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Review

Recent developments in liquid chromatography–mass spectrometry and related techniques

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ABSTRACT

This review summarizes the state-of-art in liquid chromatography–mass spectrometry (LC–MS) and related techniques with the main focus on recent developments in the last decade. LC–MS records an enormous growth in recent years due to the application potential in analytical chemistry, biochemistry, pharmaceutical analysis, clinical analysis and many other fields, where the qualitative and quantitative characterization of complex organic, bioorganic and organometallic mixtures is needed. Beginners and moderately experienced LC–MS users may be confused by the number of different LC–MS systems on the market, therefore an actual overview of mass spectrometers designed for the LC–MS configuration and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) from main manufacturers is compiled here together with an independent assessment of their advantages and limitations. Current trends in terms of mass analyzers, ionization techniques, fast LC–MS, LC–MALDI-MS, ion mobility spectrometry used in LC–MS, quantitation issues specific to MS and emerging mass spectrometric approaches complementary to LC–MS are discussed as well.

Contents

1. Introduction

Several reviews devoted to new trends in instrumental developments in LC–MS and related techniques were published in previous special issues of "Mass spectrometry: Innovation and application" published in Journal of Chromatography A [1,2] and in other places as well [3–5]. Due to numerous new LC–MS developments and the constant improvement of operating parameters of mass spectrometers, the situation is reviewed here again with two main goals: (1) to highlight recent innovations in LC–MS especially during the last decade, (2) to prepare a list of mass spectrometers currently offered by main manufacturers for LC–MS and MALDI-MS configurations together with their technical specifications (Tables 1–3) and the comparison of their application potential. This task could not be performed without the close cooperation with representatives of individual manufacturers and their websites [6–16], but we do our best to prepare fair and balanced scientific overview without any advertisement of individual technical solutions presented

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Table 1
Overview of commercial mass spectrometers designed for LC–MS with their technical specifications provided by individual manufacturers.[a]

Mass analyzer type	Instrument name, manufacturer	Resolving power (FWHM defined at m/z)	Resolution ($\Delta m/z$)	Mass accuracy (ppm)		m/z range	Acquisition speed (Hz)
				Internal calibration	External calibration		
Q	6150, Agilent	–	1	–	–	10–1350	10[b]
	Flexar SQ 300 MS, PerkinElmer	–	0.6	–	–	20–3000	10[b]
	LCMS-2020, Shimadzu	–	1	–	–	10–2000	15[b]
	LC/MS Purification System, Gilson	–	1	–	–	50–3000	10[b]
	MSQ plus, Thermo Scientific	–	1	–	–	17–2000	12[b]
	SQ Detector 2, Waters	–	1	–	–	2–3072	15[b]
IT	Amazon Speed ETD, Bruker Daltonics	–	0.1	–	–	50–6000	52[b]
	LCQ Fleet, Thermo Scientific	–	0.3	–	–	15–4000	12[b]
LIT	LTQ Velos Pro, Thermo Scientific	–	0.05	–	–	15–4000	66[b]
QqQ	6490, Agilent	–	0.4	–	–	5–1400	10[b]
	LCMS-8030, Shimadzu	–	0.7	–	–	10–2000	15[b]
	TQ Detector, Hitachi	–	1	–	–	2–2000	10[b]
	Triple Quad 5500, AB SCIEX	–	1	–	–	5–1250	12[b]
	TSQ Vantage, Thermo Scientific	7500 (m/z 508)	0.07	5	–	10–3000	5[b]
	XEVO TQ-S, Waters	–	1	–	–	2–2048	10[b]
Q-LIT	QTRAP 5500, AB SCIEX	9200 (m/z 922)	0.1	–	–	5–1250	20[b]
TOF	6230 TOF, Agilent	24,000 (m/z 1522)	0.06	1–2	–	25–20,000	40
	AccuTOF, Jeol	6000 (m/z 609)	0.1	5	–	6–10,000	10
	AxION 2 TOF MS, PerkinElmer	12,000 (m/z 922)	0.08	2	–	18–12,000	70
	Citius, Leco	100,000 (m/z 609)	0.006	<1	–	50–2500	200
	micrOTOF II focus, Bruker Daltonics	16,500 (m/z 922)	0.06	<2	<5	50–20,000	40
	XEVO G2 TOF, Waters	22,500 (m/z 956)	0.04	<1	–	20–16,000	30
IT-TOF	LCMS-IT-TOF, Shimadzu	10,000 (m/z 1000)	0.1	3	5	50–5000	10
Q-TOF	maXis 4G, Bruker Daltonics	60,000 (m/z 1222)	0.02	<0.6	<2	50–20,000	30 (MS), 10 (MS/MS)
	micrOTOF-Q II, Bruker Daltonics	20,000 (m/z 922)	0.05	<2	<5	50–20,000	20
	TripleTOF 5600, AB SCIEX	35,000 (m/z 956)	0.03	0.5	2	5–40,000	25 (MS), 100 (MS/MS)
	XEVO G2 QTof, Waters	22,500 (m/z 956)	0.04	<1	–	20–16,000	30
	6550 QTOF, Agilent	42,000 (m/z 922)	0.02	<1	–	50–10,000	50
Q-IMS-TOF	Synapt G2-S HDMS, Waters	40,000 (m/z 956)	0.02	<1	–	20–100,000	30
Orbitrap	Exactive, Thermo Scientific	100,000 (m/z 200)	0.002	<2	<5	50–4000	10 (at RP = 10,000)
Q-Orbitrap	Q Exactive, Thermo Scientific	140,000 (m/z 200)	0.001	<1	<5	50–4000	12 (at RP = 17,500)
LIT-Orbitrap	Orbitrap Elite, Thermo Scientific	240,000 (m/z 400)	0.002	<1	<3	50–4000	8 (at RP = 15,000)
Q-ICR	SolariX 15T, Bruker Daltonics	2,500,000 (m/z 400)	0.0002	<0.25	<0.6	100–10,000	–
LIT-ICR	LTQ FT Ultra 7T, Thermo Scientific	750,000 (m/z 400)	0.0005	<1	<1.2	50–4000	2 (at RP = 50,000)

[a] If manufacturers have more instruments in particular series, then only the instrument with the best performance is listed here. Individual manufacturers take the full responsibility for the correctness of technical specifications. Instruments in individual classes are sorted alphabetically according to the instrument name. This list contains only main manufacturers and may not be comprehensive.
[b] Acquisition speed for low RP mass analyzers is typically specified by manufacturers in Da/s, but we have recalculated these values into Hz units for the mass range of $\Delta m/z$ 1000.

in this review. Authors take no responsibility for the correctness of technical specifications provided by manufacturers. MS terms and definitions used in this paper are in agreement with the IUPAC sponsored project the Standard Terms and Definitions for Mass Spectrometry [17]. This database is now being maintained and updated by Murray to build a reference tool and a glossary of MS terms with 780 entries as of July 12, 2012 [17]. Recommended MS terms related to the separation sciences have been published in the previous special MS issue in Journal of Chromatography A [18]. Another valuable literature source on the use of correct and incorrect terms is Mass Spectrometry Desk Reference compiled by Sparkman [19], but some recommendations are not consistent with previously mentioned glossary.

2. Overview of up-to-date mass spectrometers

The market of MS and LC–MS is extremely dynamic and individual manufacturers invest into the development of new technologies. This competition has a positive effect on frequent launches of new products and technical solutions. For better

Table 2
Common parameters of mass spectrometers used in LC–MS.

Mass analyzer type[a]	Resolving power [$\times 10^3$]	Mass accuracy (ppm)	m/z range (upper limit) [$\times 10^3$]	Acquisition speed (Hz)	Linear dynamic range	Price
Q	3–5	Low[b]	2–3	2–10	10^5–10^6	Lower
IT	4–20	Low	4–6	2–10	10^4–10^5	Moderate
TOF	10–60	1–5	10–20	10–50	10^4–10^5	Moderate
Orbitrap	100–240	1–3	4	1–5	5×10^3	Higher
ICR	750–2500	0.3–1	4–10	0.5–2	10^4	High

[a] TOF, Orbitrap and ICR also include common hybrid configurations with Q or LIT as the first mass analyzer.
[b] Qs with hyperbolic rods provide mass accuracies better than 5 ppm.

