Mass spectrometry and mass-selective detection in chromatography [☆]

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Abstract

An overview of chromatography–mass spectrometry coupling is presented here, focussing mainly on possibilities offered by this detection technique. GC–MS and HPLC–MS are the two most often used variants, which have quite different characteristics. Various mass-spectrometric possibilities are briefly discussed: ionization techniques, determination of elemental formulas, structural information and the question of sensitivity and selectivity. Options for mass-spectrometric instrumentation and applications of tandem mass spectrometry are also mentioned. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chromatography combined with various detection techniques is one of the highest performance and most often used analytical methods. Among these, chromatography coupled on-line to mass spectrometry (MS) combines the advantages of chromatography (high selectivity and separation efficiency) and of mass spectrometry (structural information and further increase in selectivity), while being relatively straightforward to accomplish. Sensitivity is a prime advantage of mass spectrometry — this allows obtaining mass spectra of trace level compounds

(low sample amount and/or low concentration) in the timeframe of chromatographic elution times. Coupling of HPLC to NMR spectroscopy is also of immense importance, as described in a review paper [1]. There are many types of special detectors used in chromatography described in general textbooks on chromatography, and in an accompanying paper [2].

Mass spectrometry can be combined with various chromatographic techniques (abbreviated at present as Chrom–MS). Gas chromatography combined with mass spectrometry (GC–MS) is used probably most often, and can be regarded as a routine application. Combination with high-performance liquid chromatography (HPLC–MS) is somewhat more complex, but is dynamically developing field which is also very routinely used. Combinations with other chromatographic techniques, like with capillary electrophoresis (CE) and thin-layer chromatography (TLC) are also feasible, but are less often used. These have been reviewed in several occasions recently [3–10] a

repetition seems unwarranted and unfeasible at this point. The main purpose here is to present an overview of the chromatography–mass spectrometry combination, mainly *for chromatographers from the viewpoint of a mass spectrometrist.*

The Chrom–MS combination is often viewed in a bimodal way: Chromatographers frequently consider MS as a special (''mass-selective'') detector; on the other hand, mass spectrometrists often regard chromatography as a particular technique for introducing samples into a spectrometer. While both viewpoints have their own merits, the best results are obtained when equal emphasis is placed on chromatography and on mass spectrometry.

2. Discussion

2.1. Chrom–MS combinations: possibilities and limitations

In principle, a mass-selective detection (MSD) system means a simple mass spectrometry (MS) system. The idea of substituting mass spectrometry with the simple ''mass selective detector'' was part of a marketing strategy; and has proved to be extremely successful. It has helped to make Chrom–MS combinations widespread, and has also helped to reduce chromatographists' reluctance to use a complicated and somewhat mysterious technique. The salesman's approach to the ''mass-selective detector'' can be summarized in the following, simplistic way: (a) MSD is as easy to use as a UV or flame ionization detection (FID) detector; (b) MSD does not put much limitation on chromatography; (c) there is no real need to understand mass spectrometry, partly because MSD detects the molecular mass, partly because compounds can be identified using computer search.

There is a lot of truth or half-truth in these statements. It is true, that operating an MS (or MSD) became much easier in the last decade, and a detailed understanding of fragmentation processes occurring in MS may not be necessary for successful application of Chrom–MS. However, if one takes the simplistic ideas above seriously, there will be many pitfalls and many unrealized possibilities.

At this point it seems expedient to discuss GC,

HPLC and other combinations separately. The combination of gas chromatography and mass spectrometry is straightforward; there are no serious limitations on the GC, or on the MS side. The use of small bore and large bore capillary columns, however, may present some problems (related to sensitivity and to the vacuum system, respectively). To help the vacuum system, instead of N_2 usually He carrier gas is used in GC–MS (which also increases separation efficiency). Note that although H_2 carrier gas may also be used, this may occasionally produce chemical ionization (CI) related peaks in the mass spectrum (e.g. MH^+ instead of $M^{+ \cdot}$ ions). It is common knowledge, that mass spectrometry has high sensitivity. However, when a full spectrum is recorded during a chromatographic run, sensitivity is often inferior to that of FID. Mass spectrometry is, therefore, more important to increase the specificity or (selectivity) rather than the sensitivity of analysis. In the case of selected ion recording (when the abundance of only one particular mass is followed in time) sensitivity can be increased by one or two orders of magnitude.

