Agreement is found over the entire range of wind speeds (14 to 34 m/sec) derived for the rain-free portions of this pass. There is no evidence of any saturation of the radar cross section with increasing wind speed.

Conclusion

The accuracy of the near-surface wind speeds derived from the P-3 measurements is ± 10 percent, with errors due in part to the inertial navigation system measurement itself and to approximations inherent in the hurricane boundary layer model. There may also be small errors in the measurement and processing of the remotely sensed data. Considering these errors and possible misregistration of the two aircraft tracks, certain discrepancies are to be expected. However, when the remotely sensed wind speed, wind direction, and rain rate trends are compared with the values from in situ sources, there is close agreement.

In the future, more sophisticated beam-aiming techniques will be used to form a more optimum sampling scheme. Judicious choice of altitude and incidence angle and adjustment of the antenna azimuth sweep to compensate for the aircraft's speed will allow measurement of σ° over the desired range of azimuths for a reasonably small area of the sea surface. This would be of considerable value in a hurricane, where numerous meteorological phenomena are distributed over relatively short distances. Another improvement would be accurate detection of, and eventual correction for, atmospheric attenuation within the radar beam path.

The data presented here are based on the first of a total of approximately 20 multi–aircraft passes through Hurricane Allen's eye on 5 and 8 August. On many of these passes the AMSCAT antenna was programmed to obtain azimuth sweeps at various incidence angles other than 40°, and also to obtain elevation sweeps at fixed azimuths. The SFMR was operated in a multifrequency mode on all but one pass. In situ measurements are available for nearly all passes.

The data from most of these passes will be analyzed with a view toward optimizing the sensor operating modes and the algorithms for future hurricane experiments. Also, an attempt will be made to process on-board (in real time) at least the SFMR data to surface wind speed and rain rate.

The results of this experiment demonstrate the feasibility of microwave remote sensing techniques to obtain from high altitudes information which is presently obtained at low altitudes and at considerable risk.

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Tandem Mass Spectrometry

Fred W. McLafferty

"Needle in a haystack" analytical problems have become important in a variety of areas. Specific compounds which must be recognized in the presence of many others include drugs or disease-indicative compounds in biological fluids, pollutants in environmental samples, chemicals used in the classification of plant and animal species (chemotaxonomy), natural insect attractants (pheromones), flavor- and odor-producing compounds, petroleum and synthetic fuels, process control compounds, chemical warfare agents, and contraband agricultural products, drugs, and explosives.

Specificity, accuracy, sensitivity, and speed are the key performance characteristics that must justify the cost of the analytical technique chosen. Specificity must be sufficient to distinguish the compound sought (the targeted molecule) from all others present. For quantitation, the specific instrument reponse must be directly related to the amount of targeted compound present, independent of other components. Sensitivity is a limiting factor for many applications; enzymes, drugs, toxins, pheromones, odors, and flavors can be active in picogram concentrations. Speed and applicability are important, for a laboratory's willingness to invest in equipment and trained personnel for a technique will depend on the number and variety of analytical problems it will solve.

Radioimmunoassay (RIA) is a prime example of a technique that combines unusual selectivity and sensitivity (*1*); these properties have led to its widespread use in clinical and research laboratories around the world. For example, 10^{-13} gram of gastrin per milliliter is readily measurable, and steroid molecules differing only in the presence of a hydroxyl group can be distinguished by RIA. Its technical simplicity makes possible a low unit analytical cost and high accuracy with nonprofessional personnel, although several hours are required for an analysis and extensive development of methods may be necessary for a new targeted molecule.

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Summary. Coupling mass spectrometers in series provides a new technique that has many advantages for the analysis of specific organic compounds in complex mixtures. Sensitivity to picograms of targeted compounds can be achieved with high specificity and nearly instantaneous response. The targeted compound is selectively ionized, and its characteristic ions are separated from most others of the mixture in the first mass spectrometer. The selected primary ions are then decomposed by collision, and from the resulting products the final mass analyzer selects secondary ions characteristic of the targeted compound. Tandem mass spectrometry can achieve specificities and sensitivities equivalent to those of methods such as radioimmunoassay and gas chromatography/mass spectrometry, while performing analyses in much shorter times.

In contrast, ion-selective electrodes (*2, 3*) respond nearly instantaneously to changes in the concentration of the targeted molecule, yielding analytical information at an unusually low cost. However, their specificity and sensitivity are, in general, substantially lower than those of RIA. An electrode specific for dodecyltrimethylammonium ion gives a minimum detectable signal for $\sim 10^{-9}$ g, and to avoid interference other tetraalkylammonium ions present must differ in molecular weight by ~70 (*3*). Analytical methods based on enzymatic reactions can be unusually specific and sensitive (*4*); when combined with ion-selective electrodes, they can also provide a fast response. However, these methods are applicable only to relatively specialized chemical systems, as are a variety of other sensitive detectors (*5, 6*).

Two-dimensional chromatography is a broadly applicable method (*5*) by which selectivity can be increased. To separate a targeted molecule from 10,000 related compounds, it is usually easier to find chromatographic conditions for separating an individual fraction containing the targeted compound plus ~100 others, and then find other conditions for separating the targeted compound from these 100. Analysis for other compounds in the sample could require additional separations, increasing the sample analysis time as well as the time needed to optimize each set of separation conditions.

