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Review

10 years of MS instrumental developments – Impact on LC–MS/MS in clinical chemistry☆

Markus Himmelsbach*

Institute for Analytical Chemistry, Johannes Kepler University Linz, Altenberger Strasse 69, A-4040 Linz, Austria

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ABSTRACT

The combination of liquid chromatography and mass spectrometry (LC–MS) is a powerful and indispensable analytical tool that is widely applied in many areas of chemistry, medicine, pharmaceutics and biochemistry. In this review recent MS instrumental developments are presented as part of a special issue covering various aspects of liquid chromatography tandem mass spectrometry (LC–MS/MS) in clinical chemistry. Improvements, new inventions as well as new combinations in ion source technology are described focusing on dual or multimode sources and atmospheric pressure photoionization (APPI). Increasing demands regarding sensitivity, accuracy, resolution and both quantitation and identification guarantee on-going improvements in mass analyzer technology. This paper discusses new hybrid MS instruments that can perform novel scan modes as well as high-resolution mass spectrometers (HRMS) that finally seem to be able to overcome, or at least significantly reduce, their weaknesses in quantitative applications. Ion mobility-mass spectrometry (IMMS) itself is not an invention of the last 10 years, but a lot of progress was made within the last decade that reveals the potential benefits of this combination. This is clearly reflected by the increased number of commercially available instruments and the various designs of IMMS are covered in detail in this review. Selected applications for all these instrumental developments are given focusing on the perspective of clinical chemistry.

Contents

1. Introduction

Within the past decade mass spectrometry (MS) has entered the clinical laboratory and is now being used for a wide range of applications. The technique can be considered essential for the determination of many clinically relevant analytes in combination

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* Fax: +43 732 2468 8679.
E-mail address: markus.himmelsbach@jku.at

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with either gas chromatography (GC) or liquid chromatography (LC). The power of MS, especially when coupled to LC, is recognized by clinical laboratories worldwide and the growing versatility of these systems puts clinical laboratories in a position where they can provide a rapid response to changing clinical needs. Even though it requires some effort much needed assays can be developed in the laboratory instead of waiting for a manufacturer to respond. Furthermore it is undoubted that these techniques provide a higher level of sensitivity and specificity in many cases compared to other analytical techniques and that patient care has benefited from their use. Besides specificity and sensitivity the ability of these techniques to measure multiple analytes simultaneously is a tremendous benefit of LC coupled to MS methods since many other techniques are limited to determine one analyte at a time. Especially these multi-component methods can make the purchase of a liquid chromatography tandem mass spectrometry (LC–MS/MS) instrument cost-effective. Improvements in automation and software help clinical laboratories to deal with staffing and service issues. The scope of testing has expanded and is not focused on toxicological purposes anymore when it comes to the routine use of MS in clinical laboratories. This is underlined by the fact that books are published exclusively dedicated to the clinical applications of MS [1]. Application areas for LC–MS or LC–MS/MS in the clinical laboratory are therapeutic drug monitoring, neonatal screening, reference methods, and toxicology [2–9]. LC–MS/MS multitarget screening using (triple) quadrupole based methods allows the detection of certain analytes in the selected ion monitoring (SIM) or multiple-reaction monitoring (MRM) mode. Analytes not selected in the method before the analysis cannot be detected in that case. Nevertheless, such methods extended the spectrum of sensitive and specific MS-based methods to analytes, which are not amenable to GC–MS analysis because of their chemical or physical properties. Recently a very informative review was published by Grebe and Singh entitled "LC–MS/MS in the Clinical Laboratory – Where to From Here?" [10]. This paper describes in detail how triple quadrupole MS are utilized in clinical analysis. Basic principles as well as the frequently used modes to carry out experiments are elucidated together with a selection of applications and a future outlook. Also very recently Jiwan et al. reviewed the topic "HPLC–high resolution mass spectrometry in clinical laboratory?" [11]. They discussed why high-resolution MS (HRMS) like quadrupole time-of-flight (QTOF) and Orbitrap instruments remain almost ignored in the clinical laboratory even though they are largely used in the academic environment. In their conclusion they state that HRMS technology is now ready to enter clinical laboratories for screening applications due to the reasonable costs of the instruments at purchase and for maintenance, and there is no doubt that they will also be valuable tools for the quantitative analysis in the near future.

A triple quadrupole instrument in selected-reaction monitoring (SRM) mode is the instrument-of-choice in routine and high-throughput clinical analysis because of its outstanding performance when quantification is needed. Other mass analyzers like ion traps and HRMS like quadrupole QTOF and Fourier transform (FT)-MS based instruments underwent significant instrumental developments in the last 10 years. They offer several advantages compared to triple quadrupole instruments, such as MS^n capabilities in case of ion traps and high resolution/high mass accuracy in case of QTOF and FT-MS based instruments. As soon as they meet the quantitative analytical requirements the clinical field will adopt these instruments into their workflows. An overview of the general properties of different MS instrumentations is presented in several textbooks and papers [12–14].

Another challenge for MS producing companies to improve their instruments arises from the ever increasing speed of chromatography. Mainly progress in column technology has significantly increased the separation efficiency in LC resulting in very narrow LC peaks with peak widths at half-height below 1 s. Recently this topic was discussed by Gérard Hopfgartner in his article "Can MS fully exploit the benefits of fast chromatography?" [15].

2. Ion source developments

Coupling MS to LC was a very important motivation in the development process of atmospheric pressure ion sources. Systems where the samples are introduced via a liquid stream achieved wide acceptance and commercial importance. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the liquid introduction ion sources which had the most commercial success and enormous improvements were made in the first 15 years after their invention during the mid-1980s. Two excellent reviews were published dealing with the evolution of these ionization sources [16,17]. Based on these developments multimode ion sources were introduced on the market by various manufacturers. In addition the atmospheric pressure photoionization (APPI) ion source was developed and improved within the last 10 years. Therefore more detailed information about multimode and APPI ion sources will be presented in the following chapters and their suitability regarding their application to clinical chemistry will be discussed. Apart these commercially successful LC–MS ion sources very creative approaches were investigated for the hyphenation of LC with MS using other available ion sources. Research scientists especially set their focus on various desorption techniques. The combination of LC with matrix-assisted laser desorption ionization (MALDI) started to emerge in the mid-1990s [18,19] and continuous effort was undertaken to further improve the technique [20–25]. Very recently LC–MS methods were described using desorption electrospray ionization (DESI) [26] and direct analysis in real time (DART) [27,28] interfaces.

Signal suppression or enhancement must be regarded as a major drawback in quantitative analysis with LC–MS. These effects are mainly caused by the presence of undesired components, the so-called matrix, that are not separated from the analytes during chromatography and influence the ionization process. Matrix components can dramatically affect the method performance in terms of selectivity, repeatability, accuracy, response and limit of detection/quantification. The described ion sources are susceptible to signal suppression or enhancement at different extent because of their dissimilar ionization mechanisms. Various excellent reviews were published over the last decade dealing exclusively with that topic [29–31].

2.1. Atmospheric pressure photoionization

Although ESI and APCI are the most frequently used ionization techniques in LC–MS analysis, APPI [32] has recently expanded the range of compounds that are accessible to LC–MS. ESI is best suited for the analysis of polar molecules that contain functional groups which aid ionization or compounds which are able to form charged adducts with ammonium or alkali cations. Ionization occurs in the liquid phase, while APCI allows the ionization of less polar molecules in the gas phase via chemical ionization utilizing a corona discharge needle. APPI is a soft ionization technique that was developed with the intention to ionize those molecules that are poorly amenable to ESI and APCI. The fact that APPI is not limited to nonpolar substance and both rather polar and nonpolar substances can be ionized at the same time made this source more and more popular over the last few years [33–35]. The technical set-up for an APPI source is very closely related to that of a commercial APCI source and the scheme is depicted in Fig. 1. It consists of a heated nebulizer for spraying and evaporating the eluent and a UV lamp that induces the ionization via photons instead of the corona needle used in APCI.

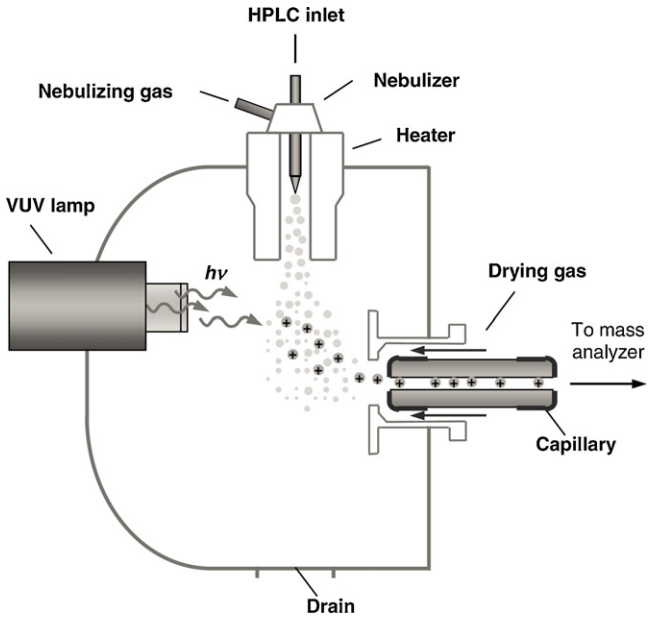


Fig. 1. Schematic drawing of the orthogonal, open-source type APPI interface. Reprinted with permission from Bos et al. [33]. Copyright 2006 Springer.

