Part I
Introduction to MS in bioanalysis

Mass Spectrometry in Medicinal Chemistry. Edited by K. T. Wanner and G. Höfner

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Mass Spectrometry in Bioanalysis – Methods, Principles and Instrumentation

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1.1
Introduction

Mass spectrometry started about 100 years ago with the work of Sir J.J. Thomson. His interest was the quantitative measurement of the mass and charge of the cathode rays (electrons). For that purpose he constructed the first mass spectrometer (parabola mass spectrograph) and he received in 1906 the Nobel Prize for Physics in recognition of his work [1]. In the next decades the major focus in the development and application of mass spectrometry was dedicated to the studies of isotopes [2]. In 1918 Dempster [3] developed an instrument in which a strong magnetic field was produced, between two semicircular iron plates, to separate positive ion rays with great resolving power. He also described the bombardment of chemical compounds with electrons forming positive ions. This technique is known today as electron impact ionization and is still widely used in modern mass spectrometry. In the early 1940s the first commercial instruments based on magnetic deflection and electron impact ionization became available. These instruments were mostly applied for the analysis of hydrocarbons in petroleum products. Beyond instrumental development the end of the 1950s saw the application of mass spectrometry for structure elucidation of natural products and the studies of fragmentation patterns. At the same time the concept of several mass analyzers was described, such as time of flight or ion cyclotron resonance.

While the first coupling of gas chromatography and mass spectrometry had been reported in the late fifties [4] one had to wait for almost another 20 years before the direct interfacing of liquid chromatography with mass spectrometry (LC-MS) was described by Arpino et al. [5]. With the direct liquid interface (DLI) the effluent of the chromatographic column was directly introduced in the electron impact source. Contrarily to gas chromatography coupled to mass spectrometry (GC-MS), LC-MS did do not catch on as rapidly. One of the reasons was that the MS interface could only handle LC flow rates of a few microliters per minute. Another limitation was that electron impact or chemical ionization was not suit-

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able for very thermolabile and high molecular weight compounds. It took almost ten years before the LC-MS analysis of larger molecules, using continuous flow fast atom bombardment (FAB), was first reported [6, 7]. For small molecules it was thermospray (TSP) [8] and particle beam (PB) [9] which allowed the routine use of LC-MS. Thermospray formed in most cases ammonium adducts, while particle beam yielded electron impact spectra. Within a few years thermospray was rapidly replaced by atmospheric pressure ionization techniques.

Quadrupole mass spectrometers [10] or quadrupole ion traps are today the most widely used mass spectrometers. The physical bases were described in the early 1950s by Paul and Steinwedel. For his work Paul received the Nobel Prize in 1989 [11]. Triple quadrupole mass spectrometers have become very popular instruments for qualitative and quantitative analysis. Yost et al. [12] built in 1978 the first instrument and it took four years before this type of instrument was commercialized. The coupling with liquid chromatography or gas chromatography is well established and benchtop ion traps or quadrupoles are nowadays part of the standard equipment of many analytical laboratories.

For the analysis of macromolecules and in particular for proteins a major milestone was achieved with the development in 1987 of matrix assisted laser desorption ionization by Karas and Hillenkamp [13] and in 1988 of electrospray ionization by J. Fenn (Nobel Prize in 2002) [14].

Over the past decade progress in mass spectrometry and its hyphenation with separation techniques has made these tools essential in life sciences. The present chapter will describe current ionization techniques as well as mass analyzers.

1.2 Fundamentals

Mass spectrometry is a sensitive analytical technique which is able to quantify known analytes and to identify unknown molecules at the picomoles or femtomoles level. A fundamental requirement is that atoms or molecules are ionized and analyzed as gas phase ions which are characterized by their mass (m) and charge (z). A mass spectrometer is an instrument which measures precisely the abundance of molecules which have been converted to ions. In a mass spectrum m/z is used as the dimensionless quantity that is an independent variable. There is still some ambiguity how the x-axis of the mass spectrum should be defined. Mass to charge ratio should not lo longer be used because the quantity measured is not the quotient of the ion's mass to its electric charge. Also, the use of the Thomson unit (Th) is considered obsolete [15, 16]. Typically, a mass spectrometer is formed by the following components: (i) a sample introduction device (direct probe inlet, liquid interface), (ii) a source to produce ions, (iii) one or several mass analyzers, (iv) a detector to measure the abundance of ions, (v) a computerized system for data treatment (Fig. 1.1).

Most mass analyzers operate under high vacuum or at low pressure, so that the charged particles do not deviate from their trajectories due to collision with resid-

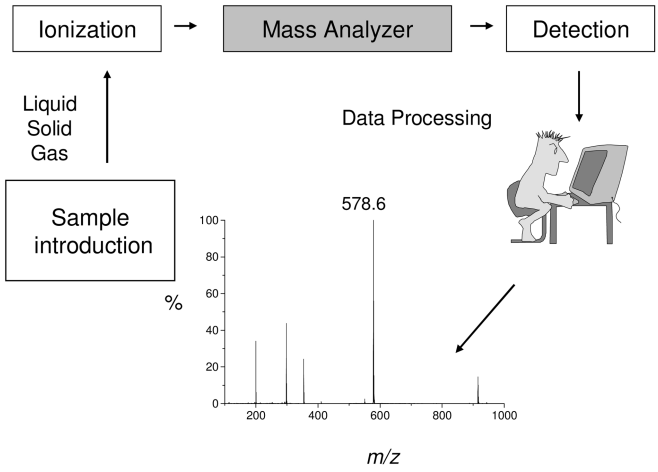


Fig. 1.1 Principle of a mass spectrometer, the outcome of an analysis is a mass spectrum with *m/z* in the *x*-axis and ion intensities in the *y*-axis. The ion intensities can be given in percentages (relative intensity) or in counts or in counts per second (absolute intensity). The most abundant peak at *m/z* 578.6 is called the base peak.

ual gas and thus never reach the detector. Mass spectrometers can be grouped into different types of operation mode: continuous mode (magnetic sector, quadrupole), pulsed mode (time of flight), and ion trapping mode (quadrupole traps, Fourier transform ion cyclotron, orbitrap). In the source, positive or negative ions are produced either under vacuum or at atmospheric pressure. Depending on the ionization technique either molecular ions ($M^{+\cdot}$) with an odd electron number or protonated ions ($[M+H]^+$) with an even electron number are formed. In the mass spectrum when no fragmentation occurs, in general the most intense peak represents the molecular ion, the protonated molecule or a molecule with an adduct ion followed by ions containing the heavier isotopes. M_r is the mass of one molecule of a compound, with a specified isotopic composition, relative to one-twelfth of the mass of one atom of ^{12}C. An important aspect is that many atoms have naturally occurring isotopes which can be differentiated by mass spectrometry. Molecules analyzed by organic mass spectrometry contain in general carbon, hydrogen, nitrogen, oxygen and sulfur. These elements have stable isotopes (Table 1.1) which have different atomic mass. Therefore, under certain conditions and for a given molecule, the isotopic contribution can be measured by mass spectrometry.

For example, carbon is composed of two naturally occurring isotopes: ^{12}C for 98.9% and ^{13}C for 1.1% abundance, respectively. For cyclohexane (C_6H_{12}) the $M^{+\cdot}$ ion composed exclusively of ^{12}C and ^{1}H atoms is observed at a nominal mass of *m/z* 84. The nominal mass is the integer of the sum of the masses calculated from the most abundant naturally occurring isotopes. The monoisotopic

Table 1.1 Isotopic abundance of common elements. Interesting to note is that chlorine and bromine have two naturally intense isotopes.

Element	Atomic mass	Symbol	Isotopic mass	Abundance (%)
Carbon	12.0110	^{12}C	12.000000	98.9
		^{13}C	13.003354	1.1
Hydrogen	1.0080	H	1.007825	99.985
		D	2.013999	0.015
Oxygen	15.993	^{16}O	15.994915	99.76
		^{17}O	16.999133	0.04
		^{18}O	17.999160	0.20
Nitrogen	14.0067	^{14}N	14.0030698	99.64
		^{15}N	15.00010	0.36
Chlorine	35.4610	^{35}Cl	34.968849	75.77
		^{37}Cl	36.999988	24.23
Bromine	79.9035	^{79}Br	78.918348	50.5
		^{81}Br	80.916344	49.5
Sulfur	32.066	^{32}S	31.97207	95.02
		^{33}S	32.971456	0.75
		^{34}S	33.96787	4.21
		^{36}S	35.96708	0.02

peak represents the exact mass of an ion or a molecule calculated from the most abundant isope of each element. The relative intensity of this ion compared to the others ions is 100%. A weaker isotopic peak ($M^{+\cdot} + 1$) is observed at m/z 85 with an abundance of 6.5% corresponding to one ^{13}C, five ^{12}C and 12 ^{1}H atoms. An even weaker peak (0.2% abundance) is visible at m/z 86 ($M^{+\cdot} + 2$) corresponding to two ^{13}C, four ^{12}C and 12 ^{1}H atoms. In this example, the contribution of deuterium can be neglected. For large molecules with increasing the number of carbon atoms, a shift of the maximum of the isotopic distribution towards higher masses can be observed, as depicted in Fig. 1.2. Above several hundred atoms of carbons, mostly a Gaussian distribution is observed. The consequence is that, in particular for protein analysis, only the relative molecular mass and not the monoisotopic mass is observed since either the monoisotopic masses can no longer be resolved or the intensity of the peak is too weak. The average mass is the calculated mass of an ion based on the relative atomic mass of each atom.

The isotopic contribution of various atoms is additive. For low molecular weight compounds, the isotopic contribution originates mainly from the carbon atom as long as no other element with a second isotope of significant abundance is present. For a molecule of M_r 192 the intensity of the m/z 194 ion represents 12% of the $[M+H]^+$ peak (m/z 193; Fig. 1.3A). Chlorine (Cl) has two intense isotopes: ^{35}Cl and ^{37}Cl (76% and 24% abundance, respectively). Replacing one H by a Cl atom results in a change of the isotopic distribution of the molecule

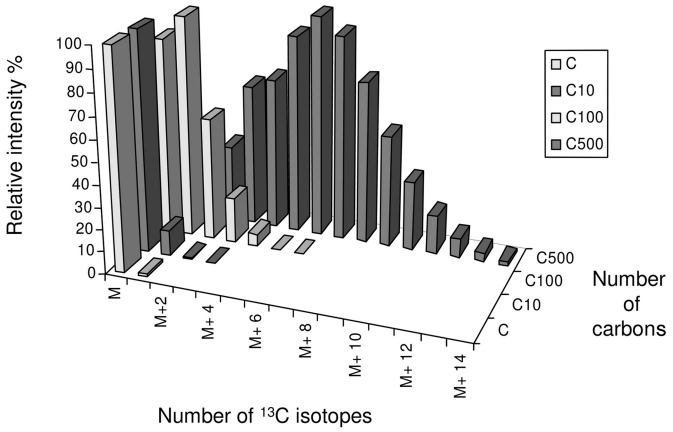


Fig. 1.2 Isotopic distribution as function of the number of carbon atoms. It can be observed that with increasing numbers of carbon atoms the maximum of the isotopic distribution shifts towards higher masses. M represents the molecular ion with only ^{12}C isotope; M+1 represents the molecular ion with only one ^{13}C isotope; M+2 represents the molecular ion with only two ^{13}C isotope; and so on.

(Fig. 1.3B). The $[M+H]^+ + 1$ peak is not affected, while the $[M+H]^+ + 2$ is increased to about 25%. The replacement of the F by a second Cl results in an increase of the $[M+H]^+ + 2$ and $[M+H]^+ + 4$ peaks (Fig. 1.3c). Chlorine and bromine have typical isotopic patterns therefore their presence in a molecule can be easily confirmed.

Mass analyzers are characterized by their mass range in m/z and their resolving power. The mass range is the m/z range where ions can be detected. The mass resolving power (R) is the ability of a mass analyzer to separate ions of different m/z with similar intensities. It is basically the m/z (m) at which the measurement was made divided by the difference (Δm_a) between the two peaks overlapping at a defined height (2 *x*%; Fig. 1.4). Because it is difficult to find two ions of equal intensities, the measure of the resolving power is often performed on a single peak. In general, the peak width is measured at 50% of its height. It is often referred to as full width at half maximum (FWHM). There is often confusion with the terms mass resolving power and mass resolution. Basically mass resolution is the smallest difference (Δm) between two equal magnitude peaks such as the valley between them is a specified fraction of the peak height. M1 and M2 are considered resolved when the valley between the two peaks represents 10% (2 *x*%) of their heights. In practice the definition of the resolution is often determined upon Δm of the a single peak at its full width at half maximum (Fig. 1.4, Δm_b).

For example for an ion measured at m/z 552 with a peak width of 0.5 m/z units (FWHM) the mass resolution would be 0.5, while the mass resolving power

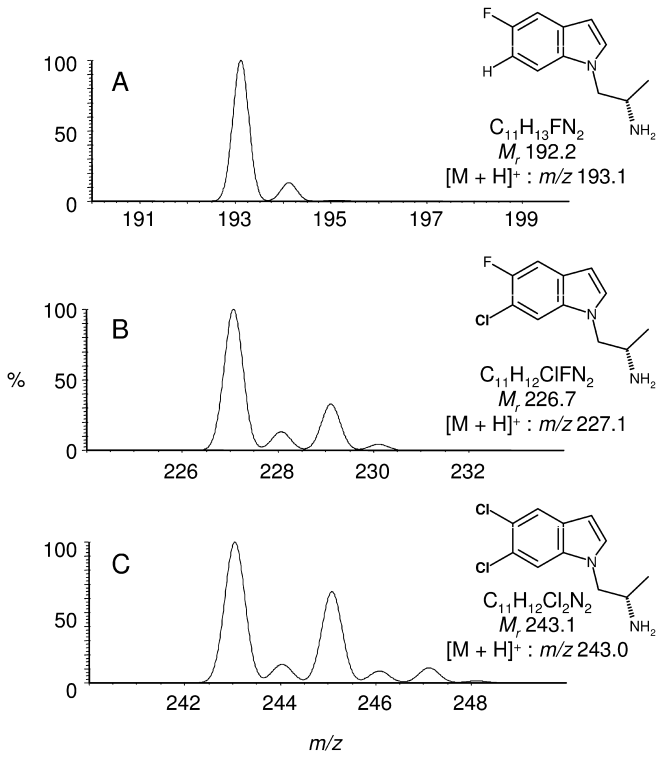


Fig. 1.3 The influence of chlorine on the isotopic distribution. (**A**) No chlorine atom, (**B**) one chlorine atom, (**C**) two chlorine atoms.

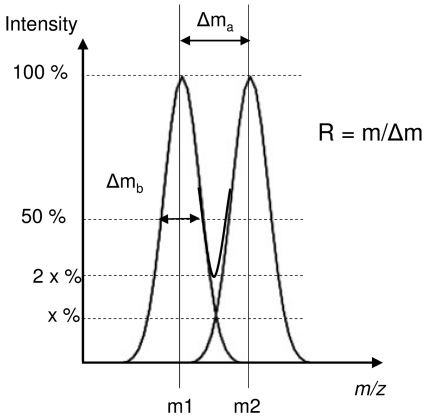


Fig. 1.4 Illustration of the mass resolution using two peaks of equal intensities (Δm_a) and a single peak (Δm_b).

would be 1104. With quadrupole and ion trap instruments the mass resolution is tuned to be constant over a defined mass range. With these instruments the term unit mass resolution is often employed to mention that the mass spectrometer is able to differentiate two ions distant by one m/z unit bearing a single charge.

While the relative molecular mass is calculated using the relative atomic mass considering all isotopes, the observed mass in mass spectrometry depends on the mass resolving power of the instrument; and various definitions are used. The exact mass represents the calculated mass of an ion or a molecule containing a single isotope of each atom. In general the lightest isotope of each atom is considered. The monoisotopic mass represents the calculated exact mass of an ion or molecule considering the most abundant naturally occurring isotopes. The accurate mass of an ion is the experimentally measured mass that is used to determine an elemental formula. The accurate mass is generally measured with at least three significant figures. The accuracy of the measure, corresponding to the difference between the measured mass and the calculated mass divided by the mass of the molecule, is indicated in parts per million (ppm).

Figure 5A, B shows the isotopic distribution, of protonated bosentan ($C_{27}H_{30}N_5O_6S$, M_r 552.6) with a mass resolution of 0.5 and 0.1 at FWHM, respectively. It is worthwhile to observe the mass shift of the most abundant ion from m/z 552.2006 to m/z 552.1911. This value does not change with a mass resolving power of 15 000 (Fig. 1.5C) or even 500 000 (Fig. 1.5D). Accurate mass measurements are essential to obtain the elemental composition of unknown compounds or for confirmatory analysis. An important aspect in the calculation of the exact mass of a charged ion is to count for the loss of the electron for the protonated molecule $[M+H]^+$. The mass of the electron is about 2000 times lower than of the proton and corresponds to 9.10956×10^{-31} kg. The exact mass of protonated bosentan without counting the electron loss is 552.1917 units, while it is 552.1911 units with counting the loss of the electron. This represents an error of about 1 ppm.

With time of flight instruments, a mass accuracy better than 5 ppm can be achieved, while with Fourier transform ion cyclotron resonance or orbitrap mass spectrometers mass accuracies better than 1 ppm have been reported. It is obvious that, for good mass accuracies, the peaks must be baseline resolved and resolution plays an essential role. For the present example, a mass resolving power of 5000 seems to be quite acceptable. In the case of the $[M+H]^+ + 1$ isotope peak, the situation becomes somewhat more complex for molecules containing nitrogen, sulfur or carbon. Figure 1.5D illustrates at a mass resolving power of 500 000 the contribution of ^{15}N, ^{33}S.

