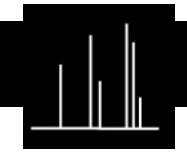
MASS SPECTROMETRY



K. L. Busch, Kennesaw State University, Kennesaw, GA, USA

Perspective of the Field

Despite the emphasis in this article on the combination of chromatography with mass spectrometry (MS), the first applications of MS in organic analysis used a direct insertion probe (from which only a crude sample mixture fractionation could be achieved), or a vapour inlet system in which the volatile samples from a glass bulb were introduced simultaneously into the ion source of the mass spectrometer. Before this, MS was not used for the analysis of organic compounds at all. Pioneers such as Aston (who won a Noble prize for the development of MS and associated instrumentation) and Dempster worked in the early part of the twentieth century on the determination of masses and relative abundances of isotopes. Preparative MS was used in the Manhattan project (carried out by the United States during World War II) to separate the isotopes of uranium, and accumulate enough fissionable material to create the first atomic bombs. A short description of the Calutrons that were used, which are based on sector instruments and still used for isotopic enrichment, is given later.

If each and every compound provided a unique retention time (or a unique set of retention times on different columns) when subjected to column chromatographic separation, only the most general detection method would be required, since the retention times alone would suffice to establish compound identity. Conversely, if MS provided a differentiating mass spectrum for each and every compound, then no separation of mixture components prior to spectral analysis would be required. Any measured mass spectrum could simply be deconvoluted as the sum of individual mass spectra. Reality destroys these idealistic dreams, as even simple mixtures can sometimes confound analytical methods used to characterize them. Approaches to mixture characterization are always based on many independent analytical data sources. The more complex the mixture, the greater the individual differentiating abilities of the methods applied must be, and the greater their independence should be. Modern column chromatography (meaning capillary column gas chromatography, microcolumn liquid chromatography, and capillary electrophoresis) provides extraordinary separations for complex mixtures. There must still be a detector to trace the elution of separated components as they elute from the column, and a nonspecific detector would suffice if the separation were perfect and distinctive. But even with the capabilities of modern chromatography, given a completely random set of compounds in a mixture, overlaps in retention time can be expected for as few as 15–20 components. For a set of related compounds, the overlap could reasonably be expected to occur at a lower number of components. At this point, the analyst must turn to the results of measurements provided by the independent detector to provide some differentiation. Consider the various detectors used in gas chromatography (GC). The thermal conductivity detector, the flame ionization detector, the electron-capture detector, and the nitrogen/phosphorous photometric detectors represent a graduated series from the more general to the more specific. But use of these detectors in and of themselves does not provide the data needed to identify a compound that elutes at any given time. They provide information that is necessary, but not sufficient, for compound identification. GC coupled with infrared (IR) or nuclear magnetic resonance (NMR) spectroscopy provides more specific information. However, the highest degree of differentiating power, clear independence from chromatography, and the greatest ease of interfacing to the chromatographic column, is provided with the use of MS. The analytical authority of combined chromatography–mass spectrometry is reflected in the pervasiveness of the method and its standing in legal and regulatory venues. This article provides a rational overview of the analytical principles of these methods as a combined analytical tool.

Technology Overview

Technological secrets are the most fleeting of all, as both scientists and engineers are inquisitive about and insistent upon the latest analytical instrumentation. The ubiquity of combined chromatography–mass spectrometry results in a competitive commercial market for such instruments. Manufacturers strive continuously for both substantive and incremental improvements. Hardware/software configurations that do not compete effectively, or do not meet

the current analytical need, are soon relegated to obscurity. This rush to sophistication often obscures the history of instrumental/methods development. However, the end result of this evolving but continually renewed market is an installed base of instrumentation that can be described in broad brushstrokes, and that is the purpose of this section.

Separation Methods

This section begins with a brief overview of the aspects of separation methods that are of general concern in the interface to MS. Then each subsection covers traits that are specific to particular methods. As a sample introduction system, the purpose of the chromatography column is to transport single, separated components of the mixture efficiently and completely into the source of the mass spectrometer. The relevant questions are therefore simple. How much material is transported? How fast is it coming through? In what form are the sample components? How well is each component separated in time from other components of the mixture? To summarize, these issues are scale, flux, phase, and purity. Therefore, the descriptions that follow will not be comprehensive overviews of how the various chromatographic interfaces to the mass spectrometer were developed or how they are operated, but will concentrate on these four central issues.

Gas chromatography The characteristic of GC that makes the interface to MS (electron ionization (EI) and chemical ionization (CI)) especially straightforward is the fact that the sample molecules are already in the gas phase, and that they are transported into the source of the mass spectrometer by a carrier gas with substantially different physical characteristics from those of the sample molecules themselves. In packed column GC, the flow of helium carrier gas was so high under the conditions normally used for separation that an 'enrichment' device had to be used to remove most of the helium, and therefore increase the concentration of sample molecules in the gas flow entering the source. The higher diffusivity of helium gas formed the basis for most of these separators. As higher pumping speeds became available with improved vacuum technology, and as the use of capillary column GC cut the flux of helium into the source of the mass spectrometer by a factor of ten, it was found that the flow of helium gas (and entrained sample molecules) could be handled directly by the improved pumping in the source of the mass spectrometer, maintaining the pressure at 10^{-5}–10^{-6} torr.

The helium was present in the source in excess, but the low mass of helium was an advantage in that most mass spectra were recorded only to a lower mass limit of about m/z 45. So the ions from helium were not recorded, and neither were ions from nitrogen, oxygen, argon, water, and carbon dioxide, all of which constituted residual molecules in the vacuum system. As there was no separate enrichment device, the efficiency of sample transport into the source was 100%, and the sample molecules were in the gas phase. As packed columns were replaced by capillary columns in GC, the influx of sample molecules changed from nanogram–microgram levels of sample per peak to picogram–nanogram levels of sample per peak. Higher amounts of sample overloaded the capillary column, and compromised separation, but these picogram–nanogram amounts of sample material were still within the detection range of the mass spectrometer. As the widths of the peaks in capillary columns were decreased relative to the widths generated by packed column chromatography, the flux in terms of amount of material(s) was still similar, even though the total amount of material was reduced. Of course, with reduced peak widths and higher separation resolution, the chances of any given peak being completely resolved were also increased. Issues that remain relevant are the need to scan the mass analyzer fast enough that representative mass spectra of a narrow peak can be recorded, and the increased demands upon a data system that is called upon to record thousands of mass spectra for hundreds of resolved sample mixture components.

Liquid chromatography Interfaces for liquid chromatography–mass spectrometry (LC-MS) must deal with transport issues that are additionally complicated by the fact that the sample is a solute in the liquid phase, the transfer into the gas phase produces large volumes of solvent vapour, and the samples are likely to be those that are relatively nonvolatile in the first place (otherwise GC would be used). Although the separation resolution may not be as high as in the best capillary GC, peaks are usually still only a few seconds wide and the amount of sample to be transported is also in the picogram–nanogram range, so the flux of material into the source is similar to that in GC-MS. The purity of the sample assessed relative to other mixture components is also similar, with separations designed to produce clean, well-resolved peaks. However, the solvent is often a mixture (as in reversed-phase gradient LC) and buffers and additives may be added to the solvent system. There is a background signal contribution from these components, and this contribution may change during the course of a chromatographic separation. As detailed in the appropriate sections, EI and CI MS act upon sample molecules in the gas phase. This is not the

form in which the sample molecules are found in LC, and it is difficult to transfer nonvolatile sample molecules into the gas phase without thermal degradation. Several ionization processes have been developed that do not rely on the sample being in the gas phase. Thermospray ionization, continuous flow fast atom bombardment, and discharge ionization sources have been developed and optimized. However, the most widely used ionization method is electrospray ionization (ESI). In this technique, a combination of progressive desolvation and field-assisted ion extraction creates a series of multiply charged ions from the sample molecules, even if those sample molecules are 'nonvolatile' and thermally fragile. The flow and flux ranges accommodated by the ESI sources overlay the range of flow and flux in modern LC, endorsing the combination.

