Ion Scanning or Ion Trapping: Why not Both?

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ABSTRACT

The present contribution describes analogies and differences between the quadrupolar ion trap and the quadrupole mass analyzers, shows the potentialities of their combination in a single instrument and presents a review of applications of such a technology in different fields. The first section describes the Quadrupole Mass Filter (QMF), outlining its principles of operation and the ion sorting procedure according to the use of oscillating electric fields inducing stable trajectories to the ions allowing them to reach the detector. Multiple quadrupole systems (normally triple quadrupoles) are then explained, showing their use in tandem mass spectrometry in space experiments (MS/MS-in-space). Quadrupolar Ion Trap (QIT) principles of operation are then examined, pointing out that in this case the use of the same combination of oscillating electric fields takes advantage of unstable ion trajectories for their sorting. Substantially, analogies and differences between QMF and QIT come out, which consist in the fact that QMF is a scanning mass analyzer, whereas QIT is a sequential mass analyzer. In addition, the section underlines that QIT is capable to perform tandem mass spectrometry in time experiments (MS/MS-in-time). Later, the possibility to use a quadrupole as a trapping system with a prevailing dimension (Linear Ion Trap, LIT) is taken into consideration, and the possibility to combine both QMF and LIT in a single instrument, a QTrap mass spectrometer, is illustrated. In this frame, a lot more experiment types are possible with respect to both standalone triple quadrupoles and LIT, and they are described as well. Several combinations of these QTrap features can be used in IDA (Information Dependent Acquisition) mode, allowing the high versatility of this instrumental configuration. The second section deals with a review of applications in different fields. These are organized by kind of QTrap and IDA features and cover different topics in biological, medical, agrochemical, nutritional and environmental fields.

1. INTRODUCTION

The quadrupole mass filter, often referred as linear quadrupole analyzer, is probably the most used analyzer in mass spectrometry (Douglas, 2009; Gross, 2017). Its simplicity, as well as its relatively low cost, allows the production of somewhat cheap instruments, such as entry-level GC-MS devices. On the other hand, the use of new materials and the possibility of a high precision machining of hyperbolic surfaces provide the possibility to obtain very good results (Gershman et al., 2011; Gershman et al., 2012). QMF is a low-resolution analyzer, but taking advantage of the above technologies, it is possible to get a better separation than nominal mass, even if not enough for a reliable measurement of the accurate mass, but capable to get a higher selectivity when selecting or monitoring ions. In addition, the scan speed (in principle, QMF is a "slow" mass analyzer) can take advantage of the most recent developments in electronics. These performances induce higher price instruments, but increase a lot the versatility of this kind of mass spectrometers. In the field of tandem mass spectrometry (MS/MS or MS^2) can be achieved by the use of three devices in a triple quadrupole geometry (also a cost increase) and allows the accomplishment of MS/MS-in-space experiments (McLafferty, 1981; Gross, 2017).

The quadrupole ion trap (QIT) is a widespread used analyzer as well (Todd & Penman, 1991; Paul, 1993; March, 1997; March & Todd, 2005). Again, it can take advantage by simplicity and cheapness, but can become quite sophisticated, offering higher performances and versatility, with a consequent cost increase. Its resolving power may be, in principle, also relatively good, up to about 30,000, simply lowering the scan speed, which is, however, much higher with respect to that of a QMF (Schwartz et al., 1991). The basic operative principles are indeed practically the same for both analyzers, and this is obvious, considering that Wolfgang Paul originated the classic (tridimensional) ion trap by a quadrupole section rotation around one of the axes, and that a standard quadrupole can act as an ion trap (linear ion trap). QMF and QIT use completely different approaches for ion sorting, as better explained later. As far as tandem mass spectrometry is concerned, ion trapping allows the accomplishment of MS/MS-in-time experiments, giving up some experiments allowed by MS/MS-in-space, but offering the possibility of multiple stage MS/MS fragmentation (MS^n) without any requirement of increasing the number of devices.

Both analyzers provide advantages and drawbacks, and both may be used in any mass spectrometry applications. QMF, in its triple quadrupole configuration, is able to provide a better performance for quantitative analysis, whereas QIT performs better for qualitative analysis. The main reasons for this dualism is that QMF is a slow scanning device, but very effective in performing selected ions monitoring. In contrast, QIT is a fast scanning device, so that it can perform several scans in a relatively short period, with a better ion statistics, and a higher signal

intensity. On the other hand, the required steps for selected ions trapping (ion cooling, ion isolation, ion excitation and fragmentation and, finally, product ion isolation and trapping) lead to an overall low efficiency for ion monitoring. Nevertheless, both QMF and QIT can reliably operate in qualitative and quantitative analysis .

In 2002, Sciex proposed a joined approach, presenting the API 2000 QTrap on the market. The idea is quite simple: such an instrument is a "standard" triple quadrupole mass spectrometer but when needed, the third quadrupole can act as a linear ion trap (Hager, 2002). This configuration allows to place together in the same machine both technologies, making this kind of instrument a highly versatile one (maybe the most versatile configuration among the different MS instruments available on the market). For instance, this configuration allows the accomplishment of MS/MS experiments both in-space and in-time. It is just too bad that accurate mass measurements are not possible.

2. QMF: PRINCIPLES OF OPERATION

Paul and Steinwegen described the principle of the QMF (Paul & Steinwedel, 1953; Paul & Raether, 1955; Paul, Reinhard, & von Zahn, 1958; Douglas, 2009). The QMF analyzer is a device that takes advantage of the stability of the trajectories in oscillating electric fields to separate ions according to their *m/z* ratios. They are made up of four rods of circular or, ideally, hyperbolic section (Figure 1), that must be perfectly parallel (Dawson, 1986). Ions pass through an oscillating electric field obtained by applying to the four bars a composite potential comprising a direct current voltage (DC) and an alternating voltage, in the region of radiofrequencies (RF), such as that instantaneously the voltage on a pair of bars is the opposite with respect to the other two bars, as illustrated in the Figure 2 (Gross, 2017). In other words, the RF frequency applied on a pair of bars is out of phase of 180° with respect to the other.