In GC–MS most often electron impact (EI) ionization is used, and positively charged ions are detected. In an ideal case an abundant molecular ion ($M^{+ \cdot}$) is observed and the spectrum (a set of fragment ions with characteristic abundance) identifies the compound studied. Sometimes the molecular ion is of very low abundance (occasionally it is completely absent), in such cases the molecular mass can not be reliably determined from the EI spectra. Identification of unknowns is often performed by computer search comparing the spectrum obtained with library spectra. There are two main problems related to this approach. One is that different compounds many yield similar mass spectra, so compound identification is often uncertain. Note that computer search results even over 95% ''accuracy'' should be regarded only as possibilities and never as a positive identification! These can be isomers, homologues or altogether different compounds having a common structural unit. The second problem is, when the compound is not present in the library, which may not be immediately obvious from the search results. In such case homologues, isomers or related structures will be indicated as the most likely structure. Note that search results below 80% ''ac-

curacy'' nearly always mean that the unknown is not present in the library. While not sufficient to provide positive identification of compounds, computer search is very useful to give hints about the possible structure of an unknown, and it is always worth checking the structure of the top 5–10 scored compounds. In spite of these complications, mass spectrometry can be often used to identify the structure of unknowns. This, however, usually requires knowledge on the fragmentation processes, special experiments (like tandem mass spectra) and comparison with spectra of standards obtained under identical conditions (not only 70 eV EI, but also the same instrument, the same ion sources temperature, etc.). Of course, the retention time (or index) is also a very valuable information. In most cases identical (or very similar) mass spectra and identical retention time reliably indicates that the sample and the standard is identical. In critically important cases, however, further checks may be necessary, as there are several examples of co-eluting compounds (typically, but not necessary isomers) having the same (or very similar) mass spectra.

It is worth knowing that beside simple EI spectra, there are other possibilities to detect, identify and quantify compounds by mass spectrometry. The most important ones are the following:

(a) *Chemical ionization* (CI) is very useful to detect the molecular mass, when the M ion is absent from the EI spectrum. In CI most often the protonated molecular ion, (MH^+ or $[M+H]^+$) is observed. Note that occasionally $[M-H]^+$, $[M+NH_4]^+$ etc. ions may also be present. CI usually gives fragment ions of low abundance — this may be advantageous when trace level compounds are analyzed, but reduces the structural information obtained.

(b) *Negative chemical ionization* (NCI or CI–) usually produces negatively charged $M^{-\cdot}$, $[M-H]^-$ or $[M+halogen]^-$ ions with little fragmentation. For some compounds (e.g. aromatic hydrocarbons, polyhalogenated compounds, nitro-derivatives, etc.) NCI increases sensitivity by several orders of magnitude — very useful for selective detection of trace level compounds in mixtures.

(c) *Accurate mass measurement* (usually with 1–10 ppm accuracy) makes it possible to determine elemental formulas for unknown compounds, and can be used to increase selectivity as well. This technique requires high resolution (in the order of 10.000) which is, however, not available on most simple MSD-s.

(d) *Tandem mass spectrometry* introduces many possibilities to increase selectivity, to reduce ''chemical'' noise and to increase structural information obtained. This technique will be discussed in some detail later.

