Kirk-Othmer Encyclopedia of Chemical Technology.

ANALYTICAL METHODS, HYPHENATED INSTRUMENTS

In 1990, Chemical Abstracts' Service listed over 10 million substances in their Registry. Moreover, the growth of new compounds is exponential, leading to a doubling of known chemicals every eleven years. Thus there is an ever increasing need to efficiently identify substances and quantitate material with high confidence. Hyphenated instruments, combinations of accepted instrumental techniques where the sample is passed from one instrument directly into another, were developed to aid in solving this problem (1).

Hyphenated analytical methods provide more complementary information in a shorter time period leading to faster and more reliable results, than data obtained from traditional instrumental methods. The types of analytical instruments that can be joined is very large depending only upon the nondestruction of samples after the initial analytical procedure and the ability of the manufacturer to interface the instrumental techniques. Combinations include separation–separation, separation–identification, and identification–identification techniques (see Analytical methods, survey).

Many of the more challenging analysis problems involve mixtures in a complex matrix. Thus an important first step is the ability to separate the components of these mixtures. Some of the earliest hyphenated techniques were those that coupled two separation methods, eg, gas chromatography/gas chromatography (gc/gc), and liquid chromatography/gas chromatograph (lc/gc) (see Chromatography). For example, using lc/gc, small quantities of pesticides extracted from a crop can be determined. Samples are first injected into the lc where the majority of the matrix is separated out, then the "heart cut" of liquid containing the pesticide analyte is routed directly to the gas chromatograph for further separation and quantitation (see Trace and residue analysis).

Table 1 lists the most significant of the hyphenated instruments that were commercially available as of 1990. The instruments and methods discussed in detail herein all have both separation and identification capability.

1. Gas Chromatography/Mass Spectrometry

Mass spectrometry (qv) is the technique by which, utilizing electric and magnetic fields, ions are separated according to mass-to-charge ratios. The method of introducing an analyte into a mass spectrometer can be varied to accommodate gaseous, liquid, or solid samples. The combination of a gas chromatograph and a mass spectrometer (gc/ms) is particularly important because it can accommodate a wide variety of samples. The history of gc/ms dates to the late 1950s; many advances in this technique were made in the 1960s (2).

The combination of gas chromatography and mass spectrometry offers several advantages over the use of these techniques individually. Capillary gas chromatography provides very high separation efficiency for complex organic mixtures such as those found in environmental, foods and flavors, and general industrial applications. The mass spectrometer adds to this separation capability the unique ability to provide molecular structural information leading to both identification and quantitation of the separated components. Thus the gc/ms is a significant labor and time-saving device, accomplishing separation, component transfer, identification, and quantitation, often accompanied by a printed final report, all in a single instrument.

Table 1. Commercially Available Hyphenated Instruments

Instrument[a]	Manufacturers	Price range, $\$ \times 10^3$	Estimated sales 1990[b], $\$ \times 10^6$
gas chromatograph/gas chromatograph (gc/gc)	Siemens AG, Carlo Erba	25	
gas chromatograph/liquid chromatograph (gc/lc)	Carlo Erba	35	
gas chromatograph/mass spectrometer (gc/ms)	Hewlett-Packard, Finnigan, VG Instruments, Varian	50–500	175
gas chromatograph/infrared spectrometer (gc/ir)	Hewlett-Packard, Nicolet, Digilab, Perkin-Elmer,	70–250	26
gas chromatograph/atomic emission spectrometer (gc/ae)	Hewlett-Packard	90	
liquid chromatograph/mass spectrometer (lc/ms)	Hewlett-Packard, Finnigan, VG Instruments	130–300	25
mass spectrometer/mass spectrometer (ms/ms)	Finnigan, VG Instruments, Kratos, Extrel	250–500	258[c]
gas chromatograph/mass spectrometer/infrared spectrometer (gc/ms/ir)	Hewlett-Packard	115	
super critical fluid chromatograph/infrared spectrometer (scfc/ir)	Nicolet, Digilab	100–200	
inductively-coupled plasma/mass spectrometer (icp/ms)	VG Instruments, Perkin Elmer	150–600	23

[a]Whereas analytical techniques are often abbreviated using capital letters, *Encyclopedia* style is to use the lower case.
[b]Strategic Directions International, Inc., Los Angeles, Calif.
[c]Includes all quadruple and magnetic sector ms (no gc/ms) of which ms/ms is a subset.

1.1. Instrumental Interfaces

The basic objective for any coupling between a gas chromatograph (gc) and a mass spectrometer (ms) is to reduce the atmospheric operating pressure of the gc effluent to the operating pressure in the ms which is about 10^{-3} kPa (10^{-5} torr). Essential interface features include: the capability to transmit the maximum amount of sample from the gc without losses from condensation or active sites promoting decomposition; no restrictions or compromises placed on either the ms or the gc with regard to resolution of the components; and reliability. The interface should also be mechanically simple and as low in cost as possible.

There are three common types of gc/ms interfaces: molecular separators, open split couplings, and capillary direct interfaces. Of the molecular separators, the two most often used are the jet separator (3) and the membrane separator (4, 5). The jet separator shown in Figure 1 produces a jet spray out of the gc column effluent. Upon exiting the jet the effluent enters an evacuated chamber where the lower molecular weight components, including the majority of the gc carrier gas, are pumped away to the vacuum pump. The higher molecular weight components, having more momentum, are able to maintain a straight trajectory into the ion source of the mass spectrometer.