In qualitative analysis, the isotopic distribution remains an important information. For example in the case the parent drug contains Br or Cl, metabolites or decomposition products can be easily identified by considering the isotopic distribution. With accurate mass measurements a list of elemental compositions can be proposed for a compound for a given accuracy range. Because the intensity of the isotopic distribution is also dependent on the elemental composition of the molecule it can be used to reduce the list of possible elemental formulas [17].

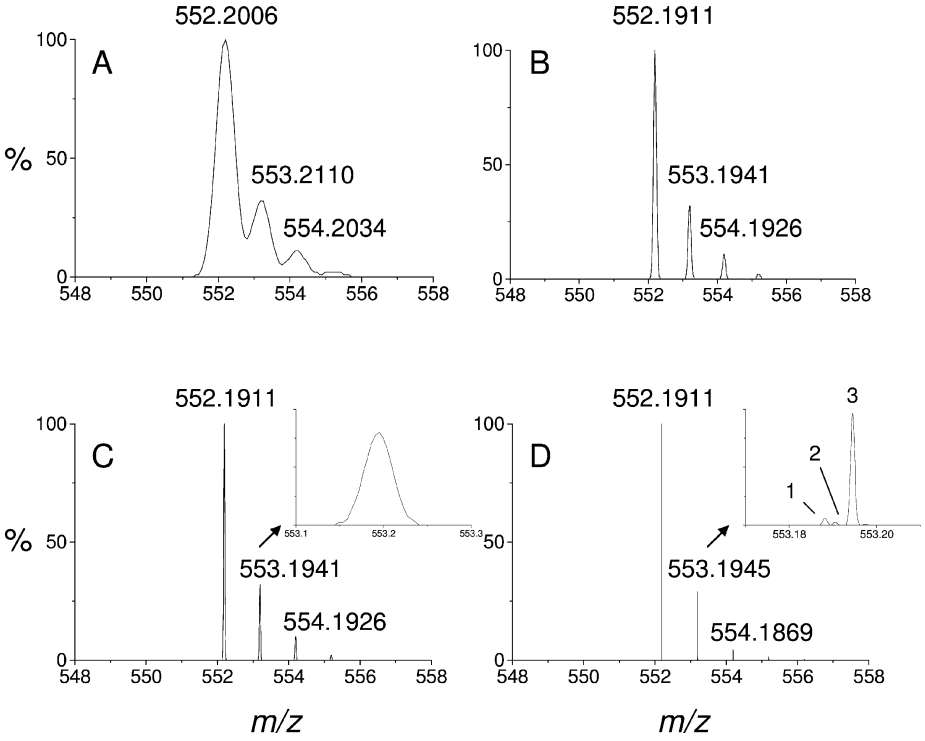


Fig. 1.5 Simulated isotopic distribution of the protonated bosentan ($C_{27}H_{30}N_5O_6S$) at mass resolving power: (**A**) R = 1104, with a peak full width at half maximum (FWHM) of 0.5 u. (**B**) R = 5520, FWHM = 0.1 u. (**C**) R = 15 000. (**D**) R = 500 000 with isotopic contribution of ^{15}N (peak 1), ^{33}S (peak 2) and ^{13}C (peak 3).

1.3 Ionization Techniques

1.3.1 Electron Impact and Chemical Ionization

Electron impact (EI) ionization is one of the most classic ionization techniques used in mass spectrometry. A glowing filament produces electrons, which are then accelerated to an energy of 70 eV. The sample is vaporized into the vacuum where gas phase molecules are bombarded with electrons. One or more electrons are removed from the molecules to form odd electron ions ($M^{+\cdot}$) or multiply charged ions. Solids, liquids and gases can be analyzed by EI, if they endure vaporization without decomposition. Therefore the range of compounds which can be analyzed by EI is somewhat limited to thermally stable and volatile compounds. The coupling with gas chromatography has been well established for

decades. The ionization energy of most organic compounds to form a radical cation is below 15–20 eV. The excess of energy transferred to the molecules causes reproducible fragmentation. Fragmentation of odd electron ions has been extensively studied but remains still a challenging task for non-experts. Under standard conditions at 70 eV, EI spectra are reproducible and instrument independent. Large commercial libraries are available to rapidly identify compounds present in a sample [18]. A limitation of the use of EI is that similar spectra can be obtained for isomers. Most analytical applications use EI in the positive mode but negative mode operation is also possible. EI is mostly combined with single quadrupole mass analyzers because often in the same spectrum, the molecular ions as well as fragment ions are present. Figure 1.6A shows the electron impact spectrum of a compound with a relative molecular mass of 355. The radical cation ion at m/z 355 as well as many fragments can be observed. Chemical ionization would generate the protonated molecule ion at m/z 356 (see Fig. 1.6B). To obtain structural information requires tandem mass spectrometry. Interestingly, odd and even electron ions undergo different fragmentation pathways, as observed in Fig. 1.6. This information is complementary, underlining that electron ionization remains an important technique for structural elucidation.

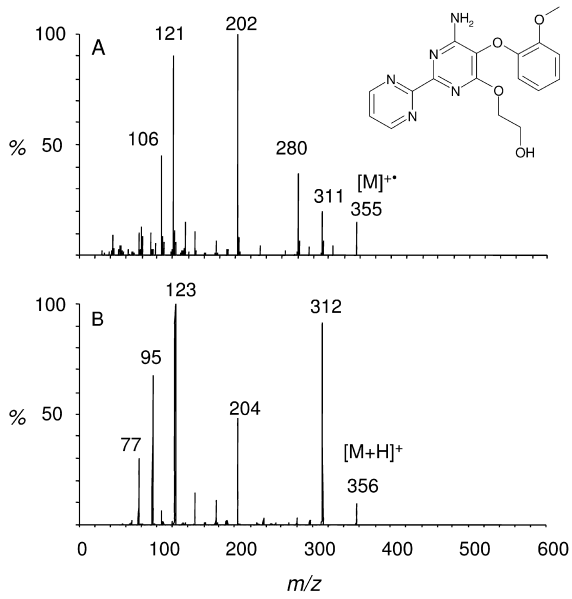


Fig. 1.6 (**A**) Electron impact spectrum obtained on a single quadrupole mass spectrometer of a compound with $M_r = 355$. (**B**) Product ion spectrum after atmospheric pressure ionization obtained on a triple quadrupole instrument. Chemical ionization and atmospheric pressure ionization give in both cases protonated precursor ions, which is ideal for tandem mass spectrometry.

Protonated or deprotonated molecules can be generated by chemical ionization (CI) sources with similar design to the classic EI sources [19]. The principal difference between CI and EI mode is the presence of a reagent gas which is typically methane, isobutane or ammonia. The electrons ionize the gas to form the radical cations (in the case of methane, $CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2e^-$). In positive chemical ionization (PCI) the radical cations undergo various ion–molecule reactions to form "CH_5^+" and finally lead to the formation, after proton transfer ($CH_5^+ + M \rightarrow [M+H]^+$), of protonated molecules. Negative chemical ionization (NCI), after proton abstraction, leads to deprotonated molecules $[M-H]^-$. Negative ions can be produced by different processes, such as by capture of low energy electrons present in the chemical ionization plasma. The major advantages of negative CI over positive EI or CI are higher sensitivity, the occurrence of the molecular ion and less fragmentation. Due to its high sensitivity NCI is mainly used in quantitative analysis after derivatization of the analyte [20].

1.3.2 Atmospheric Pressure Ionization

In atmospheric pressure ionization sources (API) the ions are first formed at atmospheric pressure and then transferred into the vacuum. In addition, some API sources are capable of ionizing neutral molecules in solution or in the gas phase prior to ion transfer to the mass spectrometer. Because no liquid is introduced into the mass spectrometer these sources are particularly attractive for the coupling of liquid chromatography with mass spectrometry. Pneumatically assisted electrospray (ESI), atmospheric pressure chemical ionization (APCI) or atmospheric pressure photoionization (APPI) are the most widely used techniques.

API offers unique opportunities for the implementation of new sources or to develop new applications. Atmospheric pressure matrix assisted laser desorption (AP-MALDI) [21] can be mounted on instruments such as ion traps which were originally designed only for electrospray and LC-MS. New API desorption techniques such as desorption electrospray (DESI) [22] or direct analysis in real time (DART) [23] have been described and offer unique opportunities for the analysis of surfaces or of solid samples.

The sampling of ions from atmospheric pressure into to the high vacuum region of the mass analyzer region requires significant pressure reduction. A gas stream introduced into a vacuum system expands and cools down. When this gas stream contains ions and solvent vapors the formation of ion–solvent clusters is observed. To obtain good sensitivities and high quality spectra one of the key roles of the interface is to prevent cluster formation. Different instrument designs have been proposed, including single stage pumping or differential stage pumping. Figure 1.7 depicts a typical single stage interface with curtain gas. The space between the orifice and the curtain plate is flushed with heated pure nitrogen. Ions are moved through the curtain gas into the mass analyzer with the help of an electric field formed between the curtain plate and the orifice. In this way, neutral solvent molecules cannot penetrate into the high vacuum region, which prevents

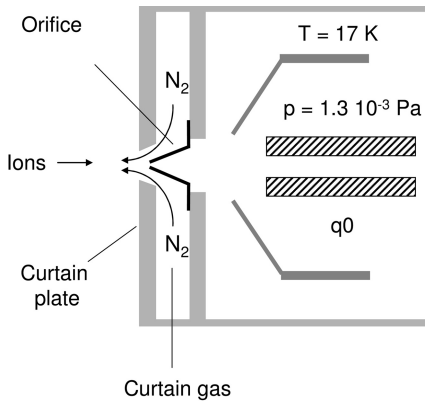


Fig. 1.7 Single stage pumping atmospheric pressure ionization interface with curtain gas. The size of the orifice is ca. 100 µm, q0 acts as a focusing quadrupole and the nitrogen curtain gas prevents neutral molecules being introduced into the mass spectrometer. T = Temperature of the cryoshells (in Kelvin); p = pressure.

the formation of cluster ions. In a single-stage pumping interface, as described in Fig. 1.7, the size of the orifice is ca. 100 µm and to maintain a high vacuum cryogenic pumps are mandatory. Declustering can also be performed by applying a potential difference between the orifice and quadrupole q0 [24]. If the value of

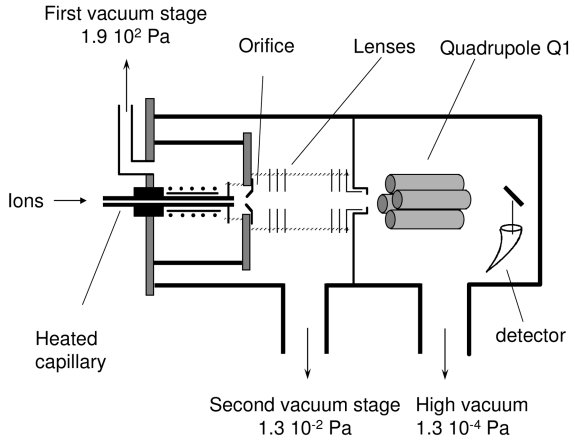


Fig. 1.8 Differential pumping design with heated capillary. This configuration requires a dual stage pumping system before the ions are introduced into the quadrupole mass analyzer which needs to be operated at high vacuum. The role of the lenses is to focus ions. In some systems the lenses are replaced by hexapoles or octapoles.

the declustering potential is set too high ''in source'' or ''up front'' collision-induced dissociation can be observed. Cryogenic pumps have high pumping capacity (10 000 L s^{-1} and more) but they need to be recycled every 48 h, which jeopardizes automated use of the instrument. Turbomolecular or diffusion pumps have much lower pumping capacities (50–800 L s^{-1}). To achieve the desired vacuum in the mass analyzer, differential pumping designs were developed. An instrument design using differential pumping with a heated capillary interface is illustrated in Fig. 1.8. In a first step ions flow through a heated capillary (T = 150–300 °C) which helps desolvatation. The internal diameter of the capillary is typically 0.5 mm. A reduced vacuum is achieved in the first pumping region with the help of a rotary pump. Ions are then pushed through a skimmer or an orifice into a second vacuum chamber where the vacuum is produced by a turbo molecular pump and then analyzed in the mass analyzer. Most modern instruments use differential pumping either with capillary skimmer or with an orifice skimmer setup with or without curtain gas.

1.3.2.1 Electrospray

A spray of small droplets at atmospheric pressure can be generated by: (i) a nebulizing gas, (ii) the application of heat, (iii) the application of ultrasounds iv) the application of an electric field. Electrospray ionization (ESI) is a process were charged droplets result from the nebulization of a solution in an electric field. The liquid flows through a stainless steel or a fused silica capillary while the potential (typically 3–6 kV in positive mode) is applied directly on the capillary or on a counter electrode. In negative mode to avoid discharge, the range is somewhat lower (typically 3–4 kV). After nebulization the charged droplets reduce their size and subdivide, up to a point where gas phase ions escape from the droplets. A stable spray can be obtained at flow rates of 1–10 μl min^{-1}. When performing LC-MS with standard bore LC columns (4.6 mm i.d.) the LC effluent must be split. To overcome this limitation, the spray process can be assisted by a nebulizing gas such as nitrogen or air [25] (Fig. 1.9). This way of operation was originally named ionspray but the term is less and less used. With liquid chromatography most sources use air or nitrogen to assist the electrospray process (pneumatically assisted electrospray). Stable sprays can be observed with flow rates above 1 ml min^{-1}, allowing direct interfacing of LC with MS. Most modern commercial instruments operate with pneumatically assisted electrospray placed orthogonally to the entrance of the MS. The nebulizing process can be further assisted with the use of heat, where either the sprayer is heated or a hot stream of nitrogen is directed orthogonally towards the formed droplets.

Very low flow electrospray is called nanoelectrospray [26] where the samples are infused into the mass spectrometer at the nanoliter flow rate range. The infusion of a few microliters will result in a stable signal for more then 30 min, using pulled capillaries or chip-based emitters [27]. With infusion, signal averaging allows to improve the limit of detection in tandem mass spectrometry. Nanoelectrospray is particularly important in combination with nanoflow liquid chromatography or chip-based infusion for the analysis of peptides and proteins.

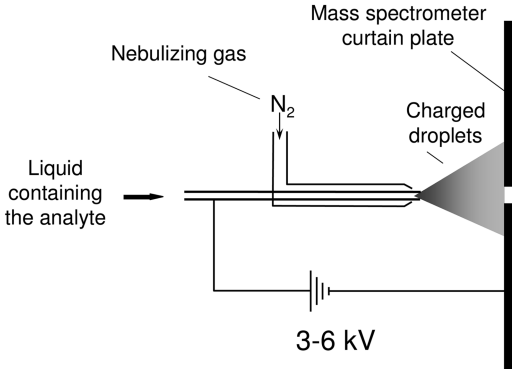


Fig. 1.9 Pneumatically assisted electrospray. The coaxial nitrogen gas assists the electrospray process allowing to operate at flow rates of several hundred microliters.

ESI is a condensed phase ionization process and the ions have to be already present in solution. To generate ions, the pH has to be adjusted in such a way that ionizable groups are either protonated or deprotonated. In some cases neutral molecules can be analyzed by the formation of adducts with ions such as ammonium, sodium, potassium, acetate or silver.

Peptides and proteins have several ionizable sites, resulting in the formation of multiply charged ions [14]. Figure 1.10 shows the ESI spectrum of human gamma interferon ($M_r = 16\,908.50$). The mass spectrum of the protein corresponds to a distribution of multiply charged ions obtained through protonation ($[M+zH]^{z+}$). The ion at $(m/z)_1$ 846.4 corresponds to human gamma interferon protonated 20 times [$z_1 \cdot (m/z)_1 = M_r + z_1 \cdot m_p$], M_r being the relative molecular mass of the protein, z_1 the number of charges and m_p the mass of the proton. Because each pair of ions differs by one proton [$(m/z)_2$ 806.1 bears 21 protons] the charge state (z_i) of any ion and therefore the relative molecular mass of an unknown protein can be determined with the following equations:

$$z_2 = \frac{(m/z)_1 - m_p}{(m/z)_1 - (m/z)_2} \quad (1)$$

$$M_r = z_2 \cdot [(m/z)_2 - m_p] \quad (2)$$

where z is charge, m is mass and m_p is proton mass.
The relative molecular mass determination of an unknown protein is generally performed automatically using various deconvolution algorithms, but the procedure is limited to relatively simple mixtures.

Electrospray ionization can be considered as an electrolysis cell (Fig. 1.11) where, in the positive mode, cations are enriched at the surface of the solution and negative ions move inside the capillary. Oxidation of the analyte has been observed at certain occasions, in particular at very low flow rates. Also in the case of

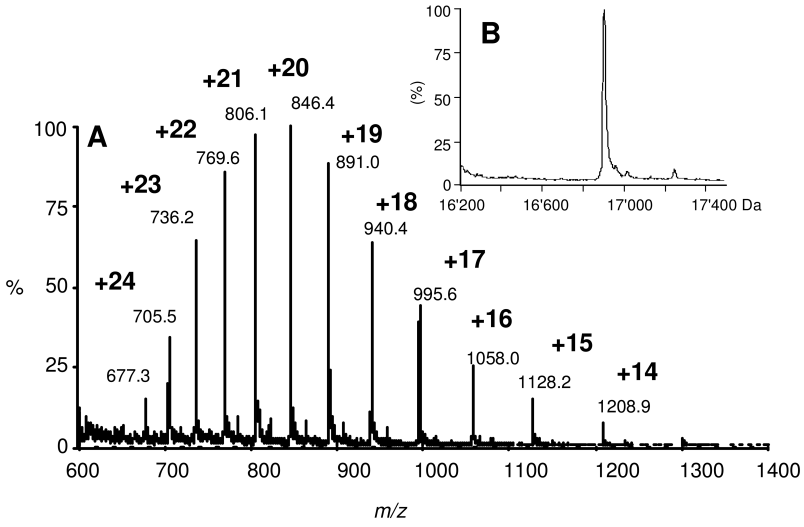


Fig. 1.10 (**A**) Positive mode electrospray spectrum of human gamma interferon on a quadrupole mass analyzer. (**B**) Deconvoluted spectrum of human gamma interferon. The molecular mass was measured at 16 908 $\pm$ 2 Da.

stainless steel sprayers nickel or iron ions can be released and form positively charged complexes with certain types of analytes.

