Pure & Appl. Chem., Vol. 65, No. 5, pp. 1021–1027, 1993.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION
COMMISSION ON MICROCHEMICAL TECHNIQUES
AND TRACE ANALYSIS*
WORKING GROUP ON ORGANIC TRACE ANALYSIS†

ANALYTICAL TECHNIQUES FOR
TRACE ORGANIC COMPOUNDS–IV

TANDEM MASS SPECTROMETRY FOR ORGANIC TRACE ANALYSIS

(Technical Report)

Prepared for publication by

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Analytical techniques for trace organic compounds–IV: Tandem mass spectrometry for organic trace analysis (Technical Report)

Abstract – Tandem mass spectrometry is a very sensitive method for the identification of organic compounds in mixtures. The first mass spectrometer serves to ionize all components of the mixture and to select out a major ion, most often the molecular ion of the compound(s) of interest, which is then fragmented by injection into a region containing a neutral gas at intermediate pressure and the resulting fragment ions are then separated and mass analyzed in the second mass spectrometer. This approach makes quantitative analysis more specific and reliable by monitoring a specific fragment ion from the precursor ion of interest. It also allows qualitative analysis by interpretation of the fragment ion spectrum of a particular component independent of the presence of the others. Tandem mass spectrometers consist of either three quadrupoles, the second one serving as the collision region; two magnetic instruments; or a combination of both. Some of the measurements can be carried out with one double-focusing mass spectrometer but at the expense of resolution.

INTRODUCTION

The process by which a molecule is first ionized and mass separated in a mass spectrometer, fragmented, and the resulting fragment ions mass analyzed in a second step is generally called tandem mass spectrometry (MS/MS). Since two-consecutive analytical steps are involved, a much higher degree of specificity or structural characterization is achieved compared to a single step, such as that provided by a conventional mass spectrum. To a certain degree, a tandem mass spectrometer system can be compared to the combination of a gas or liquid chromatograph with a mass spectrometer (GC/MS or LC/MS) because the first mass spectrometer (MS-1) separates components of a mixture by their mass-to-charge ratio, m/z (which is also measured at the same time), while the second one (MS-2) provides the mass spectrum of the particular component. All three concepts are particularly suited to the analysis of mixtures as they provide a mass spectrum of a single component (or of a highly simplified mixture if the resolution of the first step is not sufficient for complete separation). Using the first mass spectrometer of a tandem instrument to separate components is, of course, much faster than a chromatographic system. However, it is also limited to simple mixtures or, for complex ones, to a few selected components therein. The principles and earlier applications of tandem mass spectrometry have been summarized in a comprehensive book edited by F.W. McLafferty [1] and, more recently, in another one by Bush *et al.* [2].

Tandem mass spectrometry serves chiefly three purposes: (1) the detection of a specific compound in a mixture; (2) the quantitation of a specific compound in a mixture; and (3) the structure determination of a compound for which the conventional mass spectrum does not contain sufficient information for the purpose; this is often the case when the compound has to be ionized by one of the so called "soft" ionization techniques, such as chemical ionization (CI), field emission or desorption (FI, FD), or fast atom bombardment (FAB).

INSTRUMENTATION

Tandem mass spectrometry can be carried out principally in two ways: consecutive in space by using two separate spectrometers (multiple sector or multiple quadrupole mass spectrometers) or consecutive in time by using the same mass resolving system twice (ion cyclotron resonance mass spectrometers or ion traps). The former principle is schematically shown in Fig. 1. A mixture of compounds (e.g.,

P_1–P_5) is ionized in the ion source of MS-1, preferably using soft ionization to generate chiefly molecular ions (P_1^+–P_5^+), which may actually represent protonated molecules. These are separated by MS-1 which is set to transmit only one of the ionized components into a region where additional energy is imparted, sufficient to cause fragmentation. This is generally accomplished by passing the ion beam through a region containing a neutral gas, such as helium or another inert gas, present at relatively high pressure (~0.1 Pa) in a differentially pumped collision region or collision cell (CC). The resulting fragment ions (F_1–F_6 from P_4^+ in the example, Fig. 1) are then mass analyzed in MS-2. As implied in the figure, selecting the monoisotopic precursor ion has the advantage of producing a product ion spectrum that is devoid of isotopic clusters, an important advantage when working with large molecules that therefore contain a large number of carbon atoms.