Table 3
Overview of commercial mass spectrometers designed for MALDI-MS with their technical specifications provided by individual manufacturers.[a]

Mass analyzer type	Instrument, manufacturer	Resolving power, FWHM (defined at m/z)	Resolution ($\Delta m/z$)	Mass accuracy (ppm)		m/z range	Laser (wavelength, frequency)
				Internal calibration	External calibration		
LIT	LTQ XL, Thermo Scientific	15,000–30,000 (depending on scan speed)	–	–	–	15–4000	N_2 (337 nm, 60 Hz)
TOF	Autoflex Speed, Bruker Daltonics	26,000 (m/z 3147)	0.1	<2	<10	100–500,000	Nd:YAG (355 nm, 1000 Hz)
	Axima Confidence, Shimadzu	15,000 (m/z 3660)	0.2	<10	<100	1–500,000	N_2 (337 nm, 50 Hz)
IT-TOF	Axima Resonance, Shimadzu	8000 (m/z 2465)	0.3	3	5	100–12,000	N_2 (337 nm, 10 Hz)
Q-IMS-TOF	MALDI Synapt G2-S HDMS, Waters	32,000 (m/z 3495)	0.1	<1	–	20–100,000	Nd:YLF (349 nm, 1000 Hz)
TOF/TOF	Axima Performance, Shimadzu	20,000 (m/z 3660)	0.2	<5	<50	1–500,000	N_2 (337 nm, 50 Hz)
	JMS-S3000 SpiralTOF, Jeol	60,000 (m/z 2093)	0.03	1	10	10–30,000	Nd:YLF (349 nm, 250 Hz)
	TOF/TOF 5800 System, AB SCIEX	33,000 (for m/z range 1200–3700)	0.07	<1	<5	–	Nd:YLF (345 nm, 1000 Hz)
	UltrafleXtreme, Bruker Daltonics	40,000 (m/z 3147)	0.08	<1.5	<5	100–500,000	Nd:YAG (355 nm, 1000 Hz)
LIT-Orbitrap	MALDI LTQ Orbitrap XL, Thermo Scientific	100,000 (m/z 400)	0.004	<2	<3	50–4000	N_2 (337 nm, 60 Hz)
Q-ICR	SolariX 15T, Bruker Daltonics	2,500,000 (m/z 400)	0.0002	<0.25	<0.6	100–10,000	Nd:YAG (355 nm, 1000 Hz)

[a] If manufacturers have more instruments in particular series, then only the instrument with the best performance is listed here. Individual manufacturers take the full responsibility for the correctness of technical specifications. Instruments in individual classes are sorted alphabetically according to the instrument name. This list contains only main manufacturers and may not be comprehensive.

overview of current LC–MS systems, we have compiled a list of commercial mass spectrometers designed for LC–MS (Table 1) and for MALDI-MS (Table 3) with their technical specifications, such as resolving power, resolution, mass accuracy specified for internal and external calibrations, mass-to-charge (m/z) range and acquisition speed. These tables will become obsolete quite rapidly, but we still believe that such current snapshot of MS technology as of spring 2012 is useful. Table 2 shows typical operating parameters for five basic types of mass analyzers used in LC–MS.

This review is primarily intended for low to moderately experienced LC–MS users, therefore basic MS terms used in Tables 1–3 are explained here. More detailed information can be found in several excellent textbooks devoted to MS [20–23]. The basic parameter for the characterization of mass analyzer ability to resolve peaks in mass spectra is a resolving power (RP), which is defined as the m/z value of particular peak divided by the peak full width at half maximum (FWHM): $RP = (m/z)/\Delta m/z$. The RP must be always defined for the particular m/z value (*e.g.*, RP is 20,000 at m/z 922), because the RP grows with increasing m/z value on condition of identical peak width. The older definition of RP (established for magnetic sector analyzers) based on two neighboring peaks of identical heights and 10% valley is not used in the current LC–MS practice. The resolution is the inverse of RP expressed as $\Delta m/z$ for a given m/z value [19]. For the above-mentioned example, the resolution is calculated as $\Delta m/z = (m/z)/RP = 922/20{,}000 = 0.046$. Better quality of mass analyzer is associated with lower values of resolution and higher values of RP (see Tables 1 and 3). Mass accuracy (MA) is defined as the relative difference between the experimental m/z value and theoretical m/z value related to this theoretical value including the sign (plus or minus) and expressed in ppm: $MA = 10^6 \times ((m/z)_{exp} - (m/z)_{theor})/(m/z)_{theor}$. The best values of MA are achieved with the internal calibration, *i.e.*, the sample and the internal calibrant are introduced into the ion source at the same time. The introduction of internal calibrant during LC–MS may be impractical in some instances (*e.g.*, interference of calibrant with the chromatographic separation or identical masses of calibrant and analyte). The "lock-mass" calibration is based on well-defined ion with known elemental composition coming from the background (known impurities occurring from previous samples, mobile phase, air components, *etc.*), introduced by the second sprayer or high-confidence ions from the sample, which is used for the correction of previously done external calibration by locking particular m/z value [24]. Another example of lock-mass internal calibration is the use a subset of high confidence peptide identifications from a first pass database search [25]. Further improvements of MA can be achieved by software tools to eliminate a lower dynamic range of time-to-digital converters in some mass analyzers [26]. Alternative way of calibrant introduction is the use of dual sprayer in ESI-MS using a rapid modulation (switch in less than 70 ms) between ESI emitters [27]. The external calibration is the procedure, where the sample and the calibrant are not present in the ion source at the same time. If the mass spectrometer is stable enough without any mass drift, the external calibration may provide almost comparable results, but the time difference between the sample and the calibrant introduction should be as low as possible, *e.g.*, the calibrant can be introduced between the LC injection time and the void time or immediately after the elution of last peak in the chromatogram. The m/z ranges of individual mass analyzers in Tables 1 and 3 show the maximum possible measurement span of mass detectors. The acquisition speed is typically expressed in technical specifications in Da/s for low-resolution and in Hz for high-resolution mass analyzers. We have re-calculated specifications for low-resolution analyzers from Da/s to Hz for the typical measurement range of 1000 m/z to allow the comparison among all instruments (Table 1). By the way, numerical values of acquisition speed expressed in kDa/s and Hz for 1000 m/z range are identical.

2.1. Mass spectrometers in LC–MS coupling

The standard resolution of Q analyzer is a unit resolution, while somewhat better resolution can be obtained at cost of a lower ion transmission and therefore lower sensitivity, as understood from the stability diagram [22]. The resolution of spherical ITs and especially linear ion traps (LITs) is slightly higher compared to the Q analyzer, but the inverse relation between the resolution and the sensitivity based on the three-dimensional stability diagram is again valid. The typical MA of low-resolution mass analyzers is below 100 ppm (calculated for $\Delta m/z$ error 0.1 at m/z 1000), but such low MAs are not sufficient for the elemental formula

determination. Q rods with an ideal hyperbolic profiles provide a higher resolution than for regular round Q rods with the MA <5 ppm comparable to high-resolution mass analyzers (see QqQ TSQ Vantage in Table 1). Another advantage of QqQ with hyperbolic rods is a narrow isolation width of precursor ions used for the selected reaction monitoring (SRM), while the drawback is lower acquisition speed. The m/z range is typically up to m/z 2000–3000 for Qs and m/z 4000–6000 for ITs (Table 2). The special Q with significantly higher transmission for large ions (up to m/z 32,000) can be fabricated in the transmission non-resolving mode only, which means that such Qs can be used only for the transmission of all ions but not for their mass resolution.

The comparison of high-resolution mass analyzers listed in Table 1 also needs additional comments. The ICR has superior values of RP and MA among all analyzers followed by Orbitrap and then TOF based analyzers. It is also important to realize the relation between the RP and the acquisition speed, because the highest acquisition speed of Fourier transform (FT) mass analyzers ICR and Orbitrap does not correspond to their highest RP. The best parameters reported for FT analyzers require longer acquisition times due to the fact that higher number of image currents has to be recorded. The incorporation of FT mass analyzer into the fast LC–MS concept is possible, but at cost of significantly reduced RP in comparison to the best values reported for slow scan speeds in Table 1. TOF mass analyzers have the highest scanning speed among all mass analyzers and also their m/z range is theoretically unlimited (*e.g.*, measurements in hundred thousands Da in MALDI-TOF linear configuration), but the m/z range of TOF based analyzers in LC–MS systems is limited to several tens of thousands. The linear dynamic range (Table 2) depends on the particular instrument and application, but in general FT mass analyzers exhibit slightly lower linear dynamic range.

