Mass spectrometry and drug analysis an introduction.

Linda Jane. Linnell

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**LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS RECUÉ**
MASS SPECTROMETRY AND DRUG ANALYSIS

AN INTRODUCTION

BY

LINDA JANE LINNELL

A Major Clinical Chemistry Critique
Submitted to the Faculty of Graduate Studies through the
Department of Chemistry in Partial Fulfillment
of the Requirements for the Degree of
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Windsor, Ontario
Canada

1981
ABSTRACT

MASS SPECTROMETRY AND DRUG ANALYSIS
AN INTRODUCTION

BY
LINDA JANE LINNELL

In an effort to appeal to newcomers to the field of mass spectrometry, this critique provides a reasonably comprehensive, but less technical description of instrumental principles and variations along with analytical techniques commonly used. Applications of mass spectrometry to drug analysis as required in the fields of pharmacology, toxicology, and clinical chemistry have been used to provide more specific examples of the potential of mass spectrometry.

Finally, the suitability and future possibilities for the use of mass spectrometry in a routine clinical laboratory are discussed.
ACKNOWLEDGEMENTS

I would like to thank Dr. Roger Thibert for his kindness and encouragement over the many years it has taken me to reach this level of my education, and Dr. Gordon Wood, who patiently endured my frustration at the slower pace of research work relative to the hospital laboratory environment.

I would also like to acknowledge Health and Welfare Canada, Research on Drug Addiction Branch for providing the summer scholarship that introduced me to mass spectrometry and the exciting potential for its use in clinical medicine.

Finally, I am very grateful to Mrs. Valerie Walter, who did an excellent job of typing this manuscript, and to my co-workers and students at Grace and Windsor Western Hospitals and at St. Clair College for their understanding.
DEDICATION

To my dear family

ALBERT, SCOTT, and CORI

without whose patience and support I could not have succeeded in this undertaking.
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LIST OF ABBREVIATIONS AND SYMBOLS

ADC - analogue-to-digital converter
API - atmospheric pressure ionization
BANS-Cl - 5-di-n-butylaminonaphthalene-1-sulfonyl chloride
Cl - chemical ionization
COM - computer system
dc - direct current
DPH - diphenylhydantoin
EI - electron impact ionization
EMIT - enzyme-multiplied immunoassay technique (Syva)
FI - field ionization
FD - field desorption
GC - gas chromatography
GLC - gas-liquid chromatography
H - magnetic field strength
HPLC - high pressure (or performance) liquid chromatography
IIC - integrated ion current
IP - imipramine
LC - liquid chromatography
M+ - molecular ion
m - mass
m/z - mass-to-charge ratio
MS - mass spectrometer or mass spectrometry
PFK - perfluorokerosene
R - resolution
r - radius of sector curvature
rf  - radio frequency
RIA  - radioimmunoassay
SIM  - selective (selected) ion monitoring
TLC  - thin-layer chromatography
tof  - time-of-flight mass analyzer
tris-BP  - tris(2,3-dibromopropyl)phosphate
Vac  - accelerating voltage applied to ions entering the mass analyzer
CHAPTER I
INTRODUCTION

It is the intent of this paper to present a reasonably comprehensive but more general view of mass spectrometry than is found in the usual technical manuals. In addition to basic descriptions of the main instrumental principles and components, examples of its use in the field of drug analysis are presented to provide insight into the possibilities for application to an infinite array of biochemical investigations and clinical analyses. Enough references are given to provide a route for pursuing more detailed considerations. It is hoped that this approach will appeal to the non-specialist like myself, and to students with a general science background who may have occasion to consider the use of mass spectrometry for particular analytical applications, especially in the field of biochemistry and clinical chemistry.

A mass spectrometer is basically an apparatus which vaporizes compounds of widely varying volatility, produces ions from the neutral atoms or molecules in the vapor, separates the ions according to their mass-to-charge ratios, and provides output signals which are recorded to show the relative abundance of each ionic species present. Although both positive and negative ions can be studied, most instruments are used to investigate positive ions.
since they are produced in greater numbers. The mass-to-charge ratio (m/z) is the ratio of the mass (m) of the ion to the number of electrons (z) lost during ionization.

The main components of a mass spectrometer (MS) system are illustrated in Figure 1 and will be described in more detail in subsequent sections.

In Figure 2, a mass spectrum of carbon dioxide illustrates the type of result which may be obtained by mass spectrometry (MS). The m/z scale on the abscissa shows the mass-to-charge ratio of the various ions which have been detected. In addition to the carbon dioxide "parent ion" at m/z 44, most of the other peaks have been identified as fragment ions of the carbon dioxide or as fragment ions containing less abundant isotopes of carbon and oxygen. Note that the carbon dioxide ion indicated at m/z 22 is doubly charged due to the loss of two electrons during ionization and this results in an apparent mass one half that of the corresponding singly charged parent ion. The relative intensity shown on the ordinate refers to the peak heights and indicates the relative abundance or number of ions detected in each separate ion beam. In this mass spectrum, many peaks have been compressed for more convenient illustration, as indicated by the factors beside them.
FIGURE 1

DIAGRAM OF A MASS SPECTROMETER SYSTEM

Legend

Main instrumental components and sequence of analytical steps occurring during mass spectrometry.

Reprinted and modified without permission from reference 1, Chapter 1, p 10.
FIGURE 1

DIAGRAM OF A MASS SPECTROMETER SYSTEM
FIGURE 2

MASS SPECTRUM OF CARBON DIOXIDE

Legend

Positive ion fragments of carbon dioxide produced during mass spectrometry showing the relative intensity and m/z value of each.

Refer to text for further explanation.

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FIGURE 2

MASS SPECTRUM OF CARBON DIOXIDE
Mass spectrometry has been used to investigate a wide variety of problems in many areas of science and industry ranging from basic research to routine analysis and quality control. Besides providing the molecular weight and empirical formula of an unknown substance, a systematic interpretation of fragment ion peaks can yield a detailed picture of the ionization process, which in turn may be used in the determination of the molecular structure. Characteristic spectral patterns then serve as "fingerprints" for identification. This is of special importance when studying organic compounds. In addition, the heights of the peaks in a spectrum relative to each other and to standards may be used to determine the concentration of the substance under study. Further detailed investigation of ionization patterns under varying conditions are also used in the study of reaction mechanisms and thermodynamic properties. In general, however, the mass spectrometer is basically a tool for the identification and quantification of an extremely wide variety of chemicals.

Although the origins and development of MS are described only briefly here, more detail and many references of historical interest are given by Roboz (1) and White (2). In 1886, Sir William Crookes postulated the first modern concept of the isotopic structure of
chemical elements, the proof of which came with the early development of mass spectrometry. During the same year, a German physicist, Eugene Goldstein, was exploring electrical discharges at low pressure in a rudimentary precursor to a cathode ray tube and discovered some type of rays or particles that travelled in a direction opposite to the usual cathode rays. Then, in 1898, W. Wein showed that these rays could be deflected in strong electric and magnetic fields and had a positive charge.

The forerunner of the mass spectrometer or "positive ray analyzer" was built in England by J.J. Thomson in 1911. Positively charged atoms formed in a low pressure discharge tube were accelerated through a magnetic field where they were deflected in specific trajectories based on their mass and charge. These were stopped by a fluorescent screen or photographic plate that allowed direct observation of the final focal points of the various ions. By 1912, Thomson could demonstrate the separation of ions with atomic mass differences of less than ten percent and used this technique to confirm the existence of the isotopic structure of matter by showing that neon existed with a mass of 22 as well as 20.

Thomson's work was continued by his assistant, F.W. Aston, who built the prototype of the modern mass spectrometer and systematically assayed many elements to
determine their isotopic composition. At about the same
time, similar studies and further instrumental variations
were being carried out by A.J. Dempster in Chicago, and
over the next twenty years modifications by people such
as Bainbridge, Mattauch and Herzog, and Nier resulted in
greatly improved precision and mass resolving power. By
the end of the 1930's industrial research was beginning on
mass spectrometry and in 1943 Consolidated Engineering
Corporation in Pasadena sold its first commercial mass
spectrometer to the Atlantic Refining Company for use in
analysis of petroleum fractions. During World War II, MS
was used for monitoring the production of uranium isotopes
for atomic bombs and the manufacturing of synthetic rubber.
The vast potential applications for mass spectrometry
measurements became more evident during the 1950's and
several large corporations began marketing new instruments.
A large number of specialized instruments were also built
by scientists in universities and government laboratories.
In the field of biochemistry, a procedure developed by
D. Rittenberg for estimating the abundance of isotopes of
hydrogen in the mass spectrometer became available in 1952
and further advanced the study of metabolic pathways using
deuterium and isotopes of carbon, nitrogen and oxygen (3).
The founding of the first professional MS group
was sponsored by the American Society of Testing Materials
in March of 1953 in Pittsburg. Today, annual conferences and meetings with world-wide attendance are devoted exclusively to research in mass spectrometry, and many publications are available, including the periodicals *Organic Mass Spectrometry*, *International Journal of Mass Spectrometry* and *Ion Physics*, and *Biomedical Mass Spectrometry*. 
CHAPTER II

INSTRUMENTATION

A. INLET SYSTEMS

The general function of the inlet system is to supply enough sample vapor to the ion source in such a way that its composition accurately represents that of the original sample. This process must allow for the introduction of samples in various states and usually must occur over a pressure drop from atmospheric to the $10^{-5}$ - $10^{-6}$ Torr of the ion source.

Several factors must be considered when choosing the type of inlet to be used (1):

a) Sample volatility. This is one of the prime considerations. The sample must exhibit a finite vapor pressure at the temperature of the ion source (about 250°C) so that a sufficient number of molecules will diffuse into the ionization region.

b) Quantity of sample. Only a small fraction of the sample introduced will actually be ionized and eventually detected, and the amount of sample available is often limited.

c) Purity of sample. This is an especially important consideration when dealing with biological samples.

d) Qualitative versus quantitative information.
sought. This in turn will greatly affect the amount and purity of sample required.

e) Thermal stability at temperatures such as the usual 250°C of the ion source.

f) Reactivity of sample with system components.

g) Type of container sample is in and the difficulty of transfer.

General features and uses of the common types of inlet systems are briefly described in the following sections while further detail may be found in Roboz (1), White (2), Waller (3), and McFadden (4).

1. Batch Inlet

Figure 3 schematically represents a simple batch inlet system where a silicone rubber septum is usually used for liquid sample introduction from a syringe. Vacuum valves isolate and protect the various compartments and the oven temperature can be varied from 150°C to 350°C to allow for variation in sample volatility. An expansion volume of 1-2 liters assures a constant sample pressure over a considerable period (e.g., 30 minutes) while a molecular leak controls the flow of sample gas into the low pressure ionization chamber. The various principles involved in the flow of gas mixtures from the batch inlet into the source are described in McFadden (4) and in Roboz (1).
FIGURE 3

STANDARD BATCH INLET FOR MASS SPECTROMETRY SAMPLE INTRODUCTION

Legend

Sample is injected into a low pressure holding chamber and allowed to leak into the vacuum of the ion source.

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FIGURE 3

STANDARD BATCH INLET FOR MASS SPECTROMETRY SAMPLE INTRODUCTION
Most commercial mass spectrometers have a dual inlet system with separate reservoirs connected so that each may be pumped separately into the ion source. Such a setup reduces the time interval between analyses and allows for fast comparisons with other samples or with reference substances (1).

The batch inlet system is useful for quick inspection of a reaction product, a reagent, or to establish the complexity of an unknown (4). It is ideal for running authentic reference samples for spectral files and the most convenient and accurate method of metering a sample to the mass spectrometer for sensitivity determinations.

There are some limitations to the use of such a system. At least 200 μg of sample is required which is comparatively large and the sample must be both stable and volatile at the inlet temperature. The batch inlet used alone is particularly unsuitable for direct analysis of complex biological samples since any sample containing a mixture of substances gives a composite spectrum of the individual components.

2. Direct Insertion Probe

Figure 4 illustrates an inlet system whereby the sample is introduced directly into the ionization chamber on the end of a rod-like probe. A small glass vial
FIGURE 4

DIRECT INSERTION PROBE FOR MASS SPECTROMETRY SAMPLE INTRODUCTION

Legend

(a) Schematic diagram showing vacuum lock mechanism for introducing a sample on the end of a probe.

(b) Possible configurations of probe sample relative to the ion chamber entrance port.

Reprinted without permission from reference 4, Chapter 2, pp 14 - 15.
DIRECT INSERTION PROBE FOR MASS SPECTROMETRY SAMPLE INTRODUCTION

(a)

(b)
or capillary containing the sample in solid form is inserted into the end of the probe. This is then passed through a vacuum lock assembly which overcomes the necessity of breaking the internal vacuum. The probe first enters the lock chamber which must then be evacuated before a valve is opened to allow it to be pushed through into the ion source housing and up to a small hole in the source chamber for vaporization. As long as the probe tip is butted solidly against the orifice, 100% of the sample vapor will pass into the chamber (4).

The rate of sample evaporation can be controlled by varying the temperature of the probe using heating coils, water cooling or even refrigeration in the case of extremely volatile compounds. Temperature programming is available up to about 350°C and is especially useful for mixtures of compounds with varying volatilities.

The direct insertion probe inlet is most useful for the introduction of samples ranging in molecular weight from 400 to 1200. Such compounds often have insufficient thermal stability to be heated to 350°C or have very low vapor pressures even at this temperature. The low pressure in the ionization chamber aids in vaporization of these less volatile substances where a vapor pressure of about 10^{-6} Torr still allows a spectrum to be obtained on samples of 1 μg or less (5).
On the other hand, despite efforts to control the vaporization of more volatile compounds, excessive amounts of sample may occasionally flood the ion source housing and produce a contaminated source. This delays further sampling until the excess is burned off. In addition, mixtures of samples will still present a composite spectrum unless the volatilities of the components differ enough to take advantage of temperature programming. A further annoyance is evident in the fact that the changing of samples requires more time and manipulative steps than other introduction methods.

3. Gas Chromatography

Since gas chromatography (GC) is a process in which molecules are also analyzed in the vapor phase, such a tool provides a highly versatile means of separating mixtures of samples for introduction into the mass spectrometer. Any sample that is sufficiently volatile and stable to pass through a GC column is usually suitable for mass spectrometry. With a combined GC-MS system, individual mass spectra of sufficient quality are obtainable for every component that may be separated chromatographically, even though the quantities of components may be less than 1 μg and available for only a few seconds (5). Some useful data can be obtained even from sample quantities as low as 10 ng (6). The large amount of data generated from such a
system, especially when analyzing complex organic mixtures, necessitates the use of some form of computerized data collection and interpretation.

Uncoupled GC-MS systems involve the trapping and condensation of individual gas chromatographic fractions prior to introduction into the direct inlet of the mass spectrometer. If sufficiently large amounts of sample are available, this method has the advantage of allowing more time to run the individual spectra and to ascertain the best recording conditions for each component compared with the coupled systems where fast scans are taken and variations in recording conditions are not possible (5). However, many samples are so complex and/or so small that the isolation, handling, and transfer of the minor components is far too slow and impractical, if not impossible. Furthermore, where peaks overlap, the purity of collected fractions is doubtful. A sample analysis that might take weeks by an uncoupled system can often be processed in less than an hour by a combined GC-MS system (4).

The coupling of the gas chromatograph with the mass spectrometer is complicated by the vast difference in operating pressures and the dilution of the GC sample with carrier gas. To overcome these obstacles interfacing devices called molecular separators are used which allow the GC effluent to "leak" from atmospheric pressure to
the 10^{-6} Torr of the ion source while enriching its organic component by removing most of the carrier gas. The common types of separators either take advantage of the faster rate of diffusion of carrier gas relative to that of larger organic components or the preferential diffusion of organic molecules through a methyl silicone rubber membrane (4, 5).

For mass spectrometers not designed for a direct GC hookup, the direct insertion probe can be modified to serve as an inlet for the effluent from the standard interfacing devices (4). However, an important requirement for such a hookup which must be considered is the rate at which the mass spectrometer can scan a practical range of mass readings. Since the elution time of a GC peak normally varies from about 1 second to 1 minute, and since some peaks are extremely close together, the mass spectrometer must be capable of scanning at a rate of up to at least 500 mass units per second (5).

In addition, some type of GC detector is usually employed along with the MS which samples part of the GC effluent and provides a preliminary analysis of the sample in order to allow for some adjustment in MS conditions and to indicate appropriate times for initiating the MS scanning.

Another factor which must be considered is column "bleeding" from the GC. At temperatures approaching 200^\circ C, many columns normally used for GC evolve gases such as CO_2,
CO, or H₂O, and degradation of the column slowly occurs. As these substances elute into the mass spectrometer, a background to the mass spectrum is established, but since this column bleeding usually increases during sample peak elution, simple subtraction of background is not entirely satisfactory. The overall consequence is that temperatures in the GC-MS setup are kept as low as possible while using derivatization to enhance volatility (5).

For further considerations and technical details related to the use of GC-MS, refer to McFadden (4).

4. Liquid Chromatography

Whereas only volatile and thermally stable substances or their derivatives can survive GC, the development of high pressure liquid chromatographic (HPLC) systems has provided a relatively fast and sensitive (ng range) means of separating compounds that are labile, polar, and nonvolatile, including many high molecular weight substances that are important in the biochemical field. Separations of all compounds that are soluble in a liquid phase can be obtained and, with proper solvent programming, setups are possible in which columns packed with nonpolar materials linked in series to those containing anion and cation exchange resins can separate a whole spectrum of different compounds in one run (7).
However, as seen with GC, improved separations of multicomponent mixtures such as biological fluids provide extremely complex chromatograms, and in such cases the identities of many of the individual solutes are unknown. The identification of effluent fractions often requires the time consuming process of collection, concentration, and reanalysis by other methods such as MS. The direct coupling of a mass spectrometer to the HPLC system not only provides a more sensitive and versatile detector than the refractive index or ultraviolet detectors commonly used, but also supplies molecular weight and structural information resulting in positive identification of eluting fractions just as it has for GC.

The difficulties of interfacing a liquid chromatograph with a mass spectrometer include vaporizing the liquid LC effluent, separating the solvent from the sample solute molecules (although this is not always necessary), and reducing the flow of vapor to accommodate the low pressures required for MS without severely decreasing sensitivity. The most versatile method uses a "ribbon" transport system (8). The effluent passes over a moving belt, 0.32 cm wide, which carries it into a differentially pumped evaporation chamber where the solvent is removed, leaving the solute deposited on the belt. The belt then passes through a specially designed interface directly into
the ionization chamber where it may be heated if necessary under controlled conditions to vaporize the solute for ionization by conventional means. This system is independent of the solvent used for the LC separation, provides up to 40% efficiency of solute transfer into the ion source, and allows for variation in the method of ionization.

A more sensitive but less versatile system allows the continuous introduction of a small proportion of the effluent into the ionization chamber of a chemical ionization mass spectrometer where the LC solvent also acts as an ionization reagent (9, 10). (Chemical ionization will be described in a later section along with the other ionization methods mentioned below). However, this method severely limits the choices of solvent that can be used for the LC system as well as solvent programming which is often essential for proper separation. In addition, spectra obtained do not provide the structural information obtainable from electron impact ionization.

