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Applications of Tandem Mass Spectrometry: From Structural Analysis to Fundamental Studies

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1. Introduction

1.1 Mass spectrometry brief history and recent developments

The first and most important question to be asked: What is mass spectrometry?

The basic principle of mass spectrometry (MS) is to generate ions from either inorganic or organic compounds by any suitable method, to separate these ions by their mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively by their respective m/z and abundance. The analyte may be ionized thermally, by electric fields or by impacting energetic electrons, ions or photons. The ... ions can be single ionized atoms, clusters, molecules or their fragments or associates. Ion separation is effected by static or dynamic electric or magnetic fields.(Gross 2004)

Although this definition dates back to 1968, when mass spectrometry was at its childhood, it is still valid. Nevertheless, two additions should be included. Firstly, besides electrons, (atomic) ions or photons, energetic neutral atoms and heavy cluster ions can also be used to ionize the analyte. Secondly, ion separation by *m/z* can be effected in field free regions, as effectively demonstrated by the time-of-flight analyser, provided the ions possess a well-defined kinetic energy at the entrance of the flight path.

From the 1950s to the present, mass spectrometry has evolved tremendously. The pioneering mass spectrometrist had a home-built naked instrument, typically a magnetic sector instrument with electron ionization. Nowadays, highly automated commercial systems, able to produce thousands of spectra per day, are now concealed in a "black box", a nicely designed and beautifully coloured unit resembling more an espresso machine or tumble dryer than a mass spectrometer.

Mass spectrometry (MS) is probably the most versatile and comprehensive analytical technique currently available in the chemists and biochemists arsenal. Mass spectrometry measures precisely the molecular masses of individual compounds by converting them into ions and analysing them in what is called a mass analyser. This is the simplest, but somewhat reductionist, definition of mass spectrometry. The days of the simple determination of the *m/z* ratio of an organic compound are over. Today, mass spectrometry can be used to determine molecular structures, to study reaction dynamics and ion chemistry, to provide thermochemical and physical properties such as ionization energy, appearance energy, reaction enthalpies, proton and ion affinities, gas-phase acidities, and so on.

Mass spectrometry is extremely versatile and several areas of physics, pharmaceutical sciences, archaeology, forensics and environmental sciences, just to mention a few, have benefited from the advances in this instrumental technique.

The history of mass spectrometry starts in 1898 with the work of Wien, who demonstrated that canal rays could be deflected by passing them through superimposed parallel electric and magnetic fields. Nevertheless, its birth can be credited to Sir J. J. Thomson, Cavendish Laboratory of University of Cambridge, through his work on the analysis of negatively and positively charged cathode rays with a parabola mass spectrograph, the great grand-father of the modern mass spectrometers.(Thomson 1897; Thomson 1907) In the next two decades, the developments of mass spectrometry continued in the hands of renowned physicists like Aston,(Aston 1919) Dempster,(Dempster 1918) Bainbridge,(Bainbridge 1932; Bainbridge and Jordan 1936) and Nier.(Nier 1940; Johnson and Nier 1953)

In the 1940s, chemists recognized the great potential of mass spectrometry as an analytical tool, and applied it to monitor petroleum refinement processes. The first commercial mass spectrometer became available in 1943 through the Consolidated Engineering Corporation. The principles of time-of-flight (TOF) and ion cyclotron resonance (ICR) were introduced in 1946 and 1949, respectively.(Sommer, Thomas et al. 1951; Wolff and Stephens 1953)

Applications to organic chemistry started to appear in the 1950s and exploded during the 1960s and 1970s. Double-focusing high-resolution mass spectrometers, which became available in the early 1950s, paved the way for accurate mass measurements. The quadrupole mass analyser and the ion traps were described by Wolfgang Paul and co-workers in 1953.(Paul 1990) Development of the GC/MS in the 1960s marked the beginning of the analysis of seemingly complex mixtures by mass spectrometry.(Ryhage 2002; Watson and Biemann 2002) The 1960s witnessed the development of tandem mass spectrometry and collision-induced decompositions,(Jennings 1968) being a high point in the field of structural analysis, in unambiguous identification by mass spectrometry, as well as in the development of soft ionization techniques such as chemical ionization.(Munson and Field 2002)

By the 1960s, mass spectrometry had become a standard analytical tool in the analysis of organic compounds. Its application to the biosciences, however, was lacking due to the inexistence of suitable methods to ionize fragile and non-volatile compounds of biological origin. During the 1980s the mass spectrometry range of applications increased "exponentially" with the development of softer ionization methods. These included fast atom bombardment (FAB) in 1981,(Barber, Bordoli et al. 1981) electrospray ionization (ESI) in 1984-1988,(Fenn, Mann et al. 1989) and matrix-assisted laser desorption/ionization (MALDI) in 1988.(Karas and Hillenkamp 2002) With the development of the last two methods, ESI and MALDI, the upper mass range was extended beyond 100kDa and had an enormous impact on the use of mass spectrometry in biology and life sciences. This impact was recognized in 2002 when John Fenn (for his work on ESI) and Koichi Tanaka (for demonstrating that high molecular mass proteins could be ionized using laser desorption) won the Nobel Prize in Chemistry.

Concurrent with the ionization methods development, several innovations in mass analyser technology, such as the introduction of high-field and superfast magnets, as well as the improvements in the TOF and Fourier transform ion cyclotron resonance (FTICR), enhanced the sensitivity and the upper mass range. The new millennium brought new advances such as two new types of ion traps, the orbitrap in 2000 by the hands of Alexander Makarov(Makarov 2000) and the linear quadrupole ion trap (LIT) in 2002 by James W. Hager. (Hager 2002)

The coupling of high-performance liquid chromatography (HPLC) with mass spectrometry was first demonstrated in the 1970s (Dass 2007). Nevertheless, it was with the development and commercialization of atmospheric pressure ionization sources (ESI, APCI) that for the first time the combination of liquid chromatography and mass spectrometry entered the realm of routine analysis.(Voyksner 1997; Covey, Huang et al. 2002; Whitehouse, Dreyer et al. 2002; Rodrigues, Taylor et al. 2007)

A full description of ionization sources and their underlying mechanisms is outside the scope of this chapter. The reader in then encouraged to seek more information in other sources. For example, there are several excellent reviews and books that cover nearly all aspects of electrospray ionization (ESI), (Pramanik, Ganguly et al. 2002; Gross 2004; Covey, Thomson et al. 2009; Kebarle and Verkerk 2009; Cole 2010; Crotti, Seraglia et al. 2011) matrix-assisted laser desorption/ionization (MALDI), (Gross 2004; Hillenkamp and Peter-Katalinic 2007; Cole 2010) atmospheric pressure photoionization (APPI) (Raffaelli and Saba 2003) and atmospheric pressure chemical ionization (APCI),(Byrdwell 2001; Covey, Thomson et al. 2009), among others.

1.2 Tandem mass spectrometry basics

I feel sure that there are many problems in Chemistry which could be solved with far greater ease by this than by any other method. The method is surprisingly sensitive – more so even than that of spectrum analysis – requires an infinitesimal amount of material and does not require this to be specially purified:…(Thomson 1921)

J. J. Thomson made this statement about mass spectrometry, but it may be even more apt in describing tandem mass spectrometry. As such, besides being the father of mass spectrometry, Thomson can also be considered the forefather of tandem mass spectrometry. In fact, to demonstrate experimentally the processes of neutralization and collisional ionization, Thomson built the first MS/MS instrument, which consisted in a serial arrangement of two magnets, with the field on one magnet oriented perpendicular to the other.(Busch, Glish et al. 1988)

Tandem mass spectrometry (MS/MS) can be considered as any general method involving at least two stages of mass analysis, either in conjunction with a dissociation process or in a chemical reaction that causes a change in the mass or charge of an ion.(Hoffmann and Stroobant 2007)

In the most common tandem mass spectrometry experiment a first analyser is used to isolate the precursor ion (m_p^+), which then undergoes fragmentation (this could be achieved either spontaneously or by making use of some activation technique) to yield product ions (m_f^+) and neutral fragments (m_n) which are then analysed by a second mass analyser. This reaction is depicted in equation 1.

$$m_p^+ \rightarrow m_f^+ + m_n \tag{1}$$

An activation barrier must be surmounted before the general reaction depicted in equation 1 can occur. The energy to overcome this barrier can come from one of two sources (Busch, Glish et al. 1988):

i.   By the excess energy deposited onto the precursor ion by the ionization process. Nevertheless, this is valid only we dealing with electron ionization at high energies.

ii. By means of activation methods such as collision activated/induced dissociation (CAD and CID), infrared multiphoton dissociation (IRMPD), electron capture/electron

transfer dissociation (ECD and ETD) and surface induced dissociation (SID), for which the fundamental aspects will be discussed in section 2 of this chapter.

The principle of MS/MS is illustrated in Fig. 1. Tandem mass spectrometry can be conceived in two ways: in space by the coupling of two mass spectrometers, or in time by an appropriate sequence of events in an ion storage device. This consequently leads to two main categories of instruments that allow for tandem mass spectrometry experiments: tandem mass spectrometers in space or in time.(Hoffmann and Stroobant 2007)

| Isolation of the precursor ion | Fragmentation by collision with inert gas | Mass analysis of the product ions |
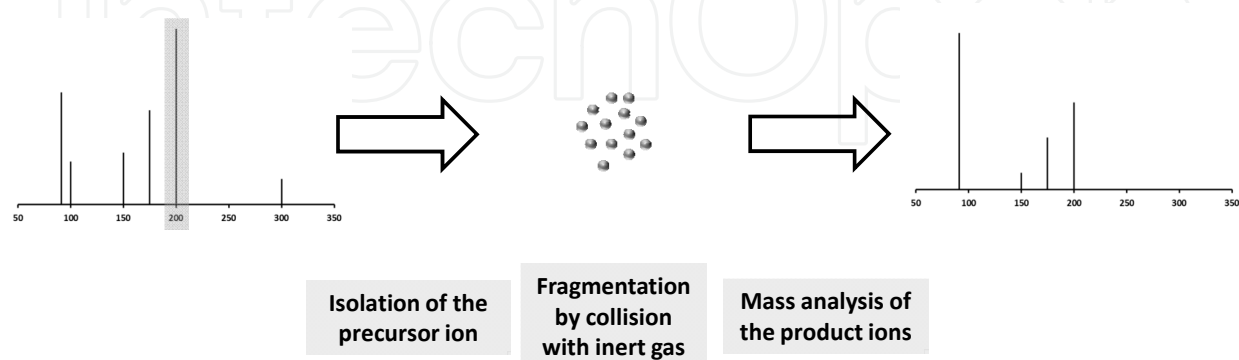
Fig. 1. Principle of MS/MS: a precursor ion is isolated by the first mass analyser, fragmented by collision with an inert gas and the products ions are analysed by the second mass spectrometer.