The combination of HPLC with mass spectrometry is significantly different to that of GC–MS. It is technically more complex, and puts much more restriction both on chromatography and on mass spectrometry. The method of ionization is also different. At present two, fairly similar ionization techniques are used, electrospray (ESI) [11] (practically the same method is occasionally called ionspray) and atmospheric pressure chemical ionization (APCI). In both cases the eluent passes through a small capillary, forms a fine spray, and the solvent is evaporated. The ions are formed during this process, and are subsequently analyzed by the mass spectrometer. Many limitations of HPLC–MS are related to this process: Non-volatile buffers (e.g. sodiumphosphate) and salts (e.g. sodium chloride, used to adjust the ion strength) are to be avoided, mainly because these can easily block the capillary (especially, if it is heated to assist evaporation) and the entrance into the mass spectrometer. In HPLC–MS most often ammonium acetate or ammonium formate buffers are used and, even with these, low ion strength is preferred. A more subtle reason to avoid non-volatile buffers and salts relates to the mechanism of ion formation in ESI and in APCI. For most compounds the best sensitivity is obtained if ion formation occurs by protonation, but it is only possible in the absence of cations. In the presence of non-volatile buffers or salts typically multiply cationized species are observed, which increases the complexity of mass spectra obtained and significantly decreases sensitivity. Luckily, volatile cations, like ammonium ions, form weak complexes and therefore cause fewer problems — this is the reason of using ammonium buffers in HPLC–MS. To complicate matters further, for the analysis of some compounds, like oligosaccharides, sodiated molecular ions may actually be preferable, so addition of *very small* amounts of sodium salt to the sample may be advantageous.

Some restrictions in HPLC–MS relate to the flow-rate which, in turn, relates to the pumping speed of the vacuum system and design of the mass spectrometer ion source. In the case of a typical, conventional ESI ion source the flow-rate should be in the range of 20 μl–1 ml/min. In the case of high water content, the maximum flow-rate may be smaller, ca. 0.2–0.5 ml/min. In the case of higher flow-rates splitting is necessary; in the case of smaller flow-rates so-called microspray or nanospray ion sources have to be used. (These produce ESI spectra, but work with much smaller flow-rates, in the range of 1 μl and 1 nl/min, respectively.) For APCI, relatively high (around 1 ml/min) flow-rates *have to be* used. This is necessary for ionization of the sample. When the flow-rate is much lower, an additional solvent flow (makeup flow) has to be added after HPLC separation. Small fluctuations in solvent flow present more problems in HPLC–MS, than in HPLC using UV detection. For this reason high-quality solvent pumps are probably even more important in HPLC–MS than in other applications. Note also, that mainly due to dead volumes between the HPLC column and the MS detector, chromatographic resolution is somewhat compromised. To reduce this problem, the HPLC and the MS detector should be physically close to each other, and narrow bore tubing is recommended.

Relative sensitivity of UV and MS detection for various compounds may differ by many orders of magnitude in either direction. This has several consequences. The purity of solvents used in HPLC is most often controlled by their UV absorption. As there are many compounds, which have low UV sensitivity (those with poor chromophores) the chemical background (due to solvent impurities) is usually far higher in MS than in UV detection. For this reason controlling solvent quality is of highest importance in HPLC–MS. Another consequence of different UV and MS sensitivities is, that chromatograms obtained by UV and by MS detection may be quite dissimilar. Probably the best solution to overcome these problems is to obtain both UV and MS spectra simultaneously by connecting the two detectors sequentially (i.e. column–UV–MS). A typical (not too bad) example is shown in Fig. 1, indicating the TIC (total ion chromatogram) and UV (at 240 nm) chromatograms of a reaction product. Note that

there is a time-lapse between the two detectors, and it is a good idea to calibrate for this difference.

There are many advantages using MS detection in HPLC. Among these the most obvious ones are the possibility of obtaining structural information (most significantly the molecular mass); to detect compounds without good chromophores; and to increase the specificity of detection. In the latter case often selected ion recording (or monitoring, abbreviated as SIR or SIM) is used: it means detection of one particular mass (more accurately the mass to charge ratio), and following its intensity (abundance) as a function of elution time. Note that selected ion recording and full mass spectra relate in an analogues way like UV detection at a given wavelength relates to array detection. In most cases the (protonated) molecular ion is detected. This approach is especially useful for quantitation. Occasionally cation adducts (e.g. $[M+Na]^+$) or fragment ions can also be used. The abundance of these peaks is usually less reproducible than that of MH^+, so special care should be taken to insure stable conditions if such peaks are to be used for quantitation.