The first results using APPI as an ionization source for MS hyphenated with LC were published in 2000 by Robb et al. [32] and Syage et al. [36]. Based on these results, only two interfaces are currently available commercially. The first is based on the prototype developed by Robb in 2000 and is sold under the PhotoSpray trademark name. The second source developed by Syage also in 2000 is called PhotoMate and differs by its orthogonal geometry.

Signal intensities can be increased significantly in APPI-MS by adding so-called dopants [32,37]. A suitable substance is added in relatively large amounts compared to the analyte either by adding it to the mobile phase or by post column addition. This can significantly increase the analyte ionization yield. A dopant is effective if it is ionized by the UV lamp and afterwards reacts with the analytes by charge exchange or proton transfer. Toluene and acetone are most frequently used to enhance the ionization, but reports describing the use of other substances for this purpose can be found in the literature [38].

In 2004 Kushnir et al. developed a simple and sensitive method for cortisol and cortisone analysis in plasma and serum using an APPI ion source [39]. The APPI ion source improved the signal-to-noise ratio for cortisol and cortisone in this method by a factor of 3, compared to APCI, the technique that was primarily used in earlier published LC–MS methods for the measurement of cortisol. Quantitative analysis was performed in the multiple reaction monitoring mode and toluene was used as a dopant to promote ionization.

APPI in negative mode with toluene as dopant was used for the determination and quantification of betamethasone in human plasma by LC coupled with tandem MS [40]. The method described was employed in a bioequivalence study of two formulations of dexchlorpheniramine/betamethasone 2 mg/0.25 mg tablets. The authors validated the method by assessment of recovery, linearity, quantification limit, precision and accuracy and concluded that the assay performance results indicate that the method is precise and accurate enough for the routine determination of betamethasone in human plasma.

In 2009 Karuna et al. used APPI to improve the sensitivity of 27-hydroxycholesterol analysis [41], compared to a published LC–APCI-MS method, allowing quantification from a very low amount of sample. The method was validated for quantification from 50 μL and 15 μL plasma, with a limit of quantification (LOQ) of 10 ng/mL and 40 ng/mL plasma, respectively. A further advantage is that no prior derivatization was needed, unlike in the case of the LC–ESI-MS or the standard GC–MS method. The authors conclude that APPI broadens the usefulness of LC–MS methods in clinical applications.

2.2. Dual or multimode ion sources

ESI is known to have serious limitations when rather nonpolar substances without functional groups or the lack of ability to form charged adducts with ammonium or alkali cations have to be ionized. To extend the range of accessible analytes the ionization technique has to be changed. If only ESI, APCI or APPI ion sources are used this requires a change in instrument hardware followed by a new optimization of the ion source parameters. This approach would result in considerably increased effort and analysis time. Since nowadays time is very critical in all areas this is not acceptable and consequently some new instrument configurations have been developed. Byrdwell has used a dual parallel MS system to acquire ESI and APCI data simultaneously from a single LC system [42,43]. This is a successful method, but it ties up two MS for a single analysis and therefore is a rather cost intensive solution to the problem. Another way to avoid the need for ion source changes is to expand developments in ion source design. The goal was to incorporate multiple ionization techniques within a single MS ion source. Two or more ion sources are essentially combined in a single unit that allows multiple analyses by selected sampling of each source or the combination of different ionization modes. Siegel et al. have developed a dual ESI–APCI source that exhibited many benefits but focused on flow injection rather than chromatographic separations [44]. Gallagher et al. developed a new combined ESI–APCI source for use in high-speed on-line LC–MS applications [45]. The combined source allows alternate on-line ESI and APCI scans within a single analysis with polarity switching resulting in a higher sample throughput. The design is characterized by clearly defined boundaries between ionization modes. This combined ESI–APCI source can generate clearly differentiated and reproducible ESI and APCI spectra while polarity-switching. The authors describe, that the qualitative performance of the combined source has been compared to the existing ESI and APCI interfaces and found to be equivalent in ESI mode and produce less thermal fragmentation in APCI mode. This data quality can be maintained over a flow rate from 50 to 1000 μL/min. Syage et al. described the combination of APPI with APCI and ESI [46]. They present three modes of operation; use of either ionizer, simultaneous use of two ionizers, and rapid switching between ionizers during a single chromatographic run. Four years later they used an electrospray photoionization source to analyze cyclodextrin and pharmaceuticals [47] and evaluated it regarding the suitability for low-flow LC–MS [48]. The authors conclude that in terms of the optimal combination of two sources, they believe that the ESI/APPI combination offers the greatest benefits relative to other dual combinations, but nevertheless the ESI/APCI dual source is now the most commonly used. Dual sources for LC–MS have the practical benefit of expanding the range of ionizable compounds that can be analyzed simultaneously without the inconvenience of manually switching spray chambers as it is conventionally done. Nowadays multimode or dual sources are commercially available from numerous manufacturers and are part of the basic equipment when a new instrument is purchased.

In 2008 Nordström et al. published a highly recommended article about multiple ionization MS strategies based on the analysis of human serum extracts [49]. The authors describe in detail how extending the ionization methods results in a significant increase in the detection and assignment of unique ions. They also conclude that their results suggest that true global metabolomics will require multiple ionization technologies to address the

inherent metabolite diversity and therefore the complexity in and of metabolomics studies.

3. Improvements in tandem mass spectrometry technology

Since LC–MS/MS technology is increasingly used for quantitation in clinical science, as well as in other fields of science, there is a need for on-going improvements of the technology. A triple quadrupole instrument in SRM mode is the instrument-of-choice in routine and high-throughput quantitative clinical analysis. Commercial triple quadrupole MS with atmospheric pressure ionization (API) sources are widely used nowadays. In the case of triple quadrupole instruments the most commonly requested improvements were defined by Bennett to be: greater sensitivity, dynamic linear range, mass resolution, wider mass range, faster acquisition cycle time and reduced cost of ownership [50]. Advances in triple quadrupole technology are challenging and focus remains in the source and interface regions to improve ruggedness and reduce matrix effects. Some minor improvements in quadrupole manufacturing processes and RF power supply stabilities enabled the production of a commercial system with enhanced mass resolution without significant losses in ion transmission [51,52]. On the other hand significant instrumental developments were achieved in the last 10 years in the fields of other mass analyzers like linear ion traps and HRMS like quadrupole QTOF and FT-MS based instruments. Therefore the future of triple quadrupoles will be determined on the variable how extensively the clinical field adopts to high resolution, high mass accuracy instruments into their workflows and analytical requirements. Up to now, in case of triple quadrupole instruments, mass resolution was typically ignored in favor of the outstanding linearity and increased sensitivity due to the selectivity offered by tandem MS. Now new tasks are gaining more and more interest where improved selectivity and full-scan data at low duty cycle times are crucial. These needs could be filled by high-resolution instruments that are now available from numerous manufacturers and are easier to operate than in the past. Especially improvements in Orbitrap and QTOF instruments have produced new generations of HRMS instruments. In the discovery process these instruments are already being frequently used for quantitative and qualitative analysis. As the cost of ownership drops and the instruments are easier to use they will find their way into routine operations of clinical laboratories. At the moment I fully agree with the statement of Bennett [50], that in the short term, it is likely that the majority of experiments requiring very high sensitivity will be performed by triple quadrupole instruments. Nevertheless, since the most substantial instrumental improvements of the last 10 years were achieved with other mass analyzers than quadrupoles the next chapters will focus on these recent developments.

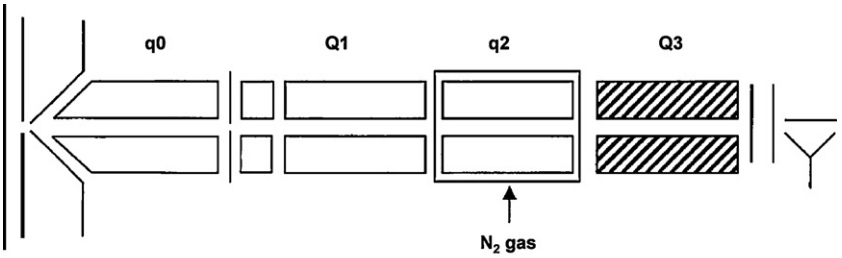
3.1. Linear ion trap mass analyzers

Linear ion trap mass analyzers (2D ion traps) use a set of quadrupole rods to confine ions radially and a static electrical potential on end electrodes to confine ions axially [53]. The linear form of the trap can be used as a selective mass filter or as a trap by creating a potential well for ions along the axis of the electrodes. Advantages of the linear trap design are increased ion storage capacity, faster scan times, and simplicity of construction. This type of mass analyzer is marketed by Thermo Fisher as LTQ (Linear Trap Quadrupole) [53]. Linear ion trap tandem mass spectrometers can perform MS/MS in the same way as 3D ion traps. Fragmentation is induced by resonance excitation that induces collisions of the parent ion of interest with a gas of sufficient energy to induce dissociation.

