Hyphenated Methods

14

Learning Objectives

- Concept of chromatographic separation
- One-dimensional and two-dimensional gas chromatography
- Liquid chromatography in various pressure regimes
- Concept and technical aspects of coupling of separation methods to MS
- Basics of quantitation by MS
- Tandem MS modes for improved selectivity
- Applications of ion mobility spectrometry-mass spectrometry
- Tandem MS as a complement for chromatography coupling
- Ultrahigh resolution as a complement for chromatography coupling

The analysis of complex mixtures generally requires the combination of both separation techniques and mass spectrometry [1–3]. The first step in this direction was made by *gas chromatography-mass spectrometry* (GC-MS) coupling [4]. Soon, GC-MS became a routine method for mixture analysis [5–7]. The aim and attempt to also handle highly polar nonvolatile compounds by *liquid chromatography-mass spectrometry* (LC-MS) [8] eventually led to the development of API methods (Chap. 12) [9, 10]. The expansion of this approach to coupling of other liquid-phase separation techniques to mass spectrometry followed as *capillary zone electrophoresis-mass spectrometry* (CZE-MS) [11–15] and *supercritical fluid chromatography-mass spectrometry* (SFC-MS) [16, 17]. Regardless of the type of separation technique, it always adds an additional dimension to the analytical measurement. The hyphen used to indicate the coupling of a separation technique to mass spectrometry led to the collective term *hyphenated methods*.

Mass spectrometry itself also offers two additional "degrees of freedom". The complexity of a sample can either be resolved by high or even ultrahigh mass resolution or by employing tandem MS techniques (Chap. 9), e.g., by separating the

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fragmentation pattern of one component from that of others in a mixture [2, 3]. In practice, the coupling of separation techniques to mass spectrometry often involves advanced MS techniques to achieve the desired level of selectivity. This altogether ensures accuracy and reliability of analytical information. (For *accuracy* and *precision* cf. Sect. 3.5.)

This chapter is about extending the range of samples that can be analyzed by mass spectrometry and about increasing the specificity of analytical information thereof. It briefly introduces the basic concepts and methodologies of the most common chromatographic techniques, handling of chromatograms, procedures for quantitation, and chromatography–mass spectrometry interfaces.

14.1 Chromatography

The term *chromatography* is derived from the Ancient Greek words for color (χρῶμα, *chroma*) and writing (γραφειν, *graphein*). Chromatography was first described about a century ago by the Russian botanist Mikhail S. Tswett as a technique for the separation of plant pigments [18].

Chromatography of any type leads to the separation of components of a mixture. The procedure relies on the equilibrium of adsorption and desorption of compounds mobilized either in the gas phase or liquid phase to and from a stationary phase [19–22].

In *gas chromatography* (GC) the mobile phase is a gas and the stationary phase is a liquid, normally presented as a thin film bound to a solid surface. This explains the original term *gas-liquid chromatography* (GLC) that was soon superseded by the shorter form *gas chromatography* (GC). In contrast to GC, the mobile phase in *liquid chromatography* (LC) is a liquid and the surface of solid particles serves as the stationary phase, i.e., the components to be separated are provided in dilute solution.

14.1.1 Chromatographic Column

Chromatographic separations are mostly performed in tubes or capillaries providing a fixed volume where the mobile phase is passed through and serving to hold the stationary phase. The tube can either be straight and several millimeters to centimeters in diameter, as in liquid chromatography, or it can be a capillary of a fraction of a millimeter in diameter that is supported on a coil, as generally the case in (capillary) gas chromatography. Such an assembly – whatever the exact form factor – represents the *chromatographic column*. The term column has been coined by the most basic setup for LC separation that is achieved by filling a vertically aligned glass pipe with silica gel or alumina and passing through the sample solution from top to bottom by virtue of gravitation. This technique is still quite common in preparative organic chemistry.

14.1.2 Equilibrium of Adsorption and Desorption

The velocity at which molecules of a compound are traveling in the mobile phase along the chromatographic column is governed by the dynamic equilibrium of adsorption to and desorption from the stationary phase.

For a compound C_i we define a *distribution coefficient* or *partition coefficient*, K_i, by the ratio of concentrations in the stationary phase, $[C_\mathrm{i}]_\mathrm{stat}$, to that in the mobile phase, $[C_\mathrm{i}]_\mathrm{mob}$:

$$K_i = \frac{[C_i]_{stat}}{[C_i]_{mob}} \tag{14.1}$$

Some compounds will adsorb more strongly to the stationary phase and thus reside for a longer time than others that only weakly interact with the stationary phase. The latter compounds move faster along the column and reach the exit earlier than those experiencing a tighter contact.

The *retention factor* k_i is used to describe the velocity of migration of a component. It is defined as the ratio of the number of molecules residing at the stationary phase, n_stat, to those migrating in the mobile phase, n_mob:

$$k_i = \frac{n_{stat}}{n_{mob}} \tag{14.2}$$

14.1.3 Dead Time and Dead Volume

Even if we assume no interaction of the pure mobile phase with the stationary phase, i.e., $k_\mathrm{i} \approx 0$, there is a minimum time needed for the mobile phase to pass the chromatographic column. This minimum time is termed *dead time*, t_0, of a given chromatographic column. No compound can leave the system before t_0.

The dead time can either be determined by dividing the length of the column by the velocity of the mobile phase or by dividing the volume of the column by the volume flow of the mobile phase. It is important to note that this relationship refers to the free fraction of the column volume, i.e., the geometric volume minus the volume of the stationary phase inside. The volume available for filling with mobile phase is therefore called *dead volume*.

The actual dead volume of a chromatographic system is somewhat larger than the dead volume of the column alone as tubing and joints from injector to column and from column to detector also contribute to the total volume.

14.1.4 Retention Time

Depending on its individual distribution coefficient, K_i, a compound experiences stronger or weaker interaction with the stationary phase. Strong interaction means slow travel along the column because the molecules spend less time in the mobile phase. In other words, the column has greater retentiveness. The time needed for a component to pass through the column is thus termed *retention time*, t_R. The retention time is given by:

$$t_R = t_0 + t_0 \times k_i = t_0 \times (1 + k_i) \tag{14.3}$$

Vice versa, k_i can be determined from:

$$k_i = \frac{t_R - t_0}{t_0} \tag{14.4}$$

The retention time is characteristic of a compound for a given chromatographic setup. It is invariable as long as the chromatographic conditions remain unchanged. Using a longer column will increase the retention time. Increase of the volume flow of the mobile phase will reduce the retention time. Stronger interaction with the stationary phase causes an elongated retention time. Finally, the retention time is always longer than the dead time.

Chromatographic separation is thus effected by elution of individual components of a mixture at different retention times.

14.1.5 Elution and Eluate

The process of leaving the chromatographic column is termed *elution*. The fluid mixture of mobile phase (either gaseous or liquid) and separated components contained therein (either vapor or dissolved) is termed *eluate*. In preparative chromatography the eluate is collected in fractions to recover purified components. In this chapter, we deal with analytical chromatography where the eluate is either consumed by detection like in MS or discarded.

14.1.6 Separation and Chromatographic Resolution

During migration along the chromatographic column the zone occupied by a component tends to expand along the column. The extent of this longitudinal expansion, essentially smearing along the column, is determined by:

- *eddy diffusion*, A, caused by the different paths molecules can travel across a porous material resulting in differences in effective path length on the molecular level,

- *longitudinal diffusion*, B, of the component back and forth in the mobile phase,
- *resistance to mass transfer*, C; a collective term for any effect related to adsorption and desorption and convection during migration.

The *height equivalent of a theoretical plate* (HETP) or just *plate height*, H, can be expressed in terms of the above parameters and the velocity of the mobile phase, u, by the *van Deemter equation* [23]:

$$H = A + \frac{B}{u} + C\,u \tag{14.5}$$

Van Deemter plots visualize the optimum flow of the mobile phase for a given system and help to understand which parameter is limiting in a specific situation (Fig. 14.1). The *chromatographic resolution* increases the lower the value of H as this results in the highest number of theoretical plates for a given chromatographic column.

In practice, an impression of chromatographic resolution is best obtained from the so-called *peak capacity*. The peak capacity is the number of peaks that can be fitted into a chromatogram without overlap, i.e., just separated from each other. The peak capacity roughly reflects the number of compounds that possibly can be separated by the system. It can range from several tens in quick HPLC runs to about one thousand in optimized column GC.

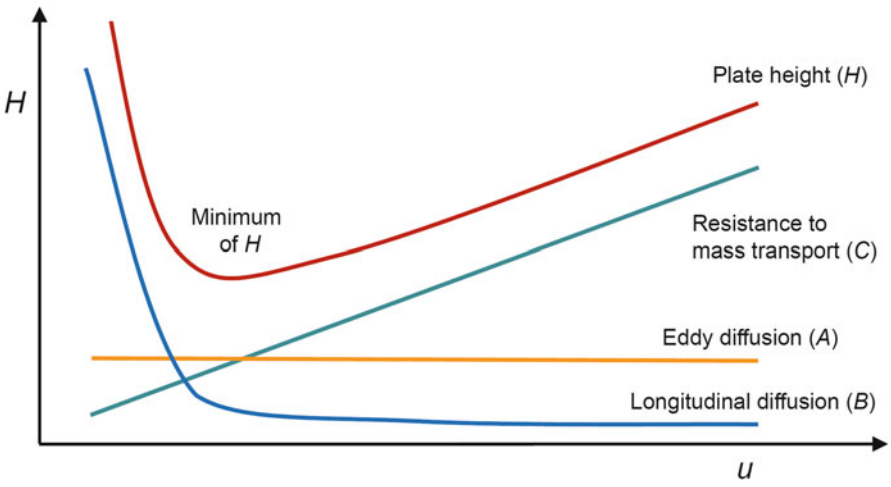


Fig. 14.1 Van Deemter plot of plate height, H, versus velocity of the mobile phase, u. The lowest value of H refers to the highest number of theoretical plates per given column length and thus marks the optimum for separation

14.1.7 Detectors

The chromatographic process effects separation of components in time. However, it does not include the means to detect whether a component is just being eluted. Even if we noticed the elution of a component there would still be no identification of this substance. With the exception of colored substances, where we may have the chance to detect the elution simply by the sudden coloration of the eluate, we need to employ some sort of *detector* to be able to judge when a colorless compound elutes.

Simple chromatographic detectors are only capable of indicating *when* a compound elutes and to provide a measure of the eluted *amount of substance* as by the relative signal intensity.

In GC, the most prominent types of detectors are *flame ionization detector* (FID), *thermal conductivity detector* (TCD), *nitrogen phosphorus detector* (NPD), and *electron capture detector* (ECD). While FID and TCD provide no or extremely low compound selectivity, NPDs only recognize nitrogen- and/or phosphorus-containing compounds and ECDs are selective for compounds with high electron affinity.

In LC, the change in UV light absorption of the eluate is normally taken as an indication of compound elution. Simple *UV detectors* rely on a single wavelength, often 254 nm, while more sophisticated *diode array detectors* (DAD) are capable of delivering a UV/Vis spectrum of the eluting component.

Mass spectrometers can serve as highly compound specific chromatographic detectors as they are capable of delivering a complete mass spectrum of every eluting compound. Occasionally, simple mass spectrometers like linear single quadrupole analyzers are referred to as *mass-selective detectors* (MSD). Here, we will certainly not follow this inappropriate habit.

More than just an inlet system
From an MS-centered view, any chromatographic system simply is another type of sample inlet, whereas from the chromatographer's point of view mass spectrometers are just detectors for their separation technique. Here, we deal with the peculiarities of those analyte-separating inlets and the associated implications for the operation of the attached mass spectrometers.

14.1.8 Chromatograms

The result of a chromatographic separation is presented as a *chromatogram* displaying the relative peak intensities versus retention time (Fig. 14.2). Ideally, all components are separated from each other and the baseline is flat. In real-world chromatography, we have to deal with overlapping peaks due to insufficient resolution, peak tailing (of polar components), baseline drift, mostly upward, e.g.,

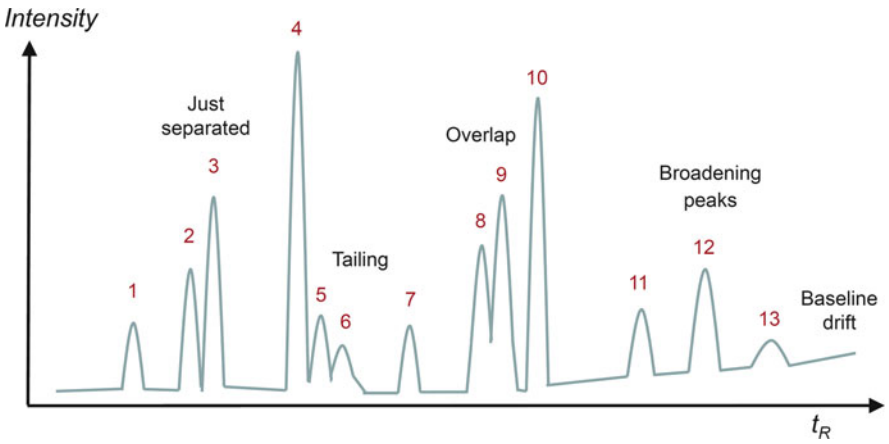


Fig. 14.2 General appearance of a chromatogram. This run shows 13 identified peaks and some frequent weaknesses like peak tailing of #6, insufficient resolution of the pair of #8, #9, and some peak broadening plus an upward baseline drift toward the end of the run

by column bleed (elution of stationary phase), and often some general peak broadening toward the end of the separation due to diffusion (cf. van Deemter plot).

14.1.9 Gas Chromatography: Practical Considerations

In *gas chromatography* (GC) the sample is evaporated and admixed to an excess of the mobile phase, i.e., an inert *carrier gas*. Normally, the sample is injected as a dilute solution in a volatile solvent. To do so, a microliter syringe is inserted into the *injector* by poking the needle through a silicone septum. The injector, essentially a hot glass tube, effects sudden evaporation and admixes the sample vapor to the carrier gas flow. Helium is frequently employed as carrier gas as it conveniently combines fast separations with perfect inertness and safety of operation. Nonetheless, the high cost of helium makes it more attractive to use nitrogen (slower separation) and hydrogen (fast separation but potentially explosive mixtures) instead.

Today, analytical GC is exclusively performed by use of *capillary columns*. Capillary columns for GC are drawn from fused silica that is transformed into a composite material by coating it with polyimide on the outside. This converts the extremely brittle quartz glass into a highly flexible material that can be wound onto a support coil (Fig. 14.3). The inner walls of the capillary are coated with a thin film of the stationary phase, mainly alkyl and aryl polysiloxanes. Typical GC capillaries are 20–60 m long, with a 0.10–0.50 mm inner diameter, and bear a film of 0.2–1.5 μm of stationary phase inside.

When the carrier gas is provided at the injector at about 1 bar, the high flow resistance of the capillary results in a gas flow in the order of 1 ml min^{-1}.

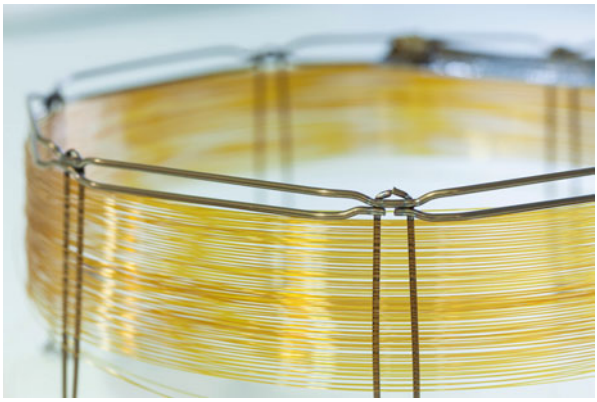


Fig. 14.3 Fused-silica capillary column for gas chromatography on a coil of about 20 cm in diameter. The golden color is caused by the outer polyimide coating of the silica capillary. While fused silica alone is extremely brittle, the composite material can easily be coiled and bent even at much smaller radius than required here

In principle, GC separations can be run at constant column temperature. However, isothermal GC separations are more time-consuming and suffer from reduced chromatographic resolution towards the end of the GC run, because elongated dwell time enhances the detrimental effect of diffusion. It is thus common to apply temperature programs that lead from low to high temperature. A gas chromatograph therefore closely resembles a convection oven wherein the column is operated under temperature control. Injector and detector are normally installed on the inside top of the housing (Fig. 14.4). As opposed to convection ovens for household use, much care is taken to achieve homogeneous temperature in the column compartment and to ensure precise and reproducible temperatures during the entire separation process. Also GC ovens reach up to at least 350 °C.

Often, the heating rate is split into a fast segment at the beginning and a slow segment covering the temperature range most efficient for the separation of the compounds of interest (Fig. 14.5). The injector and the transfer line to the detector need to be kept at higher temperature than the highest temperature of the program. Otherwise, the separated components could be condensed and thus be remixed at the column exit. Furthermore, care has to be taken that the maximum temperature specified for the actual column is not exceeded at any point of the system.

14.1.10 Comprehensive Gas Chromatography

Even though gas chromatography provides high resolution separations, this can, in some cases, be insufficient to resolve all components of complex mixtures like hydrocarbon fuels and lubricants, their combustion products, or other products of pyrolytic processes. Natural flavors and fragrances relevant for food and perfumes, aroma of spirits and wines, or coffee roasting, for example, present another field of enormous complexity.