Capillary electrophoresis In capillary electrophoresis (CE) the movement of sample molecules (often charged, but neutral molecules move through the column as well) is induced by a combination of electrophoretic and electroosmotic flow. The small differences in mobility exhibited by molecules result in different retention times within the 0.5–1-m-long columns usually used. These columns have a small diameter (50 μm) capillary to efficiently dissipate the heat produced by the high potential difference (30 kV, for example) between the front and the back of the column. The flow profile in the column is not parabolic (as in pressure-driven systems) but is essentially flat, leading to very high resolution separation. There are few instances of overlapped peaks. The small column diameter limits the amount of material that can be loaded onto the column, with loadings 10–100 times lower than in LC. Peak widths are still a few seconds wide, so the instantaneous concentration of sample is lower than in GC or LC. A small volume of sample solution (picogram–nanogram levels of sample in 10 nL of solvent) is injected at the positive end of the capillary and the separated components are detected near the negative end of the capillary. Detection is accomplished with all of the same detectors as in LC, including mass spectrometers. However, the dynamic ranges of CE and MS are not as extensively overlapped as in GC or LC coupled with MS. Despite the general assumption that MS is the most sensitive detection method in use, laser-induced fluorescence detection provides lower limits of detection than MS, but not, of course, with the same specificity. As in LC, the sample molecules of interest are not amenable to evaporation, and so ESI is most often used with CE. In fact, the electrical requirements of the capillary electrophoretic separation often dovetail nicely with the requirements for the ESI source (*vide infra*). As noted, sample peak purity is usually high because of the extraordinary resolution achievable with this method, and detection may be simplified so the solvent (often methanol) background contribution is simple and often suppressed relative to the signal from the sample.

Ionization Methods

The mass analysis step in MS requires the interaction of charged ions with magnetic or electrical fields, and therefore a means must be found either to create ions from neutral molecules, or to extract ions from a sample solution and transfer them to (or isolate them in) the gas phase. The ionization source in the mass spectrometer accomplishes this task. In mass spectrometers that interface to various methods for separation, particularly column chromatographic methods, only three ionization methods are used for the majority of applications, and will form the focus of the discussion here. EI dates back to the first developments of MS, and is the basis for the extensive mass spectral libraries available. CI was developed in the mid-1960s and is a powerful adjunct to EI. It is especially useful for the determination of molecular masses of compounds that fragment extensively under EI conditions. ESI is more recent in origin, and produces a different type of mass spectrum. Matrix-assisted laser desorption ionization (MALDI) is an even newer ionization method with special applications to high mass biomolecules. As separation methods are developed for separation of truly high mass biomolecules, and mixtures of such compounds, MALDI may become as common an ionization method as the others described in this section. Note that both EI and CI deal with sample molecules in the gas phase, while ESI brings charged species directly out of a liquid solution, and MALDI generates sample-related ions from a mixture of the solid sample and an energy-absorbing matrix.

Electron ionization EI was the first ionization method developed for MS, and it remains the most widely used. The term 'electron impact' is still also used, and the acronym EI covers both terms. Importantly, the most extensive mass spectral libraries assembled are those of EI mass spectra recorded under a 'standard' set of conditions (70 eV electron energy). The gas that flows into an EI source (helium from a gas chromatograph, for example) is confined so that the gas-phase sample molecules interact with the electrons emitted from a metal filament. A high conductance of un-ionized, neutral sample molecules out of the source must also be maintained to minimize cross-peak contamination. EI sources are maintained at

a temperature of about 200°C to prevent condensation of sample molecules on the source walls.

The ionization process is the direct result of the interaction of an energetic electron with the sample molecule. The electrons are emitted from a filament through which 3–4 A of current is passed to heat the filament to about 2000°C. Electrons are accelerated into the source by maintenance of the electron filament at a potential more negative than that of the source itself; a potential difference of 70 V (therefore 70 eV) is standard. Then electrons travel across the source to the trap where they are collected and the current is amplified. The trap is used as part of the feedback loop to maintain a constant emission of electrons from the filament. This current is usually about 100 μA (about 6.25×10^{14} electrons per second). Only a small fraction of the electrons passing through the ion source participates in ionization of sample molecules, and only about 1% of the sample molecules are ionized in EI. The EI process can be written for the gas-phase sample molecule M:

$$M(gas) + e^-_{filament} \rightarrow M^{+\cdot} + e^-_{filament} + e^-_{molecule}$$

The molecular ion $M^{+\cdot}$ (the dot denotes an unpaired odd electron) may subsequently dissociate, since EI imparts more energy to the gas molecule M than is required for ionization alone. The excess energy can cause the dissociation of the molecular ion $M^{+\cdot}$, or it can be retained in the ion as excess internal energy. Since an electron is far too light to transfer kinetic energy to the sample molecule in a collisional process, the process of EI involves only electronic excitation of M. The molecular ion $M^{+\cdot}$ retains the original structure of the molecule M, at least for a short time after its formation. If dissociations of the molecular ion are prompt, therefore, we can assume that the dissociations represent those of the original molecule and not a structurally reorganized isomer. Some molecular ions formed will be stable enough to pass through the mass spectrometer and reach the detector. Their measured m/z ratio is a direct indication of the molecular mass of the sample molecule itself. For those molecular ions that dissociate, the fragment ions that form are produced from a structure that is a direct analogue of the molecular structure. Clues to the original structure can thus be obtained by piecing together or rationalizing the processes that lead to fragmentation.

Chemical ionization In EI, if too much energy is deposited into the $M^{+\cdot}$ ion during the ionization process, or if the molecule is especially prone to dissociate, fragment ions may be seen in the mass spectrum, but the $M^{+\cdot}$ may be reduced to such a low intensity that it is indistinguishable from the background signal level. Without the molecular ion, the determination of molecular mass is difficult. CI was developed to overcome this difficulty and provide molecular ions for such compounds. CI involves a collision and reaction between an ion and a gas-phase sample molecule. The ion is called the reagent ion and the molecule is the gas-phase neutral sample molecule. There is no common and standard set of operating conditions for measurement of CI mass spectra such as exists with the EI source. As a result, there is not a large CI spectral library, and interpretation of CI mass spectra depends more heavily on the skill and experience of the user.

The CI source is a variation of the standard EI source, with modifications required to achieve a higher source pressure (about 1 torr) while keeping the mass analyser pressure within acceptable limits. Methane is a common CI reagent gas, and the reagent ion CH_5^+ will transfer a proton to the gas-phase sample molecule to form $(M + H)^+$. The protonated molecule is relatively stable, and can usually be observed in the mass spectrum of a compound for which the molecular ion $M^{+\cdot}$ formed by EI cannot be distinguished. The source filament is still heated to a high temperature so that electron emission occurs, but in CI the electron energy is usually 250–500 eV; the higher energy allows the electrons to penetrate through the high pressure in the source. The gas pressure caused by sample molecules is still about 10^{-5}–10^{-6} torr, just as it was in the EI source. As the pressure of methane is 1 torr, in an equal source volume, the electron emitted from the filament is much more likely to encounter a methane molecule. When it does, an EI process occurs, namely:

$$CH_4 + e^-_{filament} \rightarrow CH_4^{+\cdot} + e^-_{filament} + e^-_{methane}$$

The $CH_4^{+\cdot}$ ion does not travel far before it encounters a neutral gas molecule, and at 1 torr of methane and 10^{-6} torr of sample, the molecule it encounters will most likely be a methane molecule. The next reaction creates CH_5^+ and $CH_3^{\cdot}$. Several other reactions occur, and the final distribution of ions depends explicitly on the source temperature and pressure. The primary reactant ion for methane reagent gas is usually CH_5^+, and this ion acts as a strong gas–phase acid that protonates anything more basic than methane. The sample molecules are sufficiently basic to accept a proton to form the protonated molecule. The protonated molecule then fragments in accordance with the amount of internal energy it contains. In most cases not all of the protonated molecules fragment, and since there is an observable signal for the protonated molecule in the mass spectrum, the molecular mass of the sample compound can be established. The

fragmentation processes in CI are different from those observed in the EI mass spectrum, since the $(M + H)^+$ ion is an even-electron rather than an odd-electron species. The pattern of fragment ions is still interpreted to support a structure for the sample molecule. Other reagent gases form ions that transfer protons to the neutral sample molecules, forming an ion of the same mass but with a different amount of internal energy. The protonated molecule therefore fragments to a different extent. The degree of fragmentation of the sample molecule can be 'tuned' by choice of the reagent gas, and this experiment can be useful in interpretation of CI mass spectra.