Tandem mass spectrometers in space are either double focusing mass spectrometers (Fig. 2) or instruments of the quadrupole type (Fig. 3).
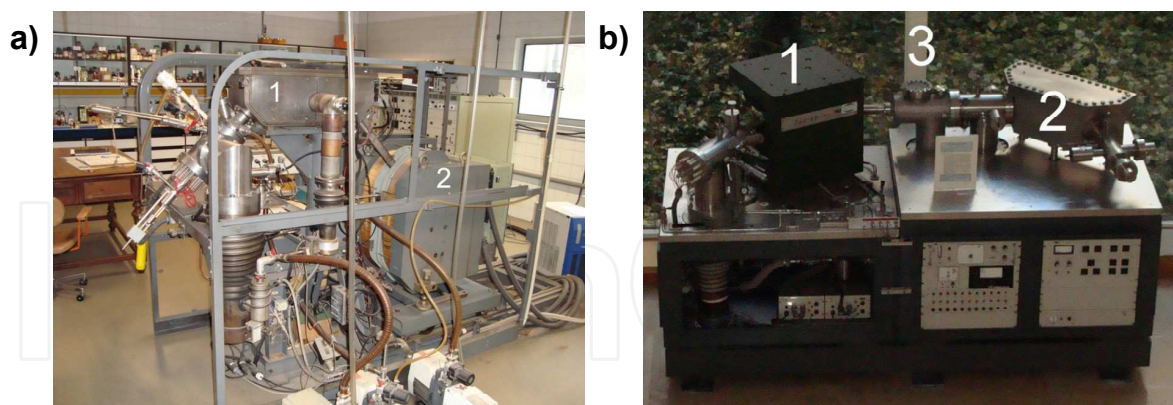

Fig. 2. A)Double focusing mass spectrometer MS9 (AEI) installed at the Forensics Biological and Environmental Mass Spectrometry Laboratory of the Faculdade de Ciências da Universidade de Lisboa, Portugal. It is a Nier-Johnson geometry mass spectrometer, electric sector-E- (1) is followed by the magnetic sector-B- (2) - EB - hence it is possible to study metastable ions through linked scans. B) Double focusing mass spectrometer ZAB 2F (VG Analytical) on display at the Faculdade de Ciências da Universidade de Lisboa, Portugal. It is a reverse geometry mass spectrometer, magnetic sector (1) followed by an electric sector (2), and it is equipped with a collision cell (3) making possible to perform CID experiments.
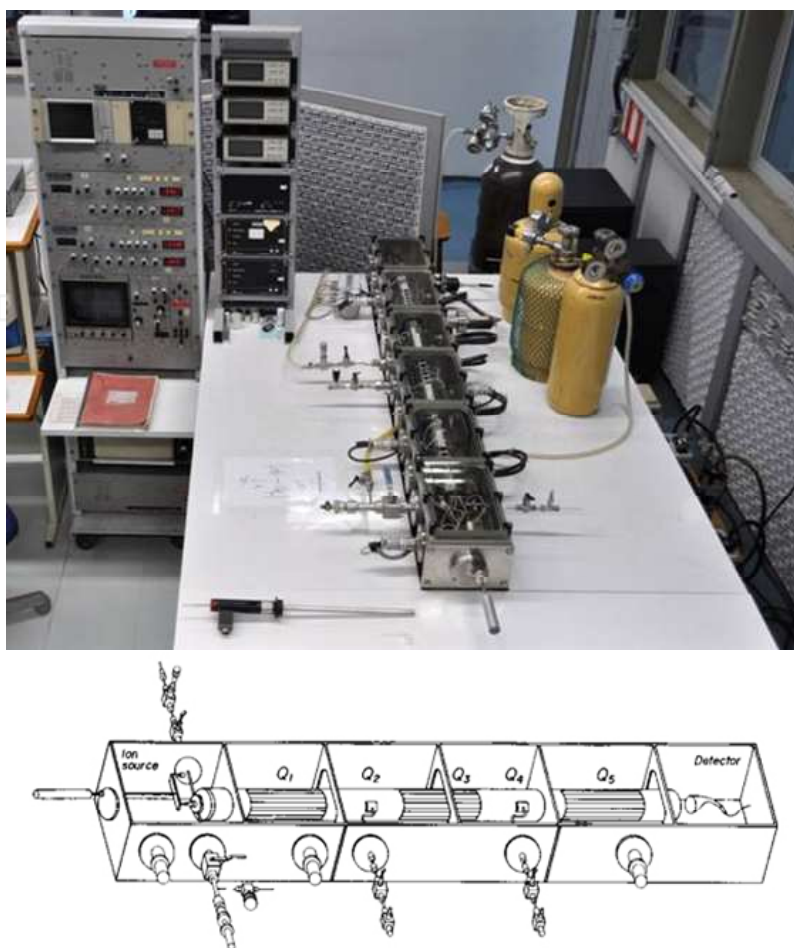
Fig. 3. Extrel Pentaquadrupole QqQqQ instaled at the ThoMSon Lab, Universidade Estadual de Campinas, Brazil. The photo was kindly supplied by Professor Marcos N. Eberlin.

Tandem mass spectrometers in time are ion traps (Fig. 4), Fourier transform ion cyclotron resonance mass spectrometers (Fig. 5) and orbitrap.

To note that the theory underlying each mass analyser will not be addressed in this chapter so the reader is encouraged to seek this information in other sources. There are several reviews on the principle of quadrupole mass spectrometry,(Douglas 2009) ion traps, (Louris, Cooks et al. 1987; Douglas, Frank et al. 2005; March 2009) FTICR, (Comisarow and Marshall 1974; Marshall, Comisarow et al. 1979; Marshall, Hendrickson et al. 1998; Barrow, Burkitt et al. 2005) time-of-flight (Price and Williams 1969; Guilhaus, Selby et al. 2000) and information on sector mass spectrometry that can easily be found in mass spectrometry textbooks.(Chapman 1985; Gross 2004; Dass 2007; Hoffmann and Stroobant 2007)

Common tandem mass spectrometers in space have, at least, two mass analysers. Quadrupoles are frequently used as mass analysers. For example, the QqQ configuration indicates an instrument with three quadrupoles, where the second one (indicated by the lower case q) is the reaction region working in RF-only mode (i.e. serving as a lens for all the ions). Other instruments combine electric and magnetic sectors (E and B) or electric and magnetic sectors and quadrupoles (E, B and qQ). Time-of-Flight (TOF) instruments equipped with a reflectron or a combination of quadrupoles with TOF instruments are also used to perform tandem mass spectrometry in space. (Hoffmann and Stroobant 2007)

Higher order MS^n spectra can be acquired by combining more analysers; nevertheless, this will certainly increase the complexity of the instrument and consequently its cost. Theoretically, any number of analysers can be sequentially combined, but since the fraction of ions transmitted in each step is low, the practical maximum, for the particular case of beam instruments, is three or four.(Hoffmann and Stroobant 2007) Nevertheless, there is always room to improve; researchers of the ThoMSon Lab (University of Campinas, Brazil) built a one-of-a-kind instrument, the pentaquadrupole (Fig. 3A), QqQqQ. In fact, the ThoMSon lab is best known as "The home of the pentaquadrupole". This instrument is still working and it is used mainly for the study of gas-phase ion-molecule reactions.

Tandem mass spectrometry experiments can also be performed through time separation with analysers such has ion traps, FTICR and orbitraps, programmed so that different steps are successively carried out in the same instrument. The maximum number of steps for these instruments is generally seven to eight (even though manufacturers claim to go as far as 10). In these instruments the proportion of ions transmitted is high, but, at each step, the mass of the fragments becomes lower.(Hoffmann and Stroobant 2007) Considering ion traps and FTICR, there are some differences among them. The most important is that in an ion trap the ions are expelled from the trap to the detector in order to get a signal (i.e. they can be observed only once at the end of the process), while in a FTICR the ions are detected in a non-destructive manner and can be measured at each step in the sequential fragmentation process.(Hoffmann and Stroobant 2007)
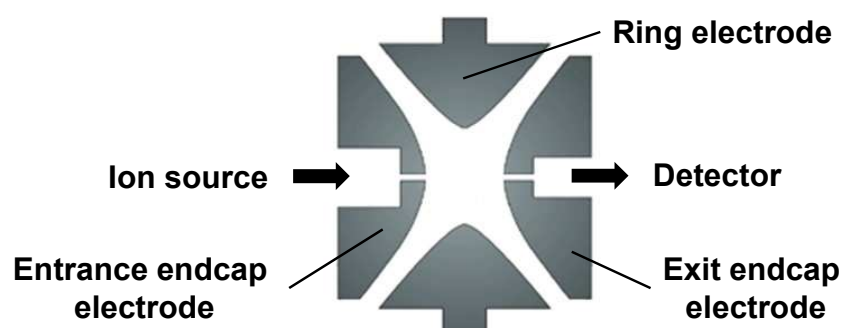

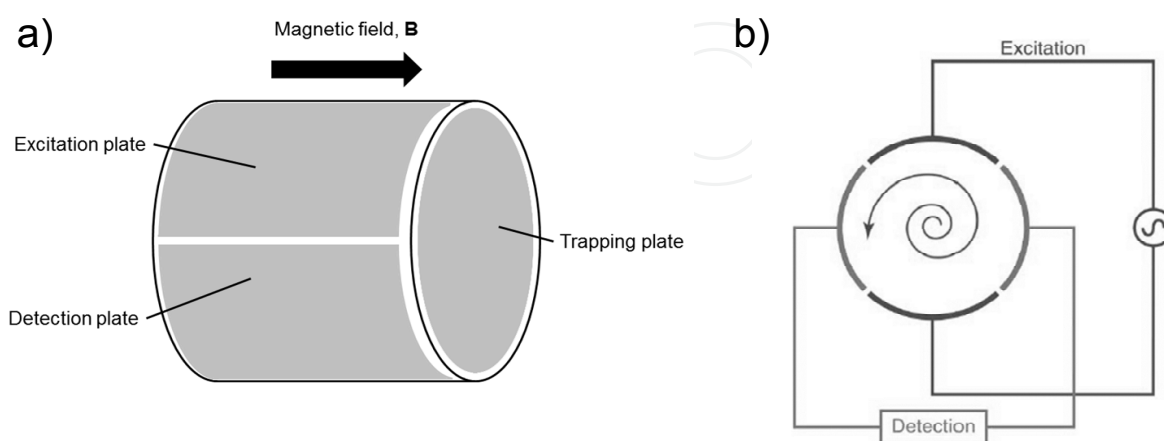Fig. 4. Schematic representation of an ion trap mass analyser.

Fig. 5. Schematic representation of: a) an ion cyclotron resonance cell (to note that the cell is composed of 6 plates, 2 for excitation, 2 for detection and 2 for trapping of the ions), b) excitation of an ion packet to higher orbit radius to allow detection.

