Journal of Chromatography A, 1218 (2011) 2700–2711

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Thin layer chromatography/mass spectrometry

Sy-Chyi Cheng[a], Min-Zong Huang[b], Jentaie Shiea[b,c,d,*]

[a] Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan
[b] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan
[c] National Sun Yat-Sen University–Kaohsiung Medical University Joint Research Center, Kaohsiung 80424, Taiwan
[d] Cancer Research Center, Kaohsiung Medical University, Kaohsiung 80424, Taiwan

ARTICLE INFO

Article history:
Available online 2 February 2011

Keywords:
TLC–MS
Ambient ionization
Vacuum-based ionization
Desorption/ionization
Direct sampling
Indirect sampling

ABSTRACT

Thin layer chromatography (TLC)—a simple, cost-effective, and easy-to-operate planar chromatographic technique—has been used in general chemistry laboratories for several decades to routinely separate chemical and biochemical compounds. Traditionally, chemical and optical methods are employed to visualize the analyte spots on the TLC plate. Because direct identification and structural characterization of the analytes on the TLC plate through these methods are not possible, there has been long-held interest in the development of interfaces that allow TLC to be combined with mass spectrometry (MS)—one of the most efficient analytical tools for structural elucidation. So far, many different TLC–MS techniques have been reported in the literature; some are commercially available. According to differences in their operational processes, the existing TLC–MS systems can be classified into two categories: (i) indirect mass spectrometric analyses, performed by scraping, extracting, purifying, and concentrating the analyte from the TLC plate and then directing it into the mass spectrometer's ion source for further analysis; (ii) direct mass spectrometric analyses, where the analyte on the TLC plate is characterized directly through mass spectrometry without the need for scraping, extraction, or concentration processes. Conventionally, direct TLC–MS analysis is performed under vacuum, but the development of ambient mass spectrometry has allowed analytes on TLC plates to be characterized under atmospheric pressure. Thus, TLC–MS techniques can also be classified into two other categories according to the working environment of the ion source: vacuum-based TLC–MS or ambient TLC–MS. This review article describes the state of the art of TLC–MS techniques used for indirect and direct characterization of analytes on the surfaces of TLC plates.

Contents

* Corresponding author at: Department of Chemistry, National Sun Yat-Sen University, No. 70, Lienhai Road, Kaohsiung 80424, Taiwan.
Tel.: +886 7 5253933; fax: +886 7 5253933.
E-mail address: jetea@fac.nsysu.edu.tw (J. Shiea).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.01.077

1. Introduction

With its advantages of simplicity, economy, easy operation, and the need for only small amounts of solvent, thin layer chromatography (TLC) is used widely in various fields to separate or purify mixtures of chemical and biological compounds. Because a new plate is used in each separation, the "memory effect" problems that occur with many other chromatographic techniques are not occurred in TLC separation, which makes TLC often used for the direct analysis of crude samples with minimal purification procedures. Because it is performed under ambient conditions, TLC is one of the most suitable separation methods for high throughput analysis. For example, many samples can be spotted on a TLC plate and separated simultaneously, or many plates can be analyzed in a tank containing the same mobile phase.

The stationary phases for TLC are typically organic or inorganic thin layer, such as silica, alkyl-silica (C8 or C18), cellulose, and monolithic polymer coated on metal, plastic, or glass sheets; the mobile phases for TLC analysis are typically organic solutions spanning a wide range of hydrophobicities [1–7]. During the separation, the edge of the TLC plate is immersed in the mobile phase, which is developed through capillary force. The diversity of interactive forces among the analyte molecules, mobile phase, and stationary phase cause different analytes to move at different rates on the TLC plate. The separation of the chemical compounds on a TLC plate is quantified in terms of the value of R_f (distance of analyte migration/distance of mobile phase migration).

Unlike high performance liquid chromatography (HPLC), TLC is a simple and rapid chromatographic technique, but its separation efficiency is usually low. Many different TLC techniques have been developed to increase the separation, resolution, and reproducibility. High performance thin layer chromatography (HPTLC) uses gel particles having small diameters (4–6 μm) as the stationary phase, thereby increasing the number of interactions with the analyte molecules [1,4,5]. Ultrathin layer chromatography (UTLC) uses a thinner stationary phase (10 μm) to reduce lateral diffusion of the analytes [5]. Overpressured layer chromatography (OPLC) uses a liquid chromatography (LC) pump to drive the mobile phase through the stationary phase [4,5]. Microemulsion thin layer chromatography (ME-TLC) uses a water-in-oil microemulsion as the mobile phase [5].