In membrane separators a thin sheet of organic elastomer is used to selectively absorb organic compounds in the gc effluent and transmit them to the ion source. The permeability of an effluent component depends on its solubility in the membrane material and its diffusion constant. Gases such as helium, a gc carrier gas, have very low solubility in the membranes as compared to a substance such as decane, so the elastomer is generally an effective barrier for gc carrier gas. Membrane separators are often used for applications involving low molecular weight analytes because of the potential loss of these species in using the jet technique. For higher molecular weight compounds, the jet separator is most often used to avoid possible low solubility in the membrane.

One method of interfacing without the use of a molecular separator is employing an open split where a portion of the gc effluent is pumped away before entering the ms ion source (6). The open split technique has

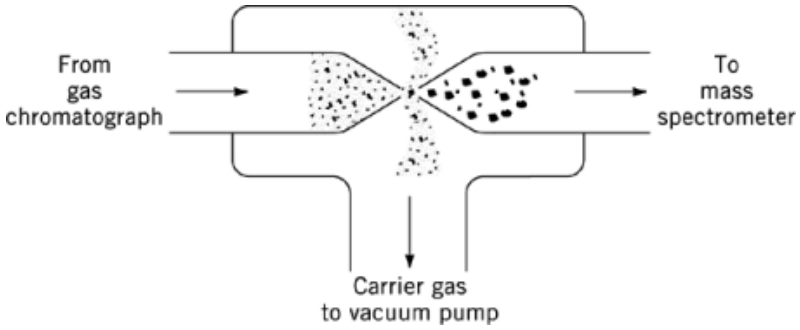

Fig. 1. Jet separator gc/ms interface.

some advantages over the molecular separators including compatibility with all column sizes, easy column handling, wider choice of carrier gases including hydrogen, and lower cost. Disadvantages for the open split approach include line broadening that results from dead volume, potentially active surfaces, variable split efficiency with some discrimination favoring compounds of higher molecular weight, and sensitivity loss resulting from the split. The introduction in the 1980s of higher pumping capacity coupled with the widespread use of fused silica capillary gc columns, have led to another interfacing approach that is widely used, called the capillary direct interface. In this system, the gc capillary column is positioned directly into the ion source so that all of the gc effluent enters the ms. Advantages are high sensitivity, low dead volumes, and minimal sample decomposition or loss because of system inertness. A disadvantage of the capillary direct approach is that the choice of columns and carrier gas is dependent on the ms vacuum system pumping capacity. Additionally, changing gc columns becomes more time-consuming because of the requisite cooling and venting of the ion source.

1.2. Economic Aspects

The costs of gc/ms systems depend primarily on the type of mass analyzer used and the sophistication of the computer controlling the system (see Computer technology). Low end benchtop models controlled by a DOS-based personal computer cost around $50,000. Midrange ($100,000–250,000) machines may add higher mass range, a wider variety of ionization techniques, more pumping capacity, and more powerful multitasking computers. Then, at the high end, there are the magnetic sector-based systems which offer high resolution, mass spectra, and a price tag of $250,000–500,000.

1.3. Applications

One of the most frequently used tools for systems requiring both qualitative and quantitative chemical analysis is gc/ms. It has been particularly valuable in the environmental field where laboratories responsible for the monitoring of hazardous compounds depend heavily on gc/ms to separate components of a usually complex mixture present in an environmental sample and then to identify and quantitate each component. One example is the analysis of semivolatile organics in drinking water (see Groundwater monitoring). This method, designated by the U.S. Environmental Protection Agency as Method 525 (7), requires full scan sensitivity down to 100 pg for 41 out of 43 target compounds. Drinking water contaminants are first concentrated using liquid–solid extraction cartridges which are eluted with a small volume of methylene chloride (see Extraction, liquid–solid). The concentrated extract is then injected into the gc/ms to produce a total ion chromatogram (TIC) as shown in Figure 2 which also lists the target compounds for Method 525.