Comprehensive gas chromatography, commonly known as GC × GC, employs a serial connection of two columns, where the first column is about 20–30 times longer than the second. The eluate of the first column is collected in an intermediate

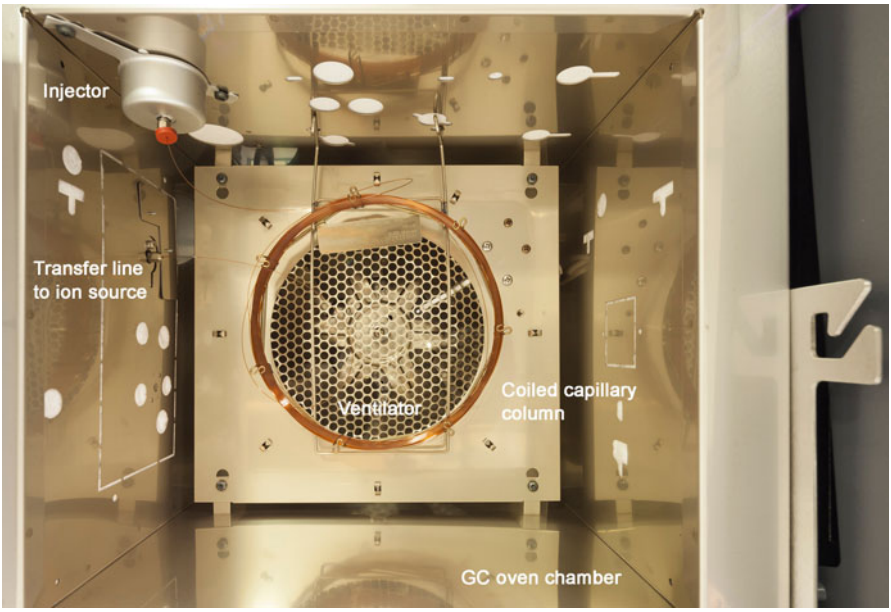


Fig. 14.4 GC oven for capillary gas chromatography with open front door. The GC column wound onto a coil is suspended to hang freely in the heated air. In this Agilent 7890B GC, the injector is on the *top left side* and the exit of the capillary to the transfer line is in the middle of the *left side lining*

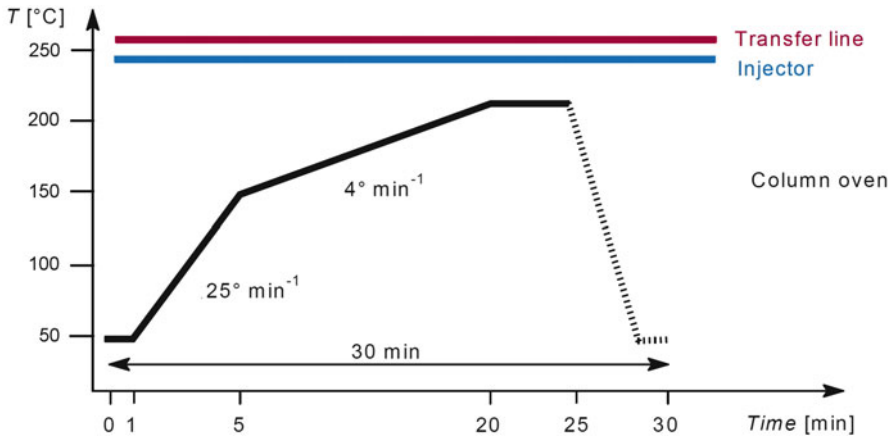


Fig. 14.5 Typical temperature program for GC operation. This program starts at 50 °C, holds that temperature for 1 min, ramps up at 25 ° C min^{-1} to 150 °C, then heats at 4 °C min^{-1}–210 °C, and holds this for 5 min before cooling down and temperature settling to get ready for the next run. Overall, this takes 30 min. Both transfer line and injector are constantly kept at about 50 °C above the highest temperature of the GC program to avoid condensation of sample

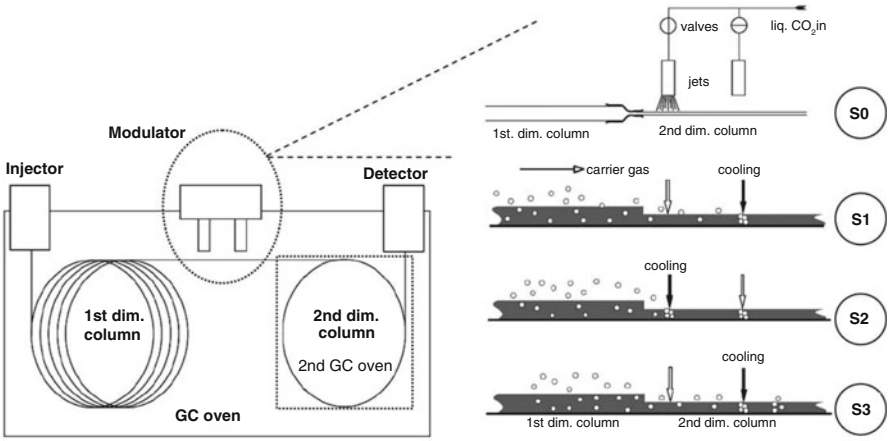


Fig. 14.6 Schematic of GC × GC instrumentation (*left*) and operation (*right*). The eluate from the first column is collected, e.g., by a dual-jet cryogenic modulator. The modulator switches from trapping to evaporation mode on two short adjacent segments at the junction of the columns (Reproduced from Ref. [24] with permission. © Elsevier, 2006)

condenser or trap [24, 25] for several seconds and then passed on in a pulsed manner to the second column (Fig. 14.6). When columns of different polarity are used, this results in an orthogonal separation, i.e., in *two-dimensional chromatography* [24–28]. Thus, co-eluting components from the first-dimension column may be separated on the second-dimension column.

GC × GC requires very fast data acquisition. Also, the amount of analytical data requires software tools capable of presenting the wealth of information. Basically, the chromatograms delivered by the high-speed second-dimension column are plotted versus the retention time of the first-dimension column (Fig. 14.7). Often, the dataset is processed further for improved visualization, e.g., for display as 2D contour plot or 3D plot. As thousands of peaks are still difficult to examine, it is common to show only zoomed-in views of the region of interest or to employ statistical tools like *principal component analysis* (PCA) to reveal common features as well as differences between samples.

14.1.11 High-Performance Liquid Chromatography

Liquid chromatography (LC) started with Tswett's work based on simple manually filled columns. The chromatographic resolution – or speed of separation at fixed resolution – can dramatically be improved by using fine and homogeneously sized particles as stationary phase [19–22]. Then, the liquid flow can only be sustained by applying high pressure in the order of 100–200 bar. This led to the development of *high-pressure liquid chromatography* (HPLC), also known as *high-performance liquid chromatography* (HPLC). HPLC was further improved when the particle size

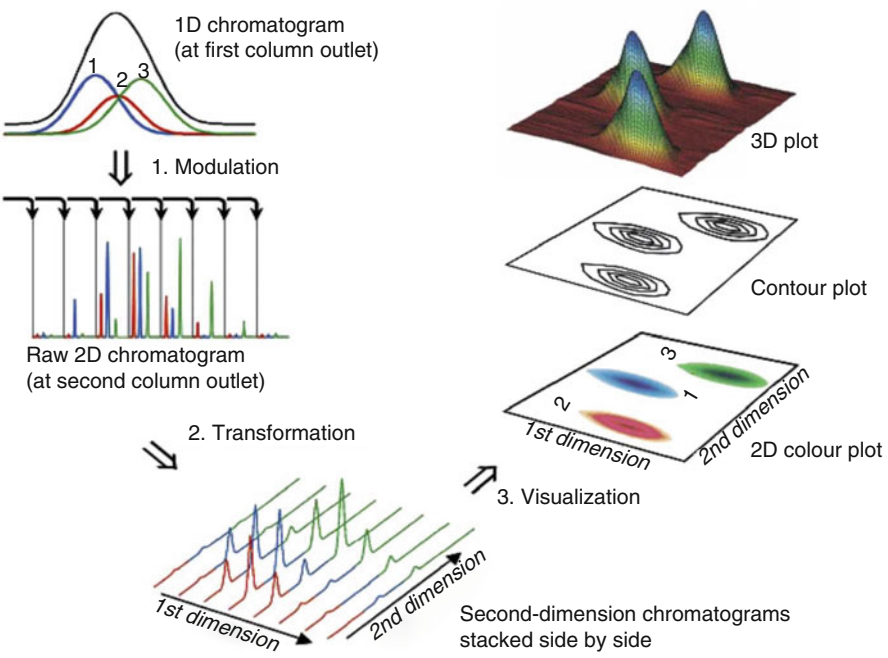


Fig. 14.7 Concept of GC × GC and data analysis. A large number of chromatograms from the second-dimension high-speed column is aligned along the retention time of the first-dimension column. Often, the data is further processed for improved visualization as contour plot or 3D plot (Reproduced from Ref. [24] with permission. © Elsevier, 2006)

could be reduced to a few micrometers causing columns to now demand pressures of up to about 1000 bar to effect flow rates of 0.5–1.0 ml min^{-1} that are useful in practice. The technique was introduced by Waters as *ultra-performance liquid chromatography* (UPLC, Fig. 14.8) and is now available from several vendors as *ultrahigh-pressure liquid chromatography* (UHPLC) [29, 30].

Most of the HPLC and UHPLC work is done on so-called *reversed phase* stationary phases. Classical LC on silica or alumina is considered as normal phases. In reversed phase chromatography the surface of the silica particles is coated with an organic layer, mostly siloxanes with various alkyl or aryl end groups. The end groups determine the interaction of the surface with the analyte molecules. Long alkyl chains, typically C_{18}, bind stronger to organic molecules of low polarity while highly polar or even ionic analytes pass by. Shorter alkyl chains like C_8 show less retentiveness to low-polarity analytes. On reversed phase columns, ionic and highly polar hydrophilic compounds may even be mobilized by pure water or water : acetonitrile = 90 : 10 (v/v). Nonpolar hydrophobic molecules require a higher fraction of organic solvent to move along the stationary phase. Overall, reversed phase chromatography leads to retention times in the order nonpolar > polar > ionic.

Fig. 14.8 UPLC column. The actual column (*right*), here shown with the two end caps still in place, is quite small as by comparison to the pencil. This particular column is a Waters BEH C18 reversed phase type of 50 mm length and 2.1 mm inner diameter filled with 1.7-µm-sized particles. The column is locked to a transponder (*left*) that is used to identify the column by the data system

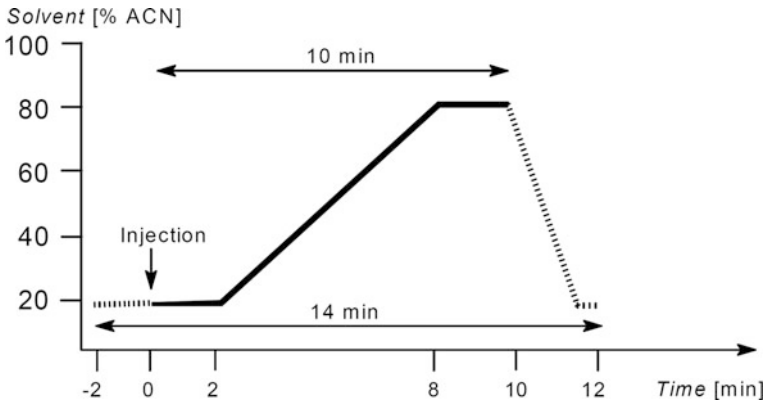


Fig. 14.9 Simple program for separation with a gradient in reversed phase UHPLC. Injection is performed after 2 min of column equilibration using a mobile phase of acetonitrile : water = 20 : 80 (v/v). After 2 min of holding this composition, the gradient leads to acetonitrile : water = 80 : 20 (v/v). Data acquisition could either stop at the end of the gradient at $t_R = 8$ min or at $t_R = 10$ min at the latest

While HPLC and UHPLC can be run at constant solvent composition, so-called *isocratic* operation, it is by far more common to employ gradually increasing concentrations of organic solvent in water to mobilize analytes of decreasing polarity. A typical *gradient* reverses the relative concentrations of two solvents (Fig. 14.9). Like the temperature program in GC, the concentration gradient in LC

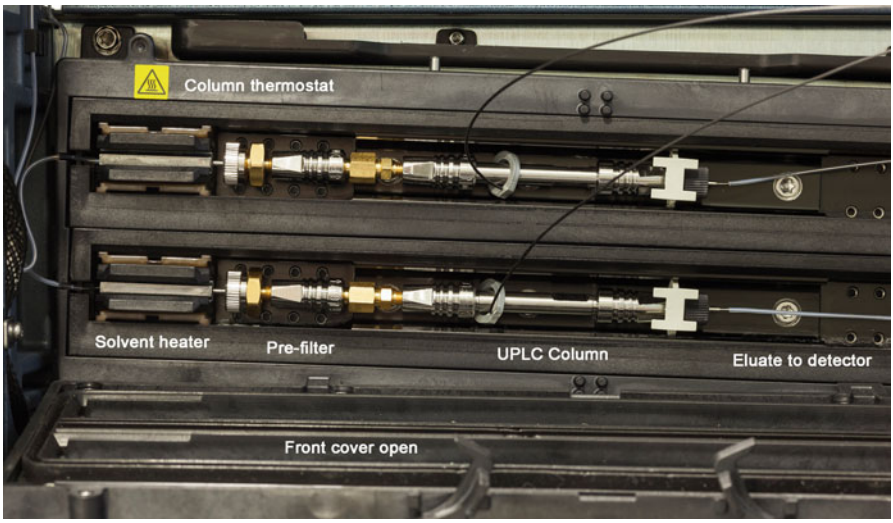


Fig. 14.10 A column thermostat of a Waters Acquity system with two UPLC columns mounted inside. The sample solution first passes a heater cartridge to ensure constant temperature equal to that of the column, then a pre-filter to prevent the column from clogging, and finally enters the UPLC column. The column compartments of the thermostat are inclined to direct solvent towards a drain in case of a leakage

serves to *i*) speed up the separation and to *ii*) preserve the level of chromatographic resolution until the end of the run.

To ensure best reproducibility, HPLC and UHPLC columns are operated at constant temperature in a thermostat (Fig. 14.10). In practice, this is most easily achieved at 30–50 °C. For practical reasons, they are run at constant flow, and thus, column pressure changes due to viscosity changes of the mobile phase.

From HPLC to UHPLC The method for transferring from conventional HPLC to UHPLC was demonstrated for the case of separating a pharmaceutical formulation containing a main product (#6) and eleven impurities (#1 – #5, #7 – #12, Fig. 14.11). The sample was analyzed in gradient mode with HPLC and UHPLC, respectively [29]. The original HPLC method used a C_{18} column of 150 × 4.6 mm with 5 µm particles at a flow rate of 1.0 ml min^{-1} that took 27 min. After transfer to UHPLC on a BEH C_{18} column of 50 × 2.1 mm with 1.7 µm particles at a flow rate of 0.6 ml min^{-1} the gradient time was shortened to 3 min. Further optimization of the UHPLC method at a flow rate of 1000 µl min^{-1} cut down the run time to 1.6 min without sacrificing resolution. In addition, the injected volume was reduced from 20 µl for HPLC to 1.4 µl for optimized UHPLC.

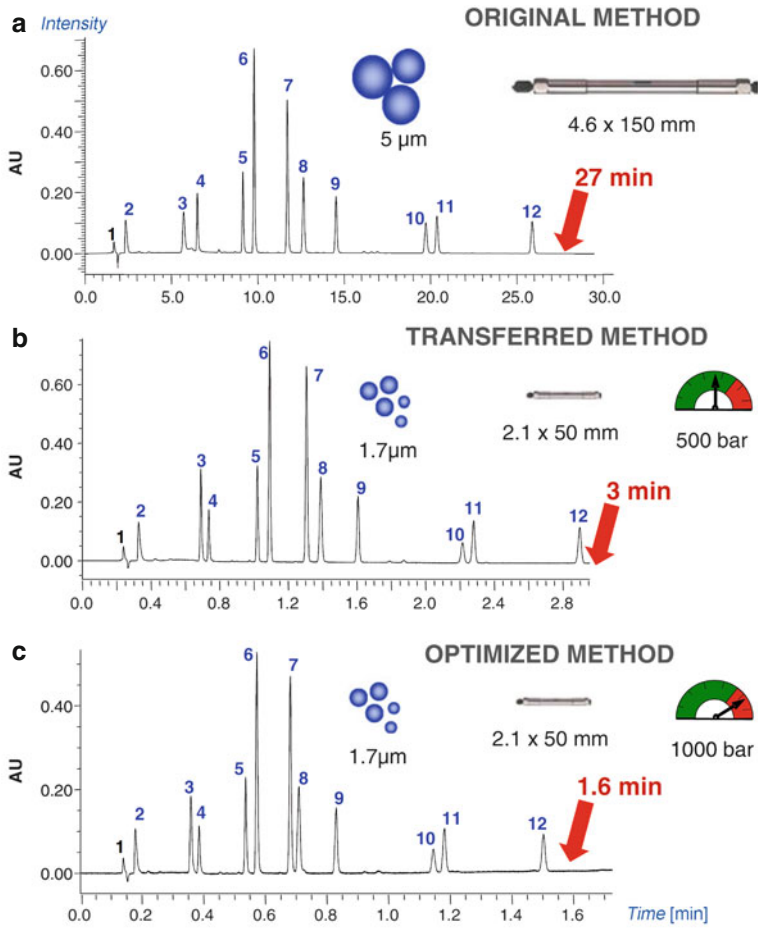


Fig. 14.11 Method transfer from conventional HPLC to UHPLC. (**a**) Original HPLC method on a C18 column of 150 × 4.6 mm lasting 27 min. (**b**) Transferred UHPLC method on BEH C18 column of 50 × 2.1 mm lasting 3 min. (**c**) Optimized UHPLC method on the same column delivering the separation in 1.6 min (Adapted from Ref. [29] with permission. © Springer, 2010)

14.2 Concept of Chromatography-Mass Spectrometry

Simple gas chromatographic detectors like the frequently employed FID deliver a chromatogram that represents the mass flow eluting from the chromatographic column. Using a mass spectrometer instead, adds a third dimension of information, i.e., the mass spectra associated to any of the eluting components (Fig. 14.12). When a mass spectrometer is employed as the chromatographic detector (also cf. Sects. 5.4 and 5.5) its output must somehow represent the chromatogram that

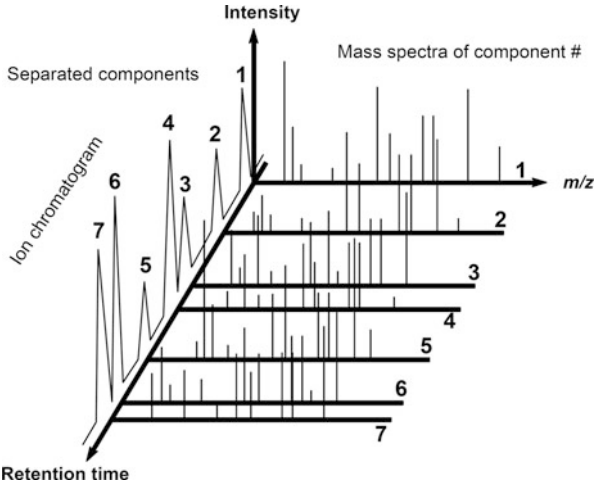


Fig. 14.12 Three dimensions of chromatography-mass spectrometry: retention time, intensity, and *m*/*z*. Each of the compounds No. 1 to No. 7 eluting from the chromatographic column is characterized by its own mass spectrum. The sum of all peak intensities of a mass spectrum at any point in retention time is used to construct the ion chromatogram

would have been obtained with other chromatographic detectors. The chromatogram as produced by the mass spectrometer is composed of a large set of consecutively acquired mass spectra. Because mass spectral chromatograms represent ionic abundances as a function of retention time, these are termed *ion chromatograms*.