The price parameter in Table 2 is intended only as a rough guide for typical configurations, but the real price strongly depends on the particular configuration and individual offers from the manufacturer. In general, Q analyzer is simplest and cheapest device followed by spherical and linear ITs. TOF analyzer is the cheapest high-resolution mass analyzer with some impressive characteristics in terms of acquisition speed, m/z range and relatively good RP and MA. FT mass analyzers, Orbitrap and especially ICR, are high-end MS technologies with the best operational parameters, but the instrumental complexity is obviously reflected in increased investment costs. Table 2 should be understood as an overview of common operating parameters but excluding extreme values obtained at specific conditions, *e.g.*, slow scan speed, reduced m/z range, *etc.*

Fig. 1 shows an overview of installed LC–MS systems in the Czech Republic. The world statistics would be more representative, but reliable data does not exist unlike to our statistics for this local central European market, where we monitor and annually update the situation [28] starting from the first LC–MS system installed at the University of Pardubice in 1995. The local statistics could be affected by regional differences, but Fig. 2 confirms a reasonable agreement with trends in the world. Some differences between Figs. 1 and 2 can be explained – at least in part – by the different way of the preparation of these graphs. Fig. 2 shows the world statistics based on the Web of Science search, while Fig. 1 is prepared from exact numbers of installed LC–MS systems in the Czech Republic. In our opinion, certain overestimation of top-class expensive instruments occur in Fig. 2, because new technologies are purchased primarily for research purposes yielding a higher number of papers compared to low-cost and low-resolution mass analyzers used mainly for routine analyses and quantitation in industrial and clinical laboratories with a lower publication activity.

Prevailing LC–MS configurations (see Figs. 1 and 2) are based on IT, TOF and Q mass analyzers. QqQ tandem mass analyzer is dominant in the quantitative analysis, because SRM scan typical for this type of analyzer is a golden standard for any LC/MS or shotgun MS based quantitation. Hybrid Q-TOF instrument is the most common in the structural characterization due to the possibility of measurements of high-MA in both full-scan and MS/MS modes. In recent years, the shift from low-resolution toward high-resolution systems including TOF based mass analyzers and ultrahigh-resolution (RP > 100,000) FT mass analyzers (Orbitrap and ICR) occurs, because ultrahigh-RP and ultrahigh-MA values open new possibilities in both qualitative and quantitative analyses, *e.g.*, SRM or even SIM approaches based on ultrahigh-RP and therefore the possibility of very narrow widths for precursor ion isolation, which leads to increased selectivity and sensitivity.

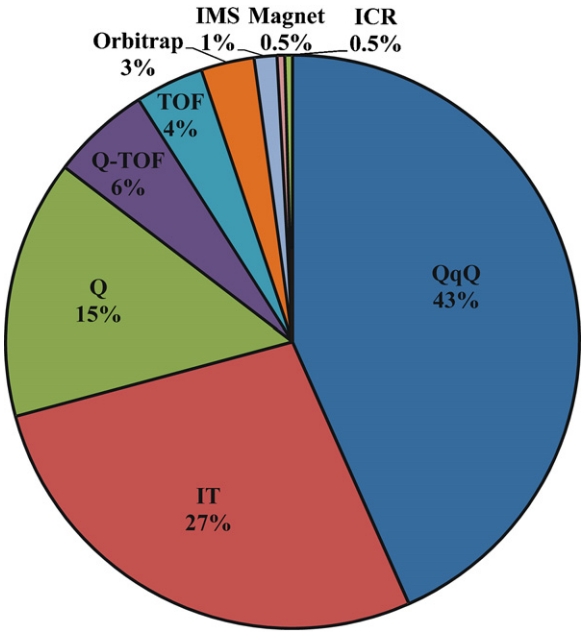

Fig. 1. Overview of installed LC–MS systems in the Czech Republic according to the type of mass analyzer (in total 233 systems, update September 2011).

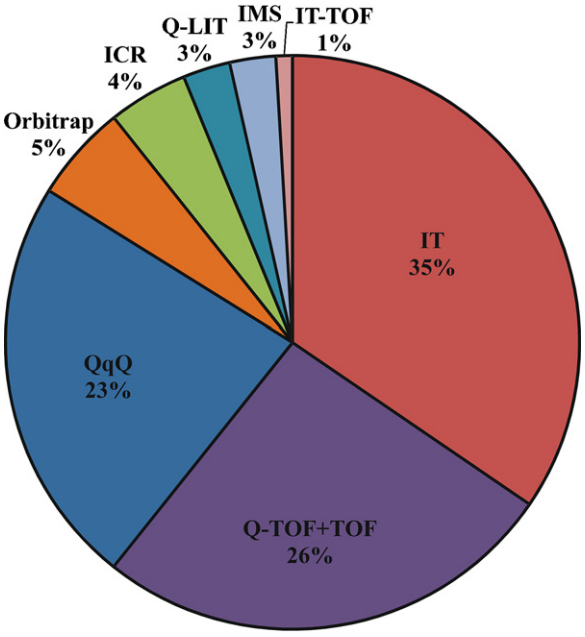

Fig. 2. Relative use of individual types of mass analyzers in LC–MS papers based on the Web of Science search from March 1, 2012.

Some papers compared a real performance of different types of modern tandem mass analyzers for particular applications, which provides valuable complementary information to Tables 1–3. LC–MS with QqQ and IT have been compared for the determination of 6 pesticides in fruits [29]. QqQ provides better linear dynamic range, higher precision, less matrix interferences and better robustness, while IT provides an excellent sensitivity for product ion measurements. Four LC–MS systems equipped with Q, QqQ, IT and Q-TOF have been compared [30] in the quantitative analysis (sensitivity, precision and accuracy) of carbosulfan and its main transformation products. QqQ provides at least 20-fold higher sensitivity compared to other mass analyzers and better linear dynamic range. The repeatability (within-day) is slightly better for Q (5–10%) and QqQ (5–9%) compared to IT (12–16%) and Q-TOF (9–16%). Although the QqQ is more sensitive and precise, mean values obtained by all instruments are comparable. QqQ, TOF and Q-TOF were compared for the qualitative and quantitative analyses of 10 anabolic steroids in human urine [31]. QqQ allowed the detection of all analytes at the minimum required performance limit established by the World Anti-Doping Agency (between 2 and 10 ng/mL in urine). TOF and Q-TOF approaches were not sensitive enough to detect some analytes. Most compounds were detected by all techniques, however QqQ was necessary for the detection of some metabolites in a few samples. TOF-based analyzers showed a benefit to detect non-target steroids and their metabolites in some samples. Human liver microsomal incubations with amitriptyline and verapamil were used as test samples, and early-phase "one lab visit only" approaches were used with different instruments [32]. TOF was the only approach detecting all metabolites, shown to be the most suitable instrument for elucidating as comprehensive metabolite profile as possible leading also to lowest overall time consumption together with the LIT-Orbitrap approach. The latter however suffered from lower detection sensitivity and false negatives, and due to slow data acquisition rate required slower chromatography. Approaches with QqQ and Q-LIT provided the highest amount of fragment ion data for the structural elucidation, but they were unable to provide high-MA data, suffered from many false negatives, and especially with QqQ, from very high overall time consumption.

The 2002/657/EC European Commission Decision established the need to obtain at least three identification points in order to confirm organic contaminants in animal products, which was applied for pesticide analyses in environmental matrices using LC–MS with QqQ, TOF and Q-TOF [33]. QqQ instrument allowed the confirmation of detected pesticides even at very low concentrations (ng/L) achieving between four and five identification points when adding confirmatory transitions. The direct confirmation with a TOF instrument was only feasible for those compounds showing sufficient sensitivity, isotopic pattern, or easy in-source fragmentation. Q-TOF provided up to 20 identification points in a single run at relatively high concentrations (sub-mg/L). Moreover, TOF-based mass analyzers allow to finding additional non-target organic contaminants.

2.2. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

The widely accepted standard in the MALDI technology is the coupling with TOF or TOF/TOF mass analyzers, because both devices are working in a pulse regime and such connection is straightforward. TOF mass analyzer can be preceded by Q or IT analyzer, where the limitation of such configurations is the ion transmission and the scanning speed of first Q/IT analyzer. Other alternatives are the coupling of MALDI source with different types of mass analyzers than TOF, either low-resolution (LIT and QqQ) or high-resolution (Orbitrap and ICR). MALDI-QqQ-MS configuration is designed for the sensitive quantitation similarly as for LC-QqQ-MS.

Table 4
Suggested definitions of low, high and ultrahigh resolving power and mass accuracy of mass analyzers.