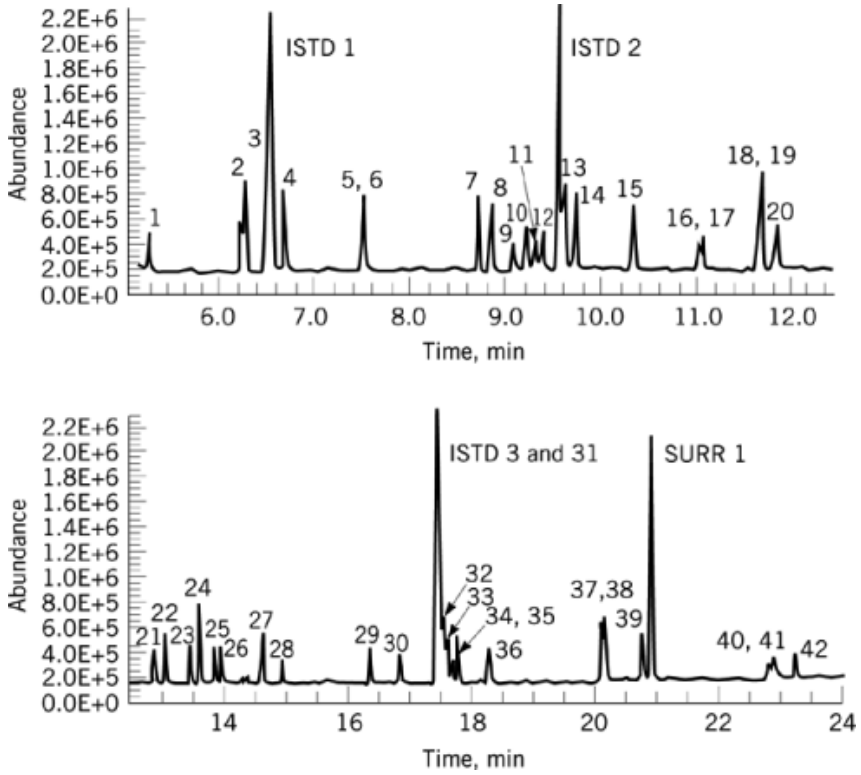

Fig. 2. Total ion chromatogram of drinking water. Analysis by EPA Method 525 using gc/ms. $_{ISTD1=}$ d_{10}-acenaphthene, $_{ISTD2=}$ d_{10}-phenanthrene, and $_{ISTD3=}$ d_{10}-chrysene are internal standards; $_{SURR1=}$ d_{12}-perylene is a surrogate splitless peak; and peaks 1–42 are 1. hexachlorocyclopentadiene, 2. dimethylphthalate, 3. acenaphthylene, 4. 2-chlorobiphenyl, 5. diethylphthalate, 6. fluorene, 7. 2,3-dichlorobiphenyl, 8. hexachlorobenzene, 9. simazine, 10. atrazine, 11. pentachlorophenol, 12. lindane, 13. phenanthrene, 14. anthracene, 15. 2,4,5-trichlorobiphenyl, 16. heptachlor, 17. alachlor, 18. di-*n*-butylphthalate, 19. 2.2′,4,4′-tetrachlorobiphenyl, 20. aldrin, 21. heptachlor epoxide, 22. 2,2′,3′,4,6-pentachlorobiphenyl, 23. gamma-chlordane, 24. pyrene, 25. alpha-chlordane, 26. *trans*-nonachlor, 27. 2,2′,4,4′,5,6′-hexachlorobiphenyl, 28. endrin, 29. butylbenzylphthalate, 30. di(2-ethylhexyl)adipate, 31. benz[*a*]anthracene, 32. chrysene, 33. 2,2′,3,3′,4,4′,6-heptachlorbiphenyl, 34. methoxychlor, 35. 2,2′,3,3′,4,5′,6,6′-octachlorobiphenyl, 36. di(2-ethylhexyl)phthalate, 37. benzo[*k*]fluoranthene, 38. benzo[*b*]fluoranthene, 39. benzo[*d*]-pyrene, 40. indeno[1,2,3,c,d]pyrene, 41. dibenz[*a,h*]anthracene, 42. benzo[*g,h,i*]perylene.

Important to environmental analysis is the ability to automate the injection, as well as the identification and quantitation of large numbers of samples. Gc/ms systems having automatic injectors and computerized controllers have this capability, even producing a final report in an unattended manner. Confirmation and quantitation are accomplished by extracting a specific ion for each of the target compounds. Further confirmation can be obtained by examining the full scan mass spectrum.

A technique that is related to gc/ms and starting to grow significantly in use is that of inductively coupled plasma/mass spectrometry (icp/ms). In icp/ms the inductively coupled plasma serves as a good interface and ion source for the quadrupole mass spectrometer (see Plasma technology). Trace elemental analysis of very complex mixtures are possible using icp/ms. Anything that is introduced to the plasma is totally and cleanly ionized to elemental form, then passed into the mass spectrometer for identification. Gold has been determined at the femtogram level in seawater using flow injection icp/ms (8).

2. Gas Chromatography/Infrared Spectroscopy

Gc/ir instruments are all of the gc/ftir variety that utilize an interferometer and require a Fourier transform of the signal as opposed to employing a monochrometer and mechanical scanning. At least 3 scan/second are needed across the capillary gc peaks which are from 3 to 6 seconds wide.

2.1. Instrumental Interface

Gc/ftir instrumentation has developed around two different types of interfacing. The most common is the on-the-fly or flow cell interface in which gc effluent is directed into a gold-coated cell or light pipe where the sample is subjected to infrared radiation (see Infrared and raman spectroscopy). Infrared transparent windows, usually made of potassium bromide, are fastened to the ends of the flow cell and the radiation is then directed to a detector having a very fast response-time. In this light pipe type of interface, infrared spectra are generated by ratioing reference scans obtained when only carrier gas is in the cell to sample scans when a gc peak appears.