14.2.1 Ion Chromatograms

Several decades ago, the *total ion current* (TIC) used to be measured by a *hardware TIC monitor* before mass analysis (nA to μA range). Today, its equivalent can be *reconstructed* or *extracted* after mass analysis [31]. Both adjectives, *reconstructed* and *extracted*, serve to illustrate that the chromatogram was obtained from a set of spectra by a computational process selecting user-defined signals to build the trace. For convenience, let us here again define some of the relevant terminology introduced in Chap. 1: The TIC represents a measure of the overall intensity of ion production as a function of time. The TIC obtained by means of *data reduction* [32], i.e., by summation of peak intensities of each mass spectrum as successively acquired during analysis, is termed *total ion chromatogram* (also abbreviated TIC). For this purpose, the sum of all ion intensities of each of the spectra is plotted as a function of time or scan number, respectively (Fig. 14.13). The term *total ion current chromatogram* (TICC) refers to a chromatogram obtained by plotting the total ion current detected in each of a series of mass spectra recorded as a function of retention times of the chromatographically separated components of a mixture (which essentially is implicated by: TIC). Unfortunately one also finds combinations such as *reconstructed total ion current* (RTIC), *reconstructed total ion current chromatogram* (RTICC), and *extracted ion chromatogram* (EIC, Table 14.1) [33].

The term *reconstructed ion chromatogram* (RIC) was and still is used by many to describe the intensity of a given *m*/*z* or *m*/*z* range plotted as a function of time or scan number. Recently, the term *extracted ion chromatogram* (EIC) has been used

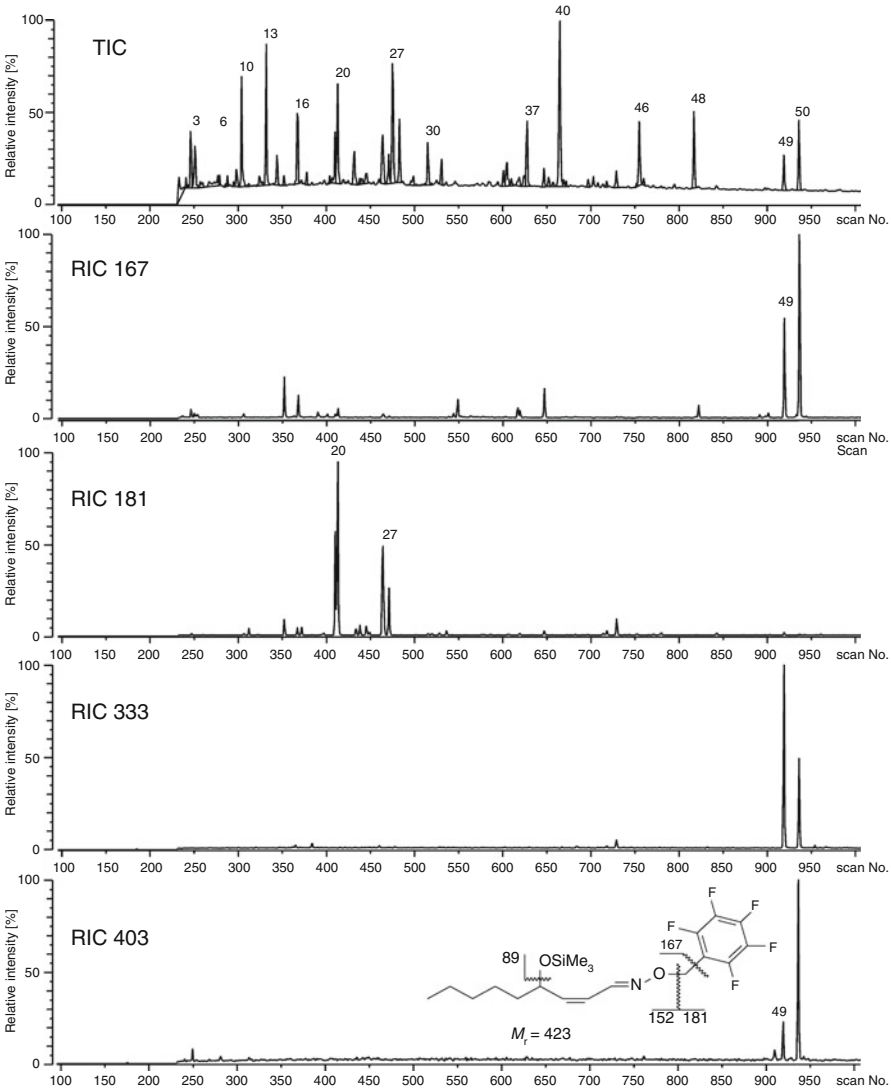


Fig. 14.13 TIC and RICs (*m*/*z* 167, 181, 333, and 403) obtained from a GC-EC-MS run of a plasma sample spiked with 4-HNE [38]. Chromatographic peaks are usually labeled using consecutive numbers to allow for their correlation to mass spectra. (The EC spectrum of the 4-HNE derivative is show in Fig. 14.14)

to describe a chromatogram created by plotting the intensity of the signal observed at a chosen *m*/*z* value or set of values in a series of mass spectra recorded as a function of retention time. Plotting RICs or EICs is especially useful to identify a target compound of known *m*/*z* from complex GC-MS or LC-MS data. In other words, the RIC allows to retrieve the retention time of the target compound. RICs

Table 14.1 Types of ion chromatograms

Acronym	Full name	Comment
TIC	Total ion chromatogram	Sum of peak intensities per spectrum vs. run time, retention time, or scan number; in practice used interchangeably. TIC is recommended here as the least complicated form
(TICC	Total ion current chromatogram)	
(RTIC	Reconstructed total ion chromatogram)	
(RTICC	Reconstructed total ion current chromatogram)	
RIC	Reconstructed ion chromatogram	Signal intensity at selected *m*/*z* vs. retention time; in practice used interchangeably
EIC	Extracted ion chromatogram	
BPC	Base peak chromatogram	Base peak intensity of each spectrum plotted vs. retention time or scan number

can also be used to elucidate the relationship of certain *m*/*z* values to different mass spectra obtained from the measurement of a single (impure) sample. Thus, RICs (EICs) often reveal valuable information on impurities accompanying the main product, e.g., remaining solvents, plasticizers, vacuum grease, or synthetic by-products (Appendix A.9).

Finally, the *base peak chromatogram* (BPC) is a chromatogram obtained by plotting the signal of the base-peak ions detected in each of a series of mass spectra recorded as a function of retention time. BPCs are particularly useful in conjunction with soft ionization methods as then the molecular mass is correlated to a chromatographic peak.

Plotting versus time or scan number
The abscissa of the TIC or RIC can either be plotted on the time scale, i.e., in units of seconds or minutes, or be labeled with scan numbers. Scan numbers are useful during data processing, while the time scale is better suited for comparison with other chromatography-mass spectrometry data.

14.2.2 Repetitive Acquisition of Mass Spectra During Elution

A widespread mode of chromatography-mass spectrometry operation is to repetitively scan the mass analyzer over the *m*/*z* range of interest during the chromatographic run [34–36]. As explained above, this generates a relationship between chromatogram and mass spectra of the eluting components. Knowledgeable handling of the TIC and suitable RICs presents the key to the effective assignment of chromatographic peaks to target compounds.

Detection of 4-hydroxynon-2-enal in plasma 4-Hydroxynon-2-enal (4-HNE) is a major aldehydic product of lipid peroxidation (LPO), its products being indicators for

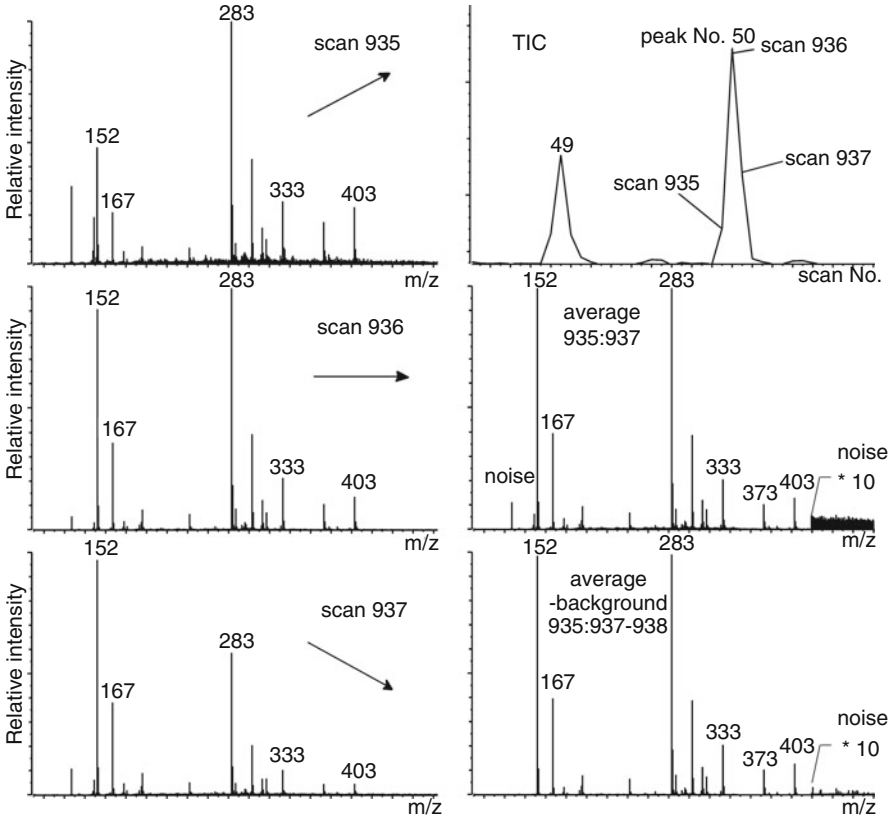


Fig. 14.14 Extraction of spectra from chromatographic peak No. 50 in the TIC of a plasma sample spiked with 4-HNE. *Left column*: single scan spectra from scans No. 935, 936, and 937 show changing relative intensities; averaging scans 935:937 levels intensity but leaves noise; background subtraction reduces noise additionally. (For explication, TIC, and RICs see preceding example and Fig. 14.13)

oxidative stress. In order to introduce LPO products as biomarkers, a GC-MS method for 4-HNE detection in clinical studies [37] was developed using a sample volume of 50 µl of plasma. For improved GC separation and subsequent mass spectral detection the aldehyde is converted into the pentafluorobenzyl-hydroxylimine and the hydroxy group is trimethylsilylated [38]. The TIC acquired in electron capture mode (EC, Sect. 7.6) exhibits 50 chromatographic peaks (Fig. 14.13). Those related to the target compounds can easily be identified from suitable RICs. The choice of potentially useful *m/z* values for RICs is made from the EC mass spectrum of the pure 4-HNE derivative (Fig. 14.14). In this case, $[M–HF]^{-\bullet}$, *m/z* 403, $[M–HOSiMe_3]^{-\bullet}$, *m/z* 333, and $[C_6F_5]^-$, *m/z* 167, are indicative, while $[CH_2C_6F_5]^-$, *m/z* 181, is not.

When the experiment is performed in the repetitive scanning mode, each point of the TIC corresponds to a full mass spectrum. The time for the acquisition of a mass

spectrum has to be shorter than the time to elute a component from the chromatographic column. In capillary GC-MS, this requires scan cycle times in the order of one second. A 20-min run at one scan per second, for example, delivers a set of 1200 mass spectra. Nonetheless, the concentration of the eluting components still varies rapidly in time as compared to the time for a scan cycle. This affects the relative intensities of mass spectral peaks, i.e., high-mass ions are emphasized in an upwards mass scan at the onset of elution, but are underrepresented when elution fades out (Fig. 14.14). *Averaging* or *accumulation* of the scans contributing to a specific chromatographic peak compensates for that source of error. Additional *background subtraction* can substantially improve the signal-to-noise ratio (Sect. 1.6) of the final spectrum [35]. It is also obvious from this procedure that the number of final spectra is always lower than the actual number of scans.

Background subtraction
High-quality mass spectra suitable for interpretation and/or data base search (Sect. 5.9) are only obtained from summing/averaging plus subsequent background subtraction. Particularly for components of low concentration the value of background subtraction cannot be overestimated.

14.2.3 Selected Ion Monitoring and Targeted Analysis

The operation of mass spectrometers in the repetitive scanning mode is useful for the identification of the components of a mixture. If *quantitation* is a major issue (below), *selected ion monitoring* (SIM) is preferably employed; the term *multiple ion detection* (MID) and some others are also in use [31]. In the SIM mode, the mass analyzer is operated in a way that it alternately acquires only the ionic masses of interest, i.e., it "jumps" from one m/z to the next [39–44]. The information obtained from a SIM trace is equivalent to that from a RIC, but no mass spectra are recorded in SIM mode. Thus, the scan time spent on diagnostically useless m/z ranges or even on mere noise in the gaps between peaks is almost reduced to zero while the detector time for the ions of interest is increased by a factor of 10–100 (Fig. 14.15) [45]. An analogous improvement in detection limit is also observed.

Targeted approach
A precondition for selecting a set of m/z values to be exclusively observed during an analysis is that the *target analytes* are well defined prior to the run. In turn, the targeted approach sacrifices all data outside the selected window (s) in favor of lower detection limits for the target compounds. This explains why relevant compounds, yet unknown at the time of analysis, can be overlooked.

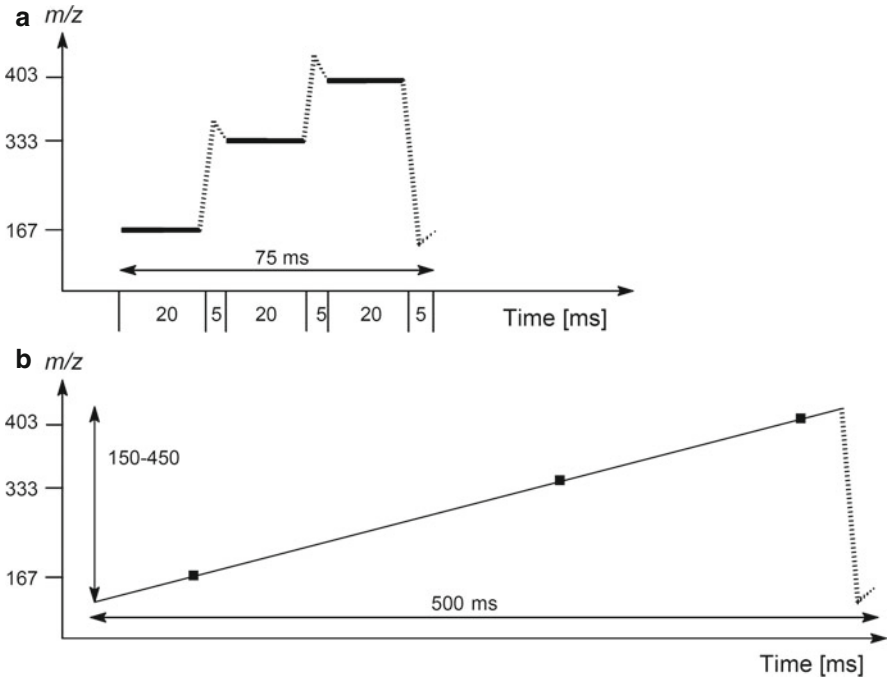


Fig. 14.15 SIM advantage over scanning mode. (**a**) Hypothetical SIM experiment to analyze 4-HNE in a plasma sample by *m*/*z* 167, 333, and 403 compared to (**b**) 500 ms-scan over the *m*/*z* 150–450 range. Only about 1.5 ms would be spent on each of the three signals during a 500 -ms-scan while 20 ms are achieved for each in a much shorter 75 ms-SIM cycle with 5 ms settling time. (Time axes not to scale.)

As the monitored *m*/*z* values are selected to best represent the target compound, SIM exhibits good selectivity that can be further increased by *high-resolution SIM* (HR-SIM) because HR-SIM almost eliminates isobaric interferences [46–49]. To ensure precise and drift-free positioning on narrow peaks, HR-SIM requires one or several *lock masses* to be employed although those are rarely explicitly mentioned in the literature [49, 50]. The role of the lock mass is to serve as internal mass reference for accurate mass measurement. (For examples cf. Sect. 14.3.)