Mass spectrometry (MS) (*7–9*) has been for many years an analytical tool of unusually high sensitivity and speed coupled with good specificity for compounds with molecular weights up to ~1000, and recent research has raised this limit substantially (*10–12*). The electron multiplier makes possible the detection of a single ion, so that sensitivities of 10^{-13} to 10^{-15} g are attainable with commercial instruments (*8*). Response can be virtually instantaneous, as ion formation and transmission require $< 10^{-3}$ second; the speed of an analysis is determined by the time required for sample introduction and collection of statistically meaningful data. Relatively high specificity can be achieved by use of the hundreds of possible mass positions that are completely resolved from their neighbors, combined with selective ionization of targeted compounds. However, a complex mixture such as a blood plasma can produce a peak at virtually every mass position from *m/z* (mass-to-charge ratio) 50 to 500, interfering with the detection of almost any trace component with a peak in this mass range.

One of the most broadly applicable techniques for specific trace analysis in complex mixtures is GC-MS, the tandem coupling of gas chromatography and mass spectrometry (*8*). This combines the complementary specificities of the chromatographic and MS separations with the subpicogram sensitivity and quantitative accuracy of MS. Only compounds that are sufficiently volatile for GC can be used in GC-MS, but substituting a liquid chromatograph (LC) (*9*) extends the sample volatility range to that of MS. The high instrument and personnel training costs are offset by the ability to analyze one or more samples per hour for a multiplicity of components per sample. Many of the problems arising from the technical complexity of the instrumentation have been met by using a dedicated computer to handle data reduction and instrument control, which also simplifies application to new problems. In addition to targeted compound analysis, GC-MS has been uniquely applicable to the identification of total unknowns in complex mixtures such as pollutants, pheromones, and flavors (*8*). The additional specificity provided by a chromatographic preseparation greatly reduces interference without appreciably reducing the sensitivity of MS, but the speed of analysis is limited by the time required for the chromatograph.

A preseparation can also be provided by use of an additional mass spectrometer, and this technique is known as tandem mass spectrometry, or MS-MS (*13, 14*). Such tandem mass spectrometers (Figs. 1 and 2) can have much greater selectivity than a single mass spectrometer without serious loss of sensitivity, and retain the unique advantage of a response time $< 10^{-3}$ second. Except for scattered early reports (*15–18*), most research activity in this field has occurred in the last 5 years (*19–25*). In this article I discuss basic aspects of the technique and recent developments.

Principles

The hypothetical complex mixture ABCD (the molecule targeted for analysis), BCDA, CBDA, EFGH, IJKL, . . . is introduced continuously into the MS ionizing region to produce a complex

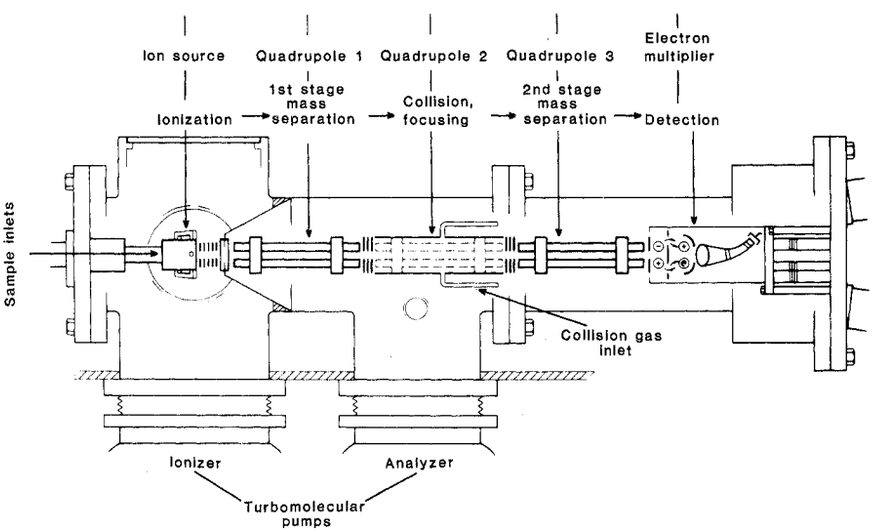


Fig. 1. Schematic of a tandem quadrupole MS-MS instrument. [Courtesy of the Finnigan Corporation]

mixture of ions, among them the isomers $ABCD^+$, $BCDA^+$, and $CBDA^+$. The first mass spectrometer (MS-I) is set to transmit only ions whose *m/z* value corresponds to $ABCD^+$, so that these and $BCDA^+$ and $CBDA^+$ ions will exit continuously from MS-I. Energy is added to these ions by collision, causing them to decompose; the secondary ions produced are then separated by MS-II. If only $ABCD^+$ primary ions (not $BCDA^+$ or $CBDA^+$) yield the secondary ions AB^+, MS-II separation and detection of the latter will provide a direct measure of the original concentration of ABCD in the sample mixture. As in multiple ion monitoring in GC-MS, other peaks in the secondary mass spectrum of a primary ion can be used to increase the specificity of measuring its corresponding component. For example, if the abundances of the secondary ions ABC^+ and AB^+ are in the ratio found when pure ABCD is introduced into the MS-MS, it is much less likely that these ions were formed by components other than ABCD in the original mixture. Analysis of other components of the mixture is straightforward; measuring EFGH would involve separating the $EFGH^+$ ions by MS-I and separating and detecting collision-produced secondary ions such as EFG^+ and FGH^+ by MS-II.