Another type of gc/ftir interface involves trapping the components as they elute from the gc column. The gc effluent is deposited onto a zinc selenide plate at liquid nitrogen temperature, then the deposited trace of effluent is moved into the optical path of a standard ftir spectrophometer. A variation of this approach is referred to as matrix isolation: the gc effluent is trapped in a matrix of argon on a gold-coated drum at liquid helium temperature. Advantages of isolation interfaces over the flow cell design are about an order of magnitude increase in sensitivity (in the range of tens or a hundred picograms for a given component), and the ability of the user to search condensed phase spectral libraries that are larger than vapor phase libraries (see Databases; Information retrieval). Disadvantages include the complexity of the systems leading to lower sample throughput and much higher price.

3. Gas Chromatography/Mass Spectrometry/Infrared Spectroscopy

Gas chromatography/fourier transform infrared spectroscopy (gc/ftir), itself a very useful analytical technique, is especially powerful when combined with mass spectrometry (9, 10). One of the factors in making the ternary gc/ir/ms system a viable analytical tool was the development of faster, more powerful computers to acquire, analyze, and report the large quantities of data generated by the mass spectrometer and the ftir. At first pyrolysis gc was carried out by trapping the effluents and analyzing them by both ir and ms (11, 12). Then, as a result of the growth and enhancement of computer hardware throughout the 1970s, the first gc/ir/ms system was demonstrated in 1981 (13).

3.1. Instrumental Interface

Gc/ir/ms systems are possible because the infrared technique is nondestructive of sample. Thus several options are available for interfacing the ir and ms instrumental components including both serial and parallel methods. For the serial configuration the gc effluent must first go through the ir flow cell and then into the ms. Parallel methods include utilizing a splitter at the end of the gc column which directs a portion of the effluent to each of the ir and ms instruments. Another parallel approach employs two capillary gc columns in a single injection port such that the effluent of one column is directed into the ir, the effluent of the other into the ms.

Each interfacing technique has certain advantages that depend on the application, but the serial approach is a good first choice because it allows the maximum amount of sample into the ir which generally has a lower sensitivity than the ms. In addition, matching corresponding ir and ms peaks in complex multicomponent chromatograms is more straightforward in serial instruments because each peak in the ms chromatogram

(TIC) trails by a few seconds the peak in the ir chromatogram also known as the Gram-Schmidt or total response chromatogram (TRC).

3.2. Economic Aspects

Costs for gc/ir/ms instruments vary widely depending on the sophistication of the components. At the lower end of the scale is the flow cell type gc/ftirs connected to a benchtop mass spectrometer. These are available for about $115,000–150,000. The isolation type gc/ftir can also be interfaced with a benchtop mass spectrometer. The prices range from about $200,000–300,000.

3.3. Applications

The capabilities of a gc/ir/ms in separating and identifying components in complex mixtures is very high for a broad spectrum of analytical problems. One area where ir information particularly complements ms data is in the differentiation of isomeric compounds. An example is in the analysis of tricresyl phosphates (TCPs) used as additives in a variety of products because of their lubricating and antiwear characteristics (see Lubrication and lubricants). One important use of TCPs is in hydraulic fluid where they tenaciously coat metal surfaces thereby reducing friction and wear. Tricresyl phosphate [1330-78-5], $C_{21}H_{21}O_4P$, exists in a variety of isomeric forms and the commercial product is a complex mixture of these isomers.

Animal feeding studies showed that TCPs containing an ortho-substituted aromatic ring are much more toxic than the other TCP isomers (14). Thus to check for a potential health and safety hazard it was necessary to analyze the TCPs in the hydraulic oil. The gc/ir/ms technique proved very useful. The gc separated the bulk of the oil from the TCPs which appeared as four small peaks late in the chromatogram at a temperature of about 250°C; ms data confirmed that the four small peaks were indeed TCPs as evidenced by a strong molecular ion (M-1) peak at 368 m/z. However, the fragmentation pattern for each of the isomers was nearly identical. The ir data, on the other hand, provided the substitution pattern information given in Figure 3. Ir absorption rules for aromatic substitution clearly identify the TCP isomers present in the hydraulic oil as trimeta (TMCP), metametapara (MMPCP), metaparapara (MPPCP), and tripara (TPCP) without the need for analytical standards. Thus the question of the presence of any of the toxic ortho-isomers was answered clearly, conveniently, and with high confidence using gc/ir/ms.

Another analysis handled effectively by use of gc/ir/ms is essential oil characterization which is of interest to the foods, flavors, and fragrances industries (see Oils essential). Even very minor components in these complex mixtures can affect taste and aroma. Figure 4 shows the TRC and TIC for Russian corriander oil which is used extensively in seasonings and perfumes (15). The ir and ms are serially configured. Spectra can be obtained from even the very minor gc peaks representing nanogram quantities in the ir flow cell.

The confidence level in the identification capability for gc/ir/ms is enhanced by the ability to perform computer library searching of large spectral databases. Unknown spectra are searched against reference databases and a hit quality number, indicating how well the unknown spectrum matches the library spectra, is generated. For the very small peak at 19 min in Figure 4, peak 6 in the TIC, the library search identified the component as α-phellandrene [99-83-2], $C_{10}H_{16}$, for both the ir and ms data. Because these data are complementary and generated from two completely independent principles of detection, this method provides virtually irrefutable evidence as to a component's correct identity. The complementary nature of the data is further demonstrated by another component in this essential oil. Late in the chromatogram is a long-chain aliphatic alcohol. The mass spectral data confirmed an unsaturated aliphatic chain of specific length, but because of dehydration in the ion source of the ms no alcohol group was evident. On the other hand, the infrared spectrum indicated the presence of an alcohol functionality, but gave little evidence with regard to the length of the aliphatic chain. Thus the combination of complementary ir and ms data was the key to the correct identification of this compound.