How many ions in one run?
Normally, three to ten *m*/*z* values are monitored 20–100 ms each in one SIM cycle. Some settling time is needed for the mass analyzer after switching to the next value, e.g., 1–2 ms for pure electric scanning, 20–50 ms for a magnet scan. Due to the comparatively slow axial ion velocity in (triple) quadrupole instruments, the time to empty the quadrupole from one ionic species prior to setting it to the next has to be taken into account.

14.2.4 Retrospective and Non-targeted Analysis

The improvement of SIM mode over full spectral acquisition mode in terms of detection limits exclusively applies to scanning instruments like magnetic sector and (triple) quadrupole mass spectrometers. Quadrupole ion traps (QIT and LIT) might be operated as to deliver a SIM output. The time to achieve ion selection is, however, essentially identical to their full scan cycle time, thus canceling out the sensitivity advantage.

With any type of TOF analyzers, the SIM mode can neither be realized nor can it offer any improvement over retrieving RICs from the spectra. As many modern TOF instruments allow for both high resolution and accurate mass, this actually becomes an advantage in that the information for HR-SIM is already contained in the dataset. Thus, any RIC, even within a narrow m/z window, can be extracted in post-processing [51–56]. It is Therefore it is no longer necessary to restrict the analysis to a limited set of HR-SIM traces while having to discard all other information. This approach is known as *retrospective analysis*. Other comparatively fast high-resolving mass analyzers like Orbitrap and FT-ICR instruments bear the same advantage, the only difference to TOFs being somewhat slower spectral acquisition rates at, in turn, even higher resolving power and mass accuracy [54].

One can roughly compare these different instrumental concepts and their modes of operation to get an idea of where the one or the other approach will work best in terms of signal-to-noise ratio versus the number of components that are to be analyzed in one cycle (Fig. 14.16). The exact position of the lines will vary depending on the actual mass spectrometer. Also, if we were to ask instrument manufacturers, the relative positions would be shifted depending on the manufacturer's perspective.

Sometimes, compounds may only appear relevant in the aftermath of an analysis, e.g., in doping control and other forensic fields as well as in environmental analyses. Retrospective analysis offers the opportunity of investigating analytes that were not considered at the time of spectral acquisition, i.e., to retrieve *non-targeted analytes*.

Non-targeted analysis
The ability to collect as much data as possible in one run without losing neither sensitivity nor selectivity permits a *non-targeted analysis* to be performed. Even though the standard procedure may be designed to detect any one of a predefined number of compounds, the data offers the chance for re-examination to look after another set initially not considered to be relevant.

Drug residues in wastewater Drugs and their metabolites are excreted and end up in wastewater. The TIC obtained by LC-HR-TOF-MS analysis of a wastewater sample mostly showed severely overlapping chromatographic peaks (Fig. 14.17)

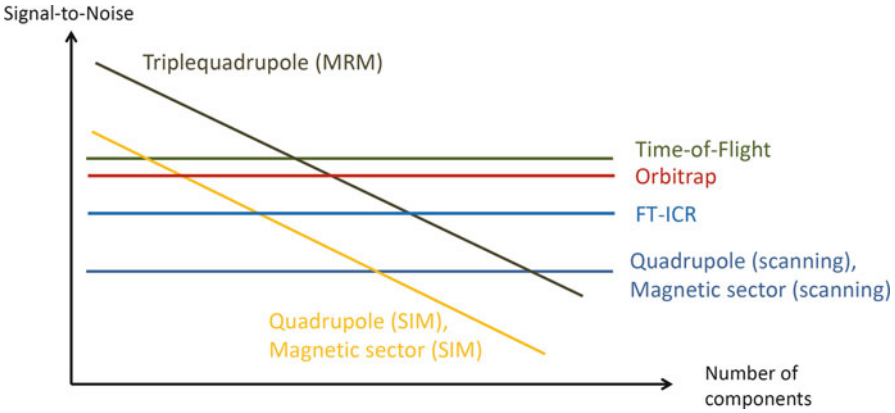


Fig. 14.16 Qualitative comparison of signal-to-noise ratios of scanning instruments, their operation in SIM or MRM modes, and nonscanning instruments versus the number of components to be analyzed per cycle

[52]. While it was impossible to detect a metabolite of the antirheumatic and antipyretic drug dipyrone from the TIC, the application of a narrow window of m/z 246.12 $\pm$ 0.02 characteristic of the $[M+H]^+$ ion revealed the presence of the compound. Having identified the retention time, 20.58 min, the corresponding mass spectrum could be obtained after background subtraction. It delivered a signal of the $[M+H]^+$ ion, $[C_{13}H_{16}N_3O_2]^+$ at m/z 246.1237, and two fragment ions, the formulas of which were also assigned by accurate mass. This demonstrates that a nontargeted analysis offers deliberate selection of target analytes during post-processing.

14.2.5 Selected Reaction Monitoring

Tandem MS adds a fourth dimension to the chromatography-MS experiment in that it allows to acquire a spectrum selectively, revealing the fragmentation of one specific ion generated of the target compound (Fig. 14.18). Like SIM, the approach of *selected reaction monitoring* (SRM, Table 14.2) requires useful ionic fragmentation pathways to be explored before their use in SRM analyses. If two or more ionic fragmentations are covered in one cycle the experiment is termed *multiple reaction monitoring* (MRM). Triple quadrupole instruments are preferred for SRM and MRM operation as they combine straightforward tandem MS operation and fast switching of m/z with a high linear dynamic range (10^4–10^5). (Examples of MRM and SRM are given in Sects. 14.6 and 14.8, respectively. Another procedure of four-dimensional analysis is discussed in Sects. 14.4.6.)

LC-APCI of polybrominated diphenyl ethers Hydroxylated and methoxylated polybrominated diphenyl ethers were isolated from simple organisms such as algae,

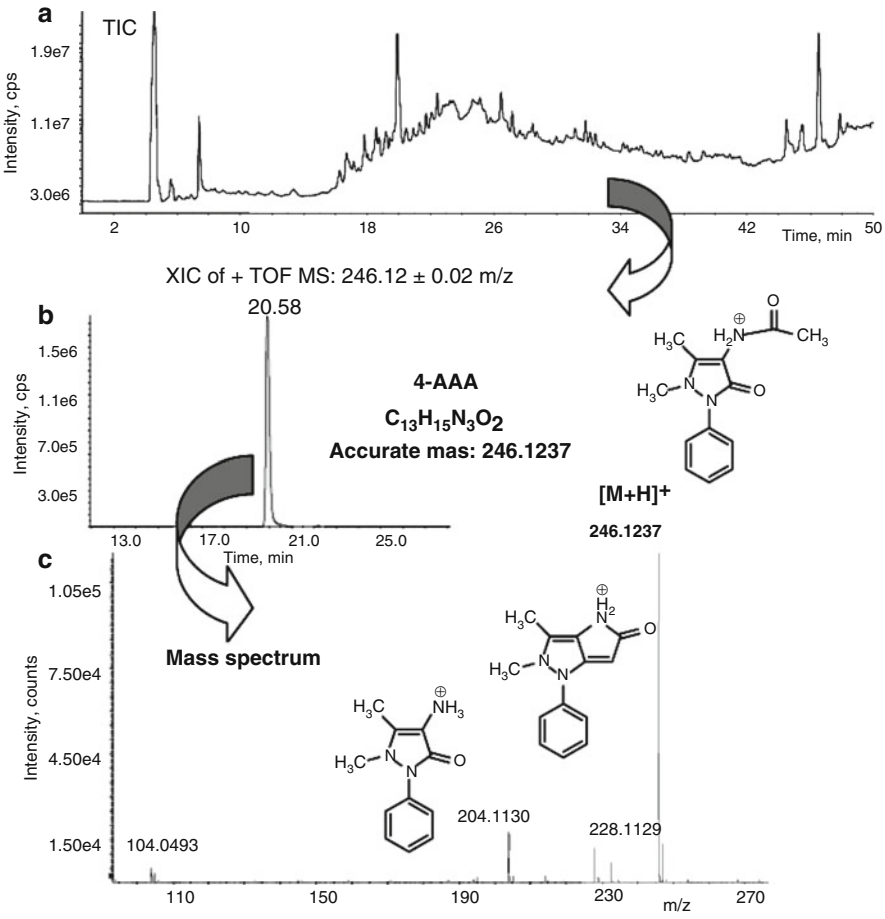


Fig. 14.17 Dipyrone metabolite in wastewater detected by LC-HR-TOF-MS. (**a**) The TIC shows severe overlap of peaks from LC analysis. (**b**) Extraction of a chromatogram at the expected *m/z* of the $[M+H]^+$ ion, *m/z* 246.12 ± 0.02, reveals the elution of the target compound, here abbreviated as 4-AAA, at 20.58 min. (**c**) Accurate mass TOF spectrum of 4-AAA as obtained after background subtraction (Adapted from Ref. [52] with permission. © American Chemical Society, 2007)

sponges, and bacteria [57]. The compounds could be separated and detected by coupling liquid chromatography to negative-ion APCI. The phenol-type compounds underwent deprotonation, and thus, formed $[M-H]^-$ ions. For higher selectivity, the compounds were not detected using full scans but by scanning for the precursor ion of their Br^- fragment ions (Fig. 14.19). As the polybrominated diphenyl ethers obviously were the only brominated compounds in these extracts, the occurrence of Br^- fragments from any of the diphenyl ether $[M-H]^-$ ions turned out to be highly characteristic for their identification. The dimethylether analogs showed a different behavior, presumably as the transient negative molecular ions

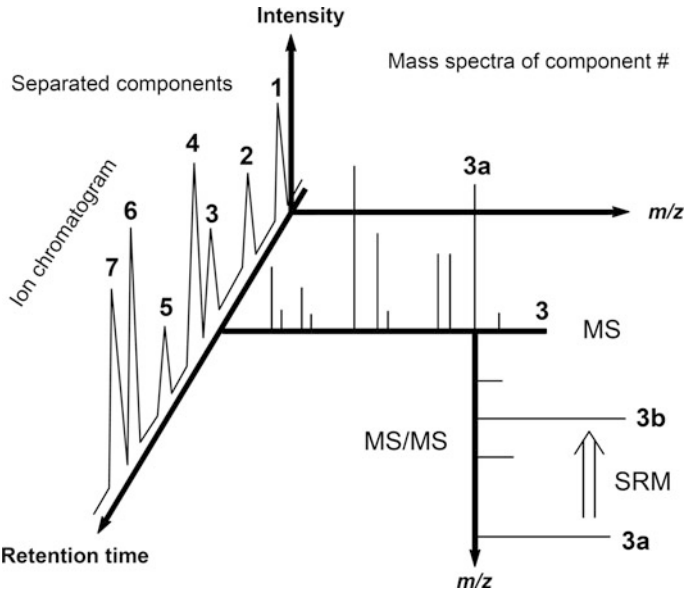


Fig. 14.18 The fourth dimension added by chromatography-tandem MS. Compound *3* has been chosen to illustrate the effect of selectively measuring the fragmentation of one precursor ion, *3a*, defined by its *m*/*z*. If the instrument is operated as to only detect one characteristic product ion, *3b*, the mode is termed *selected reaction monitoring* (SRM)

Table 14.2 Discontinuous modes of operation

Acronym	Full name	Comment
SIM	Selected ion monitoring	In practice used interchangeably; the term SIM is recommended.
MID	Multiple ion detection	
HR-SIM	High-resolution selected ion monitoring	To avoid isobaric interferences
SRM	Selected reaction monitoring	SIM in a tandem MS mode
MRM	Multiple reaction monitoring	More than one reaction is monitored per cycle

dissociated to yield nonetheless characteristic $[M-Br+O]^-$ and $[M-CH_3]^-$ ions. The bromine content resulted in clearly visible isotopic patterns.

14.3 Quantitation

Every ionization method exhibits compound-dependent ionization efficiencies (Sect. 2.3). Whether a specific compound is rather preferred or suppressed relative to another greatly depends on the ionization process employed to deliver the ions to the mass analyzer. These circumstances require a careful calibration of the

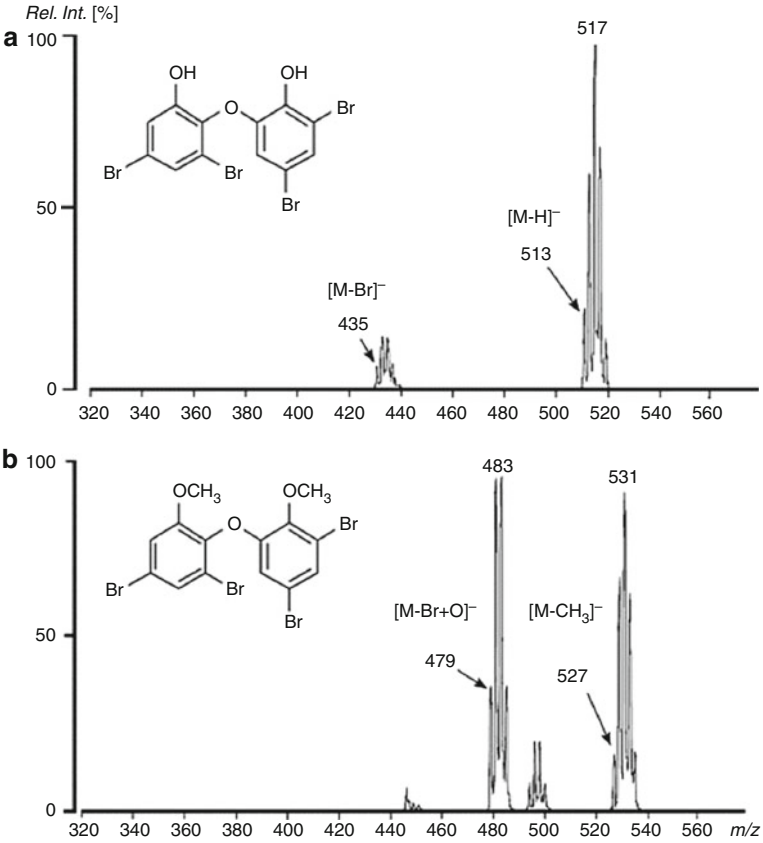


Fig. 14.19 Negative-ion Br^- precursor ion scan APCI spectra of (**a**) hydroxylated and (**b**) methoxylated tetrabromodiphenylether. The four bromine atoms are revealed by the corresponding isotopic patterns. The peak labels point at the peaks of the respective monoisotopic ions (Adapted from Ref. [57] with permission. © Springer, 2012)

instrument's response versus the sample concentration for correct *quantitation* [5, 7, 58]. While relative signal intensities are perfect for qualitative analysis, i.e., for compound characterization, some means of measuring absolute intensities would be preferred in quantitation. Basic considerations on how to approach a quantitative analysis by mass spectrometry are given below [59–62]. Readers interested in a treatment of all aspects of quantitative analysis by mass spectrometry may refer to the highly recommended book by Boyd, Basic, and Bethem [58].

14.3.1 Quantitation by External Standardization

External standardization is obtained by constructing a *calibration curve*, i.e., plotting measured intensities versus rising concentration of the target compound. Calibration curves are generally linear over a wide range of concentrations. When the concentration approaches the detection limit (Sect. 1.6) the graph deviates from linearity, either towards underestimation or towards overestimation. Underestimation can be due to losses by adsorption, overestimation may either be due to "memory" from previous injections or result from chemical background. In the regime of high concentration, saturation of the detector or of the ion source cause an upper limit (Fig. 14.20). The preparation of a calibration curve requires repeated measurements which can be very time-consuming in case of a slow chromatographic separation. In addition, drifts in instrument sensitivity, e.g., due to ion source contamination, can deteriorate the quantitation result. Depending on the instrument's dynamic range, the range of linear response is in the order of two to four orders of magnitude.

LOD versus LOQ

In practice, four or five runs at increasing concentration of the standard, preferably from well above the detection limit to close to saturation, should be run in a sequence to obtain the data for the construction of a calibration curve. Extrapolation to concentrations outside the range covered has to be strictly avoided. One should also bear in mind that the *limit of quantitation* (LOQ) of a compound is found at higher concentration than its *limit of detection* (LOD).

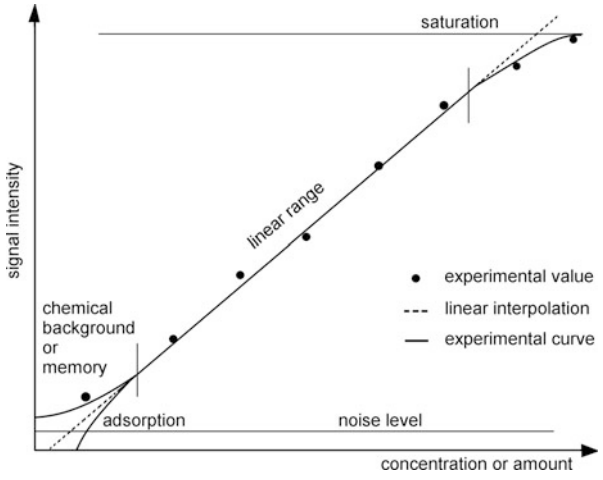


Fig. 14.20 General appearance of a calibration curve. The upper limit of the linear range is defined by saturation, the lower by memory and chemical background or adsorption. In addition, the noise level plays a role for the detection limit

14.3.2 Quantitation by Internal Standardization

Internal standardization circumvents the effects of time-variant instrument response, but does not compensate for different ionization efficiencies of analyte and standard. For internal standardization, a compound exhibiting close similarity in terms of ionization efficiency and retention time is added to the sample at a known level of concentration, e.g., an isomer or a homolog eluting closely to the analyte may serve that purpose.