The mechanisms for the formation of gas phase ions from droplets are not fully understood and two therories have been proposed: the ion evaporation model (IEV) and the charge residue model (CR) [28]. The IEV model proposes that the ions are directly emitted into the gas phase when, after evaporation and

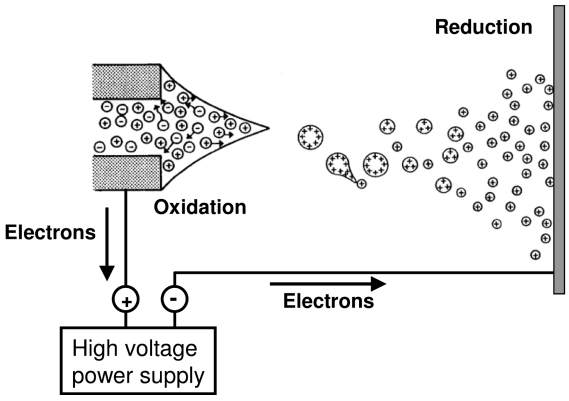


Fig. 1.11 Electrospray as an electrophoretic cell. Adapted with permission from reference [28].

coulomb droplet fission, the droplets reach a certain radius. In the case of the CR model it is assumed that gas phase ions are produced when no further solvent evaporation is possible. In the case of small molecules it is believed that the IEV model predominates while for the proteins the CR model is assumed to occur.

A very interesting characteristic of electrospray MS is that it behaves, under controlled settings, like a concentration-sensitive detector [29]. This means that the MS response is directly proportional to the concentration of the analyte. A direct consequence is that LC post-column splitting does not affect the intensity of the MS signal. Another important point is that the reduction of the internal diameter of the column results in an increase in the MS response proportional to the squared ratio between the internal diameters of the greater i.d. column to the smaller i.d. column. Assuming that the same amount of analyte is injected onto a 0.3 mm i.d. column instead of a 2.0 mm i.d. column, a 44-fold increase in response is observed. Or the same response is obtained using a 44 times smaller sample volume. The use of smaller sample volumes is attractive for qualitative analysis where sample consumption can be critical. Because the injection volumes have also to be much lower with smaller i.d. columns, column-switching approaches become mandatory to really benefit from the gain of sensitivity in quantitative analysis [30]. Generally the trapping column is of a larger i.d. than the analytical column, allowing the rapid injection of 50–100 μL of sample.

1.3.2.2 **Atmospheric Pressure Chemical Ionization**

Atmospheric pressure chemical ionization (APCI) is a gas phase ionization process based on ion–molecule reactions between a neutral molecule and reactant ions [31]. The method is very similar to chemical ionization with the difference that ionization occurs at atmospheric pressure. APCI requires that the liquid sample is completely evaporated (Fig. 1.12). Typical flow rates are in the range 200–1000 μL min^{-1}, but low flow APCI has also been described. First, an aerosol is formed with the help of a pneumatic nebulizer using nitrogen. The aerosol is directly formed in a heated quartz or ceramic tube (typical temperatures 200–500 °C) where the mobile phase and the analytes are evaporated. The temperature of the nebulized mobile phase itself remains in the range 120–150 °C due to evapo-

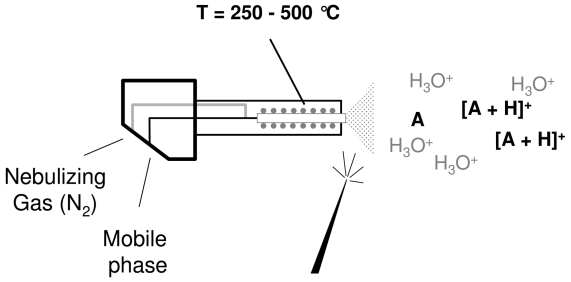


Fig. 1.12 Atmospheric pressure chemical ionization source. A Analyte.

ration enthalpy. In a second step, the evaporated liquid is bombarded with electrons formed by corona discharge. In positive mode primary ions such as $N_2^{+\cdot}$ are formed by electron impact. These ions react further with water in several steps by charge transfer to form H_3O^+. Ionization of the analyte A occurs then by proton transfer. In negative mode ions are formed either by: (i) resonance capture ($AB \rightarrow AB^-$), (ii) dissociative capture ($AB \rightarrow B^-$) or (iii) ion–molecule reaction ($BH \rightarrow B^-$). Generally APCI is limited to compounds with $M_r < 2000$ which do not undergo thermal decomposition. Singly charged ions $[M+H]^+$ or $[M-H]^-$ are predominantly observed. While electrospray is a condensed phase ionization process, APCI is a gas phase ionization process where the analyte ionization efficiency depends on its gas phase proton affinity. APCI ionization has become very popular for liquid chromatography coupled with mass spectrometry because it can handle very easily liquid flow rates from 200 μL min^{-1} to 1 mL min^{-1}. In contrast to electrospray, the application of heat may generate thermal decomposition of the analyte. At atmospheric pressure, ionization occurs with the high collision frequency of the ambient gas and rapid desolvation and vaporization limits the thermal decomposition of the analyte. Figure 1.13A shows the electrospray full-scan spectrum of the sulfuric acid monoester of 3-hydroxy retinoic acid, which is a phase II metabolite of 3-hydroxy retinoic acid without any degradation. In the APCI spectrum of the same analyte (Fig. 1.13B) several intense ions at m/z 315 and m/z 297 can be observed. These ions are not generated by collision-

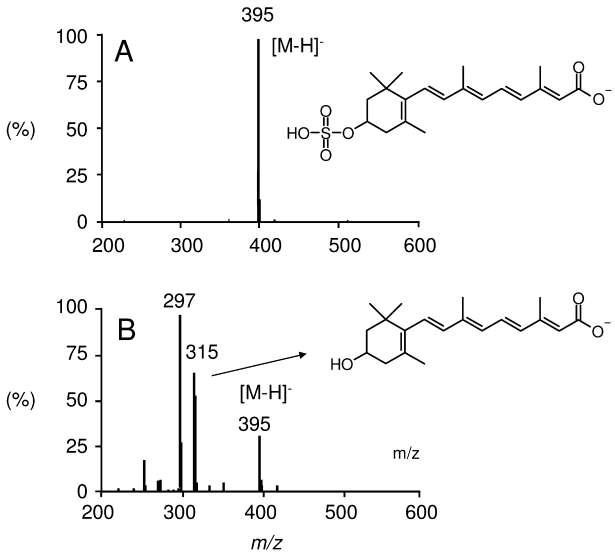


Fig. 1.13 Negative mode single quadrupole MS spectra of sulfuric acid monoester of 3-hydroxy retinoic acid: (**A**) electrospray, (**B**) atmospheric pressure chemical ionization.

induced dissociation but by thermal degradation. The product ion spectrum of the precursor ion at m/z 395 shows only a strong ion at m/z 97, corresponding to the HSO_4^- ion (data not shown). The ion at m/z 315 corresponds to 3-hydroxy retinoic acid generated in the source by the loss of SO_3. The second ion at m/z 297 corresponds to the loss of an additional 18 units (H_2O). At a first glance thermal degradation in APCI sounds detrimental, but because it is quite reproducible it can provide further structural information in qualitative analysis.

1.3.2.3 **Photoionization**

The setup for atmospheric pressure photoionization (APPI) [32–34] is very similar to that for APCI. Only the corona discharge is replaced by a gas discharge lamp (krypton, 10.0 eV) that generates vacuum ultraviolet photons. The liquid phase is also vaporized by a pneumatic nebulizer. Most analytes have ionization potentials below 10 eV while HPLC solvents have higher ionization potentials (water 12.6 eV, methanol 10.8 eV, acetonitrile 12.2 eV). The absorption of a photon by the molecule and the ejection of an electron forms a radical cation. Better sensitivities have been reported with the addition of dopants such as toluene or acetone. The mechanism of ionization is not fully understood but two different mechanisms can occur: (i) dopant radical cations react with the analyte by charge transfer or (ii) the dopant radical cation ionize the solvent molecules by proton transfer which can then ionize the analyte. APPI can also be performed in the negative mode. Like APCI, APPI can handle a large range of analytes. The performance of APPI is flow rate-dependent; and better sensitivities, compared to APCI, have been reported at lower flow rates. It appears also that APPI is less sensitive to matrix suppression and source contamination. Atmospheric pressure photoionization proves to be particularly attractive for the analysis of steroids and quinones.

1.3.2.4 **Multiple Ionization Source**

With atmospheric pressure ionization the signal response is strongly analyte-dependent. To combine more than one ionization source (ESI, APCI, APPI) is particularly attractive to extend the range of compounds that can be analyzed simultaneously. Most pharmaceutical compounds can be analyzed automatically with positive or negative ESI mode using standard conditions [35]. Those compounds which give no signal require special attention, such as optimized solvent conditions or a change in ionization method resulting in a significant loss in time. Gallagher et al. [35] have developed a combined ESI-APCI (ESCi) source for high speed online LC-MS analysis. The combined source allows alternate online ESI and APCI scans with polarity switching within a single analysis. During the LC-MS run the high voltage power supply can be switched within 100 ms from the electrospray capillary to the APCI discharge needle. Figure 1.14 shows the LC-MS analysis of a mixture of daidzein and acetophenone with the ESCi source. In this case daidzein shows the best response with ESI while acetophenone gives a strong signal with APCI.

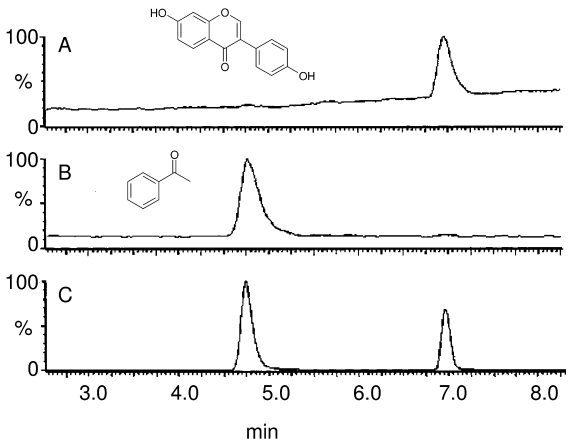


Fig. 1.14 LC-MS analysis of a mixture of daidzein and acetophenone with a ESCi source: (**A**) ESI, (**B**) APCI, (**C**) photo diode array detection. Adapted with permission from reference [35].

An other approach has been described by Syage et al. [36], who investigated the potential of various ionization sources (ESI, APCI, APPI) either in simultaneous or in switching mode. They suggest that ESI/APPI is the best combination because APPI covers a broad range of analytes while ESI covers the larger molecules.

1.3.2.5 **Desorption Electrospray and Direct Analysis in Real Time**

Direct analysis of solid samples or analytes present on solid surfaces without any sample preparation has always been a topic of interest. Desorption electrospray ionization (DESI) is an atmospheric pressure desorption ionization method introduced by Cooks et al., producing ions directly from the surface to be analyzed, which are then sampled with the mass spectrometer [22, 37]. DESI is based on charged liquid droplets that are directed by a high velocity gas jet (in the order of 300 m s^{-1}) to the surface to be analyzed. Analytes are desorbed from the surface and analyzed by mass spectrometer (Fig. 1.15).

Compared to atmospheric pressure MALDI (see Section 1.3.3.), no matrix is needed to perform the experiment. Direct analysis in real time (DART), a method related to DESI, has been reported by Cody et al. [23]. This technique is based on the reactions of metastable helium atoms generated by corona discharge with oxygen/water (negative mode) or water clusters (positive mode). The formed reactant ions ionize the analytes either by cluster assisted desorption or proton exchange. Both methods generate mostly protonated or deprotonated molecular ions. Various applications of both techniques for the analysis of the mass spectrometric profiling of intact biological tissue nicely demonstrated the characterization of the active ingredients in pharmaceutical samples formulated as tablets, ointments, or the sampling of plant material [38].

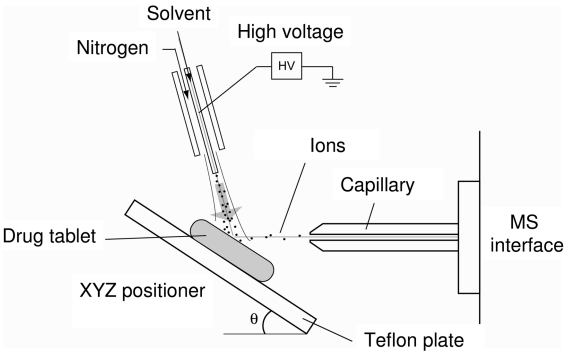


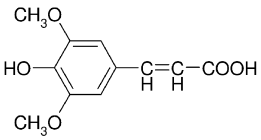
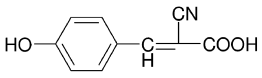
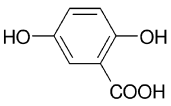
Fig. 1.15 Desorption electrospray ionization interface. The sample, in this case a pharmaceutical pill, is placed in front of the orifice and is hit by nebulized droplets. Desorbed ions are then sampled into the mass spectrometer.

1.3.3
Matrix Assisted Laser Desorption Ionization

Matrix assisted laser desorption ionization (MALDI) has grown from the efforts to analyze macromolecules by mass spectrometry. Two groups were able, in the late 1980s, to obtain mass spectra of proteins. The first group was led by T. Tanaka [39] (Nobel Prize 2002) and developed MALDI where the analyte is mixed in a matrix of glycerol and cobalt and ionized with a laser. The second group formed by M. Karas and F. Hillenkamp [13] developed MALDI where the analyte is mixed with a matrix solution containing UV-absorbing molecules (Table 1.2). A few microliters of solution are spotted onto a MALDI target where the sample crystallizes.

After introduction of the target into the vacuum, an UV laser pulse is used to desorb and ionize the sample. Nitrogen laser emitting at 337 nm and Nd:YAG laser emitting at 355 nm are the most widely used. MALDI is a very powerful technique for the analysis of synthetics and natural biopolymers. It has completely replaced former techniques such as fast atom bombardment (FAB). In

Table 1.2 Commonly used matrices for matrix assisted laser desorption ionization.

Sinapinic acid **Proteins, imaging**	**α-Cyano-4-hydroxycinnamic acid** **Peptides, small molecules**	**2,5-Dihydroxybenzoic acid** **Proteins**
		

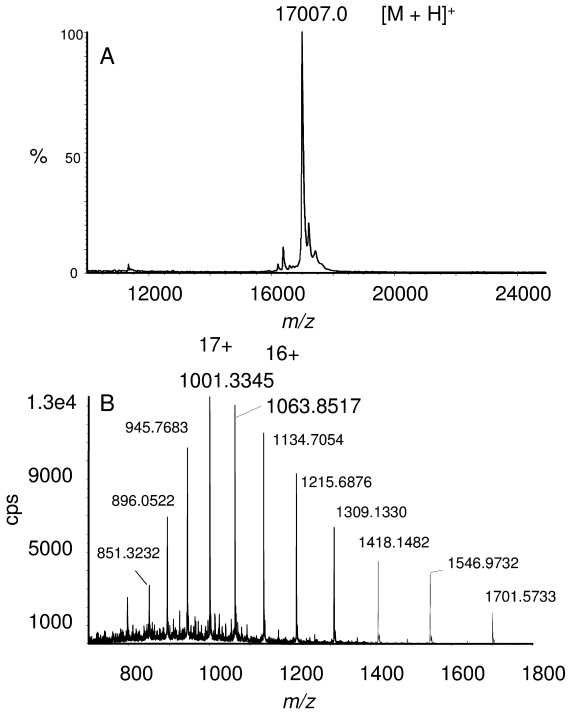


Fig. 1.16 Mass spectra of a recombinant protein obtained by: (**A**) matrix assisted laser desorption ionization–time of flight, (**B**) electrospray–quadrupole time of flight. *cps* Counts per second.

most cases singly charged ions are predominantly detected while very little fragmentation or multiply charged ions are observed. MALDI is commonly used for the analysis of high molecular weight compounds such as peptides and proteins [40], synthetic polymers [41], DNA [42] and lipids [43].

MALDI has the intrinsic advantage over ESI-LC-MS in that it can achieve a high sample throughput. Sample preparation and separation can also be decoupled from the mass spectrometric analysis. The MALDI target plate can be easily archived, which allows simple reanalysis of selected samples. MALDI or ESI are suitable for the analysis of proteins, as depicted in Fig. 1.16. One of the key advantages of ESI over MALDI is the formation of multiply charged ions which allows the analysis of proteins on almost any type of mass analyzer while MALDI requires a time of flight mass analyzer in the linear mode to cover the high mass range.

The high throughput capability of MALDI and the different ionization mechanisms make this technique also an attractive alternative to electrospray ionization for the analysis of low relative molecular mass compounds (LRMM) [44]. However, interferences of matrix ions and the ionization of the low relative molecular mass compounds are the challenges of this technique [45, 46].

Desorption/ionization on porous silicon (DIOS) without any matrix has been described for the analysis of LRMM compounds with no chemical background [47, 48]. The use of MALDI for the analysis of small molecules was recently reported. Particularly attractive is the coupling of a MALDI source with a triple quadrupole mass analyzer for quantitative analysis in the selected reaction monitoring (SRM) mode due to very high analysis speed.