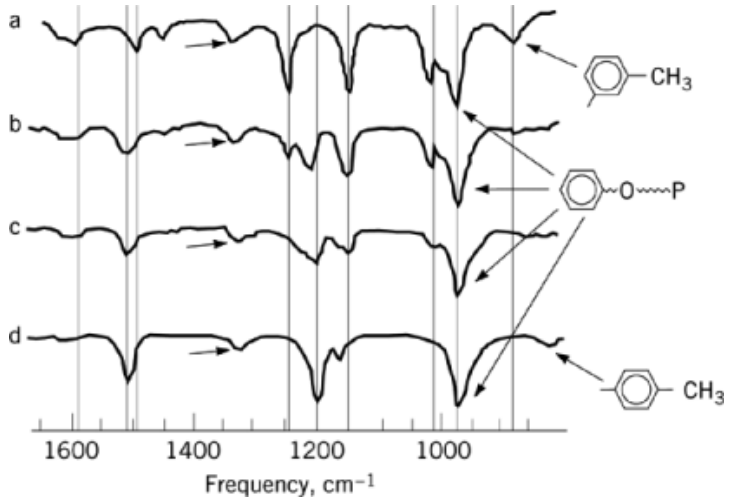

Fig. 3. Infrared absorption data for the four tricresyl phosphate isomers present in hydraulic oil. a, Trimetacresyl phosphate; b, metametaparacresyl phosphate; c, metaparaparacresyl phosphate; d, triparacresyl phosphate. The $_{P=O}$ stretch between 1300 and $_{1350\ cm^{-1}}$ is marked as is the phenyl–phosphate vibration around $_{965\ cm^{-1}}$. Also included are the metacresyl ($_{875\ cm^{-1}}$) and paracresyl ($_{820\ cm^{-1}}$) frequencies.

4. Liquid Chromatography/Mass Spectrometry

The replacement of a gas chromatograph with a liquid chromatograph (lc) substantially enhances the separation of compounds which might decompose at the operating temperatures of the gc or which are not readily volatilized. However, lc/ms coupling presents a problem in terms of removing the large quantities of lc solvent. The lc/ms technique had its beginnings as early as 1973–1974 (16–18). In the early stages of lc/ms, there was much debate on whether the combination should be an off-line or on-line process because the quantity, about 1–2 mL/min, of solvent is far more than the ms pumping system can handle. Newer instruments utilize direct introduction, on-line techniques.

4.1. Instrumental Interfaces

The ideal lc/ms interface should place no compromises on either the lc or the ms. Decomposition or loss of analyte should be avoided and efficient transfer provides for optimum sensitivity.

One direct lc/ms interface is referred to as the particle beam approach based on the Monodisperse Aerosol Generation Interface for lc (MAGIC) (19, 20). The MAGIC is actually a rather simple transport device, similar to a two-stage jet separator, where the solvent vapor is pumped away and the analyte particles are concentrated in a beam that is allowed to enter the ms ion source to be vaporized and ionized by electron impact. The particle beam interface consists of four main sections: nebulizer, desolvation chamber, momentum separator, and transfer probe. In the nebulizer the lc effluent is joined by a stream of helium and converted into an aerosol of droplets having a narrow range of diameters. As the droplets move through the desolvation chamber, which is held close to ambient pressure and temperature, the solvent is vaporized creating a mixture of helium, solvent vapor, and analyte particles. As the mixture advances toward the lower pressure, two-stage momentum separator, it is focused into a narrow beam that expands at supersonic speed as it exits the jet nozzle entering the momentum separator. In the separator, the lower mass solvent vapor is pumped and skimmed away while