More important than the linear ion trap as standalone instrument, regarding the application in clinical laboratories, is a hybrid tandem mass spectrometer which combines the capabilities of a triple quadrupole and an ion trap [54–56]. Such an instrument is distributed by AB Sciex as QTRAP and it features the ion path of a standard triple quadrupole, but the final quadrupole can be operated either as a conventional transmission RF/DC quadrupole mass analyzer or as an axial ejection linear ion trap MS. This unique feature allows the instrument to be operated either as a triple quadrupole or as an ion trap MS and perform novel scan modes not available on other instruments. Fig. 2 shows a schematic of a quadrupole linear ion trap and a description of the various triple quadrupole and trap operation modes. When a survey MS scan is acquired the Q1 quadrupole is operated as an ion guide, the Q2 collision cell is set to a low energy and the Q3 linear ion trap is used to trap the ions. Subsequent the trapped ions are scanned out of the Q3 ion trap in an axial direction toward the ion detector to yield a highly sensitive MS scan, the so-called “Enhanced MS”, or EMS scan. MS/MS experiments can be performed as with 3D ion traps but this would result in the same limitations that are experienced by 3D ion traps like reduced dynamic range und low mass cut-off. A better way to acquire MS/MS scans with this hybrid instrument is the “enhanced product ion scan” (EPI) which employs the tandem-in-space capabilities of the ion path with the high sensitivity ion trap mass scan. The precursor ion is selected in the first quadrupole, Q1, fragments are generated in the pressurized Q2 collision cell and they are trapped in Q3 prior to detection. In this mode the ion selection and fragmentation are performed as in a triple quad instrument which results in the same fragmentation pattern. A variety of novel scans are possible with this unique instrument setup that are not possible with other mass spectrometers. In addition, the ability to perform both the very selective MS/MS scans of a triple quadrupole MS instrument and the extremely sensitive product ion scans of an ion trap reduces the limits of identification and detection. The various methods of operating the commercial QTRAP hybrid instrument along with applications are described in detail by Hopfgartner et al. [57]. Challenges, new concepts and developments of MS based multi-target metabolome profiling in the field of clinical diagnostics and research were described by Ceglarek et al. 3 years ago [58]. The authors evaluated novel hyphenated technical approaches like the combination of tandem MS combined with linear ion trap regarding their ability for identification and quantification of known and unknown metabolic targets.

In the following paragraphs selected applications of linear ion trap mass analyzers will be presented to demonstrate their benefits regarding analysis related to the science of clinical chemistry. Only a representative selection of publications will be given since a comprehensive listing would be beyond the scope of the present paper.

By applying the new operating modes provided by hybrid triple quadrupole linear ion trap mass spectrometers a comprehensive method for the screening of drugs and toxic compounds in blood or urine was developed by Sauvage et al. [59]. The mass spectrometer was operated in the information-dependent acquisition (IDA) mode, switching between a survey scan acquired in the enhanced MS mode with dynamic subtraction of background noise and a dependent scan obtained in the enhanced product ion scan mode. A library of 1000 enhanced product ion-tandem MS spectra in positive mode and 250 in negative mode, generated using 3 alternated collision energies during each scan, was created by injecting pure solutions of drugs and toxic compounds. Analysis of 36 clinical samples from specific clinical cases highlighted the advantages and limitations of the method. Compiling libraries of tandem MS spectra still represents a big challenge and is not as straight forward as most people would expect. The conditions during the fragmentation process are not standardized as it is the case for electron-impact ionization used for GC–MS. MS/MS spectra generated with different tandem instruments can vary considerably and



Mode of operation	Q1	q2	Q3
Q1 Scan	Resolving (Scan)	RF-only	RF-only
Q3 Scan	RF-only	RF-only	Resolving (Scan)
Product Ion Scan (PI)	Resolving (Fixed)	Fragment	Resolving (Scan)
Precurser Ion Scan (PC)	Resolving (Scan)	Fragment	Resolving (Fixed)
Neutral Loss Scan (NL)	Resolving (Scan)	Fragment	Resolving (Scan Offset)
Selected Reaction Monitoring mode (SRM)	Resolving (Fixed)	Fragment	Resolving (Fixed)
Enhanced Q3 Single MS (EMS)	RF-only	No frag	Trap/scan
Enhanced Product Ion (EPI)	Resolving (Fixed)	Fragment	Trap/scan
MS^3	Resolving (Fixed)	Fragment	Isolation/frag trap/scan
Time delayed fragmentation (TDF)	Resolving (Fixed)	Trap/No frag	Frag/trap/scan
Enhanced Resolution Q3 Single MS (ER)	RF-only	No frag	Trap/scan
Enhanced Multiply Charged (EMC)	RF-only	No frag	Trap/scan

Fig. 2. Schematic of a quadrupole linear ion trap and description of the various triple quadrupole and trap operation modes. Reprinted with permission from Hopfgartner et al. [57]. Copyright 2004 John Wiley and Sons.

even if the same analyzer type is used notable differences can be observed between instruments provided by various manufacturers. Due to the strong demand of customers MS producing companies offer library programs together with their software packages but these tools provide not yet the convenience and quality known from well-established GC–MS databases.

Metabolites present in a complex matrix were characterized and quantified by a LC–MS method using the LTQ linear ion trap MS system [60]. Results showed excellent selectivity and high precision and sensitivity in the determination of the biomarkers creatinine and cortisol. The serum concentrations of creatinine and cortisol ranged from part per million (ppm) to part per billion (ppb) levels. Authors concluded that the high precision and good reproducibility of the LTQ linear ion trap MS system qualify it as an excellent instrument for the measurement of metabolites in complex matrices, even when such analytes are present at ultra-trace levels.

A hybrid triple quadrupole linear ion trap tandem mass spectrometer using multiple reaction monitoring was applied to clinical endometrial tissue homogenates in an effort to quantify two endometrial cancer biomarkers, pyruvate kinase and polymeric immunoglobulin receptor [61]. The feasibility of this approach was successfully demonstrated on 20 individual samples and further verified the differential expressions of these two biomarkers in endometrial carcinoma. This study confirmed qualitatively the differential expressions previously observed but also showed that the actual relative differential expressions in these samples were much higher than those reported in the discovery study [62].

Drees et al. developed a semi-quantitative LC–MS/MS assay to detect 12 chemically diverse drugs implicated in drug-related seizures [63]. Serum and plasma samples from patients who had seized were analyzed using a hybrid triple quadrupole linear ion trap mass spectrometer after solid-phase extraction. The authors used a scoring system to determine whether the results of the seizure panel would have affected patient treatment in each case where a drug was detected. Overall 157 samples from patients who seized were analyzed. 17 (11%) of these samples were found to be positive for a drug on the seizure panel. It was determined that the test results probably or definitely would have affected treatment in 7 (41%) of these cases.

In order to determine twelve tetrahydrocorticosteroid glucuronides in human urine a method for their direct analysis has been developed [64]. The analytes were 3- and 21-monoglucuronides of tetrahydrocortisol, tetrahydrocortisone, tetrahydro-11-deoxycortisol, and their 5α-stereoisomers. Regioisomeric glucuronides could be distinguished by collision-induced dissociation and tandem MS using a linear ion trap instrument operating in the negative-ion mode. The method was applied to determine the 12 analytes in urine from healthy subjects and from patients with excessive cortisol production and appears to be useful for clinical and biochemical studies.

In a considerable effort a multi-target screening method was set up that allows the simultaneous detection and identification of 700 drugs and metabolites in biological fluids in a single analytical run by a scheduled survey MRM scan, followed by an information-dependent acquisition using the sensitive enhanced product ion scan of a QTRAP hybrid instrument [65]. The identification of the compounds in the samples analyzed was accomplished by searching the tandem MS spectra against the library that was collected, which contains ESI-MS/MS spectra of over 1250 compounds. Screening was successfully applied for the analysis of post-mortem and traffic offense. Another fully automated toxicological LC–MS screening system was presented by Mueller et al. in the same year using a LXQ linear ion trap mass spectrometer from ThermoFisher Scientific [66]. Fig. 3 shows an example of a real patient urine sample chromatogram together with the MS^3 spectra of a positive identification.

3.2. Quadrupole time-of-flight mass analyzers

In order to perform MS/MS with a TOF instrument, the TOF analyzer has to be combined with another mass analyzer to form a so-called hybrid instrument. The most successful and most widely applied among these hybrids is the QTOF instrument [67,68]. QTOF tandem mass spectrometers consist of a mass analyzing quadrupole, an RF-only hexapole collision cell and a TOF analyzer in sequence [69–71]. Fig. 4 displays the technology features of a AB Sciex TripleTOF. In this configuration the third quadrupole in a triple quadrupole is replaced by a TOF mass spectrometer. This yields high sensitivity, mass resolution, and mass accuracy in both precursor and product ion modes. Excellent full-scan sensitivity over a wide mass range together with a fast duty cycle is achieved in both modes by the parallel detection feature of TOF mass analyzer. So overall, they combine the high performance of TOF analysis in both the MS and the tandem MS mode, with the