Surface enhanced laser desorption/ionization (SELDI) is a distinctive form of laser desorption ionization where the target plays an active role in the sample preparation procedure and ionization process [49]. Depending on the chemical or biochemical treatment, the SELDI surface acts as solid phase extraction or an affinity probe. Chromatographic surface is used for sample fractionation and purification of biological samples prior to direct analysis by laser desorption/ionization. SELDI is mainly applied for protein profiling and in biomarker discovery by comparing protein profiles from control and patient groups.

Because MALDI is a desorption technique, it is particularly suited for the analysis of surfaces such as biological tissues [50]. In this application, the matrix is applied on the complete surface of the tissue. The laser resolution is about 100 μm and complete analyte distribution images (low molecular weight compounds, peptides, proteins) can be recorded [51, 52].

1.4 Mass Analyzers

1.4.1 Quadrupole Analyzers

A quadrupole mass analyzer is made of four hyperbolic or circular rods placed in parallel with identical diagonal distances from each other. The rods are electrically connected in diagonal. In addition to an alternating radiofrequency (RF) potential (V), a positive direct current (DC) potential (U) is applied on one pair of rods while a negative potential is applied to the other pair (Fig. 1.17). The ion trajectory is affected in x and y directions by the total electric field composed by a quadrupolar alternating field and a constant field. Because there is only a two-dimensional quadrupole field the ions, accelerated after ionization, maintain their velocity along the z axis.

The motion of ions in the quadrupole (x, y) is quite complex and can be described by the Matthieu equations. The solution of the Matthieu equations generate two terms, a and q, which are proportional to the RF and DC potentials, respectively. For a detailed description of Matthieu equations, please see reference [53]. The trajectories of ions are stable when the ions never reach the rods of the quadrupole. To reach the detector an ion must have a stable trajectory in the x and y directions. With a quadrupole mass analyzer a mass spectrum is obtained by increasing the magnitude of U (DC) and V (RF) at a constant ratio. In a quadrupole mass analyzer when the DC voltage of a quadrupole is set to zero and

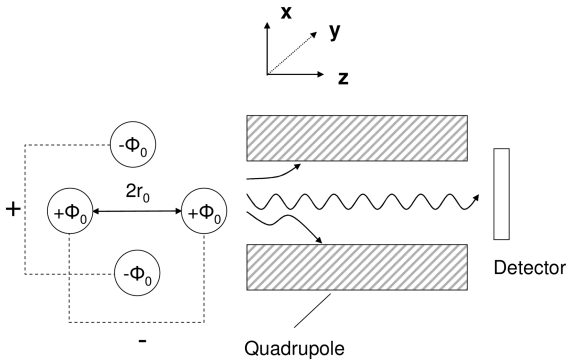


Fig. 1.17 The quadrupole mass analyzer is formed by four circular or hyperbolic rods placed in parallel. Φ Quadrupolar potential.

the RF voltage is maintained, the ions remain focused with no mass selectivity. Therefore, RF quadrupoles are ideal as ion guides or as a collision cell. Typically, quadrupole mass analyzers operate at unit mass resolution (FWHM 0.6–0.7 m/z units). There is a strong relation between resolution and transmission. In general higher mass resolution results in a decrease of transmission, but mass resolution corresponding to peak width of 0.1 m/z units without significant loss in sensitivity have also been reported. The mass range of quadrupoles is typically between m/z 5 and m/z 4000. Most common ionization sources are available on quadrupole instruments, including EI, ESI, APCI, APPI and MALDI.

1.4.2
Triple Quadrupole Mass Analyzer

A triple quadrupole instrument (QqQ) is a combination of two mass quadrupole mass filters (tandem mass spectrometry) separated by a collision cell which is also a quadrupole operating in RF-only mode (Fig. 1.18). A common nomencla-

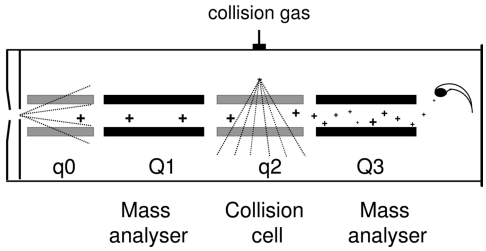


Fig. 1.18 Schematic of a triple quadrupole instrument. Stage q0: focusing quadrupole; Q1, Q3: mass analyzing quadrupoles; q2: collision cell. In the present configuration the collision energy (CE) is determined by the potential difference between q0 and q2.

ture is to use (Q) to describe a quadrupole which is operated in RF/DC mode and (q) for a quadrupole which is operated in RF only mode. Tandem mass spectrometry is particularly attractive to obtain additional mass spectral information. In a first step, a specific m/z ion (precursor ion) is selected in the first mass analyzer (Q1). Collision induced dissociation (CID) occurs in the collision cell (q2) which is filled with a neutral gas such as argon or nitrogen. The fragment ions (product ions) are then sorted according to their mass to charge ratio in the second mass analyzer (Q3) and recorded by the detector. This way to obtain MS/MS data is called MS/MS in space, contrasting with quadrupole ion traps where MS/MS experiments are performed in time. On triple quadrupole mass spectrometers the potentials used to carry out collision induced dissociation are in the range 0–250 V. The collision energy is defined in electrons volts (eV) and is therefore dependent on the charge of the ions. For a potential difference of 30 volts the collision energy for a singly charge precursor ion would be 30 eV, and 60 eV for a doubly charged precursor ion. The nature of the collision gas (N_2 or Ar) does not affect the product ion spectrum. The gas pressure in the collision cell mainly influences the sensitivity while collision energy influences the nature of the spectrum.

Depending on how the mass analyzers are operated, various types of MS and MS/MS experiments can be performed on a QqQ and these are summarized in Table 1.3. To normalize the description of various MS/MS or multi-stage MS^n experiments a symbolism has also been described [54, 55].

A product ion scan can obtain structural information of a given precursor ion while a precursor ion scan is more suited to find structural homologues in a complex mixture. Bosentan ($M_r = 551$, Fig. 1.19) has two metabolites corresponding to the tert-butyl hydroxylation product ($M_r = 567$) and the dealkylation of the methoxy group to form the phenol ($M_r = 537$). Bosentan (Tracleer, Actelion Phramaceuticals) is an oral duel endothelin receptor antagonist approved for the use in arterial hypertension [56]. Selection of the fragment at m/z 280 can fish out precursor ions corresponding only to bosentan and these two metabolites (Fig. 1.19C). A similar result is obtained with the constant-neutral loss scan mode (Fig. 1.19D) which is based on neutral loss of 44 units.

Table 1.3 Settings of the Q1 and Q3 quadrupoles for the various scan modes of a triple quadrupole mass spectrometer.

Mode	Q1 quadrupole	Q3 quadrupole
Full scan Q1/single ion monitoring (SIM) Q1	Scan/fixed	Rf mode
Full scan Q3/single ion monitoring (SIM) Q3	Rf mode	Scan/fixed
Product ion scan (PIS)	Fixed	Scan
Precursor ion scan (PC)	Scan	Fixed
Neutral loss (NL)	Scan	Scan: neutral loss offset
Selected reaction monitoring (SRM)	Fixed	Fixed

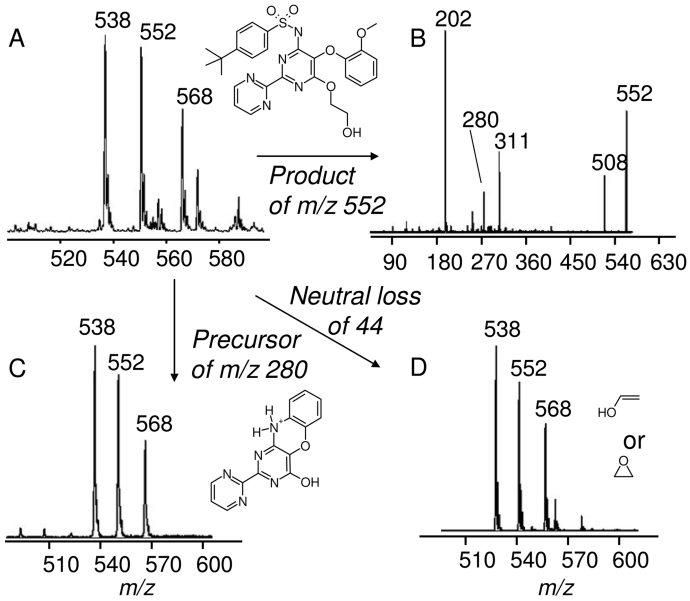


Fig. 1.19 (**A**) Q1 full-scan spectrum of bosentan [$(M+H)^+$, *m/z* 552], its demethylated metabolite [$(M+H)^+$, *m/z* 538] and its hydroxylated metabolite [$(M+H)^+$, *m/z* 568], (**B**) product ion spectrum of bosentan, (**C**) precursor ion spectrum, (**D**) neutral loss spectrum. Electrospray ionization is in positive ion mode.

Precursor ion and neutral loss scans are efficient on QqQ to identify structurally related compounds in a mixture, using either a common fragment with the parent compound or the specific neutral loss such as glucuronid or sulfate for phase II metabolites. These selective scan modes do not require any knowledge of the molecular weight or the structure of the compounds. In the selected reaction monitoring (SRM) mode, Q1 is set at the mass of the precursor $[M+H]^+$ (*m/z* 552) and Q3 at *m/z* 202, which is the most important fragment of bosentan. Because in SRM mode both quadrupoles are not scanning, better detection limits can be achieved compared to full-scan acquisition. Therefore, this mode has become the working horse for quantitative analysis. Typical dwell times are in the range 5–250 ms. Because with quadrupole mass analyzers transmission is dependent on the mass resolution, it is always mandatory, in SRM mode, to indicate the mass resolution of quadrupole Q1 and Q3. In general, full width of the peak at half maximum (FWHM) is indicated. Analysis in single ion monitoring mode can also be performed on a QqQ either using Q1 and Q3. Generally when performing a SIM analysis in Q3 mode, the collision cell is filled with collision gas and serves as a further declustering device to improve signal-to-noise.

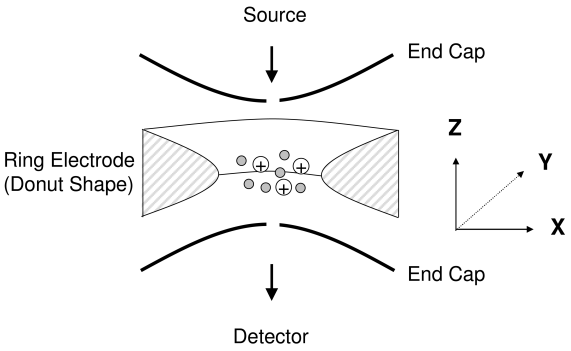


Fig. 1.20 The quadrupole ion trap. A fundamental RF potential is applied onto the ring electrode to trap ions. The gray circles represent helium gas.

1.4.3 Ion Trap Mass Spectrometry

The ion trap is a device that utilizes ion path stability of ions for separating them by their m/z [53]. The quadrupole ion trap and the related quadrupole mass filter were invented by Paul and Steinwedel [57]. A quadrupole ion trap (QIT or 3D-IT) mass spectrometer operates with a three-dimensional quadrupole field. The QIT is formed by three electrodes: a ring electrode with a donut shape placed symmetrically between two end cap electrodes (Fig. 1.20).

By applying a fundamental RF potential, the QIT can be described as a small ion storage device where ions are focused toward the center of the trap by collision with helium gas. In the QIT, because of the cylindrical symmetry of the trap, the x and y components of the field are combined to a single radial r component, where $r^2 = x^2 + y^2$. The motion of ions in the trap is characterized by one radial and one axial frequency (secular frequencies). Like quadrupoles, the motion of ions can be described by the solutions of Matthieu's equations (a, q). Ions with various m/z can be stored in the trap with the condition that trajectories are stable in r– and z– directions. Each ion of a certain m/z will be trapped at a certain q_z value. The higher m/z ions will be located at lower q values while the lower m/z will be located at the higher q_z values. The quadrupole ion trap can store only a limited number of ions before space charging occurs. To circumvent this effect, most instruments have an automatic gain control procedure (AGC). This procedure exactly determines the adequate fill time of the trap to maximize sensitivity and minimize resolution losses due to space charge. A mass spectrum can be obtained by mass-selective ejection where the amplitude of the RF potential is continuously increased at a certain rate. Ions with the lowest m/z are ejected first. The mass-selective axial instability mode requires that the ions are confined at the center of the trap and at a limited mass range. Resonant mass

ejection is another procedure which can generate a mass spectrum with a higher mass range. Ion motion can be modified either by exciting the radial or the axial frequencies by applying a small oscillating potential at the end cap electrodes during the RF ramp. In both mass-analyzing modes, the resolution of the spectrum is strongly dependent on the speed at which the RF amplitude is increased. Higher resolution can be obtained with slower scan speed. Compared to quadrupole instruments with the quadrupole ion trap, high sensitivity can be obtained in full-scan mode due to the ability of ion accumulation in the trap before mass analysis. Rapid mass analysis with the mass instability scan allows scanning at a speed of several thousand m/z units per second. There are several important components which affect the time necessary to obtain a mass spectrum (duty cycle): (i) the injection time (within 0.5–500.0 ms), (ii) the scan speed (in the range 5000–20 000 m/z units s^{-1}), (iii) isolation of the precursor ion and fragmentation in tandem MS or MS^n. Contrarily to the triple quadrupole, MS/MS is not performed in *space* but in *time*. Another significant difference is the use of helium as collision gas. Because the trap is permanently filled with gas, the instrument can switch very rapidly from single MS to MS/MS mode. High sensitivity can be achieved in the QIT because of ion selective accumulation of the precursor. Another advantage compared to the triple quadrupole is the short duty cycle for an MS/MS experiment. A typical MS^n (MS^3) sequence is illustrated in Fig. 1.21. To obtain a MS^2 spectrum the precursor ion is isolated and then excited while fragments are trapped. The next step to obtain an MS^3 spectrum is to isolate a fragment ion again and to perform CID fragmentation. Because MS/MS is performed in time in the same physical device, the operation can be repeated several times. Most commercial instruments can perform MS^n to the tenth or 11th level. A difficulty is to excite the precursor ions efficiently and trap the product ions in the same device. Generally, solely the precursor is excited in a specific window corresponding to 1–4 m/z units. The consequence is that fragment ions are not further excited and cannot produce second generation fragments. In many cases,

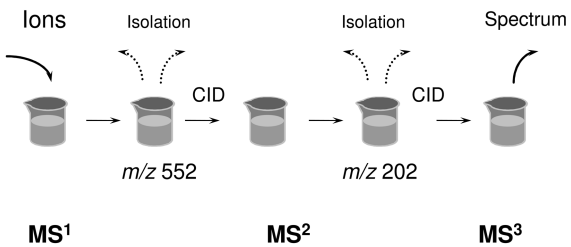


Fig. 1.21 Typical MS^3 scheme m/z 552 → m/z 202 →. In a first step the protonated bosentan molecule at m/z 552 is isolated and fragmented (MS^2). The fragments are trapped. In a second step the fragment at m/z 202 is isolated and fragmented and the spectrum is recorded.

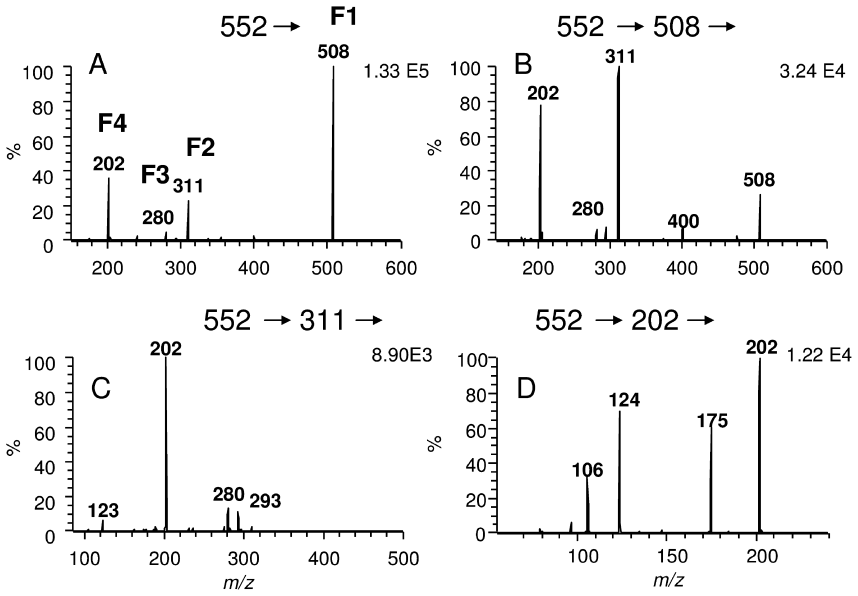


Fig. 1.22 Various MS^2 and MS^3 spectra of bosentan: (**A**) MS^2, (**B**) MS^3, (**C**) MS^3, (**D**) MS^3. *F1* to *F4* correspond to the main fragments of bosentan obtained also on the QqQ.

MS^2 trap CID generates similar spectra than quadrupole CID, but there are cases where the spectra differ significantly.

For molecules which can easily lose water or ammonia, the most abundant fragment observed in MS^2 is M-18 or M-17, which is not very informative. To overcome this limitation, wide band excitation (range 20 m/z units) can be applied. Another difference compared to QqQ is that QIT have a low mass cutoff of about one-third of the mass of the precursor ion. However QIT is particularly attractive to follow fragmentation cascades as illustrated for bosentan in Fig. 1.22. It can clearly be concluded that the fragment at m/z 175 originated from the precursor at 202 and not from the precursor at m/z 311.

