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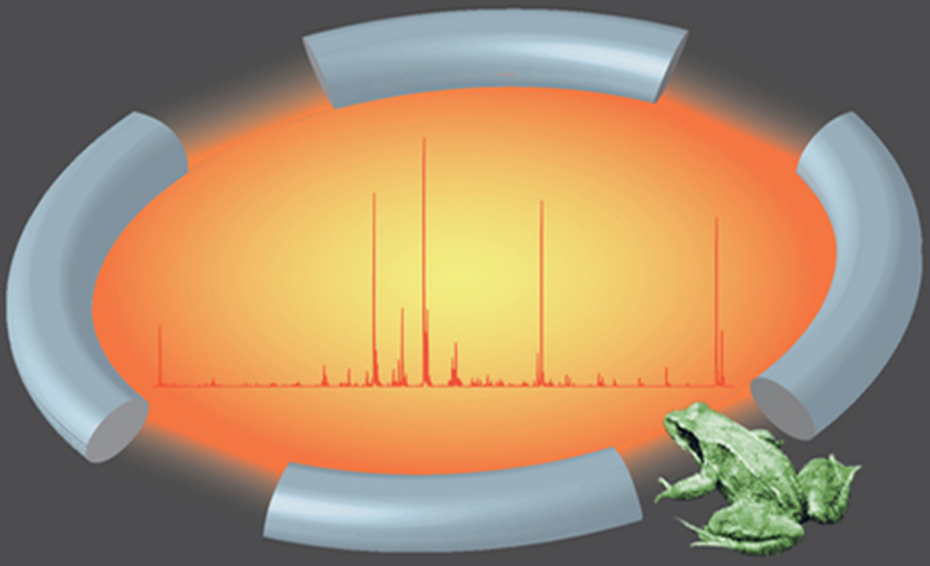

# Mass Spectrometry

## An Applied Approach

Second Edition

Edited by
Marek Smoluch | Giuseppe Grasso
Piotr Suder | Jerzy Silberring

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**Mass Spectrometry**

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An Applied Approach

*Edited by*
*Marek Smoluch, Giuseppe Grasso, Piotr Suder, and Jerzy Silberring*

Second Edition

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*In memory of Nicolaas Martinus Maria Nibbering (Nico)*
*29.05.1938 – 29.08.2014*

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# Preface

Rapid development of genomics, proteomics, combinatorial chemistry, and medical/toxicological diagnostics triggered the rapid development of various mass spectrometry techniques to fulfill requirements of many disciplines, such as biomedical sciences, toxicology, forensic research, and pharmacology. Mass spectrometry (MS) is a unique method that not only allows mass measurement but also provides detailed identification of molecules and traces the fate of compounds in vivo and in vitro. Among others, mass spectrometry may, at least partially, identify amino acid sequence of peptides and proteins, assign sites of posttranslational modifications, identify bacterial strains, and verify structures of organic compounds. The latter is particularly useful for detection of novel drugs of abuse, explosives, etc. A yet another challenge is rapid selection of combinatorial libraries, containing vast number of elements, and a novel place of mass spectrometry in nanomedicine, being a combination of diagnostics and therapy (theranostics).

MS has been proven as an efficient tool to analyze complex biological mixtures by applying hyphenated techniques, such as GC/MS, LC/MS, CE/MS, and TLC/MS, where mass spectrometer acts as a sensitive and highly specific detector. Such approaches may find their applications in, e.g. genomics, functional proteomics to reveal the role of entire pathways in biological systems (systems biology). Another interesting capability of MS is identification of the low molecular mass compounds that are not coded by genes. This aspect is a basis of metabolomics and remains, together with proteomics, a complementary way to study functions of the genes (transcriptomics).

Our goal was to offer you a book, which is written in an understandable language, avoiding complex equations and advanced physics, bearing in mind that the most important aspect for the readers are practical aspects, potential applications, and selection of proper methodologies to solve their analytical and scientific problems.

# 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.

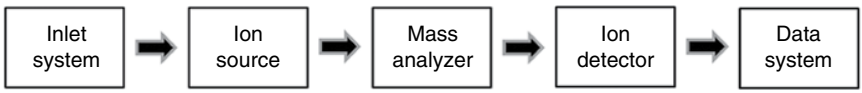


**Figure 1.1** Components of the mass spectrometer.

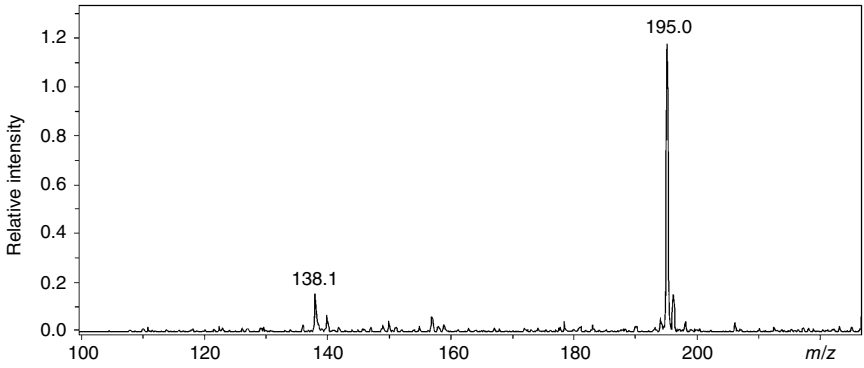


**Figure 1.2** Exemplary mass spectrum of caffeine obtained by electrospray ionization mass spectrometry (ESI-MS) technique. Signal at *m/z* 195.0 corresponds to the protonated molecule of caffeine.