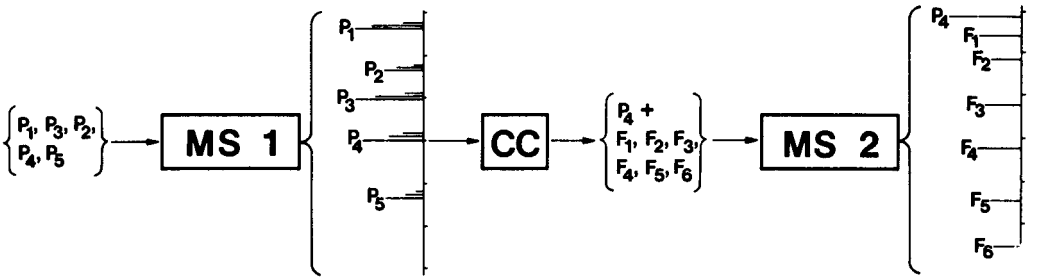

Fig. 1. Principle of a tandem mass spectrometer (from ref. 24)

Collision with a neutral gas is the most common method to produce fragment ions. However, fragmentation can also occur spontaneously in the region between MS-1 and MS-2 (so called "metastable" spectra, because the fragments are produced by the unimolecular decomposition of metastable precursor ions) or by other means of energy transfer, such as photodissociation [3,4].

In the case of magnetic deflection mass spectrometers, a tandem system may consist of electric (E) and magnetic (B) fields (sector) combined in the EB or BE configuration, three sectors (EBE or BEB) or four sectors (EBEB, BEBE, BEEB or EBBE) [1]. These vary greatly in the resolution of the precursor and product ion spectra, both increasing in this order. Historically, the EB configuration was the first commercially available system that could be used as a tandem mass spectrometer. In that case, the precursor ion is fragmented by collision right after the ion source and before the electric field (E) which serves as MS-1. We shall indicate the position of the collision cell by an asterisk, in this case *EB. Mass separation is achieved by scanning E, which transmits one product ion after the other by decreasing m/z because the kinetic energy of the fragment is proportional to the ratio of m_2/m_1, where m_1 is the mass of the precursor ion and m_2 is the mass of the product ion, assuming no change in charge state (generally +1). Mass analysis is achieved by scanning the magnetic field in such a manner that the B/E ratio remains constant ("B/E linked scan") to focus the ion beam on the collector slit placed after the magnetic field. Such a system is characterized by low resolution of the precursor ion because at a certain value of E, a range (generally ± 0.25–2%) of m_2/m_1 ratios are transmitted through the electric sector. However, unit mass resolution is achieved for the product ions because the magnetic sector functions as a single-focusing mass spectrometer.

Alternatively, in the B*E configuration the magnetic field serves as a single-focusing mass spectrometer to separate the precursor ions with good resolution (unit mass to about m/z 1000), but the product ions formed by fragmentation between B and E are only poorly resolved (resolution about 1:100) in the electric field E, which is scanned to transmit ions of different m_2/m_1 ratios. In this system, B remains constant (to select the desired precursor ion). These various scan modes are described in detail by Jennings [5].

In an EB*E tandem mass spectrometer, the first two sectors (EB) are used as a double-focusing instrument providing high resolution of the precursor ion at high ion currents but, as in the BE case discussed above, the product ions are resolved only by the second electric field (E_2) on the basis of their

kinetic energy difference. Thus, as with the B*E configuration, product ion resolution is about 1:100. The other three-sector combination, BE*B, which also utilizes a double-focusing mass spectrometer (BE) as MS-1 followed by the collision region, has somewhat better resolution for MS-2 because B_2 functions as a single-focusing mass spectrometer. However, the high energy spread (due to loss of some kinetic energy during fragmentation) of the product ions limits their resolution to about 1:250. Alternatively, such a system can be used in the B*EB configuration with moderate resolution and ion currents for the precursor ions (typical for a single-focusing MS) and good resolution for the product ions because MS-2 is now a double-focusing spectrometer.