The four main scan modes available using MS/MS, product ion scan, precursor ion scan, neutral loss scan and selected reaction monitoring – SRM – (also known as multiple reaction monitoring, MRM) are depicted in Fig. 6. A symbolism proposed by Cooks and co-workers (Schwartz, Wade et al. 1990) to easily describe the various scan modes is also depicted in Fig. 6.
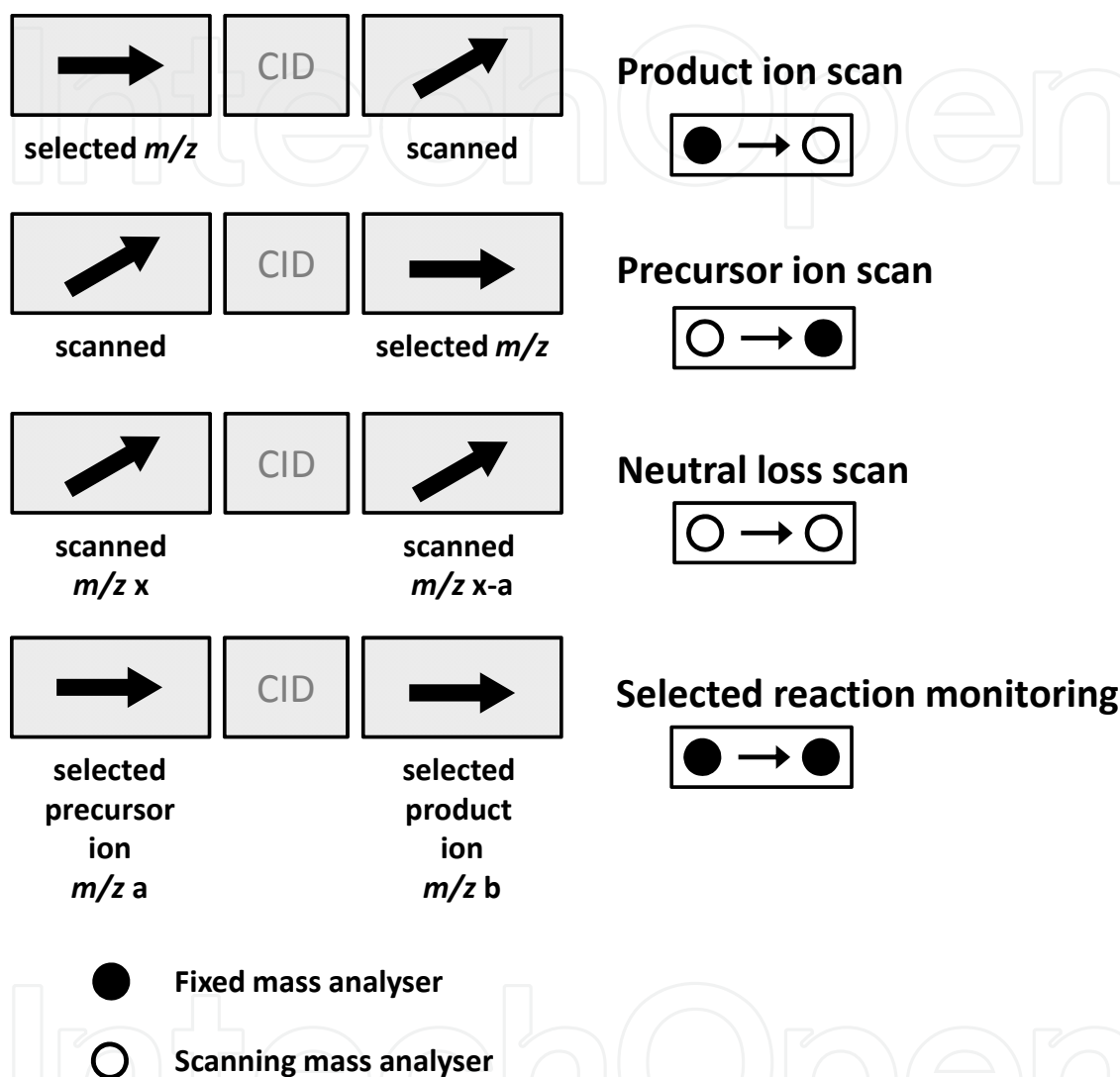

Fig. 6. Tandem mass spectrometry main scan modes, CID stands for Collision induced dissociation, and symbolism proposed by Cooks and co-workers (Schwartz, Wade et al. 1990) for the easy representation of the various scan modes.

Product ion scan, consists of selecting a precursor ion of a given *m/z* ratio and determining the product ions resulting from fragmentation.

Precursor ion scan consists of selecting a given product ion and determining the precursor ions. This scan mode cannot be performed with time-based mass spectrometers (ion traps and FTICRs).

Neutral loss scan consists of selecting a neutral fragment and detecting the fragmentations that lead to such loss. As in the case of precursor ion scan, this scan mode is not available in time-based mass spectrometers.

Selected reaction monitoring consists of selecting a fragmentation reaction. In this scan mode the first and second mass analysers are focused on selected *m/z* ratios thus increasing sensitivity.

Even though the precursor and neutral loss scans are not available in time-based instruments, these can perform quite easily MS^n experiments.

2. Fragmentation methods (Activation methods)

Early mass spectrometers were capable of only one stage of mass analysis and structure elucidation relied on the dissociation of the molecular ion in the course of its formation and within the ion source.

Soft ionization techniques (FAB, ESI and MALDI), that extended the range of application of mass spectrometry to polar, thermally labile compounds yield primarily protonated (or deprotonated) molecules with little or no fragmentation, limiting also the structural information to a single stage mass spectrum. To circumvent this limitation, tandem mass spectrometry emerged as an essential technique for structural analysis of a wide range of compounds with a clear focus on biologically relevant compounds (drugs, peptides and proteins, nucleic acids among others) over the past years.(Sleno and Volmer 2004)

Tandem mass spectrometry involves the activation of a precursor ion formed in the source and the mass analysis of its fragmentation products. The ion activation step is crucial and ultimately defines the type of product ions that are observed.(Sleno and Volmer 2004)

Method	Energy range	Instruments	Description
Collision Induced Dissociation (CID)	Low (1-100eV)	QqQ, IT, QqTOF, QqLIT, FTICR	Collision-induced dissociation by collision of precursor ions with inert target gas molecules.
	High (keV range)	Tandem TOF, Sector instruments	
Infrared Multiphoton Dissociation (IRMPD)	Low	IT, FTICR	Continuous-wave low-energy infrared laser activates the precursor ions by multiphoton absorption with consequent fragmentation
Electron Capture Dissociation (ECD)	Low	FTICR	Low-energy beam of electrons resulting in electron capture at protonation, or cationic site, with subsequent fragmentation following radical ion chemistry.
Surface Induced Dissociation (SID)	Low	Hybrid (BqQ), QqQ, IT, FTICR	Collisions between precursor ions and a solid target surface with or without a self-assembled monolayer causing fragmentation as well as other side reactions.
	High	Tandem TOF, RETOF	
IT- ion trap; FTICR – Fourier transform ion cyclotron resonance; TOF – time-of-fligth; QqQ – triple quadrupole			

Table 1. General description of the activation methods to be presented in this chapter.

2.1 Collision Activated and Collision Induced Dissociations (CAD/CID)

Collision-Induced Dissociation (CID also called Collision Activated Dissociation – CAD) remains the most common ion activation method available in present day instruments.(Jennings 2000; Shukla and Futrell 2000) Collisions between the precursor ion and a neutral gas target are accompanied by an increase in internal energy. This increase in internal energy induces decomposition with a higher fragmentation probability than, for example, metastable unimolecular dissociations.(Sleno and Volmer 2004)

The inelastic collision of an ion (with high kinetic energy) with a neutral, results on the conversion of part of the kinetic energy into internal energy leading to the decomposition of the ion.(Levsen 1978)

The conversion of kinetic energy into internal energy can be explained by the laws of physics involving a mobile species (ion) and a static target (gas). In order to simplify the description it is best to work in the center-of-mass (cm) framework instead of in the laboratory reference frame, simply because the center-of-mass momentum is always zero.(Sleno and Volmer 2004) The energy transfer in collisional activation and the kinetic energy release during ion dissociation are deduced by applying, to the process, the conservation of momentum and conservation of energy. The entire system is treated as a whole and the ion and neutral velocities are stated as velocities relative to each other.(Busch, Glish et al. 1988; Sleno and Volmer 2004)

The total energy available for conversion from kinetic to internal energy (E_{cm}) can be determined by equation 2

$$E_{cm}=[N/(m_p+N)]\times E_{Lab} \tag{2}$$

Where N is the mass of the neutral, m_p is the mass of the precursor ion and E_{lab} is the ion's kinetic energy. Equation 2 also tells us that the CID process is highly dependent on the relative masses of the two species. For example, E_{cm} increases with the target's mass, allowing more of the ion's kinetic energy to be converted into internal energy. It decreases, however, with $1/m_p$, meaning that larger precursor ions have less internal energy available for fragmentation through collision processes.(Busch, Glish et al. 1988)

Collisional activation mechanisms have been extensively studied for diatomic ions with neutral target atoms; but they are not well defined for polyatomic ions.(Sleno and Volmer 2004) The subsequent dissociation of activated ions is adequately described by the Rice, Ramsperger Kassel and Marcus (RRKM) theory (Marcus 1952) and less well by the quasi-equilibrium theory (QET).(Rosenstock, Wallenstein et al. 1952)

The overall CID process, which is depicted in equation 3, is assumed to occur by a two-step process encompassing the excitation of the precursor ions and subsequent fragmentation.

$$m_p^+ + N \rightarrow m_p^{+\neq} \rightarrow m_f^+ + m_N \tag{3}$$

Since the activation of fast-moving ions is much faster than their dissociation, it is reasonable to consider that the two processes are separate in time.

Based primarily on the kinetic energy of the precursor ion it is possible to separate the CID processes into two categories (Table 1): low-energy CID and high-energy CID.

Low-energy CID occurs in the 1-100eV range and is common in ion traps, quadrupoles and FTICR instruments. High-energy CID occurs in the kiloelectronvolt range and can be performed in sector and TOF/TOF instruments. There are important differences in the resulting CID spectra under low and high collision energy some of which will be briefly described in this chapter. In order to get more information the reader is encouraged to read

an excellent review on the subject published in the Journal of Mass Spectrometry.(Sleno and Volmer 2004)

Low-energy CID is mostly performed in quadrupole instruments (such as triple quadrupoles) and trapping instruments (such as ion traps and Fourier transform ion cyclotron resonance instruments).