Selective ionization. For mass spectrometry, convenient and sensitive ionization methods have been developed that are selective for a variety of compound types (*26*). Ionization with 70-eV electrons (electron ionization or EI), the method used most commonly for compound identification in GC-MS, is relatively undesirable for MS-MS analysis of targeted compounds, as it produced dozens of primary ions from most compounds. Chemical ionization (CI) is probably the most versatile selective ionization technique; a wide range of ionizing reagent gases and conditions that produce negative as well as positive ions have been recommended for many common classes of organic compounds (*26, 27*). For example, compounds with a high affinity for protons, such as neurotransmitter amines, are selectively protonated by ammonia. Negative ion CI often makes possible greatly enhanced sensitivities for electronegative compounds, such as those containing fluorinated, carboxyl, or nitro groups. Charge-exchange CI can provide selectivity based on ionization energy requirements; with benzene as the ionizing reagent gas one can selectively ionize aromatic compounds. The optimum concentration of reagent gas relative to that of the sample is $\sim 10^3$; thus for solutions a sensitivity increase of approximately this amount can be obtained by using the solvent as the ionizing reagent gas (*28*), introducing the sample through an interface used for liquid chromatography–mass spectrometry (LC-MS) (*9*). Atmospheric pressure ionization (API) provides high sensitivities for trace components in air by a similar mechanism (*29*).

Selectivity in primary ion dissociation. Decomposition of the separated primary ions to produce the secondary mass spectrum can occur spontaneously; however, such a metastable-ion MS-II spectrum contains at most a few peaks, the largest of which represent ions present in only 10^{-2} to 10^{-4} times the abundance of the precursor ion. To increase the number and absolute abundance of peaks in the secondary mass spectrum, it is necessary to add energy to the separated primary ions; collisionally activated dissociation (CAD) is the most convenient and widely used method for doing this (*30, 31*). Collision of the ions with gas molecules (at a pressure of $\sim 10^{-4}$ torr) converts part of the translational energy of the ions into internal energy. The subsequent unimolecular decomposition of these excited ions is similar to that of excited ions formed initially in the ion source, and in general follows correlation rules developed for normal EI mass spectra (*7*). For ions with energies of many kilovolts, excitation results from a grazing collision. For instruments such as the quadrupole MS, in which the ions have very low translational energies (5 to 20 eV), relatively large scattering angles are necessary (billiard ball collisions). Yost and Enke (*21*) found an ingenious method to direct these scattered secondary ions into MS-II by using a quadrupole with only a radio-frequency field, the heart of the tandem quadrupole MS-MS instrument.

The ions of the selected *m/z* value exiting from MS-I may still represent many components of the original sample, so that selective excitation of the desired ions in the collision process can also be helpful. The average amount of energy added by collision can be increased by increasing the ion translational energy

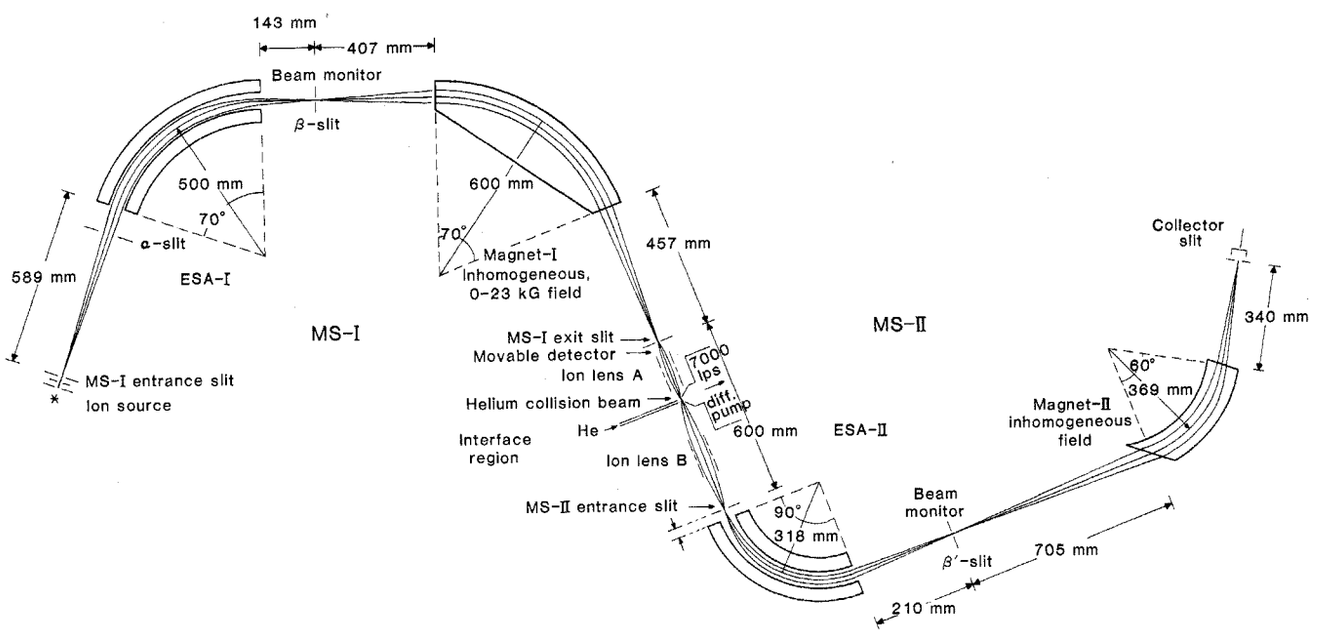


Fig. 2. Tandem double-focusing MS-MS instrument constructed at Cornell.

(*32*) or number of collisions (*33*), thus increasing the relative abundance of secondary product ions from reactions with higher energy requirements. Collisions can convert negative ions to positive ions, or singly charged ions to doubly charged ions. Cooks and co-workers showed that daughter ions requiring similar energies for their formation can be selected by collecting ions scattered at a specific angle in multikilovolt collisions (*34*). Bimolecular products of reactions between the primary ion and the collision gas can be observed when low-energy collisions take place, but these could lead to matrix effects in targeted compound analysis. Use of tailored collision conditions to obtain additional selectivity in MS-MS is a subject worth further investigation.

