Chapter 1
Introduction

Learning Objectives

- To understand the need to interface liquid chromatography and mass spectrometry.
- To understand the requirements of an interface between liquid chromatography and mass spectrometry and the performance of the combined system.

The combination of chromatography and mass spectrometry (MS) is a subject that has attracted much interest over the last forty years or so. The combination of gas chromatography (GC) with mass spectrometry (GC–MS) was first reported in 1958 and made available commercially in 1967. Since then, it has become increasingly utilized and is probably the most widely used 'hyphenated' or 'tandem' technique, as such combinations are often known. The acceptance of GC–MS as a routine technique has in no small part been due to the fact that interfaces have been available for both packed and capillary columns which allow the vast majority of compounds amenable to separation by gas chromatography to be transferred efficiently to the mass spectrometer. Compounds amenable to analysis by GC need to be both volatile, at the temperatures used to achieve separation, and thermally stable, i.e. the same requirements needed to produce mass spectra from an analyte using either electron (EI) or chemical ionization (CI) (see Chapter 3). In simple terms, therefore, virtually all compounds that pass through a GC column can be ionized and the full analytical capabilities of the mass spectrometer utilized.

This is not the case when high performance liquid chromatography (HPLC) and MS are considered where, due to the incompatibilities of the two techniques, they cannot be linked directly and an interface must be used, with its prime purpose being the removal of the chromatographic mobile phase. Unfortunately, no single

interface exists which possesses similar capabilities to those available for GC-MS, i.e. one that will allow mass spectra to be obtained from any compound that elutes from an HPLC column, and thus LC–MS has not been guaranteed to provide the required analytical information. In addition, the complexity of the mass spectrometer has meant that the majority of chromatographers have not had direct access to the instrumentation and have had to rely on a service facility to provide results. They were therefore unable to react rapidly to the results of an analysis and consequently found it a particularly inconvenient detector to contemplate using. The different interfaces that have been made available commercially, and the applications to which they have been put, are the subjects of the following chapters.

Before discussing these in detail, it is appropriate to consider a number of general questions, namely:

(1) What are the advantages of linking HPLC with mass spectrometry?

(2) What capabilities are required of such a combination?

(3) What problems, if any, have to be addressed to allow the combination to function, *and function effectively*?

1.1 What are the Advantages of Linking High Performance Liquid Chromatography with Mass Spectrometry?

In order to answer the first question, the limitations of the individual techniques must be considered and whether the combination will allow all or some of these to be overcome. Before doing this, however, the analytical tasks to which the combination will be applied must be defined.

In many analyses, the compound(s) of interest are found as part of a complex mixture and the role of the chromatographic technique is to provide separation of the components of that mixture to allow their identification or quantitative determination. From a qualitative perspective, the main limitation of chromatography in isolation is its inability to provide an unequivocal identification of the components of a mixture even if they can be completely separated from each other. Identification is based on the comparison of the retention characteristics, simplistically the retention time, of an unknown with those of reference materials determined under identical experimental conditions. There are, however, so many compounds in existence that even if the retention characteristics of an unknown and a reference material are, within the limits of experimental error, identical, the analyst cannot say with absolute certainty that the two compounds are the same. Despite a range of chromatographic conditions being available to the analyst, it is not always possible to effect complete separation of all of the components of a mixture and this may prevent the precise and accurate quantitative determination of the analyte(s) of interest.

The power of mass spectrometry lies in the fact that the mass spectra of many compounds are sufficiently specific to allow their identification with a high degree of confidence, if not with complete certainty. If the analyte of interest is encountered as part of a mixture, however, the mass spectrum obtained will contain ions from all of the compounds present and, particularly if the analyte of interest is a minor component of that mixture, identification with any degree of certainty is made much more difficult, if not impossible. The combination of the separation capability of chromatography to allow 'pure' compounds to be introduced into the mass spectrometer with the identification capability of the mass spectrometer is clearly therefore advantageous, particularly as many compounds with similar or identical retention characteristics have quite different mass spectra and can therefore be differentiated. This extra specificity allows quantitation to be carried out which, with chromatography alone, would not be possible.

The combination of HPLC with mass spectrometry therefore allows more definitive identification and the quantitative determination of compounds that are not fully resolved chromatographically.