Electrospray ionization In GC, helium carrier gas that enters the source along with the vapours of the sample does not disrupt the EI or CI process. However, both LC and CE separate species in a solvent, and the sample enters the ionization source along with a continuous flow of solvent (aqueous or organic) that generates a tremendous amount of solvent vapour. If EI or CI are to be used, the bulk of the solvent must be removed without loss of the sample, as the great excess of solvent vapour will certainly affect the ionization process. Various means have been devised to accomplish this task, but the efficiency is low and the process cumbersome and subject to many complicating factors. ESI allows ions to be created directly from the sample solution, and conveniently at atmospheric pressure. In ESI, the mechanical need for solvent removal is greatly reduced, albeit at the cost of allowing only a small continuous flow of solution to enter the mass spectrometer.

In ESI the sample solution is passed from the LC column or CE column through a connection junction into a short length of stainless steel capillary. A high positive or negative electrical potential, typically 3–5 kV, is applied to this capillary. There is clearly a need for electrical isolation in the LC connection, and potential management in CE. As the solution is forced to flow through the capillary tip, the solution is nebulized into a spray of very small droplets. This spray is formed at atmospheric pressure. The mass spectrometer operates at a vacuum of 10^{-5}–10^{-6} torr. The pressure must therefore be reduced before the droplets (and the sample species that they contain) enter the mass spectrometer. The spray of droplets is usually directed through a skimmer that provides a differential pressure aperture, and also acts as a momentum separator. As the droplets move through this region, neutral solvent molecules evaporate rapidly and the droplets become progressively smaller. As droplets leave the charged capillary needle, most of them retain an excess of positive or negative electrical charge, corresponding to the potential applied to the capillary. This excess charge resides on the surface of the droplets. As the droplets get smaller, the electrical surface charge density increases until the natural repulsion between like charges causes ions as well as neutral molecules to be expelled from the droplets. This field-induced evaporation also forces the droplets to become progressively smaller. Note that ions themselves cannot evaporate from the droplet. However, if the charge density is high, a Coulomb-force-induced 'explosion' can expel them from the droplet. As solvent molecules evaporate from the droplets they diffuse in all directions, while the higher momentum, charged droplets are directed towards the first skimmer, and then (usually) through a second concentric skimmer that lowers the pressure even further, by a combination of momentum separation and steering potentials applied to the skimmers. In some ESI sources a drying gas (nitrogen) flows along and past the end of the capillary and skimmer to assist with evaporation of the solvent from the droplets. The end result of the electrospray and progressive desolvation process is a stream of ions that have been extracted directly from the solution in which they were originally found.

If sample ions are already present in the solution then it is clear that these ions can be sampled directly. Solvents used in LC and CE also have appreciable ion concentrations, especially as buffers and ionic modifiers are often present in the solutions. In most cases, there is a substantial free proton population. Protons will not evaporate from the droplet as it becomes smaller; the 'pH' rises inexorably as the droplet becomes smaller. During the last stages of solvent evaporation, the protons will be forced to associate with the most basic molecules remaining in the droplet. This is not necessarily an acid–base equilibrium situation, because the dynamics of desolvation and sampling play a large role. However, the situation can be considered as one in which a free proton (a strong acid) protonates the sample molecule, which is forced to act as a proton acceptor. Other Lewis acids (cations) present in the droplet act similarly. There is a transition from the lower concentrations of ionic species present in the bulk solution to the near 100% ionic population present in a nanodroplet. The 'pH' drops to such a low value that multiple protonation is common.

The unique nanodroplet environment from which ions are drawn in ESI provides a route to highly protonated, multiply charged ions. It is the formation of multiply charged ions that makes ESI valuable for examination of sample molecules of high molecular masses. In EI and CI, most ions are formed with a single positive or negative charge. The x-axis of the

mass spectrum is the m/z ratio, and z is one. Therefore the mass on the 'm/z' axis is directly indicative of the sample molecular mass. In ESI, values of z greater than one are commonplace. ESI-derived positive ions are found as $[M + nH]^{n+}$, where n ranges from 2 to 30, and is sometimes as high as 100. Several factors contribute to the propensity of ESI to create multiply charged ions. The first is the strongly acidic environment of the nanodroplet. The second is the fact that higher molecular mass molecules are, quite naturally, large molecules, and larger molecules can accommodate a greater number of protons. For a protein, for example, a basic amino acid residue will be the site associated with the proton. Basic amino acid residues will be far enough apart in a typical protein that the protons add independently, and there is minimal Coulombic repulsion between the charged sites. The higher order structure of the protein will therefore determine what sites are accessible for protonation, and this characteristic is the basis for some of the most intriguing and revealing ESI experiments.

Suppose that M, the molecular mass of the sample molecule, is 10 000 Da. In ESI, the $(M + 20H)^{20+}$ ion may be formed. This ion has a mass-to-change ratio of $(M + 20H)^{20+}$, and therefore $m/z = 10\,020/20 = 501$. This mass is well within the range of the mass spectrometer, and can be determined accurately. Usually several different forms of the multiply charged ions are found, namely $(M + nH)^{n+}$, with a distribution of intensities. Each successive molecular ion contains one more proton and therefore one more charge. The series is easy to identify, and the value of n need only be determined for any one ion for the entire ion series to fall into place. The value of n can be determined from the spacing of isotope peaks in the molecular ion isotopic envelope, and the derivation has now been fully automated. The mass spectrum that contains the array of multiply charged ions is plotted in terms of the m/z values of those ions. But M is the same for each of those ions, and each ion is a slightly different pointer to that value of M. Having determined the masses of each of the multiply charged ions, the series of equations can be solved to determine the value of M. The data can now be presented as a transformed spectrum with one molecular ion, M. If there is more than one sample molecule M, the m/z spectrum can appear extraordinarily complex, but the transformed mass spectrum clearly shows the presence of multiple components (although the relative intensities may not accurately reflect the solution concentrations of the sample molecules).

Matrix-assisted laser desorption ionization The title of this section reveals MALDI as the most 'matrix-dependent' of the ionization methods discussed in this overview. This should not be surprising, as the ionization usually occurs from a solid-phase mixture of sample molecules in a large excess of energy–absorbing matrix molecules. Therefore, MALDI would appear to be the most ill-suited of the techniques discussed for interfacing to column chromatographic methods. However, effluents from LC separations have been deposited onto collection surfaces, and then the trail in space (along the x-dimension, for example) analysed by MALDI to provide the corresponding trace of sample elution in time. MALDI has also been applied to planar separation methods such as thin-layer chromatography (TLC) and gel electrophoresis. As interface technology improves, a miniaturized solid surface may be used to intercept effluents from columns. This may be a particularly attractive interface since MALDI, in conjunction with a time-of-flight mass analyser, has been shown to be a very capable ionization method for the production of simple mass spectra of very high mass biomolecules. Column chromatography will increasingly be used to perform separations of mixtures of such molecules, although not necessarily in the forms described previously in this section, and MALDI may be used increasingly in such applications.

Mass Analysis Methods

Each of the ionization methods discussed here has characteristics that affect the design and operation of the interface between chromatography and MS. Similarly, methods of mass determination affect the overall analysis, but not as directly. In most standard operating protocols, the data processing has assumed primary importance, and the details of the instrument on which the mass spectra were measured are hidden. This current state of affairs is in direct contrast to the situation 20 years ago, when there were distinct differences in mass spectra measured with quadrupole and sector instruments, and specific means were undertaken to normalize the mass spectra for purposes of library matching and identification. Similarly, scanning speed advantages of the quadrupole over the sector were an early boost for the former's incorporation into GC-MS instruments. Additionally, the high source potential of sector instruments made some of the early LC–sector MS designs problematic. Each of these technological hurdles has been overcome. With the introduction of the ion trap mass analyser, benchtop instruments for both GC-MS and LC-MS are becoming still smaller and even vehicle-portable. It is useful to briefly review attributes of each of the common methods of mass analysis. Simplified schematics for each of the mass analysers discussed are presented in **Figure 1**.