	Resolving power (RP, FWHM)	Mass accuracy (MA, ppm)
Low	<10,000	>5
High	10,000–100,000	<5
Ultrahigh	>100,000	<1

The instrument characteristics with their advantages and limitations are more or less identical as for the LC–MS coupling. The m/z range of MALDI-TOF analyzers in hundred thousands is valid only for the linear mode, while it is limited to *ca.* 100,000 or less for the reflectron mode. Another issue is the sensitivity, which can dramatically decrease for very large m/z values in the range of hundred kDa. Measurements of large proteins in the MDa range have been reported in the linear mode [8,14,34]. The interesting configuration of TOF analyzer has been reported recently by Jeol, where the traveling path of ions is increased approximately up to 17 meters due to the multiple reflections [15] with the declared RP = 60,000. MALDI sources can be equipped with 2 basic types of lasers in UV/vis region (Table 3): gas-phase laser (nitrogen laser is used in all commercial applications) or solid-state lasers (neodymium-doped yttrium aluminum garnet, Nd:YAG, and neodymium-doped yttrium lithium fluoride, Nd:YLF). Nitrogen lasers are used as a golden standard in MALDI-MS especially due to the lower price and good performance with a wide range of matrices, but they have certain limitations, such as the maximum repetition rate only up to 60 Hz and the average life span about 10^7 shots. The advantage of solid-state lasers is the higher repetition rate (>1000 Hz, see Table 3) and longer life time (10^9 shots). The combination of advantages of nitrogen and solid-state lasers is a Nd:YAG laser with a modulated beam profile [35] with the superior performance in MALDI imaging and LC-MALDI-MS coupling. Infrared lasers in MALDI were proposed as a valuable alternative to UV/vis lasers due to increased life time and absorbance of virtually all (bio)organic compounds in the infrared region [36], but infrared lasers are still not yet available in commercial MALDI setups.

3. Current trends in LC–MS

Basic characteristics of the quality of mass analyzer are RP and MA. At present time, definitions of high-RP and also high-MA are not sufficient to differentiate between high-resolution and ultrahigh-resolution mass analyzers, therefore we suggest updated definitions for low, high and ultrahigh RP and MA (Table 4). We suggest to distinguish three basic categories of RP: low-RP (<10,000), high-RP (10,000–100,000), and ultrahigh-RP (>100,000). In fact, it means that most Q and IT mass analyzers belong to the low-RP category, TOF based analyzers to the high-RP, and the ultrahigh-RP contains two FT mass analyzers—Orbitrap and ICR. The Orbitrap has started to approach closer to parameters typical for ICR mass analyzers after the launch of new type of Orbitrap with 240,000 RP [10], but recently Nikolaev et al. published a new ICR cell design [37], where they demonstrated 24 millions RP at m/z 609 recorded over 3 min for only 7 T magnetic field. This new development in the ICR cell technology will again widen the gap between the ICR and Orbitrap. It should be kept in mind that such values of RP cannot be achieved in the LC–MS time scale.

The conventional definition of high-MA is 5 ppm and better. Technical specifications of most recently launched high-RP mass analyzers report MA 3 ppm and better even for the external calibration, for FT mass spectrometers with the internal calibration or the lock-mass approach below 1 ppm (ultrahigh-MA). The best

specification for 15 T ICR instrument is reported better than 0.25 ppm for the internal calibration (Table 1). FT-ICR mass analyzers are rather expensive, so their application is typical in the most demanding analytical tasks, such as proteomics [38], petroleomics [39,40], and metabolomics [41,42]. The reasonable definition of potential ranges for individual expected elements and the inclusion of isotopic ratios in the searching algorithm may further improve the reliability of elemental composition determination [43]. In fact, the level of MA required for the reliable elemental composition determination strongly depends on the actual m/z value, because the number of possible combinations of given elements exponentially grows with the m/z value [19]. For small m/z values (*ca.* below m/z 200), the MA <5 ppm is satisfactory, but such MA bring too many possible combination in the m/z range of 500–1000. For biomolecules with MW > 1000 Da, MA better than 1 ppm may not be sufficient for the unequivocal determination of elemental formula.

The miniaturization is an important issue considered in all fields of analytical instrumentation including both parts of LC–MS coupling. The trend in LC is a reduction of the column diameter from standard bore (3–4.6 mm ID) or narrow bore (1–3 mm ID) to capillary columns (<1 mm ID) or even separations on chips [44,45]. Two commercial solutions for chip-based separations are offered by Agilent Technologies [6] and Waters [11]. The reading of specialized reviews is recommended for more details [44–46]. In terms of LC–MS, capillary columns and chips work with flow rates in the range of nL/min, which is ideally suited for the coupling with nanoelectrospray ionization [47].

The two-dimensional (2D) LC either in off-line [48,49] or on-line mode [50–53] improves the separation of highly complex mixtures, such as in proteomics [54], lipidomics [49,55] or the analysis of natural compounds [50]. The multidimensional option is considered not only on the LC side of the LC–MS system, but parallel (multidimensional) use of different types of mass spectrometers have been described in LC–MS coupling with the goal to obtain complementary information from various MS configurations [56,57]. The most comprehensive LC–MS system reported so far has been developed by Byrdwell for the parallel use of three different mass spectrometers (Q-LIT, QqQ and IT) plus three additional non-MS detectors (UV detector, evaporative light-scattering detector and corona charged aerosol detector) [58]. The goal of this "dilute-and-shoot" approach is to obtain the maximum amount of analytical information in a single run, as illustrated on examples of vitamin D_3 and triacylglycerols. Each mass spectrometer is used for obtaining complementary information from different scan types, Q-LIT is used for recording SIM, SRM and enhanced MS scans in APCI mode, QqQ operates in the full-scan APCI mode, and IT provides information on ESI-MS^4.

Nowadays, important tasks in LC–MS and the analytical chemistry in general are the sample throughput, automation and non-supervised system operation, because the clinical studies often have an enormous number of samples to be analyzed. The robotic NanoMate TriVersa system can be used for this purpose in various operation modes including the automatic liquid extraction from the tissue surface followed by ESI-MS analysis [59], the automated fraction collection followed by ESI-MS analysis in supervised or non-supervised mode [60], *etc*.

For many decades, the role of chemical derivatization was fully recognized in gas chromatography–mass spectrometry (GC–MS), where the derivatization enabled the analysis of analytes with insufficient volatility [61,62]. Now the potential of derivatization is also realized in LC–MS [63] due to increased sensitivity [64], improved bioanalytical quantitation [65], improved retention behavior of problematic analytes [66] and possible integration of derivatization of polar analytes and their extraction followed by LC–MS determination [67]. At present, planar separation techniques can be coupled to MS as well, as illustrated in recent works on thin-layer chromatography (TLC) coupled to MALDI-MS [68] or other atmospheric pressure surface sampling/ionization techniques as well [69]. TLC is simple and cheap technique still used for the routine analysis, for example in lipidomics [70].

3.1. Fast LC–MS

The group of fast LC–MS techniques comprises various approaches with the common goal to achieve the highest sample throughput and the good separation efficiency. The most widespread and well established approach is UHPLC [71], which is based on the use of small particle size (sub-two μm particles) at ultrahigh-pressures (up to 1300 bars) yielding fast analyses and narrow chromatographic peaks. On the other hand, it requires higher acquisition speed of mass spectrometer to obtain enough sampling points for the reliable peak integration. Typical peak widths in routine UHPLC–MS bioanalyses are 3–10 s [71–74], while peak widths in the fast/ultrafast LC–MS are generally in the range 1–3 s, but they can be narrower than 1 s under well optimized conditions [75,76]. For good reproducibility and precision in LC–MS quantitation, at least 12–15 points per chromatographic peak are recommended, but the current practice often rely on the lower number of data points per peak (8–10) in the qualitative and semi-quantitative analysis, but it may compromise the peak shape. The minimum acquisition speed to acquire 10 sampling points per peak is 3–10 Hz for the average peak width 1–3 s and 10–20 Hz for the average peak width 0.5–1 s, but of course higher scanning speeds are useful to generate more sampling points per peak for a better quantitation. Such acquisition speeds are achieved by modern TOF based mass analyzers and also some ion traps (see Table 1 for the acquisition speed of individual analyzers).

Other approaches used in the fast LC–MS are the use of core-shell particles [77,78], high-temperature LC (HTLC) [79,80] and monolithic columns [77,81,82]. Several specialized reviews and book chapters on these novel approaches have been published recently [71,72]. In our opinion, the use of core-shell particles with ID <3 μm is highly promising area [83], because comparable results as for UHPLC–MS can be obtained on conventional LC–MS systems without the need of additional investments. Fig. 3 shows the direct comparison of individual approaches in terms of maximum peak capacity *vs.* throughput. Detailed comparison and discussion of related aspects is available in the original paper [83].

The fastest mass analyzer is obviously the TOF analyzer with common acquisition speeds 10–50 Hz, which fits well with fast/ultrafast LC requirements. The technical specification of fastest Q-TOF instrument on the market declares 100 Hz for tandem mass spectrometry (MS/MS) measurements (Table 1), which allows numerous parallel SRM scans even in fast/ultrafast LC–MS. The fastest TOF instrument for LC–MS reports 200 Hz acquisition speed (Table 1). The additional parameter important for the quantitation is the linear dynamic range, which is at least 5 orders of magnitude or better for modern Q, LIT, TOF instruments and their combinations (Table 2).