Another system with similar limitations allows the entire eluting stream from the LC to pass into an atmospheric pressure ionization reaction chamber where it is vaporized and the solvent again becomes the ionizing agent (11, 12).

Despite the obvious merits of HPLC for sample introduction, many substances which are easily separated by
LC may still be too involatile or unstable to achieve the vaporized state required prior to ionization. In such cases, the only means of obtaining a representative mass spectrum may lie in the use of field desorption techniques for field ionization on individually collected fractions.

B. IONIZATION METHODS

The purpose of the ionization step is first to produce as many ions as possible from the neutral vapor molecules entering the ion source chamber from the inlet. Although negative ions are produced along with the positive ions and can be analyzed, the efficiency of negative ion formation is about a thousand times lower and they are only measured under certain conditions (to be discussed later). Molecular and fragment ions resulting from ionization are then shaped or focused by electrostatic optics into an ion beam, the composition of which accurately represents that of the sample. During this focusing process, the ions are first directed through a slit by means of a small positive (repeller) potential and then ejected out of the source and into the mass analyzer by a series of negatively charged plates and slits kept at various accelerating potentials. (See Figure 5 in the section on electron impact ionization to follow.)
At least thirteen distinct methods have been devised for the production of ions in mass spectrometry but only the electron impact ionization process is widely applied in organic analysis. The other two methods to be discussed here, chemical ionization and field ionization, have special applications which are also gaining prominence. A fourth method being developed for use in this area, atmospheric pressure ionization, uses a radioactive source and will be described briefly. However, other processes such as spark discharge, high voltage discharge, glow discharge, thermal, photo and laser ionization are not practical for use in organic MS.

The choice of an appropriate ionization method is the main factor in determining the overall success of an analysis. This, in turn, is dictated by the nature of the sample and the type of information being sought. Such considerations as sample volatility, stability and quantity which were shown to influence the choice of inlet system also affect the selection of a suitable ion source, as will be described. In addition, it will be seen that where an ion source which causes considerable fragmentation is valuable for structural analysis, it may not be the best choice when determining molecular weights or when dealing with complex mixtures. Thus, no one type of source is best for all applications.
In the previous section on inlet systems, it was noted several times that one of the main problems involved with the introduction of the sample to the ionization chamber was making the transition from the external atmospheric pressure to a vacuum maintained at about $10^{-6}$ Torr in the source and analyzer. There are several reasons for this requirement, but the first one to be described is the most important (1, 4):

a) When gaseous particles pass each other so that the distance between the particles is comparable to atomic dimensions, interaction occurs either by chemical reaction which changes the fragmentation pattern or by deflection which scatters the ion beam. The chemical reaction effects may in some cases be desirable but the scattering causes difficulties in focusing, peak broadening, and loss of sensitivity. At the lower pressures, these particles are obviously further apart and interactions are therefore reduced.

b) Residual gases in the ionization chamber are ionized along with sample material resulting in interfering spectral patterns and a high background which decreases sensitivity. Flushing the system with, and introducing the sample in, an inert carrier gas or appropriate volatile solvent can reduce this problem.
c) Significant amounts of oxygen burn out the source filament which provides the energy for ionization. However, this may be overcome by the same means as described in b).

d) With high pressure in the ion source chamber or housing, the several thousand volts used for ion acceleration into the analyzer may cause electrical discharge.

e) For electron impact ionization, high pressures interfere with normal regulation of the electron beam.

Many details and considerations regarding the vacuum technology involved are discussed in McFadden (4) and Roboz (1). However, examples of exceptions to this low source pressure requirement are seen in chemical ionization where the pressure in the immediate area of ionization is maintained at about 1 Torr, and in atmospheric pressure ionization where the production of ions occurs in an external source chamber.

1. Electron Impact Ionization

The ionization potential of a compound corresponds to the energy required to remove an electron from the highest occupied orbital, thereby forming a positive ion, and for most organic molecules falls in the range of 6-12 eV. In the process of electron impact (EI) ionization, the sample vapor is bombarded by a beam of electrons, the
energy of which exceeds the sample ionization potential and results in electron removal from sample molecules. Any excess energy transferred to these molecules may then cause fragmentations and rearrangements, and any of the resulting positive particles are detected along with the remaining molecular or parent ions.

Figure 5 is a schematic diagram of one type of EI source where electrons are emitted from a tungsten or rhenium filament which is heated by direct current. They are then accelerated through a slit in the source block and across the ion chamber to be collected on an anode or trap which measures the beam intensity. The accelerating voltage (or ionization energy) of the electrons is varied by changing the potential between the filament and the source block. The positive ions formed are directed away from the electron beam at a right angle and into the mass analyzer by the combined effects of a positive repeller electrode and the negative accelerator plates.

Since the total amount of positive ion current and the nature of the fragmentation pattern depend on the energy of the electron beam, some control over the ionization and fragmentation process can be achieved by varying this energy. This is illustrated in Figure 6. Lower energies show a high percentage of the total ion current in the parent ion with less fragmentation, while at 60 eV, more
FIGURE 5

SCHEMATIC DIAGRAM OF AN ELECTRON IMPACT SOURCE

Legend

As sample enters the source chamber, it is bombarded by the electron beam, and the resulting positive sample ions are propelled by the positive ion repeller and accelerated by negative accelerating plates into the mass analyzer.

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SCHEMATIC DIAGRAM OF AN ELECTRON IMPACT SOURCE

- Accelerating plates
- Beam centering plates
- Source block
- Electron trap
- Filament
- Electron beam
- Ion repeller
- Sample entrance
- To mass analyzer
FIGURE 6

MASS SPECTRUM OF ETHYL ACETATE AS A FUNCTION OF ELECTRON BOMBARDING ENERGY

Legend

(a) At 14 eV, the number of fragments is small and a large amount of parent ion at m/e 88 remains.
(b) At 20 eV, the abundance of fragments at lower mass is greater.
(c) At 60 eV, still more fragmentation is evident and the amount of parent ion remaining is minimal.

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FIGURE 6

MASS SPECTRUM OF ETHYL ACETATE AS A FUNCTION OF ELECTRON BOMBARDING ENERGY

(a)

(b)

(c)
fragment ions are present and the parent ion has almost disappeared. As long as experimental conditions are kept constant, the mass spectra of individual compounds exhibit a remarkable constancy even in the presence of other components in a mixture. This is especially true for spectra obtained with 60-80 eV electron beams because of an apparent plateau in the ionization and fragmentation process. Thus, most existing spectral catalogues contain mass spectra obtained within this energy range. In GC-MS where helium is the most common carrier gas, the beam is often operated at about 20 eV, which is about 4 eV below the ionization potential of helium, to eliminate its interference. However, this makes comparison with the usual spectral catalogues difficult (4).

Even when dealing with unknown compounds which have not been catalogued, the EI ionization process can be extremely useful. Since internal bond cleavage and rearrangement of a proportion of molecules is directly related to the atomic structure of the molecule, the pattern of ions formed contains information necessary for determining this structure. A discussion of the relationship between fragmentation pattern and structure may be found in Williams and Howe (5). However, the energies necessary for causing the required amount of fragmentation may result in very low abundance, or absence, of the parent ion. As a result, it may be necessary to operate the electron beam at lower
energies in order to reduce fragmentation and leave enough of the parent ion intact for molecular weight determination. Unfortunately, this will cause a considerable decrease in the absolute intensity of ions and thus a loss of sensitivity (4). It is partly for this reason that other ionization methods are often used in conjunction with, or even instead of, EI. Another reason involves the stability and volatility of the compound under investigation, which will be elaborated upon in the following sections.

2. Chemical Ionization

During the process of chemical ionization (CI), the ionization of the substance under investigation is brought about by reactions between molecules of the vaporized sample and a set of ions formed by EI which serve as ionizing reagents. The ionizing reagent gas and sample gas are admitted to the EI source at the same time but at different pressures; for example, 0.3-3 Torr for reagent gas and $10^{-6}$ Torr or lower for the sample (4). Both gases are exposed to the electron beam, but because of the very low abundance of the sample virtually all primary ionization due to electron bombardment occurs with the reagent gas. The ionized reagent then undergoes ion-molecule reactions with itself due to its relatively high pressure and forms a "steady-state plasma". For example, in the case of methane, normal electron impact products react with excess methane as follows:
\[ \text{CH}_4 + e^- \rightarrow \text{CH}_4^{++} + 2e^- \]
\[ \text{CH}_4^{++} \rightarrow \text{CH}_3^+ + \text{H}^- \]
\[ \text{CH}_4^{++} + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3^- \]
\[ \text{CH}_3^+ + \text{CH}_4 \rightarrow \text{C}_2\text{H}_5^+ + \text{H}_2 \]

The most important ions in the reaction plasma are \text{CH}_5^+ and \text{C}_2\text{H}_5^+ which make up 90% of the ionic content. These ions then react chemically with the dilute sample gas molecules, generally by proton transfer to or hydride abstraction from them, and thereby give rise to "quasi-molecular" ions from the sample with an apparent molecular weight \pm 1 the actual value, referred to as \((M + 1)^+\) and \((M - 1)^+\) (14). The most common reagent gases are simple hydrocarbons such as methane and isobutane, which produce minimum and easily subtractable interference in the mass spectrum of the sample.

The CI source is basically the same as the one used for EI (Figure 5) with a few modifications:

a) For reagent gas plasma formation and subsequent sample ionization, the pressure within the source chamber must be maintained at about 1 Torr, while the source housing where the electron beam is formed is held at less than \(10^{-4}\) Torr, and the mass analyzer is differentially pumped to about \(10^{-6}\) Torr. This requires a gas-tight system in which reagent and sample gases may only escape from the source chamber, either to the surrounding envelope through the slit for the entry of the electrons, or to the mass analyzer through the focusing and accelerating slits. The area of these slits is reduced by
as much as one hundred times, compared to that of the conventional EI source to help maintain these pressure differences, while other technical aspects involved in meeting the vacuum requirements under such high gas loads are considerable (4).

b) The energy of the electron beam must be made variable up to 500 eV to enable the electrons to penetrate sufficiently into the 1 Torr pressure of the source (3).

c) A dual inlet arrangement is a minimum requirement, because one direct inlet is used for introduction of the reagent gas, while the sample may either require introduction by a separate direct inlet or by a direct probe, depending on its volatility. However, for a GC-MS system using a CI source, only one inlet is necessary if the carrier gas used for the GC phase is also a suitable ionizing reagent. Obviously, molecular separators and interfacing to handle a large pressure drop are not necessary for this type of MS (3).

Since sample ionization in CI occurs with a much lower transfer of energy, the fragmentation process is modified and greatly reduced, and although the molecular ion is not usually abundant, a quasi-molecular ion formed by the loss or gain of one proton is often the most prominent ion in the spectrum. See Figure 7 for a comparison of CI and EI spectra of oral 1. Not only does a CI spectrum
FIGURE 7

COMPARISON OF CHEMICAL IONIZATION AND ELECTRON IMPACT SPECTRA OF ORTAL, MW 240

Legend

The chemical ionization spectrum shows much less fragmentation and a large amount of quasi-molecular ion at m/z 241. ( )

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FIGURE 7

COMPARISON OF CHEMICAL IONIZATION AND ELECTRON IMPACT SPECTRA OF
ORTAL, MW 240
usually provide information about molecular weight, but the fragmentation pattern may differ sufficiently from the EI pattern to reveal additional structural features (4). Even with the reduction in the amount of fragmentation, the total ion current remains high and results in a sensitivity comparable to conventional 70 eV EI-MS. This is due to the fact that the electron beam is more fully utilized because of the higher pressure reagent gas in the source chamber, which in turn leads to a higher probability of reactive collisions between reagent ions and sample molecules.

3. Field Ionization

Another technique that provides sample ionization at relatively low energy with reduced fragmentation is field ionization (FI) in which atoms or molecules are acted upon by an electric field. As mentioned earlier, the energy of an outer orbital electron must normally be increased by an amount equal to its ionization potential for ionization to occur. However, in the presence of a strongly positive electric field, this potential barrier is distorted such that an electron can "tunnel" through it (in terms of the laws of quantum mechanics) as it is pulled away from the molecule and into unoccupied surface orbitals of the metal electrode producing the field (15). This electrode is called the field anode or emitter. At sufficiently high field strengths of the order of 10^7 -
$10^8$ V/cm, most molecules reaching the emitter will undergo ionization but will acquire very little excess energy for fragmentation processes to occur.

The FI source, Figure 8, consists of the anode, which may be a thin wire, a sharp blade or sharp tip, and a cathode which contains a fine slit or hole. The anode is held at a highly positive potential in the range of 7,000-10,000 volts with respect to the cathode, and is often mounted on a sliding rod for easy removal through a vacuum lock, similar to the direct probe used for sample introduction. The ions produced are immediately repelled by the anode through the openings in the cathode and focusing plates, and on into the mass analyzer. In the combined EI-FI source, first developed in 1972 (16), both types of mass spectra may be obtained on samples introduced either by batch inlet or direct probe.

Emitter construction makes use of the principle that the field strength generated at an electrode at a given voltage depends on the shape of the electrode, a sphere with the smallest radius theoretically producing the strongest field. This is why fine points, thin wires and blades have all been used in order to obtain the field strength of $10^8$ V/cm for efficient FI and still remain within practical dc voltage levels. The most common anode is the tungsten wire, 10 µm in diameter, whose efficiency is
FIGURE 8

SCHEMATIC DIAGRAM OF A FIELD IONIZATION SOURCE

Legend

Sample is admitted by way of a batch inlet, direct insertion probe, or coated directly on the emitter, Ionization occurs at or on the emitter surface and positive ions are repelled out of the ionization chamber by the emitter itself.

Modified from reference 4, Chapter 2, p.32 and reference 5, Chapter 9, p.194.
FIGURE 8

SCHEMATIC DIAGRAM OF A FIELD IONIZATION SOURCE
greatly improved by a prior activation process which promotes the growth of microneedles on the smooth surface of the wire, and results in a very effective multipoint array. For example, one activation process involves maintaining the wire at 10 kV and then 6 kV in a chamber containing benzonitrile, at pressures between $10^{-3}$ and $10^{-2}$ torr and temperatures of 800-1000°C for periods of several hours (17). The temperature, pressure, voltage, time, and the activating chemical used are all critical factors in this process which ultimately affect the efficiency and consistency of ionization.

In 1969, Beckey (18) introduced a modification of the FI process called field desorption (FD), in which the activated emitter is coated with the sample before it is inserted into the source chamber for operation in the normal FI mode. This adsorption is accomplished by repeatedly dipping the wire into the dissolved sample and allowing the solvent to evaporate. The advantage of this method lies in the fact that the sample under investigation does not have to undergo the vaporization process prior to ionization, as is the case with other ionization methods, including conventional FI. Evaporation of ionized sample from the physically adsorbed state is usually achieved at moderate temperatures by gently heating the emitter and, since excess thermal energy is very small, many highly polar compounds of low
volatility, which might otherwise decompose on thermal volatilization, can be analyzed with a larger amount of parent ion left intact.

The principle feature of most FI/FD mass spectra, therefore, is the increased abundance of the parent ion of the sample. However, a quasi-molecular ion \((M + 1)^+\) is frequently observed and often exhibits a much greater intensity than the parent ion. This occurs particularly with polar molecules with basic groups and is due to surface reactions of sample ions, either with adsorbed water or unionized sample molecules on the emitter (3). Determining whether the most intense ion is actually the parent ion or \((M + 1)^+\) is sometimes difficult, but is usually accomplished by means of a study of any fragments produced, changes observed when varying emitter temperature, and some prior knowledge of the type of compound under investigation. The other feature of FI/FD methods is that the mass spectra are simpler than those obtained by EI and usually contain no fragments generated by rearrangements or multiple decompositions (5). Figure 9 illustrates these features as well as the advantage of FD techniques for less stable substances. The FD spectrum of D-glucose shows virtually no elimination of water molecules from the \((M + 1)^+\) ion, as opposed to the FI spectrum, and the EI spectrum exhibits no parent ion at all. In Figure 10, the
FIGURE 9

MASS SPECTRA OF D-GLUCOSE

Legend

(a) Electron impact spectrum shows considerable fragmentation and no parent ion peak.

(b) Field ionization spectrum shows some fragmentation but a large amount of (M + 1)$^+$ ion remains.

(c) Field desorption spectrum shows minimal fragmentation and a significant amount of parent ion at m/z 180.

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FIGURE 9

MASS SPECTRA OF D-GLUCOSE

Electron impact (70eV)  D-Glucose

Field ionization

Field desorption

M = 180
FIGURE 10

MASS SPECTRA OF TETRAHYDROCORTISONE, MW 364

Legend

(a) Electron impact, (b) chemical ionization, and (c) field desorption spectra show considerable differences. The advantage of field desorption in this instance is the large amount of parent ion remaining at m/z 364.

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FIGURE 10

MASS SPECTRA OF TETRAHYDROCORTISONE, MW 364
FD spectrum of tetrahydrocortisone is compared with the corresponding EI and CI spectra.

4. Comparison of the Common Ionization Methods

Although all of the ionization methods just discussed can be employed in a routine way with roughly comparable sensitivities at the microgram level, none is universally applicable. Many considerations for comparison are presented by Milne and Lacey (13).

EI sources are not only the most common and well established, but so far lead other ionization methods in areas such as stability, ease of operation, and efficiency of ionization. In addition, since most available catalogued spectra of organic compounds have been compiled from EI studies, it is necessary to have data from an EI source to utilize these catalogues. EI mass spectra almost always contain more fragment ions than CI or FI and its advantage in structure elucidation is well established. However, the very weak or absent parent ion sometimes makes molecular weight assignment impossible by this method, and the complexity of the spectra of biological samples often demands elaborate separation systems and computer analysis of results.

With CI and FI sources, the simpler fragmentation patterns and the larger abundance of parent ion or \((M+1)^+\) ion usually (although not always) observed can be a definite
advantage over EI, but makes these methods more suitable for confirming the presence of certain compounds in a mixture than for doing primary structural analysis. Molecular weight assignments to unknown compounds are often better achieved by CI or FI, although some confusion may still arise when distinguishing whether a particular peak is the actual parent ion rather than \((M + 1)^+\), \((M - 1)^+\), or even \((2M + 1)^+\).

The greatest advantage of CI lies in the wide range of reagent gases which may be used. Varying these gases can provide a possible means of altering the ionization and fragmentation reactions enough to obtain additional structural information to complement that obtained by EI. The advantages and limitations of the CI source for receiving GC and LC effluent streams have already been mentioned. Unfortunately, although CI appears to be ideal for compounds of moderate polarity such as amino acids and alkaloids, it has proven less satisfactory for relatively non-polar, acid-labile substances. With compounds of this sort, such as triglycerides and the simple steroids, FI or EI is preferable.