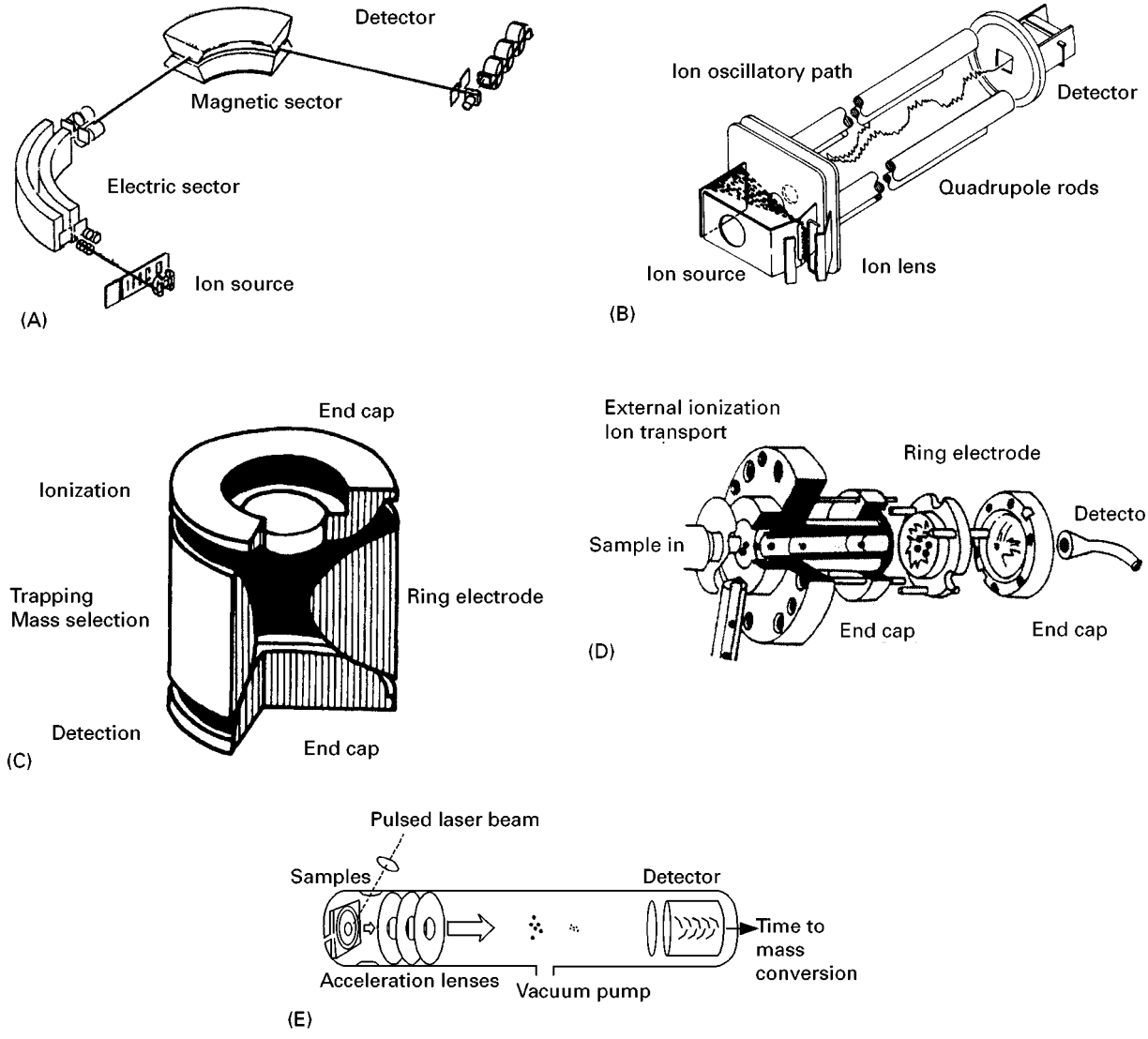


Figure 1 Simplified schematic drawings of (A) a sector mass spectrometer; (B) a quadrupole mass filter; (C) an ion trap; (D) an ion trap incorporated into a complete system; (E) a time-of-flight mass spectrometer.

Sector mass analysers The term 'sector' mass spectrometer refers to instruments in which the mass separation of the ions is accomplished by passage of the ion beam through a magnetic field directed perpendicular to the direction of ion motion. At any given magnetic field strength, ions of different mass follow trajectories of different radii. Given an equal kinetic energy for all ions, lower mass ions will trace a path of lower radius than higher mass ions. A narrow slit allows only those ions whose path follows a radius equal to that of the magnetic sector itself to pass through to the detector. Scanning the magnetic field strength passes ions of different mass to the detector. The scanning speeds of modern electromagnets are such that a mass range of 10–1000 Da is accomplished within about 0.3 s. Several complete spectral scans are therefore recorded for a typical chromatographic peak. A double–focusing sector instrument (a magnetic sector used in combination with an energy-analysing electric sector) provides higher mass resolution, that is, the m/z values of the ions can be measured to an accuracy of several decimal places (e.g., 131.12 Da). Such information can be useful in unambiguous identification of compounds as they elute from a chromatographic column. Based as they are on 'sectors' of electromagnets, these mass spectrometers tend to be larger than other types of analysers, although a significant downsizing is apparent in the current generation of sector instruments. The larger size is reflected in the higher initial costs of such instruments, and therefore sector instruments are usually not the

instrument first acquired by an analytical laboratory for chromatography–mass spectrometry. In collection of data, scanning speed is not usually an issue except for very high resolution chromatography. In selected ion-monitoring experiments, a sector instrument takes some time to hop from mass to mass, whereas a quadrupole instrument (for example) makes the transition instantaneously. Again, this is only an issue in the most exacting of experiments.

Finally, the sector-based mass analyser is used as part of a beam instrument, that is, a device in which the ion beam physically moves through the instrument from ion source to ion detector. In certain geometries the sector instrument is configured such that, at any given magnetic field strength, ions of only one mass are able to pass through the detector slit and reach the ion detector. In this characteristic the sector-based instrument is similar to the quadrupole instrument, although the latter provides a lower resolution and abundance sensitivity, and provides no transport discrimination against neutral species. The fundamental difference from ion trap and time-of-flight mass analysers will become apparent as the principles of operation of those analysers are described. Since the sector-based instrument provides a physical separation of an ion beam of one mass from an ion beam of a different mass, it can be used for the separation and collection and subsequent enrichment of that mass-selected isotopic species. The Calutron is a physically large magnetic-field mass spectrometer built to separate isotopes, with a mass throughput orders of magnitude greater than in a conventional analytical instrument. Preparative-scale MS was used at Oak Ridge as part of the Manhattan project during World War II. Several of these mass spectrometers were constructed and operated in parallel, with production reaching 200 g per day of 88% enriched ^{235}U. Two stages of enrichment were carried out on two different series of 180° sector preparative mass spectrometers, called the alpha and beta series, arranged in racetracks. These instruments maintained beam currents a billion times more intense than those in modern analytical mass spectrometers. More recently, electromagnetic isotope separation was at the cornerstone of an Iraqi programme to develop nuclear weapons. Estimates are that between five and ten billion US dollars was spent on this programme between 1981 and 1991 at Tuwaitha and Tarmiya. Initial devices used for electromagnetic isotope separation could develop a 0.5 mA beam of U^+ ions at 35 keV energy. Production devices were designed to operate with a 150 mA beam current through 1-m-radius magnets. Such uses represent exploitation of the same basic principles used for organic analysis using chromatography–mass spectrometry, with an emphasis on production of isotopes rather than production of analytical information.