The specificity of mass spectrometry can also be used to reduce the chemical background — very important in the analysis of samples in complex matrices (like blood, tissue extracts, environmental samples etc.) which are of prime importance in biochemical, pharmaceutical and environmental chemistry. The resolution of chromatographic separations can also be improved significantly, if neighboring peaks in the chromatograms have different molecular mass, or the same molecular mass but different mass spectra. In such cases ''shoulders'' or ''tails'' in chromatographic peaks can completely be resolved using mass spectrometric detection (usually by SIR or SIM techniques).

Characteristics and operating conditions of mass spectrometry (or MSD) coupled to HPLC have a large influence on the results, and it is important to have a clear understanding of the main features. As mentioned above, currently two ionization techniques are used in HPLC–MS, electrospray (ionspray, microspray, nanospray) and APCI. ESI is the preferential technique for compounds, which are ionized in solution, but it is also excellent for highly polar compounds, which can easily be ionized. On the other hand, APCI performs better for compounds
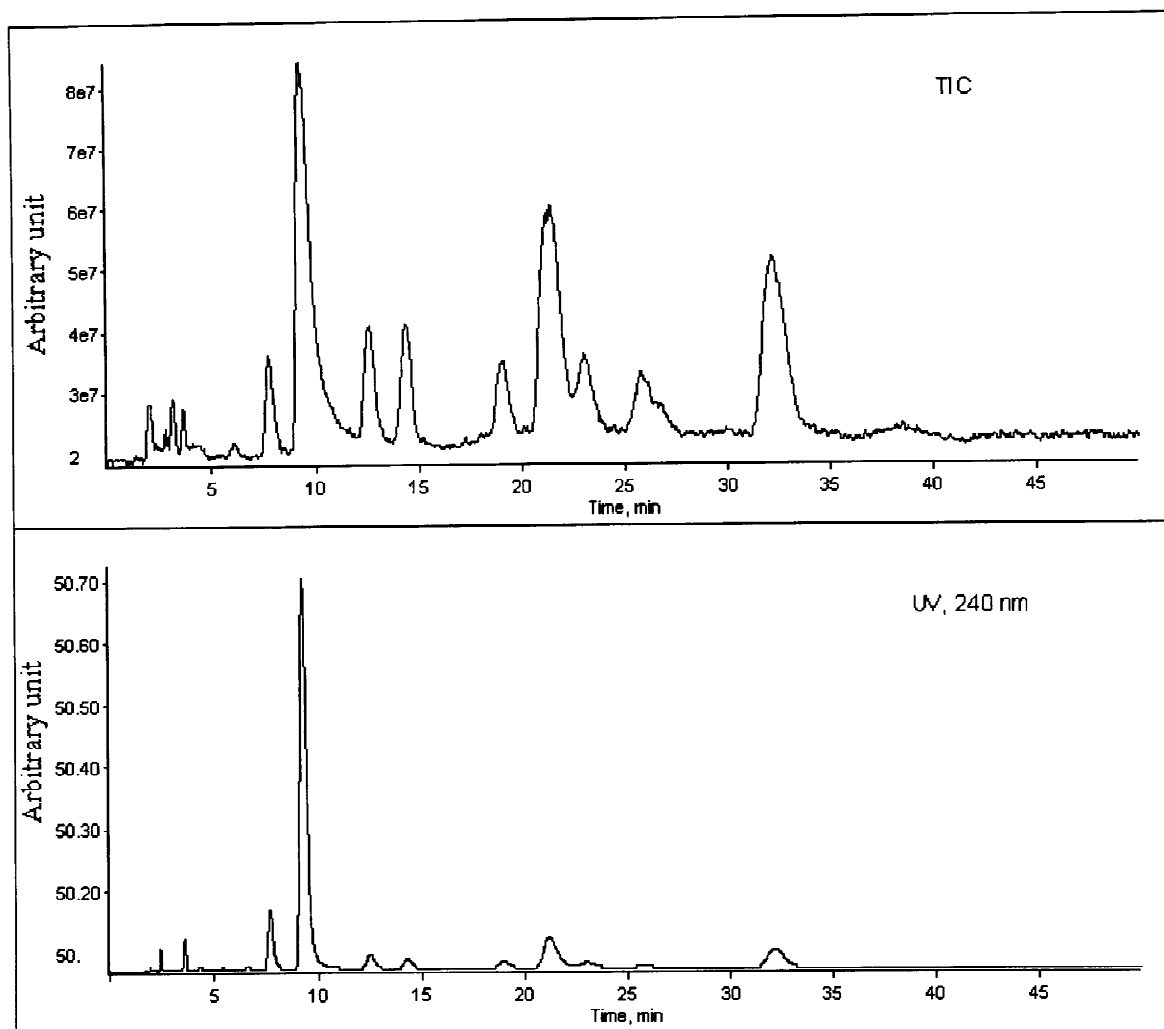
Fig. 1. Total ion and UV tracings of a synthetic mixture (by HPLC), showing a typical example of different relative sensitivities.