One of the disadvantages of FI is that ions formed leave the emitter over a wide angle and these high energy divergent ion beams are difficult to focus into the narrow entrance to the mass analyzer. This has tended to cause a
significant decrease in sensitivity which, fortunately, is usually compensated for by the increased abundance of parent ion compared to EI. Another problem with the emitter is that its surface is unstable and severely affected by previous use. Uniformity in the conditioning and activation process is difficult to obtain and severely hinders the stability and consistency of FI and especially FD. Compounding this problem is the difficulty in achieving control and uniformity in temperature throughout the shanks and tips of the emitter microneedles, which in turn affects the diffusion, ionization and desorption process for molecules adsorbed at different positions along the needles. However, improvements in design, activation and heat control of these anodes should help to increase the sensitivity and stability of this mode of ionization.

At least two drawbacks have slowed the development of widespread FD techniques for biological samples. The most serious of these is the apparent interference by metal ion impurities (e.g., Na$^+$ and K$^+$) in obtaining reproducible results. Various methods have been investigated for the removal of these ions prior to FD, such as dialysis, gel chromatography, and the use of macrocyclic ligands (20). Another difficulty is the fact that the nature of the FD technique prohibits GC or LC introduction and thus limits its general use to relatively pure samples. Nevertheless,
in cases where the compounds under study cannot be volatilized without some thermal breakdown, the only useful method described so far is FD which has opened up the area of analysis of large, polar, biologically important molecules such as proteins and nucleic acids, and their degradation products (13).

An article by Fales, Milne, et al. (21) provides comparison and discussion of mass spectra obtained by all four ionization methods on fifteen biologically important compounds. Strengths and weaknesses shown in each case further confirm that none is uniformly superior to the others and that the method of choice in any analytical problem will depend on the type of compound involved.

5. Atmospheric Pressure Ionization

Atmospheric pressure ionization (API) has already been mentioned as a means of generating ions in a reaction chamber external to the low pressure region of the mass analyzer. Development was stimulated by the need to find a more efficient and sensitive means of ionizing sample molecules in the flowing effluent streams of gas and liquid chromatographs (11, 12, 22, 23). Samples may be introduced into the ionization chamber directly in a GC effluent stream or by vaporization of LC effluent or single sample into a stream of preheated carrier gas. The ionization reactions are usually initiated by electrons from a $^{63}$Ni foil at
atmospheric pressure. Both positive and negative ions then result from a complex series of ion molecule reactions and are sampled continuously through a 0.025 cm aperture into the high vacuum of the mass analyzer. The ions produced in the source depend upon the chemical properties of the sample in the gas phase and on the nature of the reaction conditions created by solvent and/or carrier gases in the source, and spectra obtained are most closely related to the type of spectra generated from CI-MS. The API system may become invaluable for the routine handling of certain types of samples; for example, substances which can be efficiently separated by LC using solvents compatible with the API source, and at the same time are sufficiently volatile and stable to undergo this form of ionization.

C. MASS ANALYZERS

The mass analyzer component of the mass spectrometer has two main functions. The first is to resolve or disperse the beam of positive ions formed in the ionization chamber according to their m/z ratios. The second function is to maximize the intensities of the resolved ion beam components by focusing or converging each one at a separate well-defined point. Magnetic and electric fields are usually used to accomplish these objectives.

The resolution of a particular instrument is an important consideration and refers to its ability to separate
and detect ions of very close masses. In organic analysis, higher resolutions are often desirable especially for exact molecular weight determinations. However, the advantages of increased resolution must be weighed against such disadvantages as the corresponding increase in cost and decrease in sensitivity due to the higher amount of sample required. Intermediate to lower resolutions are quite adequate for many routine types of analysis where extremely precise mass determinations are not necessary.

Of the possible methods of mass analysis, only two are commonly used in commercial mass spectrometers. These are the magnetic deflection and the quadrupole instruments to be described here. Less common mass separation methods include monopole and time-of-flight (tof) spectrometry.

1. Magnetic Deflection

The basic setup for a typical single-focusing magnetic sector analyzer is illustrated in Figure 11. Ions formed in the source are accelerated through the source exit slit, S1, toward a homogeneous wedge-shaped (sector) magnetic field. A beam of ions of the same mass is shown to diverge as it approaches this field, due to the natural variations in angular motion of the molecules. However, as it passes through the magnetic field and is deflected, a process called direction-focusing occurs in which this
FIGURE 11

SCHEMATIC DIAGRAM OF A 90° MAGNETIC SECTOR SHOWING DIRECTION-FOCUSBING OF DIVERGENT ION BEAM

Legend

As ions tend to disperse upon leaving the source chamber, the sector magnetic field causes the beam to converge again before reaching the collector.

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FIGURE 11

SCHEMATIC DIAGRAM OF A 90° MAGNETIC SECTOR SHOWING DIRECTION-FOCUSING OF DIVERGENT ION BEAM
diverging beam is now caused to converge so that a focal point is reached at the collector slit, \( S_2 \). At the same time, for ions of different masses, the magnetic field acts as a prism to disperse the various mass components as they are deflected and effect direction-focusing for each at a separate point. This direction-focusing is a property of the magnetic field, and instruments which rely on this alone for focusing are called single-focusing mass analyzers. The resolution of such instruments is adequate for most types of analysis and is adjustable to some extent by varying the source and collector slits.

The principle of mass separation by magnetic analyzers is based on the fact that, as the accelerated ions are passed through the magnetic field the extent of deflection will vary with the masses of the beam components, with the heaviest ions adopting paths of the largest radii as shown in Figure 12. The other factors affecting this deflection are the strength of the magnetic field, the charge on the individual ions, and the kinetic energy previously imparted to the ions by the accelerating voltage. All of these factors are related in the equation (developed in references 1, 3-5),

\[
\frac{m}{z} = \frac{H^2 r^2}{2 V_{\text{acc}}}
\]

so that at a given magnetic field strength \( H \) and accelerating
**FIGURE 12**

**MASS DISPERSION AND FOCUSING IN A 180° MAGNETIC ANALYZER**

**Legend**

Ions of different masses in the diverging beam are brought to separate focal points by the sector magnetic field. By altering either the magnetic field strength or the accelerating voltage, different segments of the ion beam are brought to focus at the collector for detection.

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FIGURE 12

MASS DISPERSION AND FOCUSING IN A 180° MAGNETIC ANALYZER
voltage $V_{acc}$, ions of a given $m/z$ value will follow a particular path of radius $r$. Thus, by varying either the magnetic field strength (magnetic scanning) or the accelerating voltage (electrical or voltage scanning), the ions of different $m/z$ ratios can be deflected in turn through a fixed radius to the collector and in this way the mass spectrum is scanned.

For most applications, magnetic scanning is preferable for obtaining the mass spectrum because the transmission of ions in the important higher mass regions is more efficient using this mode. However, electric scanning is more practical for special applications such as GC-MS scans processed by computer and selected ion monitoring methods (described later) which require fast jumps from one mass to another (4).

For situations requiring precise mass measurements, a much higher resolution is required than can be obtained by the single-focusing mass analyzer just described. Even though the magnetic field provides direction-focusing to correct for angular divergence of ions, there is an energy divergence factor which is not compensated for in this system. Theoretically, all ions accelerated out of the source receive the same kinetic energy regardless of mass, so that all ions of the same mass should have exactly the same velocity. However, in actual fact, the beam does
possess some energy dispersion which results from factors such as differences in the positions at which various ions are formed in the source chamber, and from the natural kinetic motion of the molecules. This results in slight velocity dispersion even of ions of identical mass, and subsequent broadening of each individual peak (4). Velocity dispersion is counteracted by passing the ion beam through a radial electric field (electrostatic analyzer) prior to the magnetic field. The electric field causes velocity-focusing, and by placing a slit between the electrostatic and magnetic analyzers, ions of a closely defined kinetic energy may be selected prior to mass analysis and direction-focusing (5). Systems incorporating such an arrangement are described as double-focusing mass spectrometers and are capable of attaining much higher resolutions than single-focusing instruments.

Two different geometries for the double-focusing components are commonly used, as shown in Figure 13. In the design of Mattaugh and Herzog (24), ions of all m/z ratios are separated and brought to a double focus in a plane, and a photographic plate positioned at this focal plane records all the ions all the time. The geometry used by Nier and Johnson (25) allows only ions of one m/z ratio to meet the double-focusing condition at any one time. An electrical detector is placed at this focal point where the entire mass spectrum is obtained by scanning the magnetic field.
FIGURE 13

ARRANGEMENT OF ELECTROSTATIC AND MAGNETIC ANALYZERS FOR DOUBLE-FOCusing
MASS SPECTROMETER SYSTEMS

Legend

(a) Mattauch-Herzog geometry which brings all parts of the
ion beam to focus in a plane at one time.

(b) Nier-Johnson geometry in which different segments of the
beam are focused at different times.

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p 5.
FIGURE 13

ARRANGEMENT OF ELECTROSTATIC AND MAGNETIC ANALYZERS FOR DOUBLE-FOCUSING MASS SPECTROMETER SYSTEMS

(a)

(b)
For many applications involving very small samples, where peaks may be obscured by the peaks of calibrating or reference compounds, a double-beam instrument is useful. The system consists of two independent inlet and ion source chambers, one electrostatic and magnetic analyzer, and two independent amplifiers and recorders as shown in Figure 14. The sample and reference beams are produced and detected independently, but because they pass through the same electric and magnetic fields, simultaneous double-focusing of the two ion beams occurs, and the mass scale for each is precisely matched (4). The spectra are not superimposed but recorded with opposite peak deflections on the same record. Calibration or reference compounds can be constantly maintained in one of the sources for direct comparison at all times and operating conditions, while allowing greater sensitivity by not interfering with the sample peaks (3).

2. Quadrupole

The quadrupole mass "filter", originally developed by Paul and Raether (26), is a nonmagnetic mass analyzer which has become quite common in recent years. As shown in Figure 15, it consists of a quadrant of four circular or hyperbolic parallel rods precisely aligned to lie at the corners of a square if visualized on cross section. Opposite rods are electrically connected with one pair to a radio
FIGURE 14

SCHEMATIC DIAGRAM OF A DOUBLE-BEAM MASS SPECTROMETER

Legend

Separate inlet systems, source chambers, and collector systems handle two different samples at the same time while passing both through the same electrostatic and magnetic fields.

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FIGURE 14

SCHEMATIC DIAGRAM OF A DOUBLE-BEAM MASS SPECTROMETER

ION SOURCES

SAMPLE 1
SAMPLE 2

ELECTROSTATIC ANALYSER

BEAM 1
BEAM 2

MONITOR SLITS

MAGNETIC ANALYSER

RECORDED SPECTRA

COLLECTOR SYSTEMS AND AMPLIFIERS
FIGURE 15

SCHEMATIC DIAGRAM OF A QUADRUPOLE MASS SPECTROMETER

Legend

Insert at upper left shows the spatial arrangement of the quadrupole rods in which one opposing pair is connected to a radio frequency power source and the other to a direct current source. As voltages are increased while keeping the rf/dc ratio constant, ions of increasing masses are allowed to pass from the source chamber to the detector without hitting the rods and becoming neutralized.

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FIGURE 15

SCHEMATIC DIAGRAM OF A QUADRUPOLE MASS SPECTROMETER
Mass separation is achieved by varying the dc and radio frequency voltages in unison to maintain a constant ratio, which sets up an oscillating field of varying strength. Positive ions extracted from the ion source are focused electrostatically into the quadrupole filter and through this field along the longitudinal axis of the rods. At a specified radio frequency, only ions of a given mass undergo stable oscillation through the filter and are able to avoid collision with the rods to reach the detector. Ions of lower or higher mass undergo oscillation of increasing amplitude until they collide with and are collected on one of the rods, and thus never reach the detector. By sweeping the voltages from zero to their maximum values at a constant radio frequency/dc ratio, the entire mass spectrum is scanned.

The quality of mass spectra obtained, especially in the lower mass ranges, is good but is strongly dependent on precise pole alignment and freedom from the accumulation of any nonconducting film that would distort the symmetric field such as excess sample condensation, pump oil creep, or column bleed. Unfortunately, the workable mass range is limited to values up to about m/z 800, and even above m/z 250, the ion intensities have a lower relative abundance compared with magnetic analyzers, mainly because of mass discrimination effects.

Nevertheless, despite its limitations for handling
high-molecular weight substances, the quadrupole system is quite useful for simple routine analysis. Not only is it relatively inexpensive, but it requires little experience to operate and maintain. Unlike magnetic analyzers, it has a linear mass scale closely related to the applied voltage, which is extremely useful both for data handling and for controlling the fast jumps between masses required for selected ion monitoring (3). Another attractive feature, especially for GC and LC work, is that good quality output can be maintained at higher pressures than other conventional mass spectrometers due to the fact that separation does not depend on focusing ions of the same mass into narrow beams. However, pressures below $10^{-4}$ Torr are still required to reduce the loss of ions to the poles caused by inter-ionic collisions, which then result in a loss of sensitivity.

D. DETECTION AND RECORDING

In many samples analyzed by mass spectrometry, the actual number of charged particles in each fraction of the resolved ion beam is extremely small. For example, typical ion current values encountered range from $10^{-10}$ amp down to minimum values between $10^{-16}$ and $10^{-19}$ amp where a current of $1.6 \times 10^{-19}$ amp corresponds to just one singly charged ion per second (4). The arrival of these ions at a specified
point must be detected and presented to show the relative intensity and m/z value of each fraction of the original beam. This is accomplished either electrically, where the variation in current with time is amplified and recorded; or photographically, by the position and density of the ions striking a photographic plate.

Considerations for choosing detection and recording devices include the following factors which are interdependent: accuracy, dynamic range (ability to record large variations in intensity with equal accuracy), ion intensity, sensitivity, scan rate, response time, convenience and cost (especially in routine laboratories).

For example, the process of "scanning" the mass spectrum, as described in the previous section, usually involves varying certain parameters of the mass analyzer so that ions of various m/z values reach the detector at different times. Consequently, the rate at which the scan is carried out will affect the amount of time allotted for detection of each fraction of the total beam. This in turn, combined with the original amount of sample present, determines the total number of ions at each m/z value being detected. Slower scan rates, such as are used in high resolution mass spectrometry, will allow more ions at each m/z value to reach the detector, thus allowing less sensitive, slower response devices to be used which are
generally more accurate. Fast scanning techniques such as used in GC-MS will, therefore, require fast response systems which are sensitive to lower ion currents (fewer ions per second). On the other hand, for the "no-scan" mode, the analyzer parameters are set so that the fraction of the ion beam at just one m/z value is directed toward the detector. In this case, the detection system required is governed mostly by the total amount of sample available and the range of intensities expected.

In conventional electrical detectors the positive ions leaving the mass analyzer first pass through a series of carefully designed resolving slits and "suppressors" which further define the part of the beam to be detected, slow it down, and neutralize secondary ions and electrons emitted as a result of the impact of the arriving ions (1). As shown in Figure 16, the final collector electrode is mounted in an open-ended box called a Faraday cup or cage, and the metal surface is slanted so that reflected ions or ejected secondary electrons cannot escape. The ions striking this electrode are neutralized by electrons arriving from ground through a high ohmic resistor. The voltage drop across this resistor is directly proportional to the ion current and is connected to a commercial electrometer amplifier circuit. In quadrupole mass spectrometers, the defining-slit is usually absent and the beam area is larger
FIGURE 16

SCHEMATIC DIAGRAM OF A CONVENTIONAL FARADAY CAGE ION BEAM COLLECTOR

Legend

As positive ions reaching the collector electrode are neutralized, the resulting voltage drop is a measure of the ion current. The metal surface of the collector electrode is inclined so that reflected ions or ejected secondary electrons cannot escape from the cage.

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FIGURE 16

SCHEMATIC DIAGRAM OF A CONVENTIONAL FARADAY CAGE ION BEAM COLLECTOR
than in magnetic instruments. High-transmission grids are used to provide the required suppressor and retarding effects (1).

The basic Faraday cup collector has several advantages over other detectors to be described. It is rugged, reliable, inexpensive, and has a very low electrical noise level. The measured current is directly proportional to the number of ions and the number of charges per ion, and its response is equal regardless of the energy, mass, and chemical nature of the ions. Its principle disadvantage is the relatively long response time inherent in its amplification system which makes it impractical for fast scanning techniques.

Despite some limitations, another type of detector, the electron multiplier, is more suitable for studies in such areas as GC-MS, high resolution, or reaction kinetics. As illustrated in Figure 17, this device consists of a series of dynodes electrically connected through a resistive network. The ion beam emerges from the analyzer so that it strikes the first dynode, called the conversion dynode, and causes a shower of electrons to be emitted. These electrons are then accelerated toward the second dynode where their impact causes even greater electron emission. Thus the process is repeated down each stage of the multiplier (usually 12-20 dynodes in all) to the final collector, or
FIGURE 17

SCHEMATIC DIAGRAM OF AN ELECTRON MULTIPLIER

Legend

The impact of the ion beam at the first dynode causes the emission of 1 - 2 electrons per positive ion. These electrons are then accelerated toward the second dynode where their impact causes additional electron emission. The process is repeated down each stage of the multiplier so that a typical gain of $10^6$ is achieved.

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FIGURE 17

SCHEMATIC DIAGRAM OF AN ELECTRON MULTIPLIER

Ion beam

Electrons

Dynodes

Amplifier
anode, which is connected to a conventional electrometer. This system is capable of producing an extremely rapid current amplification (or gain) of $10^3$ to $10^8$ with virtually no noise or dark current, and can, therefore, present a measurable signal from ion currents as low as $10^{-19}$ amp (4). Unfortunately, accurate quantitative measurements are difficult and require frequent and careful calibration due to factors such as:

- discrimination effects in which response varies with mass, energy, charge and chemical nature of impinging ions;

- desensitization resulting from very large ion currents or repeated exposure to air;

- instability in which the gain achieved on each run depends on previous operating conditions.

Despite these limitations, this device is indispensable in modern organic MS and many instruments are equipped with both an electron multiplier and a Faraday cage collector for maximum versatility.

Among the various recording devices employed, the strip-chart recorder is the most basic but its uses are limited to large samples with few peaks. It can provide a wide range of sensitivity and very accurate results in quantitative analysis when the desired gain is known in advance, but the method of altering the sensitivity is far
too slow and inconvenient when the number of peaks is large and their magnitudes change frequently (1). The cathode ray oscilloscope is another basic device which is mainly used in cases where fast qualitative identification is required. Such uses may include monitoring GC-MS elutions and selecting the best time for starting the scan of each peak, or selecting a specific portion of a spectrum to be scanned, or even investigating transient fragmentation and reaction phenomena (1). Its main advantage is its speed of response but it is convenient only when a small number of scans are needed, and it obviously does not provide a permanent record.