Taking as example a triple quadrupole, QqQ, the collision cell is the second quadrupole (denoted q) and it is only operated in r.f.- mode (allowing the ions to be focused). This collision cell is filled with a neutral inert gas (usually N_2 or Ar) and ion activation is achieved by multiple collisions. Both the nature of the collision gas and its pressure inside the collision cell are important factors. At higher collision gas pressures the number of ions undergoing collisions and the probability for an individual ion to collide increases. In fact, increasing the collision gas pressure is a convenient means of increasing the degree of dissociation of higher mass ions (for which E_{cm} is low) and ions that are particularly stable.(Sleno and Volmer 2004)

In an ion trap, the precursor ions are isolated and accelerated by on-resonance excitation causing collisions to occur and product ions are detected by subsequent ejection from the trap. With on-resonance excitation, the isolated precursor ion is excited by applying a small (tickle) a.c. potential across the end-caps. Ion activation times of the order of tens of milliseconds can be used without significant ion losses, hence multiple collisions can occur during the excitation period. Because of this relatively long time-scale, this excitation technique falls into the category of the slow-heating processes.(McLuckey and Goeringer 1997) Nevertheless, for a slow-heating process, excitation in an ion trap is still fairly fast, due to the high pressure of gas (normally He at ≈ 1 mTorr) present in the trap. There are other slow-heating methods with much longer excitation times, such as sustained off-resonance irradiation (SORI) which will also be described in this chapter.

Much like ion traps, in FTICR instruments the isolation and excitation phenomena take place in the same confined space where ions are trapped for a specific time, in a combined magnetic and electrostatic field. On-resonance excitation can be achieved by using a short (hundreds of µs) high-amplitude a.c. signal at the natural cyclotron frequency of the precursor ion. This excites the ion rapidly via multiple collisions and deposits a large amount of energy in the ion. The short irradiation time is necessary to minimize precursor ion losses.

Off-resonance irradiation, the SORI technique, is usually applied for collisional activation of precursor ions in FTICR instruments. In SORI, the precursor ion is excited at a frequency slightly higher than the natural cyclotron frequency. Ions undergo multiple acceleration/deceleration cycles as they repeatedly increase and decrease their orbital radii in the FTICR cell before dissociation take place. The ion translational energy is small compared with on-resonance excitation and much longer activation times (from hundreds of milliseconds to seconds) are used without ion loss.(McLuckey and Goeringer 1997) Consequently, a large number of collisions take place and the ion sequentially absorbs more and more collision energy until the collision threshold is reached.

The mass of the neutral target has a more important role for low-energy CID. With heavier targets more energy is transferred. Even though the average energy deposited per collision is lower than in high-energy CID, product ion yields are very high due to the occurrence of multiple collisions which are allowed by the collision gas pressure employed and the length of the collision cell (in triple quadrupoles) or the time allotted for CID (in ion traps).

Collisional activation in the keV range occurs in sector and TOF instruments, where the precursor ions have very high translational energies. In both types of instruments, a collision cell is placed between the two mass analysers. The precursor ion beam, with a kinetic energy of a few keV, can enter the collision cell, usually causing single collisions before mass analysis of the product ions. High-energy CID usually employs He as collision gas, nevertheless, the collision yield can be increased by using a heavier gas (such as Ar or Xe).

At high kinetic energies, ion excitation is mainly electronic, (Hitoshi, Pham et al. 1969) however, vibrational and rotational energies can also play an important role in excitation.(Franchetti, Freiser et al. 1978) The conversion of kinetic into internal energy is most efficient when the interaction time coincides with the period of the internal mode which is undergoing excitation.(Beynon, Boyd et al. 1986)

The two possible CID regimes often yield different products and this can be used to our advantage since structurally important fragment ions can be formed in both techniques. For unimolecular dissociations, direct bond cleavages require energies higher than rearrangement reactions do.(Levsen 1978)

2.2 Infrared multiphoton dissociation (IRMPD)

Gaseous ions can be excited and subsequently fragmented by the absorption of one or more photons. This type of activation has been performed with a wide range of photon energies by using lasers of different wavelengths. In the early days, UV and visible lasers were used whereas in recent years there has been an increase in the use of IR lasers. When using an UV laser the absorption of only one photon provided enough energy to initiate the dissociation of an isolated precursor ion. The same does not apply for the particular case of IR lasers. These are of energies lower than the UV lasers hence, multiphoton processes are needed to sufficiently excite ions for efficient fragmentation to occur. With the rise in popularity of trapping instruments (such as ion traps and FTICR) there has been an increase in the number of applications for IRMPD as an activation technique in tandem mass spectrometry. Typically, the stored ions are activated by a low power (< 100W) continuous-wave CO_2 (10.6 μm) laser during a selected irradiance time (usually in the order of tens to hundreds of milliseconds), followed by the detection of the resulting product ions.

Generally, photodissociation can be viewed by the mechanism depicted in equation 4.

$$m_p^+ + N \xrightarrow{nh\upsilon} m_p^{+\neq} \xrightarrow{k_{dis}} m_f^+ + m_N \qquad (4)$$

Where n is the number of absorbed photons, $h\upsilon$ is the photon energy and k_{dis} is the rate constant for photodissociation.

The activation mechanism is assumed to occur through absorption of IR radiation by IR active modes present in the ion. After photon absorption, rapid energy redistribution over all vibrational degrees of freedom occurs, resulting in a statistical internal energy distribution, similar to CID. The dissociation occurs by low energy pathways, often the lowest possible.

There are several requirements for photodissociation to occur: 1) the precursor ion must be able to absorb energy in the form of photons producing excited species above the dissociation threshold for the ion of interest; 2) the energy gained by photon absorption

must overcome the energy lost by photon emission from the excited ions and deactivation by collisions.

Ion activation by absorption of photons is rather non-selective, i.e. product ions derived from the precursor ion can be further excited into dissociative states. Nevertheless, this outcome can be disadvantageous if too many ions are formed and the resulting spectrum becomes a complex collection of peaks.(Sleno and Volmer 2004)

The advantages of IRMPD are numerous. The amount of energy available is well defined, e.g. for a 10.6 μm CO_2 laser, the absorption of one photon corresponds to 0.117 eV of energy.(Sleno and Volmer 2004) If, for ion activation, enough time is given, the dissociation efficiency is good. In the case of FTICR, there is no need to add gas to the cell to promote activation and dissociation. Nevertheless, despite these advantages, it is a costly fragmentation technique.

2.3 Electron Capture and Electron Transfer Dissociations (ECD/ETD)

Electron Capture Dissociation (ECD) was developed by McLafferty's group in 1998.(Zubarev, Kelleher et al. 1998) Briefly, it involves the capture of low-energy electrons by multiply charged ions, with charge state reduction and subsequent fragmentation. ECD is the result of several important observations from ion-electron reactions which are all described in an excellent review by Zubarev et al.(Zubarev, Haelmann et al. 2002) The study of dissociative recombination (DR) correlates well with ECD and involves the fragmentation of gaseous positive ions following electron capture. The excited neutral then dissociates into two neutral (one radical and one even-electron species). Stabilization of the captured electron is faster than electron emission. Therefore, bond dissociation occurs faster than a typical bond vibration.

The main advantage of ECD is its ability to cause dissociation of very large biomolecules (≈40 kDa) at many sites, where other fragmentation methods are less effective.

ECD employs a tungsten filament (or a indirectly heated cathode) to emit a beam of very low-energy electrons (< 0.2 eV) for activation of the precursor ion. FTICR mass spectrometers are ideal for the application of ECD (McLafferty, Horn et al. 2001) and, accordingly, all the currently commercial FTICR instruments are equipped with this dissociation technique. The main reason for this is that the ICR cell traps the ions due to a combination of a strong magnetic field and a weak electrostatic field, without having any influence on the kinetic energy of the electrons during their interaction with the precursor ions.(Zubarev 2003) However, it remains difficult to perform ECD in ion traps, where the ions are trapped by strong RF potentials applied to the ring-electrode, thus affecting the movement of electrons inside the trap. To solve this problem, electron transfer dissociation (ETD) was developed by Hunt's group at the University of Virginia (Syka, Coon et al. 2004). This will be briefly described at the end of this section.

Charge neutralization occurs when a singly charged ion undergoes electron capture and the resulting neutral cannot, obviously, be detected by the mass spectrometer. As such, ECD is only applicable to multiply charged cationic species. The dissociation mechanism involves the fragmentation of an odd-electron ion resulting from electron capture by an even-electron species. The dissociation of the ion is restricted to specific protonation (or cationic) sites where the electron is captured. As a result, radical ion chemistry governs the bond cleavages.(McLafferty and Turecek 1993). Nevertheless, the electron is not necessarily captured directly at the charge site. The electron can land far from the cationic site with subsequent electron transfer to the highest charge density site. The intramolecular potential

difference, which causes this secondary electron transfer, is especially apparent in multiply charged metal ion complexes. Metal ions, such as Fe^{3+} and Zn^{2+}, serve as an electron sink and less fragmentation of the regions close to the metal is observed.(Zubarev, Horn et al. 2000) The landing of an electron on a multiply charged precursor ion creates an hypervalent (hydrogen-excess) unstable species. Groups with high affinity for the hot hydrogen atom, such as carbonyl or disulphide bonds, are principal sites for capture. The exothermicity of charge reduction and H• capture leads to bond cleavage initiated at the radical site.

ECD has been described as a non-ergodic process,(Turecek and McLafferty 1984) where the energy is not redistributed over the whole molecule and weakest bonds are not preferentially broken following ion activation. ECD assumed to occur much faster than other methods (CID and IRMPD) permitting the occurrence of direct bond cleavages only. As a result, it has been reported that the strong backbone $N-C_\alpha$ bonds of peptides are cleaved, affording c and z-type ions (see Fig. 7). Disulfide bonds, which are fairly stable under other activation methods, are also preferentially broken.
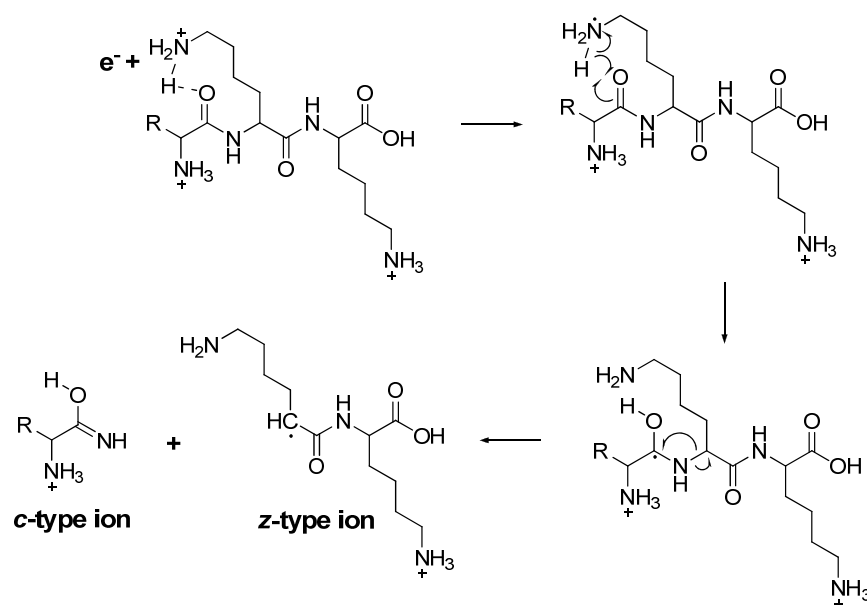
Fig. 7. Fragmentation scheme for the formation of c- and z-type ions after reaction of a low-energy electron with a multiply charged protonated peptide.