Selectivity versus resolution of MS-I and MS-II. When double-focusing mass spectrometers are employed as MS-MS instruments, an electrostatic analyzer is used as MS-I (*35, 36*) or as MS-II (*16*). The collisions produce secondary ions with a range of translational energies, so that the energy-analyzed spectra often show incomplete resolution of neighboring peaks even at relatively low masses [this problem has been solved with 40-kV postcollision ion acceleration (*37*)]. The newer tandem quadrupole instruments using low-energy collisions achieve unit mass resolution in both MS-I and MS-II (*21*). The separation of isobaric multiplets such as N_2 (*m/z* 28.0061), CO (27.9949), and C_2H_4 (28.0313) with a high-resolution mass spectrometer is well known; the use of such an instrument as MS-I (*22*) or MS-II (*38*) or both (*39*) can give a dramatic improvement in MS-MS specificity for particular problems.

Sensitivity. The subpicogram sensitivity possible in normal mass spectrometry is not substantially degraded in MS-MS; the ion losses in CAD and MS-II separation are in part compensated by the reduction in "chemical noise" achieved by the second analyzer. The efficiency of CAD for converting multikilovolt primary ions into secondary ions is 1 to > 10 percent (*39*) while efficiencies of 5 to 100 percent have been reported (*40, 41*) for the quadrupole-confined low-energy CAD method. The transmission efficiency of the added MS approaches 100 percent for magnetic and electrostatic analyzers, and for the quadrupole analyzers with ions of lower mass. Although there have been few studies to date of the ultimate sensitivities possible with MS-MS, subpicogram detection limits have been reported (*21, 41*).

Speed. Even with the added collision process and second analyzer, the time between ion formation and collection in MS-MS is $< 10^{-3}$ second for most instruments. With a dedicated computer, data acquisition times are on the order of 10^{-3} to >1 second per targeted component, depending on the accuracy and sensitivity desired as well as the efficiency of the data system. Sample handling can be the limiting factor in time requirements. With a direct introduction probe, solid samples can often be introduced at a rate of more than one per minute, although the pump-out time for marginally volatile sample components can seriously reduce this rate. Gaseous and liquid (*9, 28*) samples can be introduced directly, with sample streams analyzed continuously or many batch samples analyzed per minute with efficient sample changers.

Instrumentation

Despite the newness of the MS-MS field, certain types of instruments now appear to be preferable for particular analytical applications. For the determination of targeted compounds of low or medium molecular weight in complex mixtures the tandem quadrupole has substantial advantages: unit resolution in MS-I and MS-II, efficient CAD conversion of primary to secondary ions, efficient computer control for multiple-ion monitoring and GC-MS-MS, and, with mass production, possibly a future price comparable to that of GC-MS.

For problems involving high molecular weight compounds, the ion transmission of a magnetic instrument (8- to 10-kV ions) is an order of magnitude higher than that of a quadrupole at high mass (*m/z* 500 to 1000 at unit resolution), an effect that is multiplied for MS-MS. However, this advantage is lost through incomplete resolution if only an electrostatic analyzer is used as MS-I or MS-II, without postcollision acceleration (*37*). The tandem double-focusing instrument (Fig. 2) (*39*) has the additional advantage for high molecular weight samples of high resolution in MS-I and MS-II, but it is the most expensive MS-MS instrument. New methods (*10–12*) make possible the ionization of compounds of molecular weight >3000; unit mass resolution in this range requires the use of a double-focusing instrument. In addition, instruments employing multikilovolt ion collisions appear at present to be more suitable for molecular structure elucidation (*22*).

Magnetic and electrostatic analyzer combinations. Double-focusing mass spectrometers used for high resolution and exact mass measurements contain a tandem combination of magnetic (B) and electrostatic (E) analyzers. These can also be used as separate mass analyzers for MS-MS, and most early studies were performed with B-E ("reversed geometry") instruments of this kind (*16, 30*). The magnetic analyzer is used as MS-I to separate the desired primary ions, which are then collisionally dissociated. The kinetic energy of the resulting secondary ions is directly proportional to their mass, so that the electrostatic analyzer can serve as MS-II. The most serious deficiency of this system is the less-than-unit resolution of the secondary ion spectra; this can be improved by increasing the ion translational energy before (*39*) or after (*37*) collision. Linked scanning (*35, 36*) of the two analyzers, either B-E or E-B, makes possible unit mass resolution in the secondary ion mass spectra, but then the primary ion is poorly resolved from its neighboring mass peaks. The high kinetic energies of the ions used lead to relatively high ion transmission efficiencies for such analyzers. Data collection efficiency is substantially increased by the use of new laminated field magnets that are capable of scanning a mass decade in 0.2 second (*39, 42*).

Triple-sector and tandem double-focusing instruments. The capabilities of two-sector (B-E or E-B) instruments can be increased by the addition of one or two B or E sectors in tandem. Triple-sector instruments of the configuration E-B-(CAD)-E (*22*), B-E-(CAD)-B (*38*), or B-E-(CAD)-quadrupole (*42*) make possible high-resolution separation of the primary ions in MS-I; B-(CAD)-E-B gives unit resolution in MS-I and high resolution in MS-II (*38, 42*). Use of a fourth sector, as in an E-B-(CAD)-E-B configuration (Fig. 2) (*39*), gives high resolution in both MS-I and MS-II. A double-focusing MS focuses for energy as well as divergence; thus the MS-II of this instrument brings secondary ions of different energies but the same mass together at the same focal point, which greatly narrows the resulting peaks and increases their height and signal-to-noise ratio. Commercially available multiple-sector instruments cost $500,000 to $700,000 (*42, 43*).