Following TLC separation, the separated compounds on the plate are visualized and, in some instances, characterized. The sample spots are usually detected by spraying or dipping the TLC plate so that the analytes come into contact with chemical or biological reagents, which then react or interact with the functional groups of the analyte molecules. Although optical and spectroscopic methods can be used to sensitively detect the sample spots in terms of their visible color, UV absorption, and fluorescence excitation spectra, these methods are not comprehensive and such detections are restricted by the chemical or optical properties of the analytes. Even if the values of R_f and the spectroscopic characteristics of the sample and standard are compatible, the capability to determine molecular structure of the analyte by these detection techniques remain low. For this reason, it remains a challenge to couple TLC with more-efficient detection techniques for better characterization.

Mass spectrometry (MS) is one of the most practical techniques for characterizing chemical and biochemical compounds. By measuring the mass-to-charge (m/z) ratios of ions, it allows elucidating the chemical structures of molecules in terms of the species formed through fragmentation in the ion source or mass analyzer. Many column-based separation techniques, including liquid and gas chromatography (LC and GC), have been combined with mass spectrometry to efficiently characterize analytes ranging from small organic to large biological compounds. The compounds eluted from the GC or LC column are typically introduced directly into the ion source of the mass spectrometer.

Interfacing TLC with mass spectrometry is not as easy as interfacing GC–MS or LC–MS systems because the analyte molecules remain adsorbed on the TLC gel bed after separation, rather than being eluted from the column by a gas or liquid flow. Therefore, using mass spectrometry to successfully characterize analytes on TLC plates requires that the analyte molecules first be separated or sampled from the surface of the adsorbent. The analyte molecules are subsequently entering the ion source of a mass spectrometer in gas or liquid phase. Several TLC–MS techniques have been developed that differ based on the methods used to separate or sample the analytes from the adsorbent and also based on the mass spectrometric ion sources used [7–11]. In terms of their sampling methods, the existing TLC–MS techniques can be classified simply as (i) "indirect sampling TLC–MS" and (ii) "direct sampling TLC–MS" techniques. Fig. 1 provides a schematic representation of both approaches, together with the various mass spectrometric ionization techniques that have been used to characterize chemical compounds on the surfaces of TLC plates.

The name, "indirect sampling TLC–MS," indicates that certain labor-intensive sample pretreatment processes must be performed prior to mass spectrometric analysis [12–14]. The pretreatment processes may include identifying the spot position, scraping the gel from the spot, extracting the analyte from the gel, purifying the extraction solution, and concentrating the analyte solution. Because the analyte-adsorbed gel particles are scraped from the plate surface for further analysis, visualizing the sample spots

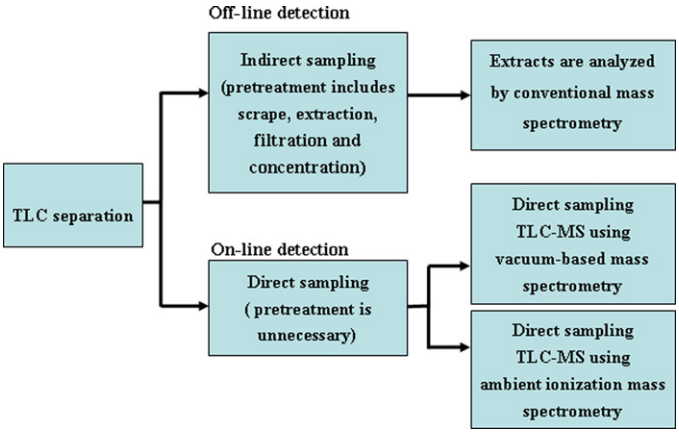

Fig. 1. Flow chart of various mass spectrometric techniques that have been used to characterize chemical compounds on the surfaces of TLC plates.

Table 1
The detection limit, repeatability, and abbreviation of the ionization methods used in TLC–MS.

