Separations by MS Speed Up, Simplify Analysis

Tandem mass spectrometry separates complex mixtures without chromatography, gives more information about molecular fragmentation

Analytical chemistry has two main functions: separation of the components in a mixture and identification of each component. One of the most powerful tools for product identification is mass spectrometry (MS). The mass spectrum typically provides the molecular weights of a compound and its fragmentation products. With this information alone, it is frequently possible to identify unknown compounds. In recent years, MS has been used in conjunction with two high-performance separation techniques, gas chromatography (GC) and liquid chromatography (LC), and the combinations, GC/MS and LC/MS, are two of the most widely applicable and successful analytical techniques. More recently, though, a new analytical technique has been developed in which both separation and identification of the constituents of a mixture are performed by mass spectrometers. This tandem mass spectrometry promises to eliminate many of the problems involved in linking a chromatograph to a mass spectrometer.

Tandem mass spectrometry is known by a number of other names, including MS/MS, MIKES (mass-analyzed ion kinetic energy spectrometry), collision-induced spectrometry, collision-activation spectrometry, and multiple quadrupole spectrometry. In each case, though, the fundamental process is the same. One mass analyzer, known as MS-I, ionizes and separates the components of a mixture, and the second one, known as MS-II, produces a mass spectrum of each component. Probably MS/MS is the most appropriate name for the technique because it emphasizes the close similarity to GC/MS and LC/MS.

For separation of mixtures, MS/MS has several advantages over both GC/MS and LC/MS. Much larger and more polar molecules can be analyzed by MS/MS than by GC/MS because the new technique works with much lower sample vapor pressures. There is also no problem of separating the sample from the solvent or carrier gas, as is the case with chromatography. MS, moreover, is a virtually instantaneous separation method, says Fred W. McLafferty of Cornell University, while chromatographs require minutes to hours for high resolution separations. This rapid separation, he says, greatly enhances the applicability of MS/MS for routine analyses and on-line monitoring of reactions.

A simple reversed-geometry MS/MS is capable of separating hundreds of peaks, says McLafferty, a resolution comparable to that of both GC and LC chromatograms. Some of the newer instruments can increase the possible number of peaks by two orders of magnitude. The sensitivity of MS/MS is also comparable to the best sensitivities achieved with GC/MS; many materials can be detected by both systems in subpicogram quantities.

Among the first to construct MS/MS instruments were R. Graham Cooks and his associates at Purdue University and McLafferty and his colleagues at Cornell. Both did so by reversing the geometry of a conventional double-focusing mass spectrometer. Such an instrument has two stages, an electrostatic analyzer and a magnetic analyzer. The electrostatic analyzer separates ions according to their kinetic energy (which is directly proportional to mass); the magnetic analyzer separates ions according to their mass-to-charge ratio. Operating together, the two segments sharply increase the signal-to-noise ratio and produce mass spectra of high resolution.

Reversing the geometry of the conventional instrument, Cooks and McLafferty put the magnetic analyzer first because it can separate the mixture of monoenergetic ions emitted by the ion source. Generally, a "soft" ionization technique, such as chemical ionization, is used so that each component of a mixture is ionized to produce only the parent or molecular ion. The parent ions are separated by their mass-to-charge ratio in the magnetic analyzer so that only one ionized compound enters the second stage of the instrument at a time.

In the second stage, the parent ions pass through a chamber where they undergo near collisions with molecules of an inert gas such as helium. These near collisions excite the ions electronically, causing them to fragment. The kinetic energy of each fragment is measured by the electrostatic analyzer to produce a mass spectrum—hence the name MIKES. Spectra obtained in this manner are similar in appearance to spectra obtained on a conventional mass spectrometer in which electron impact ionization is used and can be interpreted by the same rules.

The types of samples that can be studied with the first MS stage for separation are represented by complex hydrocarbon mixtures such as those encountered in petroleum refining. McLafferty and Frank Bockhoff of Cornell have shown, for example, that MS/MS can readily distinguish among C_9H_{12} isomers, such as trimethylbenzenes, isopropylbenzene, and ethylmethylbenzenes, all of which have a mass of 120. *n*-Propylbenzene, for instance, exhibits a large peak at mass 91 arising from benzylic cleavage, but the other isomers do not. Detection of the mass 91 peak of *n*-propylbenzene in the normal mass spectrum of gasoline is impossible because this peak arises mainly from other hydrocarbons, particularly C_7H_8 isomers and C_8H_{10} isomers.