Tandem quadrupole instruments. These mass analyzers (Fig. 1) provide unit mass resolution in both MS-I and MS-II, and the radio frequency–only quadrupole collision region provides a

high-efficiency interface between them. The unique characteristics of quadrupoles which led to their widespread use in GC-MS systems are similarly advantageous in MS-MS. Quadrupoles are compact, relatively easy to maintain, and can be efficiently controlled by an on-line computer or microprocessor. Such instruments are available commercially from several sources (*44–46*), and perhaps the rapidly growing market will substantially reduce their price; at present, the instrument with a data system costs $350,000 to $450,000.

Other MS-MS instruments. Mass spectral information can actually be obtained from metastable or collisionally activated decompositions that occur between the accelerating region and the magnetic field of a single-focusing mass spectrometer, but the resolution is poor and interferences can be serious. Louter *et al.* (*37*) built a special instrument with greatly improved resolution for MS-II spectra; a channel plate array detector in MS-II allows continuous measurement and display of these secondary spectra, which is valuable for monitoring fast experiments such as pulsed laser ionization. Glish *et al.* (*47*) constructed a magnetic-quadrupole system with considerable versatility, which is useful for research—for instance, in studies of the effect of energy on the low-energy collision process. McIver (*48*) and Freiser (*49*) have proposed MS-MS instruments based on the ion cyclotron resonance (ICR) principle. On the basis of experience with our earlier instrument (*50*), we have designed a tandem time of flight mass spectrometer with a two-dimensional array detector. A variety of tandem mass spectrometers, including magnetic-ICR and tandem double-focusing instruments, have been constructed for other purposes, such as studies of ion-molecule reactions and trace analysis for nuclidic species (*22–24*).

Table 1. Examples of MS-MS applications.

System studied	Principal investigator
Chemotaxonomy of cacti	R. G. Cooks, Purdue University
Drugs in raw urine	D. F. Hunt, University of Virginia
Polynuclear aromatics	C. G. Enke, Michigan State University
Ion photodissociation	J. D. Morrison and D. C. McGilvery, LaTrobe University, Melbourne
Tetrachlorodibenzodioxin	M. L. Gross, University of Nebraska
DNA pyrolysis	K. Levsen, Bonn University
Marine sterols	A. Maquestiau, Mons University, Belgium
Polymer sequencing	P. J. Derrick, LaTrobe University, Melbourne
Aromatic amines	K. R. Jennings, University of Warwick, England
Steroid mixtures	C. Djerassi, Stanford University
Saxotoxin	A. L. Burlingame, University of California, Berkeley
Phosphate ester chirality	J. R. Knowles, Harvard University
Ion structures	H. Schwarz, Technical University of Berlin, West Germany
Nitroaromatics	J. H. Beynon, University College of Swansea, Wales
Stereoisomers	J. Marsel, University of Ljubljana, Yugoslavia
Natural product pyrolyses	H. L. C. Meuzelaar, University of Utah
Pyrolysis of bacteria	A. J. H. Boerboom, FOM–Instituut voor Atoom- en Molecuulfysica, Amsterdam
Short-lived radicals	C. N. McEwen, DuPont
Alkaloids in plants	W. F. Haddon, U.S. Department of Agriculture
Insect pheromones	C. G. MacDonald, M. T. Lacey, CSIRO, Canberra
Isobaric mixtures	D. Stahl, Federal Technical Institute, Lausanne
Protein digests	H. R. Morris, Imperial College, London
Penicillins	J. Occolowicz, Eli Lilly Co.
Trace contaminants in air	R. A. Yost, University of Florida
Polychlorodibenzodioxins	J. R. Hass, National Institute of Environmental Health Sciences
Benzopyrenes	G. A. McClusky, Frederick Cancer Research Center
Petrochemicals	R. W. Kondrat, Shell Development Co.
Metriprananol in serum	M. Senn, Boehringer-Mannheim
Substituted aromatics	D. H. Russell, Oak Ridge National Laboratory
Metabolic profiling	M. Anbar, State University of New York, Buffalo
Polychlorinated biphenyls	R. K. Boyd, University of Guelph
Drug metabolites	A. Tatematsu, Meijo University, Japan
Detection of explosives	J. Yinon, Weizmann Institute, Israel
Enkephalins	R. M. Caprioli, University of Texas Medical School
Polymer additives	I. Sakai, Toray Industries, Japan
Coal liquids	B. W. Wilson, Battelle Northwest Laboratories
Prostaglandins	W. K. Duholke, Upjohn Co.
Steranes in crude oil	E. J. Gallegos, Chevron Research
Peptide mixtures	R. R. Ragakov, Tashkent State University, U.S.S.R.
Testosterone in tissue	S. J. Gaskell, Welsh National School of Medicine
Diesel exhaust	D. Schuetzle, Ford Motor Co.
Odors in air	V. J. Caldecourt, Dow Chemical Co.
Concealed drugs, fruits	J. B. French, Sciex, Inc., Toronto
Parathion in lettuce	J. R. B. Slayback, Finnigan Corp.
Ion plasmas	M. W. Siegel, Extranuclear Laboratories, Inc.
Sequences of mixed peptides	F. W. McLafferty, Cornell University

Applications

There has been a great increase in the last 2 years in the number of laboratories utilizing MS-MS and in the number and types of problems to which it has been applied. A number of examples are shown in Table 1; many more are given in recent reviews (*17–25*).