1.2 What Capabilities are Required of the Combination?

Ideally, the capabilities of both instruments should be unaffected by their being linked. These include the following (adapted from Snyder and Kirkland [1]):

- The interface should cause no reduction in chromatographic performance. This is particularly important for the analysis of complex multi-component mixtures (although the specificity of the mass spectrometer may, in certain circumstances, compensate for some loss of performance – see Chapter 3).
- No uncontrolled chemical modification of the analyte should occur during its passage through the interface or during its introduction into the mass spectrometer.
- There should be high sample transfer to the mass spectrometer or, if this takes place in the interface, ionization efficiency. This is of particular importance when trace-level components are of interest or when polar and/or labile analytes are involved.
- The interface should give low chemical background, thus minimizing possible interference with the analytes.
- The interface should be reliable and easy to use.
- The interface should be simple and inexpensive (a subjective assessment).
- Operation of the interface should be compatible with all chromatographic conditions which are likely to be encountered, including flow rates from around 20 $\mathrm{nl\,min^{-1}}$ to around 2 $\mathrm{ml\,min^{-1}}$, solvent systems from 100% organic phase to 100% aqueous phase, gradient elution, which is of particular importance in

the biological field in which mixtures covering a wide range of polarities are often encountered, and buffers, both volatile and involatile.

- Operation of the interface should not compromise the vacuum requirements of the mass spectrometer and should allow all capabilities of the mass spectrometer to be utilized, i.e. ionization modes, high resolution, etc.
- The mass spectrum produced should provide unambiguous molecular weight information from the wide range of compounds amenable to analysis by HPLC, including biomolecules with molecular weights in excess of 1000 Da. The study of these types of molecule by mass spectrometry may be subject to limitations associated with their ionization and detection and the mass range of the instrument being used.
- The mass spectrometer should provide structural information that should be reproducible, interpretable and amenable to library matching. Ideally, an electron ionization (EI) (see Chapter 3) spectrum should be generated. An interface that fulfils both this requirement and/or the production of molecular weight information, immediately lends itself to use as a more convenient alternative to the conventional solid-sample insertion probe of the mass spectrometer and some of the interfaces which have been developed have been used in this way.
- The interface should provide quantitative information with a reproducibility better than 10% with low limits of detection and have a linear response over a wide range of sample sizes (low picograms to μg).

1.3 What Problems, if Any, Have to be Addressed to Allow the LC–MS Combination to Function, and Function Effectively?

It is possible to carry out a chromatographic separation, collect all, or selected, fractions and then, after removal of the majority of the volatile solvent, transfer the analyte to the mass spectrometer by using the conventional inlet (probe) for solid analytes. The direct coupling of the two techniques is advantageous in many respects, including the speed of analysis, the convenience, particularly for the analysis of multi-component mixtures, the reduced possibility of sample loss, the ability to carry out accurate quantitation using isotopically labelled internal standards, and the ability to carry out certain tasks, such as the evaluation of peak purity, which would not otherwise be possible.

There are two major incompatibilities between HPLC and MS. The first is that the HPLC mobile phase is a liquid, often containing a significant proportion of water, which is pumped through the stationary phase (column) at a flow rate of typically 1 ml min^{-1}, while the mass spectrometer operates at a pressure of around 10^{-6} torr ($1.333\,22 \times 10^{-4}$ Pa). It is therefore not possible simply to pump the eluate from an HPLC column directly into the source of a mass

spectrometer and an important function of any interface is the removal of all, or a significant portion, of the mobile phase. The second is that the majority of analytes that are likely to be separated by HPLC are relatively involatile and/or thermally labile and therefore not amenable to ionization by using either EI or CI. Alternative ionization methods have therefore to be developed.

In the following chapters, the basic principles of HPLC and MS, in as far as they relate to the LC–MS combination, will be discussed and seven of the most important types of interface which have been made available commercially will be considered. Particular attention will be paid to the electrospray and atmospheric-pressure chemical ionization interfaces as these are the ones most widely used today. The use of LC–MS for identification and quantitation will be described and appropriate applications will be discussed.

Summary

In this chapter, the reader has been introduced to the analytical advantages to be gained by linking high performance liquid chromatography to mass spectrometry with particular regard to the limitations of the two techniques when they are used independently.

The characteristics of an ideal liquid chromatography–mass spectrometry interface have been discussed, with emphasis having been placed upon the major incompatibilities of the two component techniques that need to be overcome to allow the combination to function effectively.

References

1. Snyder, L. R. and Kirkland, J. J., *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974.