An obvious advantage of ECD is the possibility to identify post-translational modifications which remains difficult for the very labile γ-carboxyl, o-glycosyl and sulfate linkages when using other methods.(Zubarev 2003) Furthermore, even non-covalent interactions are stable under ECD conditions,(Horn, Ge et al. 2000) which allows the study of specific interaction sites and the determination of secondary and tertiary protein structures in the gas-phase.

The development of an ECD-like dissociation method for use with low-cost, widely accessible mass spectrometers, such as ion traps and linear ion traps, would be of obvious utility for protein sequence analysis. As stated above, storage of thermal electrons in a RF ion-containment field is, at best, problematic. To circumvent this, Hunt and co-workers (Syka, Coon et al. 2004) investigated the possibility of using anions as vehicles for delivering electrons to multiply charged peptide cations. They relied on their experience on negative ion chemical ionization (Hunt, Stafford et al. 1976; Hunt and Crow 1978) and concluded that anions with sufficiently low electron affinities could function as suitable one-electron

donors. Electron transfer to protonated peptides should be exothermic by 4-5.5 eV, trigger the release of a hydrogen radical and initiate fragmentation via the same non-ergodic pathways accessed in ECD.(Syka, Coon et al. 2004)

2.4 Surface Induced Dissociation (SID)

Surface Induced Dissociation (SID) is an activation method that is similar to CID, except that a solid surface is used as collision target instead of an inert gas. It was first developed by Cooks and co-workers in the 1970s and has been studied by several research groups, including those of McLafferty (Chorush, Little et al. 1995), Wysocki (Dongré, Somogyi et al. 1996; Nair, Somogyi et al. 1996; Schaaff, Qu et al. 1998) and Futrell.(Laskin, Denisov et al. 2002; Laskin, Bailey et al. 2003; Laskin, Beck et al. 2003).

The main idea behind this activation method is that energy transfer in CID is limited by the energy available in the center-of-mass reference frame (E_{cm}) which depends on the mass of the target gas. By increasing the mass of the target, E_{cm} becomes larger and energy transfer can be improved. Assuming that collisions occur with the entire surface, instead of with individual surface molecules, the mass of the target is effectively infinite. In theory, energy conversion should be more efficient in SID.(Laskin and Futrell 2003)

Cooks and co-workers classified the collision events at surfaces according to the collision energy (velocity) of the impacting projectile (neutral molecules, ions, atoms).(Verena, Jianwei et al. 2001) SID reactions fall in the 'hyperthermal' regime with energies ranging from 1eV to 100eV.

Hyperthermal ions can scatter on a surface in an elastic, inelastic or chemically reactive fashion (Table 2).
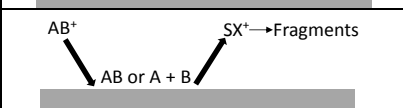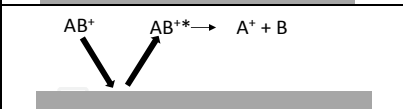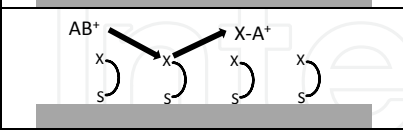
	Elastic scattering	$AB^+ + SX \rightarrow AB^+ + SX$
AB^+ → AB^+		
AB^+ → $SX^+ \rightarrow$ Fragments / AB or A + B	Charge transfer	$AB^+ + SX \rightarrow SA + SB + SX^+$
	Chemical sputtering	$AB^+ + SX \rightarrow AB + SX^+ \rightarrow S^+/X^+$
AB^+ → $AB^{+*} \rightarrow A^+ + B$	Surface-induced dissociation	$AB^+ + SX \rightarrow AB^{+*} + SX \rightarrow A^+ + B + SX$
AB^+ → $X\text{-}A^+$	Ion-surface reactions	$AB^+ + SX \rightarrow AX^+ + SB$

Table 2. Elementary processes in ion-surface interactions (SX^+ stands for surface ions).

Inelastic and reactive scattering events are the most common processes observed at the collision energies normally used in SID and reactive scattering reactions are undesired side reactions that lead to reduced efficiency and sensitivity.

Given that in general SID and CID afford similar products, it is reasonable to rationalize the activation mechanism for SID as a two-step process. Initially, the incident ion collides (inelastically) with the solid surface, forming an internally excited ion, which then undergoes unimolecular dissociations. The interaction time with the surface is of the order of 10^{-12} s, a short period compared with the dissociation time for polyatomic ions.(Sleno and Volmer 2004)

It is believed that the SID experiment involves collisional activation at the surface, followed by delayed gas-phase dissociation of the scattered projectile ion. Nevertheless, at higher collision energies, shattering of the projectile ion can occur at the surface, (Laskin and Futrell 2003) resulting in fragment ions with the same kinetic energy, since they all originate at the surface.

The internal energy distributions of excited ions for SID are relatively narrow (Wysocki, Ding et al. 1992), which offers an advantage over CID; where the internal energy of ions is not as narrow. In general, all incident ions collide directly with the surface, whereas most collisions in CID are glancing. Hence, the energy transferred can easily be varied, simply by changing the impact energy.

The center-of-mass collision energy for SID is difficult to determine, and it has been shown that several experimental factors[1] influence the amount of kinetic energy converted into internal energy of the ion.(Meroueh and Hase 2002) Due to the relatively narrow range of internal energies, SID has a potential application in isomer distinction.(Schaaff, Qu et al. 1998)

Several different instruments have been employed for SID of polyatomic ions however, several important mass spectrometer modifications are necessary. Nevertheless, for FTICR in particular, SID would be especially advantageous, given that to perform CID in these instruments it is necessary to introduce a gas in the ICR cell, which has a negative effect on mass resolutions. In fact, SID in an FTICR has been reported to be effective for the dissociation of large peptides.(Williams, Henry et al. 1990)

This activation method remains a very promising technique especially for high mass ions with relatively high dissociation thresholds, e.g. peptides and proteins. Nevertheless, it still awaits incorporation into a commercial instrument.

3. Applications

In the next sections we will present some examples where tandem mass spectrometry together with collision induced dissociations were helpful, namely for the identification of isomers (Section 3.1) and for the determination of thermochemical quantities, proton affinities and gas-phase acidities, in particular (Section 3.2).

3.1 Structure elucidation

We will present some examples of the application of tandem mass spectrometry together with collision induced dissociation to study the gas-phase behaviour of some aniline derivatives (Madeira 2010), α,β-unsaturated γ-lactones fused to sugars(Madeira, Rosa et al. 2010) and isoflavone aglycones.(Madeira, Borges et al.)

Gas-phase behaviour of aniline derivatives

The structures of the aniline derivatives studied are depicted in Fig.8.
The electrospray ionization MS[2] spectra of the protonated haloanilines studied are depicted in Fig. 9.

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[1] These are surface composition, projectile structure, collision energy and incidence angle, just to name a few.
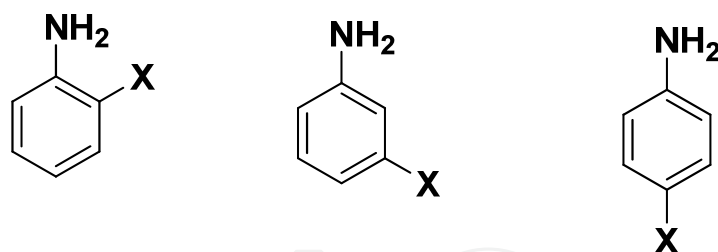
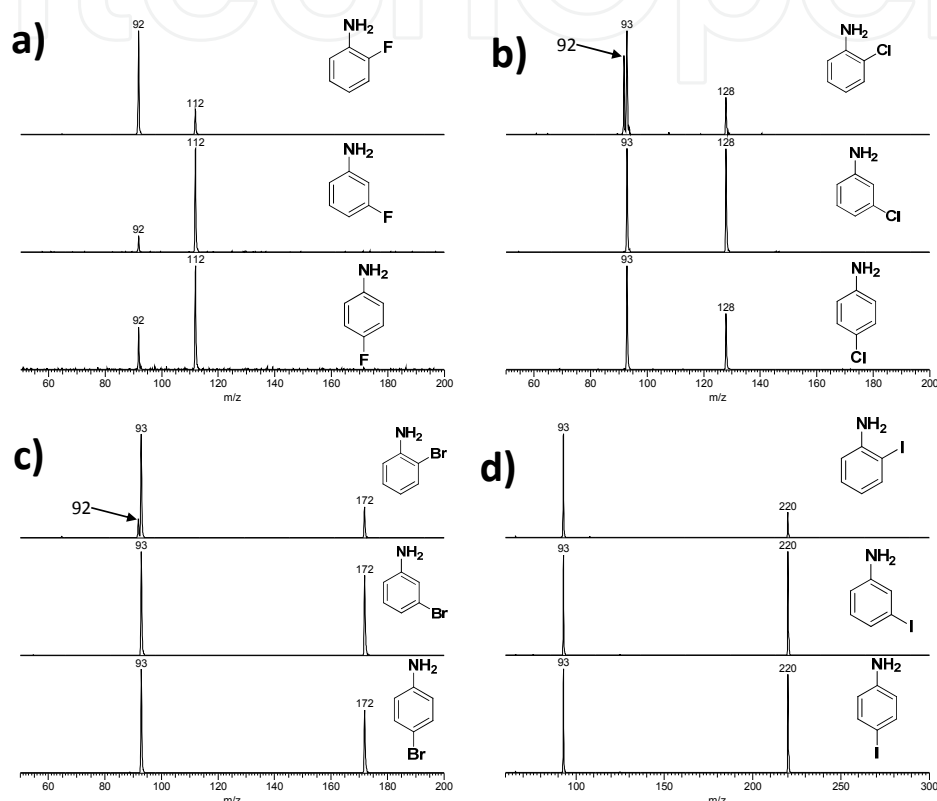Fig. 8. Structures of the aniline derivatives studied. (X=F, Cl, Br, I and NO$_2$).

Fig. 9. MS^2 spectra at 30% collision energy level of: a) fluoroanilines, b) chloroanilines, c) bromoaniline, d) iodoanilines.

It is clear that the fragmentation is similar for all, however, for the particular case of 2-chloroaniline and 2-bromoaniline, the formation of an ion at *m/z* 92, attributed to the loss of HCl, for 2-chloroaniline, and HBr, for 2-bromoaniline, allows for the differentiation of the *ortho* from the *meta* and *para* isomers.