Technique	Acronym	Environment	Detection limit	Reproducibility	Spatial resolution	Reference
Gas chromatography/mass spectrometry	GC/MS	Vacuum				
Liquid chromatography/mass spectrometry	LC/MS	Ambient				
Electron impact	EI/CI	Vacuum				
Fast atom bombardment	FAB	Vacuum				
Laser desorption ionization	LDI	Vacuum	50 pg		5 μm	[31]
Secondary ion mass spectrometry	SIMS	Vacuum	50–500 ng		50 μm × 50 μm	[35,67]
Matrix-assisted laser desorption ionization	MALDI	Vacuum	<1 ng		120 μm × 80 μm	[40,45]
Surface-assisted laser desorption ionization	SALDI	Vacuum	500 pg			[50,52]
Electrospray ionization	ESI	Ambient				
Liquid extraction junction sampling	N/A	Ambient	<20 pg	RSD ≦ 3.75%	2–4 mm[a]	[71,73]
Electrospray laser desorption ionization	ELDI	Ambient	10^{-6} M		100 μm×150 μm[b]	[63]
Laser-induced acoustic desorption electrospray ionization	LIAD/ESI	Ambient			0.35 mm	[64]
Desorption electrospray ionization	DESI	Ambient	~5 ng		400 μm × 400 μm[b]	[92,98]
Rotation planar chromatography atmospheric pressure chemical ionization	RPC–APCI					
Laser desorption/atmospheric pressure chemical ionization	LD/APCI	Ambient			0.05 mm[a]	[105]
Direct analysis in real time	DART	Ambient	<128 pg	RSD <± 5.4%	<3 mm	[62,110]
Atmospheric pressure matrix-assisted laser desorption ionization	AP-MALDI	Ambient	1–7 pmol	RSD ± 22%	0.5 mm	[54]
Laser desorption/inductively coupled plasma	LD/ICP	Ambient	3 pg		120 μm[a]	[60]

[a] Diameter of desorption area.
[b] Desorption area.

on the TLC plate, using nondestructive chemical and biological reagents or optical illumination, is necessary to mark the positions of invisible chemical species. Different types of mass spectrometers have been used to ionize and identify the extracted compounds, including ion trap, quadrupole, and time-of-flight (TOF) apparatuses equipped with vacuum-based or ambient ion sources, including electron impact ionization (EI), chemical ionization (CI), matrix-assisted laser desorption/ionization (MALDI), and electrospray ionization (ESI) systems (Table 1) [12–17].

The direct sampling approach allows the direct analysis of chemical compounds on TLC plates without the need for tedious pretreatment processes. Because TLC separation is performed on a planar plate, it is rational to develop mass spectrometric techniques capable of direct surface sampling and ionization. Based on the working pressures of the sampling devices and ion sources, "direct sampling TLC–MS" technologies can be further divided into vacuum-based and ambient TLC–MS systems. The mass spectrometry and interfaces for direct sampling TLC–MS have been reported in several recent reviews [9–11]. In this article, we review the available TLC–MS techniques that are performed using indirect and direct sampling, covering recent developments in both vacuum-based and ambient TLC–MS.

1.1. Indirect sampling TLC–MS

TLC allows the separation and optical measurement of the compounds on a chromatographic plate. Invisible analytes on a TLC plate are detected through either optical illumination (through UV, fluorescence or fluorescence quenching) or staining with a nondestructive chemical or biological reagent. Spots of interest are then scratched and collected for solvent extraction to recover the adsorbed components. After filtration and concentration, the mass spectrometric systems equipped with EI or CI sources are commonly used to characterize the extracted analytes introduced into the ion source through a direct insertion probe (DIP) [15]. One example is characterizing diazepam extracted from silica gel particles. The sample is deposited in a small tube at the tip of the DIP and is evaporated through rapid heating in the ion source. The analyte molecules are subsequently ionized through EI in the source [15].

Because different analytes might not be completely separated after TLC, GC–MS and LC–MS are often used to further separate and characterize the chemical compounds in the extracts [18–23]. Derivatization is usually required for polar compounds to increase (i) the resolution of GC separation, (ii) the volatility and stability of the analyte, or (iii) the sensitivity of the detection [19–21]. Because the extracts are evaporated through heating during GC–MS analysis (e.g., in the injector, column, transfer line and ion source), the analytes are limited to those that are volatile and thermally stable. The combination of TLC and GC–MS has been used to characterize small organic compounds such as phytosterol oxidation products, acylglycerols, peroxidized cholesterol or cholesteryl ester hydroperoxide [18–21].