Such a combination results in a constantly opposite potential on the horizontal bar pair with respect to the vertical one: when the vertical pair is at its maximum positive value, the horizontal one is at its minimum negative value. The scanning function consists in varying the DC potential and the amplitude of the alternating potential so to keep their ratio constant. Ions move along the z-axis, and follow stable trajectories in the (x,z) and/or the (y,z) planes according to the Paul equations (Gross, 2017):

$$\frac{d^2x}{dt^2} + \frac{ze}{mr_0^2}\,(U + V\cos\omega t)\,x = 0$$

$$\frac{d^2y}{dt^2} - \frac{ze}{mr_0^2}\,(U + V\cos\omega t)\,y = 0$$

where e is the electron charge, z is the charge number, thus ze is the charge, m is the ion mass, r_0 is the distance of the center edge of each bar from the center of the QMF, U is the DC voltage, V is the RF amplitude with frequency ω and t is time. For any combination of U, V, and ω, only a certain *m/z* value or *m/z* range allows stable trajectories both in the (x,z) and (y,z) planes, so that the ion can reach the end of the QMF along the z-axis.

The Paul equations can also be written in a dimensionless form as the Mathieu equations, with a change of variables (Dawson, 1986; Gross, 2017):

$$\frac{d^2x}{d\tau^2} + (a_x + 2q_x\cos 2\tau)\,x = 0$$

$$\frac{d^2y}{d\tau^2} + (a_y + 2q_y\cos 2\tau)\,y = 0$$

where $a_x = -a_y = \frac{4zeU}{mr_0^2\omega^2}$, $q_x = -q_y = \frac{2zeV}{mr_0^2\omega^2}$, $\tau = \frac{\omega t}{2}$.

Suitable a and q values identify a stability region where the ion moves according to stable trajectories and no collision with the bars happens. This makes possible its mass spectrometry analysis.

If we want to use the QMF for ion monitoring (Selected Ion Monitoring, SIM), the a and q parameters can be kept constant on a particular value, so that only an ion with a particular *m/z* value can follow a stable trajectory. This function is very useful for quantitation purposes because it strongly increases the signal-to-noise ratio and, hence, the sensitivity.

RF-only quadrupoles, that is without the application of the direct potential, can act as very effective ion guides, capable of focalizing the ion beam in the center of the device. For this reason, they are quite often used in the ion optics of some instruments. Moreover, when a suitable collision gas is introduced inside, they can act as effective collision cells, where collisionally induced dissociation (CID) of ions takes place: the resulting fragment ions are well focused to the center by the RF potential, so that possible scattering phenomena can be minimized (Dawson, 1986).

The combination of at least three QMFs (Q1 and Q3 scanning, q2 RF-only) allows configuring one of the most used MS/MS systems: the triple quadrupole that is schematically represented in Figure 3 (Yost & Enke, 1978, Yost & Enke, 1979).

This instrumental arrangement allows performing MS/MS-in-space experiments. The different scan mode, Scan or SIM mode of Q1 and Q3 (the analyzing QMFs) and the use of q2 as a collision cell, allows four types of MS/MS experiments, which are shown in Figure 4 (Gross, 2017).

In product ions scan, Q1 is used in SIM mode to select the desired precursor ion, which is fragmented in q2. Q3 is scanned to sort the product ions formed in the collision cell. Precursor ions scan is performed by using Q3 in SIM mode to select the desired product ion, whereas Q1 scans the possible precursor ions. When the precursor ion passing Q1 produces the selected precursor ion, the signal can reach the detector providing a signal. This mode is used to select, in a complex matrix, a family of compounds having a common, charged, sub-structure. For instance, a precursor ion scan of *m/z* 149 can be useful to reveal all the alkyl phthalates present in the mixture. In neutral loss scan, both Q1 and Q3 scan the desired mass range, but they are not in phase: there is a constant *m/z* difference. A signal can reach the detector only when the ion passing in Q1 fragments losing a neutral moiety corresponding to the *m/z* difference between Q1 and Q3. This mode is used to select, in a complex matrix, a family of compounds having a common, neutral, sub-structure. For instance, a neutral loss of *m/z* 162 can help in the identification of glucosides present in the mixture. Of course, due to the lack of accurate mass, both the phthalates and glucosides examples illustrated should require further investigations. For Selected Reaction Monitoring (SRM), Q1 is tuned to select the desired precursor ion, and Q3 is tuned to select the desired product ion. Hence, both Q1 and Q3 operate in SIM mode, significantly increasing the selectivity with respect to SIM on a single QMF. The monitoring of several precursor-product couples is possible, so that often this experiment is denoted as Multiple Reaction Monitoring (MRM). This operation mode offers very high selectivity and sensitivity and is presently the "gold standard" for quantitation.

3. QUADRUPOLE ION TRAP: PRINCIPLES OF OPERATION

Wolfgang Paul is also the creator of the quadrupolar ion trap (QIT) (Paul & Steinwedel, 1953). Quadrupole Ion Trap could be considered as coming from the rotation of a section of a quadrupole around an axis (in the Figure 5 scheme, the horizontal z axis).

The resulting device is something whose section is depicted in the Figure 6. The ring electrode acts as a diagonal bar pair of a quadrupole, whereas the endcap electrodes behave as the other pair. The application of the correct combination of DC and RF voltages allows, in this case, ion trapping inside the device, which takes advantage also of the presence of an inert gas (helium in GC-MS,

nitrogen when using atmospheric pressure ionization sources) that cools down the ions to a stable level. The motion equations are the same involved in the QMF operation.

The scanning function consists, in this case, in the increasing the amplitude of the alternating potential, together with a supplementary AC voltage, so that ions increase their energy, start oscillating along the z axis until their trajectories become unstable and exit from the ion trap, reaching the detector. This process is very fast, so that scan times of the order of microseconds may be used. This make the QIT a much faster analyzer with respect to the QMF.

For tandem mass spectrometry experiments, QIT does not need multiple devices as in the case of the triple quadrupole. The use of DC and RF voltages in the opportune combination allows trapping just a particular ion, the precursor ion, which can be excited by the use of a pulsed supplementary voltage that makes possible its fragmentation. The trap then scans normally to separate such fragment ions. However, if we like, we can select and trap a particular product ion that becomes the "new" precursor ion, which fragments also giving up to "new" fragment ions. The process can be repeated, in principle, several times, provided that enough signal is remaining. This refers as MS^n (when normally referring to MS/MS as MS^2). This multiple stage tandem mass spectrometry is one of the principal features of MS/MS-in-time with respect to MS/MS-in-space. On the other hand, ion trap is not capable to perform precursor or neutral loss scan experiments.

4. QMF AND ION TRAP: ANALOGIES AND DIFFERENCES

Quadrupole mass filter and quadrupole ion trap are two similar and at the same time different devices, although their operation originates from the same physical principles. Both make use of quadrupolar electric fields obtained by a combination of DC and RF potentials, but in a very different way.