Quadrupole mass filters A quadrupole mass analyser (more accurately a mass filter) consists of four rigorously parallel rods of hyperbolic or circular cross-sections, and lengths of about 20–25 cm. The ions pass through this rod structure. Opposite pairs of rods are connected electrically. A voltage composed of a direct current (DC) component and a radio-frequency (RF) component is applied to the rods. Relatively slow-moving ions (kinetic energies of 10–50 eV) move from the source into the quadrupole. They are attracted first to one rod, and then to the adjacent rod as the voltages applied to the rods change (the RF frequency). This induced oscillation in the ions results in a spiral trajectory of increasing xy magnitude as the ions move through the rods in the z direction. At a given ratio between the DC and RF voltages ions of only one mass pass completely through the quadrupole rod structure to the detector, while ions of all other masses follow trajectories that result in their collision with the rods. Scanning the magnitude of the DC and RF voltages, while keeping the ratio of the DC and RF voltages constant, allows the mass range to be scanned at a constant mass resolution.

Both the resolution and the scanning speed of the quadrupole mass filter are controlled electronically (rather than physically as with sector instruments) and can be changed quickly. In the 1970s, the much faster scanning speed of quadrupole mass filters compared with the sector instruments catalysed the development of the first viable commercial GC-MS instruments. Since that time, quadrupoles of ever increasing performance have been developed, and many entry-level instruments are based on their use.

Ion traps The ion trap consists of two end caps and a ring electrode to which DC and RF voltages are applied. Ions are formed during a short ionization pulse, after which they are forced to oscillate in stable orbits in the interior volume of the trap. The ions are kept near the centre of the trap by repeated collisions with a low pressure of helium 'buffer' gas, which removes any excess energy and relaxes the ions into stable orbits. Ion traps can be used to trap ions for extended periods of time for various purposes, including ultra high resolution accurate mass measurement. In an ion trap used in a chromatography–mass spectrometry instrument, however, the ions are trapped for only a short period of time. To scan the mass spectrum, the ions are forced out of their stable orbits to a detector by increasing the amplitude of the RF. Ions of appropriate mass (e.g. orbits of a matched

frequency) reasonantly absorb that energy. These ions become 'unstable', and trace an orbital trajectory of increasing radius until they are ejected into the detector located just outside the ion trap. As with the quadrupole mass filter, resolution and scan speed (and ion excitation for other experiments) are adjusted electronically. The ion trap itself is very small (contained within a 10 cm × 10 cm × 10 cm volume), and its associated vacuum and hardware components are assembled into a compact, low-cost benchtop unit.

Time-of-flight mass analysers The time-of-flight mass analyser is a racetrack for ions. In its most simple linear form, it is a tube approximately 1 m long. The ions are formed in a short pulse and then accelerated into the flight tube with a potential drop of a few thousand volts. Given equal kinetic energy, ions of different mass will travel at different velocities. The lighter ions are speedy, while the heavier ions lag behind. Ion arrival time at the detector is converted into a mass value by a simple equation. The resolution is relatively low because of spreads in ion velocity (and therefore flight time) derived from other sources, but the mass analyser is exceedingly simple and robust, and provides a complete mass spectrum with every pulse of the ionization source. There is no scanning involved. While other mass analysers (sectors, quadrupole mass filters, and ion traps) are used with every form of column chromatography, the time-of-flight mass analyser is used almost exclusively with MALDI and therefore has been used mostly with planar chromatographic methods of separation.

Data Processing in Chromatography–Mass Spectrometry

A chromatography–mass spectrometry file contains far more data than is usually extracted and displayed to solve a specific analytical problem. However, the specific analytical problem may be restructured, or additional questions may be asked based on the answer to the first question. It is not uncommon for a data set to be interrogated several times. Further, there may be good laboratory practice or regulatory issues that require data storage for extended periods of time. The analyst usually expends far more time in data processing than in data acquisition, especially as the latter may be automated, and the former should never be. Therefore, the focus here is on elements of data processing and interpretation that are affected by the conjunction of chromatography and MS, and in how the information derived from one augments the other.

Synergism in a Hyphenated Method

The result of the linkage between two independent procedures in a hyphenated method can be treated rigorously through information theory. In essence, both methods provide a means to characterize the components in a mixture, but the information provided by one method is independent of that provided by the other. In GC-MS, for example, the retention time of a particular component does not determine the distribution of ions in the mass spectrum. There are special traits that characterize the combination of chromatography with MS, many of which will become apparent in the following discussion. Oddly, the nomenclature for hyphenated methods sometimes includes the solidus rather than the hyphen, so one finds 'GC/MS' rather than GC-MS. The end user seldom redesigns instrumentation, makes any fundamental changes in standard operating procedures, or argues the correctness of nomenclature. The end user spends most effort gathering, analysing, and interpreting data.

Time Basis of Mass Spectral Data

Column-based chromatographic methods continuously elute sample into the ionization source of the mass spectrometer. For the most part, ionization sources also operate in a continuous fashion, generating an uninterrupted stream of ions for mass analysis. However, most mass analysers do not provide continuously measured mass dispersion. The mass range selected by the user is scanned. Usually, the mass analyser scans from a high mass to a low mass. At any given instant, ions of only one mass-to-charge ratio are passed through the mass analyser to the detector, which registers the arrival of the mass-selected ion as an electrical signal. Ions that do not possess the 'correct' mass-to-charge ratio at that particular instant follow a flight path that brings them into collision with some part of the instrument. The ions that are not selected are neutralized at the surface, and the deposited material eventually desorbs from the surface, diffuses through the vacuum, and is removed from the instrument by the vacuum pumps. The consequence of analyser scanning is that only a small fraction of the molecules that are ionized in the source proceed successfully through the mass analyser to the detector to produce a measured signal. It is the extraordinarily high gain (10^6–10^8) available with modern electron multiplier detectors that compensates for the inherent 'transmission inefficiency' associated with a scanning mass analyser.

Therefore, in a hyphenated chromatography–mass spectrometry method, the time behaviour of the data as established by the chromatography is convoluted

with the time behaviour of the data as established by the operation of the mass spectrometer. The latter is usually determined by scanning, but in the past was sometimes delineated by how fast the data could be captured and stored. In the ideal analytical design, the chromatography time base would predominate, and the time evolution of the data would be biased at least 100 : 1 in favour of the chromatographic determinant. In practice, the ratio is much closer to 10 : 1, and the time bases for operation of the mass spectrometer should be of constant concern in analysis of the data.

Procedures for Data Processing in Chromatography–Mass Spectrometry

A data set acquired with a GC-MS device will be used as the base to describe the common procedures of data processing in chromatography–MS. In GC-MS, samples are eluted into the source of the mass spectrometer at their characteristic retention times. For a Gaussian-shaped peak, the concentration of the sample in the source starts at a low value, increases to its maximum value, and then decreases symmetrically. Part of the challenge of MS is to record a characteristic mass spectrum for this sample of constantly changing concentration, given that a certain amount of time is required to scan the mass filter across its mass range. Modern mass spectrometers (considered here to be quadrupole, sector, or ion trap mass spectrometers) scan across the usual mass range in a short time. The instrument specifications for a sector instrument may show that the scan speed of the mass analyser can be as fast as 0.1 s decade^{-1}. In this context a decade is the mass range between 10 and 100 Da, or similarly between 100 and 1000 Da. In a sector instrument the scanned parameter is the magnetic field strength, and the ion m/z passing through the instrument varies with the square of this value. In a quadrupole or ion trap instrument, the scan parameter is linearly proportional to the mass of the ion in the analyser.

Approximately ten scans of the mass analyser are required to characterize any GC peak. This requirement stands regardless of the width of the GC peak, and, as the peaks in GC narrow with the achievement of higher separation resolutions, the requirements on the scan speed of the mass analyser become more onerous. Changing concentration of the sample in the source will distort the true intensities of the ions observed in the measured mass spectrum in each individual scan, so mass spectra recorded across the entire width of the peak are averaged together to create a mass spectrum that contains more accurate ion intensities. To ease the burden, the mass analyser can be forced to scan faster and faster, but eventually a fundamental hardware constraint limits the scan speed. Faced with these limits, the analyst can narrow the mass range across which the analyser must scan, or choose to monitor only certain significant ions from within the mass range. In either case, the analyst risks losing the ability to accurately identify unexpected compounds in a complex mixture.