Desorption/ionization (DI) mass spectrometry can be employed to characterize nonvolatile and thermally labile compounds present in the extracts. These DI techniques, including fast atom bombardment (FAB), liquid secondary ion mass spectrometry (LSIMS), laser desorption (LDI), and matrix-assisted laser desorption/ionization (MALDI), use accelerated ions (8–10 kV), atoms, or a pulsed laser beam to impact or irradiate the surface of the extracted sample (in the presence or absence of a matrix) and thereby desorb and ionize its chemical components. The sample is usually introduced into the DI source, operated under vacuum, through an insertion probe or stainless-steel sample plate [12,16,24–27]. Glycerol is a common matrix used for FAB and LSIMS analysis. The sample is simply prepared by mixing the extract with glycerol at the probe tip. No matrix is required for LDI analysis. For MALDI analysis, the extracts, such as lipids, polymers, and lipopolysaccharides, are mixed with matrix solution (e.g., sinapinic acid, 2,5-dihydroxybenzoic acid, or α-cyanosinapinic acid) on a stainless-steel plate; after drying, the surface of the sample is irra-

diated with a pulsed laser (commonly in the UV range) to generate analyte ions [12,16,24–27].

Solution-based ionization techniques, such as electrospray ionization mass spectrometry (ESI/MS), atmospheric pressure chemical ionization mass spectrometry (APCI-MS), and atmospheric pressure photoionization mass spectrometry (APPI-MS) can be used to characterize the chemical compounds in the extracted solutions under an atmospheric pressure environment [12–14,28–30]. The sample solution (prepared by mixing extracts with solvent) is introduced into the ESI, APCI, or APPI source through infusion or nebulization. All these ionization techniques are suitable for the analysis of polar or less polar compounds (e.g., phospholipids, glycolipids and alkaloids) except APPI can also be used to ionize aromatic compounds.

The advantage of using indirect TLC–MS for routine sample analysis is that the solutions are clean, concentrated, and free of gel particles. A concentrated sample makes it easier to detect an analyte present in trace amounts. The maintenance of a mass spectrometer is simpler if it always encounters clean samples; in addition, it will be better protected (especially for turbo vacuum systems) if the sample is free of gel particles. Even though the chemical compounds on the TLC plate are readily characterized through indirect sampling TLC–MS methods (e.g., systematic extraction), the sample preparation procedures are tedious, labor-intensive, and time-consuming. In addition, the results from indirect sampling TLC–MS analysis do not accurately provide chromatographic or quantitative information regarding the analytes on the TLC plates. Furthermore, the unknown sample spots that cannot be observed using visualizing or optical methods are not marked for analysis except the extraction of TLC plate area by area. The development of TLC–MS techniques capable of directly characterizing chemical compounds on TLC plate surfaces would overcome these shortcomings.

1.2. Direct sampling TLC–MS

Because TLC separations are performed on planar plates, mass spectrometric techniques capable of direct surface sampling and ionization have been developed. The direct sampling approach allows the direct analysis of chemical compounds on TLC plates. Based on the working pressures of the sampling devices and the ion sources, the “direct sampling TLC–MS” technology can be further divided into vacuum-based and ambient TLC–MS.

2. Direct sampling TLC–MS using vacuum-based ionization techniques

With their capability of surface analysis, desorption/ionization techniques that are operated under vacuum, such as FAB, LSIMS, LDI, and MALDI, can be used to sample and ionize nonvolatile or thermally labile compounds directly from TLC plate surfaces [17,31–33]. The advantage of integrating these surface sampling/ionizing mass spectrometric methods with TLC is that the tedious sample pretreatment processes used in indirect TLC–MS are avoided. Direct sampling TLC–MS using vacuum-based ionization techniques evolved in late 1980s, immediately after the development of the DI techniques. Due to the limitations necessitated by the use of the vacuum, however, these DI techniques are not suitable when analyzing highly volatile compounds. In addition, unless the dimensions of the ion source are enlarged to include the transport system required to move the TLC plate, high throughput analysis is difficult. Therefore, most of these systems are not commercially available. Vacuum-based direct sampling/ionizing TLC–MS can be further divided into two categories, where DI is performed using (1) fast atom or ion bombardment or (2) pulse laser irradiation.