It is important to add the internal standard before any clean-up procedure in order not to alter the concentration of the analyte without affecting that of the standard in the very same way. For reliable results, the relative concentration of analyte and standard should not differ by more than a factor of about ten.

14.3.3 Quantitation by Isotope Dilution

Virtually identical ionization efficiency for a pair of compounds is only given for isotopologs. As these differ from the nonlabeled target compound in mass, they can be added to the mixture at known concentration to result in a special case of internal standardization, hence the term *isotope dilution* [63]. The ratio of intensities of the peaks corresponding to target compound and labeled standard as delivered by the RICs, SIM, or MRM traces is then taken as the relative concentration of labeled internal standard and target compound. As the absolute concentration of the standard added before the analysis is known, the concentration of the analyte can reliably be calculated.

Drug metabolism A potential drug (M) and its metabolite (M') in a liver sample are quantified by LC-ESI-MS with internal standardization using trideuterated standards for each analyte (Fig. 14.21) [64]. Under these conditions it neither presents a problem that both analytes and their isotopic standards are almost co-eluting from the LC column, nor does the completely unspecific TIC play a role. If required for sensitivity reasons, this analysis could also have been performed in the SIM mode using the m/z values of the RICs shown.

Time windows in SIM The number of m/z values to be monitored in SIM is limited. Such limitations are more severe when additional lock mass peaks have to be included in case of HR-SIM. Therefore, it is commonplace to monitor different sets of SIM traces during consecutive time windows leading to a sequence of different SIM setups during a single chromatographic separation. The quantitation of halogenated dibenzo-*p*-dioxins in municipal waste incinerator fly ash at concentrations in the ppb to low-ppm range requires such a setup (Fig. 14.22) [48]. Here, a combined approach of external standardization for the $BrCl_3$-species and internal standardization for the Cl_4-species has been realized.

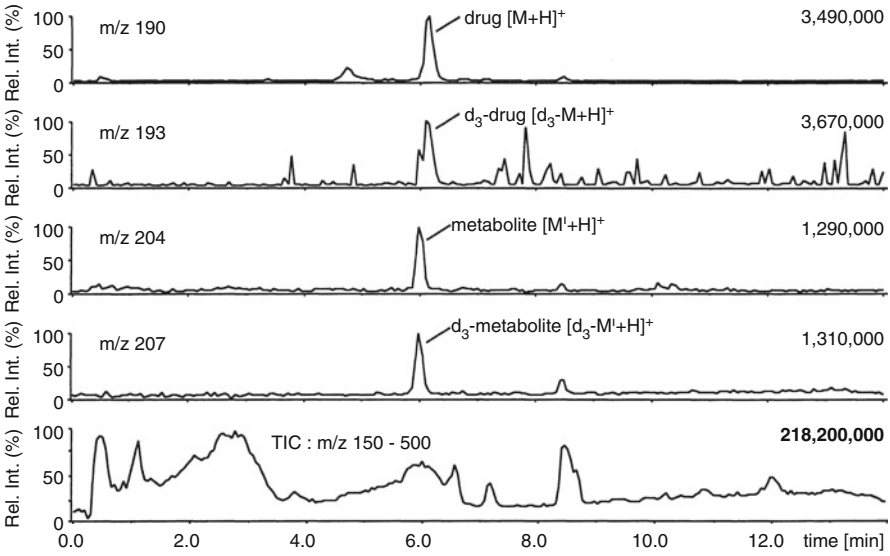


Fig. 14.21 RICs and TIC of an LC-ESI-MS quantification of a drug, $[M+H]^+$ at *m/z* 190, and its metabolite, $[M'+H]^+$ at *m/z* 204, by isotope dilution with trideuterated standards. The numbers on the top right of each trace give their absolute intensity values (Reproduced from Ref. [64] by permission. © Elsevier Science, 2001)

Getting isotopically labeled standards

According to the enormous usage of isotope dilution, ^{2}H- (D) and ^{13}C-labeled standards [65] are commercially available for a wide range of applications. Often, a methyl is exchanged for a trideuteromethyl as this safely avoids overlap with isotopic peaks of the analyte. It is also important not to select compounds with acidic hydrogens exchanged for deuterons. Other restrictions for internal standards also apply.

14.3.4 Retention Times of Isotopologs

Isotopologs exhibit somewhat different retention times in chromatography. Deuterated compounds, for example, elute from chromatographic columns at slightly shorter retention times than their nonlabeled isotopologs. Normally, the difference is less than the peak width of the corresponding chromatographic peaks, but may still be large enough to require their integration over separate time windows (Fig. 14.23).

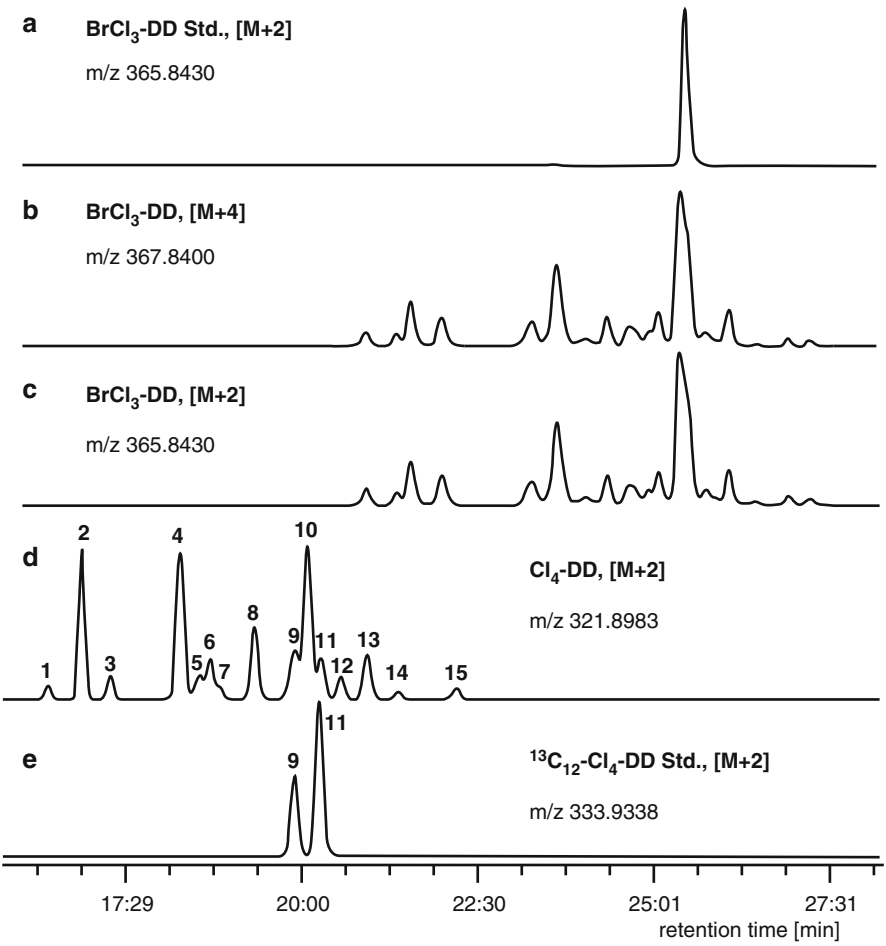


Fig. 14.22 Quantification of $BrCl_3$- and Cl_4-dibenzodioxins by HR-SIM. A $^{13}C_{12}$-labeled internal standard is added for the Cl_4-congener (Adapted from Ref. [48] by permission. © American Chemical Society, 1991)

14.4 Gas Chromatography-Mass Spectrometry

14.4.1 GC-MS Interfaces

The advent of capillary GC had a strong influence on the further development of GC-MS [66–70]. Capillary columns are operated at gas flow rates in the order of 1 ml min^{-1}, and therefore can be directly interfaced to EI or CI ion sources [71, 72]. Although the pressure inside the ion source rises due to the continuous gas load, the conditions for EI are still properly maintained. Sufficient pumping

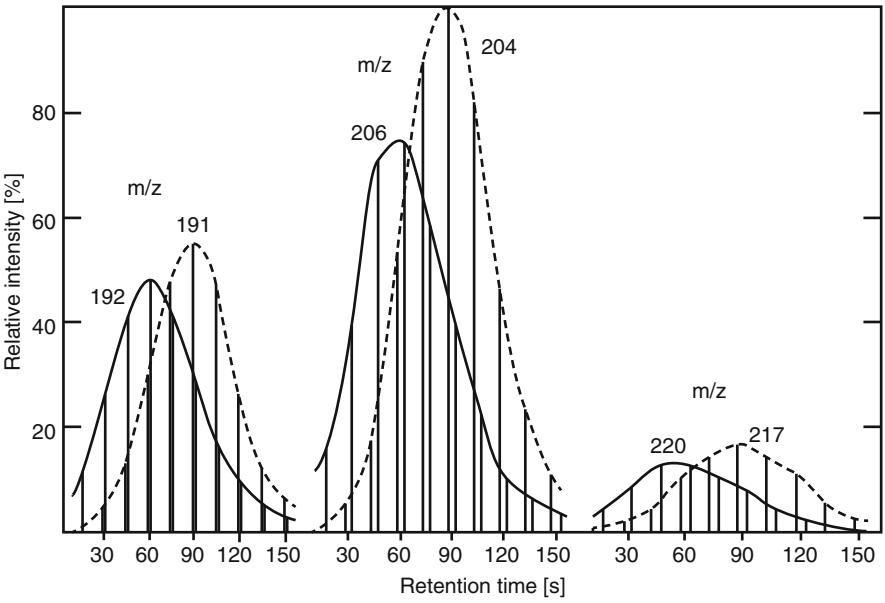


Fig. 14.23 Shorter retention times are observed for [D_7]glucose than for glucose eluting from a SE30 GC column as demonstrated for three pairs of peaks (Reproduced from Ref. [40] by permission. © American Chemical Society, 1966)

speed ($\approx 200\ l\ s^{-1}$) is recommended to keep the pressure in the ion source housing below 10^{-5} mbar. Helium is the preferred carrier gas for GC-EI-MS. If CI is intended, hydrogen or methane may be used instead as these can at the same time serve as reagent gas.

To interface the GC column to the ion source the capillary column is guided by a heated glass line (Fig. 14.24). The evacuated glass line is sealed toward the atmosphere by a graphite ferrule where the GC capillary leaves the oven. Guided by the glass line the exit of the capillary column is positioned at the entrance of the ionization volume. Most EI or CI ion sources have a dedicated entrance port opposite the direct probe port for that purpose (Sect. 5.2). Thus, a modern GC-MS interface basically consists of a heated glass line bridging the distance ($\approx$ 30 cm) between GC oven and ion source. The interface should be operated above the highest temperature employed in the actual GC separation or at the highest temperature the column can tolerate (200–350 °C). Keeping the transfer line at lower temperature causes condensation of eluting components inside the end of the column.

In contrast to sample introduction via direct probe, the components eluting from a GC capillary are quantitatively transferred into the ion source during a short time interval just sufficient to acquire about five mass spectra. Consequently, the partial pressure of the analyte is comparatively high during elution for sample amounts in the picogram range to be analyzed by capillary GC-MS.

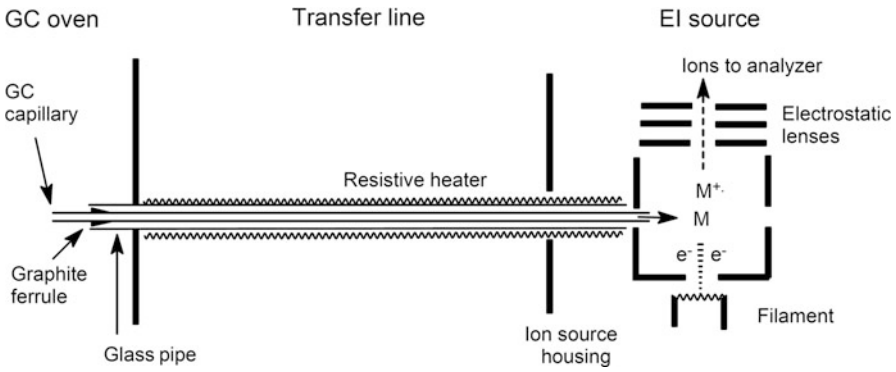


Fig. 14.24 Interfacing a capillary GC column to an EI ion source

While the market share of mass spectrometers with atmospheric pressure interface still increases, the fraction of instruments with EI sources is diminishing. It is therefore of interest to devise a means of coupling gas chromatography to APCI or APPI, for example. This can readily be accomplished by mounting the exit of the GC column into the APCI source and releasing the GC eluate into the ionization zone, which is exactly the way how ASAP is performed (Sect. 13.7) [73–75]. In recent years, GC-APCI and related techniques have found widespread application [76].

14.4.2 Volatility and Derivatization

Gas chromatography requires a certain level of volatility and thermal robustness of the analyte. Both injection block of the gas chromatograph and interface to the mass spectrometer ion source are always at high temperature even while the column oven is not. In order to adapt an analyte to these needs, *derivatization* is well established [77, 78], the most frequent derivatization procedures being silylation, acetylation, methylation, and fluoroalkylation. As derivatization transforms XH groups into $XSiR_3$, XC=OMe, XMe, or $XCOCF_3$ groups, for example, polarity of the molecules largely decreases. This causes an improved volatility even though the molecular weight increases upon derivatization. Derivatization suppresses thermal decomposition, e.g., it protects alcohols from thermal dehydration. In particular fluoroalkyl and fluoroaryl groups are extremely useful to improve detection limits in EC-MS [38, 79, 80]. There is a complete series of reviews by John Halket covering the different derivatives and their uses in mass spectrometry [81–88].

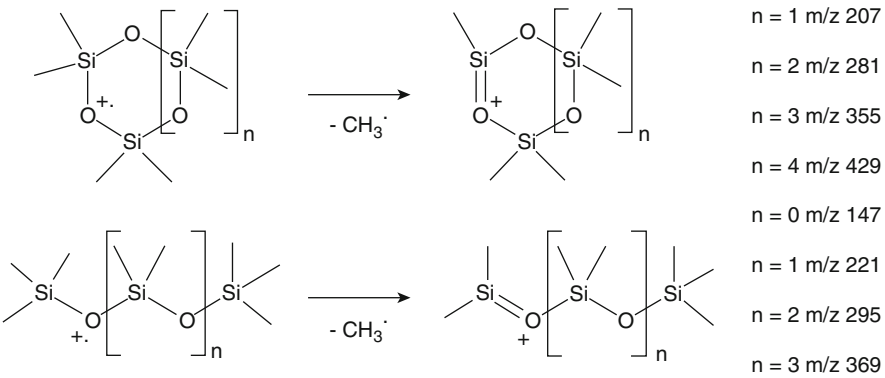
Derivatization can also introduce functional groups that induce a controlled fragmentation of the ion [89], e.g., by α-cleavage at the new functional group. This way, 3-picolinyl esters of fatty acids reveal double bond positions because the charge localizing substituent largely reduces double bond rearrangement prior to

fragmentation. The alternative use of dimethyldisulfide adducts for double bond localization has already been explained in Sect. 6.5.2.

14.4.3 Column Bleed

Rising temperature of the GC column not only assists transport of less volatile components, it also causes the slow release of the liquid phase from the inner wall of the capillary. As a result of slow thermal degradation, even chemically bonded liquid phases show such *column bleed* at elevated temperature. It is a characteristic of column bleed that it continuously rises as the temperature of the GC oven is raised and it falls again upon cooling of the system. Of course, the peaks from column bleed observed in the mass spectrum depend on the liquid phase of the GC capillary in use. In case of the frequently employed methyl-phenyl-siloxane liquid phases, abundant ions at *m*/*z* 73, 147, 207, 281, 355, 429, etc. are observed. Within one series the peaks are aligned at $\Delta(m/z) = 74$ ($OSiMe_2$) and exhibit characteristic silicon isotopic patterns. Fortunately, column bleed is easily recognized and can be removed by careful background subtraction. Similar background ions are also obtained from *septum bleed* and silicon grease [7, 90] (Scheme 14.1).

Subtracting column bleed A portion of the TIC as obtained by GC-EI-MS of an unknown mixture on a 30-m HP-5 capillary column shows a rather small chromatographic peak at a retention time of 32.6 min (Fig. 14.25), while the chromatogram shows a rather high baseline due to continuous elution of column material. Extracting scan No. 2045 at the GC peak's apex yields a spectrum that is dominated by background ions from column bleed. This becomes obvious by averaging scans 2068 to 2082, which delivers the spectrum of column bleed alone. Averaging of all scans covering the GC peak followed by background subtraction ((2035:2052)–(2013:2020)) delivers a mass spectrum of reasonable quality, even though some background peaks are still present.