The discussion that follows is software-independent. Each computer system will handle the operations described in its own unique fashion, and there may be specialized procedures beyond those that are described here. It is expected that the analyst will understand the general principles involved, and then take the time to explicitly explore these functions on the particular instrument to be used for analysis.

Background subtraction Resolution in chromatography is established by the time between adjacent peaks in which no sample components are eluting into the source of the mass spectrometer. Generally, the higher the chromatographic resolution, the more 'empty' space there is in the chromatogram. The mass spectrometer is recording mass spectral data even during the times when nothing is eluting from the chromatograph. These scans become the mass spectra of the background. The background mass spectrum is not constant. During a GC-MS run the background changes because of increased bleed from the column at increased temperatures encountered during a temperature ramp, low-level, highly retained components, and the continual desorption of organic compounds and contaminants absorbed throughout the system. As background is not constant, the details of background subtraction are not constant, although the correct procedure is generally accepted.

There is no error-free method of arbitrarily reducing the contributions of background in a mass spectrum to zero. However, based on the assumption that the sample background and ionization is independent of the elution and ionization of the sample component itself, then the number of scans averaged together to create a background mass spectrum should be equal to the number of scans averaged together to provide the mass spectrum of the sample. Simplistically, if ten mass spectral scans are necessary to completely characterize a peak, and these ten scans will be averaged together, then the 'background' mass spectrum should also be the average of ten scans. The scans taken for the background should be taken from as close before the peak elution, and from as close after the peak elution, as practical. There is no guarantee that the intensities of background ions in the mass spectrum will be reduced to zero, but they

will be minimized relative to those ions that are derived from the sample component. Simple mathematical subtraction may lead to negative relative intensities for some ions; these are arbitrarily set to zero.

When two sample components elute very close to one another, it is not possible to follow the before/after procedure just outlined. In the simple identification of a background mass spectrum, advantage is taken of the fact that the background does not change rapidly. The portion of the chromatogram from which the background mass spectrum, is taken is simply the first encountered as one moves to a point in the chromatogram when it is assumed that there is no contribution from the eluting compound. A more enlightened use of background subtraction involves creating a high quality mass spectrum from each of two closely eluting or even overlapped components. In this case advantage is taken of the fact that peak intensities that are reduced in intensity to zero do not distort the mass spectrum, and that the probabilities that ion masses overlap are small. In the case described, one can enter as background mass spectra those scans that are clearly from the second of two components with the assurance that the ions from this component will be effectively removed from the mass spectrum of the first.

The intensity behaviours of ions that belong to the background and those that belong to an eluting peak are different. This difference can be highlighted mathematically. The first derivative of the values for ion intensities that are changing will have a nonzero value. However, the first derivative of the ion intensities in an eluting peak will be characteristic in crossing zero at the retention time. This difference in behaviour can be used to identify ions from the sample as opposed to ions from the background. The mathematical result is an 'enhanced' mass spectrum. Note that the first derivatives for different ions will cross zero at slightly different times, depending on the rate of scan of the mass analyser.

Data averaging In the perfect chromatographic separation, the peak has a Gaussian shape. In the perfect spectroscopic detection system, the signal can thus be plotted as a convoluted function of mass analyser scan time and the number of scans across the peak profile, and the assumed constant level of noise recorded in the mass spectrum, from either chemical or electronic sources. None of these ideal assumptions is true. To increase the signal-to-noise (S/N) ratio of the mass spectrum recorded for a chromatographic peak, it is common practice, as described, to sum or average all of the mass spectra gathered as the peak elutes.

The need for data averaging is most apparent when the scan time is a significant fraction of the peak width. Given that ten scans across a peak are needed to establish the elution profile, the example described here is for just such a situation. Further, we will make the assumption that (as is commonly the case) the analyser scan is from high mass to low mass. The first scans across a peak are recorded as the concentration of the sample in the source is increasing. There is a bias in ion intensities towards the low-mass end of the mass spectrum. If a steady state sample concentration is reached, a mass spectrum with accurate and true ion intensities is recorded. However, such a situation is unlikely. After the instantaneous maximum in sample concentration is reached, the amount of sample in the source starts to decrease, and the remaining scans are recorded as the concentration of the sample is decreasing. The trailing scans will also be biased in that the ion intensities at the higher mass end of the spectrum will be too high relative to their low-mass counterparts. If the peak is symmetrical, and there are enough scans on either side of the peak maximum, the bias can be muted by simply averaging all the spectra together. This processed mass spectrum should be approximately the same as if the sample concentration in the source was constant, and it can therefore be searched against a mass spectral library.

Intuitively, the analyst wants to average all the mass spectra that are recorded for an eluting peak, even at the leading and trailing edges of the peak where the signal is first and last discernible against background. Even though a simple summation is the most common practice, it is not optimal in terms of providing the maximum S/N ratio in the processed mass spectrum. Recent work by Chang shows that only the mass spectra for which the ions are at least 38% of the maximum recorded ion abundance should be included in the summation. Furthermore, the use of a matched filter (such as used in NMR experiments) provides an additional increment in S/N ratio, provided that the shape of the matched filter parallels that of the chromatographic peak itself. The data processing applies to any combination of chromatography and detection, but is demonstrated specifically with the combination of LC and MS. The widespread use of processed mass spectrometric data to provide enhanced chromatographic resolution, based on the independence of the mass spectrometric data, makes this study particularly worthwhile. It is revealing that background subtraction has been used in mass spectral data processing for decades, and essential elements of its character are still being deduced.

Reconstructed ion chromatograms A peak eluting from a chromatographic column exhibits a characteristic retention time. When using MS as a detection

technique, that retention time is determined by the point at which all the ion intensities in the mass spectrum reach their maximum value. As described previously, the first derivative of the ion intensities crosses zero. That statement was carefully crafted to differ from the statement that the retention time is the point at which the total ion current (TIC) trace reaches a maximum. The TIC summed intensity is derived from both sample-related and background ions. The two statements are usually, but not always, identical in meaning. Using the more accurate description also provides the underlying basis for introduction of the reconstructed ion chromatogram (RIC) procedure.

A data file contains mass spectra recorded as a function of time; the mass spectrum is a table of m/z values and intensities. Therefore the data file is a collection of intensities of all of the m/z channels recorded as a function of time. Any m/z value can be specified, and the intensity data can be extracted from the data set and plotted as a function of time. In the elution of a sample peak from a column into the mass spectrometer, all of the sample-related ions should follow a similar time profile as the concentration rises. It is assumed that if an ion properly 'belongs' in the mass spectrum, then its intensity profile should track in time all of the other sample-related ions. Therefore, the converse should hold. If an ion intensity trace follows the same profile as ions that are known to be in the mass spectrum, then it 'belongs' in the mass spectrum. More powerfully, if it does not follow that trace exactly, then it does not belong. The RIC is nothing but an independent series of intensity versus time traces that graphically establish spectral propriety, and provide hints when something is amiss. The plots are independently calculated, and the absolute intensity of the ion is normalized. The graphical appearance of correctness is striking in the alignment of peak maxima and in the duplication of peak shape on both leading and trailing edges. When there is an unresolved peak component, ions that belong in that mass spectra show a strikingly different trace. A peak that belongs in the background will show a slowly changing trace with multiple maxima.

If the sample analysed by GC-MS is a mixture of closely related compounds, the mass spectra of each member of that compound class will generally contain characteristic ions of the same mass. As the analyst recognizes that a correspondence exists between the ion mass and the compound class, the entire data set can be interrogated for those characteristic ions. The RIC trace should exhibit multiple maxima that correspond to the retention times of each of the individual compounds in that class. The relative areas or peak heights for each trace do not directly represent the quantitative distribution of those class members, since they reflect the relative intensity of that mass-specific ion in the mass spectrum. However, the power of the RIC in highlighting compounds within a homologous series is evident.