of medium polarity. Both techniques can be used equally well in positive and in negative ion mode, although most often positive ions are used. Negative ion detection is best for negatively charged ions, highly aromatic compounds, halogen and nitro-de-rivatives. In APCI typically singly charged proton-ated or cationized molecular ions are observed ([M−H]$^-$ or [M+halogen]$^-$ in negative ion mode); the upper molecular mass limit is usually 1500–2000. In ESI ionization protonation, deprotonation or cation attachment is observed, but formation of multiply charged species is very common, especially for

higher mass molecules. For high mass compounds the mass to charge ratio is usually in the m/z 500–2000 range and a distribution of variously charged species is observed. This makes it possible to detect molecular masses up to 10^5–10^7 using ESI on conventional instruments.

One of the most important parameters in ESI or APCI is the cone (or skimmer) voltage. Increasing the cone voltage increases the internal energy of molecules, so the probability of fragmentation also increases. At low cone voltage often only the molec-ular ion is observed — a favorable feature for

studying complex mixtures or trace level compounds, and for quantitation. At higher cone voltages fragment ions may appear in the spectrum (mainly for compounds with molecular masses below 2000–3000), which yield valuable structural information. Like in GC–MS, accurate mass measurements and tandem mass spectra yields further structural information. At present there are no good ESI or APCI spectral libraries, so computer search for structural identification is unavailable in HPLC–MS.

Combinations with other chromatographic techniques are possible, but these are not as common, as GC–MS or HPLC–MS. Capillary zone electrophoresis can be coupled to MS, in a way very similar to HPLC–MS. Due to the much lower flow-rate, usually micro or nanospray ionization is used. Combination of TLC with MS is possible, but it is used usually in an off-line manner (first the TLC separation is performed, which is followed by analysis). It is more labor intensive, and is used only occasionally [12].

2.2. Instrumentation

There is a wide range of mass spectrometers and mass selective detectors, and it is not always easy to orient oneself among them. First, it is reiterated that there is no fundamental difference between MS and MSD; the name MSD is part of a marketing strategy, and implies a simple, relatively cheap instrument. The most important difference among various mass spectrometers is the type of the analyzer. It is a fundamental characteristic of an instrument, and can not be modified. The main analyzer types are the following: quadrupole, ion trap, time-of-flight (TOF), sector and Fourier-transform (FT) type instruments. Some analyzers are inherently capable for MS–MS (like ion trap or FT-MS), some others can be connected ''in tandem'', offering MS–MS capabilities. The most common analyzers (quadrupoles, sectors and in most respect ion traps) pass one ion (a given m/z ratio) at any one time, and filter out all other ions (which are lost for analysis). To obtain a mass spectrum, the analyzer scans through the mass range of interest. The consequence of this ''scanning'' is, that the sensitivity of detecting a given ion is much less compared to the case, when only a given mass is monitored (when the analyzer stands still to pass only one ion). These analyzers can be used in two ways: Either monitoring a given ion (with ''full'' sensitivity), or scanning the mass range (with loss of sensitivity) to obtain a mass spectrum. Other analyzer types (TOF, FT-MS, in some respect ion traps and special sector instruments) can simultaneously detect all ions in a mass range, so there is no need for scanning. In this case the ''full'' sensitivity is obtained irrespectively if one ion or a full mass spectrum is studied. Although this is a simplistic description, it can still be used to understand some basic features of, and differences between instrument types.