- 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

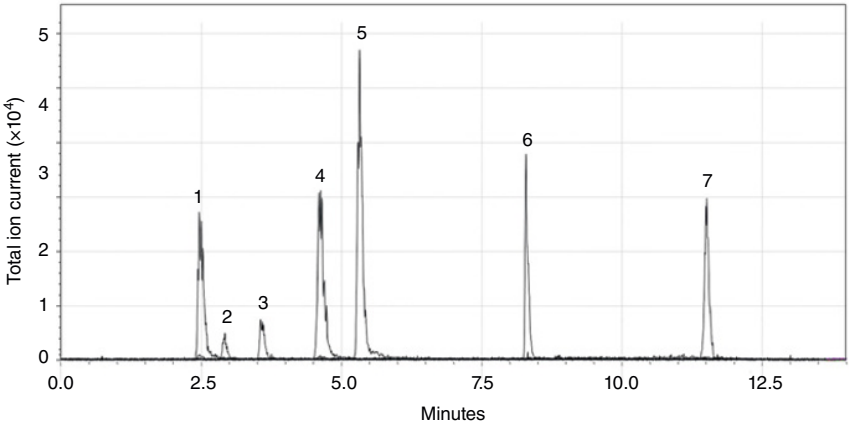


**Figure 1.3** Mass chromatogram of several designer drugs separated by the reversed-phase liquid chromatography (LC)-ESI-MS.

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.

# 2

# A Brief History of Mass Spectrometry

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Mass spectrometry (MS) is almost 120 years old, but despite its age, it is still an extremely attractive technique. Let us compare here its birth and development to the fate of the title character of the novel *The Curious Case of Benjamin Button* by F.S. Fitzgerald. The main character becomes younger and younger over time. This is somehow similar to MS, still showing new faces and possibilities.

The origin of development of this technique is considered to be 1897, when the British physicist J.J. Thomson conducted research on tubular radiation and, at that time, he experimentally confirmed the existence of an electron by estimating its *m/z* value. This resulted in the construction, in 1912, of a device called *the parabola spectrograph* for spectra measurements of $O_2$, $N_2$, CO, and $CO_2$. F.W. Aston, Thomson's assistant, applied this technique for studying isotopic compositions and, in 1919, presented another construction called a *mass spectrograph*. From this time, there was a significant increase in the awareness of the equipment, which can quickly determine isotopic composition of the elements. The breakthrough in MS dates back to the Second World War. In 1939, the Manhattan Project management asked A. Nier for help to solve an important issue: which uranium isotope is responsible for the fission reaction and how to gather the necessary material for further work. The answer to this question was crucial to enable a possibility to construct the atomic bomb. Nier had a machine built by E. Lawrence, which was based on a magnetic analyzer, a technique that was not efficient in separation of isotopes for military purposes. Around the same time, MS was used in petrochemical industry to evaluate the components of crude oil. This branch of industry had a lot of money, which was always driving the advancement of technology. The first

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spectrometers offered relatively small measuring capacities, the *m/z* range was about 70, and the spectrum recording time was about 20 minutes. During this period, the instruments were complicated, and only the chosen were able to manage those "black boxes." With the development of computers driven by the very complex operating systems (e.g. UNIX), they became increasingly incomprehensible to scientists. The basis for further and rapid development became commercialization of the production. The apparatus no longer had to be built on its own, but it could simply be purchased.

Postwar applications of MS were focused on the analysis of the low molecular weight compounds due to the lack of ionization techniques suitable for the studies of higher molecular masses, predominantly peptides and proteins. The primary source of ions was electron ionization (EI). However, it is worth mentioning here that gas chromatography–mass spectrometry (GC-MS) systems were also utilized to analyze amino acids and peptides.

The breakthrough came in 1981 when Michael Barber from Manchester developed the *fast atom bombardment* (FAB). For the first time, scientists could analyze biological compounds (including peptides and lipids) in solutions and not only in the gas phase. An additional advantage of this technique was the spontaneous fragmentation leading to assignment of the amino acid sequence. The problem was the presence of glycerol, effectively contaminating the ion source that had to be thoroughly cleaned at least once a week, and in the case of a source connected to the liquid chromatography (LC) (continuous-flow FAB), cleaning routine had to be carried out daily. In parallel, the analysts had at their disposal the thermospray ionization, the protoplast of the electrospray method, but the source was operated under high vacuum.

Another breakthrough in the development of ionization techniques was made in the mid-1980s of the previous century by the introduction of electrospray technique (J. B. Fenn with the team, 1984) and matrix-assisted laser desorption/ionization (MALDI) in 1985, with the name given by the creators of the source (M. Karas and F. Hillenkamp). It was further developed by K. Tanaka, who applied it to the analysis of higher molecular compounds and obtained the Nobel Prize for his achievements, together with J.B. Fenn (for electrospray ionization [ESI]) and K. Wüthrich (for NMR). ESI and MALDI are complementary techniques, and their main advantage is the ability to analyze compounds in a very wide range of masses. ESI was the first method operating at atmospheric pressure, enabling direct coupling of separation techniques and introduction of the sample in solution.

Initially, quadrupole or sector analyzers were used, and ion traps were introduced in 1983. Ion traps were unwillingly accepted by the world of scientists, because of the very low resolution, which then reached the value of only 50–100! The promising designs included the time-of-flight (TOF) analyzers most commonly linked to the MALDI source. The initial TOF constructions were also characterized by a low resolution on the order of 50; however, the