The oscillographic recorder, or "flying-spot" detector, acts like a photographic strip-chart recorder with the fast response of the cathode ray oscilloscope. Three to five galvanometer assemblies with separate input terminals are inserted in a magnetic block, as shown in Figure 18 (4). As current from the detector passes through the small coils, it causes a deflection of the tiny mirrors which are reflecting an intense beam of light from a mercury arc lamp onto ultraviolet-sensitive paper, and thus the displacement of the light beam is proportional to the ion current. Each galvanometer receives the same signal but at different attenuations for various sensitivities, a feature which provides a wide range of intensities on each
FIGURE 18

SCHEMATIC DIAGRAM ILLUSTRATING THE OPERATION OF AN OSCILLOGRAPHIC RECORDER

Legend

Detector current passing through the small galvanometer coils causes deflection of miniaturized mirrors which reflect an intense light beam from a mercury arc lamp onto ultraviolet-sensitive paper. Each galvanometer receives the same mass spectral signal but at different attenuations so that a wide dynamic range of ion intensities is recorded.

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FIGURE 18

SCHEMATIC DIAGRAM ILLUSTRATING THE OPERATION OF AN OSCILLOGRAPHIC RECORDER
scan with high accuracy. The small size of the galvanometers allows quite a fast frequency of response and, despite a relative insensitivity in the system, the high current gain of an electron multiplier detector makes up for this deficiency. The main disadvantage is that care must be taken to protect the chart paper from exposure to sunlight or prolonged use in fluorescent light, since irreversible fading occurs, and although the traces can be fixed photographically for more permanent records, the process is inconvenient.

The method of photoplate recording is the most unpopular of all the methods used but it is still a good method for obtaining high-resolution data and precise mass measurements. This technique can be used only with a double-focusing mass analyzer of Mattauch-Herzog geometry, described earlier, in which all ions are brought to a focus at the same time in a plane (Figure 13). Fifteen to thirty individual spectra can be obtained on a single ion-sensitive photoplate (5 cm by 25 cm) by manually shifting the plate to a new position in the focal plane of the ion beam (3). The mass spectrum is not scanned because all ions are simultaneously striking the plate. After the plate is developed, the mass "spectrograph" appears as horizontal rows of well-defined, short, vertical lines (Figure 19). The relative positions of all lines in each spectrum are measured with a micro-
FIGURE 19

MASS SPECTRUM DISPLAYED ON PHOTOGRAPHIC PLATE

Legend

The position and density of each line respectively represents the m/z value and relative abundance of the ion beam focused at that point.

(a) Actual size.
(b) Section between the arrows of (a) magnified 9 times.
(c) Section between the arrows magnified 54 times the actual size.

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densitometer, and when a computerized comparator is used to process the data from unknown and reference lines, the masses of the ions can be determined with sufficient accuracy to allow the assignment of their elemental compositions.

The photoplate provides a permanent, compact record of the complete high-resolution mass spectrum. The ability to record all components of the ion beam simultaneously is particularly advantageous when the sample is only available for a brief period, such as in GC-MS. The precision for mass measurements is excellent and variabilities inherent in electronic detection and recording are eliminated. Unfortunately, the disadvantages of this system cause it to be avoided whenever possible. The data recorded is not available until the image is developed and the lines are measured, and the comparator necessary for precise measurement is quite expensive. The method is less sensitive than an electron multiplier and does not provide accurate quantitative and relative intensity measurements. Despite these problems, photographic detection is still a valuable procedure for precise high-resolution studies (4).

E. RESOLUTION AND SENSITIVITY

The term resolution, or resolving power, as already described during the discussion of mass analyzers,
refers to the ability of the mass spectrometer system to separate and identify ions of nearly the same mass. The most common method of expressing resolution uses the "valley" definition, which refers to the highest mass at which two adjacent peaks of equal height, and differing in mass by one unit, exhibit a valley between peaks not greater than a certain percentage of the peak height (1). Figure 20 illustrates a 10% valley resolution for two peaks, m and m + Δm. This resolution, R, is determined by the relationship:

\[ R = \frac{m}{\Delta m} \]

where m is the mass of the first peak and Δm is the difference in the masses of the two peaks. If the two peaks are at 500 and 501 m/z values, then the resolution is 500. But if, for example, the two peaks are both at m/z 99 with actual masses of 99.08098 and 99.11737, the resolution required to distinguish the two accurately is 99.08098/(99.11737 - 99.08098) or about 2800 (4).

The resolution attainable by a mass spectrometer is determined mainly by the basic optical design of the mass analyzer which, once selected, can seldom be altered (1). However, in magnetic sector instruments, some control over resolution can be obtained by altering the sizes of the source and collector slits. The principle objectives
FIGURE 20

TEN PERCENT VALLEY RESOLUTION OF ADJACENT PEAKS

Legend

Using the 10% valley definition of resolution, peak \( m \) is considered first resolved from peak \( m + \Delta m \) when the height of the valley between them is 10% of the peak height \( h \). The resolution is then expressed as the value of \( m/\Delta m \).

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FIGURE 20

TEN PERCENT VALLEY RESOLUTION OF ADJACENT PEAKS
of high resolution measurements are to separate peaks only
millimass units apart and to perform precise mass determi-
nations when necessary to derive the structural formula of
an unknown substance. The higher the resolution desired,
however, the higher the cost of the instrument, and since
this capability is not necessary in many types of analysis,
the extra cost is not always justified.

The use of photoplate recording with high
resolution instruments has already been described as one
method for extremely accurate mass measurements despite its
disadvantages. Spectra of calibration standards with peaks
of known masses are interspersed at exposure positions
between those of the unknown. Careful (preferably
computerized) measurements of the distance between known
and unknown mass peaks can yield the exact mass of the
unknown to within one millimass unit (3). To achieve
similar results with instruments designed for electrical
recording, there is a "peak matching" technique which
requires an oscilloscope with a highly phosphorescent
"memory" screen (3). Once the reference or the unknown
peak is established on the screen, the accelerating voltage
is quickly changed to cause the second peak to be super-
imposed on the previous image of the first peak. The change
in accelerating voltage required to match the peaks is
proportional to the difference in masses of the two ions
which, when correlated with the exact mass of the reference ion, permits the calculation of the unknown mass. In both of these methods, low resolution spectra are usually run first in which peaks are selected for further analysis. A suitable reference compound is chosen with ions close to the unknown m/z values, and high resolution runs are then used for precise comparisons.

Sensitivity is another important parameter used in the evaluation of the performance of a mass spectrometer. Unfortunately, because of the many different types of analysis performed by MS, several different definitions of sensitivity have arisen to fit various situations, thus making comparisons between methods and instruments very difficult. In general, however, the sensitivity of a mass spectrometer refers to the response of the instrument to ions of a particular substance at an arbitrary m/z value (1). This response is evaluated under controlled conditions and is measured by the charge received at an electrical measuring device or by the degree of blackening of an ion-sensitive photographic plate caused by the introduction of a known amount of a sample via the batch inlet or the direct probe (4).

There are three main areas of consideration which affect the overall sensitivity of an analysis. The basic instrument sensitivity is dependant on the type and design
of each of its components, as described in previous sections. The method of sample handling determines the efficiency of sample utilization and includes purification and transfer steps, method of introduction, and scan parameters. Even factors such as the type and mass of the sample under consideration, and the presence of other substances will influence the output signal obtained for a given quantity of sample.

Sensitivity is expressed in several ways, depending on the type of analysis. For example, when a sample is evaporated from the direct probe into the ion source, the instrument response per unit weight of material consumed per second may be employed. GC-MS may use sample flux in nanograms per second. In trace analysis, sensitivity may be expressed as the minimum absolute amount of a substance, or the minimum concentration of a substance in a matrix of another material, that can be detected under given experimental conditions. Here, instrument noise must be considered and a signal-to-noise ratio of at least 2:1 is required to ascertain the presence of the substance being measured. However, sensitivity is expressed for a particular analysis, the definition should state: the chemical substance, the mass peak measured, the resolution used, the quantity of sample used per second, the electron beam intensity or emitter current, and the ion current
arriving at the detector (4). It must also be remembered that detection of a substance and its identification are not the same, and the minimum amount of sample required for identification cannot be specified, since the type of sample and amount of information needed varies so widely.

Although it might appear that the highest possible resolution combined with maximum sensitivity are the most desirable characteristics of a mass spectrometer, it must be noted that these two parameters are approximately inversely proportional to each other. Instruments capable of the highest resolutions are not only the most expensive but must impose certain conditions which are detrimental to the sensitivity attainable. For example, the narrower source and collector slits used to isolate smaller segments of the ion beam decrease the number of ions reaching the detector at each point along the spectrum. In addition, because of the slower scan rates which are necessary, an increased amount of sample must be used to maintain a constant sample pressure throughout the scan (3). This is particularly a problem in GC-MS where the peaks eluting from the GC column provide a constantly changing sample concentration, and a relatively fast scan must be obtained at the top of each peak in order to avoid distortion of the relative ion abundances measured. Thus, for very small samples, resolution and scan rate must be
kept as low as is practical to achieve high sensitivity (4). The ideal situation is, therefore, to be able to afford an instrument with adjustable slits, and interchangeable inlets, detectors, and even sources so that a wide range of resolution and sensitivity measurements can be employed during an investigation. In the fields of organic chemistry and biochemistry, the need for high resolution capability is obvious when considering that, for any nominal mass number, many possible combinations of carbon, oxygen, nitrogen, and hydrogen exist, and that this number of possibilities increases with mass (1). However, low resolution measurements are more sensitive, easier to obtain, and quite adequate for preliminary investigations and in cases where the identity of a compound is already known and interferences from other compounds do not pose a problem.

Throughout all of the preceding sections, many references to sensitivity and resolution have been made. Table 1 presents a brief review list of the instrumental components discussed along with comments on these aspects of their performance.

F. PROCESSING RAW DATA

1. Manual

Once a suitable set of mass spectra has been
### TABLE 1

**GENERAL REVIEW OF MASS SPECTROMETER COMPONENTS**

#### A SAMPLE INLETS:

1. **Batch Inlet**
   - Good for quick sample inspection; running authentic reference compounds for spectral files; metering sample to analyzer for sensitivity determinations
   - Requires at least 200 μg of compounds which are volatile and stable at inlet temperature

2. **Direct Insertion Probe**
   - 1 μg or less
   - Good for high molecular weight, less volatile, less stable compounds
   - Least convenient

3. **Gas Chromatograph**
   - Most efficient for small amounts of volatile components in mixtures
   - Good spectra from 1 μg samples present for only brief periods
   - Useful data obtainable for 10 ng or less depending on monitoring method (see later)
   - Components must be volatile and stable at temperatures used

4. **Liquid Chromatograph**
   - Best for high molecular weight, labile, non-volatile, polar compounds in mixtures
   - Sensitivity to equal GC as adequate interfacing becomes available

#### B ION SOURCES

1. **Electron Impact**
   - Most efficient and sensitive ionization method
   - Most fragmentation but possible loss of molecular ion; lower energy EI leaves more molecular ion but sensitivity is reduced
   - Best for structural determinations

2. **Chemical Ionization**
   - Good for less stable substances
   - Decreased amount of fragmentation and thus more prominent parent ion
   - Sensitivity comparable to EI

3. **Field Ionization**
   - Good for less stable substances
   - Significantly less overall sensitivity but compensated for by abundance of parent ion
TABLE 1 - Continued

4. Field Desorption - best for high molecular weight, unstable, involatile substances
   - sensitivity comparable to FI
   - least convenient

5. Atmospheric Pressure Ionization - most sensitive for samples in flowing gas or liquid streams
   - pg range of sensitivity

C MASS ANALYZERS

1. Magnetic - Single Focusing - low to medium resolution - 300 - 10,000

2. Magnetic - Double Focusing - capable of resolution up to 100,000 for most precise measurements
   - the higher the resolution used, the lower the sensitivity

3. Quadrupole - most suitable for masses below 250
   - resolution up to 1,000
   - linear mass scale
   - easiest to operate

D DETECTORS

1. Faraday Cup - low noise, good sensitivity, slow response
   - precise and accurate for high resolution

2. Electron Multiplier - very sensitive, fast response
   - less stable; response varies with type of compound

E RECORDERS

1. Strip-chart Recorder - good for large samples with few peaks
   - accurate for quantitative measurements
   - slow response and low but wide sensitivity range

2. Oscillographic Recorder - very sensitive when coupled with electron multiplier
   - wide sensitivity range, fast, and accurate

3. Oscilloscope - good for fast qualitative study and for transient phenomena

4. Photoplate - less sensitive but no scanning required
   - very accurate and precise
   - best for high resolution measurements
   - resolution up to at least 70,000
obtained from a sample, the first task in the interpretation process is to assign mass numbers to each of the peaks. Basic manual mass-marking involves identifying peaks of known mass, and then interpolating visually to derive mass numbers for the unknown peaks. For example, the unavoidable introduction of some air with the sample produces a nitrogen ($N_2^+$) peak at m/z 28 and a smaller oxygen ($O_2^+$) peak at m/z 32. The inclusion of a simple reference compound or, in the case of GC-MS, background peaks due to column bleed, also provide useful guides, as long as they do not interfere with the sample peaks. In the lower mass ranges, this extrapolation procedure is reasonably accurate, even for magnetic sector instruments which have a non-linear scan function. However, above m/z 200, the frequency of peaks decreases, and manual mass-marking can be very difficult, unless a quadrupole instrument has been used which has a linear scan function (4).

To simplify the mass-marking process, a separate calibration chart may be prepared from the spectrum of a known material which is obtained immediately after completion of the sample run, or even at the same time if a double-beam instrument is used. This chart is then used as an overlay for the marking of unknown peaks. The spectrum of perfluorokerosene (PFK) is frequently employed for this purpose because of the considerable mass range and convenient
periodicity of its peaks, as shown in Figure 21 (4). Unfortunately, the accuracy of this process for non-linear scans also diminishes as the mass increments become very close together in the higher ranges. However, many mass spectrometers can now be equipped with a mass calibration system that provides a simultaneous galvanometer trace, below the sample spectrum, showing mass units marked automatically with every tenth and hundredth mass mark accentuated (4). The "mass-marker" is a device capable of producing a potential proportional to the magnetic field which is then converted to a digital output calibrated in mass units and recorded in parallel with the detector output. Such a system not only reduces the time involved in mass-marking, but provides much more reliable calibration in the higher mass ranges (4).

Once the various peaks of a spectrum have been assigned mass numbers, the information sought may often be observed without any further work. However, for careful study which may involve comparisons with standards, catalogued spectra or with spectra from another instrument or source, each spectrum of interest should be reduced to a graphical or tabular form, showing quantitative measurements of peak intensities "normalized" to a common base. The most popular approach is to use the most intense peak in the spectrum as the "base peak", and assign to it a
FIGURE 21

MASS SPECTRUM OF PERFLUORINATED KEROSENE (PFK)

Legend

The considerable range and repetition of mass peaks every 100 mass units makes the PFK spectrum a useful overlay calibration chart for the mass marking of unknown spectra.

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FIGURE 21

MASS SPECTRUM OF PERFLUORINATED KEROSENE (PFK)
value of 100. The intensities (or heights) of all other peaks are then expressed as percent fractions; or relative abundances, compared to this base peak (4). In another method of normalization, the sum of the intensities of all peaks is used as a base and individual-peak intensities are expressed as percent fractions of the total ionization. The main disadvantages of this method are the necessity of measuring the intensities of all spectral peaks, even when only a few are of interest, and also the contribution of any impurities to all peak values. For both methods of normalization, the information obtained may be presented in the form of a bar graph or in a table, as illustrated in Figure 22 as well as in several previous figures.

The interpretation of mass spectra requires an understanding of ion fragmentation processes which can only be gained by experience, and it is often the identification of fragment ions that leads to the final determination of the structure of a compound, and even the true molecular weight in cases where the molecular ion is absent. Williams (5) and Waller (3) both present relatively concise accounts of many of these processes, although there are several texts devoted solely to the interpretation of spectra (27-30). Once postulated, the positive identification of an unknown requires a comparison with a known spectrum. There are now several sources of catalogued spectra
FIGURE 22

COMPARISON OF DIFFERENT FORMS OF MASS SPECTRAL DATA

Legend

(a) Raw unmarked oscillographic chart.

(b) Normalized bar graph showing peaks as percentages of the total ion intensity on the left side, while the right scale shows the relative abundance of all peaks, with the most abundant peak equal to 100.

(c) Tabulated relative abundance data. The peak at m/z 298 is indicated as the molecular ion, p.

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FIGURE 22

COMPARISON OF DIFFERENT FORMS OF MASS SPECTRAL DATA

(a)

(b)

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<th>\text{RA}</th>
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</table>
presented in a form that allows a fairly rapid search and comparison (31-33). The number of spectra in most listings ranges from 10,000 - 20,000, usually indexed in 4 - 6 ways; for example, by molecular weight, compound name, order of ion intensity, empirical formula. See Figure 23 for an example of a listing based on the m/z value of the most intense ion. Files of compounds from body fluids, metabolic studies, poisons, and drugs are still rather limited but are gradually increasing (examples, 34-36). Although most compilations consist so far of data collected from EI studies, files of spectra from other ion sources will also become available. In addition, each laboratory should build its own catalogue of spectra related to its own work. It should also be noted that, even when a positive identification cannot be made through a file search, the identification of fragments and a preliminary classification of the compound, along with knowledge of its origin is often sufficient for a reasonable conclusion.

2. Computer

From the previous description of the manual routine for processing a raw spectrum, it is apparent that, as long as the number of spectra acquired for the samples run each day is relatively low, the task is not insurmountable. For example, a mass spectrometer used for qualitative analysis on batch samples might put out 10-20 spectra per day (4). However, if the same instrument is operated in GC-MS style,
FIGURE 23

SAMPLE PAGE FROM A CATALOGUE OF KNOWN MASS SPECTRA

Legend

In this example, each compound is listed according to the 10 most intense ions in its mass spectrum starting with m/z 105 as the most abundant.

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### Figure 23

#### Sample Page from a Catalogue of Known Mass Spectra

<table>
<thead>
<tr>
<th>Composed Name</th>
<th>Mass Spectrum</th>
<th>C</th>
<th>H</th>
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<th>N</th>
<th>S</th>
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<th>M/Z</th>
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<tr>
<td><strong>Naphthalene</strong></td>
<td>114 77 51 50 30 146 79 37 149 50 7 9 1</td>
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the output might be 100-500 spectra per day, and the mass marking, normalization, interpretation, and file search process becomes virtually impossible if undertaken manually. Furthermore, the use of high resolution measurements for the study and identification of complex organic compounds also demands many hours of work if the data presented is to be thoroughly processed. The advantage of employing some type of computer for collection, reduction and even interpretation of such large volumes of data at a much faster rate than is possible by manual methods, cannot be over-emphasized, despite some weaknesses described by McFadden (4), which include possible loss or distortion of sharp peaks and exclusion of small peaks which may be important for interpretation.