Interestingly, the protonated molecules of the three isomers of fluoroaniline afforded the ion at *m/z* 92, attributed to the loss of HF, which was already reported for fluoranilines under electron ionization conditions.(Tajima, Ueki et al. 1996) This loss was proposed to occur via a "ring-walk" mechanism, where the fluorine atom migrates to the *ortho* position. A similar behaviour has also been reported for 4-fluorotoluene and 4-chlorotoluene.(Parry, Fernandez et al. 1992) Taking into account that the typical ion residence time within the ion trap is a few hundred milliseconds (the maximum ejection time for these MS^2 experiments was set to 200 ms) and that this phenomenon was detected in a sector mass spectrometer,(Tajima, Ueki et al. 1996) for which the typical ion residence time within the ion source is ca. 10^{-6} s, it is

reasonable to assume that this ring walk is possible in ion trap mass spectrometry conditions. With this in mind, it is understandable that the m/z 92 for 2-fluoroaniline has the highest relative abundance of the three. The proposed fragmentation pathways for the haloanilines are presented in Fig. 10.
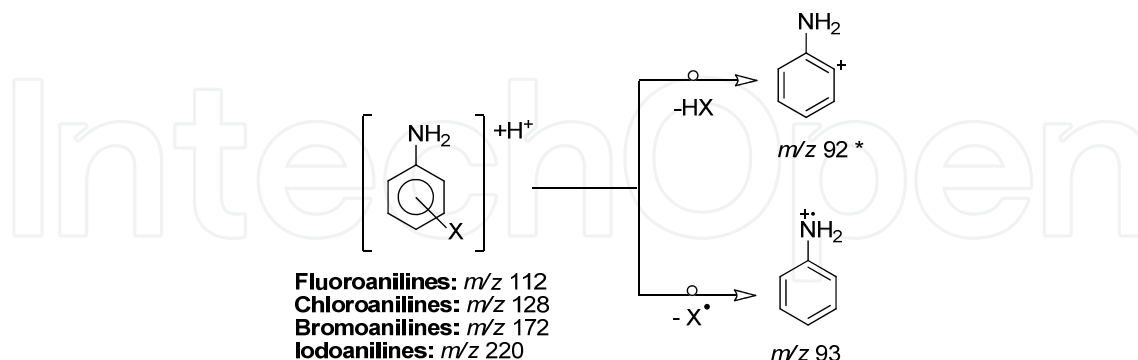

Fluoroanilines: m/z 112
Chloroanilines: m/z 128
Bromoanilines: m/z 172
Iodoanilines: m/z 220

Fig. 10. Proposed fragmentation pathways for the protonated molecules of the haloanilines (*- the m/z ion was only detected for the fluoroanilines, 2-chloroaniline and 3-bromoaniline).

The electrospray ionization MS² spectra of the nitroanilines studied are depicted in Fig. 11. It is quite clear that the three isomeric forms can easily be distinguished from the fragmentation pattern alone.
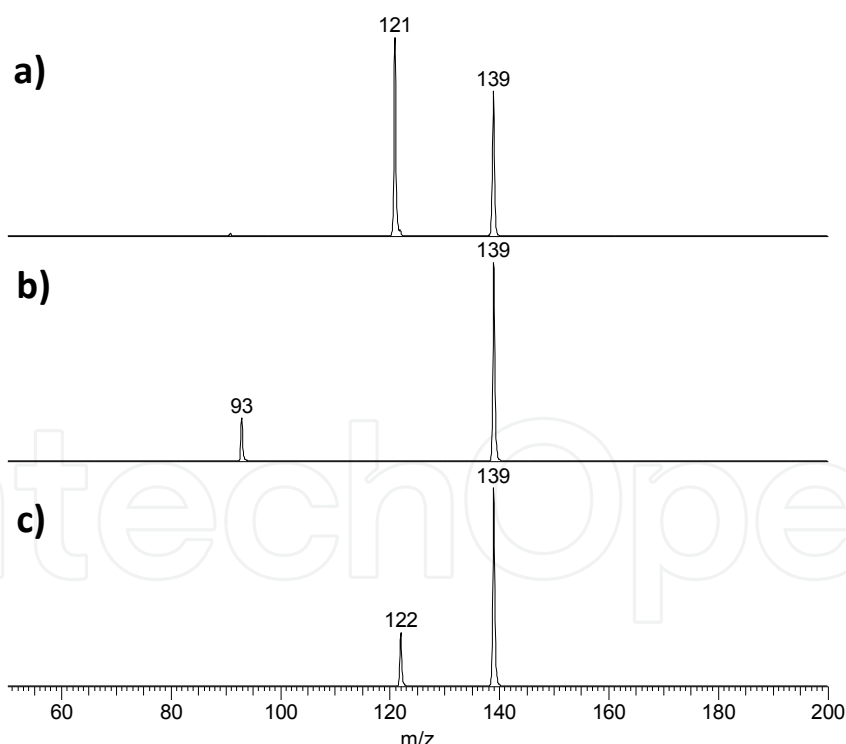
Fig. 11. MS² spectra at 30% collision energy level of: a) 2-nitroaniline, b) 3-nitroaniline, c) 4-nitroaniline.

The protonated molecule of 2-nitroaniline, Fig.11a, loses 18 Da, attributed to H_2O, affording an ion at m/z 121. Since this loss is not detected for the other two nitroanilines, it can be attributed to an *ortho* effect. This effect is well documented in the literature for electron

ionization,(Schwarz 1978; Bobyleva, Kulikov et al. 1989; Attygalle, Ruzicka et al. 2006; Jariwala, Figus et al. 2008), but it was also observed when using electrospray ionization.(Holman, Wright et al. 2008) A possible pathway for this fragmentation is depicted in Fig. 12 and involves the formation of a six-membered ring intermediary.
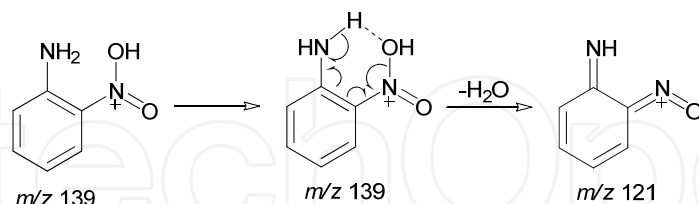

Fig. 12. Possible fragmentation pathway to afford the *m/z* 121 ion from the protonated molecule of 2-nitroaniline (*m/z* 139).

Gas-phase behaviour of α,β-unsaturated γ-lactones fused to sugars

In this study (Madeira, Rosa et al. 2010) we came across an interesting behaviour of two of the compounds studied (structures depicted in Fig. 13), that are isomers. The MS² spectra of the deprotonated molecules of these compounds are depicted in Fig. 14.
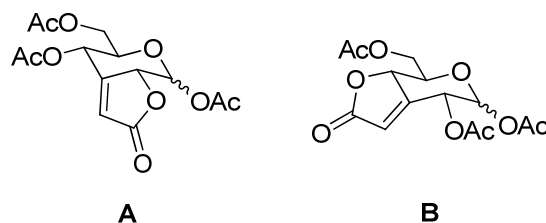
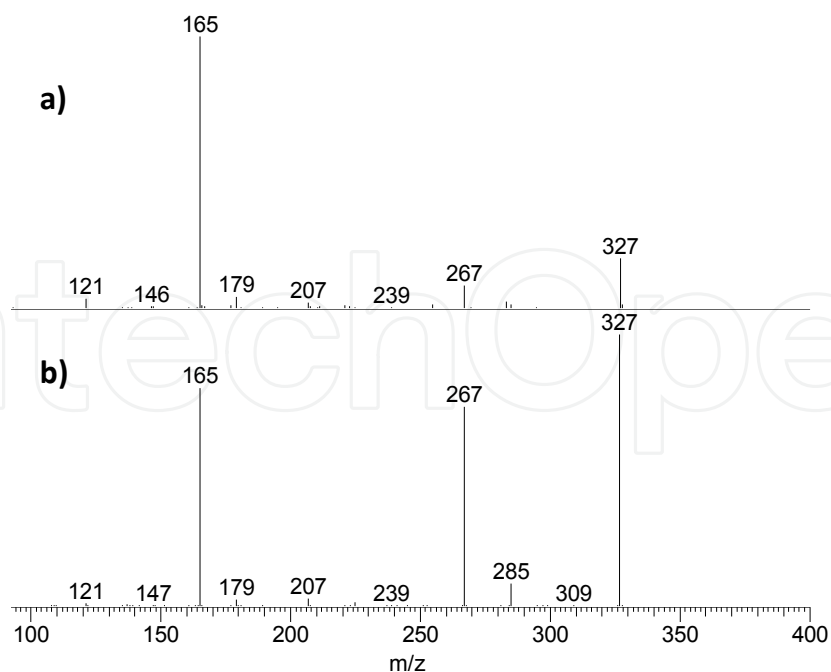Fig. 13. Structures of the two isomers included in the electrospray ionization study.

Fig. 14. Negative ion mode ESI MS² spectra at normalized collision energy of 15% of the deprotonated molecules of: a) compound **A**; b) compound **B**. (Reprinted with kind permission of John Wiley & Sons)

These compounds are isomers that differ in the positions of the lactone moiety and of one of the acetyl groups within the sugar ring. Regarding their fragmentation, the first significant difference is the loss of 42 Da, ketene (H_2CCO), from the deprotonated molecule to afford the ion at *m/z* 185. This loss is more pronounced for compound **B** than for **A**, and this behaviour might be related with the different substitution patterns of these two compounds. Both compounds lose 60 Da, CH_3COOH, affording the product ions at *m/z* 267 and, again, this loss is more pronounced for compound **B** than for **A**. This behaviour was attributed to the fact that compound **B** possesses an acetyl group at C^2 which is more labile than the other substituents. In this particular case the MS^2 experiments allowed the distinction between both isomers.