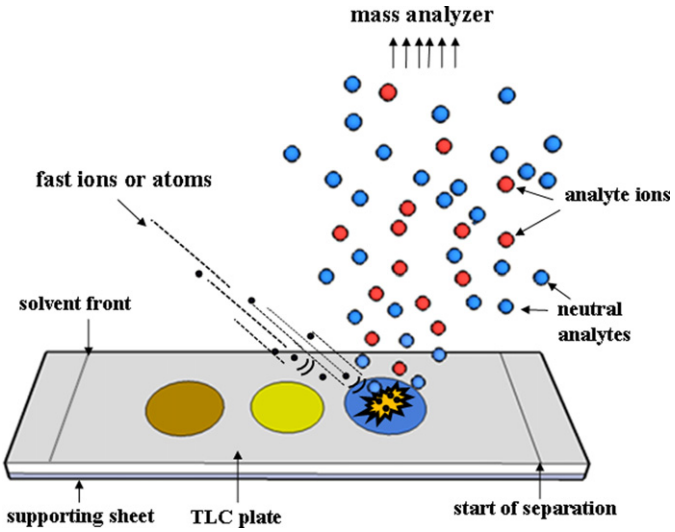

Fig. 2. Schematic representation of FAB and LSIMS analyses of chemical compounds on the surface of a TLC plate.

2.1. Direct sampling TLC-MS using fast atom or ion bombardment for desorption/ionization

FAB and LSIMS use fast atoms and ions to impact the sample surface for desorption and ionization. The plate is covered with a viscous matrix solution (e.g., glycerol) prior to MS analysis. The functions of the viscous matrix include (1) absorbing, dissipating, and transferring the energy provided by fast atom or ion bombarding to the analyte, (2) self-cleaning the bombarded sample surface, (3) providing protons for ionization, and (4) extending the ion signal duration. Fig. 2 displays a schematic representation of FAB and LSIMS system used to directly characterize chemical compounds on a TLC plate. A liquid matrix solution (e.g., a methanol solution containing glycerol, *m*-nitrobenzyl alcohol, triethanolamine, or thioglycerol) is manually deposited on the TLC plate surface to promote the concentration of the analyte on the TLC plate. When the TLC plate is introduced into the ion source operated under vacuum, the methanol is immediately pumped away, leaving the analyte/matrix on the plate surface. A stream of fast atoms (Ar) or primary ions (Cs^+) accelerated at high potential (8–10 kV) is then used to impact the plate surface to desorb and ionize the analytes [32–35]; the analyte ions are subsequently detected by a sector, quadrupole, or TOF mass analyzer. Lateral diffusion of compounds along the plate might be inevitable after the introduction of the matrix solution, thereby decreasing the chromatographic resolution of the compounds on the plate. Although the mass spectra obtained using FAB and LSIMS are generally nearly identical, the fast ion beam in LSIMS can be focused much better than the fast atom beam in FAB; therefore, the sampling area in LSIMS is typically smaller, meaning that higher-resolution imaging is possible. The chemical compounds of low molecular weight (less than 1000 Da) such as morphine, and coccidiostats separated on TLC plates have been characterized by FAB in the vacuum [32,34].

An analytical method that couples TLC with TOF-SIMS without using a liquid matrix has been reported: the sample spots that had been separated on a TLC plate were developed in the second dimension to enter the interfacial region formed by depositing silver vapor or aqueous mercury (II) chloride solution on one side of the TLC plate; this interfacial region was then cut off and affixed to the sample target for TOF-SIMS analysis [36,37]. Although the presence of the interfacial region did not decrease the chromatographic integrity or ability to identify the analytes, scanning of the TLC plate’s surface was not demonstrated.

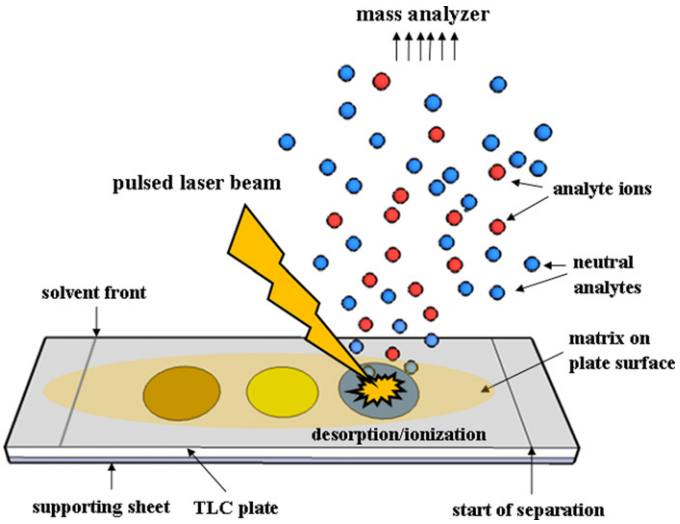

Fig. 3. Schematic representation of LDI, MALDI, and SALDI analyses of chemical compounds on the surface of a TLC plate.