The choice of the type of mass spectrometer-computer (MS-COM) system adopted is governed, not only by the basic requirements of a laboratory, but also by economic factors, thus accounting for the wide variety of systems in use which have been automated to different extents. Several examples of individualized setups are described in Waller (3). Nevertheless, the principles of the most basic computer-linked system are relatively simple. The continuous output (analogue signal) from either an electrical detector or a microdensitometer reading photoplate light transmittance is sampled at precise intervals and then
converted into digital (pulsed) form by an analogue-to-digital converter (ADC). These digital signals now consisting of amplitude and either time or count number (Figure 24) are passed directly to a computer, or may be stored on tape for processing later. All signals having an amplitude below a certain value are then rejected in a process called "thresholding", so that only data for peaks are handled further. Peak centers and areas are computed, and accurate masses are then calculated by relating the scan times of unknown peaks to those of a marker compound whose spectrum and exact peak masses and times are known. Normalization of peak intensities is carried out, and the information is displayed on an oscilloscope and/or printed out in standard graphic or tabular form.

In addition to the basic acquisition, processing and output steps described above, various other fairly simple data manipulations are also possible, examples of which are:

- presenting a summation plot of all scans made during a run (total ion chromatogram);
- correcting mass spectra for background;
- enhancing selected sections of a spectrum by a chosen multiplication factor;
- presenting difference mass spectra by subtracting any specific scan;
FIGURE 24

ANALOGUE-TO-DIGITAL CONVERSION FOR AUTOMATIC DATA PROCESSING

Legend

The continuous electrical output (analogue signal) from the detector of the mass spectrometer is sampled at precise intervals and then converted into digital (pulsed) form. Digital samples having an amplitude below a certain threshold limit are rejected so that only digitized data for peaks are further processed.

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FIGURE 24

ANALOGUE-TO-DIGITAL CONVERSION FOR AUTOMATIC DATA PROCESSING
presenting a summation plot of only certain selected ions in the spectrum of a run (mass chromatogram).

With proper interfacing, a computer may also be used to monitor and even control such instrument parameters as temperature, scanning, and electron beam intensities. More sophisticated operations, for which programs are fairly well-established, include file searches for comparison of the unknown with known stored spectra (37), and interpretive analysis where unknown ion masses are converted into all possible elemental compositions and likely structures suggested (38). More detail, references, and illustrations of computer capabilities in the MS field are presented at a fairly basic level of understanding by McFadden (4), and recent improvements in computer matching and interpretation are described by McLafferty, et al. (39).

G. ADDITIONAL INSTRUMENTAL TECHNIQUES FOR ACQUIRING DATA

1. Repetitive Scanning

The basic mass scan, as described in earlier sections, is achieved by the continuous alteration of certain instrumental parameters so that ions of various (usually increasing) m/z values reach the detector at different times. In magnetic sector analyzers, this involves varying either the accelerating voltage or the magnetic field strength, whereas in quadrupole instruments the radio frequency and direct current voltages of the rods are altered
while maintaining a constant ratio.

Following sample introduction for a batch analysis, a typical routine might consist of observing the arrival of sample ions at the detector via an oscilloscope, setting the appropriate instrumental parameters for optimum results, and manually activating the scan circuits at a time selected by the operator. During one run, several scans are usually performed, with or without further adjustments, until the sample is depleted.

With the advent of GC-MS for the study of complex mixtures, a more systematic approach to repetitive scanning was introduced (40) in which a complete mass spectrum was scanned every few seconds throughout the elution of the entire GC sample, or a fraction of interest. This cyclic scanning process can now be fully automated and is indispensable for GC-MS. Although the type of instrument, the mass range, and the resetting time are all factors which govern the scan cycle time, a typical scan cycle may take one second, and quadrupole instruments are generally capable of faster and more stable recovery than magnetic sector instruments (4).

Despite the widespread use of cyclic scanning with GC-MS, there are circumstances in which manual control of scan circuits is preferable, even for long runs. For example, by using a good oscillographic display, the best
starting time for a scan can be easily selected for very sharp peaks or for shoulder peaks which might otherwise be missed or poorly scanned by the automatic cyclic mode.

If a typical 30 minute GC-MS run is scanned every 5 seconds, the necessity of some form of computer for the recording and processing of the 360 mass spectra obtained is again obvious. Since many GC-MS runs take much longer than 30 minutes, automatic scanning, data acquisition and processing steps save countless man hours while providing complete mass spectra of all component peaks of the sample. These may then be inspected in a variety of ways. For example, Figure 25 illustrates a computer-generated total ion chromatogram compiled from all scans of a urinary steroid extract, whereas Figure 26 shows the computer-printed partial spectra of scans 5175 and 5189 of Figure 25. Figure 27 represents a computer summation plot of only selected ions of the total run on the same sample.

As seen in the sample illustrations, cyclic scanning coupled with computer processing has many advantages, but it is obviously limited by the size of data storage unit available, and by the capability of the mass spectrometer and its detector system for fast, reproducible scanning. In cases where 2-3 seconds may be critical for obtaining the best scan position for a particular sample component, valuable information may be lost, unless the
FIGURE 25

COMPUTER-PLOTTED TOTAL ION CHROMATOGRAM OF A URINARY STEROID FRACTION

Legend

Following repetitive scanning, the total ion intensity of each scan is plotted against the scan number. Refer to text for further explanation.

FIGURE 25

COMPUTER-PLotted TOTAL ION CHROMATOGRAM OF A URINARY STEROID FRACTION
FIGURE 26

COMPUTER-PRINTED MASS SPECTRA OF SELECTED SCAN NUMBERS

Legend

Partial spectra of scan numbers 5175 and 5189 are recalled from the repetitive scan data obtained from the same sample as in Figure 25. Refer to text for further explanation.

FIGURE 26

COMPUTER-PRINTED MASS SPECTRA OF SELECTED SCAN NUMBERS
FIGURE 27

COMPUTER-GENERATED SELECTED MASS CHROMATOGRAM

Legend

The intensities of ions at m/z 255, 273, 271, and 287 are plotted against scan number for comparison with the total ion chromatogram. Note that these ions are characteristic of the two steroid extract components displayed in Figure 26. Refer to text for further explanation.

FIGURE 27

COMPUTER-GENERATED SELECTED MASS CHROMATOGRAM
operator reverts to manual scan activation, as described previously.

2. Selective Ion Monitoring

One alternative to conventional scanning methods which has already been mentioned is the no-scan mode. This technique was described for photoplate detection where all ions are recorded simultaneously, and for electrical detection where analyzer parameters are set to direct the fraction of the ion beam at only one m/z value toward the collector. The process of selective ion monitoring (SIM) falls between conventional scanning methods and the no-scan mode, and involves the selective recording of the signals of only a few specified masses. This technique has also been called multiple ion detection (MID) and mass fragmentography.

By using prior knowledge of the unknown, a special voltage alternator can be programmed to cause the mass analyzer to switch between preset values which alternately direct the various ions of interest to the detector in a stepwise manner (3). The data are then recorded by a multichannel recorder using one channel for each mass selected, and the resulting pattern is called a mass chromatogram or mass fragmentogram. Figure 28 illustrates the mass chromatogram of two derivatives of the drug chlorpromazine which has characteristic ions at m/z 232, 234,
FIGURE 28

MASS CHROMATOGRAM OF CHLORPROMAZINE DERIVATIVES RECORDED BY SELECTIVE ION MONITORING

Legend

The ions at m/z 232, 234, and 246 were monitored to determine the presence of the drug chlorpromazine. The abundance of these ions showed a simultaneous rise and fall for the two compounds which eluted at 5 and 8 minutes, and these substances were therefore strongly suspected of being derivatives of chlorpromazine. This was subsequently confirmed. Refer to text for further explanation.

FIGURE 28

MASS CHROMATOGRAM OF CHLORPROMAZINE DERIVATIVES RECORDED BY SELECTIVE ION MONITORING
and 246. By selectively monitoring a few ions in the spectrum of a compound, the presence of that compound is usually confirmed if the ions appear in the correct relative amounts and at the correct time during a GC elution. The usefulness of this technique for quantitative analysis will be described later.

Although any mass spectrometer can be modified for SIM, most magnetic sector instruments are restricted in the mass range and the number of ions that can be selected without causing the ion beam to become defocused (4). The monitoring of 2-4 ions with a maximum range of 30% of the lowest mass value is typical. On the other hand, quadrupole instruments can be adapted for monitoring up to 8 selected ions over any mass range, and are capable of faster switching between masses than are magnetic analyzers. The relative merits of quadrupole versus magnetic sector instruments for SIM are discussed further by Falkner, et al. (41).

It may be recalled that a computer is easily capable of generating a mass chromatogram from repetitive full-scan data (Figure 27). However, the SIM technique provides a particular advantage when the sample is very small. Because a rapid scan of the entire spectrum is not performed, the time spent at each mass peak can be increased to as much as 1 second, thus providing a considerable increase in sensitivity and lower detection limits (4).
The actual sensitivity reached, however, obviously depends on factors such as the number of ions monitored, the fraction of total ion current carried by these ions, the ionization process used, and interference by ions from other compounds. It must also be remembered that the amount of structural information obtainable from SIM is considerably reduced, and some prior knowledge of the compound is necessary in order to select the appropriate masses to be monitored.

3. Integrated Ion Current

The integrated ion current (IIC) technique is another application of the no-scan mode, and has proven to be a simple and useful method for the identification and assay of components of crude biological extracts (42-44). Quantitative information down to the pg range is obtainable without the use of a gas chromatograph, even in the presence of gross contamination.

For this procedure, one prominent ion is selected from the spectrum of the compound to be assayed. The mass spectrometer is then set to continually record the ion current at the selected m/z value as the sample is evaporated from the direct insertion probe. The result appears as a series of lines representing ion abundance readings taken at short intervals during sample evaporation (See Figure 29). If the sample is pure, a symmetrical "envelope" enclosing the
FIGURE 29

INTEGRATED ION CURRENT PATTERNS PRODUCED BY p-TYRAMINE

Legend

(a) Symmetrical envelope enclosing recorded ion currents at m/z 137 obtained during the evaporation at 220°C of 1 ng of pure p-tyramine from the direct insertion probe.

(b) Ion current recorded at m/z 137 at 2-second intervals from a rat brain extract. The p-tyramine envelope is no longer symmetrical.

(c) Ion current recorded at m/z 137 from a mixture of the isomers α-, m-, and p-tyramine in which components evaporate from the probe at different rates.

Refer to text for further explanation.

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INTEGRATED ION CURRENT PATTERNS PRODUCED BY p-TYRAMINE
ion current readings is obtained, as shown for pure
p-tyramine monitored at m/z 137 in Figure 29 (a). However,
when the IIC technique is performed on crude extracts, the
envelope ceases to be symmetrical, as seen in Figure 29 (b).
Nevertheless, when sample handling and operating conditions
are identical in subsequent runs, the area under the curve
(or integrated ion current) is proportional to the concentra-
tion of the sample component under investigation. In
addition, it is even possible to identify isomers by this
method due to differences in rates of evaporation from the
probe (43). Figure 29 (c) shows a separation of o-, m-, and
p-tyramine.

Despite its apparent simplicity, the IIC procedure
requires an instrument capable of providing constant ion
source conditions and reliable calibration in order to ob-
tain accurate results, especially when applying it to crude
extracts. The use of reference compounds for checking
calibration and detecting instrument variation during IIC
measurements is described by Majer and Boulton (44). Another
obvious pitfall occurs if the ion beam being monitored arises
from more than one component of a mixture. Such a problem
may be alleviated by measuring the IIC for a different ion
in the sample spectrum, or else by converting the substance
to a suitable derivative.
4. Negative Ion Mass Spectrometry

During the last five years, a resurgence of interest in the detection of negative ions has been stimulated by the observation that far more negative ions are produced in the higher pressure ion sources, namely CI and API, than in the conventional EI source (45). The negative ions are formed by two main routes: strong gas phase acids ionize to \((M-H)^-\) in the presence of basic reagent gas ions, a process called negative chemical ionization; or negative molecular ions, \(M^-\), may be formed by direct electron attachment, called secondary electron capture. Although most organic molecules do not form stable negative ions at the higher pressures used, and thus do not appear, many environmentally hazardous compounds do, and can be detected and quantitated at remarkably low levels, even in mixtures (46). In some cases, for substances which do not ordinarily produce negative ions, the proper choice of a derivatizing agent may selectively enhance the negative ion current of the compound of interest without increasing the ion current due to other molecules in the mixture. Fragmentation, and thus structural information, is generally less than that obtainable with positive ion MS, but since parts of organic molecules which stabilize a negative charge are usually not the same as those that stabilize a positive charge, any fragment ions that are produced can provide complementary information.
The separation and measurement of negative ions is accomplished simply by reversing the polarity of the repeller, accelerator, focusing, and detector elements of the instrument and, of course, the magnetic field in sector analyzers. Although conventional electron multipliers with a positive potential at the first dynode have been used, better sensitivity and much lower noise levels have been achieved by accelerating the negative ions into a separate metallic surface (conversion dynode) held at a high positive potential (47). Positively charged sample ions and/or metal ions generated as a result of negative ion impact on the metal surface are then collected on the first dynode of a conventional positive ion electron multiplier placed several millimeters away.

Hunt, et al. (48) were among the first to achieve simultaneous recording of both positive and negative ion CI mass spectra using a quadrupole instrument. By "pulsing" the potentials at a controlled rate, packets of positive and negative ions are ejected from the ion source in rapid succession and enter the quadrupole filter. Unlike the situation in magnetic sector instruments, ions of identical m/e, but of different polarity, traverse the quadrupole filter with equal ease and exit the rods at the same point. Detection of positive and negative ion beams is accomplished
using two detectors of opposite conversion dynode potential, placed side by side at the exit aperture. Thus, positive ions are attracted to one and negative ions to the other, and the result is that both are recorded simultaneously as deflections in opposite directions with a conventional oscillographic recorder. Recently, Siegel (49) has reported simultaneous recording of positive and negative ion CI spectra plus conventional EI spectra. Examples of results obtained by negative ion mass spectrometry will be presented in CHAPTER III.

H. SAMPLE

This section deals with further discussion of various sample considerations introduced earlier, and more specific illustrations of results obtained will then be presented in the following chapter.

1. Pure Samples and Mixtures

In general, any spectroscopic, chemical or physical method of analysis is best performed on a pure substance, even though high levels of purity may be difficult or impossible to obtain with certainty when dealing with unknowns. However, pure samples of a compound under study are invaluable as references for either qualitative or quantitative comparison with unknowns, and also for sensitivity studies in which instrument response to known
amounts is evaluated. For pure unknown compounds, the interpretation of a mass spectrum involves the assumption that all ions present in significant amounts derive from a single molecular ion species and by careful study of fragments present, the structure of the unknown may first be postulated and then compared with the spectrum of a pure sample of the substance. One or two abundant fragment ions from a low-level impurity could misdirect the interpretation process, and although techniques such as low-voltage and soft-ionization MS may reveal the existence of impurities, it is preferable to ensure that pure samples are introduced whenever possible to increase the certainty of interpretation in such studies. There are, however, many types of analysis in which the information sought does not require a high degree of purity, as will be illustrated later.

The amount of sample available obviously has a direct bearing on the manner in which it is handled and its amenability to elaborate purification schemes. For extremely small samples, the isolation of minor components may be impractical, but adequate results may still be possible by intelligent manipulation of instrument parameters such as choice of sample introduction, mode of ionization, temperature controlled evaporation techniques, selective ion monitoring, negative ion measurements, high resolution measurements, and computer processing.
Samples of biological material which are among the most complex of mixtures are also characterized by minute amounts of analyte. For mass spectral analysis, these problems are further compounded by thermal instability, low vapor pressure due to high molecular weight and/or the polar chemical properties, and instability to the ionization process itself. With hundreds of compounds contributing to the mass spectrum of an unpurified biological sample, it is only in rare instances where the time saved by omitting some form of purification may justify the analysis of the extremely complex data that results.

As described earlier, the attractiveness of softer ionization techniques for studying biological mixtures is obvious because of the larger molecular ion peaks and reduced amounts of fragmentation. Frequently a CI or FI/FD analysis on a complex mixture will give information in a rapid and convenient fashion without separation. Such an example is shown in Figure 30 which illustrates the rapid and "certain" identification of barbiturates in the gastric contents of a drug victim. However, many other peaks are observed in this spectrum and so-called identification is really only a strong confirmation of a fact already suspected (4). It should also be remembered that some fragmentation is usually desirable as it reveals more confirmative structural information.
FIGURE 30

CHEMICAL IONIZATION MASS SPECTRUM OF GASTRIC CONTENTS FROM A DRUG VICTIM

Legend

The rapid identification of barbiturates from this direct probe crude extract is only a strong confirmation of a fact already suspected. For certain identification, techniques such as high resolution measurements, repetitive scanning during fractional evaporation, GC purification, and/or EI examination would be necessary.

FIGURE 30

CHEMICAL IONIZATION MASS SPECTRUM OF GASTRIC CONTENTS FROM A DRUG VICTIM

Pentobarbital
C₁₁H₁₈N₂O₃
M = 226

Seconal
C₁₂H₁₈N₂O₃
M = 238
Another technique which is particularly suited to the analysis of known compounds in highly complex mixtures, while requiring a minimum of sample "cleanup", is selective ion monitoring. Since one or more ions known to be diagnostically or structurally important are recorded, rather than the entire mass spectrum, interferences from other compounds may be virtually eliminated. High resolution SIM allows an even greater increase in selectivity by monitoring ion current only at a m/z value equal to the exact mass of the selected ion. For example, in analyzing extracts of biological samples for a 17-\(\beta\)-estradiol derivative, Millington, et al. (50) monitored ion current at m/z 416.257 with a resolving power of 10,000 (10% valley), thereby avoiding interference from other materials that produced an ion current at nominal m/z 416. It must be remembered, however, that SIM is only applicable when the identity of at least some characteristic fragments of the compound under investigation are already known.

2. Pretreatment

Despite the attractive possibility of being able to analyze some samples without purification, the quality of an analysis is usually substantially improved by the use of some form of initial cleanup. Especially with regard to specificity, it has been shown that the more sophisticated the isolation steps, the more reliable the results, even
when dealing with very small amounts (for example, ng/ml), and even if serious losses of sample are involved (51). Even a preliminary isolation of the chemical class desired by acid, base, neutral, or polarity extractions can lead to significant enrichment of the analyte under study. Thin-layer chromatography (TLC) and elution of desired zones is also widely used as an isolation procedure.

Although crude cleanup procedures are often adequate, the development of more complex techniques may be critical to some types of analysis, especially when studying unknowns. A typical sample pretreatment usually involves a two-phase extraction with a water immiscible organic solvent. More recently, column extraction techniques using ion exchange resins are being used, especially for polar and charged components (51). Further purification is achieved by using back extraction techniques (based on the same principle as the preliminary extraction but changing pH and polarity of the extractant), column chromatography, and TLC. Concentration by solvent evaporation under a stream of nitrogen gas, or under reduced pressure, then precedes introduction into the mass spectrometer either as a solid or dissolved in a suitable solvent.