Gas-phase behaviour of isoflavone aglycones

Regarding the gas-phase behaviour of isoflavones (Madeira, Borges et al. 2010) it was also possible to differentiate two isomers (prunetin and biochanin A) since their fragmentation afforded different diagnostic ions for each. The ESI-MS^2 spectra of the protonated molecules of formononetin, prunetin and biochanin A are depicted in Fig. 15.
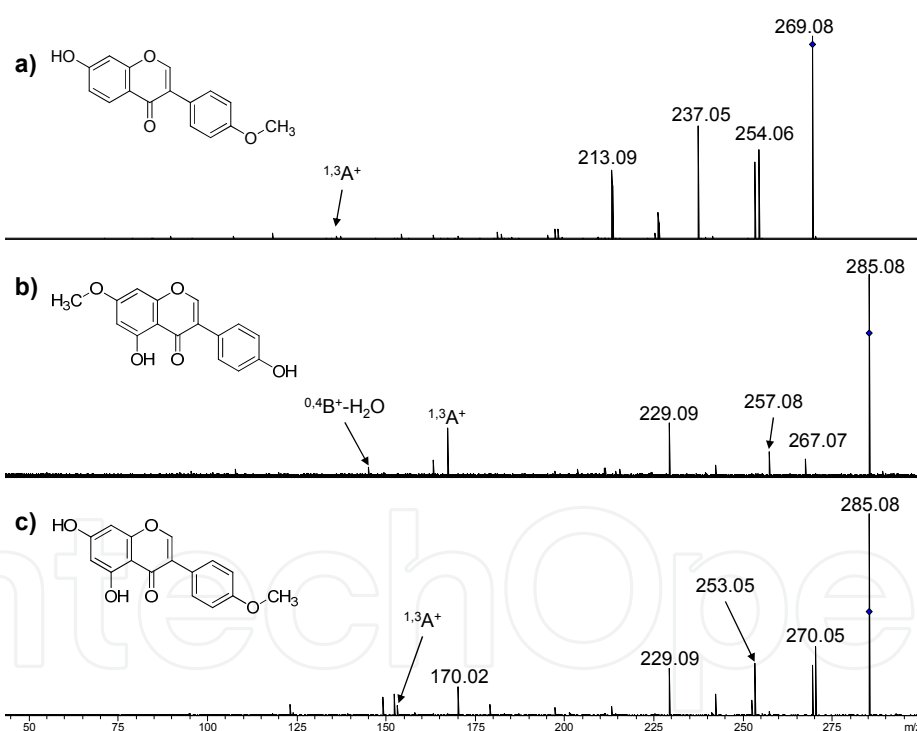

Fig. 15. ESI-MS^2 spectra at collision energy of 17 eV of the protonated molecules of: a) formononetin; b) prunetin; c) biochanin A. (Reprinted with kind permission of John Wiley & Sons)

The position of the methoxy group also influences the fragmentation pattern. For instance, the losses of $^\bullet CH_3$ and CH_3OH were only detected for formononetin and biochanin A,which have the methoxy group attached to the B-ring. The ions afforded by these fragmentations can also serve to access the B-ring substitution.

3.2 Gas-phase ion thermochemistry

Tandem mass spectrometry has other uses than the frequently and currently used structural analysis or the identification of proteins. MS/MS techniques can, for example, also be used to determine gas-phase thermochemical properties of several classes of molecules (biomolecules included).

The kinetic method was developed in order to determine thermochemical properties whenever the equilibrium method was either not applicable or the instrumentation was not available, and was based on the rates of competitive dissociation of mass-selected cluster ions.(Cooks and Wong 1998) Over the years, the kinetic method has been subjected to a great deal of discussion, for example see the Special Feature issue of volume 34 of Journal of Mass Spectrometry.(McLuckey, Cameron et al. 1981; Armentrout 1999; Capriolli 1999; Cooks, Koskinen et al. 1999; Drahos and Vékey 1999).

Proton affinities and Gas-phase basicities of aniline derivatives

We will start with a simple example using only compounds with known thermochemical properties. In this example we are going to deal only with proton affinities (PA) and gas-phase basicities (GB) which can be determined in the positive ion mode.(Madeira 2010) The kinetic method can, however, be used to determine other thermochemical properties. For example, the gas-phase acidity is determined in the negative ion mode.

The thermochemical properties of several aniline derivatives are well known (Table 3) and we will use these to illustrate how to apply the kinetic method in its simplest form.

	PA (kJ mol^{-1})	GB (kJ mol^{-1})
4-Nitroaniline	866.0	834.2
3-Fluoroaniline	867.3	835.5
3-Chloroaniline	868.1	836.3
4-Fluoroaniline	871.5	839.7
3-Bromoaniline	873.2	841.4
4-Chloroaniline	873.8	842.0

Table 3. Proton affinities (PA) and gas-phase basicities (GB) of haloanilines and 4-nitroaniline. (Hunter and Lias) These values were assumed to have an error of 8.4 kJ mol-1.

For this example we will determine the proton affinity and gas-phase basicity of 4-nitroaniline by pairing it with the other reference compounds presented on Table 3. The mass spectrum of a mixture of 4-nitroaniline and 4-fluoroaniline is presented in Fig. 16a and the MS^2 spectra at different normalized collision energies are depicted in Fig. 16b-e.

In the MS^2 spectra presented earlier (Fig. 15b-e) it is clear that the ion at *m/z* 112 (protonated 4-fluoroaniline) has a higher abundance than the ion at *m/z* 139 (protonated 4-nitroaniline). It is therefore reasonable to say that 4-fluoroaniline has a higher proton affinity (and gas-phase basicity) than 4-nitroaniline. The abundance ratio between the reference ion (4-fluoroaniline) and the unknown ion (4-nitroaniline) is a key element for the application of the kinetic method. Repeating the procedure for the other references in Table 3 we obtain the data presented in Table 4.
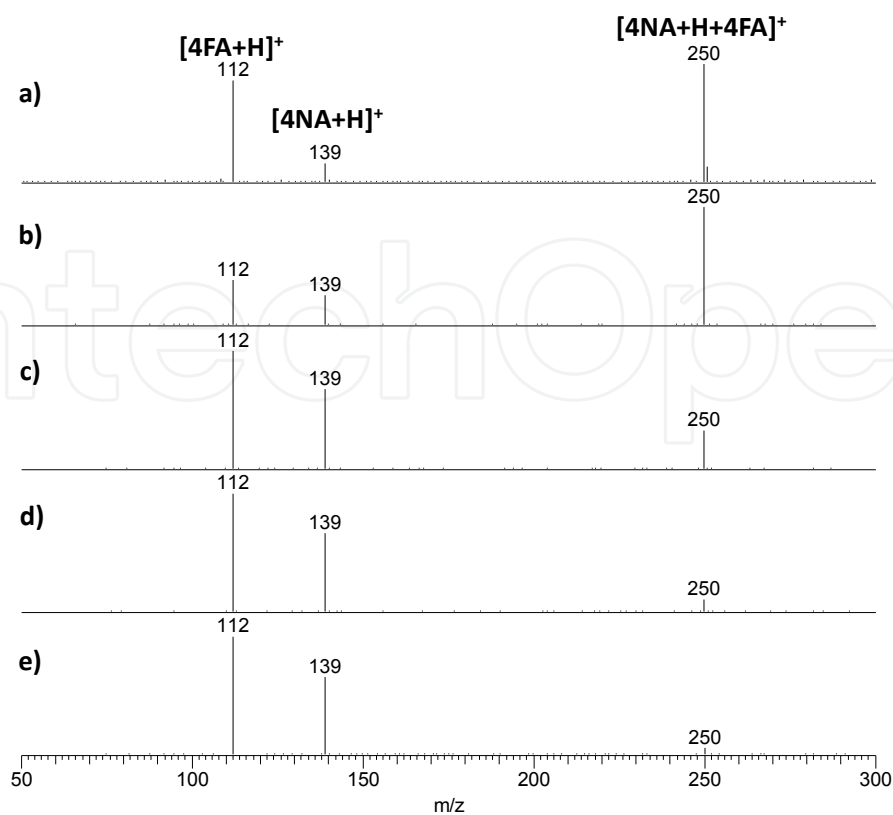
Fig. 16. Full scan mass spectrum of a mixture of 4-nitroaniline (4NA) and 4-fluoroaniline (4FA); b) MS² spectrum of the heterodimer [4NA+H+4FA]⁺ (*m/z* 250) at a normalized collision energy (NCE) of 5%; c) MS² spectrum of the heterodimer at NCE 7%; d) MS² spectrum of the heterodimer at NCE 10%; e) MS² spectrum of the heterodimer at NCE 15%.

	PA (kJ mol⁻¹)	GB (kJ mol⁻¹)	Ab(AH⁺)/Ab(BH⁺)	Ln [Ab(AH⁺)/Ab(BH⁺)]
3-fluoroaniline	867.3	835.5	2.3	0.8
3-chloroaniline	868.1	836.3	2.7	1.0
4-fluoroaniline	871.5	839.7	0.7	-0.4
3-bromoaniline	*873.2*	*841.4*	*2.6*	*1.0*
4-chloroaniline	*873.8*	*842.0*	*2.3*	*0.8*

Table 4. Values of Ab(AH+)/Ab(BH+) and Ln[Ab(AH+)/Ab(BH+)]. (AH+ reference ion, BH+ unknown ion). 3-bromoaniline and 4-chloroaniline are presented in italic because they were not considered when constructing the ln(abundance ratio) vs GB or PA graphical representation. See explanation in the text.

3-Bromo and 4-chloroanilines were removed from the data, since their behaviour was somewhat unexpected (Fig. 17a and b). Indeed, according to the gas-phase thermochemical data presented in Table 3, after fragmentation of the heterodimer the abundance of the protonated molecule of 4-nitroaniline should have been lower than that of 4-chloroaniline and 3-bromoaniline. The MS² presented in Fig. 17 show the opposite thus, these two systems were not considered when calculating the proton affinity and gas-phase basicity of 4-nitroaniline.

For these two systems, isomerization reactions could be responsible for this behaviour. For the particular case of 4-chloroaniline, the migration from the *para* to the *meta* position should be energetically favourable. This type of migration has already been reported by Parry et al.(Parry, Fernandez et al. 1992) for *para*-halotoluenes and it seems to be a reasonable explanation for this observation. In fact, the branching ratio found for 4-chloroaniline is comparable to the one found for 3-chloroaniline, which supports the assumption of isomerization.
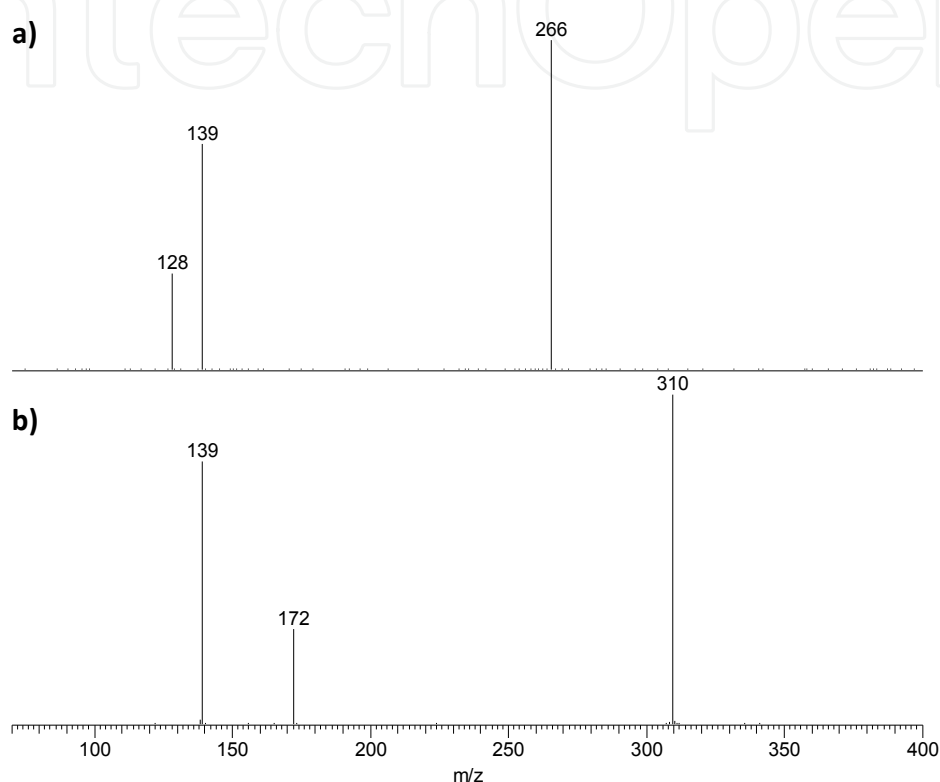
Fig. 17. ESI-MS² spectra at NCE 5% of a) 4-chloroaniline + 4-nitroaniline proton-bound heterodimer (*m/z* 266); b) 3-bromoaniline + 4-nitroaniline proton-bound heterodimer (*m/z* 310).