QMF uses them to stabilize a particular *m/z* ion, so that it can take a stable trajectory, reaching the end of the device and, hence, the detector. The continuous variation of the DC and RF potentials, keeping constant their ratio, stabilize the next *m/z* ions so that a complete scan affords the mass spectrum. QMF is a "scanning" analyzer: at any moment all the ions without the correct value of *m/z* are discarded. QMF, hence, takes advantages of stable trajectories through the device.

QIT uses DC and RF potentials to trap ions inside the device. Their excitation induces unstable trajectories so that ions are sequentially ejected out of the trap. In other words, QIT takes advantage of unstable trajectories. QIT is hence a sequential analyzer: it allows the simultaneous transmission of all ions, sorted one after the other. All the ions entering the analyzer are collected onto the detector.

As far as resolving power is concerned, both QMF and QIT are normally considered unit resolution mass analyzers. This is correct, but, actually, QIT can operate at higher resolving power (Schwartz et al., 1991). QMF is a relatively slow mass analyzer. A scan speed of 200 Da/s is a good compromise between scan time and signal quality. If necessary, it can operate at a higher speed, 1000 or 2000, even up to above 10000 Da/s, but ion statistics become very poor, and the resulting spectra are satisfactory only if the signal is relatively high (that is, sensitivity gets worse). When the cycle time, that is the time between one scan and the next, is not a problem (for instance, when performing analysis by continuous infusion with a syringe pump) the spectrum quality can be improved using a lower scan speed (down to 10 Da/s). Scan speed, however, does not affect the resolving power, but only the definition of the chromatographic peaks in term of number of data points. Figure 7 compares the $[M-H]^-$ ion of catechin, at *m/z* 289, acquired on a QMF using 200 (a) and 10 (b) Da/s. The peak width, and hence the resolving power is practically identical. Looking to the T.I.C. chromatograms (analysis of a standard mixture of catechin and epicatechin, Figure 8) we can observe that the trace obtained scanning at 10 Da/s is not acceptable for quantitation purposes: the peaks of catechin and epicatechin are distorted because they are described only by 5 data points (at least 12 are required for a good integration).

QIT, on the contrary, is a relatively fast mass analyzer. It can readily operate at 10000 Da/s, while a lower scan speed can be used without any problem. In this case, however, a lower scan speed improves the resolving power, as clearly shown in the Figure 9. Resolving power, defined as Full Width at Half Maximum (FWHM) divided by the relative m/z value, at 10000 Da/s is substantially the same as a QMF, even if slightly better, but reaches the value of over 5000 at 50 Da/s. Unfortunately, such a performance is not useful for accurate mass measurements, due to the typical mass shift associated with the ion trap (Traldi & Favretto, 1992; Murphy & Yost, 2000). This is also evident from Figure 9 itself, where the *m/z* value of the ion is different in the four experiments.

In summary, even if QMF and QIT take advantage of the same physical principles, they are quite different mass analyzer: differences are more significant than analogies. In the original idea of Wolfgang Paul, the QIT is a tridimensional device derived from the rotation of a section of a quadrupole around an axis. A standard quadrupole, however, is also able to trap ions, making use of opportune trapping potentials. In this case, there is a dominant direction in the sense that the x, y and z dimensions are not the same as in a tridimensional QIT, so that this kind of approach refers as "Linear Ion Trap" (LIT) (Tolmachev et al., 2000). Linear ion traps provide high capacities as far as the number of trapped ions is concerned, because the ion cloud can expand along the entire device. The scanning can operate in two possible modes: one employs excitation of the ions to achieve

mass-selective ejection in radial direction, the other uses mass-selective axial ejection by application of an auxiliary electric field to the rods of the LIT (Welling et al., 1998; Hager, 2002). LITs operate as the "traditional" tridimensional ion traps, also including the capability of precursor ion selection for MS/MS experiments (Collings et al., 2003). After their introduction, LITs become very widely used in mass spectrometry (Douglas et al., 2005).

5. COMBINATION OF QUADRUPOLE AND ION TRAP IN A SINGLE INSTRUMENT

The use of a standard quadrupole as a LIT, when operating in the axial ejection mode, makes the motion of the ions along the trap similar to what they do along the quadrupole under normal conditions. This suggests the possibility to drive a quadrupole in standard or ion trap mode, "on demand". In principle, in a triple quadrupole, any of the QMFs could operate in its "normal" scanning function or in trapping mode. Because of this consideration, a new mass spectrometer configuration, named QTrap was proposed (Hager, 2002). The name, QTrap reflects the fact that the instrument combines quadrupole(s) operating as QMFs and quadrupoles operating as LITs in the same platform. Figure 10 shows the schematic representation of the triple quadrupole instrument used for developing such an apparatus. As in standard triple quadrupole systems, curtain plate acts as the counter-electrode for the electrospray voltage, orifice plate allows the ion entrance through the orifice, skimmer is used to select the core of the expansion plume. The ion source (not shown in the scheme) can be Electrospray (ESI), Atmospheric Pressure Chemical Ionization (APCI) or Atmospheric Pressure PhotoIonization (APPI). In principle, both Q1 and Q3, and, if desired, also q2 can act as a LIT, but in the usual configuration, just Q3 was driven in such a way.

This approach provides all the classical scan functions of a triple quadrupole, and additional experiments related to the use of Q3 (and/or q2) as a LIT. Moreover, tandem mass spectrometry experiments can be performed by selection of the precursor ion in Q1, fragmentation in q2 and ion sorting in Q3 (MS/MS-in-space) but also using Q3 in LIT mode for isolating, exciting and fragmenting a precursor ion (MS/MS-in-time). Table 1 summarizes the different scan modes and the related role of the different devices, Q1, q2 and Q3 (Hopfgartner et al., 2003; Hopfgartner et al., 2004). The upper part of the table lists the classic triple quadrupole functions, while the lower part shows the possible experiments when LIT is operative.

Table 1. Description of the various scan modes for Triple Quadrupole and LIT-related in a QTrap mass spectrometer. Adapted from figure 2 in Hopfgartner et al., (2003) with permission. Copyright © 2003, Wiley.