Probably the most efficient and widely used technique for sample pretreatment is the gas chromatograph. As previously described, mass spectra of sufficient quality
are obtainable for every component that can be vaporized from the GC effluent even though the quantities may be very small and available for only a few seconds. GC has proven especially important for the isolation of substances not previously amenable to conventional separation techniques because of sample size or of sensitivity to hydrolysis, oxidation, etc. Introduction of the newer capillary columns has enabled even smaller and more complex samples to be characterized, and because of the smaller volumes used it has, in some cases, eliminated the need for the usual interfacing devices described earlier (52). Improvements in the control of SIM coupled with GC-MS have also made it possible to study more components in a mixture at one time with improved sensitivity. Although a total of 8-12 ion currents may be monitored during a run, the voltage alternating device may be programmed to monitor 2-4 ion currents during the given time interval that certain compounds are expected to elute, then switch to different ions in the next elution period, and so on, without the loss in sensitivity resulting from monitoring a large number of ions at one time (52). Despite the apparent ease with which GC separation combined with automatic data processing techniques appear to be able to select and present only the MS information on selected components of interest in a complex mixture, some type of extraction procedure, no matter how crude, prior
to GC introduction still substantially improves the quality of the results while decreasing the amount of data that must be processed.

For many components of mixtures which are not amenable to GC, the use of HPLC is rapidly growing. So far, because of the practical difficulties and/or expense encountered in obtaining on-line LC-MS systems, as described earlier, HPLC is widely used in off-line sample cleanup procedures prior to direct insertion or even GC-MS. DeRidder and Van Hal (53) have developed an automated HPLC system by attaching an auto-sampler and a time-controlled fraction collector which allows for unattended operation, handles 45-135 samples in 24 hours, and provides for recoveries and reproducibilities comparable to the manually operated system.

The use of derivatization in GC methodology is well-established as a chemical pretreatment technique for enhancing sample volatility by reaction with compounds that combine with the polar groups such as hydroxyl, carboxyl, amine and amide. In MS, derivatization may also result in a more pronounced parent ion peak, smaller chemical degradation products more suitable for MS analysis, or useful information on fragmentation. For GC alone, the goal in derivatization is simply a more efficient separation, but when used for MS the derivative group added should have a relatively small mass and should not undergo easy fragmentation which might
confuse the mass spectral interpretation (5). Esterification, methoxylation, acetylation, and reduction have all been applied along with exotic combinations of reagents for special applications. Some prior knowledge of the analyte is required and since each chemical system is different, general rules cannot be applied in every case. A textbook by Knapp (54) provides a wealth of information on derivatives of a wide variety of functional groups, together with practical details of their preparation, and references to their MS characteristics. Specific examples of derivatization are described among the papers presented in CHAPTER III.

3. Quantitation

The use of MS in quantitative analysis has proven to be, not only highly sensitive and specific, but continues to show increases in precision and accuracy which now rival radioimmunoassay (RIA) techniques (to be discussed later). The success of quantitation depends mainly on the careful choice of an internal standard, related in some way to the compound being quantitated, which is added at the earliest possible stage in the sample pretreatment. This is done in order to compensate for any variations in sample handling, ionization, and instrument response occurring within and between runs. In certain types of biological studies the internal standard may even be ingested by (or injected into) the subject before the sample is collected.
Quantitative results are then obtained by comparison of peak heights (or areas) of specific analyte ions in the mass spectrum with those resulting from the known amount of internal standard added.

As with any quantitative method, calibration curves are essential for determining linearity of detector response and the range of concentrations which provide reliable results. For biological material, this usually involves "spiking" aliquots of the sample with various known amounts of the analyte under study along with a constant amount of internal standard. A "blank" spiked only with the internal standard is used for the base line. Resulting peak height or area ratios between analyte and standard are plotted against the initial analyte concentration added. In addition recovery studies to determine sample losses during pretreatment are easily carried out by adding the same amount of standard to different samples but at a different stage along the way for each. Details of these and other techniques used in quantitative analysis by MS are described in a textbook by Millard (55), and a review of the topic as applied in biochemistry and medicine has been written by Lehmann and Schulten (56).

Compounds labelled with stable isotopes were first used for biological studies in 1940 (57) and are currently the most widely used reference standards for quantitative MS.
In general, a labelled analog of a substance under study will not be separated from the normal compound by any chemical or physical manipulations involved in sample preparation, but will be distinguishable in the mass spectrum. Standard curves are necessary, as when using any standards to calibrate the mass spectrometer, since significant isotope effects may be seen in MS between labelled and unlabelled compounds, leading to differences in ion abundances for a given fragment. One disadvantage in the use of isotope labels is the high cost of acquiring the isotope and placing it into a suitable position on the molecule. Although $^{13}$C, $^{15}$N, $^{18}$O, and $^{37}$Cl have all been used, deuterium has been the most popular because of its relatively lower cost and its availability at high isotopic purity in a wide variety of reagents and organic compounds, which thus facilitate its incorporation into standards. The standard ion peaks then selected for comparison with the normal ion peaks must obviously contain one or more labelled atoms, and the isotopic enrichment should be as great as possible to minimize any contribution from the standard ions to the intensities of the normal ion.

In many cases, however, when an isotopically labelled standard is prepared, it is not possible to achieve complete enrichment of the compound with the number of label atoms desired. This means that any molecules that have not acquired the label will contribute to the ion current at
the m/z value of the compound being measured. Conversely, the unknown may contain a significant amount of naturally occurring heavier isotope that will contribute to the standard ion currents. Thus, a simple ratio of the intensities of standard versus unknown ions will not provide an accurate quantitation. The isotope dilution technique, originally developed for biological studies in 1940 by Rittenberg and Foster (57), provides a means by which quantitation is still accomplished. The concentration of the unknown is determined from the change produced in the ion intensities resulting from its natural isotopic composition, by the addition of a known quantity of labelled compound for which the isotopic abundance has been previously determined. The calculations can be rather complicated but the method is quite accurate and is often used in metabolic studies, for example, of turnover rates and "pool" sizes. This method, along with many other aspects of the synthesis and use of stable isotopes in MS analysis, are presented with many references by Caprioli and Bier (58), and by Klein and Klein (59).

Other types of internal standards sometimes used for quantitation are radioactively labelled compounds, chemical analogs of the compound of interest, and compounds having the same general features but different structure. The latter two are the least expensive, but also the least
satisfactory due to the risk that some separation of the analyte and standard will occur because of differences in solvent solubilities, chemical reactivity to derivatizing agents, etc.

For the quantitation of known substances in biological fluids, selective ion monitoring has become invaluable by providing greater sensitivity and specificity than scanning methods. Using the internal standard techniques just described, one or more ions of a compound are monitored along with corresponding internal standard ions, and resulting peak height or area ratios are used for quantitation. The stable isotope standards are most appropriate since the corresponding peaks of ions containing the isotope are close to the normal ions, and thus facilitate quick and accurate switching between masses. Several examples of SIM and stable isotope quantitation are described in the following chapter. The integrated ion current technique introduced earlier is similar to SIM but applies more specifically to the continuous monitoring of ions of a sample as it evaporates from the direct probe. Here also, the area of the resulting evaporation profile of a sample ion is then compared to the corresponding peak area of the internal standard for quantitation.
CHAPTER III

DRUG ANALYSIS BY MASS SPECTROMETRY

It is obvious that in the fields of biochemistry and clinical chemistry alone the range and number of physiological compounds that are, or will be, amenable to mass spectrometry is almost infinite, but in order to demonstrate its potential at a more specific level this chapter will present examples of work in which the various instrumental techniques previously described have been used for the analysis of drugs in the fields of pharmacology, toxicology, and clinical chemistry. Examples presented will be limited to drug analysis in humans, and have been chosen to illustrate either a specific instrumental or sample handling technique or a specific application that may be of interest to the clinical chemist or biochemist. Much more information and many references on the analysis of specific drugs by MS may be found in several recent publications (60-63). In general, it will be seen that for most problems in qualitative analysis, such as structure determinations, investigating the metabolic fate of a drug, or identifying the cause of a toxic reaction or overdose, EI-MS techniques predominate because of the added information obtainable from the increased fragmentation that results. However, for rapid "screening", or for monitoring an already known drug or metabolite, softer ion-
ization methods are preferred, with CI being the most commonly used. For sample pretreatment, GC and LC are usually best when dealing with unknowns, whereas crude extracts combined with direct probe introduction are common for quick inspections or for quantitative measurements. In addition, SIM techniques used with stable isotopically labelled standards provide the most sensitive and accurate means for the quantitation required in therapeutic monitoring.

A. STRUCTURE ELUCIDATION

The task of determining the structure of a drug or its metabolite may range from a basic exercise to an extremely complex correlation of results obtained from a variety of sophisticated spectral techniques (64). Among the most difficult areas of structure proof is that of new drugs such as antibiotics and antitumour agents isolated from natural sources. In such cases, probably the single most valuable piece of information obtainable from MS is the molecular weight, or empirical formula if high resolution measurements are possible, for molecular and fragment ions produced under different ionization conditions. When dealing with complete unknowns, the interpretation of information obtained by MS usually requires an experienced organic chemist. Papers dealing with such interpretations involve discussions of fragmentations and rearrangements which are beyond the scope of this critique.
The structural elucidation of the metabolite of a known drug is usually a more straightforward exercise in terms of MS interpretation because the metabolism of a drug frequently involves only slight modifications of the original structure. The investigator knows the structure of the drug which was initially administered, has some idea of what kinds of structural changes to expect and, hopefully, a selection of standards or similar compounds available for comparison. The ideal approach is first to obtain spectra of the parent drug and any similar compounds available and to correlate the fragmentation with the structure in each case (65). Then, when spectra of the metabolites are obtained, comparable fragmentation patterns may be sought, leading by analogy to structural determination.

One of the difficulties initially encountered in any of these processes is determining which of the many compounds present in a biological sample are actually metabolites of the drug being studied. One direct approach to this problem, which is not often used in human studies, involves the administration of radioactively labelled drugs and a radioactivity detector in a split stream GC-MS (66). The total ion monitor provides a complete chromatogram of the mixture, while the radioactivity detector registers peaks only when drug-related material passes through. At these points in the chromatogram, the mass spectrometer may be
scanned and spectra obtained only for the labelled compounds.

Another approach which is used more often to identify drug metabolites is called the isotope cluster or "twin ion" technique. Administration of an equimolar mixture of unlabelled and stable-isotope labelled forms of the drug leads to the formation of metabolites whose mass spectra exhibit isotope clusters or doublets, thus facilitating their detection. This technique is illustrated in a paper by Horie and Baba (67) in a study of the metabolites of a central muscle relaxant, 3-phenylpropyl carbamate. An equimolar mixture of the normal drug and drug labelled with 5 deuterium atoms attached to the aromatic ring was administered. Urine was treated with $\beta$-glucuronidase to hydrolyze metabolites conjugated with glucuronic acid by the liver, and extracts were derivatized by trimethylsilylation and analyzed by GC-MS (EI). Spectra of fractions showing characteristic doublet patterns, as shown in Figure 31, were recognized as drug-related compounds. The postulation of the structure of these metabolites and their confirmation by comparison with the spectra of authentic samples of the suspected compounds is also described in this paper, along with an illustration of the isotope dilution technique for quantitation.
FIGURE 31

ISOTOPE CLUSTER OR "TWIN ION" TECHNIQUE FOR METABOLITE RECOGNITION

Legend

Ingestion of an equimolar mixture of the drug 3-phenylpropylcarbamate plus its $\left[^{2}H_5\right]$-labelled analogue results in metabolites whose spectral fragments exhibit paired peaks differing in 5 mass units. The mass spectra shown resulted from the trimethylsilyl derivatives of:

(a) 3-phenylpropanol
(b) 3-hydroxy-3-phenylpropanol
(c) 3-hydroxy-3-phenylpropylcarbamate
(d) 2,3-dihydroxy-3-phenylpropylcarbamate

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FIGURE 31

ISOTOPE CLUSTER OR "TWIN ION" TECHNIQUE FOR METABOLITE RECOGNITION
Another example of the use of MS in a structure elucidation problem is presented in a reasonably concise description by Barry and Petzinger (68). The authors were involved, for litigation, in the confirmation by MS of the presence of ephedrine in adulterated powdered ipecac and ipecac fluid extracts. The products had been found to be subpotent and apparently diluted with ephedrine in order to elevate the total alkaloids content so that they would conform to government standards. MS did confirm the presence of ephedrine but as a result of the study some other information was obtained. Although FI, FD, and CI techniques had all shown an abundant (M + 1)$^+$ ion for ephedrine, EI spectra either exhibited no molecular ion at all, or one of insignificant abundance that could not be readily distinguished from background peaks. The fragment ion with the highest mass was found at m/z 146 or (M - 19)$^+$ and the structure of this ion had not yet been confirmed. In this paper, the authors illustrate how, by studying the spectra of deuterated and normal ephedrine and its analogs, a proposal for the fragmentation pathway of ephedrine and the structure of the ion at m/z 146 is derived, and how a previously proposed structure is disproved.

In a similar exercise, Smith, et al. (69) derived the structure of a newly discovered metabolite of 5,5-diphenylhydantoin by studying differences in the fragmentation patterns that result from using several different derivatizing reagents.
B. METABOLISM

The study of the activity of a drug in the body is the task of the pharmacology laboratory and may incorporate such factors as rates of absorption and excretion, and any metabolic transformations undergone which may involve the production of reactive or toxic intermediates. Structural determinations and quantitation of the drug itself and/or its metabolites in body fluids and tissues are usually essential. Mass spectrometry has proven to be invaluable in providing the sensitivity and specificity necessary for obtaining the type of qualitative and quantitative information required from the small amounts of sample often available.

In a paper by Midha, et al. (70), the value of HPLC for sample cleanup and its advantage over GC for some problems is demonstrated in the determination of metabolites of caffeine in human plasma. Previously proposed metabolic pathways for caffeine had been impossible to prove by conventional MS techniques because of difficulties in producing characteristic volatile derivatives that would separate adequately by GC. The use of HPLC allowed adequate separation of metabolites without derivatization, and resulting mass spectra were able to provide positive proof that theophylline, theobromine, and paraxanthine are metabolites of caffeine, and confirmation that the known
metabolic pathways of theophylline catabolism are also applicable to caffeine.

Plasma samples plus ammonium sulfate were extracted with a chloroform/isopropanol mixture. The organic layer, separated by centrifugation, was evaporated to dryness under nitrogen, redissolved in HPLC solvent and put through two different HPLC systems for purification and fractionation. Effluent fractions were collected manually, dried, and introduced into the mass spectrometer by direct probe for EI. Confirmation of proposed structures was then achieved by comparison of mass spectra obtained with those of plasma samples spiked with known compounds.

A somewhat related metabolic study by Brazier, et al. (71) involved the use of theophylline for treating apnea in premature newborns. Normal drug combined with \([1,3-^{15}N]\), \([2-^{13}C]\) theophylline was administered to newborns and timed blood and urine samples were collected. Crude chloroform/isopropanol extracts were treated to form N-pentyl derivatives and the ion cluster technique was employed for identifying the metabolites following GC-MS. TLC was then used to purify some of the metabolites for structure identification. Results showed the presence of caffeine in amounts running lower than, but parallel to, the plasma theophylline levels, and that the metabolism of theophylline in newborns is the inverse of the metabolism of caffeine in adults.
Specialized applications of stable isotope labelling and detection by MS which also show great promise in metabolic studies include such areas as comparisons of bioavailability of different formulations or routes of administration and studies on stereospecific aspects of drug metabolism and activity (61).

In a study of the comparative bioavailabilities of two commercial drug preparations, for example, Heck, et al. (72) used a combination of HPLC and direct probe FI-MS along with stable isotopes to quantify the drug. Imipramine (IP), the active ingredient in Tofranil, is widely prescribed for the treatment of depression. It is readily absorbed from the intestine and is extensively metabolized, a major product being the monomethylated derivative desipramine, which is also pharmacologically active. IP is usually administered to depressive patients on a daily basis over a period of several weeks, and under such conditions a very wide range of plasma concentrations has been found. Patient recovery is reported to be highly correlated with total plasma levels of IP and desipramine that exceed 180 ng/ml, and the purpose of this study was to see if there is any difference in the bioavailabilities of Tofranil and generic IP.

Each of eight subjects was given a 25 mg tablet of imipramine (either Tofranil or generic IP) along with 25 mg
of $\left[ \text{H}_2 \right]$ IP hydrochloride which was used to compensate for inter- and intrasubject variability. Blood samples were then drawn at regular intervals and analyzed for the drug. One week later, the study was repeated on the same subjects but those who had received Tofranil in the first trial received generic IP in the second, and vice versa.

Before the rather lengthy extraction procedure, a known amount of a $\left[ \text{H}_6 \right]$ IP hydrochloride reference standard was added to each plasma sample. The samples were then made alkaline with sodium carbonate and extracted three times with a hexane/iso-amyl alcohol mixture using centrifugation to separate the layers. Combined organic extracts were then back-extracted into dilute HCl. Further purification was achieved by extracting the drug into $\text{Na}_2\text{CO}_3$ and hexane, back again into HCl, and finally into the $\text{Na}_2\text{CO}_3$/hexane. Despite all of the steps involved, later measurements of the $\left[ \text{H}_6 \right]$ IP remaining showed a 72% recovery.

Still further cleanup was accomplished using HPLC. The hexane was evaporated under nitrogen and the residue dissolved in HPLC solvent composed of methylene chloride; iso-propanol, and n-propylamine. IP emerging at about 6 minutes was collected and the solvent evaporated under nitrogen.

For MS, the residue was resuspended in 15 μl of purified hexane saturated with methanol and applied to the
outside of a sealed glass capillary. After solvent evaporation, the capillary was placed in a larger capillary and dropped into the tip of the direct insertion probe of the mass spectrometer. The probe was then cooled to 0°C with a freon aerosol and inserted through the vacuum lock. All of these final steps served to prevent the sample from evaporating too rapidly into the ion source.

Finally, the probe tip was heated gradually from 0-110°C and the evaporating sample was subjected to FI-MS during which repeated scanning was performed over the mass region m/z 278-292 for computer processing and comparison of peaks at m/z 280, 282, and 286 representing IP, $[^{2}\text{H}_2]\text{IP}$, and $[^{2}\text{H}_6]\text{IP}$, respectively.

The results of the study showed that both forms of the drug attained almost identical maximum levels in each subject, and that levels as low as 0.41 ng/ml could be accurately quantitated, which was an order of magnitude more sensitive than previous methods using GC-MS with SIM.

In another study by Matin, et al. (73), the potential of MS as a sensitive and specific method for studying the effects of stereocheristry in drug activity and metabolism is demonstrated. Amphetamine is optically active, the D isomer being a more potent central stimulant and sympathomimetic agent than the L isomer, and this work describes a method for the simultaneous measurement of D- and L-amphetamine.
at the ng/ml level in plasma and even saliva when racemic amphetamine is administered to the patient.

