1

Introduction

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Mass spectrometry underwent a rapid and dynamic development during recent years. Innovative solutions brought highly advanced instruments that fulfill user demands with respect to sensitivity, speed, and simplicity of operation.

Mass spectrometer, independently on its construction, measures the ratio of mass of a molecule to its charge, *m/z*. While interpreting data obtained during analysis, it should be carefully noted that not always the *m/z* value can be directly related to the molecular mass of the analyzed compound. This happens when multiple charges are being attached to the molecule (multiple ionization), which results from the attachment or depletion of a proton or several protons. Even a popular electron impact ionization generates radicals, depleted of one electron. Typically, we tend to neglect this electron while analyzing spectra at low resolution. However, this lack of one electron will be clearly seen during high-resolution analysis using Fourier transform ion cyclotron resonance (FT-ICR) instrument. The multiplicity of ionization depends on the ion source used and their different types. These are described in the following chapters of this book.

The principle of operation of the apparatus can be compared to the sensitive balance, by which we weigh mass of molecules. Another association implies a comparison of a mass spectrometer with electrophoresis in a vacuum because the analyzed molecules, in the form of ions, are accelerated in the device under the influence of applied potential.

Until recently, the mass spectrometer consisted of elements traditionally associated with various ionization methods. For example, matrix-assisted laser desorption/ionization (MALDI) was combined with the time-of-flight (TOF) analyzer, and electron impact/chemical ionization (EI/CI) was typically used

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with quadrupole or magnetic and electrostatic analyzers. Today's constructions are built of "blocks" that can create combinations not yet very common, such as MALDI with ion trap, electron ionization with TOF, inductively coupled plasma (ICP) with TOF, and electrospray with TOF (qTOF). New constructions like Orbitrap are relatively cheap and affordable by many laboratories.

The mass spectrometer consists of several basic elements, presented schematically in Figure 1.1.

The basic requirement for a substance to be analyzed in a mass spectrometer is its ability to ionize. Ions can move in a vacuum under the influence of an applied electric field. It is important to note that vacuum is necessary inside mass spectrometer, where ions are analyzed. Ion sources, in many cases, do not require vacuum at all. The heterogeneous ion beam is separated in the analyzer depending on the *m/z* values for the individual ions. Separated ions are then introduced into a detector that converts quantum ion current into electrical current. The system control software transcribes the intensity of these signals as a function of the *m/z* value and presents these data as a mass spectrum, as shown in Figure 1.2.

Spectra are derived from the substances that are present in the sample. The mass spectrometer can simultaneously analyze the mixture (to some extent), which is extremely important in the study of complex biological material or other unknown samples. It is also possible to analyze selected substances only in the mixture. This saves analysis time, reduces the amount of data on the hard drive, and improves the signal-to-noise ratio. This method is referred to as the single ion monitoring (SIM) or multiple ion monitoring (MIM) and is mainly used for quantitative analysis of compounds and their fragment ions.

The main advantages of a mass spectrometer, compared with other techniques, are as follows:

- Speed of analysis.
- High sensitivity, reaching femto-/attomolar level.
- Simultaneous analysis of many components of mixtures.
- Ability to obtain information on the structure of the compounds (including amino acid sequence) and posttranslational modifications.
- Possibility of combining with separation techniques (e.g. gas and liquid chromatography, capillary electrophoresis, isotachophoresis).
- Quantitative analysis.

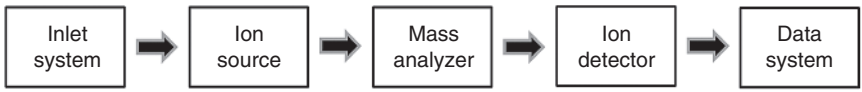

Figure 1.1 Components of the mass spectrometer.