There are several limitations when using FAB or LSIMS for TLC–MS: (1) the analytes must be polar so that they can be dissolved in and mixed well with the viscous matrix; (2) volatile compounds are difficult to detect; (3) the affinity of cation (proton, sodium, or potassium) of the compound must be greater than that of the matrix (operated in positive ion mode); (4) compounds with molecular weights greater than 2000 Da cannot be desorbed or detected. In addition, the gel particles generated upon fast atom or ion bombardment of the TLC plate surface are potentially harmful to the vacuum systems, especially for those using turbo molecular pumps.

2.2. Direct sampling TLC–MS using pulsed laser irradiation for desorption/ionization

LDI, MALDI, and surface-assisted laser desorption ionization (SALDI) are laser-based desorption/ionization mass spectrometric techniques that have been used to characterize chemical compounds after separation on TLC plates [9,17,31,38]. Fig. 3 displays a schematic representation of LDI, MALDI, and SALDI analyses of compounds on the surfaces of TLC plates. In these techniques, the developed plate is attached to the sample probe using double-sided adhesive tape and then a pulsed laser beam is used to desorb and ionize the compounds present on the plate. LDI employs a pulsed laser beam to irradiate the spots of interest; in the absence of any matrix, the laser density required to produce analyte ions from the TLC plate is greater than that from a metal surface [31]. In MALDI analysis, an analyte/matrix co-crystal is formed by applying an organic matrix solution to the spot surface; the matrix adsorbs energy from the pulsed laser beam to assist the desorption/ionization of the analytes such as ganglioside, phosphopeptide, glycosphingolipids and bacterial toxins [9,39–49]. In SALDI analysis, a suspension of carbon powder in a liquid is applied to the plate surface; this inorganic matrix adsorbs the UV laser energy to assist desorption/ionization of the separated compounds [38,50–52]. When using micrometer-sized graphite or activated carbon as the matrix, the analyte signals in the low mass range do not suffer from interference by matrix signals. Moreover, other nano- and micro-particle suspensions (e.g., Co, TiN, TiO_2, silicon) have been employed successfully as inorganic matrices for TLC–SALDI-MS analysis [53]. The innate character of laser irradiation provides MALDI and SALDI with high spatial resolution for sample scanning. Although the ionization of analytes is improved by the matrix, the addition of organic matrix solution upon the plate surface may decrease the chromatographic resolution and concentration of the analyte compounds as a result of lateral diffusion along the plate. Various organic compounds including dyes, peptides, alkaloids, gangliosides, and lipids were characterized by TLC–MALDI-MS in positive or negative ion mode and peptides, herbicide, diuretic, polymers, and porphyrins separated on TLC plate were detected by SALDI-MS [38–52].

Despite their advantages, several problems remain when using vacuum-based sampling/ionizing TLC–MS techniques: (1) to maintain the vacuum in the ion source, the sample introduction and retreatment processes are time- and labor-consuming; (2) the small dimensions of the vacuum chamber require that the TLC plate must inevitably be cut into small pieces to fit in the ion source; (3) poor sensitivity is encountered when detecting volatile or semivolatile compounds; (4) relatively poor reproducibility is encountered in quantitative analyses; and (5) interference of matrix ions occurs in low-mass range.

3. Direct sampling TLC–MS using ambient ionization mass spectrometry

The development of direct sampling TLC–MS techniques was greatly benefited by the development of atmospheric pressure ionization mass spectrometry. For example, although an organic matrix solution must be evenly spread upon the TLC plate surface, atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) has been successfully coupled with TLC for the direct characterization of chemical compounds on TLC plates [54]. Interfacing TLC with a solution-based atmospheric pressure ionization mass spectrometric technique like ESI/MS or APCI/MS has resulted in the development of several surface sampling devices to extract or elute the chemical compounds from the adsorbents and to deliver the extracted solutions to the ESI or APCI sources for further analysis [55–57].