For the study, a human volunteer was administered 10 mg of amphetamine orally at four different times. On three occasions, DL-, D-, and L-amphetamine were ingested under alkaline urine conditions. On a fourth occasion, DL-amphetamine was administered under acidic urine conditions. Controlled urine pH was achieved by the administration of 2 g of either sodium bicarbonate or ammonium chloride every 4 hours during the study. At various intervals over 48 hours both saliva and heparinized blood samples were collected. Saliva flow was induced by having the subject chew on a piece of Teflon.

To each sample was added a known amount of a racemic $\left[^2\text{H}_2\right]$ amphetamine internal standard. Samples were then alkalized, and the drug extracted with hexane followed by derivatization by reaction with $\text{N}$-pentafluorobenzoyl-$\text{S}$-(-)-propyl-1-imidazole. The reaction of the amphetamine enantiomers with this chiral reagent yields diastereomers which are easily resolved by GC. Dried extracts were reconstituted in ethyl acetate for GC-MS injection and ionized by methane CI.

The L- and D-amphetamine derivatives eluted at 13.4 and 19.2 minutes respectively and produced mass spectra in which the only abundant ions were the quasi-molecular,
\((M + H)^+\), ions at m/z 427 and 429 representing the normal and labelled forms of the drug. For quantitation, SIM at these two m/z values was performed, and good results were obtained by comparison of either peak height or area ratios. Figure 32 shows some of the selected ion recordings which resulted. Calibration curves were prepared by plotting peak height ratio versus the known amount of unlabelled derivative spiked in blank plasma or saliva. These proved to be linear from 0-80 ng/mL.

Plots of plasma and saliva drug concentration versus time yielded several conclusions. In all cases, the L isomer exhibited a slower rate of disappearance (longer "half-life") than the D isomer, probably due to slower metabolism. When DL-amphetamine was administered, the half-life of each isomer was the same as when taken alone. Under alkaline urine conditions, both forms of the drug remained longer. Finally, although saliva values were higher than plasma levels, the rate of disappearance for both was parallel, and thus demonstrated the value of saliva analysis as a non-invasive technique for monitoring the uptake of amphetamine.

In a more recent paper, Frank, et al. (74) describe the development of a chiral stationary phase for GC columns which causes the separation of enantiomers during GC without the type of derivatization just described.
FIGURE 32

SELECTED ION RECORDING OF ISOMERS OF AMPHETAMINE AND STABLE ISOTOPE-LABELLED AMPHETAMINE

Legend

SIM was performed at m/z 427 for amphetamine and m/z 429 for \([^{2}\text{H}_2]\) amphetamine after extraction from plasma and derivatization. Derivatives from both forms of L-amphetamine eluted at 13.4 minutes while D isomers eluted at 19.2 minutes.

Recordings shown derive from:
(a) 100 ng of DL-amphetamine plus 100 ng of DL-\([^{2}\text{H}_2]\) amphetamine;
(b) 50 ng of L-amphetamine plus 100 ng of DL-\([^{2}\text{H}_2]\) amphetamine;
(c) 50 ng of D-amphetamine plus 100 ng of DL-\([^{2}\text{H}_2]\) amphetamine;
(d) blank plasma;
(e) saliva containing 100 ng of DL-\([^{2}\text{H}_2]\) amphetamine only.

Refer to text for further explanation.

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SELECTED ION RECORDING OF ISOMERS OF AMPHETAMINE AND STABLE ISOTOPE-LABELLED AMPHETAMINE

FIGURE 32
C. THERAPEUTIC MONITORING

It is well known that drug dosage requirements for optimal therapeutic effect vary greatly among patients; that routine administration of an identical dosage to all patients will be ineffective in some and cause serious drug toxicity in others (75). This inconsistent relationship between dosage and pharmacologic effect is obviously the result of individual patient variability in rate and completeness of drug absorption, body size and composition, drug distribution among body compartments, drug binding at nonreactive sites, and rates of drug metabolism and excretion. Thus, for most drugs, the actual serum concentrations of the drug itself or its active metabolite reflect the availability to the target organ or cell, and correlate better with therapeutic effect or toxicity than does drug dosage. The combined versatility, specificity, and sensitivity of MS methodology can provide a quantitative method for accurately adjusting drug dosage to individual needs, especially when dealing with substances for which simpler methods have yet to be developed (for example, antitumour and antiviral agents). Furthermore, since most of the standard therapeutic monitoring procedures have been developed for individual drugs and do not distinguish metabolites from parent drug, and because multiple drug therapy is quite common, the advantage of MS in providing complete "profiles" for drugs
and their metabolites in a single analysis is also apparent.

A study by Truscott, et al. (76) demonstrates the simultaneous quantitation of 5 anticonvulsants in crude underivatized extracts using direct insertion CI-MS. The technique is rapid, simple, and sensitive, allowing for the routine analysis of 50 μl of serum in 30 minutes, and shows good within run and day-to-day precision. The successful analysis relies on both the fact that a drug or drug metabolite is often present in much greater concentration in the extract than the endogenous compounds, and also on the simpler mass spectra obtained using CI.

Known amounts of the following stable isotope-labelled standards were added to 50 μl of serum which already contained the unlabelled form of each drug: \([^{13}C_2]\), \([^{15}N_1]\) diphenylhydantoin (DPH), \([^2H_3]\) mephobarbital, \([^{2}H_5]\) carbamazepine, \([^{15}N_2]\) phenobarbital, and \([^{2}H_5]\) primidone. Acidification with HCl was followed by extraction with chloroform and evaporated to dryness under nitrogen. The residue was redissolved in chloroform for transfer to the direct probe tip and dried under a vacuum prior to undergoing CI-MS using isobutane as the ionizing reagent gas. As the sample fractionally evaporated from the probe, 30 scans were taken over 3.75 minutes and computer processed.

Figure 33 shows the evaporation profile of each drug by plotting the ion intensity at the corresponding
FIGURE 33

EVAPORATION PROFILE OF FIVE ANTICONVULSANT DRUGS UNDERGOING CHEMICAL IONIZATION FROM THE DIRECT INSERTION PROBE

Legend

Relative intensities versus scan number for quasi-molecular ions of mephobarbital at m/z 247, phenobarbital at m/z 233, carbamazepine at m/z 237, primidone at m/z 219, and diphenylhydantoin (DPH) at m/z 253, all extracted from underivatized plasma.

Refer to text for further explanation.

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FIGURE 33

EVAPORATION PROFILE OF FIVE ANTICONVULSANT DRUGS UNDERGOING CHEMICAL IONIZATION FROM THE DIRECT INSERTION PROBE.
m/z value for each versus the scan number. Figure 34 shows two CI mass spectra of the chloroform extracts corresponding to scans 9 and 18 of Figure 33, and show that the protonated molecular ions of the drugs and their internal standards are clearly the most abundant ions in the spectra.

Concentrations of the individual anticonvulsants were determined by comparing the peak height of the protonated molecular ion of the unlabelled drug with that of the corresponding labelled drug and averaging the result from a number of scans. Extraction efficiencies were determined by comparing the protonated molecular ion ratios just described with those from serum extracts in which the internal standards were not added until after the extraction step. Recoveries ranged from 78% with primidone to 96% with DPH. Similar results were obtained by extracting the drugs at neutral pH.

Interference with this type of assay procedure can arise if there are compounds present in the extract which contribute ions of the same masses as the drugs of interest. However, such compounds need not necessarily interfere if they have volatilities which differ from these drugs. For example, Figure 35, taken from the paper just described, shows the ion current profile at m/z 256. The first peak in this profile from spectra 5 through 11 arises from the molecular ion of palmitic acid which is easily extracted from serum.
FIGURE 34

SELECTED MASS SPECTRA FROM REPETITIVE SCANNING OF FIVE ANTICONVULSANTS UNDERGOING CHEMICAL IONIZATION FROM THE DIRECT INSERTION PROBE

Legend

Mass spectra result from an underivatized extract of plasma containing drugs indicated plus their stable isotope-labelled analogues, and correspond to (a) scan 9 and (b) scan 18 of Figure 33.

The ratio of peak heights between each drug and its corresponding internal standard is multiplied by the known standard concentration for quantitation.

Refer to text for further explanation.

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FIGURE 34

SELECTED MASS SPECTRA FROM REPETITIVE SCANNING OF FIVE ANTICONVULSANTS UNDERGOING CHEMICAL IONIZATION FROM THE DIRECT INSERTION PROBE
FIGURE 35

ION CURRENT PROFILE AT MASS 256 ILLUSTRATING DIFFERENT VOLATILITIES OF TWO COMPOUNDS OF THE SAME MASS

Legend

Potential interference from palmitic acid, which appears at the same mass as the internal standard for DPH, is avoided by taking advantage of their sequence of evaporation from the direct probe and using peak heights from later scans for the quantitation of DPH.

Refer to text for further explanation.

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FIGURE 35

ION CURRENT PROFILE AT MASS 256 ILLUSTRATING DIFFERENT VOLATILITIES OF TWO COMPOUNDS OF THE SAME MASS.
with chloroform. The second peak corresponds to the labelled internal standard of DPH. In this case, the two components have significantly different volatilities and consequently the presence of palmitic acid in the chloroform extract of serum does not interfere with the assay for DPH. Cholesterol, caffeine, and fatty acids are examples of endogenous compounds which appear regularly in significant amounts in organic extracts of serum.

A very similar method has been described by Garland, et al. (77) for the quantitation of the antiarrhythmic agents lidocaine, quinidine, and the metabolite, dihydroquinone, in which benzene extracts of alkaline plasma were processed by direct insertion isobutane CI-MS. In this instance, a turn-around time of 5 minutes is claimed, which probably does not include the extraction steps. Figure 36 illustrates the type of spectra obtained including the prominence of cholesterol and caffeine in plasma extracts which in this case does not interfere with the analysis.

Of particular interest in some of these studies is the use of the internal standard to correct for variability among subjects and in the same subjects at different times. Individual variation in response to drugs is often clearly demonstrated by the fact that subjects who suffer uncomfortable side effects often achieve much higher blood levels of the drug than other subjects, despite receiving the same dose.
FIGURE 36

DIRECT INSERTION CI-MS OF ANTIARRHYTHMIC DRUGS

Legend

(a) Isobutane CI mass spectrum of a benzene extract of plasma taken 180 minutes after administration of 15 mg/kg quinidine gluconate. Ion at m/z 325 represents quinidine, while ions at m/z 327 and 329 arise respectively from the dihydroquinone metabolite and the \( [2H_2] \) dihydroquinone internal standard. The base peak at m/z 369 arises from the loss of water from the quasi-molecular ion of cholesterol but does not interfere with the analysis. Most other endogenous compounds are present in much smaller amounts than the drug and its metabolite being measured.

(b) Isobutane CI mass spectrum of a benzene extract of plasma taken 60 minutes after administration of 250 mg lidocaine hydrochloride monohydrate. Ions at m/z 235 and 239 represent lidocaine and \( [2H_4] \) lidocaine internal standard respectively. Here the large peak at m/z 195 represents caffeine and was also present in the zero-time blood sample from the same patient.

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FIGURE 36

DIRECT INSERTION CI-MS OF ANTIARRHYTHMIC DRUGS

(a)

(b)
For situations in which the drug being measured cannot be easily distinguished from an interfering substance at the same mass, high resolution techniques in the mass region under investigation should provide satisfactory results if the direct analysis of extracts without a chromatographic separation is still desired. For example, Duncan, et al. (78) describe the use of high resolution measurements on crude extracts of serum, milk, sweat, saliva, cerebrospinal fluid, and synovial fluid during a study of cyclophosphamide, a widely used immunosuppressant and antitumor drug. The drug was found to be too unstable for GC-MS and a method was being sought to identify it directly from crude extracts.

Samples of the above fluids collected from patients undergoing cyclophosphamide therapy were simply extracted three times with equal volumes of chloroform. The chloroform layers separated by centrifugation each time were combined and evaporated to dryness under nitrogen. For milk, an extra extraction step was necessary to remove excess lipophilic compounds during which the chloroform residue from the routine extraction was dissolved in pentane and the drug back extracted into water, and then again into chloroform. Residues were then dissolved in chloroform for transfer to the direct probe, and subjected to EI-MS.

For samples in which the amount of the drug was fairly high, the examination of low resolution spectra and
fractional evaporation patterns, as described earlier, were sufficient for identification. However, for the sweat and 9-hour serum extracts, the small amount of drug present created difficulties in identification, but subsequent high resolution measurements of ions appearing at the usual position of the cyclophosphamide ions did permit the assignment of the elemental compositions, and confirmation that these were indeed the molecular ions and fragments of the drug.

The advantage of FD techniques for the analysis of unstable and less volatile compounds is well known. However, its use in routine quantitative drug studies has been rare to date because of the time consuming manipulations involved and variabilities in the desorption process. In recent years, the variability problems have been resolved to some extent by the use of computer-controlled emitter current-programming and signal-averaging techniques, plus the development of silicon emitters (79 and its references).

As an example of the use of FD-MS, a paper by Lehmann, et al. (80) compares FD and EI results in the qualitative and quantitative analysis of dopamine and some of its metabolites in urine. In previous studies of this drug, strong fragmentation had eliminated most of the molecular ion. However, in this work, high molecular weight fluorescent derivatives of the drug and its metabolites were
produced by reaction with 5-di-n-butylaminonaphthalene-1-sulfonyl chloride (BANS-Cl) and resulted in prominent molecular ions in both FD and EI spectra. The derivatives had been isolated by TLC and visualized by fluorescence at 364 nm. The spots were scraped off, extracted into ethyl acetate and concentrated under nitrogen. The extract was applied to the FD emitter by the "dipping" method and ions were detected by photoplate recording. Quantitative results, using $[{}^{2}H_{4}]$-dopamine as the internal standard, compared well with direct insertion EI results.

The potential of negative ion MS for the measurement of very small amounts of substances in complex mixtures has been described in the previous chapter. Because not all molecules form significant numbers of negative ions, any drugs that do will produce a spectrum with a signal-to-noise ratio that is much higher than would be seen in positive ion MS. Horning et al. (81) demonstrate this by using API negative ion MS for measuring phenobarbital and diphenylhydantoin. To plasma samples containing these drugs were added known amounts of the same drugs containing three $^{13}C$ atoms. Crude extracts were concentrated and injected directly into the API source. Since these two drugs are strong gas phase acids, the ions formed correspond to the anions $(M-H)^{-}$ and are illustrated in Figure 37 as being easily detectable above the background. A further improve-
FIGURE 37

NEGATIVE ION API MASS SPECTRUM OF PHENOBARBITAL AND DIPHENYLHYDANTOIN

Legend

Mass spectrum results from direct insertion of a crude extract of plasma following administration of these drugs. Known amounts of $^{13}_3C_3$ phenobarbital and $^{13}_3C_3$ DPH were added as internal standards before extraction.

Refer to text for further explanation.

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NEGATIVE ION API MASS SPECTRUM OF PHENOBARBITAL AND DIPHENYLHYDANTOIN

NEGATIVE IONS (M-H)^-

231
\text{\textsuperscript{12}C}

\text{PHENOBARBITAL}

234
\text{\textsuperscript{13}C}

254
\text{\textsuperscript{13}C}

251
\text{\textsuperscript{12}C}

\text{DIPHENYLHYDANTOIN}

\text{PLASMA SAMPLE}

\text{PLASMA BLANK}

m/\text{z}
ment in sensitivity, because of better compound isolation, was achieved by Horning et al. (11) by allowing the entire effluent stream from a liquid chromatograph to vaporize through the API source, followed by computer processing of multiple scans of the negative ion fractions.

Hunt and Crow (47) also demonstrated the potential of negative ion MS for drug analysis when they used conventional GC-CI-MS and SIM to detect dopamine, amphetamine, and Δ⁹-tetrahydrocannabinol derivatives at the attomole (10⁻¹⁸) level.

The analysis of saliva samples provides another example of the advantage of the sensitivity of MS for therapeutic drug monitoring. As mentioned during the description of the work of Matin et al. (73) on amphetamine isomers, there is a direct relationship between plasma and saliva concentrations of some drugs. Horning (82,83) has shown that saliva can be used instead of plasma for therapeutic monitoring if the transfer of the drug from plasma to saliva is not affected by the difference between the pH of plasma (7.4) and saliva (6.5-7.2). For weakly acidic or basic drugs this difference is not important and the concentration of the drug in saliva will correspond to the concentration of the free, pharmacologically active form of the drug in plasma. However, the transfer of more acidic or basic drugs is severely affected even by slight
changes in the pH of the saliva alone. Thus, for drugs in these groups it is difficult to predict the concentrations in plasma from measurements made on saliva.

Nevertheless, for certain drugs it was shown that saliva samples may prove clinically useful. The drugs were more easily isolated from saliva than from plasma because of the lower protein concentration. They were also able to survive a procedure whereby saliva with added internal standard was transferred to an adsorbant paper, dried for storage, and later re-extracted for quantitation by LC- or GC-MS, thus indicating the ease with which samples might be mailed to a central laboratory for analysis. Because saliva can be collected by non-invasive techniques, it is extremely useful when repeated sampling is required for therapeutic monitoring, especially in the elderly and in young children.

D. TOXICOLOGY

1. Clinical and Forensic Medicine

The necessity for rapid identification of sub-microgram amounts of unknown materials extracted from human physiological samples is one of the most difficult problems encountered in toxicological analysis. The circumstances in which the problem occurs may range from hospital emergency rooms needing accurate diagnosis of comatose patients, to urine analysis for narcotics or drugs-of-abuse on patients associated with methadone clinics and probation
departments, to forensic science analysis to detect drugs in drivers suspected of being "under the influence", and to the complex analytical problems involved in post-mortem toxicology where the cause of death is the critical issue (84). The constantly increasing involvement of multiple drugs in cases of unexplained death and in unexpected adverse clinical reactions has forced the introduction of meticulous analytical techniques which provide unequivocal qualitative and quantitative information. GC-MS-COM systems developed for this purpose represent perhaps the most sophisticated technology available to date.

Simple extraction procedures are used for blood, urine, or gastric aspirate. The extracted components are separated by GC and identified from their mass spectra by comparison with reference data. Mass spectral files containing the necessary range of compounds for automatic identification by computer matching, have been compiled by a number of workers (examples, 85-87). Computer-library searching has advantages, both because it saves time and because the operator requires no detailed knowledge of mass spectra. The spectral files are central to the MS methods, and if complete, should include: a) all prescribed drugs and their metabolites where necessary; b) household poisons; c) common artifacts and contaminants; and, d) normal body fluid constituents (6). The last category need not extend
beyond compounds that are abundant in and readily extracted from body fluids, because the toxic material is frequently present at such high concentrations that other endogenous compounds do not interfere at the sensitivities used. Any mass spectra of previously unencountered compounds found in the course of analyzing overdose samples can be added to the file. For example, Costello et al. (85) described their methods and experiences in the analysis of materials from over 600 patients. Gastric contents (10 ml), blood (5 ml) and urine (10 ml) samples were the specimens usually supplied by an area hospital. The isolation procedure consisted of filtering or centrifuging the sample, extraction of the supernatent with 5 volumes of high-purity methylene chloride and drying the combined organic fractions over sodium sulfate. This extract was then concentrated to about 100 μl, and 2 to 5 μl samples were analyzed by GC-MS. Comparison of the unknown and known mass spectra was accomplished by the computer in two phases: a presearch, and then a detailed comparison of abbreviated mass data. The output of data for this system is shown in Figure 38 for the analysis of urine from a teenage patient who had ingested the drug phencyclidine. In addition to the total ion chromatogram, a contour plot of the "closest fit" found in the library for each mass spectrum is also given. Of 600 samples analyzed, at least one toxic substance was identified in 75% of the cases which represented a range of 72 different
FIGURE 38

TOTAL ION CHROMATOGRAM AND CONTOUR LIBRARY-SEARCH PRINTOUT FROM AN OVERDOSE VICTIM

Legend

Methylene chloride extract of urine was analyzed by GC-EI-MS and results of the computer library search plotted in the same shape as the total ion chromatogram with the "closest fit" for each scan indicated. The result of the analysis indicated an overdose of phencyclidine.