The ultimate in performance is a four-sector instrument, in which both MS-1 and MS-2 are double-focusing mass spectrometers. It assures unit resolution for the precursor ions as well as the product ions to at least *m/z* 3000, the useful range for MS/MS experiments. This configuration is most useful for the structure determination of relatively large molecules (M_r >1000), where reliable mass assignment of the fragment (product) ions is extremely important. A diagram of a four-sector instrument of the EBEB configuration is shown in Fig. 2 [6]. It also incorporates the elements of a two-sector spectrometer where collision takes place after ion source 1 (*EB) and the signal is detected at collector 1; of a three-sector (EB*E) spectrometer where collision takes place between B_1 and E_2 and the signal is detected at collector 2; and the full system with collision between B_1 and E_2 (EB*EB) and detection of the product ions at detector 3. A variation of this system is the BE*EB configuration that is also commercially available [7].

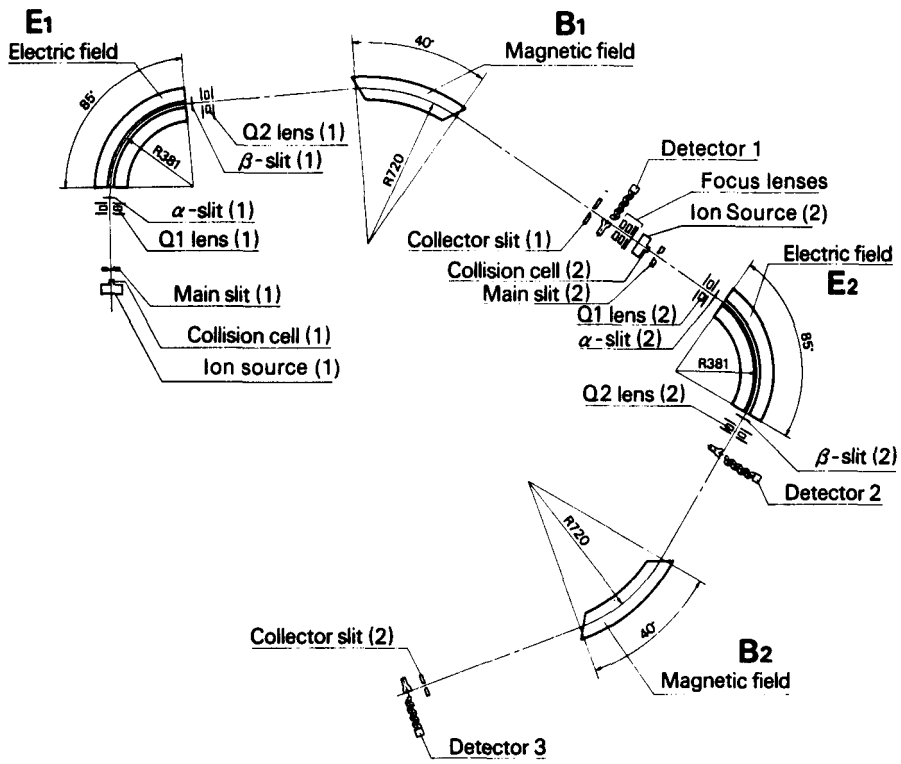

Fig. 2. Schematic of a four-sector magnetic mass spectrometer (from ref. 6)

Tandem mass spectrometers where both MS-1 and MS-2 are quadrupoles represent an alternative approach that is less complex and offers sufficient performance at a reasonable mass range and resolution to be applicable to most experiments, particularly qualitative identification and quantitative determination of compounds with molecular weights below 2000 (more reliable and sensitive below 1000). Recently, the mass range of such instruments has been pushed to *m/z* 4000 but, to date, no MS/MS spectra in this extended range have been published.

Such spectrometers consist of an arrangement of three-consecutive quadrupole systems (triple quadrupole, QQQ) in which the second one serves as the collision region (Fig. 3) [8]. Since quadrupole mass spectrometers operate at low ion kinetic energies (less than 100 eV), the collisions in triple quadrupole tandem mass spectrometers are of low energy. However, because of the low velocity of the ions and the relatively long flight path through the collision region (20-30 cm), the precursor ions undergo multiple collisions. This is in contrast to the magnetic instruments described above, which employ kilovolt acceleration potentials (therefore, high ion velocities) and short collision regions (1 cm) resulting in single collisions at high energy. In very general terms, the fragmentation processes are the same under these very different conditions, but there are some significant differences. For example, fragmentation processes that require high energies [9] are not observed in the triple quadrupole spectrometer.