At present quadrupole-type instruments are most widespread. These are simple to use, cheap and rugged, good for basic GC–MS or HPLC–MS studies and for quantitation. ''Ruggedness'' means that they are reliable, not very sensitive to impurities, and the tuning of the instrument is simple and stable. Using quadrupole instruments one ion is detected at a time and the mass spectrum is obtained by scanning over the mass range of interest. Sensitivity can be increased using single ion monitoring, when the abundance of only one particular ion is monitored as a function of time. It is also possible to monitor a few ions by switching rapidly among them. Other parameters of quadrupole instruments, like unit resolution, scan time (0.1 s or shorter) and mass to charge range (up to 600–4000, depending on the instrument) are sufficient for most applications. The so-called triple quadrupole instruments (commonly called triple quads, QqQ) have similar characteristics, but are used for tandem mass spectrometry. (Note that a tandem MS instrument is always capable of working in single stage MS mode just as well, as a single stage MS instrument). A large advantage of triple quad type tandem mass spectrometers is that all tandem scan types (to be discussed below) are easy to perform. Such HPLC–MS–MS instruments are common ''workhorses'' in many laboratories.

Ion trap instruments are also common, fairly cheap mass spectrometers. One of their advantages over quadrupole instruments is that they are somewhat more sensitive in full scan mode (i.e. when the whole spectrum is recorded). A further, more important advantage is that, depending on the price range, high resolution (offering accurate mass mea-

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surement) and tandem mass spectrometry is also
available on many ion trap instruments. While ion
trap instruments are very cost-effective for accurate
mass measurements and for tandem mass spec-
trometry, they are not always ideally suited for these
purposes. Mass measurements can be performed with
better accuracy on sector, time-of-flight or FT-MS
instruments. MS–MS in ion-trap instruments occurs
through a so-called ''slow heating'' technique [13],
which has disadvantages compared to MS–MS on
other instrument types. In spite of this, another pro
for ion traps is that not only MS–MS, but also triple
stage tandem mass spectra (MS–MS–MS, precursor
ion→fragment→further fragment) is also possible.
This is a great advantage in structural studies, like
peptide sequencing. In principle, MS–MS steps can
be repeated infinitely, called MS^n, but in practice
sensitivity limits its use to MS–MS–MS. (Note that
sensitivity in terms of the number of ions detected
per sample amount is always lower or much lower in
tandem MS, than in single stage MS. The advantage
of MS–MS comes from its selectivity, which often
over-compensates the loss of sensitivity in trace
analysis.) The main disadvantage of ion trap instru-
ments is that the pressure inside the ion source has a
critical influence on spectral quality. This is crucial
in GC–MS using (the most common) EI ionization.
If a large chromatographic peak is observed, and the
pressure inside the ion source increases, instead of EI
so-called self-CI ionization may occur. In practice
this may mean, that $M^{+\cdot}$ ions and EI spectra are
observed at the beginning and at the tail of the peak,
while MH^+ ions (and CI spectrum) is observed at the
top of the chromatographic peak. As the EI and CI
sensitivities may differ significantly in either direc-
tion, instead one peak showing the $M^{+\cdot}$ ion one may
observe two peaks (seemingly isomers), or one peak
with an erroneous molecular mass of (M+1). The
problem may be even worse in quantitation, when
with changing the sample amount the sensitivity may
change dramatically. The worst case is, when one is
monitoring a small impurity and there may be more-
or-less co-eluting compounds in varying concen-
trations, having different molecular masses (so these
impurities are not obviously observed). These co-
eluting compounds can change the effective sen-
sitivity of the instrument from sample to sample!
Such problems can be resolved or avoided by careful

analysis, but it is very important to be aware of such
possibilities. For these reasons ion trap instruments
are not always favored for quantitation.