The data on Table 4 allows us to construct the graphical representation that will enable us to determine the proton affinity and gas-phase basicity of our unknown, 4-nitroaniline. This graphical representation is depicted in Fig 18.

The proton affinity/gas-phase basicity is the intercept of the least mean squares trendline with the x-axis. For 4-nitroaniline, the proton affinity was estimated to be 866.3±0.8 kJ mol⁻¹ and the gas-phase acidity 834.5±0.8 kJ mol⁻¹. These values are in close agreement with the literature values (Table 3), which are 866.0 kJ mol⁻¹ and 834.2 kJ mol⁻¹ for the proton affinity and gas-phase basicity, respectively.

Gas-phase acidities of substituted phenols

Some antioxidant mechanisms displayed by several phenolic compounds relate with OH bond dissociation energy (DH⁰(ArO-H). One way to determine it in the gas phase is to combine the gas-phase acidity, $\Delta_{ac}H^0(ArOH)$, the electron affinity of the phenoxyl radical, $E_{ea}(ArO^\bullet)$, and the ionization energy of the hydrogem atom, $E_i(H)$ (equation 5).

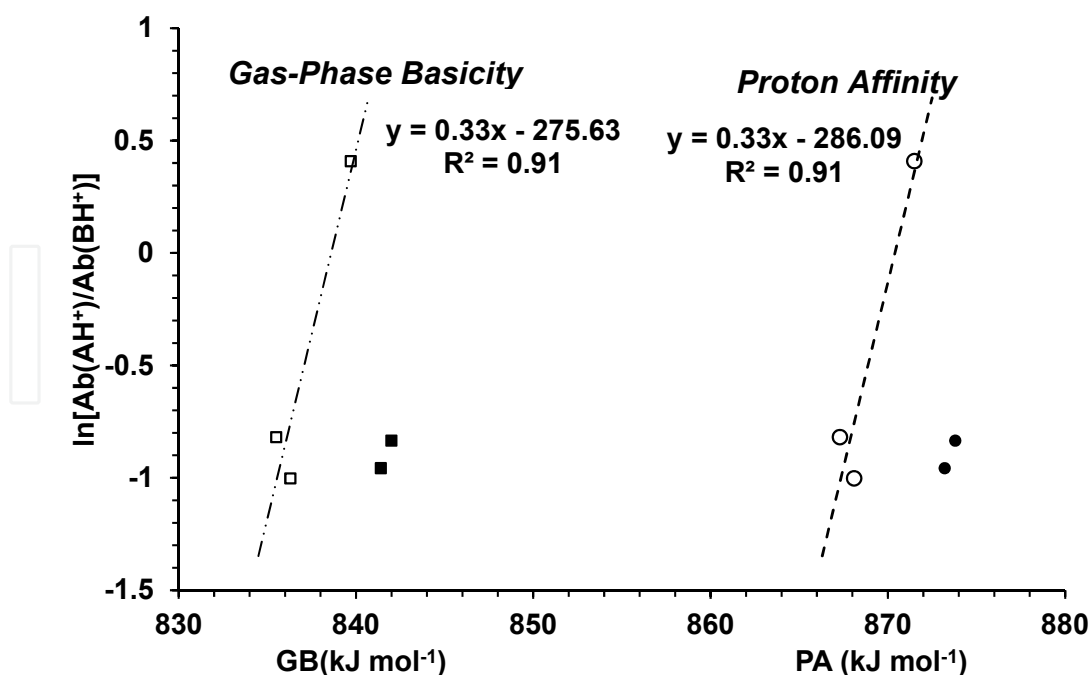Fig. 18. ln[Ab(AH+)/Ab(BH+)] vs gas-phase basicity (kJ mol^{-1}) and proton affinity (kJ mol^{-1}). (AH+ – reference ion, BH+ – unknown ion).

$$DH^0(ArO-H)= \Delta_{ac}H^0(ArOH)+E_{ea}(ArO^\bullet)-E_i(H) \qquad (5)$$

In the past years we have determined the gas-phase acidity for several substituted phenols, including dimethylphenols,(Madeira, Costa et al. 2008) dimethoxyphenols and chromanol(Madeira, Faddoul et al. 2011), with the purpose of establishing a bridge towards vitamin E.

Fig. 19a and b depicts the negative chemical ionization mass spectrum of a mixture of 3,5-dimethylphenol and 4-methylphenol and the MS^2 spectrum of the isolated heterodimer (*m/z* 229). To note that in the case of proton affinities and gas-phase basicities the work is done in the positive ion mode while for gas-phase acidities the mass spectra are acquired in the negative ion mode.
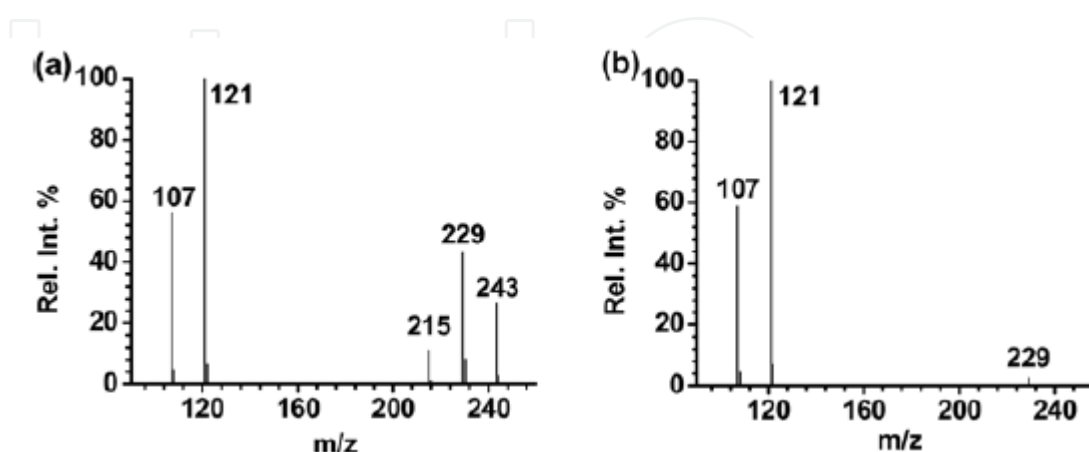

Fig. 19. (a) Chemical ionization mass spectrum of 3,5-dimethylphenol and 4-methylphenol mixture. (b) MS^2 spectrum of the isolated heterodimer (*m/z* 229, 6 ms of delay time). With kind permission from Springer Science+Business Media (Madeira, Costa et al. 2008)

Same as for the aniline derivatives presented earlier, if we pair the unknown compounds with other compounds with known gas-phase acidity we can apply the kinetic method formalism and determine its gas-phase acidity. The gas-phase acidity scales for dimethylphenols (Madeira, Costa et al. 2008) and for dimethoxyphenols and chromanol (Madeira, Faddoul et al. 2011) are graphically depicted in Fig. 20.
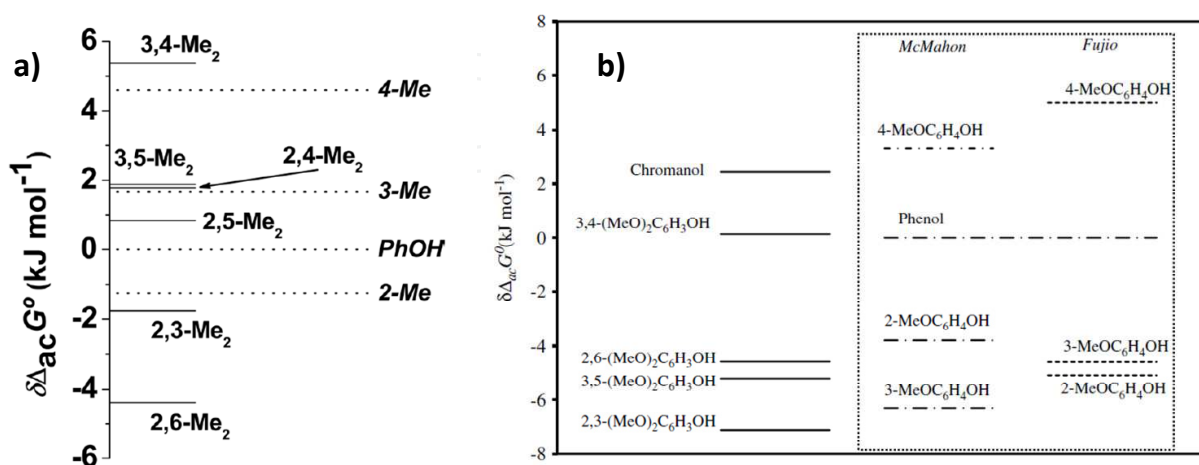

Fig. 20. Experimental gas-phase acidity scales relative to phenol for: a) dimethylphenols, with kind permission from Springer Science+Business Media (Madeira, Costa et al. 2008); b) dimethoxyphenols, with kind permission from John Wiley and Sons (Madeira, Faddoul et al. 2011).

4. Acknowledgment

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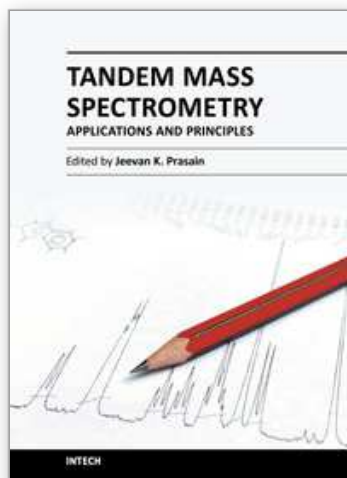
Tandem Mass Spectrometry - Applications and Principles

Edited by Dr Jeevan Prasain

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http://www.intechopen.com/books/tandem-mass-spectrometry-applications-and-principles/applications-of-tandem-mass-spectrometry-from-structural-analysis-to-fundamental-studies

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