One of the first laboratories active in MS-MS research was that of Cooks and co-workers; their work attracted widespread attention to the potential of the technique. For example, they performed direct quantitative analyses of urine for picogram amounts of homovanillic acid and testosterone; untreated samples were dried on the MS insertion probe and the components vaporized directly in the ion source (*19*). Similarly, they analyzed stems, leaves, and berries of coca plant for cocaine and cinnamoylcocaine by direct ion source introduction of 1-mm^3 samples (*20*). Recently, they showed that direct MS-MS analysis of samples of low volatility, such as saccharides and quaternary ammonium salts, is possible with laser desorption ionization (*51*).

Hunt and his colleagues have performed a wide variety of analyses with a tandem quadrupole MS-MS instrument using negative (*52*) as well as positive ions. The compounds analyzed included 90 carboxylic acids in urine, peptide mixtures with component sequencing, organosulfur compounds in crude oil and coal liquids, and pollutants present in sewage sludge at a concentration of 100 parts per billion (the total analysis time was 15 minutes per sample) (*52, 53*). Enke and co-workers (*54*) obtained results that show promise for routine analysis of the acid fraction components of priority pollutants in complex mixtures.

Meuzelaar *et al.* (*55*) have used MS-MS in the classification of complex materials such as body tissues, bacteria, and coal by analyzing the distribution of products formed on Curie-point pyrolysis of submicrogram samples. Haddon

and Molyneux (*56*) analyzed samples of *Senecio* and and similar plants for pyrrolizidine alkaloids, which are toxic to livestock, and found the method 100 times more sensitive and 30 times faster than the routine LC method used previously. Slayback and Story (*25*) obtained linear calibration curves down to 10^{-11} g for the pesticide parathion in ivy and lettuce leaves; although the negative ion CI spectrum of the crude uncontaminated leaf showed a peak at virtually every mass, there was no interference for the characteristic secondary ions at *m/z* 154 and 169 formed from the primary ion at *m/z* 291. The API technique is highly sensitive and convenient for direct sampling. Caldecourt *et al.* (*57*) used API to measure odor components in air at concentrations of parts per billion and determined the structures of unknown compounds; some matrix effects on quantitation were also noted. Davidson *et al.* (*41*) described a variety of applications of API, including analyses of vapors from drugs, explosives, and fruits in air expressed from luggage; nitrosamines in air above frying bacon or in beer; polynuclear aromatics in oil; aflatoxin B_1 in peanut butter; sulfamethazine in pork; and drug metabolites in raw urine. The quantitative response for tetrachlorodibenzodioxin in fish extracts was found to be linear to less than 10^{-12} g.

High molecular weight samples. Linscheid *et al.* (*58*) obtained structural information for polysaccharide mixtures by field desorption ionization and MS-MS; mycobacterial methylmannose polysaccharides were characterized up to *m/z* 2506. Barbalas *et al.* (*59*) applied MS-MS to polybromobiphenyls and to the cardiac glycosides digoxin and digitoxin. Fast atom bombardment ionization and MS-MS were used by Morris *et al.* (*60*) to analyze mixtures of polar and nonpolar compounds of high molecular weight, such as carbohydrates and peptides. A new high-field (2.3-tesla) magnet of radius 30 cm allowed them to use 8-kV ions up to *m/z* 3000. At Cornell we are installing a 2.3-tesla magnet of radius 60 cm on the tandem double-focusing MS-MS instrument (*39*), which should allow mass analysis of such ions up to *m/z* 12,000.

Use of MS-MS with separation systems. Tandem mass spectrometers can also act as a very specific detector for GC and other separation systems, including the crude fractionation obtained by heating the sample in the MS ion source. For such use rapid scanning and data acquisition are valuable, so that the computer-controlled tandem quadrupole instruments are often particularly appropriate. In these applications MS-MS can be used in three general modes, to supply (i) a secondary ion spectrum that is characteristic of a specific primary ion (also used for targeted compound analysis); (ii) primary ions that yield a common secondary ion (or ions) which is characteristic of a structural class; and (iii) primary ions that yield secondary ions through loss of a neutral moiety (or moieties) which is characteristic of a specific structural class.

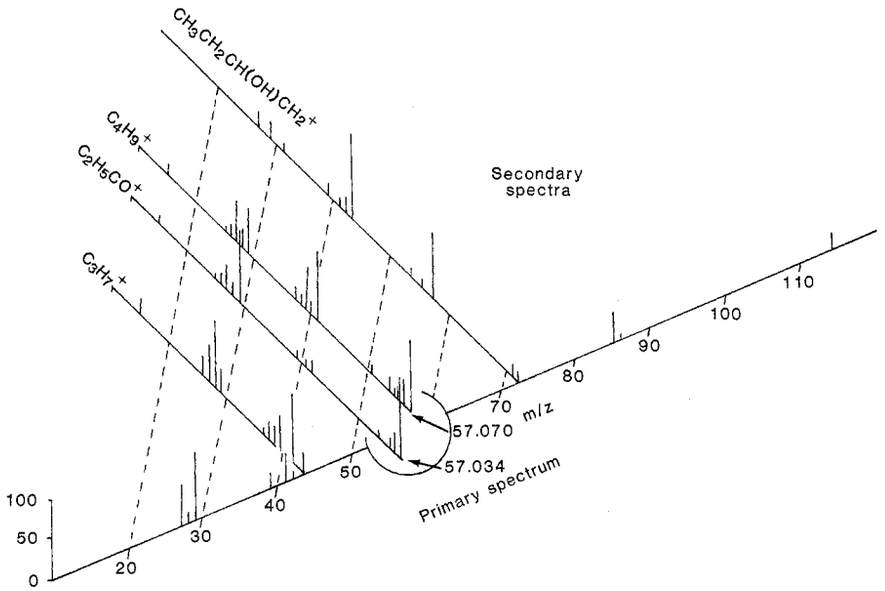


Fig. 3. Secondary collision mass spectra produced from fragment ions in the electron ionization mass spectrum of 5-methyl-3-hexanone.