A relatively new, high-performance instrument
type used for Chrom–MS are TOF instruments.
These can be connected equally well to GC and to
HPLC. They have high sensitivity (especially in
''full scan'' mode, as they do not scan but detect the
whole mass spectrum at the same time) and they are
ideal for accurate mass measurement: this obtained
simply and accurately. TOF instruments are also
ideal as a second stage in tandem MS experiments.
TOF instruments may be combined with quadrupole
(Q-TOF) or with sector type instruments. These
combinations constitute the most powerful mass
spectrometers currently available, which are suffi-
ciently simple to combine with chromatography.
When used in single-stage MS mode, the quadrupole
transfers ions into the TOF analyzer, which records
all ions in the mass spectrum simultaneously, with
high sensitivity and with high resolution. In MS–MS
mode the quadrupole (or the sector) selects the
precursor ion while to TOF analyzer records the
whole tandem spectrum, without the necessity of
scanning (and therefore with only minor loss of
sensitivity).

Sector-type instruments (also called magnetic in-
struments) are of high-performance: have high sen-
sitivity, high mass range, high resolution (accurate
mass measurement) and (usually) MS–MS capa-
bility. They are, however, also expensive and fairly
difficult to operate. Both GC–MS and HPLC–MS
connections are possible, but (especially the latter)
have many practical difficulties. For these reasons
they are less often used in Chrom–MS combinations,
only when their high-performance is really required.
Fourier-transform instruments (FT-MS, also called
FT-ICR) are very high-performance mass spectrome-
ters, but are so expensive and so difficult to use that
these are rarely coupled to chromatography.

2.3. Tandem mass spectrometry

To understand all nuances of MS–MS [14,15]
may not be necessary for a chromatographer, but
some basics are quite useful. The basic tandem MS
experiment is the following: First, ions are formed
from the sample and one of these ions (usually the

molecular ion) is selected for tandem MS. The selected ion is excited, usually by collisions with a neutral gas in the collision region of the mass spectrometer (called the collision cell). The ions excited this way undergo unimolecular reactions, forming product ions. The whole process is called collision induced decomposition (CID) [16] or collisionally activated dissociation (CAD). The range of product ions formed in CID is subsequently analyzed (by the ''second stage'' mass spectrometer) according to their mass to change ratio, and this forms the tandem mass spectrum.

Energetics, activation and fragmentation of ions [17] in tandem mass spectrometry is of central importance, but a detailed description is outside the scope of the present paper, only the main features will be discussed. Activation of ions using surface induced dissociation (SID) [18] or excitation by lasers are important options in MS–MS, but in Chrom–MS combinations practically only CID is used. Features of CID depend mainly on the collision energy which, in turn, is determined by the type of mass spectrometer. Collision energy in the keV range (''high energy collisions'') is used in sector and in TOF instruments. In this range the spectra shows a relatively large variety of fragments (which is advantageous) and changes in the collision energy have only minor influence on the spectra. Collision energies in the 0–200 eV range (''low energy'' CID) is used on quadrupole-type instruments. In this range fragmentation depends significantly on the collision energy: Between ca. 0–20 eV practically only the molecular ion is observed; between ca. 10–100 eV both the molecular ion and some fragments are seen; while in the 50–200 eV range mainly fragments (including low mass fragments) are present in the spectra. This collisional energy dependence can advantageously be utilized, as the spectra can be varied according to the needs of the analysis. In the case of ion traps, very low energy collisions are used (0.1–1 eV, called ''slow heating'' or ''tickling''), but very many collisions occur. Like in quads, the spectra change with the collision energy, but high activation energy fragmentations are absent. Further details on activation processes will not be described here; these have been adequately reviewed [14].

The conventional use of tandem mass spectrometry is to increase the amount of structural information available. When ''soft'' ionization techniques are used, which produce mainly molecular ions ($[M+H]^+$, $[M+Na]^+$, $[M-H]^-$ etc.), tandem MS of these ions may yield structural information similar to that gained from EI spectra. Tandem spectra of fragment ions may also be obtained, and may be used to identify the structure of the selected ion: This can be performed either by spectrum evaluation, or by comparison with spectra of ''standard'' structures.