Refer to text for further explanation.

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FIGURE 38

TOTAL ION CHROMATOGRAM AND CONTOUR LIBRARY-SEARCH PRINTOUT FROM AN OVERDOSE VICTIM

BODY FLUIDS 72, MALDEN HOSPITAL
TOTAL IONIZATION PLOT    RUN NO. 372
DATE = 2-9-72    NUM. OF SPECTRA = 233
drugs and 26 other toxic substances.

Gates, et al. (87) have developed a GC-MS-COM system which uses retention indices of eluting compounds as the initial basis for the computer library search and performs simultaneous qualitative and quantitative analyses of more than 100 compounds in a mixture at the rate of one compound every 6 seconds. Typical of the analytical results obtained in this study are an observed precision of retention index determination of 0.2%, a lower limit of detection of 10 ng injected, a GC-MS precision of 8% on duplicate determinations, and a 1000-fold linear range of quantitative analysis.

The recent development of an HPLC-MS-COM system by Henion (88,89) should provide similar results with an even broader scope. In the first design (88), part of the split effluent LC stream entered the EI/CI source through a glass capillary passing through the direct probe inlet. Many spectra of various underivatized drugs are presented. In the second design (89) the total effluent from a micro HPLC column enters the CI source. The interface is simple enough that the LC apparatus can be disconnected easily when not needed and daily use of the system has not affected conventional GC-MS work. Full scan spectra were obtained from 1-5 ng of drugs and their metabolites and SIM provided 20 pg detection limits.

The time required to make an analysis by GC-MS is
limited mainly by the rate of elution of the sample fractions from the GC column which typically takes 20-40 minutes. Where a faster result is required, direct insertion CI techniques have provided a means of screening samples for abnormal components. Milne, et al. (90) describe the use of direct insertion isobutane CI for screening samples for 48 commonly used drugs following a simple chloroform extraction. Figure 30 illustrates the type of spectra obtained.

Saferstein, et al. (91) describe a similar procedure for the rapid screening of urine for the presence of 14 common drugs with sensitivities ranging from 0.06-1.5 μg/ml. The method has been used for over 6 years by the New Jersey State Police for routine drug screening.

The use of SIM for the detection of a series of known drugs has value in forensic toxicology as well as in therapeutic monitoring. Jindal, et al. (92) demonstrate the use of this technique for quantitative measurements of cocaine and its active metabolite norcocaine in the urine of a drug user, using the deuterated compounds as internal standards. Figure 39 illustrates the result.

2. Perinatal Toxicology

The transfer of drugs to the fetus through maternal blood and consumed amniotic fluid, and to the newborn through breast milk, is of considerable concern in
SELECTED ION CHROMATOGRAMS FOR COCAINE AND NORCOCAINE

Legend

I. SIM carried out for:

- cocaine molecular ion at m/z 303;
- $[\text{H}_3]$cocaïne molecular ion at m/z 306;
- norcocaine-trifluoroanhydride derivative fragment ion at m/z 263;
- $[\text{H}_3]$norcocaine-trifluoroanhydride derivative fragment ion at m/z 266.

Chromatograms shown represent:

(a) control urine spiked with known amounts of drugs and standards;

(b) urine from a patient 2-4 hours after cocaine ingestion, showing 1778 ng/ml cocaine and 49.6 ng/ml of norcocaine.

II. SIM performed at several characteristic m/z values for derivatives of norcocaine and $[\text{H}_3]$norcocaine from the same patient shows doublet peaks for molecular ions at m/z 385-388 and fragment ions at m/z 263-266, 194-197, and 100-103. Large singlet peaks at m/z 105, 163, and 164 arise from common fragment ions of the two compounds and further confirm the identification. Quantitative analysis is performed using the doublet at m/z 263-266.

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SELECTED ION CHROMATOGRAMS FOR COCAINE AND NORCOCaine

I.

(a)  
Relative abundance
266  263  306  303
2  3  4  5

(b)  
10  
266  263  306  303
2  3  4  5

II.  
6
103  163  388
100  164  385
105  197  385
194  266  263
0  4  8
Retention time (min)
perinatal medicine. MS techniques allow for specific measurements of a wide variety of toxic agents in fetal and neonatal samples at sensitivities not possible with conventional methods.

Horning, et al. (93) studied the transfer of several drugs from mother to fetus or infant by GC-CI-MS and SIM techniques. Results showed that the amount of drug transferred in some instances corresponded to adult dosages when calculated in mg/Kg of body weight. In one case using SIM, phenobarbital was still detected in the infant urine 22 days after the last breast feeding from a mother who had been taking the drug.

Caldwell, et al. (94) studied the transfer of bupivacaine, an epidural anaesthetic commonly administered during childbirth. Reports of abnormal heart rate patterns and behavioral changes in babies born after epidural block had suggested the anaesthetic as the possible cause.

Six obstetrically normal mothers in uncomplicated labor were studied. During delivery, samples of maternal blood were obtained at frequent intervals through a cannula in the back of the hand, and fetal capillary blood was obtained by scalp puncture. At birth, umbilical cord, arterial and venous blood samples were taken. Neonatal blood was then obtained at 2, 6, and 24 hours after delivery by heel prick.
A structural analogue of the drug was added to all samples as the internal standard. A series of extractions into ether and back extractions while changing pH were used to purify the drug before injection for GC-MS and SIM at m/z 140 for the drug and m/z 154 for the standard.

Results showed that the drug entered the maternal circulation quite rapidly (peaking at 5 minutes) and was detectable in the fetal circulation within 10 minutes after administration to the mother. Levels eventually reached about one third of the peak level in the mother but the decline was slower. Infant urine showed only traces of the drug, which indicated less ability than adults to metabolize it. These results provided probable proof of the origin of symptoms reported in babies born to mothers given this drug.

3. Environmental Toxicology

The enormous number of potentially toxic chemicals in polluted air, water, soil, and food has been the subject of countless studies using MS (45,79,95). Only one example will be considered here to illustrate a rather significant discovery made using negative ion CI-MS and API-MS.

The flame retardant tris (2,3-dibromopropyl) phosphate or tris-BP was a major chemical used in sleepwear between 1973 and 1977, for the purpose of complying with federal regulations designed to reduce burn injuries in children. About 50 million children were exposed to this
chemical before it was banned from use in children's clothing in April, 1977, because of the results of studies like one undertaken by Blum et al. (96).

This chemical had been known to produce mutations in bacteria, cancer when fed to rats and mice, or when painted on the skin of mice, and testicular atrophy and sterility in rabbits after application to the skin. Similar chemicals had long been known to penetrate human skin because of the appearance of metabolites in the urine following skin contact. However, human skin absorption of tris-BP had not been found to occur in previous studies due to the absence of its metabolite, 2,3-dibromopropanol in the urine when screened for by a method which could detect the compound down to a level of 200 ng/ml.

Two different research teams were used to analyze all urine samples from children in various test groups. One team used acid hydrolysis and negative ion CI-MS to provide molecular identification and an order-of-magnitude estimation of quantities in the urine. The second team used enzymatic hydrolysis and negative ion API-MS for quantitative analysis. The structural analogue 1,4-dibromo-2-butanol was used as the internal standard.

SIM was carried out at m/z 79 and 81 which correspond to the two naturally occurring bromine isotopes. Figure 40 illustrates sample ion chromatograms resulting from each
FIGURE 40

SIM OF FLAME RETARDANT METABOLITE AND INTERNAL STANDARD

Legend

Negative ion API-MS was used to analyze the 2,3-dibromopropanol metabolite of tris (2,3-dibromopropyl) phosphate, a flame retardant used in children's sleepwear. SIM was performed at m/z 79 and 81 corresponding to the two naturally occurring bromine isotopes of the compound. All peaks indicated by "A" represent the tris-BP metabolite while peaks indicated by "B" arise from 50 ng of the structural analogue, 1,4-dibromo-2-butanol used as internal standard.

Refer to the text for an explanation of the origin of each of the recordings shown.

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test group. Peaks appearing at position A in each case represent the bromine isotopes from 2,3-dibromopropanol, while peaks at position B represent the bromine isotopes from 50 ng of 1,4-dibromo-2-butanol standard. Chart I of this figure is the result of a urine analysis from a child who had been wearing new treated pyjamas for 4 days. Chart II represents a urine profile on day 1, for the same child before wearing the new pyjamas. Chart III is from a child who had never been exposed to treated pyjamas. Charts IV and V are from children from different families who had been wearing treated pyjamas that had been laundered for at least 5 months. Chart VI is from a child who had stopped wearing treated pyjamas 6 months prior to testing.

Quantitative results showed up to 29 ng/ml of 2,3-dibromopropanol appearing in urine of children exposed to the tris-BP-treated sleepwear, that some chemical was still present in the cloth after many washings, and that traces of the chemical remained in the body long after exposure had ceased. Any fractions of the chemical stored in the body or excreted in feaces was not accounted for.

The results of this study suggest the obvious need for more stringent regulations for the toxicological testing of additives used in the textile industry.
CHAPTER IV

CONCLUSIONS - FUTURE OF MASS SPECTROMETRY IN CLINICAL CHEMISTRY

A. VALUE OF DRUG ANALYSIS BY MASS SPECTROMETRY IN THE CLINICAL LABORATORY

In theory, the value of a toxicological report in cases of acute poisoning or overdose lies in:

a) confirming the suspected diagnosis so other needless diagnostic tests will not be done;

b) in identifying the specific agent responsible to enable specific care to be given based on the drug's kinetics or the possible availability of an antidote;

c) in assessing the severity of the intoxication so the basic management may be altered if necessary;

d) in predicting the outcome so family members may be adequately informed of the patient's chance for survival;

e) in distinguishing symptoms caused by a drug from those of other disorders;

f) in following the patient's progress (97).

Most laboratories provide some form of screening procedure for identifying at least major drug classes, but the results lack specificity and are of little value if the toxic agent does not belong to one of these groups (a fact that has become more common in recent years). The amazing
capabilities of sophisticated GC-MS-COM systems, such as the one developed by Costello, et al. (85) for emergency toxicology, have been well documented. Many larger centers provide such a service for area hospitals on a 24-hour basis and are capable of reporting their findings within 1-2 hours.

However, there have been many arguments about the utility of such a system, when some studies have shown that knowing the drug(s) present in an overdose victim makes little difference to the clinical management of the patient (98). This may, in fact, be due to lack of confidence in the laboratory's ability to make an accurate determination. As the availability of clinical laboratory toxicology testing of high quality increases, more correlation between the lab result and patient management in such cases should also increase. The advantages of GC-MS, at least for handling samples which show equivocal results by routine screenings, should be thus restated. It will definitively identify drugs and their metabolites rather than simply the class of drug; multiple drug ingestion can be recognized; it may be possible to identify drugs and other toxic agents not previously encountered; and finally, the opportunity is provided for studying metabolism of drugs in humans at levels well above therapeutic dosage (99). In most cases, once the toxic agent has been identified, more economical and convenient methods can then be used for follow up.
In the area of therapeutic monitoring, GC-MS using SIM and stable isotope-labelled standards is unsurpassed in sensitivity and specificity for the simultaneous quantitation of a wide range of drugs and even active metabolites. Powers and Ebert (100) demonstrated its superiority over radioimmunoassay (RIA) methods in a study of amphetamine isomers, while van der Slooten and van der Helm (101) showed the same when compared with the Syva Enzyme-Multiplied Immunoassay Technique (EMIT) for the opiates, morphine and codeine in urine. On the other hand, both the RIA and EMIT methods are more convenient and inexpensive to use and prove to be far ahead of GC-MS in sample throughput capacity.

Thus, the use of MS techniques in this area are invaluable, but are only justified for measuring trace amounts of substances in small numbers of samples for which more convenient methods are not available; for example, the monitoring of antitumor or antiviral agents in certain high-risk patients.

B. IMPROVEMENTS IN INSTRUMENTATION

Until the last few years, the high cost and complexity of mass spectrometers, and the technical skill required, not only for interpreting results, but for operation and maintenance (which could be 10-20% of normal
running time) have made it necessary for most routine laboratories to avoid even considering its use. Centralized regional laboratories have been suggested as a means of grouping the technical experience required to carry out MS analysis at a reasonable cost (6). However, in general, especially in larger hospitals where there could be a wide variety of samples requiring different types of information, such analyses might be handled more effectively within the individual hospitals. For this purpose, mass spectrometers will have to be cheaper, more reliable, easier to operate, and capable of coping with a larger variety of selected assays than instruments now used for research.

With such considerations in mind, several companies have indeed developed systems which are more streamlined in design and/or simpler to operate. The important features of three such systems are outlined in Table 2 (102). The least flexible of the three is the Olfax 11A which is designed only for SIM in mass ranges below 400. The HP 5992 is the most compact, with its small quadrupole analyzer sitting on top of the GC component. The DP-102 is obviously the most versatile. In all three cases, critical instrument parameters are continuously self-monitored by microprocessors and conveyed to the user by a series of diagnostic messages, while programs for SIM, file searches, and maintenance checks provide for relative ease of operation.
<table>
<thead>
<tr>
<th></th>
<th>Hewlett-Packard HP 5992A</th>
<th>Vitek Olfax IIA</th>
<th>Du Pont DP - 102</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Analyzer</td>
<td>Hyperbolic Quadrupole</td>
<td>Quadrupole</td>
<td>Magnetic sector</td>
</tr>
<tr>
<td>Ionization Mode</td>
<td>Electron Impact</td>
<td>Electron Impact</td>
<td>Electron Impact and Chemical Ionization</td>
</tr>
<tr>
<td>Source Renewal</td>
<td>In situ alternate filament</td>
<td>Open system and replace source</td>
<td>Automatic source rejuvenation</td>
</tr>
<tr>
<td>Separator</td>
<td>Membrane</td>
<td>Dual membrane</td>
<td>Jet separator</td>
</tr>
<tr>
<td>Mass Range (amu)</td>
<td>10 - 800</td>
<td>To 400</td>
<td>To 1,000</td>
</tr>
<tr>
<td>Maximum Scan Rate (amu/s)</td>
<td>1,100</td>
<td>150</td>
<td>1,000</td>
</tr>
<tr>
<td>Pump-down Time (min)</td>
<td>60</td>
<td>60</td>
<td>45 (10 min with turbomolecular pump)</td>
</tr>
<tr>
<td>Computer Storage</td>
<td>32K (core), 250K (tape)</td>
<td>31K (core), 60K (tape)</td>
<td>64K (core), 20,000 (disc)</td>
</tr>
<tr>
<td>Cost (1979)</td>
<td>$ 49,500</td>
<td>$ 60,000 -</td>
<td>$ 135,000</td>
</tr>
</tbody>
</table>

Reprinted without permission from reference 102.
Another instrument developed by Franzen (103), the GCMS 1001, incorporates an auto-sampler capable of handling more than 100 sample vials with sample volumes as low as a few hundredths of a ml. It injects controlled amounts of liquid into a pre-column placed in the injection port of the capillary-column GC, and provides exact reproduction of absolute retention times.

Finally, Hillig, et al. (104) describes a fully automated instrument with successive introduction of up to 30 samples by means of the direct insertion probe.

The user of such instruments need not be a skilled mass spectrometrists, but should have a practical appreciation of the scope of applications amenable to MS, and of such constraints as specimen type, containers, internal standards, and derivatizing agents.

Despite the loss of the flexibility found in the larger, more complex systems, the advantage gained in reliability, ease of operation, and lower cost of the smaller instruments, should more than compensate when put to use in a routine laboratory.

C. ADDITIONAL CLINICAL APPLICATIONS OF MASS SPECTROMETRY

The number and diversity of applications of MS to clinical medicine have increased rapidly in the past few years despite their limited use to date in routine laboratories. Many examples and references to recent work in this area are
given by Caprioli, et al. (60) and by Burlingame, et al. (79). In general, in addition to emergency toxicology and therapeutic monitoring discussed earlier, the following are examples of areas which appear to be particularly suited to MS analysis in the clinical laboratory:

- metabolic profiling of organic acids, volatiles, and lipids for diagnosis of inborn errors of metabolism (23 previously unknown metabolic abnormalities of genetic origin have been discovered using GC-MS); and defined clinical conditions such as diabetes, uremia, hepatic disorders, multiple sclerosis;
- steroid profiles for detecting abnormalities in development, menstrual cycle, pregnancy, and disorders of the adrenal cortex, breast, and gonads;
- neurotransmitter studies for diagnosis of neurological and psychological disorders;
- rapid diagnosis and monitoring of viral infections from urine profiles (interesting work in this area is described by Abbott, et al. (105));
- rapid identification of bacteria and viruses.

Although blood and urine are the most common samples used, normal and abnormal profiles are also being studied for saliva (example, 106), respiratory gases (107), and other volatiles emitted from the body (108).

The superior accuracy and precision of quantitative
MS techniques using SIM and stable isotope-labelled standards may solve the urgent need for definitive reference methods and for biological controls with constituents of accurately known concentrations. This would better enable clinical laboratories to validate routine methods in use or under consideration. Such reference techniques have already been approved for the measurement of cholesterol, triglycerides, urea, creatinine, glucose, cortisol, inorganic phosphate, calcium, testosterone, progesterone, and melatonin (58).

Most routine clinical chemistry laboratories, out of necessity, have focused on the ability to perform efficient simple tests of low cost, which can be executed on vast numbers of samples. However, it seems likely that the need to evaluate certain patients in greater depth will gradually become less the responsibility of special interest groups and more a part of the laboratory routine, at least in larger centers. Such studies will involve the clinical chemist with making fewer analyses of a much wider range of substances in a variety of sample types, as just described.

Thus, it becomes obvious that, as exemplified throughout this critique, the capability of MS systems for making qualitative and quantitative measurements of an exceptionally large number of compounds with a high degree of sensitivity and specificity, can be of immense value to certain areas of clinical chemistry. However, because of
this potential, it should not be hindered by analyses that can be performed by simpler, more economical methods, but rather reserved for problems where sensitivity and specificity are of major importance and where a large series of samples are not the primary aim. With the development of instruments which are more appropriate for routine use, the future in this area is promising, to say the least.
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