A large number of other types of mixtures have been analyzed by MS/MS. Carl Djerassi and his colleagues at Stanford University, for example, have shown that simple steroid mixtures can be separated and the individual components identified. At the University of Mons in Belgium, A. Maquestiau and his colleagues have similarly shown that complex mixtures of marine sterols can be efficiently and rapidly analyzed.

In many cases, little or no sample preparation is required, giving MS/MS a distinct advantage over other analytical techniques. Cooks and his colleagues have been able to map the distribution of natural products in whole plant tissues by cutting out plant sections as small as 1 cubic millimeter, crushing the sample under liquid nitrogen, and introducing it directly into the spectrometer. The concentration of cocaine in coca leaves can thus be quantified by setting MS-I to pass ions of mass 304 (the molecular ion of cocaine) and MS-II to pass ions of mass 182, a characteristic fragment of cocaine. This dual selection ensures that it is cocaine that is being monitored. Similar pairs of masses can be used to identify many other natural products.

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Another complex mixture whose components can be identified by MS/MS is DNA. Karsten Levsen and his colleagues at the University of Bonn, for example, identified six major volatile components of the complex mixture produced by pyrolysis of herring DNA in the spectrometer's injection port. Cooks and his colleagues pyrolyzed salmon sperm DNA in the same manner and identified, among other things, 5-methylcytosine, which is a breakdown product of the rare nucleoside 5-methyldeoxycytidine. They were also able to distinguish 1-methyladenine from the 2-methyl and N^6-methyl isomers.

The technique can, of course, also be used to elucidate the structure of pure compounds. One good example cited by Chris G. Enke of Michigan State University is unknown 5.13 in McLafferty's classic text, *Interpretation of Mass Spectra*. The electron impact ionization spectrum of the unknown shows a molecular weight of 120 with major peaks at 105 and 77. Considering the 77 peak (phenyl moiety) and 105 peak (loss of methyl), there are two possible solutions, $C_6H_5COCH_3$ and $C_6H_5CH(CH_3)_2$. Relying on the 121/120 ratio and the unusually small loss of hydrogen ions from 120 and 105, McLafferty concludes that the unknown is acetophenone. Such a conclusion requires a great deal of knowledge and experience on the part of the analyst.

Companies Hop on the Bandwagon

Most of the pioneers who did the original work on MS/MS constructed their own instruments, either by reversing the geometry of a conventional spectrometer or by adding extra quadrupoles to a quadrupole spectrometer. At the recent Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, several manufacturers demonstrated that they expect the MS/MS field to grow and that they intend to grow along with it.

VG Micromass Ltd. of Cheshire, England, is the only company that offers a reversed-geometry MS/MS. The Micromass ZAB-2F is a high-resolution gas chromatograph–mass spectrometer (GC/MS) in which the geometry has been reversed at the factory to make the instrument a GC/MS/MS. The instrument can be expanded with an additional mass filter to produce a triple analyzer with a resolution as much as 10,000. The base price of the ZAB-2F is in the $350,000 to $400,000 range; this price does not include a data system.

The only other manufacturer that appears interested in reversed-geometry instruments is Kratos Scientific Instruments, Inc., which has constructed a special triple analyzer, the MS50TA, for the University of Nebraska (see story). Kratos hopes to build similar instruments for other customers. The estimated cost is $400,000 to $600,000, depending on options.

Other manufacturers, however, are more interested in the less expensive multiple quadrupole instruments. Finnigan Corporation, for example, has developed a triple quadrupole spectrometer based on its top-of-the-line GC/MS. The company already has an operating prototype and expects to sell about half a dozen instruments this year. Finnigan president T. Z. Chu says the company has spent a great deal of time developing new software for instrument control, including such refinements as push-button control of tuning. A manually operated GC/MS/MS will cost approximately $230,000, while the price of a fully automated system will be $305,000. For comparison, the GC/MS on which the instrument is based costs $170,000 in a fully automated version.