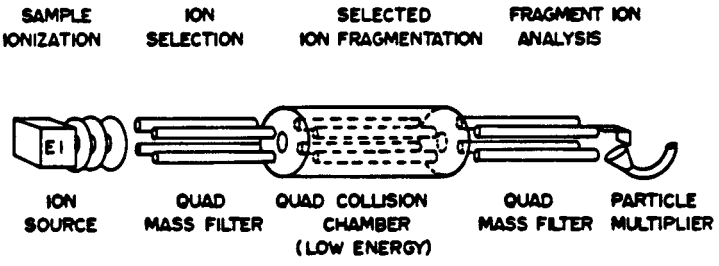

Fig. 3. Schematic of a triple quadrupole mass spectrometer (from ref. 8)

An advantage of the QQQ system is the ease by which various scan modes of MS-1 and MS-2 can be accomplished. For example, a "neutral loss" scan reveals all pairs of ions of which one is formed by the loss of a specified mass from the other one, the precursor [10]. Thus, if the scan of Q_1 is offset by +42 mass units from Q_3, the signal produced by Q_3 will indicate the molecular weight (by adding 42) of all aromatic propyl or isopropyl esters (which exhibit a characteristic loss of propene). A neutral loss scan, therefore, serves as a group-specific detector in a GC/MS or LC/MS experiment. This scan mode is more difficult to achieve in a four-sector tandem mass spectrometer because it requires a very precisely coordinated scan of two double-focusing mass spectrometers.

A magnetic deflection mass spectrometer (as MS-1) can be combined with a quadrupole spectrometer as MS-2 to form a "hybrid" tandem instrument (e.g., EBQQ) [11]. In this case, the collision cell is again a quadrupole system, just as in the QQQ configuration. For the reasons stated during the discussion of the latter, the collision energy is usually kept low by decelerating the high energy ions emerging from MS-1. The hybrid system has good precursor resolution; product ion resolution and mass range are limited to between *m/z* 1000 and *m/z* 2000 by the performance of the specific quadrupole used.

The tandem "in time" principle is used in ion cyclotron resonance (ICR) mass spectrometers, mainly in the Fourier transform version (FTMS) [12,13] or the much simpler "ion trap" [14,15]. In both, the sample is ionized first, generally either by an electron beam or laser beam, and the ions produced excited in the resonance cell of the spectrometer. Ions of all masses, except that to be fragmented (e.g., the molecular ion of the compound of interest) are ejected from the cell. The remaining ions are then exposed to a burst of collision gas or a laser beam [16] and the resulting fragment ions mass analyzed in the conventional manner. In contrast to the tandem "in space" instruments that allow the collisional activation of the molecular ions of one compound of a mixture after another in one experiment, for the "in time" tandem spectrometers a new set of ions has to be generated each time because all other ions have been ejected from the cell of the ICR spectrometer. However, the overall limit of detection is generally still lower than for the "in space" tandem systems.

An ion trap [14,15] consists of a circular hyperbolic surface (the ring electrode) and two hyperbolic electrodes (end caps) above and below the ring (Fig. 4). An electron beam enters the trap through an aperture in one of the end caps and ionizes the sample molecules introduced into the trap. The resulting ions are confined to a circular motion by the application of rf-voltages applied between the ring electrode and the end caps. Proper choice of these voltages permits the ejection of all ions below a certain *m/z* value and the retention of those of higher mass. Thus, one can either scan the spectrum by raising the

voltage and recording the consecutively ejected ions [17], or retain the molecular ion for the purpose of an MS/MS experiment. In that case, the trapped ions are subjected either to a burst of a collision gas or a laser beam to cause fragmentation, and the product ion spectrum is then recorded by the scan method mentioned above [18]. It should be noted that, unlike an ICR spectrometer, an ion trap does not require a magnetic field. This simplifies the construction of this small device, resulting in a low cost instrument. It is generally used in a GC/MS configuration but, as mentioned above, can also be used as a tandem mass spectrometer, even in the GC/MS/MS mode. Commercial versions of the ion trap have a limited mass range (generally up to *m/z* 600), but recent developments have greatly extended the range (to *m/z* 20,000 or even beyond) by using a smaller precision-machined device and axial modulation [19]. Thus, the ion trap principle may find much wider application in the near future.