3.2. Ionization techniques in LC–MS

The *status quo* in ionization techniques is that nearly all LC–MS systems are equipped with ESI, sometimes accompanied by atmospheric pressure chemical ionization (APCI) for less polar compounds and the normal-phase LC operation [84–87]. Fig. 4 shows the relative percentage of individual atmospheric pressure ionization techniques used in published LC–MS papers according to the Web of Science search, where the dominant role of ESI (82% of papers) is evident. Main application areas of ESI are in the characterization of biomolecules, ionic and very labile

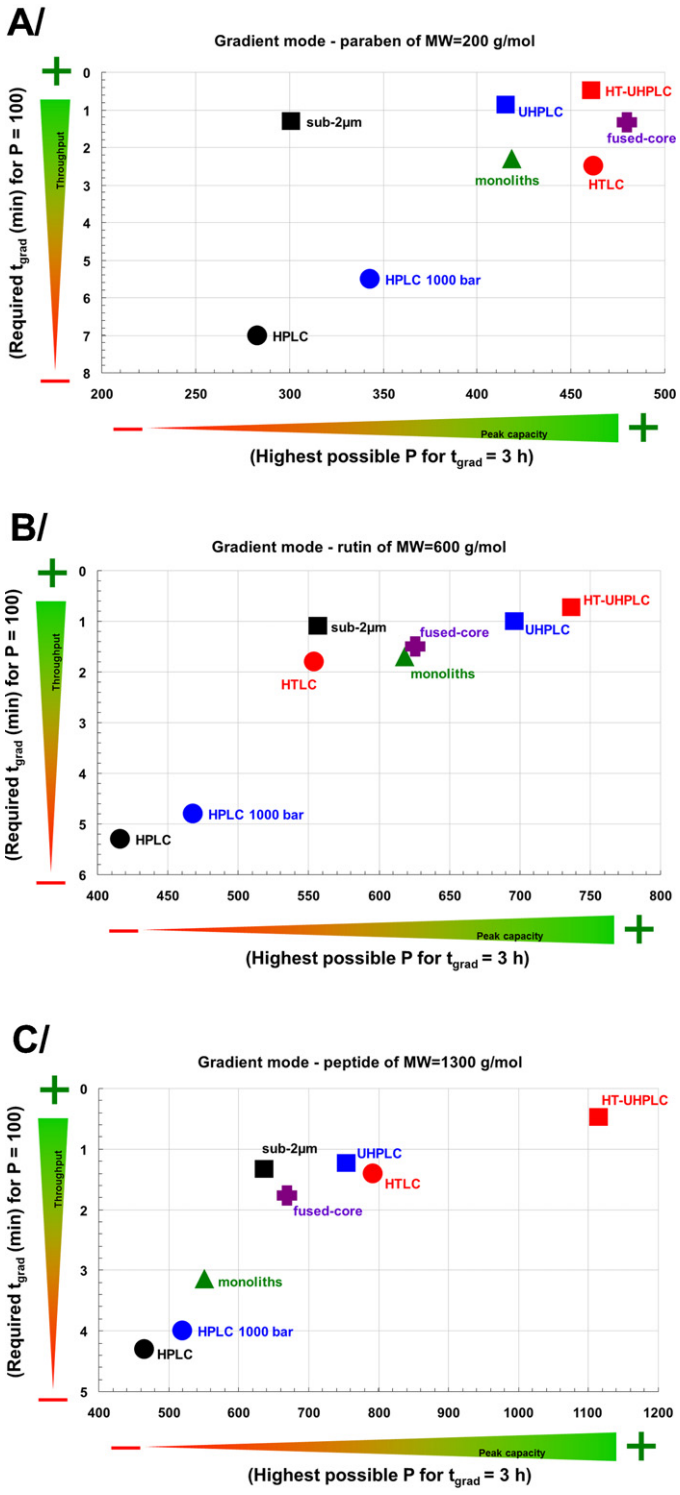

Fig. 3. Performance comparison of LC strategies in terms of throughput (t_{grad} for $P = 100$) and maximum peak capacity (P_{max} for $t_{grad} = 3$ h) in the gradient elution for model compounds: (A) butylparaben (MW = 200 Da), (B) rutin (MW = 600 Da), and (C) peptide triptorelin (MW = 1300 Da).
Adapted with a permission from [83].

organic and organometallic compounds [86,88]. Fundamentals, instrumentation and biological applications of ESI and MALDI have been described in a monograph edited by Cole [89] and recently the current knowledge on the mechanism of ion formation in ESI has been reviewed [89]. The application potential of APCI is mainly in the area of medium polar to non-polar organic compounds

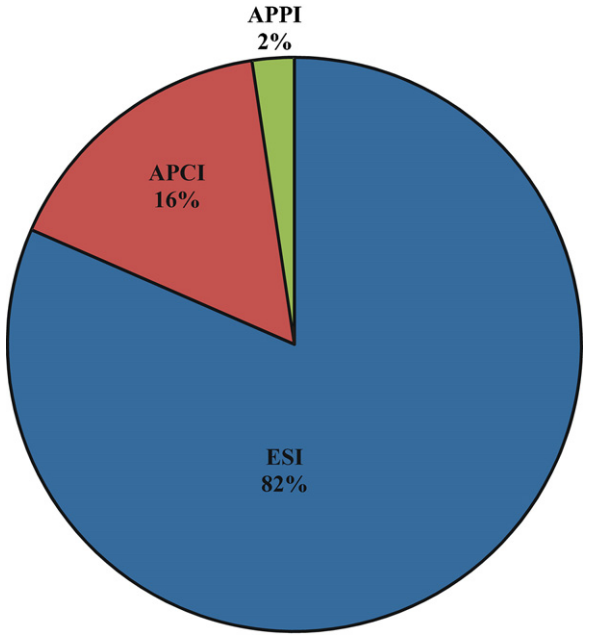

Fig. 4. Relative use of individual atmospheric pressure ionization techniques in LC–MS papers based on the Web of Science search from March 1, 2012.

and synthetic polymers (16% of papers, Fig. 4). The atmospheric pressure photoionization (APPI) is less widespread (2% of papers, Fig. 4) compared to two above mentioned ionization techniques, which can be probably explained by a comparable application range as for APCI. The application potential of ESI, APCI and ESI was compared for 5 polar pharmaceuticals [90], where ESI showed the best performance in terms of sensitivity and selectivity.

Ionization mechanisms in APPI [91,92] are rather complex consisting of two basic ionization modes: without dopant and with the assistance of dopant. The ionization process in APPI is initiated by photons emitted by a discharge lamp (typically krypton, 10 eV and minor 10.6 eV). These photons ionize compounds with ionization energies lower than their energy (10 eV), which includes analyte molecules, but not typical gases and solvents used in LC–MS separation and nebulization processes. Analyte molecules are ionized rather selectively without background interferences. The ionization of analytes is dependent on their ionization energies rather than their proton affinities unlike to ESI and APCI. Toluene and acetone are the most common dopants in APPI often providing significantly better sensitivity for low polar analytes compared to ESI/APCI techniques. Dopants are first ionized by photoionization and then they ionize target analytes by ion-molecules reactions, *e.g.*, by the proton transfer in the positive-ion mode. The presence of radical molecular ions $M^{+\cdot}$ in positive-ion APPI mode is not rare unlike to ESI/APCI and they can be formed by direct photoionization (conditions without dopant) or charge-exchange mechanisms, which depend mainly on the solvent polarity, flow rate and the presence of additives [91,92]. Recently, the experimental and quantum mechanical studies were used to revisit the mechanism of $[M+H]^+$ formation in APPI [93]. Results show that both electron transfer and hydrogen transfer can occur as a concerted reaction through the ion-molecular complex precursor state.

The present LC–MS practice moves toward fast/ultrafast LC analyses for high-throughput, which puts demands on the speed of mass spectra recording. For numerous applications, mass spectra recorded in both polarity modes provide valuable complementary information both for qualitative and quantitative analyses, because certain compound classes can be ionized only in one polarity mode, *e.g.*, (poly)sulfates and (poly)sulfonates do not provide

a signal in the positive-ion mode unless the presence of other functional groups with easy ionization in the positive-ion mode [86]. For this reason, the mass spectrometer with a fast polarity switching is desirable (<50 ms for low-resolution and <1 s for high-resolution mass analyzers), because the parallel measurement of both polarity modes can be performed within one run. The fast polarity switching in case of high-resolution mass analyzers is more demanding technical task, because the electronics usually need certain time for the stabilization of high voltages in the range of kV, so only few high-resolution systems are capable of relatively fast polarity switching, but the equilibration time of few minutes is typically recommended.