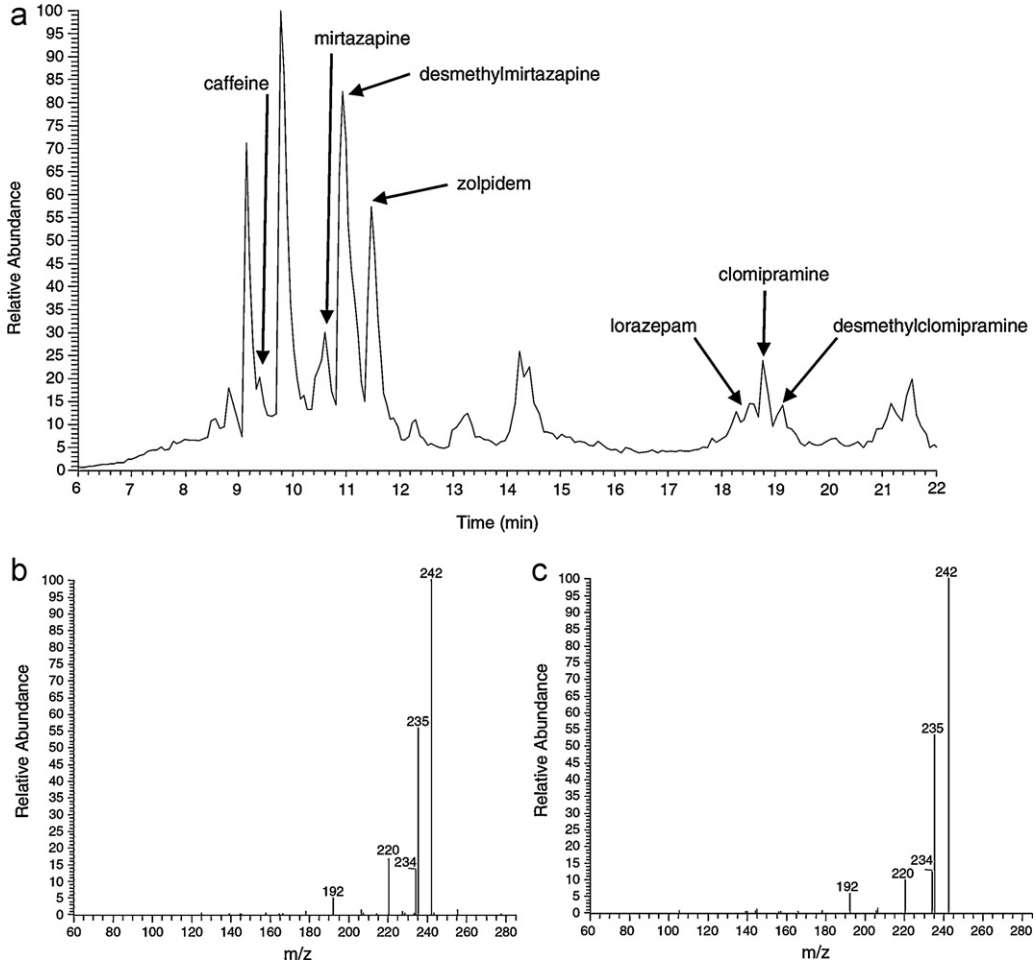


Fig. 3. Example chromatogram and spectra from Ref. [66]. (a) Chromatogram of a real patient case with identified substances annotated (total ion chromatogram). (b) Acquired MS^3 spectrum of a metabolite of the antidepressant clomipramine, desmethylclomipramine, from the chromatogram shown in (a). (c) Library MS^3 spectrum of desmethylclomipramine.

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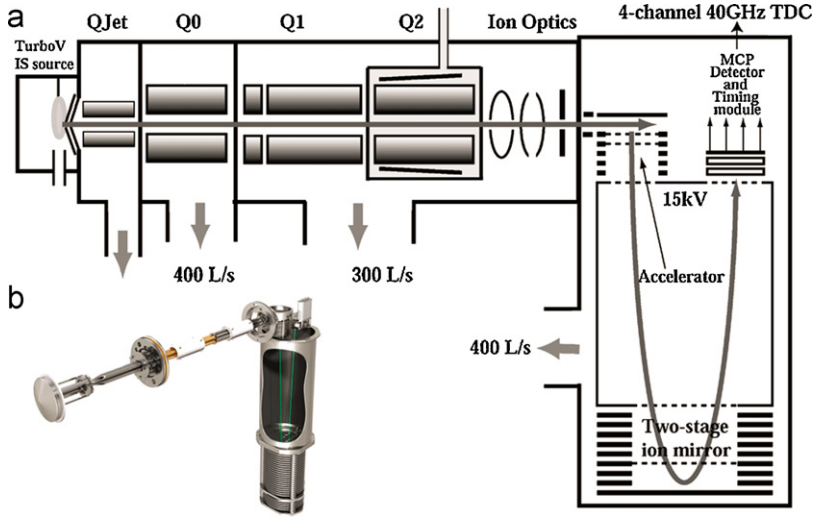

Fig. 4. The TripleTOF MS technology features diagramed. (a) A detailed illustration of the major platform features. (b) An image of the machined TripleTOF MS instrument platform.

Reprinted with permission from Andrews et al. [72]. Copyright 2011 American Chemical Society.

widely used techniques of ESI, APCI and APPI. This advantage does not inherently apply to the more specialized triple quadrupole acquisition modes like the precursor ion, neutral loss, and multiple reaction monitoring scans. If only single MS experiments are performed the mass filter quadrupole (Q1) is operated as a transmission element, while the TOF analyzer is used to record spectra. The resulting spectra benefit from the high resolution and mass accuracy of the TOF instruments, and also from their ability to record all ions in parallel, without scanning. For MS/MS experiments the first quadrupole (Q1) is used as a mass filter for the parent ion of interest. The transmitted ions undergo collision-induced dissociation in the collision cell (Q2). The generated fragment ions along with residual parent ions are then detected by the TOF mass analyzer. Initially the instrument was considered to be suitable only for qualitative analysis, but actually nothing stands against its use for quantitative applications. Improvements on issues that affect the dynamic range of QTOF instruments were made in recent years and systems are commercially available from different manufacturers that claim to allow high-resolution quantitation [72–74]. At the moment triple quadrupole systems are still the most frequently used instruments for targeted analysis, but the increased specificity provided by the higher resolution QTOF may provide benefit in some applications in clinical laboratories. As soon as the vendors of MS instruments are able to proof that QTOF systems have overcome their limitations regarding quantitation their obvious advantages like high resolution, high mass accuracy and fast duty cycle full-scan data acquisition will come to the fore. But at the moment the actual literature reflects the fact that QTOF instruments are almost exclusively in use for screening or profiling proposes in qualitative clinical research. This may be a little surprising, since already in 2008 Williamson et al. used a validated TOF-MS method to determine risperidone, its active metabolite paliperidol, haloperidol, clozapine, and olanzapine in rat plasma [75]. They made a comparison between the use of LC-TOF and LC–MS/MS using a triple quadrupole for these compounds for specificity, linearity, precision, accuracy, matrix effects, and recovery. The authors concluded that both methods were found not to be statistically different. On the other hand some other reports in the literature may induce serious concerns to purchase QTOF instruments for a routine laboratory. In 2010 Jin et al. reported a UPLC–QTOF method to determine antibiotics in urine with a linear response below one order of magnitude from 0.5 to 2 ng/mL [76]. The authors present a calibration curve in the range from 0.5 to 10 ng/mL which is still not impressive and will not convince any triple quadrupole user to purchase a QTOF instrument. Nevertheless, the following two applications reflect the strengths of modern QTOF systems.

In 2008 Garbis et al. carried out a study aimed to identify candidate new diagnosis and prognosis markers and medicinal targets of prostate cancer [77]. A total of 20 prostate tissue specimens from 10 patients with benign prostatic hyperplasia and 10 with prostate cancer were analyzed by isobaric stable isotope labeling (iTRAQ) and two-dimensional LC–MS/MS approaches using a hybrid QTOF system. The study resulted in the reproducible identification of 825 non-redundant gene products of which 30 exhibited up-regulation and another 35 exhibited down-regulation between the benign prostatic hyperplasia and prostate cancer specimens constituting a major contribution toward their global proteomic assessment. The large number and extensive biological distribution of the identified proteins supports existing knowledge and uncovers novel and promising prostate cancer biomarkers. The authors conclude that the findings of this study can be targeted either individually or on a panel basis in clinical sera specimens in the development of MS based assays in a clinical setting. The use of MS based bioassays typically exhibit over 99% confidence and constitutes a major advancement in clinical practice that may complement biochemical assay based methods.

Lysophosphatidylcholines (lysoPCs) levels can be a clinical diagnostic indicator that reveals pathophysiological changes. In the work of Dong et al. [78] a method was developed to discriminate between different types of lysoPCs using reversed phase ultra-performance LC coupled to QTOF-MS, using mass spectrometry MS^E, where E represents collision energy. MS^E is a technique in which precursor and fragment mass spectra are simultaneously acquired by alternating between high and low collision energy during a single chromatographic run [79]. Isomeric lysoPCs were distinguished based on retention time and the peak intensity ratio of product ions, and 14 pairs of lysoPCs regioisomers were identified in human plasma. The identification of lysoPCs and regioisomers requires detailed examination of the MS/MS information. The plasma samples of 12 lung cancer patients and 12 healthy persons were collected and analyzed by principal component analysis to generate metabolic profiles of the identified lysoPCs. ESI results showed that all lung cancer patients had the same five lysoPCs metabolic abnormalities. Therefore the authors conclude that the function of isomers with different fatty acyl positions may be related to lung cancer, and this may help elucidate the mechanism of the disease.

3.3. Orbitrap mass analyzers

Among the discussed hybrid instruments the LTQ-Orbitrap deserves special attention because it uses a novel mass analyzer. The Orbitrap was invented by Makarov [80] and is now commercially distributed by ThermoFisher Scientific. The schematic layout of the LTQ Orbitrap Velos mass spectrometer is depicted in Fig. 5. The Orbitrap mass analyzer employs orbital trapping of ions in its static electrostatic fields [81–85] in which the ions orbit around a central electrode and oscillate in axial direction. The electrode confines the ions so that they both orbit around the central electrode and oscillate back and forth along the central electrode's long axis. This oscillation generates an image current in the detector plates that is recorded by the instrument. The frequencies of these image currents depend on the ion m/z ratios and like in Fourier transform ion cyclotron resonance (FT-ICR) instruments the Orbitrap uses a fast Fourier transform algorithm [86] to convert time-domain signals into mass-to-charge spectra. The most important features of the Orbitrap mass analyzer are as follows: high resolution up to 240,000, high mass accuracy in the low ppm range, a mass-to-charge range of 6000, and a dynamic range greater than three orders of magnitude [82,83]. In the same way as combining a quadrupole and a TOF analyzer the Orbitrap benefits from adding an ion trap in front of the Orbitrap. This generates a hybrid instrument that has the advantages of high resolution and mass accuracy of the Orbitrap and the speed and the sensitivity of the LTQ ion trap. Furthermore the LTQ can carry out fragmentation reactions allowing high-resolution MS/MS experiments. This combination offers resolution and mass accuracy that comes close to the highest standards defined by FT-ICR instruments at considerably lower prices and a lower maintenance cost which makes it more reasonable for clinical laboratories.