Selected ion monitoring Selected ion monitoring (SIM) is a procedure used in data acquisition and processing in which the mass analyser is not scanned over a mass range, but instead hops rapidly between several preselected m/z values (this is called peak hopping). For example, instead of scanning the mass range from 35 to 1000 Da in 0.2 s, the analyser will spend some time at m/z 77, some time at m/z 91, some time on the ion at m/z 135, and finally some time at m/z 180. Usually, all of these ions are those that belong in the mass spectrum of the targeted sample component. In the scanning experiment the analyser will spend $0.2\ \text{s}/965 = 2.07 \times 10^{-4}$ s recording the signal in each nominal mass channel. In the SIM experiment the same 0.2 s (ignoring the short time that it takes to hop between peaks) is spent monitoring the ion signal in four ion channels. The detector is integrating a signal for a period that is 242 times as long in the SIM experiment. If this is indeed where the signal is to be found, then an increase in the sensitivity of the mass spectrometric analysis can be attained simply by virtue of the fact that a longer time is spent recording the signal. S/N ratios for each individual ion trace are appropriately increased as well, since this value scales with the number of independent measurements taken. Various values for the increase in sensitivity attained with the use of SIM are found in the literature. These values range from 10 to 100 fold, and depend on the width of the ion mass window monitored, and the intensity of the ions to be found within that window. Unfortunately, ions chosen for SIM are often only those that are found in the mass spectrum of the targeted sample component. It is wise to include in the selected ions a m/z value that represents a nonsample ion, so that the true S/N ratio for the experiment can be determined.

The analyst should be clear about what is gained and what is lost in the SIM experiment. Clearly there is a gain in sensitivity. The resolution of the mass analysis is not changed, so there is no tradeoff here. What is lost is the generality of the mass spectrometric detection. In short, the analyst must already know the identity of the target compound, for example, and the masses of the ions to be monitored. These are established in separate experiments that precede the selection of the SIM experiment. If a large amount of an unexpected sample is eluted from the GC during the SIM experiment, and this unexpected

adulterant does not produce ions at the monitored masses, and there is no matrix effect as a result of its presence, it will simply go undetected, even if it elutes at exactly the same time as the targeted component. Loss of such 'insurance' capability should always be carefully considered when setting up a SIM protocol.

The output of a RIC looks identical to the output of a SIM experiment. However, in this case the RIC graphical output is the result of a data processing routine. During the data acquisition process, the mass analyser is scanned across the full mass range, and each scan is a complete mass spectrum in the stored computer file. A full data set can be interrogated repeatedly with different selected ions. A SIM experiment contains ion intensities only for those ions that were selected. The RIC provides an increase in confidence of spectral propriety, but no increase in sensitivity.

Advanced computer processing The combination of chromatography with MS would not exist today were it not for the capabilities of computers in instrument control, data acquisition, data processing, and spectral manipulation and display. Advances in computer capabilities have provided more precise control, faster and more accurate data acquisition, faster and more sophisticated data processing, and higher content and more striking visual displays of chromatographic and mass spectral data. Computational power has always been applied to the interpretation of mass spectral data, and computer-assisted interpretation of mass spectral data, specifically in the area of structure/spectral relationships, continues. Computer-aided interpretation, orginally applied exclusively to EI mass spectra, is now used with success in the interpretation of CI, ESI, and MS-MS data. It is analytically compelling to support this expansion, as it is unlikely that libraries of these types of mass spectral data will grow to the size of the current libraries of EI mass spectral data. The precepts behind computer applications in the interpretation of mass spectral data have been described (Karjalainen). More recent applications have increased the speed and expanded the scope of applications, but no matter what, the progress the fundamental principles continue to apply. Pattern recognition programs can be used to recognize similarities in groups of mass spectra data. Calibration, especially in isotope ratio measurements, often involves sophisticated computer-performed mathematical algorithms. Pyrolysis MS often involves searches for similarities and differences in complex mass spectra through computer algorithms. Correlation analysis is used in many different areas of MS.

The growth in computer-assisted evaluation of chromatography–mass spectrometry data has been slower. This is surprising given the sophistication of computer hardware and software, and the proliferation of chromatography–mass spectrometry instruments. The quantitative information content of GC-MS has been described using latent variables in the context of multivariate analysis. Regression and least squares methods have been used to specifically model quantitative results obtained for GC-MS of closely eluting compounds. Procrustes analyses have been used to determine the number of significant masses in GC-MS, where significant masses represent the ions in the mass spectrum that differentiate one compound from the other. Each of these recent studies suggests that there is more information to be obtained from GC-MS than we have yet mined. The promise is that the general informational methods described will be adopted seamlessly into LC-MS and CE-MS as well.

Data Storage in Chromatography–Mass Spectrometry

Regulatory issues and requirements for good laboratory practice affect all users of analytical methods such as chromatography–mass spectrometry. Details and procedures for each user, company, or institution become part of the proper way of conducting scientific business for that organization, defined by tradition and regulation. Here, the causes and impacts of several overarching data storage and archiving issues are discussed. GC-MS is again used as the example, but of course the general issues apply to any form of chromatography–mass spectrometry.

The product of a GC-MS analysis is data. There is no collection of vials that represents a collection of compounds separated from a complex mixture. The sample aliquot analysed by the instrument is destroyed, leaving only measurement data as residue. Often, only a small fraction of that data appears in hard copy form for perusal by the analyst, who may receive a paper copy of a TIC trace, print-outs of a few selected mass spectra, and tabulated results of an automatic library search for each of these mass spectra. Each of these outputs is calculated as needed from the original data set, which is preserved within the data system, along with relevant parameters of instrument operation, calibration, and certification. Often there are several layers of safeguards that prevent post-analysis changes to the original data set. Further, in some instances a duplicate copy of the master data set is recorded remotely, while all post-run processing occurs from a local copy of the data. The backup of the data occurs automatically without

operator intervention. The rapid growth of GC-MS, coupled with the more rapid growth of regulation, implies that the sheer volume of this form of data will predictably overwhelm attempts to logically archive or access it.

Hardware Overview

GC-MS could not have been developed without the advent of the computerized data system. Conversely, the computerized data system makes it possible to exploit all the synergistic powers of the hyphenated method. It is revealing to return to early published forays into the method and read the concerns of the practitioners regarding the sheer volume of data that was available and recorded. In retrospect, the first data systems were primitive beasts. Processing speeds were very slow, and they were matched by the speed of the analogue-to-digital conversions. Data storage initially used magnetic tapes, similar to those still available today but with lower capacities. The early high-capacity data storage devices were removable platter drives. Generically, they were known as Winchester drives. The term Winchester comes from an early type of disk drive developed by IBM in 1973 that stored 30 MB and had a 30 ms access time. It was called a Winchester in honour of the 0.30-calibre rifle of the same name. For the mainframe data systems that controlled mass spectrometers, the platters (encased in cassettes with diameters of about 40 cm and thicknesses of about 5 cm) had a capacity of 10 MB. In GC-MS each platter filled up quickly; the full disk was removed from the drive, and an empty platter inserted. Winchester hard disk drives were not available for personal computers (PCs) until 1980. The PCs of today are more powerful than the mainframes used in the early days of GC-MS. Commercial systems are controlled by PCs, which is one reason that they have dropped in price. Storage capacities of 20 GBs on a single platter are available today.

Data records have a much longer life than does the original sample itself. There are rules that govern how long a sample or sample extract can be stored, at what temperature, and in what form. Outside of that time limit, that sample cannot be used for analysis. These rules exist as a result of concerns about sample degradation. In general, analysts do not worry about data degradation, although the long-term stability of magnetic-based recording media has been a topic of discussion. The relevant lifetime of data records depends on the availability of a software system that can access and manipulate the data. Analogies with commonplace computer software can be drawn readily, as each new iteration of software makes obsolete some fraction of the installed software base and its associated data. The problem is exacerbated by a competitive marketplace and manufacturers that disappear or are eager to make older systems obsolete.