The first mode is illustrated by the identification of penicillins in mixtures such as fermentation broths. When ionized by electrons, these compounds form abundant $C_7H_{12}NO_2S^+$ fragment ions from their common tetrahydrothiazole ring; after collision, these primary ions yield a secondary mass spectrum characteristic of penicillins (*61*). This allows penicillins to be distinguished from other compounds, such as dihydrocephalosporins and tetramethylthiazolidine carboxylates, which also give large $C_7H_{12}NO_2S^+$ peaks in their EI spectra. As an example of the second mode, Gallegos (*62*) reported that terpane and sterane molecular ions produce abundant secondary peaks at *m/z* 191 and 217, respectively, on metastable decomposition. A sample from Green River shale vaporized from the direct probe produced a homologous series (C_{18} to C_{35}) of molecular ion peaks yielding secondary peaks at *m/z* 191 and another homologous series yielding peaks at *m/z* 217. By assuming that relative secondary ion yields were the same for all homologs, Gallegos obtained quantitative values in agreement with those derived by a much more time-consuming analysis. Two examples of the third mode may be given. Zakett *et al.* (*63*) used the loss of mass 44 (CO_2) from primary ions $(M - 1)^-$ to indicate that those ions originated from carboxylic acids, and Shushan *et al.* (*64*) found that M^+ ions of polychlorobiphenyls and other highly chlorinated compounds are indicated by their characteristic secondary losses of Cl and Cl_2.

Molecular structure determination. Secondary CAD spectra of multikilovolt ions can be characteristic of the structure of the ion, independent of its mode of formation (*30, 31, 65*) [although in low-energy CAD spectra variations with mode of formation are observed (*54*)]. The molecular structure of a pure compound can thus be determined by identifying fragment ion structures in its normal EI mass spectrum. Figure 3 shows MS-MS information derived from 5-methyl-3-hexanone. The primary high-resolution mass spectrum shows two peaks of nominal mass 57, $C_3H_5O^+$ and $C_4H_9^+$. The secondary CAD spectrum of the former agrees well with a reference spectrum of the $C_2H_5CO^+$ ion; if a reference spectrum had not been available it might have been identified from the characteristic loss of CO to form the abundant $C_2H_5^+$ peak. The CAD spectra of the $C_4H_9^+$ isomers are the same within experimental error. However, the presence of the *m/z* 72 peak, as well as its CAD spectrum, shows that the 3-position is unsubstituted.

In collaboration with D. A. Cooper of the U.S. Drug Enforcement Administra-

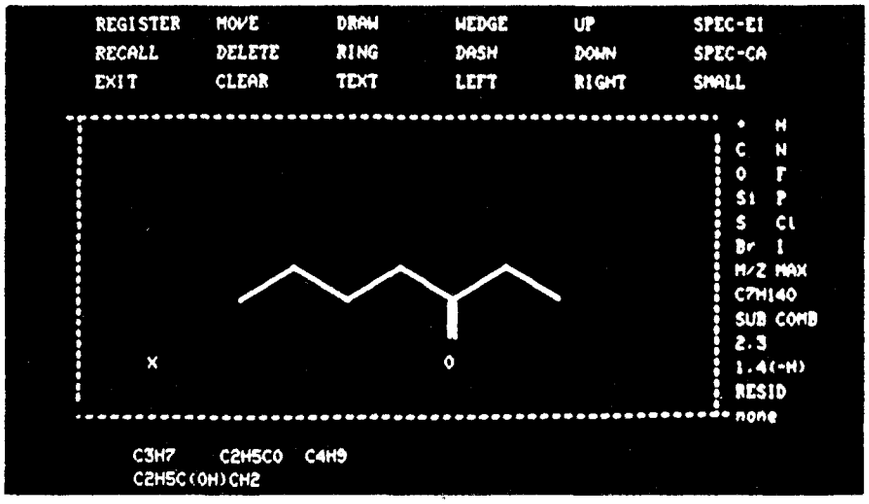
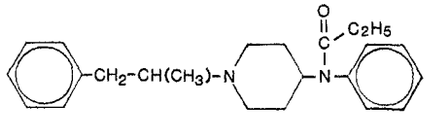


Fig. 4. Cathode-ray tube display of the results of computer interpretation of the data of Fig. 3. The fragment ion structures at the bottom of the screen were identified by probability-based computer matching of the observed CAD spectra against the reference file. The data at the lower right are possible combinations of these substructures that fit the proposed molecular formula $C_7H_{14}O$. One possible molecular structure consistent with these data, that of 3-heptanone, was assembled by the interpreter with a light pen accessory.