Scheme 14.1

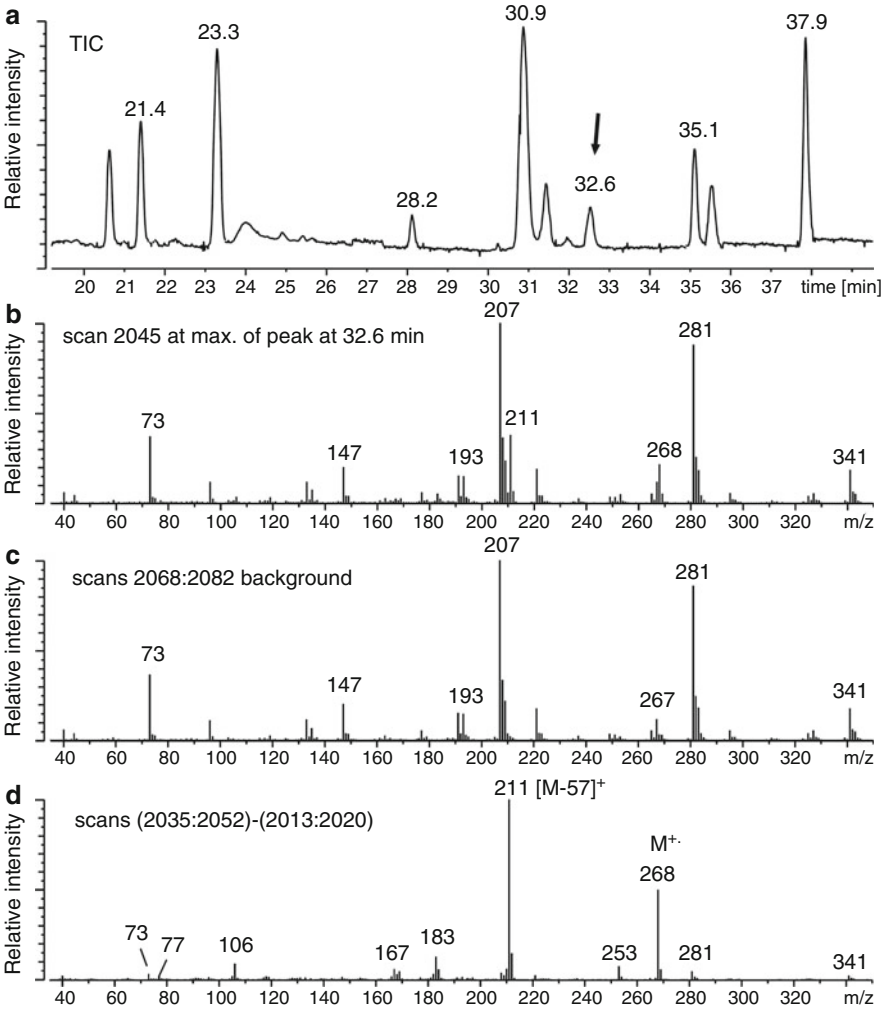


Fig. 14.25 Background subtraction to remove peaks from column bleed in GC-MS of an unknown mixture. (**a**) Partial TIC, (**b**) single scan mass spectrum from peak top at 32.6 min, (**c**) summation of scans from pure column bleed between GC peaks (cf. Scheme 14.1), and (**d**) final spectrum after averaging of scans and background subtraction

14.4.4 Fast GC-MS

When a high sample throughput is of importance, *fast GC-MS* offers a time-saving concept for mixture analysis [91–93]. The GC separation can be accelerated by replacing standard size (20–60 m × 0.25–0.53 mm i.d.) capillary columns by short narrow-bore columns (2–5 m × 50 μm i.d.) and by applying sufficient pressure

(8–10 bar) and rapid heating (50–200 °C min^{-1}). Thus, a conventional 30-min separation is compressed into a 3-min or even shorter time frame. However, in fast GC the half-life of an eluting peak is too short for use with scanning quadrupole or magnetic sector analyzers. In fast GC-MS, oaTOF analyzers (Sect. 4.2.8) are typically employed because of their ability to acquire about a hundred spectra per second. Furthermore, the high duty cycle of oaTOFs offsets the difference in sensitivity between repetitive scanning and SIM analysis. At somewhat lower rates, advanced oaTOF systems even enable accurate mass determination at good accuracy.

14.4.5 Multiplexing for Increased Throughput

Even in fast GC operation the capacity of the capillary column is not fully exploited, because gaps between loaded zones inevitably occur as the separation proceeds. Hadamard transform GC can be applied to improve the duty cycle (Sect. 4.2.10) of GC separations, i.e., the next injection onto the column is made long before all components of the preceding injection have left the column. In contrast to conventional chromatographic separation, the chromatogram then consists of several chromatograms overlapping in time. Key to mathematical deconvolution by Hadamard transform is to inject rapidly and precisely by a *multiplexing* injector according to an *n*-bit pseudorandom sequence (Fig. 14.26) [94, 95]. These pseudorandom injections are further divided into subsets of different concentrations, which may later serve for control of the time-shifted repetitive injections. Thus, a computer-controlled injection sequence combined with Hadamard transform of the detector output delivers the concentrations of all components at enhanced sample throughput. Using a multiplexing injector capable of up to 3000 injections per hour allowed for a 50% duty cycle of the chromatograph to be achieved.

Multiplexing means simultaneous runs
The term *multiplexing* refers to the use of one separation device for multiple samples at the same time. To achieve an improved duty cycle of the device multiplexing requires a complex mathematical deconvolution of the detector output. In contrast, *multiplexed* chromatographic systems are using one detector for multiple parallel chromatographs in a simple alternating mode (Sect. 14.4.2). Multiplexed systems use high-speed detection and are based on the assumption not to miss relevant information while the detector connects to other lines between sampling intervals.

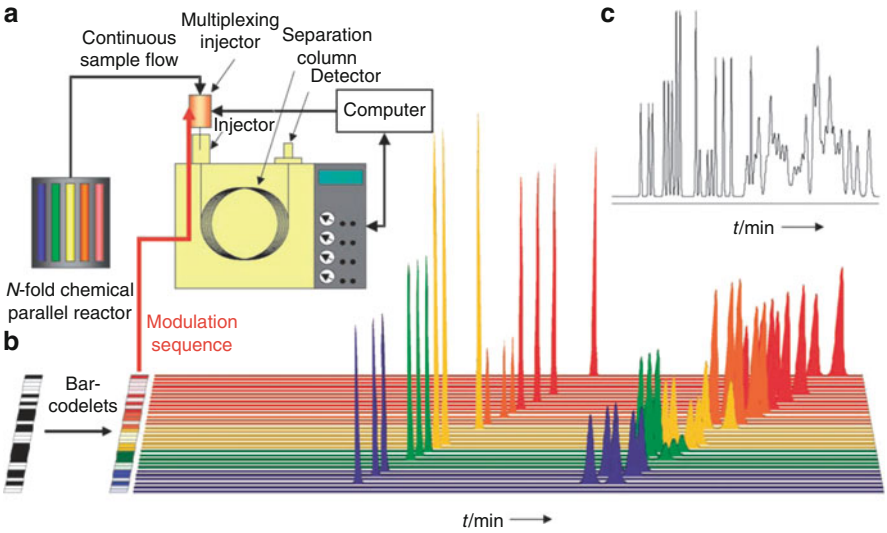


Fig. 14.26 High-throughput multiplexing gas chromatography. (**a**) Experimental setup for the analysis of the sample composition from an *N*-fold parallel reactor. The multiplexing injector is loading the column by short pressure pulses (1–5 ms) according to an *n*-bit binary pseudorandom sequence. (**b**) Temporally shifted chromatograms obtained by repetitive sample injections following the bar codelets of an *n*-bit sequence. (**c**) Convoluted chromatogram which is the sum of the chromatograms shown in (**b**) (Reproduced from Ref. [95] with permission. © Wiley-VCH, 2007)

14.4.6 Comprehensive Gas Chromatography-Mass Spectrometry

Like tandem MS, GC × GC adds an analytical dimension (Sect. 14.1.9). GC-MS/MS and GC × GC-MS are thus complementary in that they both provide four-dimensional analysis, either with an emphasis on separation or on enhanced-level mass analysis. The extremely high spectral acquisition rate required for GC × GC-MS does not permit GC × GC-MS/MS, however. As the mass analyzer for GC × GC-MS needs to be extremely fast, GC × GC-MS is the domain of TOF analyzers. Instruments optimized for high spectral acquisition rates are also available, like the LECO Pegasus 4D series.

Pyrolysis products from recycling of tires Pyrolysis of used tires is a promising recycling technology that yields pyrolysis liquids, carbon black, and steel [96–98]. These pyrolysis liquids are complex mixtures of organic compounds and can serve as a valuable resource similar to crude oil.

GC × GC-MS of a pyrolysis liquid was performed using a Pegasus 4D TOF analyzer and a gas chromatograph with a liquid nitrogen cryogenic modulator. A polar first column (Rxi-17SilMS, 30 m × 0.25 mm) and an apolar second column (SLB-5MS, 1.5 m × 0.1 mm) were used, i.e., different from common GC × GC conditions in reverse order of polarity [98]. A sample of 1 µl was injected at 280 °C. The GC oven program started at 35 °C and was held there for 5 min. Then, the

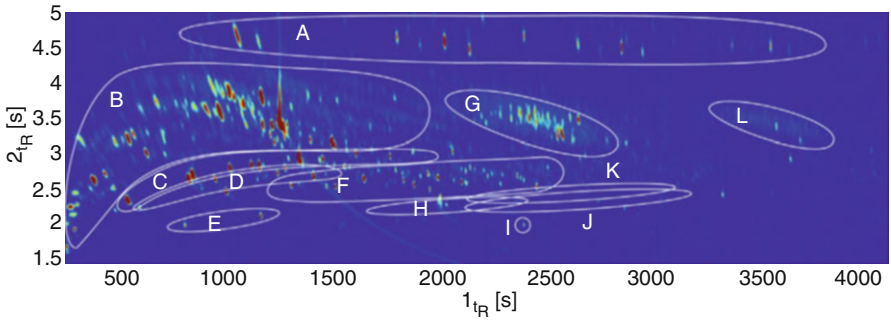


Fig. 14.27 Elution pattern of all compounds in pyrolysis liquid from tyre. The compounds are grouped as follows: *A* Alkanes, *B* cyclic hydrocarbons, *C* monocylic aromatic hydrocarbons, *D* thiophenes, *E* cycloalkanones, *F* styrenes, indanes, and indenes, *G* unidentified compounds, *H* phenoles, *I* benzothiazole, *J* benzothiophenes, *K* polycyclic aromatic hydrocarbons, and *L* unidentified compounds (Reproduced from Ref. [98] with permission. © Elsevier, 2015)

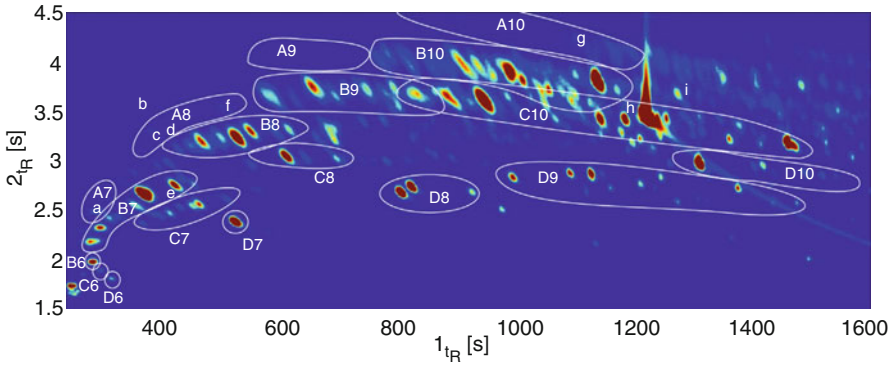


Fig. 14.28 Zoomed-in view of the elution area of hydrocarbon compounds with r + d = 1–4. The letters denote the number of r + d (A : 1, B : 2, C : 3, D : 4) and numbers denote the carbon number. The plot is based on ions of *m*/*z* 55, 67, 79, 81, 83, 91, 105, 119, 133. The positions of reference compounds are denoted by lower case letters (a: methylcyclohexane, b: octane, c: 1-octene, d: 3-octene, e: 1-methylcyclohexene, f: ethylcyclohexane, g: butylcyclohexane, h: limonene, i: *trans*-decahydronaphthalene) (Reproduced from Ref. [98] with permission. © Elsevier, 2015)

primary oven was ramped up to 300 °C at 3 °C min^{-1} and held there for another 10 min. The second dimension oven was set higher relative to the primary oven by 5 °C and the modulator higher by 40 °C.

The GC × GC-MS data can be displayed in various ways. It may reveal elution areas on the ^2t_R versus ^1t_R plane sorted by compound classes (Fig. 14.27), which, of course, requires proper selection of ions specific for each compound class of interest. Alternatively, the data can, for example, be displayed to show the elution pattern of hydrocarbons with r + d = 1–4, which is achieved by selecting ions of *m*/*z* 55, 67, 79, 81, 83, 91, 105, 119, 133 and zooming in to first-dimension retention times below 1600 s (Fig. 14.28). This demonstrates the enormous depth of

analytical information contained in a single GC × GC-MS run. It also shows the need to examine the data in a selective manner, e.g., by looking at compound classes, levels of r + d, or whatever may be of interest.

14.5 Liquid Chromatography-Mass Spectrometry

Coupling of liquid chromatography (Sect. 14.1.10) to mass spectrometry has not only led to a wide variety of interfaces, it also fostered the development of new ionization methods (Sects. 7.8 and Chap. 12) [8–10, 99–102]. ESI, APCI, or APPI are suitable for LC-MS, their selection depending on sample properties like molecular mass and polarity (Sect. 12.7).

Today, LC-MS either refers to *high-pressure liquid chromatography* (HPLC) or *ultrahigh-pressure liquid chromatography* (UHPLC). For very low sample amounts, nanoLC, i.e., capillary LC, can directly be interfaced to nanoESI.

In the analytical practice, LC-MS plays a tremendous role that could be testified here by a large number of applications [8, 58, 103, 104]. However, other than proper adjustment of the interface to the liquid flow from the chromatograph the operation of LC-MS does not require dedicated techniques on the MS side. All scanning and ion monitoring techniques discussed so far in this chapter can equally well be employed for LC-MS analyses. Three examples shall highlight representative applications of LC-MS and LC-MS/MS.

Impurities in pharmaceutical preparations UV photodiode array (PDA) and ESI-TOF detection can be combined if the LC effluent is split or the PDA precedes the ESI interface. The detection methods complement each other in that their different sensitivities towards components of a mixture prevent substances from being overlooked. RICs help to differentiate a targeted compound – an unknown impurity in this case – from others and to identify eventually present isomers. Finally, accurate mass measurement helps in the identification of the unknown, the $[M+H]^+$ ion of which was assigned as $[C_{20}H_{21}N_{10}]^+$. The mass error in the order of 5 ppm was considered perfect for the oaTOF instruments of that time (Fig. 14.29) [64].

Illicit drugs in wastewater An LC-ESI-MS/MS method for the simultaneous determination of 16 illicit drugs and their metabolites in wastewater was developed [105]. Based on tandem mass spectra of the compounds, MRM experiments were set up to detect each drug by a characteristic transition (Fig. 14.30). Among others, cocaine, amphetamines, morphine, and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol were found in substantial amounts in waste water treatment plants at Milan (Italy) and Lugano (Switzerland). Quantification was achieved by addition of several deuterated internal standards. Quantification ranges were found to be 0.2–1 ng l^{-1} for cocaine and 60–90 ng l^{-1} for 11-nor-9-carboxy-Δ9-THC. Illicit drugs were therefore considered ubiquitous contaminants, discharged into the environment together with pharmaceuticals presenting an environmental risk. Moreover, the

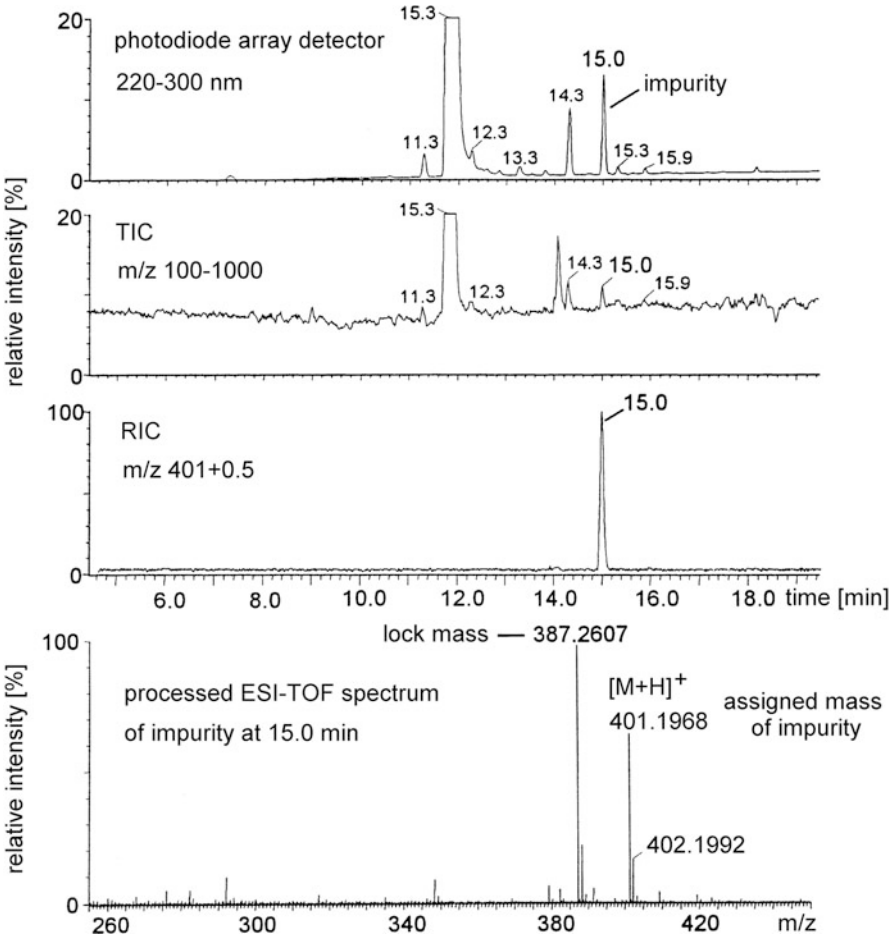


Fig. 14.29 Liquid chromatograms (*from top*) by photodiode array detection, TIC and RIC (*m/z* 401) from LC-ESI-MS, and accurate mass measurement (*bottom*) of the unknown impurity based on a one-point internal mass calibration (Adapted from Ref. [64] by permission. © Elsevier Science, 2001)

concentrations of illicit drugs in wastewater reflect their consumption by the local population.