Due to the high sensitivity in MS^n mode, ion traps are particular attractive for qualitative analysis in drug metabolism and proteomics studies. Compared to QqQ, similar sensitivities can be achieved for quantitative analysis but at the cost of precision and accuracy. A major difference is the number of transitions which can be monitored at the same time. While more than 100 SRM transitions can be recorded within one second on a QqQ, this number is much lower with the QIT (generally four to eight transitions). Ion traps have larger mass ranges (up to 50 000) than quadrupole instruments but smaller ranges than time of flight mass analyzers. Most commercial instruments use two mass ranges: (i) from m/z 50 to m/z 2000–3000 with a mass resolution of 0.7 m/z units or better and (ii) from m/z 200 to m/z 4000–6000 with a mass resolution of 2–4 m/z units.

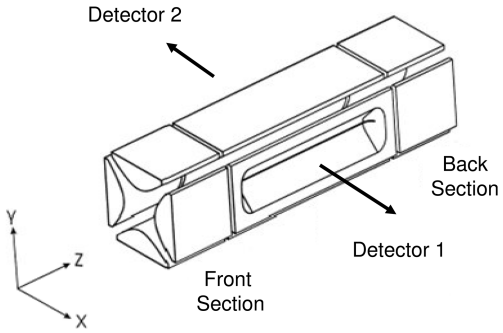


Fig. 1.23 Standalone linear ion trap. Because the ions are ejected radially two detectors are required for best sensitivity. Adapted with permission from reference [59].

Very recently linear ion traps (LIT) or two-dimensional ion traps (2D IT) have gained interest for various applications, either as standalone mass analyzers or coupled with Fourier transform ion cyclotron, three-dimensional ion trap (3D IT), TOF or orbitrap mass analyzers [58]. Physically, a linear ion trap is like a quadrupole formed by four hyperbolic or circular rods placed symmetrically. In a linear ion trap the ions are confined radially by a two-dimensional radio frequency field. To prevent ions from escaping axially, a DC potential is applied to the end electrodes. The same type of experiments which can be performed on 2D or 3D ion traps are basically the same but there are several advantages to trap ions in a 2D trap compared to 3D traps: (i) no quadrupole field along the *z*-axis, (ii) enhanced trapping efficiencies, (iii) more ions can be stored before observing space charging effects and (iv) strong focusing along the center line instead of focusing ions to a point.

Schwartz et al. [59] described a standalone linear ion trap where mass analysis is performed by ejecting the ions radially through slits of the rods using the mass instability mode. To maximize sensitivity the detection is performed by two detectors placed axially on either side of the rods (see Fig. 1.23).

1.4.4
Triple Quadrupole Linear Ion Trap

In a linear ion trap one of the most efficient ways to perform mass analysis is to eject ions radially. Hager [60] demonstrated that, by using fringe field effects, ions can also be mass-selectively ejected in the axial direction. There are several benefits for axial ejection: (i) it does not require open slits in the quadrupole, (ii) the device can be operated either as a regular quadrupole or a LIT using one detector. A commercial hybrid mass spectrometer was developed based on a triple quadrupole platform where Q3 can be operated either in normal RF/DC mode or in the LIT ion trap mode (Fig. 1.24).

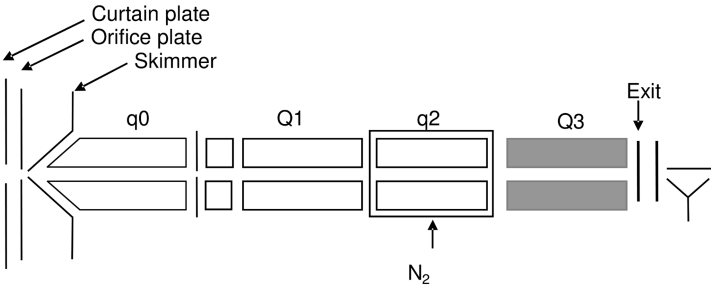


Fig. 1.24 Schematic of the triple quadrupole linear ion trap (AB/MDS Sciex). Q3 can be operated in quadrupole or trap mode. In both modes ions are detected in the axial direction.

In the triple quadrupole linear ion trap, tandem MS^2 is performed in space where the LIT serves only as a trapping and mass-analyzing device. Figure 1.25 illustrates the difference between quadrupole CID spectra and trap CID spectra for trocade.

With quadrupole CID all fragments are recorded in one experiment, while in the case of the 3D ion trap MS^2, MS^3 and MS^4 experiments are required to ob-

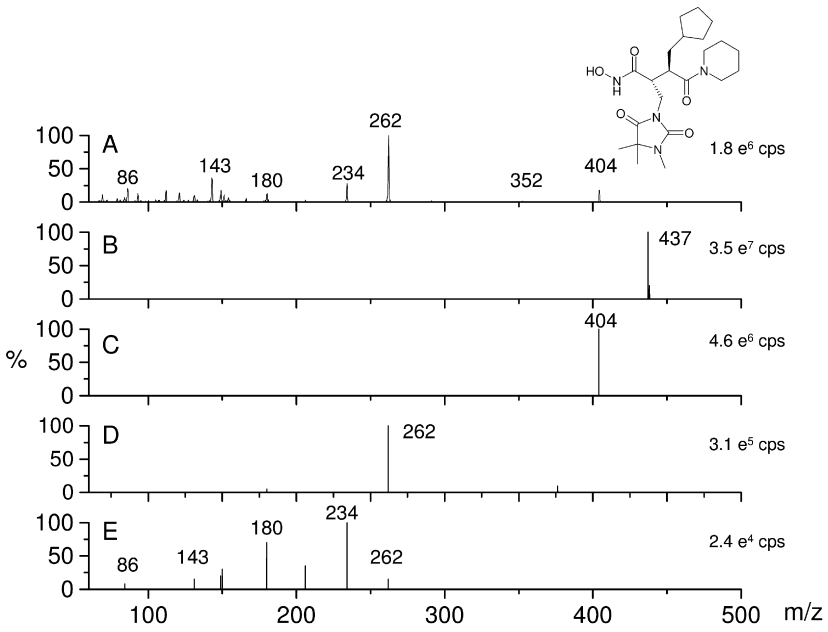


Fig. 1.25 Quadrupole CID spectra and ion trap CID spectra for trocade (M_r 403): (**A**) MS/MS on QqQ_{LIT}, (**B**) MS, (**C**) MS^2, (**D**) MS^3, (**E**) MS^4. Spectra **B**–**E** were recorded on a 3D ion trap).

tain the low mass fragments. In the triple quadrupole linear ion trap MS^3 is performed in the following manner. The first stage of fragmentation is accomplished by accelerating the precursor ions chosen by Q1 into the pressurized collision cell, q2. The fragments and residual precursor ions are transmitted into the Q3 linear ion trap mass spectrometer and are cooled for approximately 10 ms. The next generation precursor ion is isolated within the linear ion trap by application of resolving DC near the apex of the stability diagram. The ions are then excited by a single frequency of 85 kHz auxiliary signal and fragmented. The particularity of the QqQ_{LIT} is that the instrument can be operated in various ways, as described in Table 1.4 [61, 62]. MS^2 spectra are obtained in the quadrupole CID mode while MS^3 spectra are obtained in the trap CID mode.

The major advantage of this instrument is that qualitative and quantitative analysis can be performed in the same LC-MS run. As an example in a data-dependent experiment, the selected reaction monitoring mode can be used as a survey scan and the enhanced product ion mode (EPI) as a dependent scan. The consequence is that for each quantified analyte a confirmatory MS/MS spectrum can be obtained.

Table 1.4 Mode of operation of the triple quadrupole linear ion trap (QqQ_{LIT}).

Mode of operation	Q1	q2	Q3
Q1 scan	Resolving (scan)	RF only	RF only
Q3 scan	RF only	RF only	Resolving (scan)
Product ion scan (PIS)	Resolving (fixed)	Fragment	Resolving (scan)
Precursor ion scan (PC)	Resolving (scan)	Fragment	Resolving (fixed)
Neutral loss scan (NL)	Resolving (scan)	Fragment	Resolving (scan offset)
Selected reaction monitoring mode (SRM)	Resolving (fixed)	Fragment	Resolving (fixed)
Enhanced Q3 single MS (EMS)	RF only	No fragment	Trap/scan
Enhanced product ion (EPI)	Resolving (fixed)	Fragment	Trap/scan
MS^3	Resolving (fixed)	Fragment	Isolation/fragment trap/scan
Time delayed fragmentation (TDF)	Resolving (fixed)	Trap/no fragment	Fragment/trap/scan
Enhanced resolution Q3 single MS (ER)	RF only	No fragment	Trap/scan
Enhanced multiply charged (EMC)	RF only	No fragment	Trap/scan

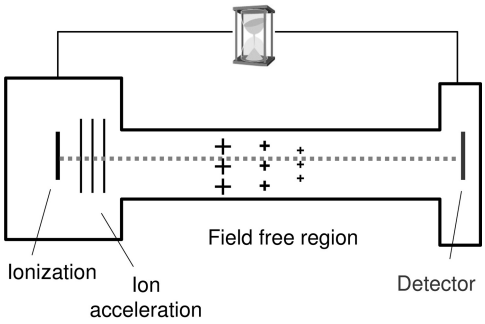


Fig. 1.26 Schematic of the simplest form of a time of flight mass spectrometer. After ionization the ions are accelerated with a strong electric field.

1.4.5
Time of Flight Mass Spectrometry

From the physical principle time of flight (TOF) may be the simplest way to perform mass spectrometric analysis (Fig. 1.26). TOF is the measure of the time that ions need to cross in a field free tube of about 1 m length [63, 64]. It is a pulsed technique and requires a starting point. The motion of an ion is characterized by its kinetic energy $E_c = 0.5m \times v^2$ (m = mass, v = speed). Therefore, the speed of ions or the time to fly through the tube is proportional to their $\sqrt{m/z}$ value. The velocity of the ions formed is generally low and they are accelerated by strong electric fields (2–30 kV) in the direction of the detector. Low mass ions reach the detector more rapidly than high mass ions. Due to the short flight time (50–100 μsec) and the good transmission, a spectrum can be generated within 100 ms over an almost unlimited mass range. Detection of the ions is performed with a multichannel plate detector (MCP, see Section 1.5) which has a relatively small dynamic range (generally two to three orders of magnitude).

With soft ionization techniques such as MALDI, ions of m/z 200 000 can be routinely detected. The mass range is mainly limited by the fact that with the detector the response decreases with increasing m/z of the ions. The mass resolution of a TOF mass analyzer is relatively poor (unit mass resolution and less) and is affected by factors that create a distribution in the flight time of ions with the same m/z. The simplest way to increase the mass resolution is to increase the length of flight tube or to reduce the kinetic energy spread of the ions leaving the source.

One way to reduce the kinetic energy spread is to introduce a time delay between ion formation and acceleration, referred to as delayed pulsed extraction. After a certain time delay ranging from nanoseconds to microseconds a voltage pulse is applied to accelerate the ions out of the source.

The second way to improve the mass resolution significantly is to use an electrostatic mirror (mass reflectron) placed in the drift region of ions (Fig. 1.27).

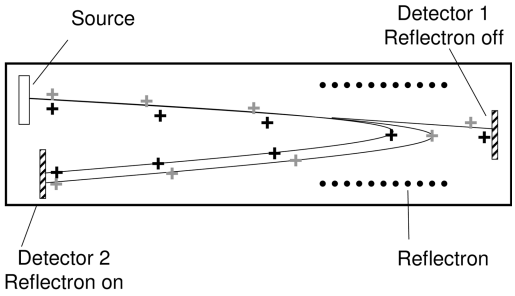


Fig. 1.27 Schematic of a time of flight mass spectrometer equipped with a reflectron. The instrument can be operated in the linear mode (reflectron off) or in the reflectron mode (reflectron on).

Briefly, the ions with high energy penetrate deeper into the ion mirror region than those with the same m/z at a lower energy. Because of the different trajectories, all ions of the same m/z reach the detector at the same time. Thus, all ions of the same m/z have then a much lower energy dispersion. With the reflectron the flight path is increased without changing the physical size of the instrument. In reflectron mode a mass resolving power of 15 000 is standard but the mass range is limited to several thousand m/z units. TOF instruments are non-scanning mass spectrometers resulting in an increased sensitivity compared to quadrupole mass spectrometers.

In general the commercial TOF instruments have two detectors; one for the linear mode and one for the reflectron mode. The combination of MALDI with TOF is ideal because both techniques are pulsed techniques. However, it is also possible to arrange a continuous beam as generated by electrospray ionization. For that purpose orthogonal acceleration was developed [65]. The ion beam is introduced perpendicularly to the TOF and packets are accelerated orthogonally (oa-TOF) at similar frequencies improving the sensitivity. While a packet of ions is analyzed, a new beam is formed in the orthogonal acceleration.

Time of flight instruments are mainly used for qualitative analysis with MALDI or atmospheric pressure ionization. With MALDI ionization one of the main applications is the identification of proteins by analyzing their peptides after trypsin digestion (peptide mass finger print; PMF). Further structural information of the peptides can be obtained from metastable transitions or collision-induced dissociations generated in the drift tube prior to entering the reflectron. This technique is called post-source decay (PSD). A metastable ion is an ion which dissociates in the free field region of the mass spectrometer. For TOF instruments the acquisition rate is in the range 10–20 Hz, making these mass analyzers best suited for the interfacing of fast liquid chromatographic separations or capillary electrophoresis using electrospray ionization.

Due to their fast acquisition rate and high resolution capabilities TOF mass analyzers are often used as the last mass analyzing stage in hybrid tandem mass

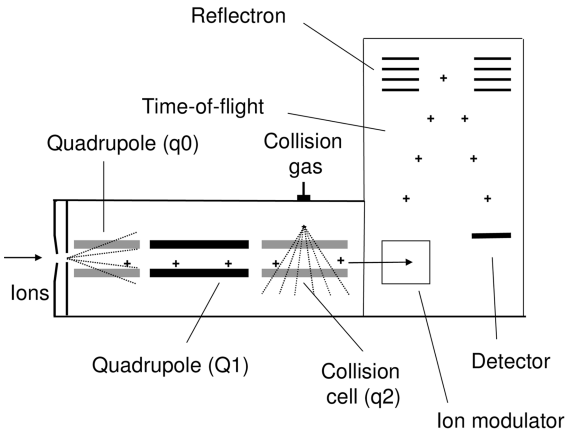


Fig. 1.28 Schematic of a quadrupole–time of flight instrument. Quadrupole q0 is used for collisional cooling and ion focusing. Nitrogen or argon is generally used as collision gas. The ion modulator pushes the ions orthogonally to their initial direction into the TOF analyzer.

spectrometers such as quadrupole–time of flight instruments. A quadrupole–time of flight instrument (QqTOF) is the result of the replacement of the last quadrupole section (Q3) of a triple quadrupole instrument by a time of flight analyzer (Fig. 1.28), a powerful combination in regards of mass range (m/z 5 to m/z 40 000), mass resolving power of 10 000 and sensitivity [66, 67]. In single MS mode the quadrupoles (q0, Q1, q2) serve as RF ion guides and the mass analysis is performed in the TOF. To accommodate ion injection a pulsed field is applied in the ion modulator to push the ions orthogonally to their initial direction into the TOF analyzer.

In tandem MS mode, because the product ions are recorded with the same TOF mass analyzers as in full scan mode, the same high resolution and mass accuracy is obtained. Isolation of the precursor ion can be performed either at unit mass resolution or at 2–3 m/z units for multiply charged ions. Accurate mass measurements of the elemental composition of product ions greatly facilitate spectra interpretation and the main applications are peptide analysis and metabolite identification using electrospray ionization [68]. In TOF mass analyzers accurate mass determination can be affected by various parameters such as: (i) ion intensities, (ii) room temperature or (iii) detector dead time. Interestingly, the mass spectrum can be recalibrated post-acquisition using the mass of a known ion (lock mass). The lock mass can be a cluster ion in full scan mode or the residual precursor ion in the product ion mode. For LC-MS analysis a dual spray (LockSpray) source has been described, which allows the continuous introduction of a reference analyte into the mass spectrometer for improved accurate mass measurements [69]. The versatile precursor ion scan, another specific feature of the triple quadrupole, is maintained in the QqTOF instrument. However, in pre-

cursor scan mode the sensitivity is lower in QqTOF than in QqQ instruments. The lack of good quality product ion spectra on conventional MALDI-TOF instruments made the use of MALDI on QqTOF instruments an interesting alternative for the sequencing of peptides. As in electrospray TOF, in the case of QqTOF the MALDI ion production needs to be decoupled from mass measurements. The technique to interface MALDI with QqTOF is named orthogonal MALDI (o-MALDI) TOF with collisional cooling. With o-MALDI the pulse is almost converted in a continuous beam equivalent to that originated from an electrospray source.

The TOF mass analyzer has a low duty cycle, and the combination with an ion accumulation device such as an ion trap is therefore very advantageous. It offers also MS^n capabilities with accurate mass measurement. In all acquisition modes, the ions are accelerated into the time of flight for mass analysis. Various other hybrid mass spectrometers with TOF have been described, including quadrupole ion trap [70] and linear ion trap [58]. High energy tandem mass spectrometry can be performed on TOF-TOF mass spectrometers [71, 72].

1.4.6 Fourier Transform Mass Spectrometry

1.4.6.1 Fourier Transform–Ion Cyclotron Resonance Mass Spectrometry

The main components of a Fourier transform ion cyclotron resonance mass spectrometer are a superconducting magnet and a cubic or cylindrical cell (Fig. 1.29). Typically, the magnet field strengths (B) are in the range 3.0–9.4 Tesla. Ions are stored in the cell according their cyclotronic motion arising from the interaction of an ion with the unidirectional constant homogenous magnetic field. A static magnetic field applied on the z direction confines ions in the $x-$ and $-y$ directions according the cyclotronic motion. To avoid the escape of ions along the z axis, a low electrostatic potential is applied to the end cap electrodes [73].