Scan Type (Triple Quad)	Q1	q2	Q3
Q1 Scan	Resolving (Scan)	RF-only	RF-only

Q3 Scan	RF-only	RF-only	Resolving (Scan)
Q1 SIM	Resolving (Fixed)	RF-only	RF-only
Q3 SIM	RF-only	RF-only	Resolving (Fixed)
Product Ion Scan (MS2)	Resolving (Fixed)	Fragment	Resolving (Scan)
Precursor Ion Scan (PI)	Resolving (Scan)	Fragment	Resolving (Fixed)
Neutral Loss Scan (NL)	Resolving (Scan)	Fragment	Resolving (Scan Offset))
Selected Reaction Monitoring (SRM)	Resolving (Fixed)	Fragment	Resolving (Fixed)
Scan Type (LIT in QTrap)	**Q1**	**q2**	**Q3**
Enhanced Q3 Single MS (EMS)	RF-only	No Frag	Trap/Scan
Enhanced Resolution Q3 Single MS (ER)	RF-only	No Frag	Trap/Scan
Enhanced Multiply Charge (EMC)	RF-only	No Frag	Trap/Empty/Scan
Enhanced Product Ion (EPI)	Resolving (Fixed)	Fragment	Trap/Scan
MS^3	Resolving (Fixed)	Fragment	Isolation/Frag Trap/Scan
Time delayed frag capture Product Ion (TDF)	Resolving (Fixed)	Trap/No Frag	Frag/Trap/Scan

The enhanced Q3 single MS (EMS) is the method to generate a conventional mass spectrum, in which ions are transmitted from the source, through the RF mode quadrupoles, into the ion trap by the RF voltage, working in the radial direction, and the DC operating in the axial direction. LIT is filled with ions in 1–500 ms, and once trapped the ions are cooled, just thermalizing their kinetic energy, typically in 10–30 ms. Then, the ions are axially ejected according to their mass in a sequential order, exploiting the fringe fields of the lenses at the end of the quadrupole. This scan type delivers a highly sensitive full scan for the detection of unknown analytes (Hopfgartner et al., 2004).

The enhanced resolution (ER) scan mode allows the obtainment of mass spectra with increased resolution, because of the slow scan rate of LIT. In a typical experiment, ions within a 30 Da region are collected in Q3 for a specified time and scanned slowly at 250 Da/s. Only a 10 Da window is displayed. Resolution of about 6000 (FWHM) can be achieved, allowing the determination of the charge state of multiply charged ions (Hopfgartner et al., 2004). These are useful when trying to determine structural information, perform database searches, and perform peptide sequencing,

among many other applications (SCIEX Technical Note, 2016). Unfortunately, the aforementioned mass shift, typical of quadrupole ion trap devices prevents the possibility to get accurate mass determination.

The enhanced multiply charged scan mode (EMC) is a unique QTrap function that can be used to improve the signal/noise ratio on ions, which are multiply charged, since it allows the removal of singly charged ions from the LIT. EMC is based on the principle that once the ion trap has been filled and the ions have been cooled for an adequate time, they have the same kinetic energy. After thermalization, the effective DC trapping barriers depend only on the charge state of the ions and not on their masses. So that, suitable settings of the DC voltage and trap emptying time allow a preferential release of selected ions, starting with ions with the lowest charge (Hopfgartner et al., 2004)

The Enhanced Product Ion (EPI) scan is a trap scan used to obtain high quality MS/MS spectrum on a specific ion. Q1 is used as a resolving RF/DC transmission quadrupole to select the precursor ion of interest, which is then accelerated into q2, where it is submitted to fragmentation and the resulting fragment and residual precursor ions are transmitted into the Q3 LIT, where they are mass selectively scanned out toward the detector. While the Q3 LIT is performing the scan, ions can be accumulated in Q0, further enhancing instrument duty cycle (Hager & Le Blanc, 2003). One of the interesting features is the lower mass cutoff compared with 3D ion traps since the fragmentation step is spatially separated from the LIT (Hager & Le Blanc, 2003; Douglas, 2009; Gross, 2017). Moreover, since precursor ion is selected in Q1 that is also spatially separated from the LIT, no critical isolation of precursor ions from the other ions, which often happens with conventional ion traps and that could lead to activation and loss of the precursor ion itself, takes place (Hopfgartner et al., 2003; Hager & Le Blanc, 2003). Finally, as expected, the fragmentation patterns of spectra obtained with Q3 working as a conventional QMF and as a LIT are nearly identical, but the EPI scan delivers higher sensitivity and faster scanning when compared to a classic basic product ion scan (Figure 11).

QTrap mass spectrometers can also carry out MS^3 (MS/MS-MS) experiments, and, consequently, they have also MRM^3 capabilities. The first fragmentation is achieved as usual by accelerating the precursor ions, selected in Q1, into q2. Both fragments and residual precursor ions are transmitted into the LIT, where they are cooled, and the next-generation precursor ions are isolated by a suitable resolving DC voltage. They are then excited by applying a suitable RF voltage and fragmented to give the sequential product ion spectrum. It is well known that MS^3 can remove the interference and enable a much lower detection of the analyte, in the meanwhile allowing the obtainment of additional structural information (Hopfgartner et al., 2004).

The time delayed fragmentation (TDF) scan mode can be used to simplify the lower half of an MS/MS spectrum, since it is able to produce product ions that arise from precursor ions with a modified internal energy distribution, so that multiple sequential fragmentations are significantly reduced. It is well known that CID of precursor ion is originated by the conversion of its translational energy, which is the consequence of its collision with a neutral gas target, into internal energy. If the latter is high, fragments from multiple reaction could be achieved. Contrarily to the classical triple quadrupole, where ion activation is carried out via Q1-to-q2 acceleration, TDF performs ion activation via q2-to-Q3 acceleration, so that fragments can be originated from cooled precursor ions, with undoubted advantages in terms of control of the fragmentation process (Hager, 2003; Hopfgartner et al., 2004).

The co-presence of both a (relatively) low speed, scanning analyzer and a (relatively) high speed, sequential analyzer in the same instrument allows the performance of multiple and combined experiments in the same scan. In principle, it is possible to set a main experiment and, when a particular condition occurs, switch the instrument to perform another function. This approach combines different scan function in the same acquisition session. This can be named "Data Dependent Scan" (DDS) or "Information Dependent Acquisition" (IDA) and so on. For instance, one can perform a single MS scan and, when a particular ion exceeds a definite intensity threshold, switch the instrument to MS/MS mode and carry out a product ion scan of that ion. A standard triple quadrupole instrument is not suitable for these combined acquisition modes as, even if one single possible precursor ion is pre-selected and hence a single MS/MS experiment takes place, the cycle time becomes too high for normal HPLC-MS analytical runs. In addition, these advanced scan functions provide their best utility, in terms of spectral information, if there is the possibility of collecting several product ion spectra from different precursor ions. This is quite affordable using the QTrap configuration, as well as a Q-TOF configuration: both LIT and TOF are fast scanning analyzers, but QTrap is more versatile as both MS/MS-in-Space and MS/MS-in-Time are available.