Another aspect of MS–MS is mixture and trace analysis, which is of increasing importance. In fact, most tandem MS applications are in this field. In some sense, the first stage mass spectrometer acts as a separation device (like chromatography, but it is based strictly on mass), the second stage as spectroscopy, giving structural information. In a simplistic way, in tandem MS compounds having a given molecular mass and containing a particular structural feature are detected, depending on the setup of the instrument. (Note that the main idea is not to substitute, but to complement chromatographic separation!) Tandem mass spectra yield very specific information, and can be used therefore to increase selectivity and to reduce chemical noise. Using the chromatographic retention time as well, identification (and/or quantitation) of compounds in complex mixtures can be performed accurately and reliably using tandem MS techniques.

In tandem mass spectrometry various scan types can be used. More detailed descriptions of these can be found in textbooks describing MS–MS [14]. For didactic reasons it is advantageous to discuss them the case of a triple quadrupole type instrument. In such an instrument the ions pass through two mass analyzers, which either let through only one ion or scan the mass range of interest. Between the two analyzers the ions fragment, usually due to a CID process. The possible scan types are the following:

(a) *Product (or daughter) ion scans.* The most common scan type, where the products of a given precursor ion are observed. In this case the first analyzer is set to pass the precursor ion, while the second one scans the mass range of interest for fragments. This scan type can be used equally well for structural studies and for analytical purposes. A common application is to select an ionization technique (or experimental condition), where only molec-

ular (or quasi-molecular) ions are formed (i.e. there is little fragmentation, so sensitivity is high and the spectrum is simple) and obtain structural information using product ion scans.

(b) *Reaction monitoring (and multiple reaction monitoring, MRM).* The idea is similar to single ion monitoring (SIM, SIR) techniques. In this case the first analyzer is set to pass the precursor ion, the second is set to pass a characteristic fragment of the selected precursor: This scan type, therefore, detects a given reaction occurring in the mass spectrometer. There is no scanning in this case, so sensitivity is significantly increased. The quadrupole analyzers can be quickly switched among several reactions to detect them with high sensitivity (either several fragmentation processes of a given precursor, or reactions characteristic of different compounds). Like SIM, MRM is particularly well suited to chromatographic applications, to quantitation and to compound type analysis.

(c) *Precursor ion scans.* In some sense these are the opposites of product ion scans. The first analyzer scans over the mass range of interest, the ions fragment between the two analyzers, and the second analyzer is set to pass only the desired product. In consequence only those ions will be detected, which fragment to yield a common product ion. This scan type is particularly well suited to mixture and to compound type analysis.

(d) *Constant neutral loss.* In this case both analyzers are scanning, but with a characteristic mass difference. The second analyzer is set to scan e.g. 18 a lower mass than the first one — so detects all ions which loose water (a common process for alcohols). This scan type is also well adapted to mixture and to compound type analysis.

The situation is somewhat different for other instrument types. Sector type MS–MS instruments are similar, although MRM and precursor in scans are more difficult, while constant neutral loss scans are not practically possible. When the second stage is a TOF analyzer ''product ions'' are detected simultaneously, i.e. without scanning and consequently with a huge gain in sensitivity. Using computerized data manipulation, data can be extracted to present them in the form of reaction monitoring, precursor ion or constant neutral loss scans, but (in a contrast to triple quads) this does not result in a sensitivity

gain. Ion traps and FT-MS instruments operate under different principles (ions are selected ''in time'' not ''in space''), have MS^n capability, but always detect product ion scans.

3. Outlook

The combined technique of chromatography–mass spectrometry is becoming ever more popular and mass spectrometers (mass selective detectors) start to be common equipment in chromatographic laboratories. In parallel with this development, mass spectrometers have become much simpler to operate. While it is simple to set up a mass spectrometer to detect a single mass, a basic understanding of mass spectrometry is advantageous to understand limitations of the technique and to exploit new possibilities. Possibilities using tandem mass spectrometry were briefly outlined above. It is the strong belief of the present author that tandem MS will soon become just as widely used in chromatography, as MSD is at present. Note that tandem MS is only marginally more complex to use, than single stage MS. Data evaluation in tandem MS is also not much more complex, than in ''conventional'' MS. On the other hand tandem MS is a very selective and versatile special ''detector'' for chromatography.

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