Peptide identification from biological samples Colorectal cancer cells have been lysed and the protein fraction thereof has been subjected to 2D gel electrophoresis. A gastrointestinal-specific A33 antigen was expected in the molecular mass range of 40–45 kDa. The corresponding slice of the 2D gel was cut out and the proteins on it were subjected to tryptic digestion. Capillary column (150 × 0.2 mm C8 reversed phase) HPLC-ESI-MS of this total tryptic digest delivered a complex and mostly unresolved TIC (Fig. 14.31). A mass spectrum of a retention time span around

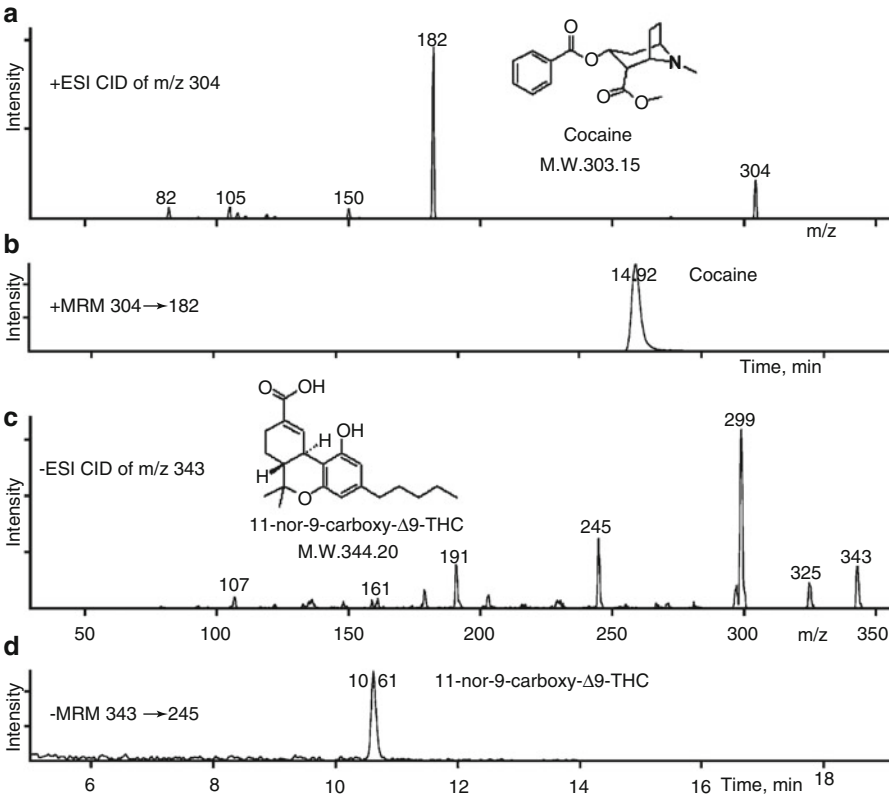


Fig. 14.30 Two examples from the analysis of illicit drugs in waste water by LC-MS/MS and MRM. (**a**) Positive-ion ESI-CID spectrum of the $[M+H]^+$ ion of cocaine and (**b**) the MRM trace derived thereof. (**c**) Negative-ion ESI-CID spectrum of the $[M+H]^-$ ion of 11-nor-9-carboxy-Δ9-THC and (**d**) the corresponding MRM trace (Adapted from Ref. [105] with permission. © American Chemical Society, 2006)

25.20 min still showed numerous peaks of protonated peptides requiring identification by mass spectrometric sequencing. One of these, an $[M+2H]^{2+}$ ion at *m/z* 1198.4, was identified as the A33 antigen peptide YNILNQEQPLAQPASGQPVSLK using SEQUEST database search.

14.6 Ion Mobility Spectrometry-Mass Spectrometry

Ion mobility spectrometry (IMS) presents an effective means of separating gaseous ions in an electric field according to their collision cross sections. From the perspective of its working principle, IMS could be regarded as gas-phase electrophoresis. As IMS is a separation method normally coupled to MS for ion detection, IMS-MS might completely be dealt with in line with GC-MS and LC-MS.

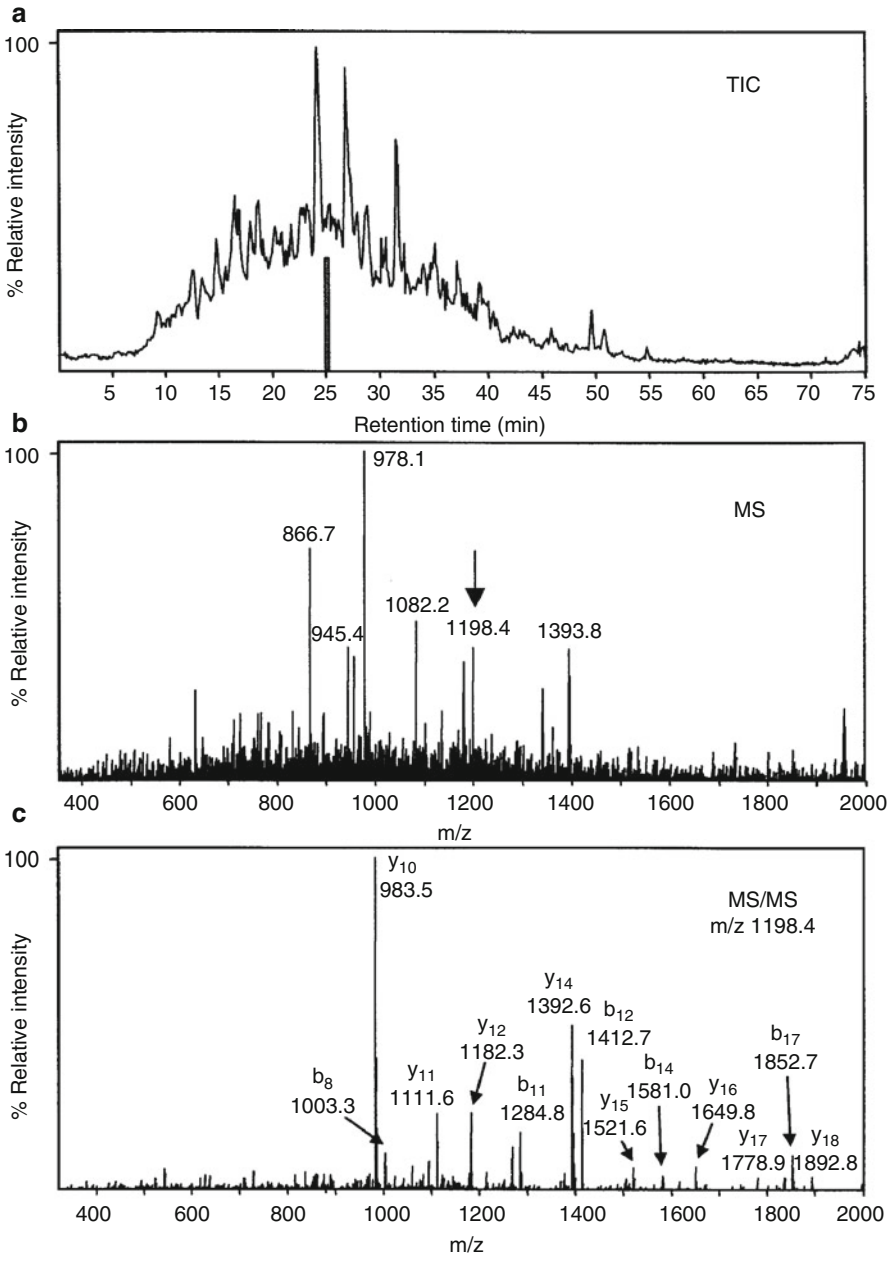


Fig. 14.31 Capillary column HPLC-ESI-MS/MS of a tryptic digest. (**a**) An unresolved TIC trace, (**b**) mass spectrum around retention time of 25.20 min, and **c**) CID spectrum of an $[M+2H]^{2+}$ ion at *m/z* 1198.4, identified as the A33 antigen peptide (Adapted from Ref. [106] with permission of publisher and author. © Wiley-VCH, Weinheim, 2000)

However, IMS is different from GC and LC in that its instrumental implementation is strongly dependent on the features of the mass analyzer attached. Accordingly, we have already discussed IMS-MS systems in the context of instrumentation (Sect. 4.10) [107, 108].

During the last decade or so, IMS-MS instrumentation has changed from custom-built to commercially available instruments. The development started with the Waters Synapt series, now available as Synapt G2-Si, In between, Agilent (Ion Mobility Q-TOF) and most recently Bruker (timsTOF) have also presented instruments in this segment and Waters also offer a second model, the Vion IMS-Q-TOF. While the Synapt G2-Si offers mass-selection in a quadrupole prior to IMS, the latter three instruments essentially have IMS-Q-TOF geometries. The Bruker timsTOF differs from the Waters Vion IMS-Q-TOF and Agilent Ion Mobility Q-TOF in that it employs the more recent technique of *trapped ion mobility spectrometry* (TIMS) [109]. This approach not only offers IMS in a compact and efficient design but also circumvents the need for an additional gas supply by employing the residual gas streaming down a modified ion funnel system for ion mobility separation. TIMS can also be adapted to FT-ICR analyzers [110].

TIMS or TIMS?
Unfortunately, the acronym TIMS is not anymore unequivocal. While TIMS once exclusively referred to *thermal ionization mass spectrometry* (Sect. 15.2), it now also serves to represent *trapped ion mobility spectrometry*. It seems as if the two communities were worlds apart.

To complement the purely instrumental approach to IMS-MS in Sect. 4.10, this section presents three selected applications. IMS-MS can be employed to:

- distinguish compound classes,
- differentiate isomers,
- separate different charge states of one molecular species,
- tell apart different gas phase conformations of otherwise identical ions [107, 111],
- separate chiral compounds when a chiral modifier is added to the inert drift gas [112, 113].

Differentiation of isomers by TIMS-MS Trapped ion mobility spectrometry-mass spectrometry (TIMS-MS) was used for the differentiation between $[M–H]^-$ ions of five isomeric hydroxylated tetrabrominated biphenyl ethers (OH-BDE) generated by negative-ion ESI [114]. TIMS provided baseline separated signals when selected binary and ternary mixtures of these compounds were injected, demonstrating ion mobility resolving power in the order of 400 (Fig. 14.32). The collision cross sections (CCS) of the isomers varied from 194.5 $Å^2$ to 197.2 $Å^2$.

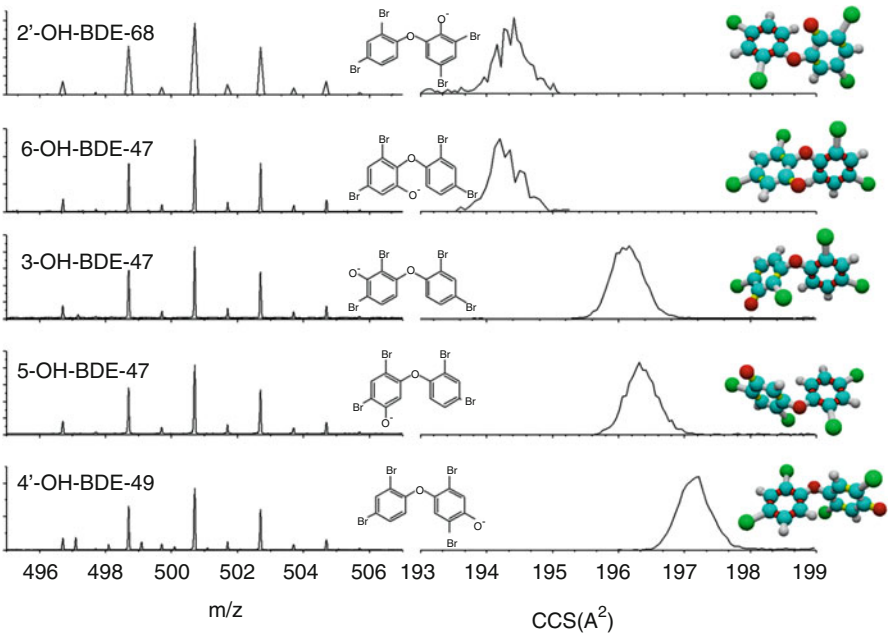


Fig. 14.32 Comparison of $[M–H]^-$ ions of five isomeric hydroxylated tetrabromobiphenyl ethers (OH-BDEs) by their mass spectra (*left column*), trapped ion mobility spectra (*center*), and correlation of IMS data with calculated structures (*right*) (Reproduced from Ref. [114] with permission. © Springer, 2016)

Biomolecule classification by IMS-MS Singly charged ions of biologically relevant molecular classes (96 oligonucleotides, 192 carbohydrates, 610 peptides, and 53 lipids) were generated by MALDI and subjected to IMS. Then a plot of this data, i.e., of their collision cross sections vs. *m*/*z* allowed for the calculation of distinct curves marking average collision cross sections vs. *m*/*z* distinct for each compound class. Figure 14.33 shows a plot of the MALDI-IMS-MS conformation space as obtained for a mixture of model species (roughly a dozen species for each class in the range up to 1500 u) [111]. Dashed lines correspond to regression curves for each molecular class in conformation space. There was some fragmentation of the parent ions causing the signals in the vicinity of the asterisk in the upper left corner of the diagram.

Separation of chiral compounds by IMS-MS Amino acids and other small chiral molecules can be separated by IMS-MS if a chiral modifier is admixed to the inert nitrogen drift gas (Fig. 14.34) [112]. To achieve separation of L- and D-tryptophan, (*S*)-(+)-2-butanol at a concentration as low as 10 ppm has been used as chiral modifier. The differences in drift times of the amino acid enantiomers are large enough to deliver two peaks if the mixture is subjected to chiral IMS.

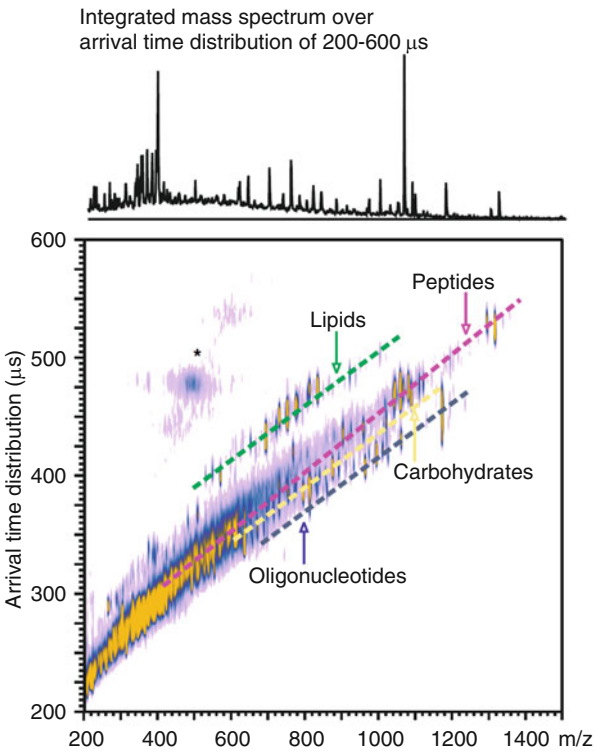


Fig. 14.33 Plot of MALDI-IMS-MS conformation space as obtained for a mixture of biomolecular model species. *Dashed lines* are for visualization purposes of where each molecular class occurs in conformation space. Signals in the vicinity of the *asterisk* arise from limited post-IM fragmentation of the parent ion species (Reproduced from Ref. [111] by permission. © Springer, 2009)

14.7 Tandem MS as a Complement to LC-MS

The principles of tandem mass spectrometry have been discussed in Chap. 9 and many different uses of tandem MS have been shown throughout this book (Table 14.3). More examples are following in this chapter showing how tandem MS offers increased selectivity [115], simplified clean-up procedures, or faster analysis as a direct result of the fourth dimension added to the analytical measurement.

Improved selectivity by tandem MS SIM is not sufficient for the LC-MS detection of 100 pg dextrometorphan (DEX) spiked into 1 ml of human plasma. The corresponding signal of the $[M+H]^+$ ion in the SIM trace at m/z 272 is barely detectable, whereas the SRM chromatogram obtained from the reaction $[M+H]^+ \rightarrow [M–C_8H_{15}N]^+$ shows a clean background and a signal-to-noise enhancement of more than 50-fold (Fig. 14.35) [64].