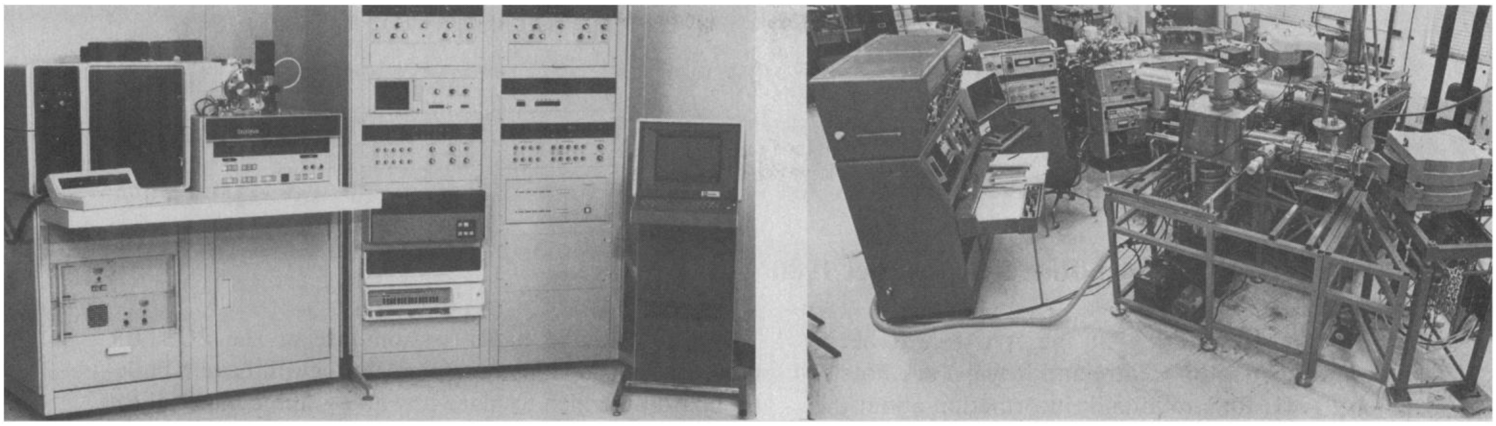
Extranuclear Laboratories, Inc., of Pittsburgh has taken a slightly different approach to multiple quadrupoles in its collision gas mass spectrometer. This instrument uses only two quadrupoles. The collision gas chamber in this instrument is enclosed not by a quadrupole, but by an axial cylinder constructed of a patented material that Extranuclear characterizes as a "leaky dielectric." This material allows the RF current, but not the d-c potential, from the two quadrupoles to pass to the interior of the cylinder so that all ions are transmitted to the final quadrupole. The company has sold one instrument so far and expects to sell several more this year. The price is about $150,000, exclusive of a data system.

Sciex Inc. of Thornhill, Ontario, has a new multiple quadrupole spectrometer that is especially good for trace atmospheric gas analysis (TAGA). The TAGA 6000 has a special, patented inlet port for atmospheric pressure chemical ionization of volatile samples, and cryogenic pumping is used to remove excess gas from the inlet port and to evacuate the collision chamber. Such pumping allows more collision gas to be used and increases the number of ions that fragment in the final quadrupole. A fully automated version of the TAGA 6000 is priced in the range of $300,000 to $450,000, depending on options.—T.H.M.

But the structure of this unknown can be obtained in a direct manner by MS/MS, says Enke. The large peak at mass 43 in the spectrum corresponds to the remainder of the molecule [CH_3CO^+ or $(CH_3)_2CH^+$] after loss of the phenyl group. A spectrum of the mass 43 ion obtained in MS-II is identical with the spectrum of CH_3CO^+ from acetone, confirming McLafferty's conclusion. Enke's group is developing a library of the spectra of common molecular fragments so that the identity of much larger structures can be determined from positive identification of their fragments.

The resolution and sensitivity of MS/MS can be enhanced by increasing the number of mass analyzers. Kratos Scientific Instruments, Inc., of Westwood, New Jersey, has added a second electrostatic analyzer to a modified Kratos MS50 high-resolution mass spectrometer to produce a so-called "Triple Analyzer" (MS50TA) for Michael L. Gross and his colleagues at the Midwest Center for Mass Spectrometry at the University of Nebraska. The MS50's magnetic and electrostatic sectors function as MS-I and the added electrostatic analyzer is MS-II. Experiments at the factory on the assembled instrument have produced MS/MS spectra with a resolution as high as 50,000. Such a resolution means that 500 peaks can be separated between masses 100 and 101.

McLafferty, Peter Todd, Michael Baldwin, and Donald McGilvery of Cornell have just constructed a high-resolution MS/MS in which each stage of the instrument is double-focusing—that is, MS-I and MS-II each have both electrostatic and magnetic analyzers. The high resolution of MS-I has been used to separate isobaric ions (those with the same nominal mass) for identification by MS-II. For example, $C_6H_5N^+$ (mass 91.0422) and $C_7H_7^+$ (mass 91.0547) ions can be separated cleanly and identified



The lower price of multiple quadrupole instruments is accounted for in part by their relative simplicity (left) compared to the greater complexity of McLafferty's double focusing MS/MS (right).

by their characteristic spectra. The additional magnetic analyzer in MS-II brings ions of the same mass but different energy to a common focus, producing spectra with a greatly increased signal-to-noise ratio and much narrower peaks.