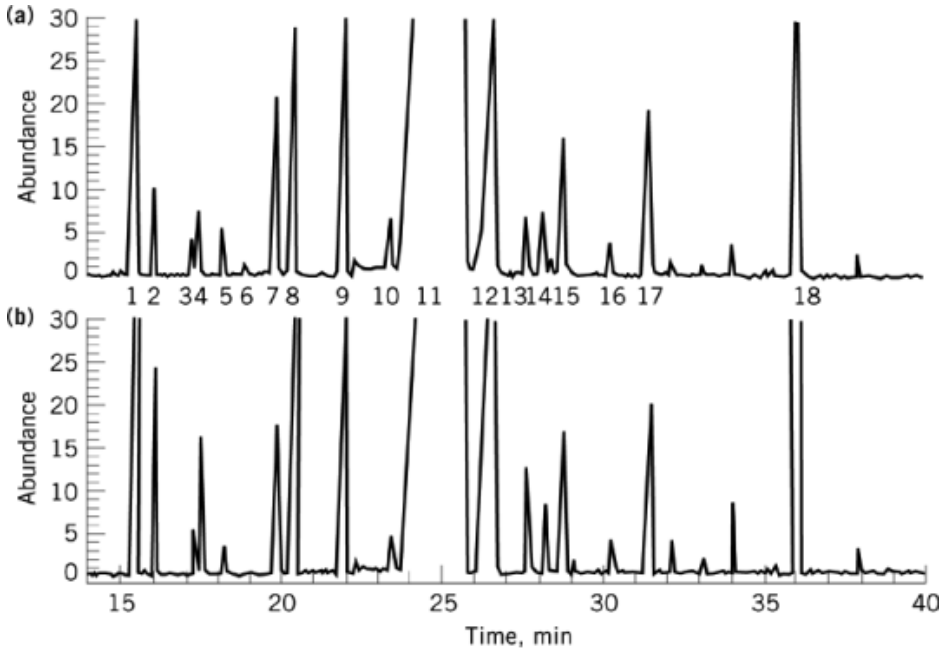

Fig. 4. Chromatograms of Russian coriander oil injected into a serially configured gc/ir/ms: (**a**) total ion chromatogram (TIC); (**b**) total response chromatogram (TRC).

the higher mass, and thus higher momentum, particles pass in a narrow beam through the transfer probe into the ms ion source where they strike the heated source wall and are vaporized.

Another popular type of on-line lc/ms coupling is called the thermospray interface. In contrast to the particle beam approach, thermospray serves as a means for both droplet formation and ionization. The lc effluent first enters the thermospray vaporizer probe where partial vaporization of the liquid occurs to form droplets. As the liquid droplets evaporate and decrease in size, ions present in the droplets are ejected. These ionized molecules then diffuse through a chamber and exit through a small sampling cone into the high vacuum chamber. The ions exit, are directed into the quadrupole analyzer, and are detected in the electron multiplier. This technique provides soft ionization, ie, formation of molecular adduct ions having minimal fragmentation and structural information. Volatile buffers such as ammonium acetate are used to enhance ionization and to provide a reagent for chemical ionization of the analyte. An electric discharge such as a Townsend discharge may be used, if desired, along with an electron filament to create additional fragmentation and to increase sensitivity. Generally, the filament-on mode produces more abundance than the filament-off mode. Temperature control of the vaporizer process is required for optimum sensitivity and reproducibility.

The particle beam and thermospray interfacing techniques are actually complementary in nature. The particle beam approach is the best choice for producing electron impact spectra which can then be library searched against spectral databases. In addition, the particle beam approach is somewhat less complex and easier to use than the thermospray technique. The thermospray approach, which produces chemical ionization spectra, is very useful for high molecular weight compounds that have ion volatility. Because there is very little fragmentation when using the thermospray, selected ion monitoring (sim), where sensitivity is enhanced usually several orders of magnitude over full scan mode, can be effectively used.

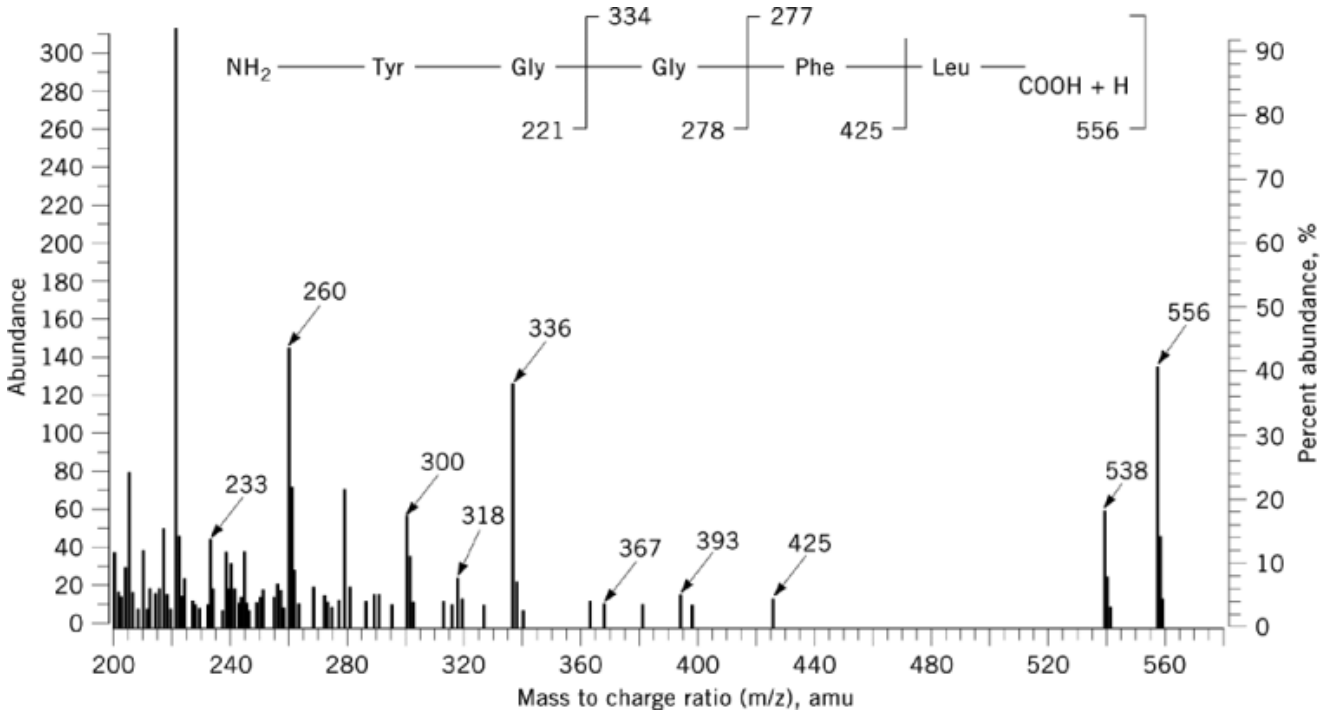

Fig. 5. Thermospray lc/ms analysis of leucine-enkephalin mol wt 555.