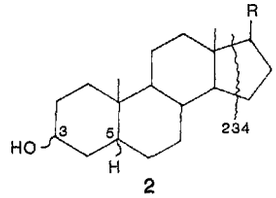
tion, M. T. Cheng and G. H. Kruppa of Cornell used MS-MS to examine the street drug "china white," which appeared in the Los Angeles area in 1980. Cooper and his associates had assigned the structure shown below (**1**) on the



1

basis of MS, NMR spectroscopy, infrared spectroscopy, and synthesis. Its mass spectrum shows prominent peaks at several masses, including 57, 91, and 146. Comparison of the peaks with reference CAD spectra indicate the substructures $C_2H_5CO^+$, $C_6H_5CH_2^+$, and $C_6H_5NH^+{=}C(CH_3)CHCH_2$ (by rearrangement), which would be sufficient to determine the structure.

Such secondary spectra can also provide stereochemical information. M. T. Cheng, M. P. Barbalas, and R. F. Pegues at Cornell studied a variety of 3-hydroxysteroids that produce a characteristic mass spectral peak at *m/z* 234 corresponding to an ion containing the A, B, and C rings as shown below (**2**).



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Using pregnane-3,20-diols, they found four different secondary CAD spectra of these *m/z* 234 ions for the four possible combinations of 3-OH group configurations and A-B ring geometry: 5α,3α; 5α,3β; 5β,3α; and 5β,3β. These CAD reference spectra are useful for characterizing the stereochemistry as well as the primary structure of unknown compounds; the CAD spectrum from the *m/z* 234 peak of 5α-cholestane-3α-ol is the same within experimental error as the 3α,5α reference spectrum.

It would be advantageous to be able to identify substructures automatically by comparison of fragment ion CAD spectra with a reference collection. To this end, we have collected a data base of 700 CAD spectra (average mass, 110) (*66*). To search this file, I. K. Mun, M. P. Barbalas, and W. Staedeli of Cornell developed an algorithm modeled after the probability-based matching system for EI mass spectra (*67*). The computer can also serve as an aid to construct possible molecules consistent with the identified substructures, molecular ion composition, and other information. Figure 4 shows a cathode-ray tube display of results of such an interpretation of the data in Fig. 3; the operator assembled the retrieved substructures by using light pen manipulation and showed that the structure of 2-heptanone is consistent with the data.

Future Prospects

Two general areas of MS-MS growth appear to be particularly promising. Use of MS-MS for routine analysis for lower molecular weight compounds in complex mixtures with a minimum of sample work-up should continue to increase. I believe that this demand will bring the price of computerized MS-MS instruments down to $50,000; the present price appears to be the major deterrent to MS-MS growth rivaling that of GC-MS and LC-MS in recent years. The versatility of such an instrument should make it applicable to a wide variety of problems in industrial, clinical, regulatory, and research laboratories, where it could perform 1000 or more quantitative analyses per day. This is already possible in normal mass spectrometry with direct liquid introduction systems (*9*) and commercial sample changers; instrument reliability will be a critical factor in achieving such a goal.

A second area in which MS-MS appears to have a high potential is the analysis and characterization of macromolecules, including biomedical samples, plant materials, crude oil, and commercial polymers. A key problem is that of obtaining mass spectral information from nonvolatile compounds (*10–12, 68*). When further progress is made in this direction it may be possible to introduce complex biological material into the ion source of a tandem double-focusing MS-MS (*39*) and use CI to selectively produce $(M + H)^+$ ions, corresponding to polypeptides and other species with a high proton affinity, for unit mass separation in MS-I; this should be possible to *m/z* 50,000 for 2-kV ions. The secondary CAD spectrum of such a polypeptide ion could provide complete information about its amino acid sequence; this can be done for peptides of molecular weight 2000 (*60*). It may be possible to do this with picomole quantities of material, although the sensitivity is highly dependent on a number of to-be-determined factors, such as ionization efficiency and computer-controlled data acquisition.

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Bioselective Membrane Electrode Probes

Garry A. Rechnitz

One of the most rapidly expanding research areas relating to analytical measurements is the development of potentiometric membrane electrodes with selectivity for ions, dissolved gases, and biological materials. Activity in this field is so intense that new publications are appearing at a rate approaching 500 per year (*1*). Entirely aside from the appealing practical possibilities for such potentiometric membrane electrodes, it appears that new research directions have been directly stimulated by the timely infusion of concepts from physics (*2*) and biology (*3*). Some of the consequences of the latter are examined in this article.

Potentiometric membrane electrodes have their conceptual origin in the ubiquitous *p*H glass membrane electrode which, in its present form, is still the most sensitive and selective of all such electrodes. During the past 20 years, a wide variety of conventional potentiometric ion-selective membrane electrodes based on glasses (*4*), crystals (*5*), and various liquid membranes (*6–8*) has been developed and commercialized. Several recent reviews (*1*) and monographs (*9*, *10*) give comprehensive accounts of this work.

Throughout the period of ion-selective electrode development, efforts were made to extend the measurement capabilities of membrane electrodes to biological materials through the use of enzyme catalysis to convert substrates to species that could be sensed by ion-selective membrane electrodes. The resulting "enzyme electrodes" represent an increasingly practical, but now largely conventional, means of effecting bioselective measurements with membrane electrodes (*11*).

A special impetus was given to research on bioselective membrane electrodes in the early 1970's when stable and reliable potentiometric sensors for ammonia, carbon dioxide, hydrogen sulfide, and other dissolved gases became commercially available on a routine basis. Such electrodes combine the technology of ion-selective membrane electrodes with that of microporous synthetic membranes (*12*). The ammonia gas–sensing membrane electrode, for example, is a potentiometric sensor in which a hydrophobic gas-permeable membrane is superimposed on a flat *p*H-type glass membrane electrode in contact with a thin layer of ammonium chloride electrolyte solution. This arrangement gives exceptional selectivity for the measure-

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