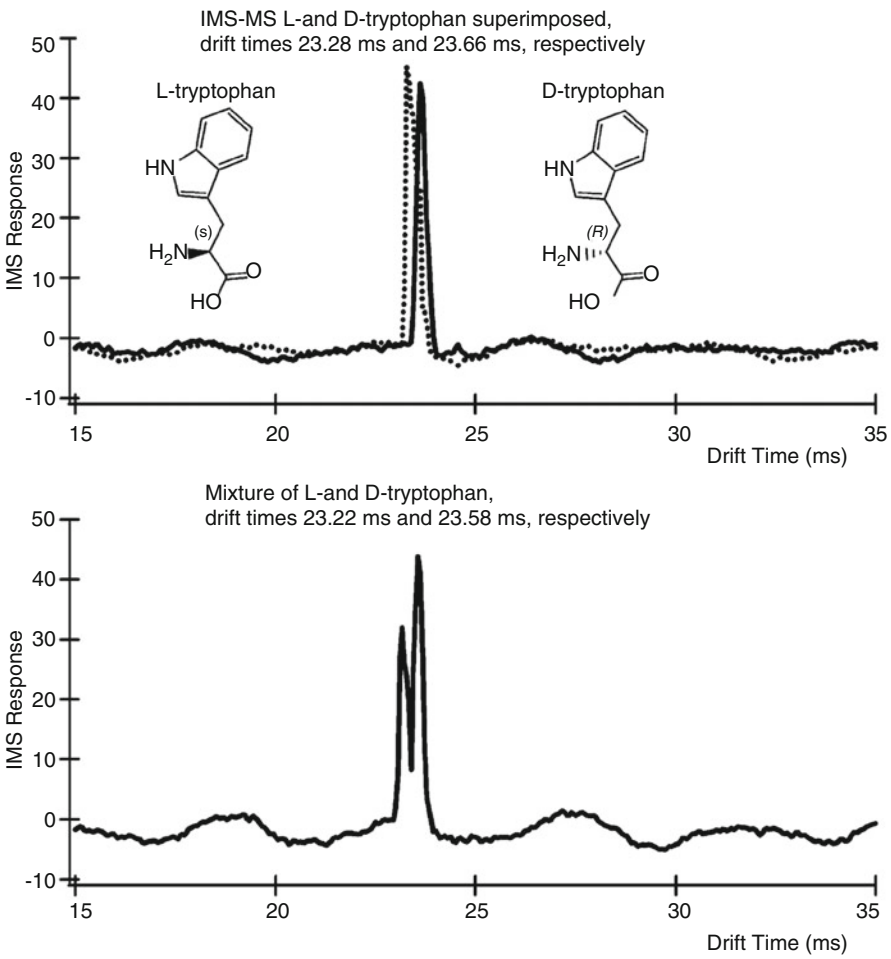


Fig. 14.34 Chiral separation of L- and D-tryptophan by IMS-MS using (*S*)-(+)-2-butanol as chiral modifier of the carrier gas (Adapted from Ref. [112] by permission. © American Chemical Society, 2006)

Suitable instruments

Triple quadrupole instruments are considered as the "gold standard" for quantification, in particular by SRM and even more so MRM. Also relevant are the high linear dynamic range, the ease of setting up SRM and MRM experiments, and the speed of switching between channels when monitoring multiple reactions. More recent QqTOF, QqLIT, and QqOrbitrap hybrids provide basically similar capabilities.

Table 14.3 Tandem MS methods and spectra referred to throughout the book

Section	Aspect of tandem MS
2.9.3	MIKES of amine molecular ions in the context of H/D isotope effects
9.3.2	CID spectrum of toluene molecular ion on a magnetic sector instrument
9.4	SID and CID of noncovalent protein complexes
9.5.1	MALDI-PSD spectrum of a peptide
9.6.5	Tandem MS with magnetic sector instruments; example: $B^2E = constant$ linked scan for caffeine quantitation using [D_3]caffeine internal standard
9.6.6	CID-FAB-MS/MS with magnetic sector instrument for peptide sequencing
9.8	Tandem MS with the quadrupole ion trap; examples: peptide sequencing by ESI-MS and LC-MS^4 to identify cyclic peptides
9.9.1	MS^4 on a QqLIT instrument for structure elucidation of a drug
9.10.1	Peptide sequencing by ESI-CID-MS/MS on LIT-Orbitrap instrument
9.10.2	Parallelized tandem MS in proteomics using a dual-LIT-Orbitrap instrument
9.11.1	Sequencing of an oligosaccharide by MALDI-SORI-FT-ICR
9.14.1	ESI-IRMPD-MS/MS of gangliosides on a FT-ICR instrument
9.14.4	ECD of doubly charged peptide ions on a FT-ICR instrument
9.15	ETD of triply protonated peptide ions on a LIT instruments
9.17	Ion–molecule reactions: in catalysis research, to elucidate peptide fragmentation, and to study short-lived species in the gas phase
11.6.3	Peptide sequencing; mechanisms and applications of MALDI-MS/MS
11.6.4	Structure elucidation of carbohydrates by PSD-MALDI-MS
11.6.7	Peptide sequencing by DIOS-TOF-MS/MS
12.3	Peptide sequencing by nanoESI-MS/MS
12.6.4	Oligonucleotide sequencing by ESI-IRMPD-FT-ICR-MS
12.6.5	Structure elucidation of a nonasaccharide by nanoESI-CID-MS/MSon a Q-TOF hybrid instrument
13.2.4	DESI-MS/MS of aspirin and of alkaloids from *Atropa belladonna* seeds

In tandem MS each stage of mass analysis provides an added step of selectivity or structural information to the analysis. Therefore, one tandem MS stage is to a certain degree equivalent to a chromatographic separation, provided the separation of isomers is not required. While chromatography separates components by their retention time, tandem MS isolates them by m/z. The next example illustrates how far MS/MS alone may go. The analysis presented in example III in Sect. 14.4, however, would have been impossible without using LC prior to MS/MS.

Tandem MS as a substitute for LC Akt is a key serine/threonine kinase controlling cellular processes such as cell survival, differentiation, proliferation, and metabolism. Three isoforms (Akt1, Akt2, and Akt3) are known in mammals. The nanoESI mass spectrum of all peptides obtained upon digestion of Akt3 with pepsin (peptic peptides) showed a large number of multiply charged ions (Fig. 14.36) [116]. The peptides were then characterized by tandem MS. The $[M+3H]^{3+}$ ions at m/z 797, for example, corresponded to a peptide of a neutral mass of 2388 u. As low-abundant peptides were difficult to identify by nanoESI-MS/MS

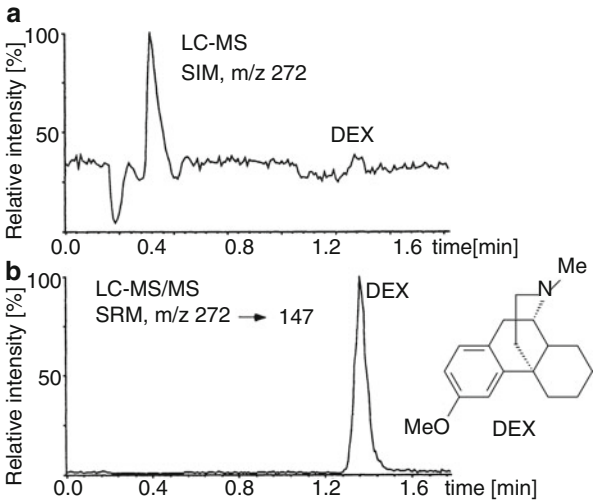


Fig. 14.35 The effect of SRM as compared to SIM in the detection of dextrometorphan. (**a**) LC-SIM and (**b**) LC-SRM (Reproduced from Ref. [64] by permission. © Elsevier Science, 2001)

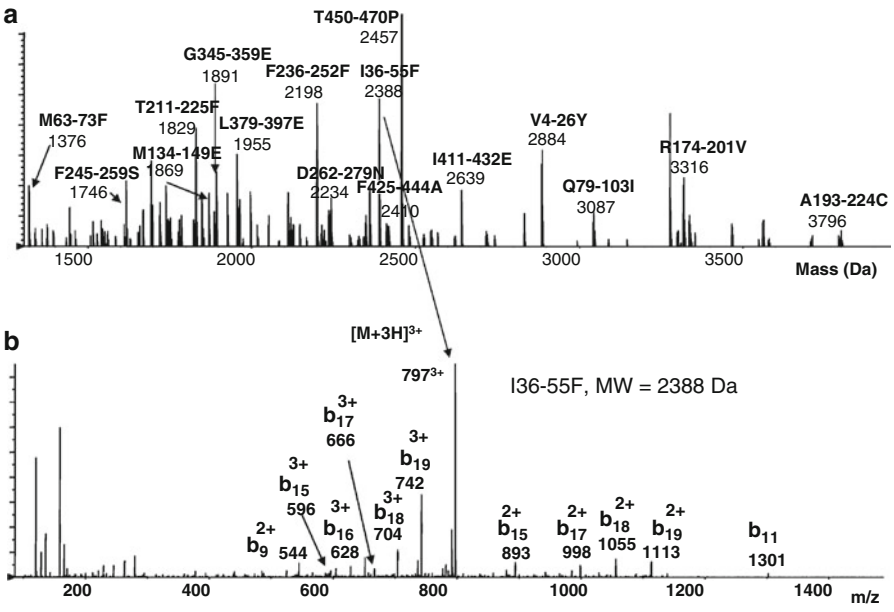


Fig. 14.36 Analysis of Akt by nanoESI-MS/MS. (**a**) Charge deconvoluted nanoESI spectrum of the complete peptic digest. (**b**) Tandem mass spectrum of the $[M+3H]^{3+}$ peptic peptide ion (I36-55F) at *m*/*z* 797 corresponding to a neutral peptide of 2388 u (Reproduced from Ref. [116] with permission. © John Wiley & Sons Ltd, 2009.)

alone, nanoLC/ESI-MS/MS was used in addition. Combining these approaches, a total of 24 peptide peaks were identified in both inactive and active Akt covering 70% of the amino acid sequence of Akt.

14.8 Ultrahigh-Resolution Mass Spectrometry

High and, in particular, ultrahigh resolution (Sect. 3.7) in combination with a soft ionization method such as ESI, APCI, MALDI, or FD present another way to achieve the separation of the molecular species contained in a mixture. Given a sufficient level of resolution, isobaric ions are displayed separately in the range of their common nominal mass value (Sects. 3.3 and 3.4).

The potential of ultrahigh-resolution mass spectrometry for the analysis of complex chemical mixtures is particularly illustrated by FT-ICR-MS, which currently is the definite standard. Ultrahigh resolution was applied to separate several thousand components in crude oil [117, 118], fuels [119, 120], explosion residues [121], brown coal [96], or aroma of Scotch Whisky [122]. Also, the attempts to analyze dissolved organic matter (DOM) and similar complex systems present another prominent field of application requiring the ultimate performance of FT-ICR instruments equipped with magnets of up to 15 T [123, 124].

South American crude oil In a sample of South American crude oil positive-ion ESI selectively delivers $[M+H]^+$ ions of the basic compounds, i.e., only a small fraction of the entire chemical composition. Nevertheless, the positive-ion ESI-FT-ICR mass spectrum exhibits more than 11,100 resolved peaks, of which >75% may be assigned to a unique elemental composition ($C_cH_hO_oN_nS_s$). Such a separation in mass is possible because the average mass resolution in the *m*/*z* 225–1000 broadband spectrum is approximately 350,000 (Fig. 14.37). This demonstrates the current upper limit for the number of chemically distinct components resolved and identified in a single step [118].

Molecular analysis of oceanic DOM *Dissolved organic matter* (DOM) substantially contributes to biomass on Earth and presents a highly complex system of mostly polar organic compounds. It requires a multi-step approach to acquire an insight into the molecular structures of DOM [124]. As a first step, reversed-phase chromatography provides bulk molecular information, but is not capable of separating individual components (Fig. 14.38). Second, the nominal mass distribution may be determined using soft ionization methods and low-resolution MS. Next, ultrahigh-resolution FT-ICR-MS separates nominally isobaric molecules, often more than a dozen per nominal mass, and delivers molecular formulas. As composition alone does still not permit the elucidation of structures, nuclear magnetic resonance (NMR) spectroscopy can be employed to reveal structural features that may, in turn, be correlated to molecular formulas.

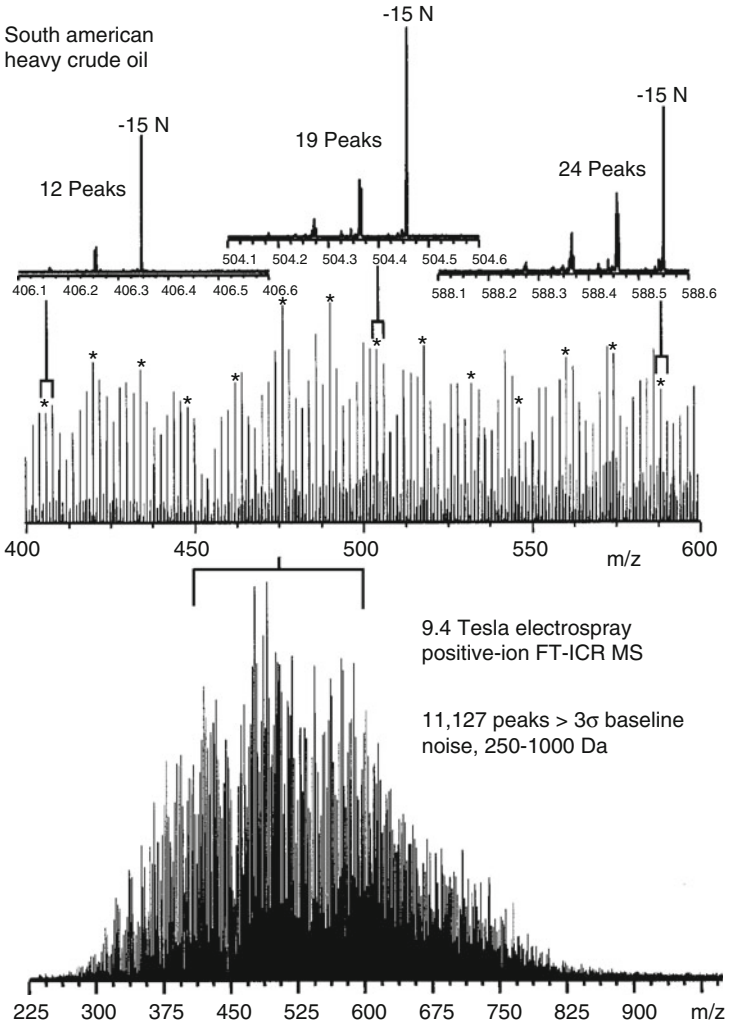


Fig. 14.37 Positive-ion ESI-FT-ICR spectrum of crude oil. The mass scale is successively expanded from the broadband spectrum to a more detailed one at three nominal mass values (Adapted from Ref. [118] by permission. © American Chemical Society, 2002.)

14.9 Summary of Hyphenated Techniques

Separation Techniques

There are numerous separation techniques available that can deal with any range of molecular mass of an analyte or its polarity, provided the analytes can either be evaporated or dissolved without decomposition. The majority of separation

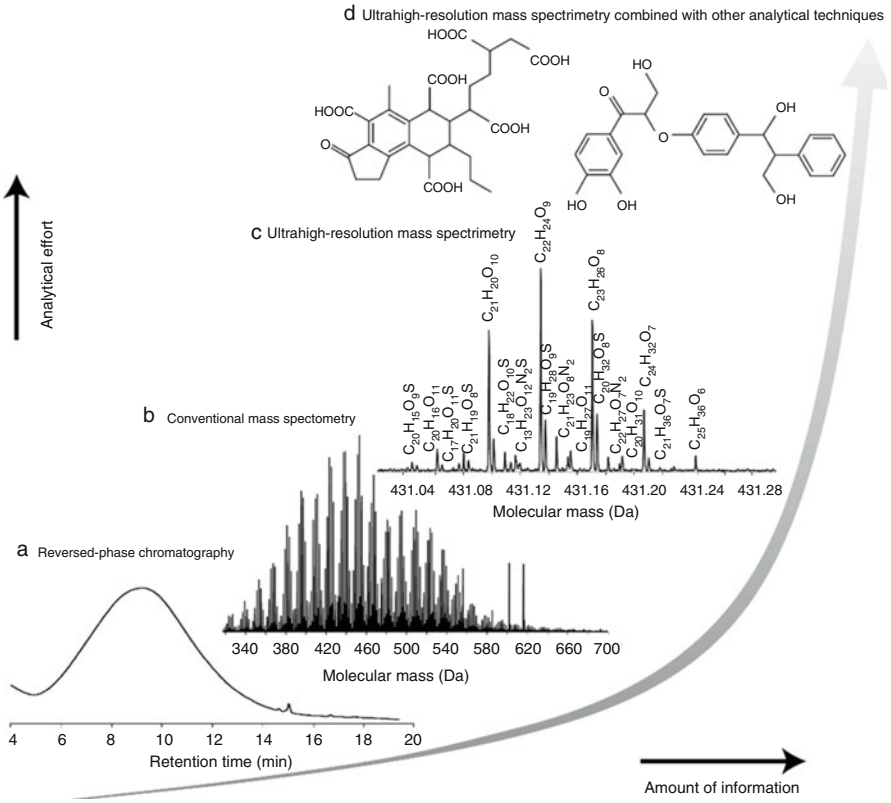


Fig. 14.38 Stepwise increasing depth of analytical information versus analytical effort in the analysis of DOM (Adapted from Ref. [124] with permission. © Springer Nature, 2009)

techniques are of chromatographic nature. A chromatographic system's ability to separate components of a mixture is expressed in terms of the height equivalent to a theoretical plate (HETP) or the number of theoretical plates. The factors influencing HETP are described by the van Deemter equation. In practice, the peak capacity of a system is more intuitive in describing its ability to resolve individual components of a mixture.

Dimensionality of an Analysis

Mass spectrometry provides a two-dimensional analysis, i.e., intensity versus m/z, as does chromatography, i.e., intensity versus retention time. Combining both techniques leads to three-dimensional analysis, i.e., intensity versus m/z versus retention time. Both tandem MS and comprehensive gas chromatography (GC × GC) add another dimension of analytical information. Thus, four-dimensional analysis can either be achieved by LC-MS/MS, GC-MS/MS, or GC × GC-MS.

Alternatives to Chromatography–Mass Spectrometry Coupling
Ion mobility spectrometry (IMS) presents a technique of separating isolated gas phase ions and can replace chromatographic separation in front of the mass spectrometer, at least to some extent. Alternatively, components of mixtures can be selected as precursor ions and analyzed by tandem MS. The tandem MS approach to mixture analysis is particularly promising in combination with soft ionization methods. In contrast to separation techniques, tandem MS cannot distinguish between isomeric precursor ions. Finally, ultrahigh-resolution MS is useful in analyzing complex mixture of related compounds.

The Method of Choice
Finding the method of choice to deliver the utmost depth of analytical information can be a tedious task as it is not easy to predict which instrumental approach will deliver the best result. Often, the techniques at hand in the particular laboratory have to be used anyway, even though others may exist. Also, different analytical approaches may be used interchangeably and experience with proven methods may counterbalance the potential of techniques used elsewhere.

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