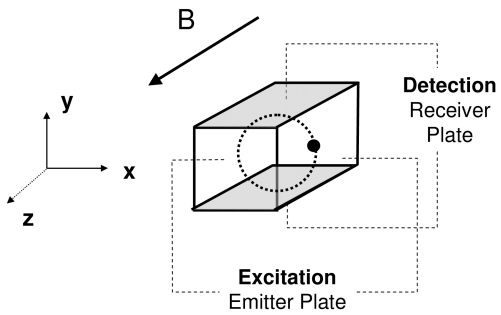


Fig. 1.29 Diagram of an ion cyclotron resonance instrument. The magnetic field is oriented along the z-axis and ions (•) are trapped according the same axis. Due to the cyclotronic motion the ions rotate around the z-axis in the x–y plane.

Cyclotron motion is characterized by its cyclotron frequency (f; from 5 kHz to 5 MHz) which depends on: (i) the magnetic field (B), (ii) the charge on the ion (z) and (iii) the mass of the ion (m). In contrast to other types of mass spectrometers, detection is performed in a non-destructive way. The ions are detected by excitation applying a coherent broadband excitation. The ions undergo cyclotron motion as a packet with a larger radius. When the ion packet approaches the detection plates it generates an alternating current named image current. The resulting signal is generally called the transient free induction decay (FID). Ions of any mass can be detected simultaneously with Fourier transform mass spectrometry (FTMS). The image current is composed of different frequencies and amplitudes which are converted by applying a Fourier transformation to frequency components and further to a mass spectrum. Mass resolution is best with high field strength, decreases when the mass increases and is dependent on acquisition time. The mass resolution is strongly dependent on the length of the transient time. Typical transient times are in the range 0.1–2.0 s. With commercial instruments a mass resolving power of 100 000 or more can be routinely achieved. Collision induced dissociation can also be performed in the FT-ICR cell. The transient signal decreases with collision of ions and neutral gas molecules. It is therefore essential to work at very high vacuum (1.3×10^{-8} Pa). The dynamic range of a FT-ICR mass spectrometer is relatively poor because the instrument suffers from the fact that the number of ions in the trap must be in a specified range. Over- and underfilling of the trap results in mass shifts towards high and low values, respectively. To have a better control of the ion population in the cell, a commercial hybrid instrument (LTQ-FTMS, Thermo) was developed by combining a linear ion trap (LIT) with a FT-ICR mass spectrometer [74]. Because the LIT is equipped with two detectors data can be recorded simultaneously in the ion trap and in the FT-ICR mass spectrometer. In this way the FT-ICR operates only as a high resolution detector for MS or MS^n experiments performed in the linear ion trap.

1.4.6.2 **Orbitrap Mass Spectrometer**

Makarov [75] invented a novel type of mass spectrometer based on the orbital trapping of ions around a central electrode using electrostatic fields named orbitrap. Kingdon had already described the orbiting of ions around a central electrode using electrostatic fields in 1923, but the device had been only used for ion capturing and not as a mass analyzing device. The orbitrap (Fig. 1.30) is formed by a central spindle-like electrode surrounded by an electrode with a barrel-like shape to create an electrostatic potential. The m/z is a reciprocal proportionate to the frequency (ω) of the ions oscillating along the z-axis. There is no collisional cooling inside the orbitrap, which operates at very high vacuum (2×10^{-8} Pa). Detection is performed by measuring the current image of the axial motion of the ions around the inner electrode. The mass spectrum is obtained after Fourier transformation of the image current. The mass resolving power depends on the time constant of the decay transient. The orbitrap provides a mass resolving power exceeding 100 000 (FWHM) and a mass accuracy ≤ 3 ppm. To be opera-

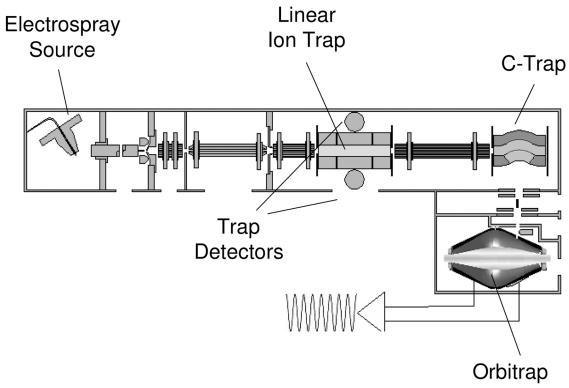


Fig. 1.30 Schematic of the linear ion trap (LIT)–orbitrap (LTQ orbitrap, Thermo). One of the specificities of the system is that the LIT has two detectors. Therefore the LIT can perform various experiments at the same time. Adapted with permission from reference [76].

tional as a mass spectrometer the orbitrap requires external ion accumulation, cooling and fragmentation. The setup of the LIT–orbitrap from Thermo is depicted in Fig. 1.30. The instrument consists of a linear ion trap with two detectors connected to the orbitrap via a C-trap. With the LIT various MS or MS^n experiments can be performed. When the orbitrap is used as a detector the ions are transferred into the C-trap where they are collisionally damped by nitrogen at low pressure. The C-trap acts as a trapping and focusing device. Injection from the C-Trap into the orbitrap is then performed with short pulses of high voltages.

The particularity of the LIT–orbitrap instrument is the independent operation of the orbitrap and the LIT. Because high resolution requires longer transient time, further data can already be collected in the LIT at the same time. As an example accurate mass measurements of the precursor ion can be performed in the orbitrap while MS^2 and MS^3 spectra are recorded with the linear ion trap. The LIT–orbitrap has less resolution than a FT-ICR instrument with similar duty cycle, but its maintenance costs are far lower than for the FT-ICR. Both instruments will have a major impact in mainly qualitative analysis of low molecular weight compounds and macromolecules.

1.5 Ion Detectors

To obtain a mass spectrum, ions need to be converted into a usable signal by a detector. The simplest form of ion detection is a photographic plate or a Faraday cup for the direct measurement of the charge. In a Faraday cup the induced current is generated by an ion which hits the surface of a dynode and emits

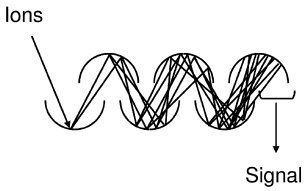


Fig. 1.31 Discrete-dynode electron multiplier. When the ions hit the surface of the detector electrons are emitted to form an avalanche of electrons which generates the signal.

electrons. This type of detector is generally insensitive and mounted in isotopic ratio mass spectrometers. The first electron multipliers mounted in mass spectrometers were discrete-dynode multipliers fabricated from beryllium copper alloy. When a positively or a negatively charged ion reaches the detector electrons are produced (Fig. 1.31).

In this type of detector the electrons are accelerated down the channel producing additional electrons to the output signal. The created cascade of electrons results in a measurable current at the end of the detector [77].

Channel electron multipliers (CEM) are fabricated from lead-silica glass (Fig. 1.32) and can have curved or straight forms. In a channel electron multiplier, when the charged particles (positive or negative) hit the surface of the electrode, electrons are produced from the surface which then generate the current.

Channel electron multipliers can be operated either in analog or pulse counting mode. The difference between the two modes of operation is that pulse counting produces output pulses with a certain amplitude while analog detectors produce a wide distribution of output pulses. Therefore, the pulse counting mode is more suitable for high sensitivity mode while analog mode is best suited for intense signals. In modern mass spectrometers, autotune procedures optimize the analog multipliers based on signal-to-noise. The tuning of pulse counting detectors is somewhat different because they operate in a different mode. The sensitivity of a detector decreases almost exponentially with the mass of the ions. One way to improve the signal in the channel electron multiplier detector sensitivity at higher mass is to use a conversion dynode (Fig. 1.33). A conversion dynode is a metal surface which is held at high potential (>3 kV). The role of the dynode

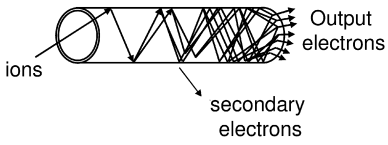


Fig. 1.32 Straight channel electron multipliers (CEM) are typically used in quadrupole-type mass spectrometers.

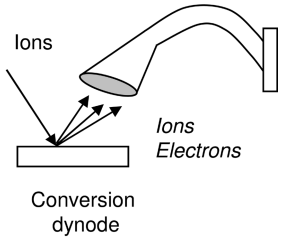


Fig. 1.33 Curved channel electron multiplier with conversion dynode. The conversion dynode acts as a post acceleration device of the ions before they hit the surface of the channel electron multiplier.

potential is to accelerate ions to a point where good conversion in secondary ions or electrons occurs.

The lifetime of channel electron multipliers is ca. 1–2 years. Neutrals or photons hitting the detector also increase the noise of the detection.

A further widely used multiplier is the photon multiplier. In this case the ions (positive or negative) elicit secondary ions formed by a conversion dynode, which are further accelerated towards a phosphorescent screen where they undergo conversion into photons detected by a photomultiplier (Fig. 1.34).

The advantage of the photomultiplier compared to the electron multiplier is the longer lifetime (several years). Channel electron multiplier and photomultiplier are mostly used in quadrupole instruments or ion traps.

Array detectors, such as the multichannel plate (MCP) detector are best suited for mass analyzers where ions are spatially dispersed like in time of flight instruments. Array detectors are detectors [78] which allow simultaneous multichannel detection. The advantages of such detectors are high sensitivity and the possibility to eliminate the accompanying noise. Array detectors are largely used with TOF mass analyzers. Generally, the array consists generally of 10^6 microscopic glass channels, ca. 5–50 μM in diameter, bound together and electrically connected with each other. Each channel operates as a continuous dynode electron multiplier (Fig. 1.35).

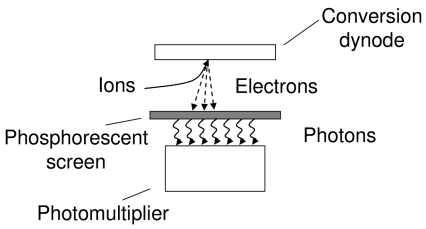


Fig. 1.34 In the photon multiplier detector ions are transformed into photons which are detected by a photomultiplier.

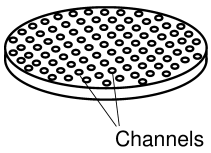


Fig. 1.35 Multi-channel plate multiplier. Each hole corresponds to a single channel detector.

1.6
Practical Aspects and Applications in Bioanalysis

1.6.1
Introduction

Mass spectrometry plays currently a major role in the qualitative and quantitative analysis of low molecular weight compounds and macromolecules in life sciences. Quantitation of pharmaceutical compounds, their metabolites and endogenous metabolites in biological matrices, such as plasma and urine, is nowadays mostly done with liquid chromatography coupled with atmospheric pressure tandem mass spectrometry (LC-MS/MS) [79]. Gas chromatography coupled with electron impact ionization mass spectrometry (GC-MS) remains an important analytical tool in forensic sciences, doping control and toxicology. For this purpose quadrupole or ion trap mass analyzers are typically used. In contrast, triple quadrupole instruments have become more the working horse for quantitative pharmaceutical bioanalysis. While quantitative analysis is already well established, many of the new developments in the field of mass spectrometry will contribute to improve metabolites identification, metabolomics and proteomics analysis. Automated computerized data handling (bioinformatics) has become mandatory to cope with the large amount of data generated by the various systems. Mass spectrometers are, from a software point of view, becoming more user friendly while the expanding analysis capabilities of hybrid systems may require more fundamental user training. Due to the enhanced scan possibilities of MS, data dependent acquisition (DDA) has become state of the art for qualitative analysis. A DDA experiment includes a survey scan, a dependent scan and a selection criterion. Typically a survey scan is a full-scan MS and the dependent scan is a MS/MS scan. The selection criterion requires to record a MS/MS spectrum of the most abundant ion in the survey scan which is above a certain threshold and taking into account the inclusion of ions of interest and exclusion of background ions.

One critical feature of mass spectrometry when combined with chromatographic or electrophoretic separation techniques remains the duty cycle of the mass analyzer. A conventional LC chromatographic peak lasts about 10 s, which is sufficient to perform various MS and MS/MS experiments on various types of instruments. In the case of fast LC, the peak width can be in the range 1–2 s which is too fast for most mass analyzers except for TOF mass spectrometers.

1.6.2
Quantitative Analysis in Biological Matrices

Due to its high selectivity and sensitivity LC-MS with quadrupole mass analyzers has almost completely replaced traditional UV detection in many bioanalytical laboratories. ESI, APCI and APPI have become the ionization techniques of choice, covering a large variety of analytes. One limitation with API techniques is that the ionization response factor is compound-dependent and thus requires the use of an internal standard. Isotopically labeled (^{2}H or ^{13}C) internal standards have become very popular because they are capable of compensating for losses during sample preparation, HPLC and ion evaporation due to co-elution with the analyte. In the early days of LC-MS, analysis was mostly performed on QqQ instruments. Quantitative LC-MS analysis can also be performed on single quadrupole instruments, in particular when the M_r of the analyte is higher than 400 and when the limit of quantification is not below the ng ml^{-1} level. Figure 1.36 shows the total ion current (TIC) chromatogram of the LC-MS analysis of a cyclohexanediol derivative analyzed in human plasma after liquid–liquid extraction. It demonstrates clearly the selectivity of triple quadrupole compared to single quadrupole MS. Because this analyte does not have an appropriate chromophore, UV detection would not have been suitable. In contrast to GC, LC is not a high resolution separation technique and co-elution with endogenous compounds may require longer analysis time or improved sample preparation.

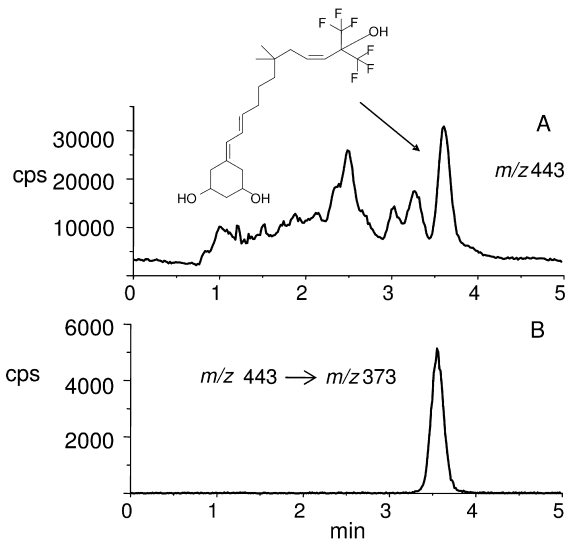


Fig. 1.36 Comparison of the LC-MS and LC-MS/MS analysis of a cyclohexanediol derivative in human plasma. (**A**) Selected ion monitoring mode *m/z* 443. (**B**) Selected reaction monitoring *m/z* 443 → *m/z* 373. Ions were detected in the negative mode.

An important issue with quantitative LC-MS analysis concerns the matrix effects which need to be addressed during method development and validation. Matrix effects are caused by the co-elution of endogenous analytes which either enhance or suppress the analyte signal [80]. The major concern is that matrix effects are sample-dependent and may vary from one sample to another. It is also believed that ESI is more prone to matrix effects than APCI. Various approaches were devised and applied to investigate matrix effects. However, adequate sample preparation and selection of an appropriate internal standard generally provide the key to success. For multicomponent assays it is also important to use the internal standards most appropriate for the respective analyte. Offline and online solid phase extraction, column switching and automated liquid–liquid extractions are the most used sample preparation techniques. Online SPE combined with column switching are particularly attractive because they allow direct analysis of plasma in an automated and high throughput setup. With the high sensitivity of modern triple quadrupole instruments, protein precipitation of plasma in 96-well plate format followed by dilution and direct injection of the eluent has also become a viable approach. Shortterm matrix effects due to different samples may be relatively simple to monitor while longterm matrix effects are very difficult to monitor. Table 1.5 shows the calibration and quality control (QC) results obtained in human plasma of a cyclohexanediol derivative analyzed by LC-MS/MS. At a first glance the calibration seems to be very good. However, when the 10 ng mL^{-1} calibration sample is reanalyzed ($n = 35$) and declared as a quality control sample the accuracy becomes disastrous.

The explanation of this result is illustrated in Fig. 1.37, which shows selected reaction-monitoring traces of the sample at 10 ng mL^{-1}. It becomes obvious that the response ratio between the analyte and the IS has dramatically changed. On one side there is enhancement of the analyte's response and on the other side suppression of the internal standard (IS) signal. These effects are mainly caused

Table 1.5 Calibration and QC data for a cyclohexanediol derivative in human plasma.

Sample	n	spiked ng/ml	found ng/ml	Accuracy %
00 Plasma	5	0	0	–
C01	6	1	0.994	99.4
C02	7	2	1.991	99.6
C03	8	4	4.124	103.1
C04	**9**	**10**	**10.19**	**101.9**
C05	10	20	19.93	99.7
C06	11	50	46.44	92.9
C07	12	100	102.1	102.1
C08	13	200	203	101.5
QC04	**35**	**10**	**5.925**	**59.3**

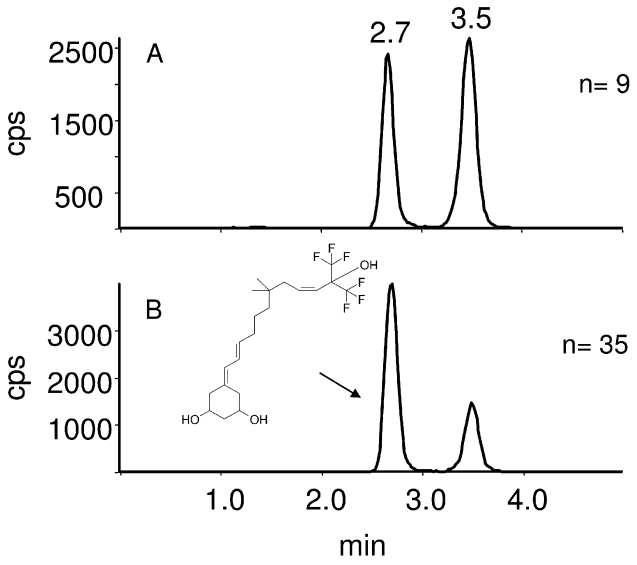


Fig. 1.37 Selected reaction monitoring mode LC-MS/MS analysis of the same human plasma sample standard at 10 ng mL^{-1} placed at different positions in the analytical sequence: (**A**) at position 9, (**B**) at position 35. The peak at RT = 2.7 min corresponds to the analyte and the peak at RT = 3.5 min to the internal standard. Detection was performed in the negative mode.

by the accumulation of endogenous compounds on the HPLC column after each run, and therefore an increasing bleed of these endogenous sample components to the effluent of the column directed to the API interface. In this case the gradient elution was obviously not effective enough to remove efficiently endogenous compounds after each analysis. The IS, a structural analogue, was not capable of compensating the matrix effect. The solution to the problem was to replace the IS by an isotopically labeled structural analogue which co-eluted with the analyte. This example exemplifies how critical appropriate method development and validation is before running real study samples.