Combined ion sources can be considered as an option merging the advantages and application ranges of atmospheric pressure ionization techniques, but on the other hand their sensitivity may be a compromise between both modes. The advantage of combined ESI/APCI [94] or ESI/APPI ion sources [95] is possible detection of both polar and non-polar analytes in one run, which can increase the number of identified components for highly complex matrices, such as traditional Chinese medicine [94] or simultaneous detection of cyclodextrins, pharmaceuticals and their binding interactions [95]. The combination of APCI, ESI and APPI can be useful for combinational chemistry and high-throughput biological screening. ESI can normally analyze around 80% of samples, which can be complemented by combined source operating with the polarity switching within a single run [96]. The combined ion source with a computer-controlled switch between MALDI and ESI modes in the ICR configuration has been developed with a possible exchange between ESI and MALDI in less than 1 min [97].

3.3. LC–MALDI-MS coupling

LC–MALDI-MS coupling has some specific advantages over LC–ESI-MS, mainly the possibility of decoupling of separation and mass analysis steps, which allows re-analysis of peaks of interest later on, lower suppression effects (possibility to use more harsh LC conditions compared to LC–ESI-MS) and high m/z range of TOF mass analyzer. On the other hand, the critical step in LC–MALDI-MS coupling is the transfer of effluent from LC exit to the MALDI plate and matrix introduction, which may be responsible for certain band broadening. Off-line and on-line approaches in LC–MALDI-MS coupling have been described for capillary LC [98–100] and separations on microfluidic chips [101–103]. Widely used off-line methods are based on the deposition of LC effluent on the MALDI plate using a continuous trace or discrete spots. The continuous sample deposition is better for preserving the chromatographic resolution. The MALDI targets with pre-coated matrix are easier for the sample preparation than mixing the LC effluent with matrix. Liquid samples can be also applied on special nanostructured surfaces used in matrix-free approaches [103]. The deposition of continuous streak (called in-line coupling) or discrete fractions can be accomplished using laboratory-built or commercial robotic spotters. The spotting on the MALDI target plate is achieved in several ways, *i.e.*, most frequently contact deposition (using T-junction), spray deposition (electrospray, nebulizer), electric pulse deposition, impulse driven deposition, heated droplet interface or piezoelectric microdispensor (Fig. 5) [99]. Off-line approach is often used in proteomics (*e.g.*, analysis of protein digests [104,105], identification of post-translational modifications [106], *etc.*), but applications in small molecule [99,107] or synthetic polymer analysis [108,109] can be also found, typically with microparticular [98,110] or monolithic columns [104]. MALDI is less prone to the ion suppression effects than ESI [111]. It has higher throughput for a large number of deposited samples and higher tolerance toward salts and buffers. In general, MALDI is known as less convenient for the quantitative analysis unlike to LC–ESI-MS, but

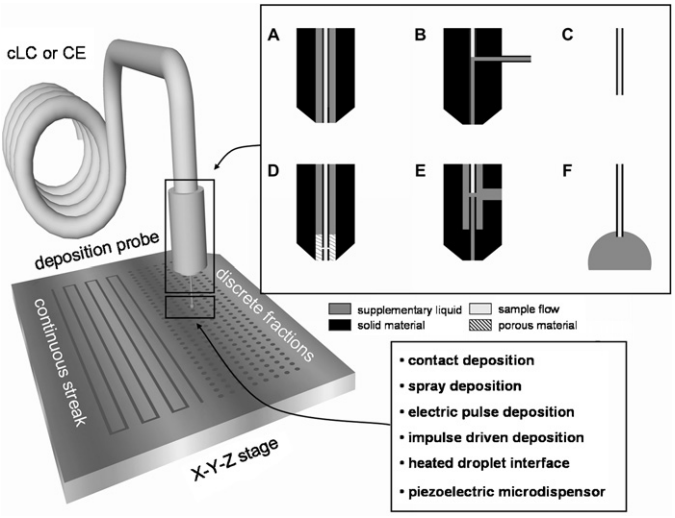

Fig. 5. Scheme of off-line LC–MALDI-MS coupling with the supplementary liquid addition and deposition mechanisms. Junctions used for coupling of microcolumn techniques include the following: (A) coaxial sheath flow, (B) T-junction, (C) sheathless interface, (D) porous junction, (E) liquid junction, and (F) droplet electrocoupling. A, B and C are typical for capillary LC–MS, while D, E and F are common in capillary zone electrophoresis–MS.
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some authors report comparable results for stable-isotope-labeled proteomic quantitation [112]. Due to the complex character of studied proteomic samples, one-dimensional chromatography is often not sufficient and multidimensional separation approaches are needed for the adequate fractionation of studied samples [98,110,113,114], *e.g.*, the combination of orthogonal separation principles of ion-exchange and reversed phase LC [110,113].

For on-line LC–MALDI-MS coupling [115,116], the effluent from LC is delivered directly to the mass spectrometer. Contrary to off-line coupling devices, on-line devices are not yet commercially available. Methods for the liquid sample introduction can be performed by continuous-flow MALDI using frits, aerosol MALDI, moving wheel or moving (rotating) ball methods, but these applications are more common for the MALDI coupling with capillary electrophoresis [99,117].

3.4. Ion mobility spectrometry

Ion mobility spectrometry (IMS) was developed over the past few decades as a method for the separation and subsequent detection of volatile and semi-volatile organic compounds. IMS enables the differentiation of ions by size, shape, charge as well as mass, which can provide important supplementary information to the chromatographic separation of molecules and mass spectrometric separation of ions. Detailed description of ion mobility principles have been discussed previously [118–122]. In principle, the separation of gas-phase ions at atmospheric pressure is based on their different mobilities in the low or high electric fields. Four methods of ion mobility separation can be combined with MS, *i.e.*, drift-time ion mobility spectrometry (DTIMS), aspiration ion mobility spectrometry (AIMS), differential-mobility spectrometry (DMS) also called field-asymmetric waveform ion mobility spectrometry (FAIMS) and traveling-wave ion mobility spectrometry (TWIMS). Only TWIMS and DMS/FAIMS are commercially available in the LC–MS coupling so far [119]. The construction of TWIMS originates from the traditional IMS analogous to the TOF separation, where formed ions are moved to the drift region *via* a shutter grid. These ions are then separated based on different ion mobilities in a weak electric field with the opposite direction of the inert gas

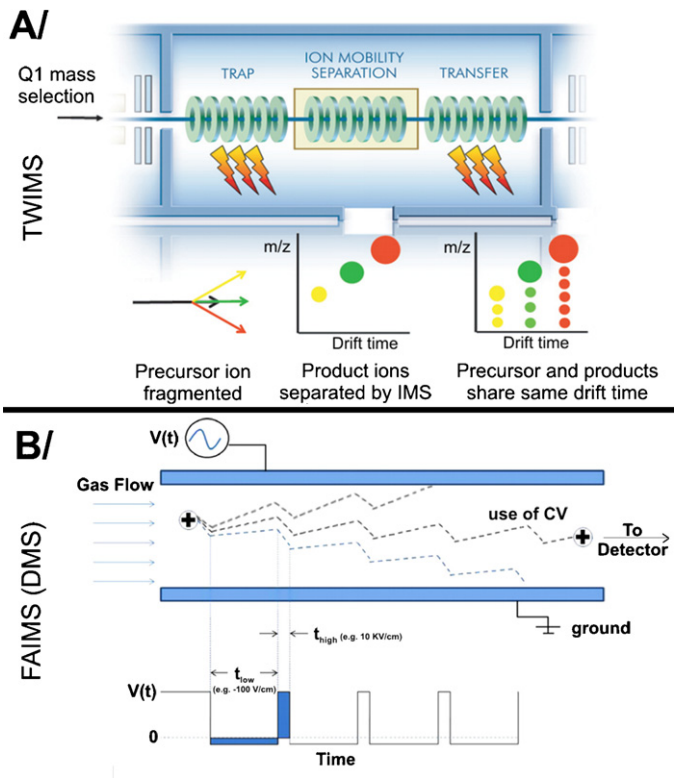

Fig. 6. Principles of ion mobility separation of ions in: (A) traveling-wave ion mobility spectrometry (TWIMS) including the scheme of time-aligned parallel (TAP) fragmentation and (B) field-asymmetric waveform ion mobility spectrometry (FAIMS).

Adapted with a permission from [122,124].

