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Mass spectrometric techniques in the clinical laboratory

F.L. Mitchell

Denefield, Overstream, Rickmansworth, Herts. England WD3 4LD

Abstract - Mass spectrometers are expensive instruments and considerable expertise is necessary both to maximise their utilisation and interpret correctly the results obtained. Therefore they are best operated as a central facility to which work may be referred. They are indispensible for many aspects of medical research and necessary for solving a range of difficult problems in the diagnostic laboratory where positive identification of analytes is required. When possible isotope dilution-mass spectrometry is the method of choice for definitive methodology, used for the control of accuracy in the clinical analytical laboratory. When single or multiple ion monitoring is used, very high sensitivity can be obtained. With ionisation by the new technique of fast atom bombardment there is minimal fragmentation and therefore compounds can be separated within relatively crude extracts by mass spectrometry alone; also with this technique work is possible with compounds of very high molecular mass.

INTRODUCTION

Since the mid 1960s, when mass spectrometry became a practical entity for clinical investigations, many clinical scientists have been hoping that the cost and complexity of instruments would bring them within the area of use by the general hospital clinical laboratory. This has not happened and shows little sign of happening; relatively simple instruments have become available but their very simplicity has meant that they have had a limited scope of application. To be of general use a mass spectrometer must be versatile and therefore expensive in itself and also have attendant expensive computer hardware and software.

However, expense apart, advances over recent years have shown that the modern mass spectrometer is an indispensible tool for many aspects of medical research and access to an instrument is highly desirable for solving many problems in the diagnostic laboratory.

With the limited time available this morning it will only be possible to indicate areas of mass spectrometry application which are of particular interest. I will endeavour to cover a few and Dr Gleisbach of Austria will mention others. Unfortunately we will have to assume that the audience is familiar with the basic principles of MS, when coupled and not necessarily coupled to a gas chromatograph.

Maximum utilisation of mass spectrometry can only be achieved by specialists concentrating on applications, interpretation and instrumentation function. Therefore centralisation of equipment has advantages in the concentration of expertise as well as in maximising the utilisation of expensive equipment.

The applications which we will give as examples have been chosen both for their importance in clinical chemistry, analytically and diagnostically, and to demonstrate the versatility of modern instruments.

Definitive methodology

In non-biological analysis, matrix effects are usually known and are minimal. Accuracy does not in general present a major problem since it may be achieved by the use of the highly purified standard materials available for the calibration of each method. In biological analysis, however, the matrix containing the analyte invariably includes a confusing collection of materials which may cause considerable interference in the measurement of the analyte itself using the analytical methods which are practical. For this reason, although basic calibration of a procedure or instrument should, whenever possible, be done by the use of pure standards, control and reference materials should ideally have the same matrix constitution as the analyte. These materials, used for the quality control of accuracy should ideally be assayed by "an analytical method that is capable of providing the highest accuracy among all methods for determining that analyte". This has been termed a definitive

method and the technique of choice when practical, is isotope dilution-mass spectrometry (IDMS) since for each stage in the procedure the factors governing accuracy are understood and can mostly either be controlled or allowed for.

As early as possible in the analytical procedure a known amount of an isotope of the analyte is added, then any subsequent losses during work-up can be disregarded since quantitation is done by comparing the measurement of ions at the mass number of the analyte with those at the mass number of the isotope. A simple reference to the amount of isotope originally added will then give the definitive measurement of the analyte.

It has to be assumed that the two isotopes behave the same in the preliminary work-up procedure and unfortunately it is known that in chromatography for example, this is not necessarily true. However, IDMS remains the technique of choice for most definitive methods. The work involved is not easy and much dedication is required both in the use of the mass spectrometer itself and in the preliminary purification technology if the necessary high degree of accuracy is to be achieved. A good organic mass spectrometer is required for organic compounds but a spark source instrument is necessary for elements such as sodium and potassium, and few centres have such an instrument together with the necessary expertise. Currently only the National Bureau of Standards in Washington DC is doing elemental work.