4.2. Economic Aspects

The lc interface to a mass spectrometer is usually provided as an option to the more sophisticated, full capability instruments. Some systems can have both lc and gc interfaces and can conveniently switch between the two modes. Lc interfaces are approximately $30,000 bringing a lc/ms that includes a data system into the $130,000–180,000 range.

4.3. Applications

The primary advantage of an lc/ms is in combining the separation of large, potentially thermally labile compounds with the qualitative and quantitative features of the mass spectrometer. As the need to analyze mixtures containing larger biological molecules increases in the fields of pharmaceuticals (qv), agricultural chemistry, and biotechnology, the demand for lc/ms should also increase.

When the compounds of interest are fragile and thermally labile, thermospray lc/ms is a good choice. Figure 5, shows the thermospray spectrum for leucine enkephalin [58822-25-6], a pentapeptide of molecular weight 555. The lc/ms approach has been very helpful in unraveling the structure of large biological molecules (21).

Lc/ms has also been effective in environmental analysis. Many compounds designated hazardous that require monitoring are either too heavy or too thermally labile to pass through a gas chromatograph. The polyaromatic hydrocarbons (PAHs), especially those having five or more rings, are an example. Figure 6 shows the particle beam lc/ms analysis of a hazardous waste sample. The compound at about 23 min in the TIC was library searched using a large database of electron impact spectra and found to be benzo[*a*]pyrene [50-32-8], $C_{20}H_{12}$, mol wt 252.3.