Data processing and manipulation routines are manufacturer-specific and sometimes proprietary. The computer for a mass spectrometer may remain in the laboratory past the time when the mass spectrometer itself is removed so that the system can be used as required to read and process old data files. Clearly this is neither a desirable nor an efficient approach to data archival management. Manufacturers of commercial mass spectrometers have become much more cognizant of this fact over the past few years, and analytical data interchange efforts have been undertaken. The issue of backwards data compatibility is significant, and should be considered as part of the yearly performance assessment of an analytical laboratory using chromatography–mass spectrometry. Hardware capabilities are not the only issue. The preferences and habits of individual analysts also affect the manner in which data are presented and interpreted. Individuals are shuttled into and out of laboratories regularly, and a record of individual proclivities needs to be maintained and understood, especially in systems as complex as chromatography–mass spectrometry. The proper time to determine whether archived data can be located, read, processed and re-evaluated is not in the midst of crisis or urgency. If the data are worth saving, it is worth going through an exercise to test one's ability to retrieve those data. If the exercise shows that retrieval and reuse is not feasible, the data should not be saved. Previously the criterion for saving the data was the amount of storage capacity that was available, as GC-MS files were 'large', and it was considered impossible to physically save all of the data that could be recorded. The files are still indeed 'large', but storage capacities have increased by a factor of 1000 over the past 20 years, while the files have increased in size by only a factor or 2–3 (the laboratory time frame has not changed in its perception, and the resolution of the recorded data has increased only slightly). Storage capacity is no longer a relevant issue. Meaningful retrieval has become the determinant factor.

Regulatory and Legal Issues

The very public disputations over drug testing of athletes is emblematic of many of the same issues that arise in defining and documenting the impact of chromatography–mass spectrometry. GC-MS, LC-MS, and CE-MS underlie analytical results in environmental testing, drug testing, pharmaceutical analysis, and forensic investigations, in addition to being core techniques in exploratory and discovery

research. Laboratory accreditation requires strict adherence to issues of sample handling, instrument calibration and operation, and data processing and archiving. Many commercial laboratories offer such services. This section touches on the broad perspectives of the field in this area.

It is important to remember that many analyses involving chromatography–mass spectrometry are protocol-driven. This means that the exact procedures and the instrumental means of analysis are prescribed in specific detail. Deviation results in an invalidation of the results. Such protocols may not represent the 'best' way of performing the analysis. Quality attributes such as fastest, cheapest, most sensitive, most accurate, or least prone to interference are comparative. Analytical laboratories are usually paid to produce results, not to provide comparisons. Regulations are prescriptive of what methodology can be used and proscriptive of anything else. Regulations most certainly lag behind state-of-the-art capabilities. A general rule is that regulations will not change until the approved methodology lags behind current methodology by a factor of 10. Further, that factor of 10 must be meaningful. A 10-fold lowering in the limit of detection is irrelevant if the current limit of detection suffices for practical needs.

The variability in legal treatment of analytical data from court-to-court and country-to-country suggests that any justification for preemptive consistency is weak. A chromatography–mass spectrometry approach generally recognized as valid by experts is a good basis on which to build an argument, and the 'value imputed by scientific consensus' argument is accepted in many legal systems. Analytical details have to be in conformance, and good laboratory practice helps to ensure this. In the final analysis, an explanation of the results of an analysis by chromatography–mass spectrometry will be presented to a jury or a judge with a minimum of scientific background. Here, clear and focused explanations of the basic principles of the analyses are of highest value; such explanations are the purpose of this overview.

Conclusions

It is dangerous to predict that all of the essential technical innovations that allow the linkage between chromatography and mass spectrometry are in place. It would be equally foolish to try and identify any combination for which the interface technology has not already been demonstrated, at least in a primitive sense. Certainly one major area of future innovation will be in the continued reduction in the size of the instruments. However, although gas chromatographic and capillary electrophoretic separations on a chip level have been demonstrated, such devices have not swamped the marketplace. There is a realistic laboratory scale for the physical dimensions of instrumentation, and the sizes of a computer keyboard and a computer monitor are quintessential examples of that scale. Further reductions in physical size may be possible, but are not in concordance with human operation. It is not unrealistic to predict that GC-MS units will soon be the size of a PC, and may be moved about and reconfigured as easily. This reduction in physical size by a factor of about two from present day instruments will require clever packaging and insightful engineering, but does not depend on the development of fundamentally new technologies. As long as the analysis of samples occurs in a single instrumental channel (serial analysis), such scale is appropriate.

What would happen if mass spectrometers could be reduced in physical scale to the chip size (*xyz* dimensions of a few centimetres) and interfaced to some form of miniaturized chromatographic separation? Assuming that the performance of each individual combination was the same, the serial analysis has the potential to become a parallel analysis. The analytical results would be the same no matter which channel of analytical instrumentation is specified. Therefore parallel analysis allows many similar analyses to be run simultaneously, with special data acquisition and processing programs designed to zero identical results (results identical to each other or to a standard result) and highlight differences. Alternatively, adjacent channels of analysis may sample a system in which a variable (temperature, pressure, time, light, reactant concentration) is systematically varied. The possibilities for such miniature analytical instrument arrays are diverse and exciting.

What technological impediments stand between the present and, as an example, miniaturized laser ionization, time-of-flight mass spectrometers? The ions themselves are small, lasers and filaments can be small, and flight tubes are nothing but channels that can be folded into compressed S shapes. Electron multiplier detectors can also be made very small, subject only to space charge effects that limit the entire system. Electrical connections to the outside world are entirely manageable on a micrometre scale. There are two other physical connections to the outside world that have to be managed. The first is the introduction of the sample to the mass spectrometer. Assuming that the sample is eluted from the column, the problem is transformed into loading the sample onto the column. Miniaturized robotic injectors or microfluidics are already available for this task. The second physical connection is the attainment and

maintenance of a vacuum. Vacuum pumps are, in general, not amenable to miniaturization, since they must possess the physical means to transport molecules from inside the system to the outside environment. The only restriction is the insistence on maintaining vacuum, with the assumption that many samples will be analysed by the same mass spectrometer. If a miniaturized mass spectrometer has a total evacuated volume of 1 mL (not outside the reasonable scale), then a vacuum reservoir of 100 mL suffices for pumping by virtue of expansion. Essentially the vacuum is a rechargeable resource. Removing of the vacuum hardware as a physical limitation to the size of the mass spectrometer will be a genuine innovation in the field. Hopefully, this same overview written ten years from now will document the applications of new miniaturized chromatography–mass spectrometry systems.

See Colour Plate 12.

Further Reading

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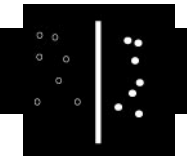
MEMBRANE SEPARATION



R. W. Baker, Membrane Technology & Research Inc. (MTR), Menlo Park, CA, USA

Introduction

Since the 1970s industrial membrane separation technology has developed into a US$1–2 billion per year business. The market is fragmented, but can be divided into six principal industrial process areas: microfiltration, ultrafiltration, reverse osmosis, electrodialysis, gas separation and pervaporation. Dialysis, another membrane separation technique, is limited to two biomedical processes, haemodialysis (artificial kidneys) and blood oxygenators (artificial lungs). The market for these two biomedical applications is another US$2 $\times 10^9$ per year. Further membrane separation applications, including membrane contactors, membrane reactors and coupled and facilitated transport, are under development. Although similar membranes and membrane module designs are used in all of these process areas, the ways by which the separations are performed and the process applications are very different. A brief overview of each process is given here; more detailed descriptions of the individual processes are given elsewhere in the encyclopedia.

History

The concept of the ideal semipermeable membrane able to separate two species with the theoretical minimum work has been used by thermodynamicists for more than 150 years, but attempts to use membranes for practical separations did not begin until the 1900s, when Bechhold devised a technique for preparing nitrocellulose membranes of graded pore size. Later workers, particularly Zsigmondy, Bachmann, Elford and Ferry, refined these preparative techniques and membranes were used to separate a variety of laboratory solutions by dialysis and microfiltration.