In 2009 Zhang et al. published a promising study in which they claim that a high-resolution mass spectrometers such as the LTQ-Orbitrap can be used efficiently for quantitative analysis [87]. The quantitative performance of a LTQ-Orbitrap was compared with that of a triple quadrupole (AB Sciex API 4000) operating in selected reaction monitoring detection mode. Comparable assay precision, accuracy, linearity and sensitivity were observed for both approaches. The concentrations of study samples from 15 drug candidates reported by the two methods were statistically equivalent. As expected this approach not only provides quantitative results for compounds of interest, but also will afford data on other analytes present in the sample. As proof an example of the identification of

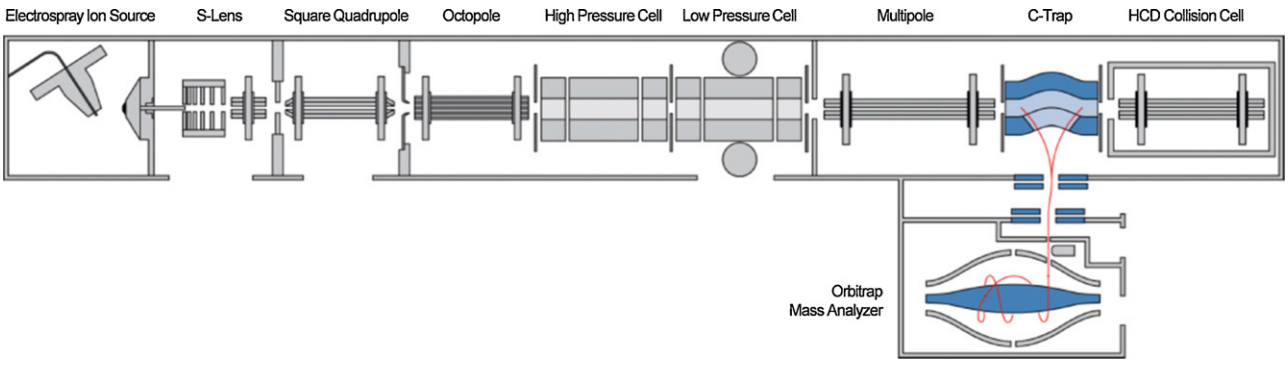

Fig. 5. Schematic layout of the LTQ Orbitrap Velos mass spectrometer.
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a major circulating metabolite for a preclinical development study is demonstrated.

Vogliardi et al. also showed that LC–HRMS can be used to identify and quantify simultaneously 28 benzodiazepines, including 6 metabolites, in hair [88]. Positive ion ESI and HRMS determination in the full-scan mode were realized on an Orbitrap mass spectrometer at a nominal resolving power of 60,000. In-source collisional experiments were conducted to obtain additional information for a more reliable identification of the investigated drugs. HRMS in full-scan mode allowed the exact determination of molecular masses of all analytes eluting from the HPLC run, so that both the immediate and the retrospective screening of results for drugs and their metabolites were available. The lowest limits of quantification (LLOQs) ranged from 1 to 10 pg/mg and the linearity was in the very acceptable range from LLOQ to 1000 pg/mg. After validation, the procedure was applied to real samples collected for clinical and forensic toxicology purposes from subjects who were assumed to have taken benzodiazepines. Fig. 6 displays extracted ion chromatograms and the quantitative results from such a case. This method clearly demonstrates the benefits of HRMS by showing the suitability for both the screening, confirmation and quantitation of target compounds in a single experiment.

The LTQ Orbitrap was applied to identify human liver microsomal metabolites of carvedilol using parent mass list triggered data-dependent multiple-stage accurate mass analysis, at a resolving power of 60,000 in external calibration mode [89]. A metabolite identification workflow was developed to utilize chemical formulas from high-resolution accurate mass measurements to confirm structures of product ions of a proposed compound. A total of 58 in vitro metabolites of carvedilol were detected using 5-ppm mass tolerance filters for theoretical m/z of protonated molecules of predicted metabolites. Mass accuracies obtained for all full scan MS and MS^n spectra were <2 ppm. The majority of the metabolites identified agreed with those previously reported except for those that have not been reported before, but several glutathione conjugates of carvedilol were reported for the first time, which may explain the reported hepatotoxicity during clinical trials and recent clinical use.

Kentsis et al. extended the current characterization of the human urinary proteome using high-accuracy mass measurements with a linear ion trap-Orbitrap mass spectrometer and LC–MS/MS of peptides generated from extensively fractionated specimens [90]. They identified 2362 proteins in routinely collected individual urine samples, including more than 1000 proteins not described in previous studies. Many of these are biomedically significant molecules, including glomerularly filtered cytokines and shed cell surface molecules, as well as renally and urogenitally produced transporters and structural proteins. Annotation of the identified proteome reveals distinct patterns of enrichment, consistent with previously described specific physiologic mechanisms, including 336 proteins that appear to be expressed by a variety of distal organs and glomerularly filtered from serum. Comparison of the proteomes identified from 12 individual specimens revealed a subset of generally invariant proteins, as well as individually variable ones, suggesting that this approach may be used to study individual differences in age, physiologic state and clinical condition. Consistent with this, annotation of the identified proteome using machine learning and text mining exposed possible associations with 27 common and more than 500 rare human diseases, establishing a widely useful resource for the study of human pathophysiology and biomarker discovery.

3.4. Fourier transform-ion cyclotron resonance instruments

When it comes to high resolution, high mass accuracy and sensitivity FT-ICR-MS is the gold standard among all mass analyzers. FT-ICR spectrometry is based on the principle of cyclotron motion in a uniform magnetic field. Ions are detected in a cyclotron cell, which is located inside a super-conducting magnet with fixed field strength. The principle of FT-ICR is described in detail in two reviews by Marshall et al. [91,92]. The technique is not as widespread as quadrupole, ion-trap, or TOF technologies, but is certainly gaining territory [93,94], mainly due to the introduction of user-friendly instruments by manufacturers and the new generation of hybrid FT-ICR instruments [95]. Despite the impressive performance of these instruments, the slow scan speed and the high costs compared to other mass analyzers will prevent their widespread use in routine clinical laboratories in the near future. Because of these limitations FT-ICR-MS will not be discussed in further detail in this review even though the use of these instruments in research facilities will further increase.

4. Ion mobility-mass spectrometry

Interfacing ion mobility spectrometry (IMS) with MS can provide significant advantages. The potential was understood early in the development of IMS, and the coupling of the two techniques is virtually as old as IMS itself. So ion mobility-mass spectrometry (IMMS) cannot be regarded as new, but after the demonstration of protein conformer separation by Clemmer et al. [96] there was a considerably increase in interest within this research area. During the last 10 years instruments became commercially available and both applications and instrumental designs of IMMS are now one of the most rapidly growing areas of MS. As a matter of fact numerous articles, reviews [97–105] and even books [106,107] are dealing with the topic of IMMS. The combination of IMS and

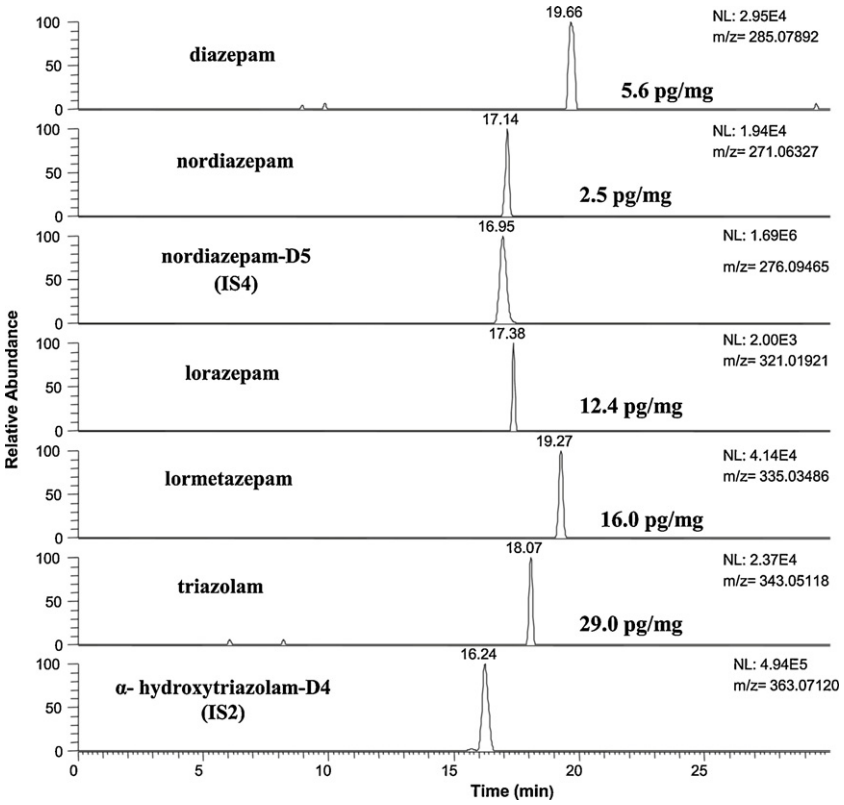

Fig. 6. Extracted ion chromatograms obtained by LC–HRMS hair analysis together with the extracted ion chromatograms of internal standards. Reprinted with permission from Vogliardi et al. [88]. Copyright 2011 Springer.