LC-MS/MS has dramatically changed the way bionalysis is conducted. Accurate and precise quantitation in the pg ml^{-1} scale is nowadays possible; however one has to be aware of certain issues which are specific to mass spectrometric detection such as matrix effects and metabolite crosstalk. With the current growing interest in the analysis of endogenous biomarkers in biological matrices, quantitative bioanalysis with MS has certainly the potential to contribute further in this field with the development of multicomponent assays. Modern triple quadrupole instruments have the feature to use very short dwell times (5–10 ms), allowing the simultaneous determination of more than 100 analytes within the timescale of an HPLC peak. Due to the selectivity of the MS detection the various analytes

do not need to be chromatographically baseline resolved. This is only true for analytes with different precursor and product ions.

1.6.3 Drug Metabolism

During drug discovery and drug development, it is important to establish how the body metabolizes a drug; therefore rapid identification of metabolites from *in vitro* or *in vivo* samples becomes essential [81]. The classic way to perform metabolic studies is to use ^{14}C or ^{3}H radiolabeled drugs. Liquid chromatography with online radioactivity detection is applied to collect the metabolites, which after further purification are identified by mass spectrometry and nuclear magnetic resonance spectroscopy (Fig. 1.38). One of the advantages of the radiolabeled parent drug is that the response of the radioactivity detector is directly proportional to the amount of metabolite. Also due to the high specificity of the radioactivity detector urine or plasma can be directly injected onto the LC system.

Metabolic stability of drugs has become an important parameter in drug discovery and hundreds of samples can be rapidly generated using *in vitro* systems such as hepatocytes and microsomes. For structural elucidation, nuclear magnetic resonance spectroscopy is the technique of choice, but it does not allow high throughput analysis and sensitivity is still in the microgram range. LC-MS has therefore become the technique of choice. Ideally one would require a mass spectrometer with fast acquisition capabilities in positive and negative mode, selective scan modes, multiple stage MS and accurate mass measurements. Such an ideal instrument is currently not available and therefore drug metabolism studies require multi-instrument strategies.

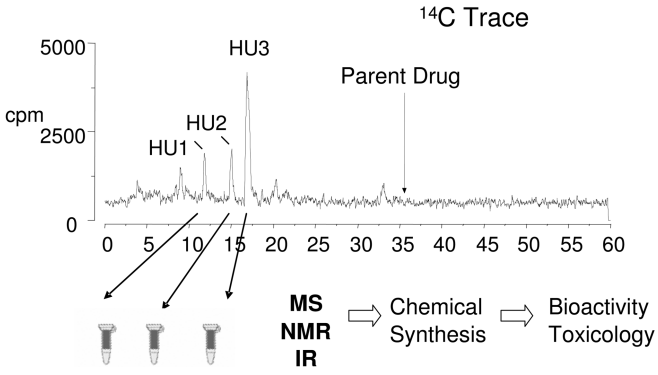


Fig. 1.38 LC separation with radioactivity detection of an urine sample. The response of the various peaks is directly proportional to the amount of metabolites present in the sample. Peaks *HU1–HU3*: human urine metabolites.

When working with non-radiolabeled drugs the major challenge is to find metabolites in the biological matrices. Because the enzymes responsible for metabolism are quite well characterized metabolic changes can partially be predicted. For example hydroxylation of the parent drug is in many cases the principal metabolic pathway. From a mass spectrometric point of view it results in an increase of 16 units in the mass spectrum. In the full-scan mode an extracted ion current profile can be used to screen for potential metabolites. In a second step a product ion spectrum is recorded for structural interpretation. Ideally, one would like to obtain relative molecular mass information and the corresponding product ion spectrum in the same LC-MS run. This information can be obtained by data dependant acquisition (DDA), as illustrated in Fig. 1.39.

In this case the survey scan was set as a full scan and the dependent scan as a product ion scan. The problem with data dependent acquisition is to determine the selection criteria. In most cases the system picks up the most abundant ion in the full scan spectrum. An inclusion list with masses of potential metabolites or exclusion list of known interferences significantly improves the procedure. In the example shown in Fig. 1.39, a procedure called dynamic background subtraction (DBS) was applied. This procedure considers chromatographic peak shapes and monitors not the most abundant signal in the spectrum but the largest increase of an ion in a spectrum. The advantage is that once a signal of a peak has

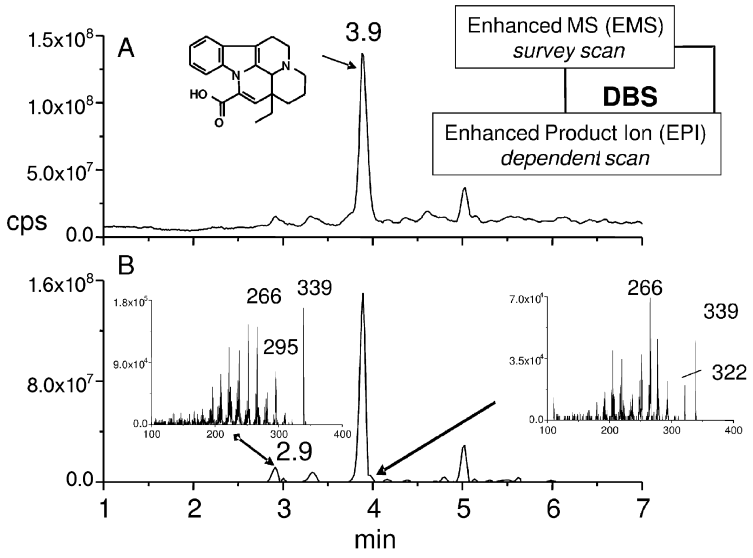


Fig. 1.39 LC-MS data dependent analysis of vinpocetin in rat urine using dynamic background substraction (DBS) on a triple quadrupole linear ion trap. (**A**) Full scan MS (survey scan) trace. (**B**) Enhanced product ion scan (dependent scan). The major peak at 3.9 min corresponds to apovinpocetin, the minor one at 2.9 min to the hydroxylation product of apovinpocetin (*m*/*z* 339).

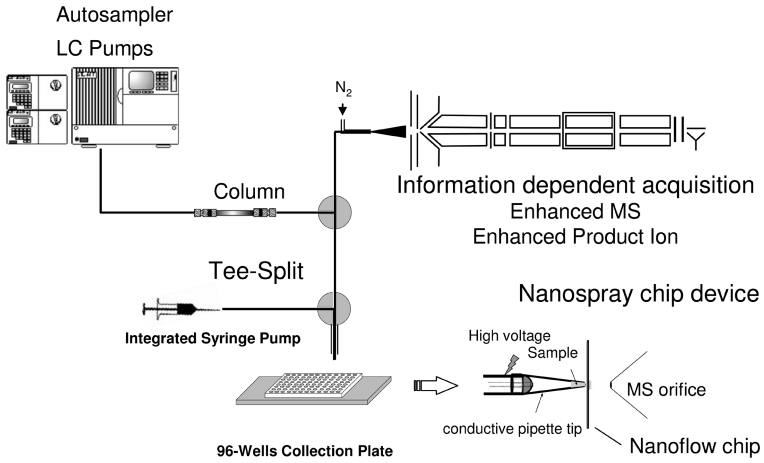


Fig. 1.40 Schematic of online LC-MS analysis combined with fraction collection into 96-well plate. Depending on the online MS data, further MS experiments are performed with chip-based infusion at 200 nL min^{-1}.

reached its maximum it switches automatically to the next mass. This is particularly important with co-eluting peaks of different intensities, as illustrated in Fig. 1.39B. It is then possible to obtain a good product ion spectrum of the small peak eluting at 4.0 min (m/z 339). In drug metabolism not only is the sensitivity of the mass spectrometer important but the selectivity is also crucial, particularly when working with plasma samples.

Most methods of metabolite identification are done with online LC-MS. As mentioned earlier there is no ideal mass spectrometer for this type of work and the sample has to be reanalyzed several times on different types of mass spectrometer. The consequence is that metabolic investigation is often time-consuming. A concept has been described by Staack et al. [82] (Fig. 1.40) where, during the LC-MS run, fractions are collected onto a 96-well plate.

Either the information obtained during the data-dependent acquisition is sufficient or a fraction of interest can be re-analyzed by chip-based infusion at a flow rate ca. 200 nl min^{-1}. Due to the miniaturization sample consumption is very low (typically 1–3 µl) and acquisition time is no longer critical. Therefore various MS experiments can be performed on various instruments, including MS^n and accurate mass measurements. An additional advantage is that the eluent can be removed and the infusion solvent can be optimized for positive or negative ion detection or for deuterium exchange measurements.

Advances in high resolution mass analyzers (TOF, FT-ICR, orbitrap) have greatly improved the detection and identification of metabolites based on accurate mass measurements. In single MS mode accurate mass determination is mainly used to differentiate between isobaric ions. Combined with LC-MS, it allows the detection of predicted metabolites by performing extracted ion current profiles

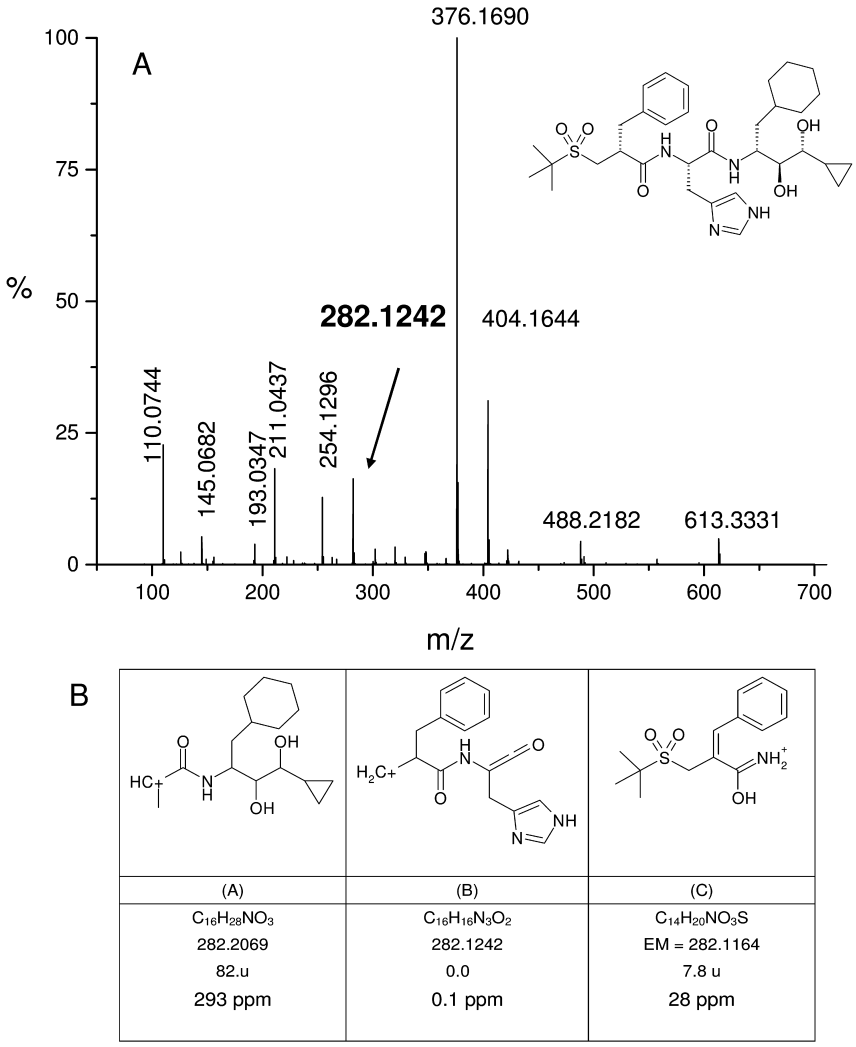


Fig. 1.41 (**A**) Product ion spectrum of remikiren obtained on a QqTOF. (**B**) Software-predicted fragments (Mass Frontier, HighChem) for the ion at *m*/*z* 282.

with much smaller mass windows than for unit mass resolution mass analyzers eliminating therefore background interferences. In MS/MS mode on hybrid systems (LIT-orbitrap, QqTOF, IT-TOF, FT-ICR) high resolution improves the interpretation of product ion spectra. As an example, in the product ion spectrum recorded at unit mass resolution spectra of bosentan and its phenol metabolites display an ion at *m*/*z* 280. When performing the accurate mass measurements of this ion on a QqTOF it was found that bosentan generates an ion at *m*/*z*

280.0835 and its phenol metabolite at m/z 280.0628 [68]. It was shown that both ions were formed through a different cyclisation mechanism involving either the phenol or the amine substituant. The mass difference of 20.7 milliunits corresponds to the mass difference betwenn NH_2 and O.

The understanding of the fragmentation mechanism of the parent drug is very important for the metabolite assignment. The product ion spectrum of remikiren is illustrated in Fig. 1.41. Conventional spectra interpretation is time-consuming and the use of predictive fragmentation software such as Mass Frontier (HighChem) can help to rationalize spectra interpretation [83]. In the case of the fragment at m/z 282, three different fragments are proposed by the software. Only accurate mass measurement with an accuracy better than 10 ppm allowed selection of the right fragment (Fig. 1.41B, middle structure).

A similar approach using accurate mass measurements and predictive fragmentation software was also applied for the examination of the human microsomal metabolism of nefazodone using a linear ion trap–orbitrap hybrid mass spectrometer. Based on a single LC-MS run, using data-dependant acquisition, 15 metabolites of nefazodone could be identified in MS and MS/MS with a mass accuracy better than 3 ppm.

Zhang et al. [84] reported a strategy using a software mass defect filter to improve the detection of expected and unexpected metabolites in accurate mass LC-MS. Metabolic structural changes in the parent drug have an effect on the mass defect of the metabolites compared to the parent drug. As an example hydroxlation changes the mass defect by –5 milliunits, demethylation by −23 milliunits and glucuronation by +32 milliunits. In fact most phase I and phase II metabolites have a mass defect window within 50 milliunits. It is therefore possible to apply a software filter which includes ions within a mass defect window relatively close to the parent drug and exclude ions, generally matrix interferences, which are outside the specified window. The application of the mass defect filter to a plasma sample spiked with omeprazole metabolites is illustrated in Fig. 1.42 [85].

For spectra interpretation and metabolite characterization accurate mass measurements become a must while it remains complementary to MS^n, precursor and neutral loss for identifying metabolites in complex biological matrices.

1.6.4
Analysis of Proteins

The analysis for proteins present in plasma or a cell extract is a challenging task due to their complexity and the great difference between protein concentrations present in the sample. Simple mixtures of intact proteins can be analyzed by infusion with electrospray ionization and more complex ones by matrix assisted laser desorption ionization. MALDI is more suited for complex mixtures because for each protein an $[M+H]^+$ signal is observed while for ESI multiply charged ions are observed. Surface enhanced laser desorption (SELDI) is a technique for the screening of protein biomarkers based on the mass spectrometric analysis of intact proteins [49]. However in most cases for sensitivity reasons mass spec-

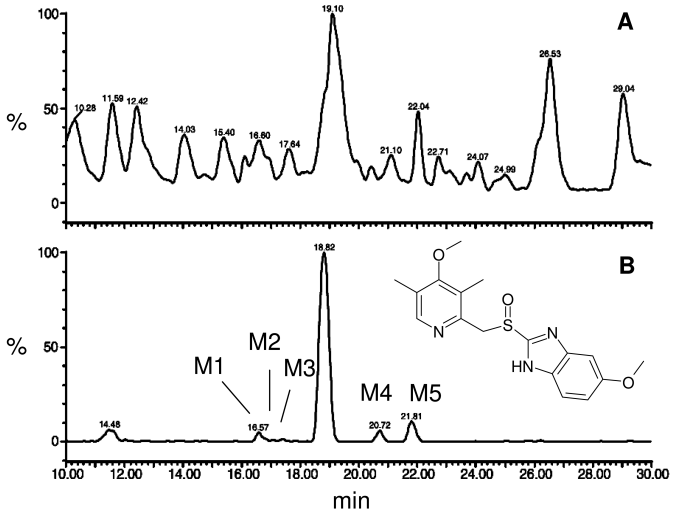


Fig. 1.42 LC-MS profile of omeprazole metabolites spiked in plasma: (**A**) without mass defect filter, (**B**) with mass defect filter. Peaks: *M1* mono-oxidation metabolite [+16 u, Mass defect (MD) +5 milliunits], *M2* reduction and demethylation (−30 u, MD +10 milliunits), *M3* mono-oxidation metabolite (+16 u, MD −5 milliunits), *M4* reduction (−16 u, +5 milliunits), *M5* mono-oxidation metabolite (+16 u, +5 milliunits). Adapted with permission from reference [85].

trometric analysis is performed at the peptide level after enzymatic digestion. Basically there are two approaches for the identification of complex mixtures of proteins (Fig. 1.43). The first is based on two-dimensional electrophoretic separation of intact proteins followed by trypsin digestion and matrix assisted laser desorption–time of flight (MALDI-TOF) detection. The second approach digests first the protein mixture and the resulting peptides are then separated by a two-dimensional chromatographic procedure using nanoliquid chromatography coupled to nanoelectrospray ionization.