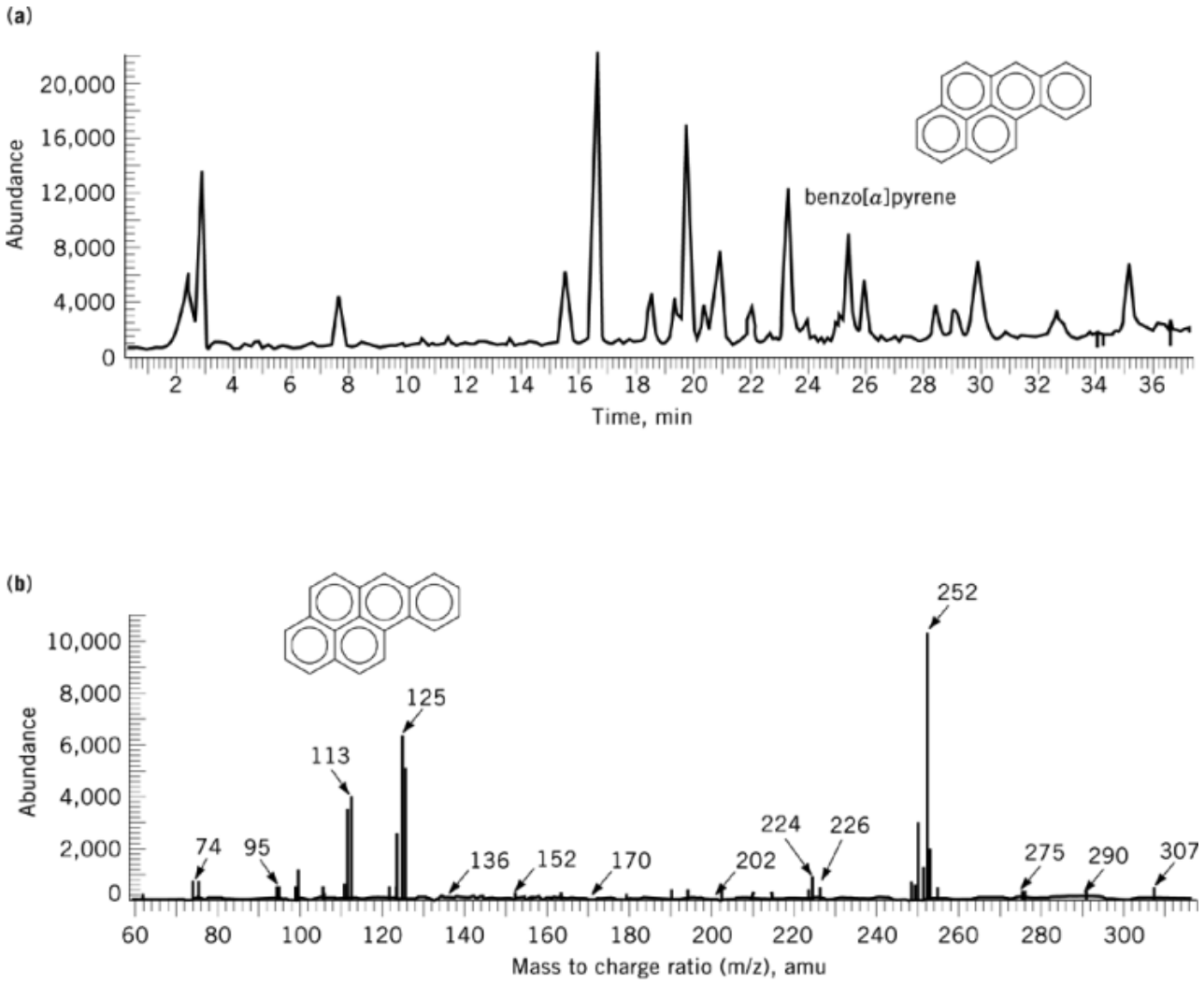

Fig. 6. Particle beam lc/ms analysis of a complex hazardous waste sample (**a**) TIC showing peak at 23.23 min; (**b**) mass spectrum of 23.23 min peak of TIC.

4.4. Other Techniques

A growing technique related to lc/ms and regarded as complementary to it is that of capillary zone electrophoresis/mass spectrometry (cze/ms) (22). Using cze/ms, high resolution separation of water-soluble compounds is accomplished by the principles of electrophoresis (qv). The sample is then coupled to the mass spectrometer by electrospray ionization (23) or a fast atom bombardment interface (fab) to produce molecular ions (24). Biotechnology applications of cze/ms have great potential (25).

5. Mass Spectrometry/Mass Spectrometry

Tandem mass spectrometry or ms/ms was first introduced in the 1970s and gained rapid acceptance in the analytical community. The technique has been used for structure elucidation of unknowns (26) and has the ability to provide sensitive and selective analysis of complex mixtures with minimal sample clean-up (27). Developments in the mid-1980s advancing the popularity of ms/ms included the availability of powerful data systems capable of controlling the ms/ms experiment and the viability of soft ionization techniques which essentially yield only molecular ion species.

Ms/ms is based on the characterization of selected ions in the mass spectrum of a sample through further fragmentation and analysis. Fragmentation can be spontaneous or induced, although most instruments rely heavily on induced ion dissociations. Collisional activation is the most common approach. The system consists of an ion source, two mass analyzers separated by a fragmentation region, and an ion detector. The principles are straightforward and can be compared to conventional gc/ms. A sample mixture is introduced into the ion source where ionization takes place producing ions characteristic of the individual components. The separation of the component of interest is then achieved by the mass selection of a characteristic ion of the analyte by the first mass analyzer. This parent ion undergoes collisionally activated dissociation through collisions with neutral gas molecules in the fragmentation region to yield daughter ions which are analogous to the fragmentation occurring in the initial gc/ms ionization step. Identification of the separated components is accomplished by mass analysis of the daughter ions in the second mass analyzer.

The most common modes of operation for ms/ms systems include daughter scan, parent ion scan, neutral loss scan, and selected reaction monitoring. The mode chosen depends on the information required. Structural identification is generally obtained using daughter or parent ion scan. The mass analyzers commonly used in tandem systems include quadrupole, magnetic-sector, electric-sector, time-of-flight, and ion cyclotron resonance. Some instruments add a third analyzer such as the triple quadrupole ms (27).

The main advantages of the ms/ms systems are related to the sensitivity and selectivity they provide. Two mass analyzers in tandem significantly enhance selectivity. Thus samples in very complex matrices can be characterized quickly with little or no sample clean-up. Direct introduction of samples such as coca leaves or urine into an ms or even a gc/lc/ms system requires a clean-up step that is not needed in tandem mass spectrometry (28, 29). Adding the sensitivity of the electron multiplier to this type of selectivity makes ms/ms a powerful analytical tool, indeed. It should be noted that introduction of very complex materials increases the frequency of ion source cleaning compared to single-stage instruments where sample clean-up is done first.

5.1. Economic Analysis

Costs of ms/ms instrumentation remain at the high end of the scale for hyphenated systems. Because more powerful computer systems are becoming available at lower cost and improvements are being made in the less expensive ms hardware, the trend in instrumental cost is downward. The current range for ms/ms systems extends from about $350,000 to about $1.4 million where the ms components are equipped with higher mass and resolution capabilities.

5.2. Applications

Ms/ms has found application in areas such as trace analysis of biological tissue (30), complex hazardous waste site samples (31), and human blood serum (32), as well as in drug testing (33). Anabolic steroids are synthetic derivatives of the male sex hormone testosterone. Although many athletic governing bodies, eg, the International Olympic Committee, have strict rules prohibiting use, these drugs are utilized for both human and equine performance enhancement in sporting events. Routine testing of participants is performed.

One anabolic steroid, the presence of which has proven difficult to analyze, is stanozolol [10418-03-8], $C_{21}H_{32}N_2O$. A metabolite of the parent drug, hydroxy stanozolol, detected in equine urine eight hours after ingestion of the parent drug is actually identified, usually at very low levels. Analysis was done by lc/ms/ms which had a shortened analysis time advantage over gc/ms procedures because of the elimination of the need for a derivatization step (33).

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