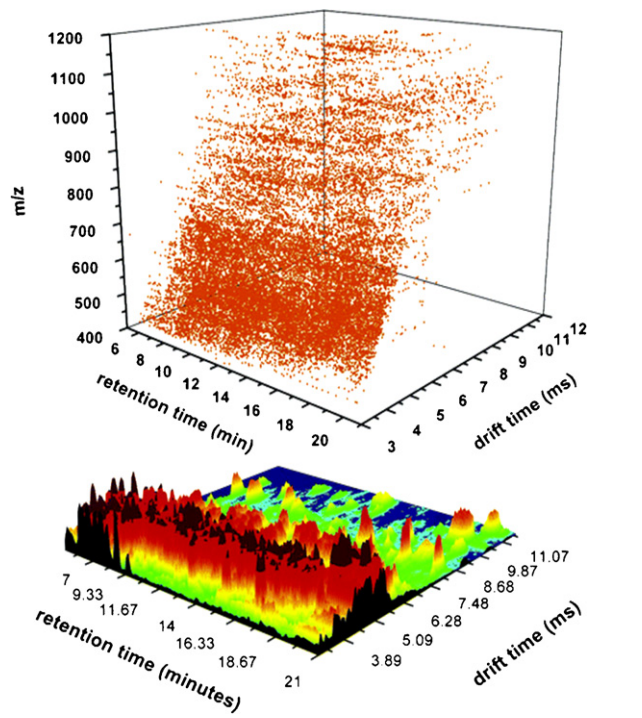

Fig. 7. 3D plot of retention times in the LC separation, m/z values in the MS separation and drift times in the ion mobility separation of proteins obtained by LC–IMS-MS experiment.

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flow. Unlike the traditional IMS, where the low electric field is applied continuously on the cell, a sequence of symmetric potential waves (high-field) is continuously applied through the series of segmented electrodes of cell in the same direction with the ion migration [118,123,124]. Ions are introduced from Q at reduced pressure, and their motion in the electric field of IMS cell depends on particular ion mobilities (Fig. 6a). On the contrary, the ion mobility device for DMS/FAIMS is placed in the ion source region and ions are separated under ambient conditions. DMS/FAIMS works as a scan filter and sorts ions by the difference between ion mobilities at high and low electric field with the opposite polarity, induced by a periodic asymmetric field (application of so called separation or dispersion voltage) orthogonal to the ion path. The different mobility of ions during the application of high and low voltages causes the ion drift toward one of two electrodes [118,120,122]. The trajectory of particular ions along the radial axis can be corrected using the application of compensation voltage to avoid ion discharge (Fig. 6b). This approach is similar to the Q filtering. DMS and FAIMS instruments are based on the same principle of ion separation, but they differ in the instrumental design. Electrodes are not segmented and the alternating electric field is placed between two electrodes (plate electrodes for DMS *vs.* cylindrical electrodes for FAIMS).

Instruments equipped with the ion mobility spectrometry (IMS) were commercially introduced by Waters in 2006 (TWIMS) and now they are also provided by Thermo Scientific (FAIMS) and AB SCIEX (DMS). IMS can be applied for the separation of isobaric compounds (on condition that their cross-sections differ at least by about 3%), the reduction of high background noise, the separation of endogenous matrix interferences from target analytes to increase the selectivity and enables the charge state screening used mainly in proteomics (the separation of unwanted singly charged background compounds and cluster ions from doubly charged ions with the aim to simplify the spectra). The reduction of interferences followed by increased selectivity can be further enhanced by the use of suitable volatile chemical modifier (called dopant) [119,120]. This advantage results in the reduced chemical noise, increased dynamic range and enhanced peak separation, and it is closely associated with chemical properties of used dopant and its concentration. MALDI and ESI are commonly used in IMS-MS. Due to the easy coupling with LC and possible ionization of less volatile compounds, the ESI is the best choice for LC–IMS-MS applications [118,119,122]. In principle, all types of mass analyzers can be used after the ion mobility separation. The commercial solution of TWIMS is followed by TOF-MS, while DMS/FAIMS forms a part of the ion source and can be combined with any type of mass analyzer.

The ion mobility separation is different compared to chromatographic and mass spectrometric separations, and the combination of these separation modes provides a better resolution for complex samples. Three-dimensional data set consists retention times, drift times and m/z values, as illustrated (Fig. 7) in the 3D separation of peptide digest of human plasma proteome [125]. The use of MS/MS provides an additional level of structural information. In addition to traditional collision-induced dissociation (CID), new fragmentation approaches have been introduced by Waters, such as energy dependent fragmentation (MS^E) and time-aligned parallel (TAP) fragmentation. MS^E can be applied in all tandem mass spectrometers, while application of TAP is restricted to IMS instruments. The TWIMS ion mobility separation plays an important role in TAP approach (Fig. 6a). The precursor ion is fragmented in the trap with subsequent separation of the first generation of product ions by IMS. The second generation of fragment ions is formed in the transfer, and they are associated to the first generation parent based on individual drift-times [118,124]. The combination of IMS and MS with high-RP and advanced fragmentation experiments (MS^n, electron transfer/capture dissociation, MS^E or TAP) is a powerful tool for the structural determination.

The ion mobility MS was mainly associated with the analysis of volatile compounds for homeland security and environmental applications, such as explosives, chemical warfare agents, chemical pollutants or drugs detection. The LC sample introduction followed by ESI has extended the range of applications to the field of biological, biomedical and pharmaceutical research [118]. A wide range of applications for LC–IMS-MS can be found in the literature, *e.g.*, profiling of plasma proteome [126], study of isomeric transformation of phenolic compounds present in foodstuff [127], profiling of indole alkaloids in yohimbe bark [124], high-throughput proteomic studies [128], characterization of rat urinary metabonome [129], improvement of separation of drug-related materials [130], drug metabolism study [131], *etc.* The 2D-LC–IMS-MS coupling has a great potential in the biomarker discovery due to the possible orthogonal character of these separation techniques in liquid and gas phase, as illustrated on the example of 8 pharmaceutical compounds with the identical nominal mass m/z 316 [132]. UHPLC can separate these molecules in a liquid-phase, ultrahigh-RP MS can separate their protonated molecules in a gas-phase according to their accurate m/z values, while IMS separates them according to their size-to-charge ratio.

3.5. Mass spectrometric quantitation

The QqQ mass analyzer with SRM scans is a golden standard in any mass spectrometric quantitation either in the LC–MS configuration or MS stand-along systems [133]. All MS quantitation approaches obviously require the use of internal standards to eliminate any possible variations during the ionization process and the mass analysis, such as the ion suppression/enhancement, the contamination of the ion source or the mobile phase, *etc.*, extraction losses or any other unpredictable reasons. The best internal standard in MS and LC–MS is the addition of isotopically labeled analogs, where all physico-chemical properties including the retention behavior, fragmentation behavior and extraction efficiency are almost identical except for characteristic mass shifts caused by the number of labeled isotopes. For higher number of deuterium atoms (at least about 5), small shifts in retention times can occur, but it does not constitute any problem in the LC–MS quantitation. The ion suppression/enhancement effects play an important role in LC–MS quantitation and the extend of these effects needs to be quantitatively assessed, as suggested in few recent works [134–136].

The novel approach for LC–MS quantitation uses [137] ultrahigh-RP in the full-scan single stage mode using reconstructed ion currents for very narrow extraction windows (*e.g.*, 5 ppm around the theoretical m/z value). This approach provided comparable detection specificity, assay precision, accuracy, linearity and sensitivity for 17 therapeutic drugs as for the conventional SRM acquisition on QqQ without the need of the optimization of MS/MS parameters for SRM transitions. The full-scan mass spectra information is still retained unlike SRM measurements, which can be beneficial for the detection of co-eluting species, unexpected adducts of analytes, *etc.*

The recent trend in MS based quantitation is an integrated quantitative and qualitative bioanalysis [133], which essentially requires the use of high-resolution tandem mass analyzers coupled to LC (preferably in fast LC mode). Hybrid FT tandem mass analyzers are convenient for this purpose due to the ability to collect full-scan high-RP mass spectra at scan speeds required for UHPLC together with routine measurements of MA <5 ppm [138]. Another possibility is the use of the following acquisition schemes on Q-TOF mass analyzer [139]: (1) information-dependent acquisition with TOF survey scan and product-ion scan as dependent scan, (2) MS^{ALL} by collecting TOF mass spectra with and without fragmentation by alternating low and high collision energy, and (3) sequential window acquisition of all theoretical fragment-ion spectra (SWATH) mode in which sequential precursor ions windows (typically 20 m/z) are used to collect the same spectrum precursor and fragment ions using a collision energy range. High-resolution SRM (HR-SRM) on a rapid acquisition (<50 ms) Q-TOF instrument with the resolving power above 20,000 leads to new possibilities in the integrated quantitative and qualitative bioanalysis.