An IDA method automatically runs experiments based on results obtained from previous experiment/s. Referring to the table 1, the most common IDA experiments that can be performed on a QTrap instrument are (here the sign ">>" indicates "switches to"): Q3 Scan >> EPI, EMS >> EPI, EMC >> EPI, very useful for untargeted analysis of complex mixtures, MRM >> EPI, useful for qualitative confirmation of analytes in "classic" targeted MRM quantitation runs, Precursor Ion/Neutral Loss Scan >> EPI, also useful for untargeted analysis when looking for specific classes of compounds (for instance, NLS of *m/z* 162 >> EPI for the analysis of glucosides, as that is the neutral glucose fragment lost). In all cases, the first scan function allows getting the first MS

spectrum (or SRM transition) where one or more ions are then selected for the secondary scan function, mostly enhanced product ion scan, taking advantage of LIT scan speed. If necessary, it is possible even the combination of two survey scan experiments, such as MRM and Q3 Scan or EMS. This allows the performance of both target and untarget analysis in the same analytical run (Figure 12).

REVIEW OF APPLICATIONS IN DIFFERENT FIELDS

Here we describe some examples of the use of the advanced QTrap capabilities of this kind of instruments, underlining their utility to solve quite sophisticated analytical tasks. Very often, a QTrap Mass Spectrometer is used merely as a "normal" triple quadrupole, but the use of specific QTrap functions is constantly increasing. IDA experiments are largely prevailing in this respect. We decided to comment just the most used, so far, methodologies. The applications are presented organized by QTrap functions.

Enhanced Product Ion (EPI)

Sphingosine-1-phosphate (S1P) is the bioactive metabolite of sphingolipid, which is considered as a critical regulator of many physio-pathological processes, such as cancer, atherosclerosis, diabetes, and osteoporosis, and, more recently, also inflammation, and Alzheimer's disease. Most of the known actions of S1P are mediated by a family of five specific G protein-coupled receptors designated S1PR1–5 (Maceyka et al., 2012). A reliable quantification of low levels of S1P in biological samples could be very useful to elucidate the pathological mechanisms, but it could be a challenge and sensitive and accurate analytical techniques are necessary. *Müller and Gräler* setup a method to assay S1P in liver and heart from C57BL/6 wild-type mice, sphingosine kinase 1 knockout mice, and sphingosine kinase 2 knockout mice, based on the use of Sciex 2000 QTrap triple-quadrupole mass spectrometer operating in MRM and EPI modes (Müller & Gräler, 2021). The sensitivity with MRM was poor and the quantification of S1P at the endogenous levels was not possible; on the contrary, the EPI mode provided a sensitivity good enough to quantify S1P also in real liver extracts. These results are clearly shown in Figures 13a, 13b, and 13c.

The method exhibited a Limit of Detection (LOD) of 1 nM, which allowed the accurate quantification of S1P in liver tissues containing as little as 14 nmol/kg.

QTRAP is also used to quantitate active principles of drugs, *i.e.*, chemical substances which induce the pharmacological activity of drugs, and simultaneously screen metabolites in in vitro and in vivo samples. IDA with QTRAP allows the simultaneous semi-quantification and structural confirmation of metabolites in a single run. A method composed of one MRM scan, one IDA

criterion, and one EPI scan, where MRM scan acts as a survey scan and IDA was used to trigger EPI scans by analyzing MRM signals, can be profitably used to provide semi-quantitative and structural confirmation of metabolites in a single run (Lee JY et al., 2015; Lee K et al., 2021). This method, which is often referred to as predictive MRM>>IDA>>EPI (pMRM>>IDA>>EPI) was used by *Lee et al.* to characterize the metabolites of LW6, an antitumor drug candidate that promotes the hypoxia-inducible factor-1α (HIF-1α) degradation, in ICR mice (Lee K et al., 2021). A total of 12 metabolites, produced by amide hydrolysis, ester hydrolysis, mono-oxidation, glucuronidation, and a combination of these reactions, were characterized based on their EPI spectra, and the retention times were compared with those of the parent compound.

In addition, the field of profiling active components in natural extracts takes advantage of the EPI scan mode. *Li et al.* used different IDA approaches to investigate the chemical constituents of Danhong injection, which is extracted from salviae miltiorrhizae and flos carthami that has been widely used to treat various diseases in China for many years (Li C et al., 2019). As shown in Figure 14, they made use of both EMS>>EPI and Precursor Ions Scan>>EPI for the identification of active principles, as well as their complete chemical characterization and classification in in-house compounds library. This novel strategy allowed the detection and the characterization of 90 components. Among them, a sum of 46 salvianolic acids and related phenolic compounds were identified systematically.

Target analysis as well can get a nice improving using the QTrap potentialities. In this case, target MRM transitions can be set for the desired analytes and this operation mode can be used both for quantitation, but also as a survey scan for an IDA switching to EPI. This is very convenient because only one MRM transition for component (instead of two, required by a classic QQQ system) may be used, where qualitative confirmation can be achieved by a full scan MS/MS spectrum and, in case, library search. In this frame, *Zeng et al.* performed the analysis of 32 toxic natural substances in herbal products (Zeng et al., 2015). Figure 15 shows the MRM chromatogram of a standard solution of the 32 analytes, whereas the Figure 16 shows an example of library search matching on the EPI MS/MS spectra. It is worthy to note that this library match can be carried out using the normal functions of the acquisition software, without any need for additional software tools.

Maldini et al, from University of Sassari, used QTrap Technology to investigate antioxidant profile and vegetal metabolomics in different plant extracts (Maldini et al., 2012; Maldini et al., 2014; Maldini et al., 2016a; Maldini et al., 2016b). IDA was used to perform the qualitative analysis. The IDA method included the following: IDA criteria (specifying the charge state and the mass range), enhanced MS scan, enhanced resolution; enhanced product ion scan or MS/MS scan.

Quantitation of target compounds was later performed using MRM as a normal triple quadrupole, but, again, IDA MRM>>EPI mode was used to confirm structures by their complete MS/MS product ions spectrum. Phenolic and glucosinolates in *Moringa oleifera* (Maldini et al., 2014), and profiling and simultaneous quantitative determination of anthocyanins in wild *Myrtus communis* L. Berries (Maldini et al., 2016a) were investigated taking advantage of the above described approaches, as well as a study of "glucosinoloma" in broccoli sprouts (Maldini et al., 2012) and a metabolomic study of wild and cultivated caper (*Capparis spinosa L.*) (Maldini et al., 2016b) **Errore. Il segnalibro non è definito.**. Figure 17 shows an example of the MS spectra obtained.