MS can produce improved data not available from mass spectra alone. Reports in the literature show the very fast separation of isomers, isobars, and conformers. Furthermore chemical noise and other interferences are reduced. IMS separates ions on the basis of their size-to-charge ratios which represents an analytical tool for investigating molecular structures if added to mass spectrometers. Another feature is the possibility to map mass-mobility correlation plots for structurally similar ions and ions of the same charge state that can be separated into families of ions. In this review the three most common designs of ion mobility separation coupled with MS will be discussed. The three available systems are drift-time ion mobility spectrometry (DTIMS), differential mobility spectrometry (DMS) marketed by AB Sciex as SelexION^{TM} technology, which is also referred to as field-asymmetric waveform ion mobility spectrometry (FAIMS) introduced by ThermoFisher Scientific and traveling-wave ion mobility spectrometry (TWIMS) as a trademark of Waters. All of these three systems have their particular benefits and limitations. The highest IMS resolving power is provided by DTIMS and it is the only IMS method which can directly measure collision cross-sections, but on the other hand it cannot compete with the other designs in terms of sensitivity due to its low duty cycle. With DMS and FAIMS high separation selectivity can be achieved and the ions are monitored in a continuous fashion which results in the ability to act as a very effective ion-filtering device. Low resolving power but good sensitivity are characteristic for the TWIMS device which is a novel method of IMS that is very well integrated into a commercial mass spectrometer system marketed by Waters. In principle each of these ion mobility devices can be interfaced to a variety of mass spectrometers; TOF analyzers are used most frequently but also combinations with quadrupole and ion trap systems can be found. To extend the possibilities even further an ion mobility cell can be interfaced to other ion mobility cells together with tandem mass spectrometers to produce IMS^n–MS^m type analyzers [108,109].

4.1. Drift-time ion mobility spectrometry

DTIMS was the first type of IMS and since it is the "traditional" form it is often referred to simply as IMS rather than DTIMS [97,101]. Ions are moving through a homogeneous, continuous electric field in a drift tube in the presence of neutral gas molecules. The time it takes the ions to migrate down the drift tube is directly proportional to the ion's collision cross-section. When low-field conditions are applied the velocity of the ion is directly proportional to the electric field. The proportionality constant is called ion mobility constant and is related to the ion's collision cross-section [97,110]. IMS fundamentals are so well-known that from the time it takes an ion to pass through the drift tube one can rather accurately calculate the average collision cross-section of the ion. At the moment DTIMS is the only form of IMS which allows the measurement of collision cross-sections of ions directly from the drift time. Fig. 7 shows a schematic diagram of a typical DTIMS-TOF mass spectrometer design commonly used today. DTIMS is mainly utilized in combination with direct infusion to determine cross-sections of large biomolecules, but also LC–IMS–MS reports can be found in the literature.

In 2009 Baker et al. evaluated a reversed-phase capillary LC–IMMS system regarding its applicability for the rapid analysis of complex proteomics samples [111]. Samples were prepared by spiking 20 reference peptides at varying concentrations from 1 ng/mL to 10 μg/mL into a tryptic digest of mouse blood plasma and analyzed with both a LC–FT-MS and LC–IMS–TOF-MS. The LC–FT-MS detected 13 out of the 20 spiked peptides that had concentrations ≥100 ng/mL. In contrast, the drift time selected mass spectra from the LC–IMS–TOF-MS analyses yielded identifications

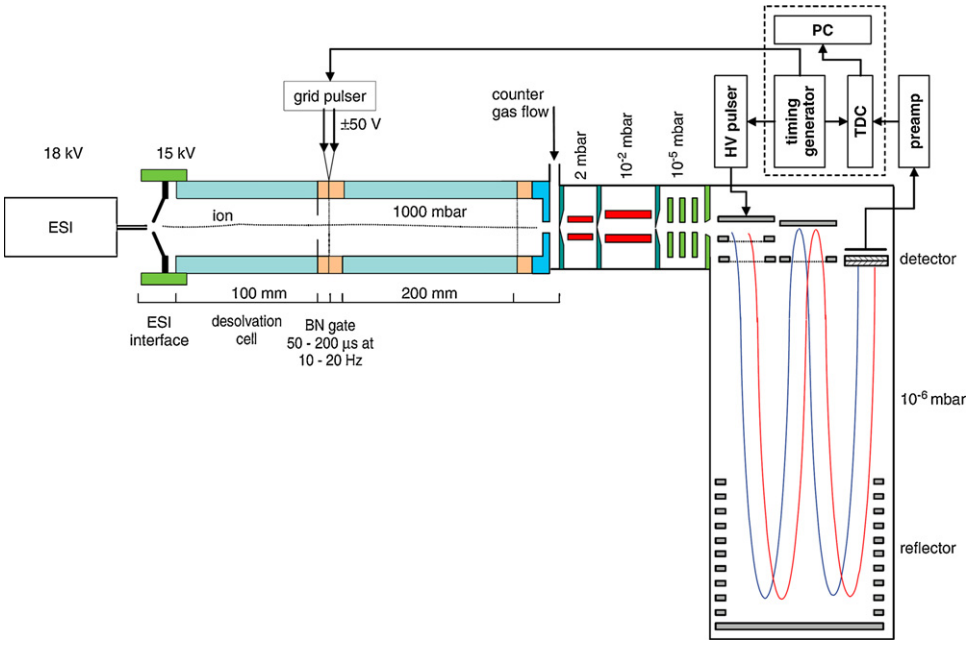

Fig. 7. Schematic of an ambient-pressure IMS(tof)MS.
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for 19 of the 20 peptides with all spiking levels present. The greater dynamic range of the LC–IMS–TOF-MS system could be attributed to two factors. First, the LC–IMS–TOF-MS system enabled drift-time separation of the low concentration spiked peptides from the high concentration mouse peptide matrix components, reducing signal interference and background, and allowing species to be resolved that would otherwise be obscured by other components. Second, the automatic gain control in the linear ion trap of the hybrid FT-MS instrument limits the number of ions that are accumulated to reduce space charge effects and achieve high measurement accuracy, but in turn limits the achievable dynamic range compared to the IMS-TOF instrument.

Zhu et al. used an ambient pressure IMS for the rapid separation of isomeric precursor ions of oligosaccharides prior to their analysis by MS with a quadrupole ion trap [112]. Separations were not limited to specific types of isomers; representative isomers differing solely in the stereochemistry of sugars, in their anomeric configurations, and in their overall branching patterns and linkage positions could be resolved in the millisecond time frame. Physical separation of precursor ions permitted independent mass spectra of individual oligosaccharide isomers to be acquired to at least MS^3, the number of stages of dissociation limited only by the abundance of specific product ions. IMS–MS^n analysis was particularly valuable in the evaluation of isomeric oligosaccharides that yielded identical sets of product ions in tandem MS experiments, revealing pairs of isomers that would otherwise not be known to be present in a mixture if evaluated solely by MS dissociation methods alone. Fig. 8 clearly demonstrates that fact and reveals the enormous potential benefits of IMMS. A practical example of IMS–MS^n analysis of a set of oligosaccharide isomers released from bovine submaxillary mucin is described.

A combination of strong-cation-exchange and reversed-phase LC with ion mobility and MS was described by Liu et al. as a means of characterizing the complex mixture of proteins associated with the human plasma proteome [113]. The increase in separation capacity associated with inclusion of the ion mobility separation led to an extensive proteome map. Plasma samples of five healthy humans were analyzed in triplicate and the identification of 9087 proteins is reported from 37,842 unique peptide assignments. An analysis of expected false-positive rates leads to a high-confidence identification of 2928 proteins. The results are cataloged in a fashion that includes positions and intensities of assigned features observed in the datasets as well as pertinent identification information such as protein accession number, mass, and homology score/confidence indicators. Comparisons of the assigned features reported here with other datasets shows substantial agreement with respect to the first several hundred entries; there is far less agreement associated with detection of lower abundance components.