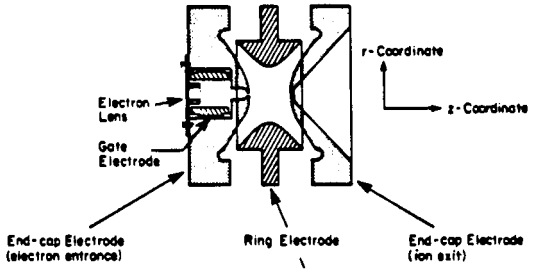

Fig. 4.
Schematic of an ion trap (from ref. 18)

APPLICATIONS

A typical example of the use of tandem mass spectrometry in organic trace analysis is the specific quantitation of tetrachlorodibenzodioxins (TCDD) [20]. Upon collision, the molecular ion fragments by elimination of CO + Cl, e.g., the precursor ion of *m/z* 320 $(C_{12}H_4O_2{}^{35}Cl_4)^{+\bullet}$ decomposes to the product ion of *m/z* 257 $(C_{11}H_4O^{35}Cl_3)^+$. With MS-1 set for *m/z* 320, the ion current of *m/z* 257 in MS-2 measures the amount of TCDD introduced into the ion source with high specificity, because interferences also giving rise to *m/z* 320 in MS-1 (such as a fragment produced from heptachlorobiphenyl, M_r = *m/z* 392, by loss of 2 x HCl) are unlikely to produce a product ion of *m/z* 257. Other isomers of TCDD are, of course, not resolved by MS-1 but generally the sample is introduced into the mass spectrometer via a high resolution gas chromatograph capable of separating the isomers. Plotting the output of MS-2 (which is set for *m/z* 257) vs. time generates a quantitative gas chromatographic profile of all isomeric TCDDs. A slight complication arises if one uses an isotopically labelled standard such as $^{13}C_{12}$–TCDD, because it requires switching MS-1 back and forth between *m/z* 320 and 332, and MS-2 between *m/z* 257 and 268. Again, this is easier with a QQQ system than with a magnetic tandem mass spectrometer.

A recent concern about other sources of TCDDs has made it important to be able to measure the unchlorinated precursor dibenzo-*p*-dioxin (DBD), which under chlorinating conditions can be converted to TCDD and other harmful polychlorinated dioxins. Because the molecular weight of DBD is only 184, its detection by GC/MS in complex mixtures, such as the effluent of the pulp and paper industry, is very difficult. However, by monitoring the transitions *m/z* 184 to *m/z* 128 and *m/z* 108, respectively, in a triple quadrupole mass spectrometer coupled to a gas chromatograph, it was possible to detect as little as 4 pg of DBD [21]. A variety of examples for other applications of tandem mass spectrometry for organic trace analysis had been summarized by Johnson and Yost [22].

The most significant use of high performance tandem mass spectrometers, consisting of two double-focusing instruments (EB*EB or BE*EB), is the determination of the amino acid sequence of peptides to determine the structure of native or modified proteins. Peptides ionized by fast atom (Xe^0) or ion (Cs^+) bombardment form $(M+H)^+$ ions, which upon collision with helium fragment characteristically along the consecutive peptide bonds. From the *m/z* value of the resulting fragments, the amino acid sequence can be deduced [23,24]. This methodology lends itself particularly well to the determination of the structure of modified peptides [25-27]. The same measurements can be carried out with a triple quadrupole mass spectrometer [28], except that for larger peptides unit resolution for the precursor cannot be achieved with acceptable limits of detection.

CONCLUSION

For organic trace analysis, tandem mass spectrometry increases the specificity of the identification, detection or quantitation of a compound. The major advantage over conventional mass spectrometry is its application to mixtures and the measurement of minor components therein. Although the ion currents involved are generally lower, the signal-to-noise ratio is increased because only fragment ions derived from the precursor selected in the first step contribute to the signal measured.

For routine measurements, particularly the detection and quantitation of target compounds, the simpler and less expensive instruments, such as the ion trap or the triple quadrupole, are most useful. For the identification or structure determination of large molecules, such as peptides generated in the course of the determination of the amino acid sequence of a protein, the large and expensive four-sector magnetic deflection mass spectrometers are the most useful instruments.

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