Steroid analysis

The possible disorders of steroid metabolism are very varied and can be very complex; they are often distressing or fatal to the patient concerned and often if an affected baby is to survive it has to be diagnosed soon after birth when the steroid metabolic picture is greatly complicated by mechanisms and happenings concerned with birth or life in utero. Many abnormalities can be detected by the colorimetric measurement of groups of steroids but others can only be elucidated by so-called steroid profiling. Such a profile is best produced by high resolution capillary column gas chromatography which however, presents a bewildering array of peaks, the interpretation of which can be baffling even to the few experts who have spent a lifetime studying such problems.

A mass spectrometer suitably computer programmed can immediately identify all peaks of known steroids and this facility has led not only to the identification of many new steroids but the elucidation of several previously unknown steroid disorders. Many clinicians have had to alter a diagnosis on the basis of a gas chromatography-mass spectrometry steroid excretion pattern.

Often compounds (of which steroids are only an example) are excreted in amounts too small to enable a mass apectrometer to derive a full fragmentation pattern. To produce its so-called stick diagrams a mass spectrometer has to have a continuous supply of ions for several seconds and even so the speed of the scan allows for the collection of ions at one mass number only for a very short time, so a relatively large amount of sample is required and sensitivity at any particular mass number is low. When sensitive detection and quantitation of a single compound is required, single or multiple ion monitoring is used. For the former, the largest peak in the fragmentation pattern is usually chosen and the mass spectrometer tuned to collect all ions with that mass number. This gives high sensitivity but low specificity since other compounds may produce fragments with the same mass number. If the mass spectrometer is arranged to switch between two or more mass number positions selected as being distinctive for the particular analyte in question, the time for the collection of ions at each position is reduced but specificity is increased since with the number of peak positions monitored, the chances of other compounds producing the same fragments decreases.

New developments in mass spectrometry are indicating exciting possibilities for the future. Fast atom bombardment known as FAB, and molecular secondary ion mass spectrometry (SIMS) can be operated on complex mixtures very satisfactorily without the previous separation of compounds by gas chromatography. The parent molecule is not fragmented to any degree and therefore the mass spectrogram shows the sort of separation of molecules more familiar with a gas chromatogram but on a molecular weight basis. FAB and SIMS are carried out by inserting the sample, supported in a glycerol matrix, into the mass spectrometer on a solid injection probe. It is then bombarded with a fast neutral argon atom beam, or by ions of caesium or atoms of zenon. The system has many advantages apart from minimal fragmentation; most of the sample can be recovered after analysis and since the bombardment releases ions of much higher molecular mass the usable mass range should theoretically go up to 10,000 Daltons. Masses up to 2,000 are presently easily analysed compared with around 1,000 for other methods of ionisation.

Drug analysis

The use of mass spectrometry in following the metabolism of drugs is well illustrated by investigating the impact upon the baby of the administration to pregnant women of the drug α-methyl-dopa, to treat hypertension. It was not known how much of the drug passed the placental barrier to affect the foetus since assays in newborn urine by fluorimetric methods

gave widely discrepant results. An internal standard for mass spectrometric assay was made by substituting deuterium for hydrogen in the methyl group, and after extraction the free and conjugated drug fractions were separated. The ions with mass numbers 542 ($-CH_3$) and 545 ($-CD_3$) were chosen for monitoring and the mass spectrometer arranged to switch between these ions to enable assay by comparison. The technique proved to be sensitive and accurate and indicated that large quantities of the drug both free and conjugated were excreted by an affected infant up to two days after birth, showing that drug effect on the foetus must obtain.

Mass spectrometry has proved invaluable in the detection of drug taking by athletes. Anabolic steroids have been widely used to enhance athletic performance but their use leads to disqualification from major athletic events. Urinary screening may be done using immunoassay but incontrovertible proof of the presence of anabolic steroid metabolites can only be obtained by mass spectrometry. For this reason for all major games including the Olympics, mass spectrometric detection systems must be readily available.

Conclusion

For many reasons therefore, mass spectrometry has become essential for a wide range of requirements in clinical analysis and though the instruments are too expensive and not suitable for use in general laboratories, the clinical chemist must know the principles and applications, and have access to instruments and expertise.