4.2. *Differential mobility spectrometry*

DMS is a variant of IMS and differs from IMS in the geometry of the instrumentation. It is a method to separate ions based on the difference between ion mobility in high and low electric fields in gases at or near atmospheric pressure [99,101,114–118]. FAIMS and DMS are the most common names used to refer to this mode of mobility spectrometry. In difference to DTIMS, ions are continuously introduced into an ion mobility spectrometer and travel between two planar parallel electrodes [119] or two concentric cylinder electrodes [120]. RF voltages, often referred to as dispersion or separation voltages, are applied across the ion transport channel, perpendicular to the direction of the transport gas flow. Ion separation occurs because of the difference between high and low field ion mobility coefficients [121]. Due to this difference ions are migrating toward the walls and a compensation voltage has to be applied to one of the electrodes to focus ions and transport them into a mass spectrometer. By scanning the compensation voltage, ions with characteristic differential mobilities are detected in the mass spectrometer. Other than an IMS instrument, which separates ions linearly in time based on physical property such as shape or size, a DMS or FAIMS device separates ions on the basis of how much their mobility changes as a function of field strengths. As a consequence, DMS or FAIMS devices act as an ion mobility filter instead of an ion mobility analyzer and therefore are best suited for certain specific uses in a targeted approach. By adding polar modifiers to the gas in the DMS cell one can enhance the formation of clusters in a

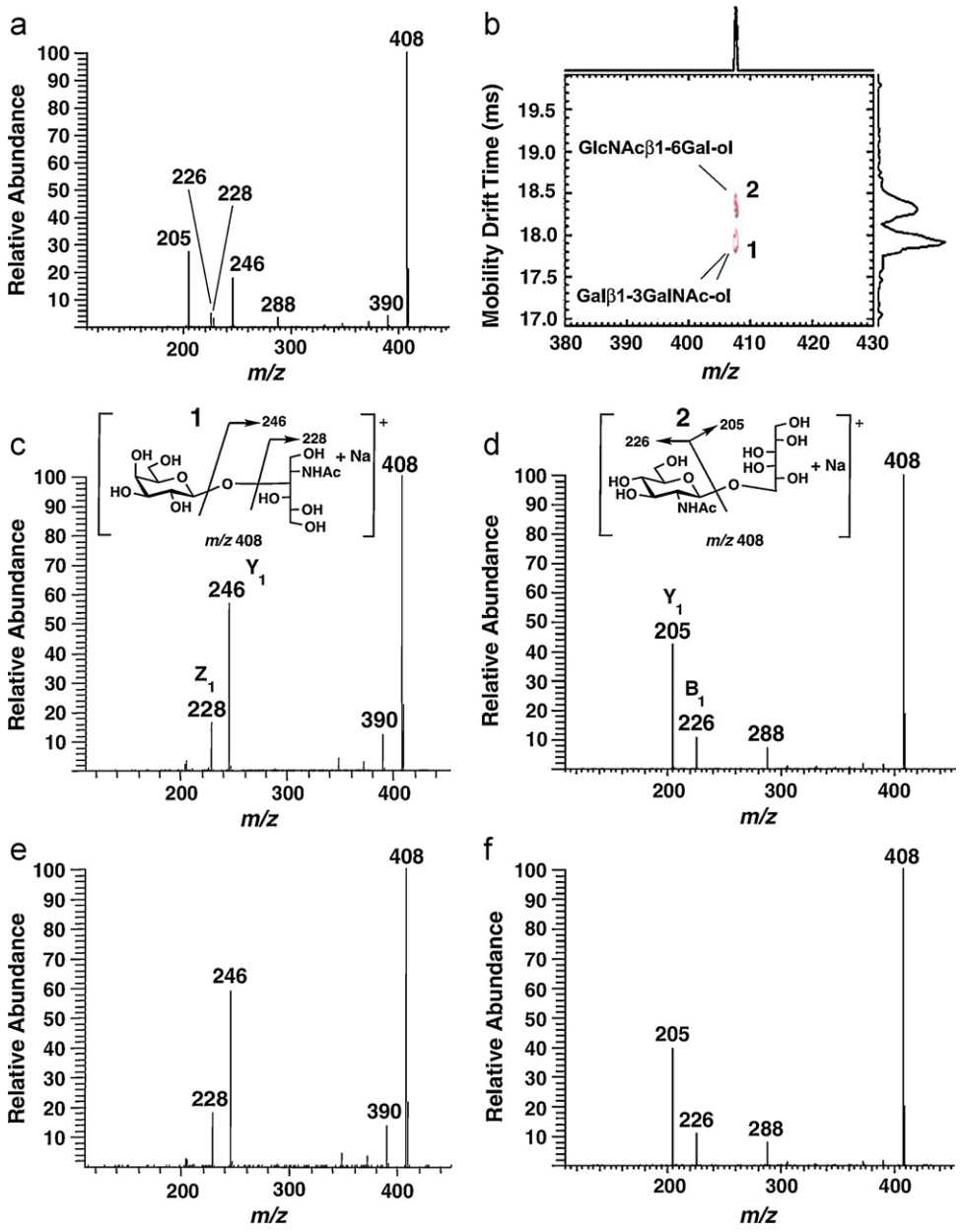

Fig. 8. Ion mobility-MS/MS of isomeric disaccharide alditols illustrating their separation and acquisition of independent MS/MS spectra for the molecules as sodiated molecules. Structural diagrams and postulated dissociation patterns for the ions are shown (*m/z* 408 precursors). (a) The MS/MS spectrum (quadrupole ion trap) of a mixture of the two disaccharide alditols Galβ1-3GalNAc-ol and GlcNAcβ1-6Gal-ol. (b) A two-dimensional ion mobility-MS spectrum performed on an APIMS-TOF instrument where mass spectra were acquired in real time during the ion mobility experiment. (c) The MS/MS spectrum of Galβ1-3GalNAc-ol (1) run individually on the IMS-ion trap instrument, selecting precursor ions within the 39.7–40.4 ms window. (d) The MS/MS spectrum of GlcNAcβ1-6Gal-ol (2) run individually on the IMS-ion trap instrument, selecting precursor ions within the 40.7–41.1 ms window. (e) The MS/MS spectrum of the 39.7–40.4 ms window on the IMS-ion trap instrument, spraying a mixture of the two isomeric disaccharide alditols. (f) The MS/MS spectrum of the 40.7–41.1 ms window on the IMS-ion trap instrument, spraying a mixture of the two isomeric disaccharide alditols. All mass spectra were accumulated using identical collision energies.

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field-dependent way which amplifies the high- and low-field mobility difference of ions, resulting in increased peak capacity and separation power [121–124]. These chemical interactions that occur between an ion and neutrals gas molecules increases the selectivity of the separation, and the depression of low-field mobility relative to high-field mobility increases the compensation voltage and peak capacity. Furthermore drift gas temperature and pressure can influence the performance by changing peak positions, heights and widths [125]. Especially FAIMS, which is commercially available and well integrated into MS instruments, is frequently used in combination with LC to improve method performance. In the following paragraphs representative examples related to bioanalysis are given.

The feasibility of developing a multi-component bioanalytical method using FAIMS coupled with LC–ESI-MS/MS is demonstrated by Wu et al. using nefazodone and its two metabolites as model compounds [126]. The performance of the bioanalytical method for the three analytes, with three different compensation voltage values, is assessed using standard curves and quality control samples, which exhibited good accuracy, precision and ruggedness. The authors show that, with a chromatographic peak width of 10 s and a dwell time of 50 ms, bioanalytical method development for three

analytes with three different compensation voltage settings (one compensation voltage value for each analyte and its internal standard) is feasible.

The effect of metabolite interference during LC–MS/MS analysis of an amine drug was investigated using FAIMS by Kapron et al. [127]. The selected reaction monitoring transition used for the drug exhibited interference due to in-source conversion of the N-oxide metabolite to generate an ion isobaric with the drug. The on-line FAIMS device removed the metabolite interference before entrance to the mass spectrometer. FAIMS was used to demonstrate the relative accuracy and precision of drug analysis even in the presence of a co-eluting metabolite that may undergo insource conversion. The authors conclude that LC–FAIMS-MS/MS is compatible with sample preparation and handling procedures currently in use, and promises to be a valuable tool in overcoming known and potential metabolite interferences.

A LC–FAIMS-MS/MS semi-quantitative method was developed by Kapron et al. [128] for the simultaneous determination of prostanoids and thromboxane B_2. Diluted samples containing prostanoids and their tetra-deuterium-substituted internal standards were analyzed by LC followed by either selected reaction monitoring or FAIMS and selected reaction monitoring. FAIMS reduced background noise, separated two isobaric ions, and separated dynamically interchanging thromboxane B_2 anomers. This is the first report of the separation of interconverting anomers by FAIMS. Without changes to sample preparation or chromatography in the traditional method, FAIMS increased selectivity, reduced background and improved signal-to-noise ratios. Application of the optimized method to the analysis of tissue extracts demonstrated the ability of FAIMS to enable low-level bioanalysis in complex matrices.

Venne et al. showed how the use of FAIMS combined with nanoscale LC–MS can improve the detection of multiply charged peptide ions from complex tryptic digests [129]. The combination of FAIMS provided a marked advantage over conventional nanoLC–MS experiments by reducing the extent of chemical noise associated with singly charged ions and enhancing the overall population of detectable tryptic peptides. Such advantages were evidenced by a 6 to 12-fold improvement in signal-to-noise ratio measurements for a wide range of multiply charged peptide ions. A comparison of nanoESI mass spectra of Glu-fibrinopeptide at different concentrations with the conventional nanoESI and the FAIMS interface is shown in Fig. 9. An increase of 20% in the number of detected peptides compared to conventional nanoESI was achieved by transmitting ions of different mobilities at high electric field versus low field while simultaneously recording each ion population in separate MS acquisition channels. This method provided excellent reproducibility across replicate nanoLC–FAIMS-MS runs with more than 90% of all detected peptide ions showing less than 30% variation in intensity. The application of this technique in the context of proteomics research is demonstrated for the identification of trace-level proteins showing differential expression in U937 monocyte cell extracts following incubation with phorbol ester.

Xia et al. developed a sensitive and selective method for the quantification of a peptide drug candidate in plasma using FAIMS coupled with LC–MS/MS to evaluate the applicability of FAIMS in quantitative bioanalysis [130]. The LC–FAIMS-MS/MS method provided significant advantage over the corresponding LC–MS/MS method by reducing chemical/endogenous background noise associated with plasma matrix, thereby improving the sensitivity via increasing the signal-to-noise ratio. Linearity was established within 1–1000 nM in rat plasma, and the overall method accuracy and precision were good meeting the generally adopted acceptance criteria for a bioanalytical method. The global selectivity of FAIMS from plasma endogenous components as a function of the compensation voltage across molecular masses that encompass small-molecule drugs was also demonstrated. Authors conclude, that FAIMS coupled with LC–MS/MS can be highly advantageous in quantitative bioanalysis.

