coupled with gas (or liquid) chromatography as a means to efficiently introduce very small samples into the mass spectrometer, and to serve as a preseparation method that removes interferences.

One of the most useful characteristics of mass spectrometry in these three areas is its very low limit of detection. Quantitation can be achieved at the low picogram or even femtogram level and identification at the nano- to picogram level. For structure determination, it is more difficult to estimate the sample requirement. If it can be done by mass spectrometry alone, a few micrograms (or even less) may suffice, depending on the complexity of the molecule. If other methods are also required, their sample requirement becomes the limiting factor.

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## GLOSSARY

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| API | atmospheric pressure ionization |
| CI | chemical ionization |
| CID | collision induced decomposition |
| EI | electron ionization |
| FAB | fast atom bombardment |
| FD | field desorption |
| FTMS | Fourier transform mass spectometer |
| GC | gas chromatograph |
| GC/MS | combination of a gas chromatograph with a mass spectrometer |
| HPLC | high performance liquid chromatograph |
| ITD | ion trap detector |
| LC | liquid chromatograph |
| LC/MS | combination of a liquid chromatograph with a mass spectrometer |
| LDMS | laser desorption mass spectrometry |
| LSIMS | liquid (matrix assisted) secondary ion mass spectrometry |
| MID | mutiple ion detection |
| MS | mass spectrometer |
| MS/MS | tandem mass spectrometry |
| $m/z$ | mass-to-charge ratio |
| PDMS | plasms desorption mass spectrometry |
| SIM | single ion monitoring |
| TOF | time-of-flight (mass spectrometer) |