MALDI couple to TOF analyzer is not a typical method of choice for the quantitative analyses due to worse scan to scan reproducibility, but the coupling of MALDI with QqQ mass analyzers combines important advantages of both approaches. MALDI is the ultrafast technique without the need of chromatographic separation, while QqQ with SRM is the best technique for the sensitive quantitation [140]. The usefulness of MALDI-QqQ configuration in the quantitative analysis has been demonstrated on the ultrafast quantitation (6 s for one sample) of selected drugs [141] and proteomic analysis [142]. Volmer et al. reported [143] that MALDI SRM quantitation on QqQ mass analyzer may be a serious alternative to established LC–ESI-MS methods in terms of linearity, limit of quantitation, precision and accuracy. However, MALDI assay was at least 50 times faster than LC–ESI-MS.

Another important issue is the sample preparation preceding LC–MS analysis [72,144]. Obviously, the internal standard must be added before any sample pre-concentration step. The LC–MS quantitation approach has a clear advantage in terms of reduced ion suppression effects, trace analysis and retention times bring an additional dimension in the selectivity. On the other hand, advantages of shot-gun approach (typically used for example in lipidomics) are mainly the analysis speed and simplicity. The fastest QqQ mass analyzers enable the determination of numerous species within few minutes without any chromatographic separation, but the information on isobaric species with the identical fragmentation pattern is lost. In the quantitation of complex protein mixtures, the isotope-coded affinity tags (ICAT) approach [145] and related tag techniques are often used [146,147]. Another alternative approach for the relative quantitation is the use of response factors determined from the calibration curves of pure standards and then applied for real samples [148,149]. The internal standard addition and response factors approach can be combined in one platform together with well-optimized chromatographic separation, as illustrated on the lipidomic class quantitation [150]. The stable isotope labeling by amino acids in cell culture (SILAC) [151] is a simple approach for the incorporation of the isotopic label into proteins for MS-based quantitative proteomics. Two cell populations are grown in the culture media that are identical except for light (non-labeled) and heavy (labeled with deuterium, ^{13}C or ^{15}N) form of a particular amino acid, which is incorporated into the protein.

4. Mass spectrometric approaches complementary to LC–MS

In the last decade, several new approaches designed for the direct mass spectrometric analysis at ambient conditions without the chromatographic separation have been introduced. The main advantage of such approaches is the fast analysis without any (or minimum) sample preparation, which significantly increases the sample throughput. On the other hand, some drawbacks must be also mentioned in terms of reduced amount of analytical information due to the absence of separation and sample preparation steps. The ion suppression and matrix effects can cause severe problems with the quantitation and the trace analysis.

The term ambient ionization technique has been first introduced by Takáts et al. [152] and now there is an explosion of new ambient ionization techniques and associated acronyms

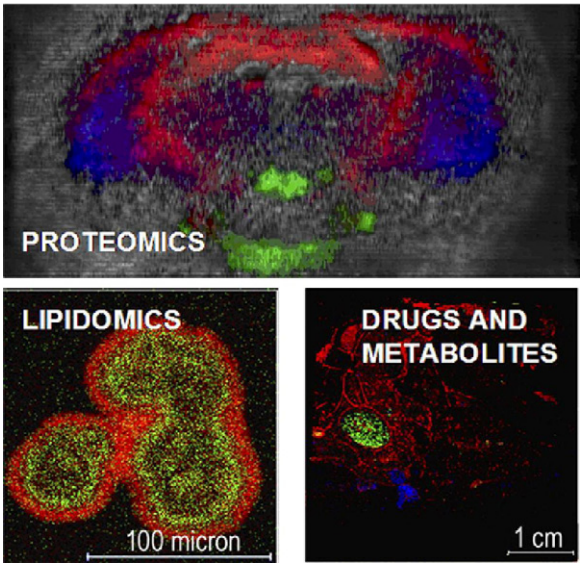

Fig. 8. Typical applications of MALDI mass spectrometry imaging in proteomics, lipidomics and drug metabolites.
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[153–155]. The main group is a family of ambient desorption ionization techniques, such as desorption ESI (DESI) [152,156], desorption APCI (DAPCI) [157], and desorption APPI (DAPPI) [158]. The direct analysis in real time (DART) is the name of ionization technique introduced by JEOL [159] for the soft ionization of analyte molecules on surfaces, in liquid-phase or gases without any sample preparation. The Penning ionization mechanism describes based on interactions between excited helium atoms and target analyte at ambient conditions. Another ionization technique applicable for different types of gaseous, liquid and solid samples is termed the atmospheric solids probe analysis (ASAP) [160]. The ASAP and DESI can be combined in one ionization source [161], which extends the range of analyte compounds in terms of their polarities and molecular weights. Recently, the paper spray ionization has been described, where the analyte (*e.g.*, the whole blood) is spotted on the paper (so called dry blood spot analysis [162]), then the selected solvent is automatically added, and the solution is electrosprayed from the paper into the mass spectrometer [163]. This technique is intended for high-throughput clinical analyses. The new ionization technique solvent based direct inlet MS [164] is suitable for both small and large molecules in solids or liquid solvents including in LC–MS configuration. The principle of this ionization technique is based on the heated inlet used for reversed-phase system with some similarity to former thermospray ionization, but author report better sensitivity and applicability to peptides.

Promising MS techniques designed for the determination of spatial distribution of analyte molecules on the surface is mass spectrometry imaging (MSI) [165–170], which is typically used for the spatial imaging of biomolecules in biological tissues. At present, the spatial resolution of MALDI-MSI is routinely in the range of tens micrometers [167,171]. Best reported values are below 5 μm [172], while secondary ion mass spectrometry (SIMS) can provide the spatial resolution even below 1 μm [167,173]. The additional level of information can be obtained by 3D spatial imaging of biological tissues using SIMS [174]. Most typical application areas of MSI (Fig. 8) are lipidomics [171,175–177], the distribution of drugs and other small molecules in tissues [178,179], while the number of MSI papers in proteomics is slightly lower due to the sensitivity limitations and essential removal of abundant lipids [170,179]. The MSI with high spatial resolution has been suggested as an alternative technique to the histological staining of

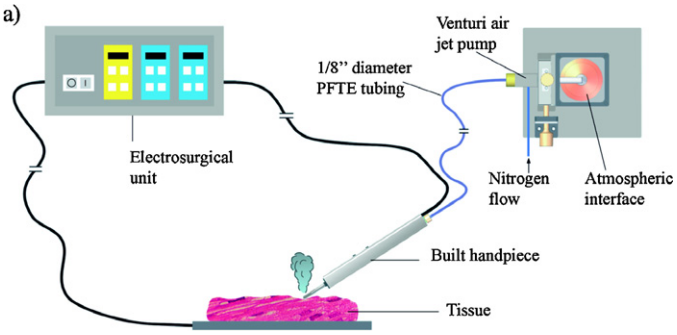

Fig. 9. Surgical mass spectrometry: scheme of ion transfer from the tissue to the atmospheric interface to mass spectrometer.
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tissues [180]. Recently, another interesting application of MS has been published and referred as rapid evaporation ionization mass spectrometry (REIMS) (Fig. 9) [181]. During the electrosurgical dissection, the tissue is locally exposed to high-frequency electric current resulting in the ionization of molecules contained in this tissue, preferably lipids. The lipidomic composition of dissected tissue can be obtained within less than second and used for the verification of tissue type (cancer *vs.* healthy tissue) [180].

5. Current state and future trends

Some trends in the area of LC–MS and related techniques are already recognized now: (A) the shift from low-resolution to (ultra)high-resolution tandem mass analyzers providing high-MA below 1 ppm, (B) the shift from conventional HPLC–MS to UHPLC–MS or other fast LC–MS techniques (core–shell particles, high-temperature LC and monolithic columns) requiring fast MS analyzers (typically TOF based systems), (C) the use of 2D-LC–MS for complex samples, and (D) other dimension also in MS, such as IMS-MS, parallel use of more mass spectrometers, ionization techniques and polarity modes. Present LC–MS systems generate huge amounts of analytical data, which is often impossible to interpret manually, so dedicated softwares can help with the automation of data processing and interpretation [182]. The significant impact on the whole mass spectrometric community had the invention [183] and commercialization of the sixth type of mass analyzer—Orbitrap. The notable improvement has been recently reported in the field of ICR cell construction, where Nikolaev et al. [37] demonstrated the RP exceeding 24 millions, which shifts the limits of MS. Groundbreaking news (such as the discovery of new type of mass analyzer) are not probable in the near future, but such discoveries cannot be anticipated. On the other hand, improvements in the area of ionization techniques, ion optics, fast electronics, dedicated scans and consequently the sensitivity and selectivity will certainly continue.

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