Two-dimensional electrophoresis [86] is a well established technique for the separation of intact proteins. In the first dimension the proteins are separated based on their isolectric point while the second dimension separates them based on their size. The presence on the gel of the proteins is revealed by Coomassie blue or silver staining. Under favorable conditions several thousand spots can be differentiated. The gel is digitized and computer-assisted analysis of the protein spot is performed. The spots of interest are excised either manually or automatically and then digested with trypsin. Trypsin cleaves proteins at the C-terminal side of lysine and arginine. In general one spot represents one protein and the peptides are analyzed by MALDI-TOF to obtain a peptide mass fingerprint. A peptide mass fingerprint involves the determination of the masses of all pep-

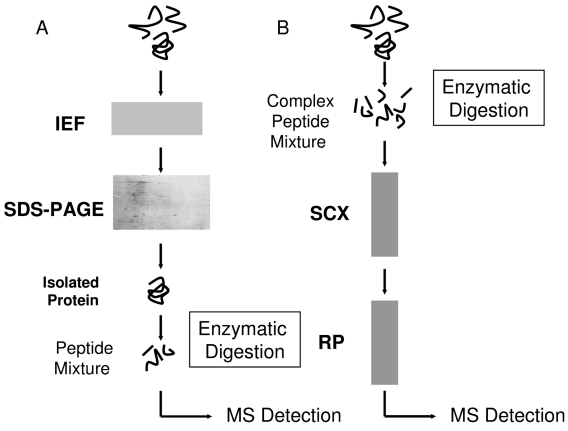


Fig. 1.43 Strategies for protein identification. (**A**) 2D gel electrophoresis approach. (**B**) 2D liquid chromatography approach. *IEF* Isoelectric focusing, *SCX* strong cation exchange column, *RP* reverse phase column, *SDS-PAGE* sodium dodecyl sulfate polyacrylamide gel electrophoresis.

tides present in the digest. The list of peptides is then submitted to a database search to identify the protein. This approach does not work if several proteins are present in the same spot or if the sample is contaminated for example with keratin. The identification of the protein can be improved by sequencing selected peptides either by post source decay (PSD-MALDI) or tandem mass spectrometry (MALDI-TOF/TOF).

High-performance liquid chromatography (HPLC) represents an attractive alternative to two-dimensional electrophoresis for the separation of both proteins and peptides because of its chromatographic resolving power, reproducibility and its compatibility with MS detection. The use of multidimensional chromatography for the separation of complex protein and peptide mixtures has consequently seen increased use in proteomics studies [87, 88]. A typical approach involves the digestion with trypsin of an extract. Furthermore the preparation and handling of peptides is less tedious than with intact proteins and the whole process can be easily automated. A typical two-dimensional LC experiment (2D-LC) involves the initial separation (first dimension) of the resulting peptide mixture by their electrostatic charge using strong cation exchange (SCX) chromatography. In the second dimension peptides are then separated by their hydrophobicity using reversed phase (RP) chromatography coupled directly to ESI-MS. In a typical analysis of a complex protein mixture from a single sample the procedure is repeated about ten times with increasing salt concentration, resulting in a total analysis time of about 12 h.

As electrospray ionization is concentration-sensitive the last LC dimension uses a nano LC column with an internal diameter of 75 μm to achieve maximum sen-

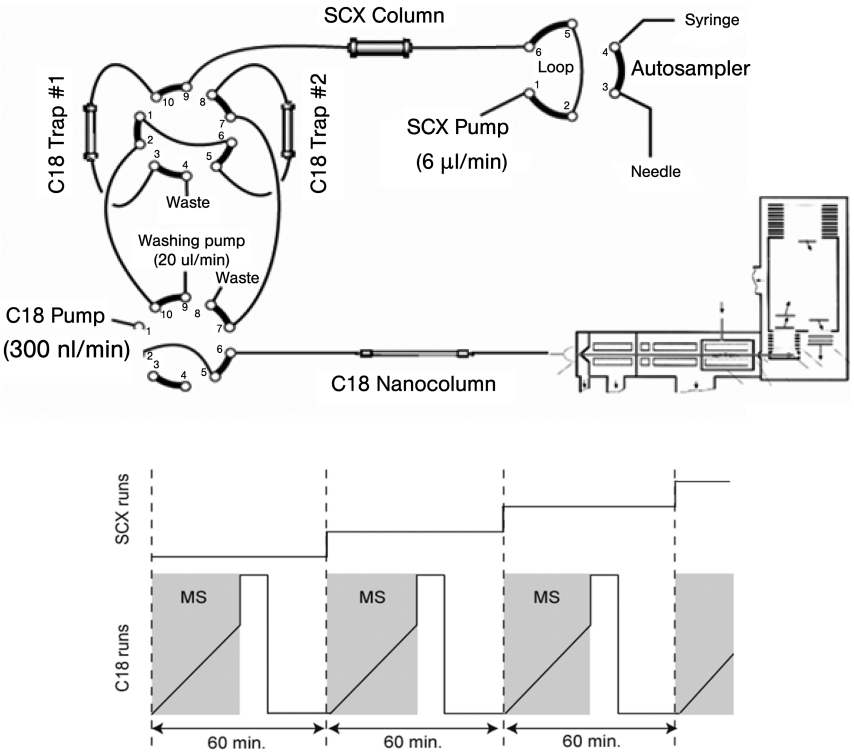


Fig. 1.44 2D-LC setup. The first ion exchange dimension is performed with a column with an i.d. of 1 mm, at a flow rate of 50 µL min^{-1} while the second dimension uses a nanocolumn with an i.d. of 0.75 mm and a flow rate of 300 nL min^{-1}. First dimension ion exchange has ten salt steps: 0, 5, 10, 15, 20, 25, 50, 75, 100, 200 mM KCl. Second dimension is typically an organic gradient: 5% to 80% acetonitrile with 0.1% formic acid in 30 min.

sitivity while larger diameters are preferred for the first ion exchange dimension to be able to inject large sample amounts and volumes. A 2D-LC system is depicted in Fig. 1.44. Ion exchange elution can be performed with ammonium acetate buffers which are MS-compatible. More efficient is potassium chloride elution, but the drawback is that it affects the detection of peptides. Therefore it is necessary to implement trapping columns for desalting the fraction before transferring it in the second reversed phase LC dimension. At the end of the analysis all the data are processed together to generate a list of several hundred proteins. For this task efficient bioinformatics tools are essential.

Figure 1.45 illustrates a typical 2D nano LC-MS/MS analysis of a *Caenorhabditis elegans* extract. For each timepoint a single MS and a product ion spectrum are

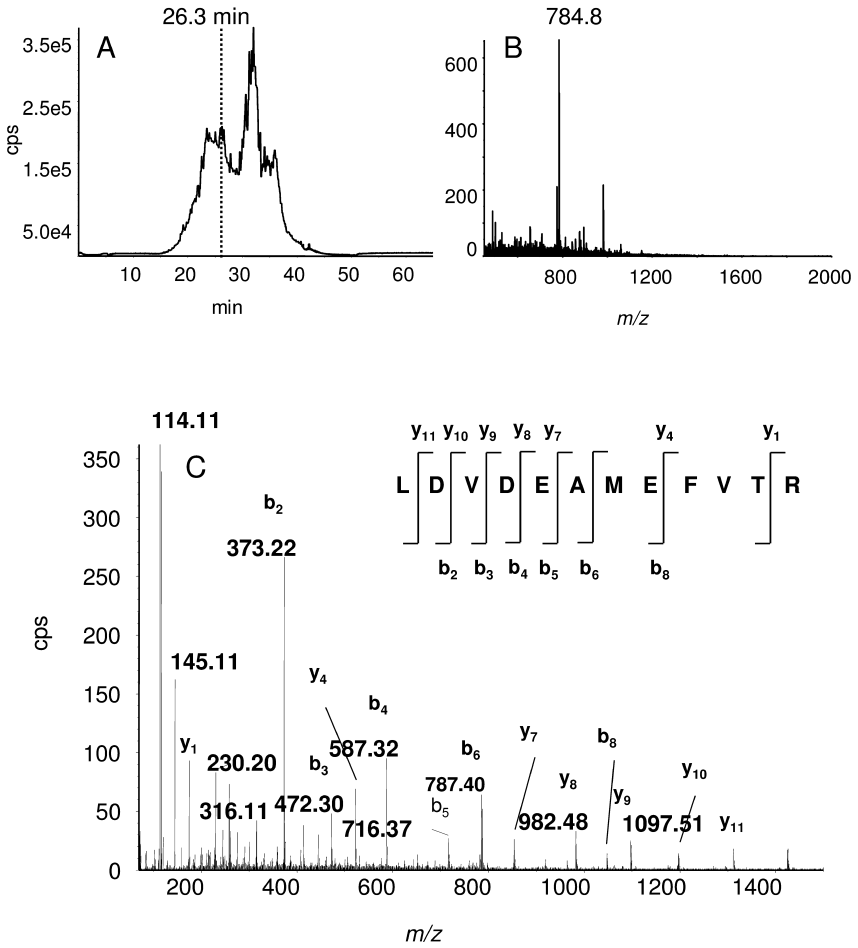


Fig. 1.45 Example of a 2D nano LC-MS/MS analysis of a *C. elegans* extract. (**A**) Fraction 2, 4 mM KCl salt elution on the strong cation exchange column. (**B**) Full scan MS spectrum of the peak eluting at RT 26.3 min in (**A**). (**C**) product ion spectrum of the doubly charged precursor of (**B**) at *m/z* 784.8. Y fragments are typical for C-terminal fragments while b ions are typical for N-terminal fragments.

recorded (Fig. 1.45B, C). With the help of bioinformatic tools the product ion spectrum can be automatically interpreted. The *y* fragments are typical for C terminal fragments, while the *b* ions are typical for N-terminal fragments.

Two-dimensional–liquid chromatography (2D-LC) approaches are much easier to automate than 2D-electrophoresis. However 2D electrophoresis has the advantage that separation is performed at the protein and not at the peptide level and

that the proteins can be visualized by staining. With 2D-LC one has to wait for bioinformatics treatment to see if the experiment was successful.

1.7 Perspectives

Mass spectrometry originated from quantitative measurements of the mass and charge of electrons [1]. Since that time the application of mass spectrometry has moved from the analysis of inorganic elements to organic molecules and finally to macromolecules. Over the past decade spectacular improvements were made in instrumental development regarding performance and new mode of operations in particular with hybrid instruments. Orbitrap, Fourier transform or triple quadrupole linear ion trap mass spectrometers could be used routinely only for the past few years and their potential is certainly not fully exploited yet. The strength of mass spectrometry lies in its sensitivity (femtomoles, atomoles); and in many applications the analyte of interest can be detected in its intact form. The challenge in life sciences bioanalysis is the diversity and the number of the molecules to analyze as well as the concentration range.

Analysis of pharmaceutical compounds in biological matrices with liquid chromatography coupled to mass spectrometry (LC-MS) has become a routine technique in many laboratories. However, certain issues such as non-standardized ionization response and matrix effects still need further investigation and improvement. The application of LC-MS for metabolomics studies [89] is gaining interest. Therefore, it is expected that accurate and high throughput quantitation of low molecular weight biomarkers will be one of the major challenges in the near future. Identification and quantification of proteins has progressed significantly; however in many cases the numbers of proteins which can be analyzed still remains limited. Electrospray ionization has been shown to be very powerful for single protein analysis but the technique is also well suited for the characterization of very large non-covalent complexes of proteins, which may lead to an increasing understanding of protein assemblies [90].

Single nucleotide polymorphism (SNP) genotyping has become a key technology in gaining a partial understanding of complex diseases or why patients react differently to drug treatment. Matrix assisted laser desorption especially with high speed laser allows real high throughput and is well suited for the analysis of oligonucleotides. MALDI is therefore an interesting approach for SNP discovery and genotyping, molecular haplotyping, methylation analysis, and RNA and allele-specific expression but needs further optimization before routine application [42, 91].

Significant progress has been realized in the miniaturization of separation sciences and mass spectrometric detection. Presently, the samples are transferred to highly specialized laboratories for analysis. But in the future it may become feasible to bring mass spectrometry as a portable technique to the bed for diagnostic or therapeutic monitoring.

Table 1.6 Common definitions and abbreviations.

General	
m/z	Symbol used to denote the dimensionless quantity formed by dividing the mass of an ion in unified atomic mass units by its charge number (regardless of sign). m/z should be written in italic and lower case. The Thomson (Th) is sometimes used as unit but it is not recommended.
$M^{+\cdot}$	Molecular ion, the ion results from the loss of one electron from the neutral molecule
$(M+H)^+$	Protonated molecule formed by the addition of a proton to a neutral molecule (teh terms pseudo-molecular ion or quasi-molecular ion should not be used)
u	Symbol for atomic mass unit
Accurate mass	Experimentally determined mass of an ion that is used to determine an elemental formula. The precision of the measure is indicated in parts per millions (ppm).
Atomic mass	The average of the atomic masses of all the chemical element's isotopes (also known as atomic weight and average atomic mass)
Average mass	Mass of an ion or molecule calculated using the average mass of each element weighted for its natural isotopic abundance
Exact mass	Calculated mass of an ion or molecule containing a single isotope of each atom, most frequently the lightest isotope of each element, calculated from the masses of these isotopes using an appropriate degree of accuracy
Mass defect	The difference between the exact mass of an atom molecule, ion and its integer mass in MS. In physics, the mass defect represents the difference between the mass of an atom and the sum of the masses of its unbound constituents.
Mass defect filter (MDF)	A software filter which allows the removal of interference ions from drug metabolites in accurate mass liquid chromatography–mass spectrometry
Mass range	Operating m/z range of a mass analyzer
Monoisotopic mass	Exact mass of an ion or molecule calculated using the mass of the most abundant isotope of each element
M_r	Relative molecular mass: mass of one molecule of a compound, with specified isotopic composition, relative to one-twelfth of the mass of one atom of ^{12}C

Table 1.6 *(continued)*

Nominal mass	Mass of an ion or molecule calculated using the mass of the most abundant isotope of each element rounded to the nearest integer value and equivalent to the sum of the mass numbers of all constituent atoms
Ion	An atomic or molecular species having a net positive or negative electric charge
Metastable ion	An ion formed with an internal energy higher than the dissociation threshold but with a sufficient lifetime that it can exit the source and enter the mass spectrometer where it dissociates
Isotope	One of several forms of an element having the same atomic number but differing atomic masses
Base peak (BP)	The most intense peak in the spectrum
Total ion current (TIC)	The sum of all the separate ion currents contributing to the spectrum
Extracted ion current (XIC)	The current of a specified m/z ion current
Mass resolving power	In a mass spectrum, the observed mass divided by the difference between two masses that can be separated: m/Δm. The procedure by which Δm was obtained and the mass at which the measurement was made should be reported.
Unit mass resolution	Means that a mass spectrometer is able to differentiate two peaks (generally the isotopes) distant of 1 m/z unit
Mass resolution	Smallest mass difference (Δm) between two equal magnitude peaks so that the valley between them is a specified fraction of the peak height
Ionization	
Even-electron ion	An ion containing no unpaired electrons in its ground electronic state
Odd-electron ion	An ion containing unpaired electrons in its ground state
EI	Electron impact ionization
PCI	Positive chemical ionization
NCI	Negative chemical ionization
API	Atmospheric pressure ionization: generic term for ionization techniques occurring at atmospheric pressure
ESI	Electrospray ionization: most commercial systems operate with pneumatically assisted electrospray (originally defined as ion spray)

Table 1.6 *(continued)*

Nano-ESI	Nanoelectrospray ionization: flow rates range from a few nanoliters per minutes to a few hundred nanoliters per minutes; nanoelectrospray is performed with pulled capillaries or on chips which serve as emitter
APCI	Atmospheric pressure chemical ionization
APPI	Atmospheric pressure photoionization
MALDI	Matrix assisted laser desorption ionization
Mass analyzer	
QqQ	Triple quadrupole: Q1 and Q3 are the mass resolving quadrupoles, q2 is the collision cell
QIT	Quadrupole ion trap: refers in general to a 3D ion trap instrument
LIT	Linear ion trap: refers in general to 2D ion trap; ion ejection is either axial or radial
QqQ_{LIT}	Triple quadrupole linear ion trap instrument. In this instrument the quadrupole Q3 is operated either in RF/DC mode or in RF mode
QqTOF	Quadrupole–time of flight instrument
TOF-TOF	Tandem time of flight instrument
FT-ICR	Fourier transform ion cyclotron resonance instrument
MS^n	Multistage mass spectrometry: applies generally for ion trap mass spectrometers
CID	Collision induced dissociation: the dissociation of ions after collisional excitation
PSD	A technique specific to reflectron time-of-flight mass spectrometers where product ions of metastable transitions or collision-induced dissociations generated in the drift tube prior to entering the reflectron are m/z separated to yield product ion spectra
NL	Neutral loss spectrum
PIS	Product ion spectrum
PC	Precursor ion spectrum
SRM	Selected reaction monitoring mode

1.8
Common Definitions and Abbreviations

The intention of this section is to provide to the reader a rapid and comprehensive reference for the most common definitions and acronyms used in mass spectrometry. Currently IUPAC has initiated a project to update and extend the definitions of terms related to the field of mass spectrometry. The definitions presented here (Table 1.6) are from the third draft document [16]. For more details and the latest updates, please consult www.msterms.com.

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