In the figure the MS^2 and MS^3 are reported for an unknown component present in Moringa oleifera (A and B), compared with the MS^2 spectrum of standard glucomoringin. While the MS^2 spectrum of the component shows only an ion at *m/z* 570, its fragmentation in the MS^3 spectrum is similar to that of the standard in C. Hence, it is possible to hypothesize that the ion at *m/z* 912 is a glucosinolate structurally correlated to glucomoringin. Further studies are needed to clearly identify this compound (Maldini et al., 2014).

This IDA function, MRM>>EPI, can be very useful in many fields. For instance, an important topic is the investigation of emerging pollutants, among which Pharmaceutical and Personal Care Products (PPCPs) that are of particular concern for their presence in different environmental samples (water, sediments, and so on) as well as in tap and drinking water. *Li C et al.* recently published a paper describing the determination of 19 anthelmintics in environmental water and sediment (Li C et al., 2020), while *Gros et al.* propose the tracing of pharmaceutical residues of different therapeutic classes in environmental waters (Gros et al., 2009). In the latter case, most analytes are determined by the classic approach, two MRM transition and their ratio for qualitative confirmation, but the IDA MRM>>EPI technique was also used, as illustrated in the Figure 18.

Despite of the high number of analytes traced in this paper the authors played a good attention to the chromatographic peak definition, above 14 points per peak for all components. EPI was carried out using 3 different values for collision energy, 24, 40 and 55 eV. Figure 19 shows an example of full scan qualitative confirmation for tylosin in an urban influent wastewater.

Food safety is another field where the targeted approach MRM>>IDA can be very useful. The higher qualitative confidence offered by the full scan Enhanced Product Ion spectra is without any doubt crucial in this case where the correct identification of any food contaminant is mandatory. *Alkadri et al.* used this approach for checking the presence of mycotoxins in wheat grains (Alkadri et al., 2014). Mycotoxins are quite dangerous food contaminants, and the confirmation of identification by the EPI spectrum really helps.

Enhanced Multiply Charge (EMC)

"Enhanced Multiply Charged" (EMC) scan on the QTrap is a tool that can be advantageously used for the structural elucidation of proteins, detected as they are or as peptides coming from trypsin digestion. They both form multiply charged ions, which are often hidden by a multitude of singly charged ions that constitutes the chemical background of the mass spectrum. Thus, the preferential release of the lowest charge state species from LIT allows multiple-charge species to be more easily identified, especially when using automated peak picking software routines (Le Blanc et al., 2003).

EMC was used by *Biswas et al.* for the qualitative characterization of the glycoforms of a recombinant human bone morphogenetic protein (rhBMP-2, a TGF-beta superfamily cytokine that plays a dominant role in bone formation and regeneration) that is released from a biodegradable absorbable collagen sponge scaffold, which is implanted to stimulate natural bone formation and remodeling, and avoids the need for harvesting bone from other parts of a patient's body (Biswas et al., 2019). rhBMP-2 is a protein with multiple isoforms and a molecular weight of 29-32 kDa, so that the ionization process produces multiple charged ions that, in the EMC scan mode of the Sciex 4000 Qtrap, were retained inside the linear ion trap (LIT), while the more abundant singly charged ions were expelled. There were some losses of multiply charged ions during the LIT fill, but the loss of singly charged ions were much more in relative comparison. The mass spectrometry characterization confirmed that the formulation is a complex mixture of isoforms of the protein, and the deconvolution of the obtained mass spectra allowed the identification of many glycoforms, with a different number of mannose units present on the intact protein. Moreover, some samples were differently stressed in order to check if the composition could change and actually, some mass shifts were noticed. For instance, in temperature stress treated samples of rhBMP-2 the main change in the structure could be related to cyclization of Glu, deamidation of Asp, and oxidation of Met and Trp (Biswas et al., 2019). It must be pointed out that conventional EMC scan on a QTrap system usually begins by filling the linear ion trap with the entire ion population generated at the source. This scan can be modified by using Q1 as a mass filter that allows ions within narrow windows (<2 mass units) to reach the LIT that essentially works as the EMC mode, which is controlled through a MS^3 modified function. This mode of operation, which is named targeted-EMC (tEMC), is a modified EMC that is particularly useful to detect peptides and proteins as intact species with increased mass resolution and dynamic range compared to conventional EMC. *Drogaris et al.* illustrate the utility of the tEMC scan mode in the detection of histone proteins from complex cellular extracts (Drogaris et al., 2009). In practice, the analytical performances of the Q3 and tEMC scan modes were compared by injecting on a nano-HPLC system coupled to a Sciex 4000

QTrap mass spectrometer a recombinant yeast histone H3 (rH3) purified from *Escherichia coli* and the extracted mass spectra are reported in Figure 20. tEMC (Figure 20b) provided 5-fold enhanced resolution and a 4-folds sensitivity in terms of signal to noise ratio compared to those of the Q3 scan (Figure 20a). The expected average mass of yeast histone H3 without the initiator Met residue is in close agreement with that observed in Figure 20, *i.e.*, 15225 Da, while the additional peak at 15268 Da probably is a carbamylation artifact due to the denaturing conditions used to isolate rH3.

However, tEMC selectivity could be lower if compared to MRM, as demonstrated by *Hao et al.* in the quantification of therapeutic peptides (Hao et al., 2011). For instance, a sample of angiotensin I in dilute horse plasma at the concentration of 1.23 nM injected into a LC-MS system, including a Sciex 4000 QTrap mass spectrometer. The instrumental performance of SIM, MRM, and tEMC provided the following results: SIM failed to detect triply protonated angiotensin I (*m/z* 432.9) due to the chemical background from singly charged ions. MRM of *m/z* 432.9 → 619.8 and *m/z* 432.9 → 647.5 detected angiotensin I, and also tEMC of *m/z* 432.9 is able to detect it. The latter again provided a greater sensitivity with respect to MRM, since only 1.2% of ions are retained from MS to MS/MS. Despite the lower selectivity for tEMC, it provided an adequate resolution and discrimination against background ions.