4.3. Traveling-wave ion mobility spectrometry

TWIMS represents a novel method of IMS that has recently been developed and introduced commercially [101,131–133]. It uses a sequence of symmetric potential waves continually propagating through a drift tube that drive ions along with velocity dependent on the ion mobility constant. Small ions collide less frequently with gas molecules and make it first to the mass spectrometer, whereas large ions collide more and get delayed. As a result different species transit the tube in unequal times. In contrast to FAIMS and DMS, TWIMS uses pulsed ion injection and is more similar to DTIMS where multiple species can be measured simultaneously and ions are separated by size. In difference to DTIMS the traveling-wave principles of this complex, but unique mobility cell are not that well characterized until now. No general valid equation is available to get an ion's size and shape, which means that the instrument has to be calibrated against a traditional IMS instrument [134]. Similar to DTIMS, TWIMS is frequently used to determine the cross-section of large biomolecules even though no direct measurement is possible and the instrument has to be calibrated. In the following some examples from the field of bioanalysis applying the T-wave IMS are given.

Carotenoids are biosynthesized in plants as their all-trans isomers, but isomerize in solution and in humans to multiple cis isomers which can have different bioavailabilities and functions. Since separation and characterization of isomeric carotenoids using HPLC or LC–MS/MS is time-consuming, the potential of IMMS to resolve and characterize carotenoid isomers rapidly without chromatography was investigated using TWIMS on a QTOF mass spectrometer [135]. The all-trans isomers of lycopene and β-carotene were separated by several milliseconds from the cis isomers which were detected as partially overlapping peaks. Furthermore the collision cross-section values of these carotenoid isomers were determined. Collision-induced dissociation MS/MS of ion mobility resolved isomers indicated that cis and all-trans carotenoid isomers can be distinguished by their fragmentation patterns. Previous MS/MS studies of cis and all-trans carotenoids had suggested that they produced identical tandem mass spectra [136], but this appears to have been the result of isomerization during ionization. Introduction of specific cis or trans isomers by infusion or HPLC resulted in cis/trans isomerization in the ion source during electrospray, and the relative levels of cis carotenoids forming in the ion source compared to the all-trans isomers were temperature dependent.

In 2007 Olivova et al. reported an improved analytical method for glycosylation structural characterizations of a monoclonal antibody using a traveling-wave quadrupole ion-mobility TOF mass spectrometer [137]. Using this method, high-resolution mass spectra were acquired to produce the overall glycosylation profile of the monoclonal antibody. Additionally, the light and heavy chains from the reduced antibody were separated in the gas phase by the ion mobility functionality of the instrument, allowing accurate mass measurement of each subunit. Furthermore, the glycan sequences, as well as the glycosylation site, were determined by a two-step sequential fragmentation process using the unique dual-collision-cell design of the instrument, thus providing detailed characterizations of the glycan structures.

Drug metabolism is an integral part of the drug development and drug discovery process. The current methodologies of choice for metabolite structural elucidation are LC–MS/MS and nuclear magnetic resonance spectroscopy. There are, in certain cases, examples of metabolites whose sites of metabolism cannot be

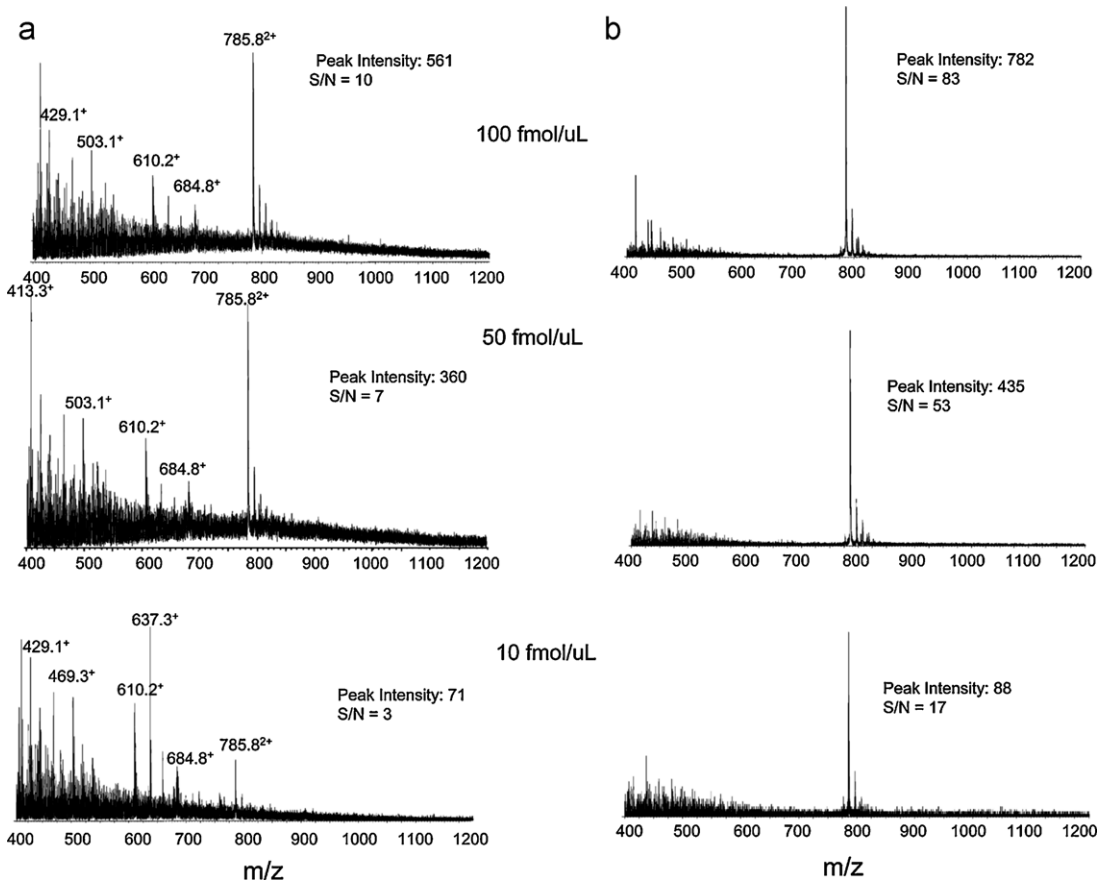

Fig. 9. Comparison of nanoelectrospray mass spectra of Glu-fibrinopeptide at concentrations of 100, 50, and 10 fmol/μL in 50% aqueous methanol with (a) the conventional nanoelectrospray and (b) the FAIMS interface at a compensation voltage of −15.5 V.
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unequivocally identified by MS/MS alone. Dear et al. utilized commercially available molecular dynamics packages and known quantum chemistry basis sets to generate the lowest energy structures for a group of aromatic hydroxylated metabolites of the model compound ondansetron [138]. Theoretical collision cross-sections were calculated for each structure. Subsequently TWIMS measurements were performed on the compounds, thus enabling experimentally derived collision cross-sections to be calculated. A comparison of the theoretical and experimentally derived collision cross-sections were utilized for the accurate assignment of isomeric drug metabolites. The authors conclude that the UPLC–IMS–MS method has the ability to measure metabolite structural isomers, which differ in collision cross-section, by IMMS and has the potential to supplement and/or complement current methods of metabolite structural characterization.

5. Conclusion

The power of MS coupled to LC is recognized by clinical laboratories worldwide and the growing versatility of these systems puts clinical laboratories in a position where they can provide a rapid response to changing clinical needs. A triple quadrupole instrument in SRM mode is the instrument-of-choice in routine and high-throughput clinical analysis because of its outstanding performance when quantification is needed. Due to the advances in the field of triple quadrupole technology, the instruments are capable of hundreds of MRM transitions in a single LC–MS/MS run and will certainly continue to be very important. Nevertheless, other mass analyzers like ion traps and especially HRMS like QTOF and FT-MS based instruments underwent significant instrumental developments in the last 10 years. Therefore the future of triple quadrupoles will be determined on the variable how extensively the clinical field adopts high resolution, high mass accuracy instruments into their workflows and analytical requirements. In case of triple quadrupole instruments, mass resolution was typically ignored in favor of the outstanding linearity and increased sensitivity due to the selectivity offered by tandem MS. As new tasks are gaining more and more interest where improved selectivity, mass accuracy and full-scan data at low duty cycle times are crucial, these needs could be filled by high-resolution instruments that are now available from numerous manufacturers. In the discovery process these instruments are already frequently used for quantitative and qualitative analysis.

ESI and APCI are the most important ion sources when it comes to the ionization of liquids. They had the most commercial success and enormous improvements were made in the first 15 years after their invention during the mid-1980s. Based on these developments multimode ion sources were introduced on the market by various manufacturers. In addition the APPI ion source was developed and improved within the last 10 years.

Ion mobility-mass spectrometry cannot be regarded as new, but there is a considerably increase in interest within this research area. During the last 10 years instruments became commercially available and both applications and instrumental designs of IMMS are now one of the most rapidly growing areas of MS. The combination of IMS and MS can produce improved data not available from mass spectra alone. Reports in the literature show the very

fast separation of isomers, isobars, and conformers. Furthermore chemical noise and other interferences are reduced.

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