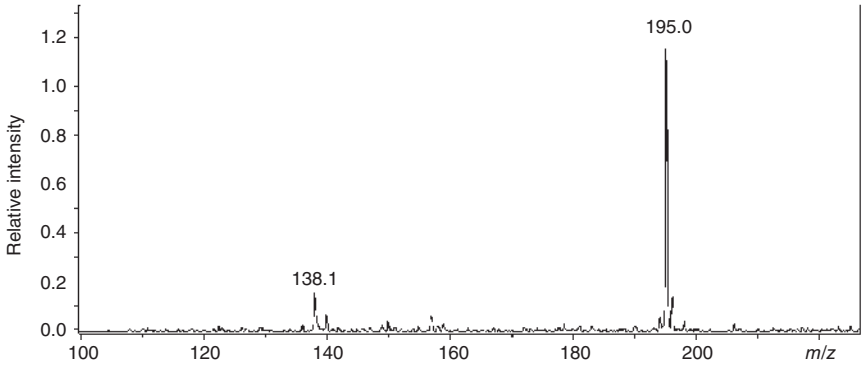

Figure 1.2 Exemplary mass spectrum of caffeine obtained by electrospray ionization mass spectrometry (ESI-MS) technique. Signal at *m/z* 195.0 corresponds to the protonated molecule of caffeine.

- Analysis of the elemental composition.
- Analysis of isotopic composition.
- Unambiguous identification of the substance.

This latter feature distinguishes mass spectrometry from other detection techniques encountered in chromatographic or electrophoretic methods. It is worth emphasizing here that the chromatogram obtained by ultraviolet–visible (UV/Vis) detection, electrochemical or other, generates only the signal intensity for the eluted fraction and the retention time. However, this is insufficient to obtain detailed information about the nature of the substance eluted from the column. The overlap of several components additionally complicates such analysis. Simply put, retention time is not a sufficient method of identification even if standards are provided. We cannot assume in such case that there is no other component in the mixture having the same retention time. The mass spectrometer, used as a detector, gives the exact mass of the substance, together with the information on its structure, hence eliminating the above problems. By analogy to the UV/Vis chromatogram, the mass spectrometer generates a mass chromatogram (Figure 1.3) having several features:

- It provides the relationship between the retention time of the substance and the intensity of the peaks on the spectrum (quantitative analysis).
- It also provides information on substances eluted from the column at the same time.
- It moreover provides detailed information about the structure of the compounds (identification of unknown components).

Figure 1.3 shows the retention time on the horizontal axis and the absolute intensity of the signals (a.i.) on the vertical axis. Individual components eluted

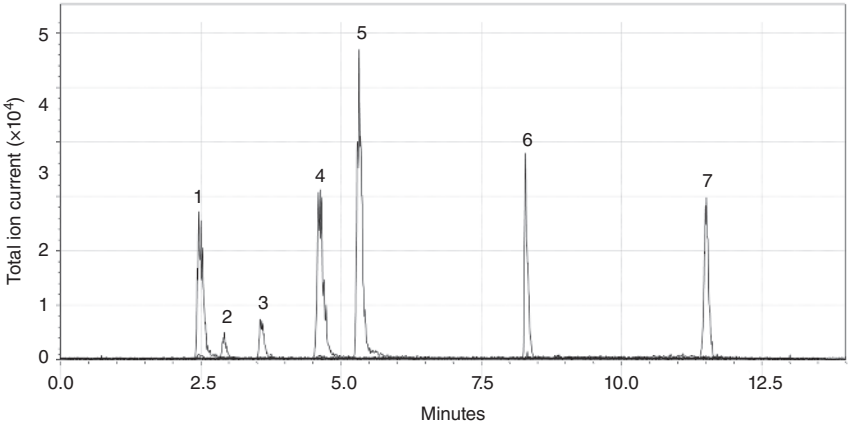

Figure 1.3 Mass chromatogram of several designer drugs separated by the reversed-phase liquid chromatography (LC)-ESI-MS.

from the chromatographic column are recorded by a mass spectrometer. Each of the detected substances is simultaneously characterized by mass spectrum and fragmentation spectrum. Surface under the peaks on a mass chromatogram is a measure of the concentration of individual elements in a sample.

An important feature of modern equipment is also very high accuracy in determining the weight of the substance, reaching the sixth decimal place. This allows to perform measurements with a resolution much higher than that necessary to determine the loss of one electron by a molecule! The following chapters of this book describe, in more details, the variety of techniques used in mass spectrometry and their applications in various areas of our life.