Both the triple analyzer and McLafferty's new instrument are expensive pieces of equipment in terms of the initial cost and the sophistication of personnel necessary for maintenance and operation (see box). They will thus most likely find application for those problems that cannot be solved in any other way. Even a simple reversed geometry MS/MS is quite expensive, so that it too will be used primarily for solving difficult analytical problems. Perhaps the brightest hope for the general use of MS/MS lies in the development of multiple quadrupole spectrometers, which should be in the same price range as GC/MS and LC/MS instruments while still offering high resolution and sensitivity.

The quadrupole is a simple mass filter constructed by placing four stainless steel rods in close proximity lengthwise to form a passageway for ions. When both a radio-frequency (RF) current and a direct-current (d-c) voltage are applied to the rods, ions passing between them travel in a complicated pathway. At any given combination of RF current and d-c voltage, only ions of one mass-to-charge ratio will follow a stable trajectory all the way through the center of the quadrupole, while all others will hit the sides of the instrument and be captured. It is possible to produce a conventional mass spectrum by scanning through various combinations of RF current and d-c voltage. Most important, a quadrupole mass spectrometer is smaller and much less expensive than a conventional double-focusing instrument, yet gives results that are nearly as good. The quadrupole is also much simpler to operate under computer control.

Quadrupole spectrometers can readily be adapted for MS/MS by increasing the number of quadrupoles. The first such instrument was constructed by Donald McGilvery and James D. Morrison of La Trobe University, although they designed it to perform laser-dissociation spectrometry. They assembled two quadrupole mass filters and placed between them a third that is operated only on RF current. The middle quadrupole acts as a dissociation chamber; since only RF current is applied, all the ions emerging from MS-I are transmitted to MS-II. A transparent window was installed in the second quadrupole so that a laser could be focused on the ion beam to induce dissociation. McGilvery and Morrison soon found, however, that laser-induced dissociation was a very inefficient process and that nearly as many ions were dissociated by traces of gas remaining in the chamber.

Enke and Rick A. Yost, now at the University of Florida, were at the same time designing a tandem quadrupole MS/MS that could use collisional dissociation. Yost went to La Trobe to try out their ideas by adapting the second chamber of McGilvery and Morrison's instrument so that an inert gas could be injected at low pressures. They obtained much higher collision efficiencies with this apparatus than Cooks and McLafferty had obtained, primarily because the RF field permits product ions from direct collisions to be transmitted to MS-II. They also found that spectra produced by the triple quadrupole instrument are remarkably similar to those obtained with other MS/MS instruments in which the dissociation is induced by near-miss collisions. Yost and Enke subsequently constructed another triple quadrupole instrument at Michigan State and, shortly, thereafter, Donald F. Hunt and his colleagues constructed another at the University of Virginia.

Multiple quadrupole instruments can be used for most of the same applications as a reversed-geometry instrument. One good example of such applications is the identification of so-called priority pollutants in industrial sludges. Hunt has shown, for example, that such toxic materials as *p*-nitrophenol, 2,4-dinitrophenol, and dioctyl phthalate can be identified in industrial sludges at the 100 parts-per-billion level. Little sample preparation is necessary; the sludge is simply freeze-dried and inserted directly into the ion source of the spectrometer. The technique could thus be used to monitor effluents from industrial processes.

One other important potential application of MS/MS is the determination of the amino acid sequence of peptides, a feat that has long been a goal of mass spectroscopists. Hunt has devised a procedure in which oligopeptides from digestion of a protein are *N*-acylated with an equimolar mixture of acetic anhydride and perdeuteroacetic anhydride, then permethylated to make them more volatile. Amino-terminal fragment ions thus give a pair of peaks separated by three mass units. This pair of peaks is separated in MS-I. The spectra obtained in MS-II then have similar pairs of fragment ions formed by peptide chain cleavage in which the amino terminus is retained. From these, it is possible to deduce the amino acid sequence. Hunt has recently reported sequencing by MS/MS 23 oligopeptides in a mixture produced by elastase digestion of glucagon. The technique has the potential to be much faster and simpler than conventional approaches.

The number of potential applications of MS/MS has been growing at a rapid rate in recent years, and many investigators, not named here, have made valuable contributions. Instrument companies have also become interested in the technique (see box), which means that many other investigators should soon have access to it. If current trends are any indication, MS/MS should become one of the most widely used instrumental techniques of the 1980's.

—THOMAS H. MAUGH II