Enhanced Q3 Single MS (EMS)

EMS is mostly used as a survey scan with IDA methods triggering EPI or different scan modes. Hence, in the literature, just a few studies that make use of EMS as a main experiment are available, since this scan type provides full scan spectra that, although highly sensitive, allow the detection of unknown analytes just with molecular weight information. Further experiments are necessary when characterization and quantification are required. One of few studies centered on EMS was carried out by *Agrahari et al.* (Agrahari et al., 2015) on a Sciex 3200 QTrap mass spectrometer that operated in positive ion EMS mode to investigate the stability of Tenofovir (TFV), a drug able to prevent the transmission of the Human Immunodeficiency Virus (HIV) through the vagina, under various storage and stress conditions. In particular, the samples were submitted to pH and temperature stressing tests and molecular ions as well as variation of peak intensities were considered suitable to get the necessary information. The effects of the treatments are shown in Figure 21. Four compounds were produced from the degradation of TFV: A_1, A_2, B_1, and W_1. The degradation products of TFV with molecular ions at *m/z* 289.2 (A_2, B_1, W_1) were proposed as 6-Hydroxy derivatives, while that with molecular ion at *m/z* 170 (A_1) as (2-hydroxypropan-2-yloxy)methylphosphonic acid.

Enhanced Resolution Q3 Single MS (ER)

The enhanced resolution (ER) scan mode is a tool that provides significant advantages when conventional HPLC-MS/MS methods, *i.e.*, chromatographic separation and unit mass resolution-based ion modes, fail in the discrimination of isobar compounds with similar structures. That was clearly pointed out by *Hernando et al.* who described the development of an enhanced liquid chromatography-mass spectrometry (LC– MS) method for the analysis in olive oil of one hundred pesticides belonging to different classes. Among them diuron and flumeturon, that have quite close retention times of 17.01 and 17.55, respectively, and the same MRM transition (*m/z* 233→72). In conventional triple quadrupole systems, if a retention time shift of a few seconds happens, each of these analytes can appear as an interferent of the other, while diuron and fluometuron could be sufficiently resolved (*m/z* 233.1 → 72.1, *m/z* 233.2 →72.1, respectively) when using the QTRAP analyzer in ER mode (Hernando et al., 2007). ER can be used profitably also in the elucidation of post-translational modifications to proteins, such as glycosylations. *Lewandrowski et al.* conducted a study focused on the determination of N-glycosylation sites on plasma membrane proteins on the model of human platelets. After a suitable sample preparation, including enrichment, purification, tryptic digestion of proteins, enrichment, and glycosidic cleavage of glycopeptides, the obtained samples were submitted to LC-MS analysis. A Sciex Qtrap 4000 mass spectrometer was used in the positive ion mode comprising an EMC scan (380–1500 amu, three spectra summed at 4000 amu/s) as survey scan, followed by ER scans of selected precursors (single spectra at 250 amu/s) that were furthermore sequenced by EPI scan (115–1500 amu, two spectra summed at 4000 amu/s), where only ions with charge states +2 and +3 were chosen for fragmentation. The method provided good results, since it was able to identify of 148 glycosylation sites on 79 different protein species. (Lewandrowski et al., 2007). However, the approach of ER scan preceding EPI scan is frequent, and it is widely used for structural characterization in metabolomics and proteomics, as well as in different fields (Sentandreu et al., 2007; Yan et al., 2014; McCloskey et al., 2015).

MS/MS Product Ions and Library Search

As stated before, Q-TOF and QTrap instruments, owing to their fast scanning, share the capability to perform more than one experiment in a very short time. When used in combination with a survey scan (normally a TOF scan in Q-TOF systems, an EMS scan on a QTrap) in a data driven experiment this allows to get MS and MS/MS data in the same chromatographic run. It is even possible to carry out more than a MS/MS scan.

These features largely increased the use of mass spectral libraries in LC-MS analysis. Some examples have already been described. Most vendors can provide general purpose as well as

custom-built libraries for particular applications. It is also relatively easy to create internal proprietary libraries dedicated to the topics normally under investigation in one's own laboratory. Wissenbach et al. tried to put some order, comparing the high reliability of the widely used GC-MS libraries with the more variable MS/MS spectra and relative libraries, suggesting some ideas to produce reliable and reproducible spectra on LC-MS/MS instruments, suitable for their inclusion in libraries. Search algorithms are discussed as well (Wissenbach et al., 2012). Figure 22 shows three examples of identification.

7. CONCLUSIONS

QTrap geometry provide a really versatile platform to perform highly reliable and sophisticated analytical tasks. The combination of relatively low speed analyzers, but very reliable for ion monitoring rounds, and relatively high-speed sequential analyzer, providing high performance for qualitative data, as well as the co-presence in the same instrument of MS/MS-in-space and MS/MS-in-time make this combination really unique. Its main competitor is the Q-TOF geometry. This last offers high resolution and accurate mass determination, but can perform only MS/MS-in-space experiments.

Different combinations of the single operation modes in an IDA run further improves the versatility of this instrumental combination, allowing to get highly reliable qualitative and quantitative data. Despite of its already long presence in the field of mass spectrometry (since 2002) the use of the additional (with respect to a "normal" triple quadrupole instrument) features is still limited, probably also because some of these features still need to be completely understood. Without any doubt, however, the use of the "new" QTrap function will increase in a short time, allowing easier and easier analytical procedures.

CAPTIONS TO FIGURES

Figure 1. Schematic representation of a quadrupole mass filter.

Figure 2. Composite potential applied to the quadrupole bars. A positive voltage plus an alternating voltage is applied to the vertical diagonal pair of bars, whereas an opposite negative voltage plus an alternating voltage 180 degrees out of phase.

Figure 3. Schematic representation of a triple quadrupole. Q1 and Q3 make use of both DC and RF voltages, so that they act as mass filters, whereas q2 is a RF-only quadrupole and serves as a collision cell.

Figure 4. Operation MS/MS modes of a triple quadrupole (MS/MS-in-space).

Figure 5. Schematic representation of a trap, ideally resulting from the rotation of a section of a quadrupole. Adapted from Patent US2939952 (June 7, 1960).

Figure 6. Quadrupole ion trap: (a) photograph of a disassembled quadrupole ion trap, and (b) schematic diagram of the ion trap, showing ring electrode, endcap electrodes, and quartz spacers. Adapted from March (2009) with permission. Copyright © 2009, John Wiley and Sons.

Figure 7. ESI-MS spectrum of catechin ($[M-H]^-$ ion) acquired on a QMF instrument at 200 (a) and 10 (b) Da/s scan speed.

Figure 8. Total Ion Chromatogram (TIC, grey line) and real data points (black dots) for the analysis of a mixture of catechin and epicatechin at 200 (a) and 10 (b) Da/s scan speed.

Figure 9. ESI-MS spectrum of catechin ($[M-H]^-$ ion) acquired on a quadrupole ion trap instrument at 10000 (a), 1000 (b), 250 (c) and 50 Da/s scan speed. An increment in the resolving power (RP) is achieved with a lower scan speed.

Figure 10. Schematic representation of a QTrap instrument. Q3 can operate either as a quadrupole or as a linear ion trap, as well as q2. Adapted from Hager (2002) with permission. Copyright © 2002, John Wiley and Sons.

Figure 11. Product ion spectra of reserpine obtained with a QTrap instrument operating as a conventional triple quadrupole mass spectrometer (upper trace), and in "enhanced product ion" (EPI) mode (lower trace). The intensity enhancements in EPI mode were of >200×. Adapted from Hager & Le Blanc (2003) with permission. Copyright © 2003, Elsevier.

Figure 12. Scheme of target MRM >> EPI, untarget EMS >> EPI and combined target/untarget EMS-MRM >> EPI experiments. Adapted from Jarvis (2011) with permission. Copyright © 2011, AB Sciex.

Figure 13. Sensitivity, selectivity and specificity of the enhanced product ion (EPI) mode. Comparison between the ion chromatograms from increasing amounts of S1P in EPI (a) and in MRM (b), where the expected retention times are indicated by an arrow, and liver extracts of C57BL/6 wild-type mice as they were and spiked with 50 nM of S1P (c). Adapted from Müller & Gräler, 2021 (CC BY).

Figure 14. Workflow of UHPLC-Qtrap-MS based integrated strategy for chemical characterization of Danhong Injection. Reprinted from Li C et al. (2019) with permission. Copyright © 2019, Elsevier.

Figure 15. TIC of MRM chromatogram of the 32 standards in one run at 25 ng/g. Adapted from Zeng et al. (2015) with permission. Copyright © 2015, Elsevier.

Figure 16. Example of library match. Upper spectra (blue) refer to experimental spectra, whereas the lower ones (green) refer to standard spectra present in the library. Reprinted from Zeng et al. (2015) with permission. Copyright © 2015, Elsevier.

Figure 17. ESI-MS/MS (A), ESI-MS^3 (B) spectra of compound at m/z 912 and ESI-MS/MS (C) of glucomoringin, reprinted from Maldini et al. (2014) with permission. Copyright © 2014, John Wiley and Sons.

Figure 18. Outline of the IDA experiment performed. Reprinted from Gross et al. (2009) with permission. Copyright © 2009, American Chemical Society.

Figure 19. Example of an IDA experiment performed for the determination of the macrolide antibiotic tylosin in an urban influent wastewater: SRM and EPI spectra recorded at the collision energies of 25, 40, and 55 eV, respectively. Reprinted from Gross et al. (2009) with permission. Copyright © 2009, American Chemical Society.

Figure 20. Comparison between Q3 scan and tEMC scan. Extracted mass spectra of a recombinant yeast histone H3 purified from Escherichia coli acquired in Q3 scan mode (a), and in tEMC (b). The relative deconvoluted spectra are also shown. Adapted from Drogaris et al. (2009) with permission. Copyright © 2009, American Chemical Society.

Figure 21. EMS of Tenofovir (TFV). pH stressing tests: unstressed sample (1 mg mL^{-1}) (A); stressed samples under Acidic (0.1 M HCl), analyzed after day one (B) and after day 5 of reflux (B insert); alkaline (0.1 M NaOH), analyzed after day 5 (C); neutral (water) hydrolytic conditions, analyzed after day 5 (D). Temperature stressing tests: stressed sample of Tenofovir (TFV) (1 mg mL^{-1}) under acidic pH 4.5, analyzed after 5 days of reflux (E); TFV samples stressed under acidic pH 4.5 at 25°C, analyzed after 10 days (F); TFV samples stressed under acidic pH 4.5 at 40°C, analyzed after 10 days (10), respectively. A_1, A_2, B_1, and W_1 are degradation products of TFV.

689 Adapted from Agrahari et al. (2015) with permission. Copyright © 2015, John Wiley
690 and Sons.
691
692 Figure 22. Mass spectra (above axis) and relative library match (below axis) for clozapine-M
693 (hydroxy-glucuronide) isomer 1 (A), amitriptyline-M/artifact (nor-hydroxy-, -H2O)
694 (B) and paroxetine (C) after centroiding. Reprinted from Wissenbach et al. (2012)
695 with permission. Copyright © 2012, John Wiley and Sons.
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ABBREVIATIONS

AC: alternate current

CID: collisionally induced dissociation

Da/s: Dalton per second

DC: direct current

DDS: data dependent scan

EMC: enhanced multi charge

EMS: enhanced mass spectrum

EPI: enhanced product ion

ER: enhanced resolution mass spectrum

GC-MS: gas chromatography-mass spectrometry

HPLC-MS: high performance liquid chromatography-mass spectrometry

IDA: information dependent acquisition

LIT: linear ion trap

MIM: multiple ion monitoring

MRM: multiple reaction monitoring

MS/MS, MS^2, MS^n: tandem mass spectrometry

Prec.: precursor ion

Q: quadrupole

q: RF-only quadrupole

QIT: quadrupole ion trap

QMF: quadrupole mass filter

RF: radiofrequency

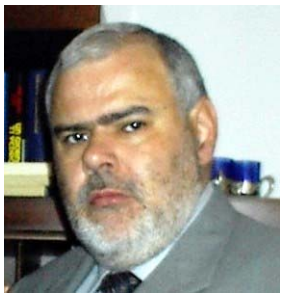
SIM: selected reaction monitoring

SRM: selected reaction monitoring

TDF: time delayed fragmentation

TOF: time of flight

BIOGRAPHIES



Andrea Raffaelli just retired from Italian National Council of Research, where he served as a Senior Researcher, but still continues his research work both as an affiliate researcher, in the Institute of Life Sciences of Scuola Superiore S. Anna in Pisa, and in Institute of Agricultural Biology and Biotechnology of National Research Council (CNR-IBBA). His research activity focus on the use of mass spectrometry in different fields, mainly organic chemistry, biochemistry, clinical chemistry and nutraceutic, with particular interest in profiling natural antioxidants in vegetal extracts.

Alessandro Saba is an Associate Professor of Chemistry and Biochemistry at the Department of Pathology of the University of Pisa. His research activity is mainly focused on the use of mass spectrometry for investigations in biochemistry and clinical chemistry, with particular interest in thyroid and steroid hormone metabolism. He currently serves also as a chemical officer at the Laboratory of Clinical Pathology of the University Hospital of Pisa, where he is in charge for the clinical diagnostics with mass spectrometry on a routine basis.

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