Ambient ionization mass spectrometry is a technique that is capable of directly characterizing analytes on solid surfaces, in solutions, or in the gas phase with minimal or no sample pretreatment under ambient conditions [58]. Several ambient ionization techniques, including laser desorption/atmospheric pressure chemical ionization (LD/APCI), laser ablation inductively coupled plasma (LA-ICP), desorption electrospray ionization (DESI), direct analysis in real time (DART), electrospray laser desorption ionization (ELDI), and laser-induced acoustic desorption/electrospray ionization (LIAD/ESI), have been developed for ionizing analytes on solid surfaces [59–64].

Because the separation of TLC is performed under ambient conditions and the analyte molecules adsorbed on the TLC plate can be regarded as “solid” samples, interfacing TLC with ambient ionization mass spectrometry is much easier than it is with vacuum-based DI mass spectrometry. Recently, several ambient TLC–MS techniques, using ESI, APCI, and ICP for ionization, have been introduced to directly characterize organic and inorganic compounds on TLC plates. Coupling TLC with ambient ionization techniques allows the atmospheric-pressure characterization of TLC plates without the need to place them within the vacuum chamber for ionization. Volatile, semivolatile, and nonvolatile analytes can all be detected; in addition, the dimensions of the vacuum system do not limit the plate’s size. In the following sections, we describe these ambient TLC–MS methods, which can be further classified into ESI- and APCI-based TLC–MS techniques.

3.1. Ambient TLC–MS using ESI-based ion sources

Directly interfacing TLC with ESI-MS is not as easy as it is with LC/MS or GC/MS because, after separation, the analyte molecules

remain on the plate, rather than eluting out of the stationary phase. This obstacle was overcome through the development of the following techniques: (a) liquid extracting (or sampling) the analyte on the plate followed by electrospray ionization from the extracted solution; (b) continuously eluting the analyte from TLC plate followed by electrospray ionization of the eluted solution; (c) sampling the analyte on the plate first and then post-ionizing the analyte molecules in the electrospray plume; (d) sampling/ionizing the analyte directly on the plate surface using the charged solvent droplets generated by electrospray ionization. Fig. 4 shows detailed schematic representations of these analytical approaches.

3.1.1. ESI-based ambient TLC–MS using liquid extraction junction for sampling

To online-extract the analyte from the plate surface, two types of the surface sampling devices have been developed to deliver the extracted solution to the ESI source for further analysis [55,56]. Fig. 5a displays a schematic representation of a coaxial tube sampling probe for extracting, delivering, and ionizing analytes on a plate surface. The probe is fixed vertically to the plate surface with its end contacting the plate surface to establish a liquid micro-junction between the probe and the TLC plate. The extracted solution is pumped by a syringe pump and delivered to the plate surface through the space between the inner and outer capillary tubes; meanwhile, the extracted aqueous sample is transferred to the electrospray emitter through the inner capillary tube [55,65–68]. By interfacing with the surface sampling probe, the TLC plate can be connected to a commercial electrospray ionization source for direct sampling and ionizing the developed dye mixtures and caffeine in diet drinks. Furthermore, this surface sampling probe has also been used to connect a TLC plate with an APCI source to analyze compounds of lower polarity [57].

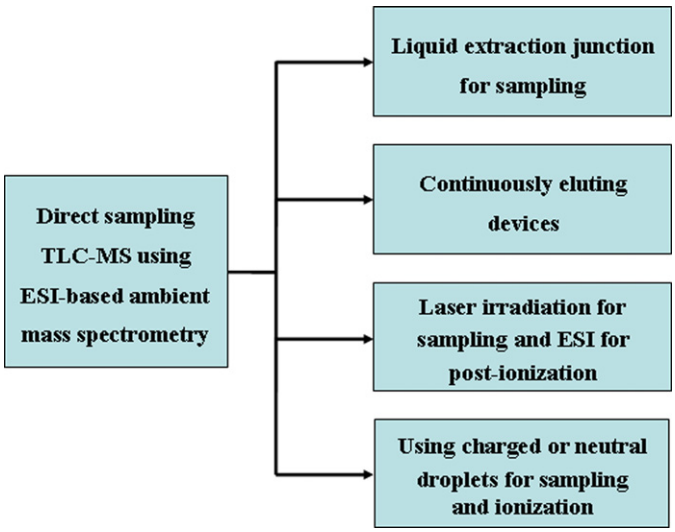

Fig. 4. Flow chart of direct sampling TLC–MS methods using ESI-based ambient mass spectrometry to characterize chemical compounds on the surfaces of TLC plates.

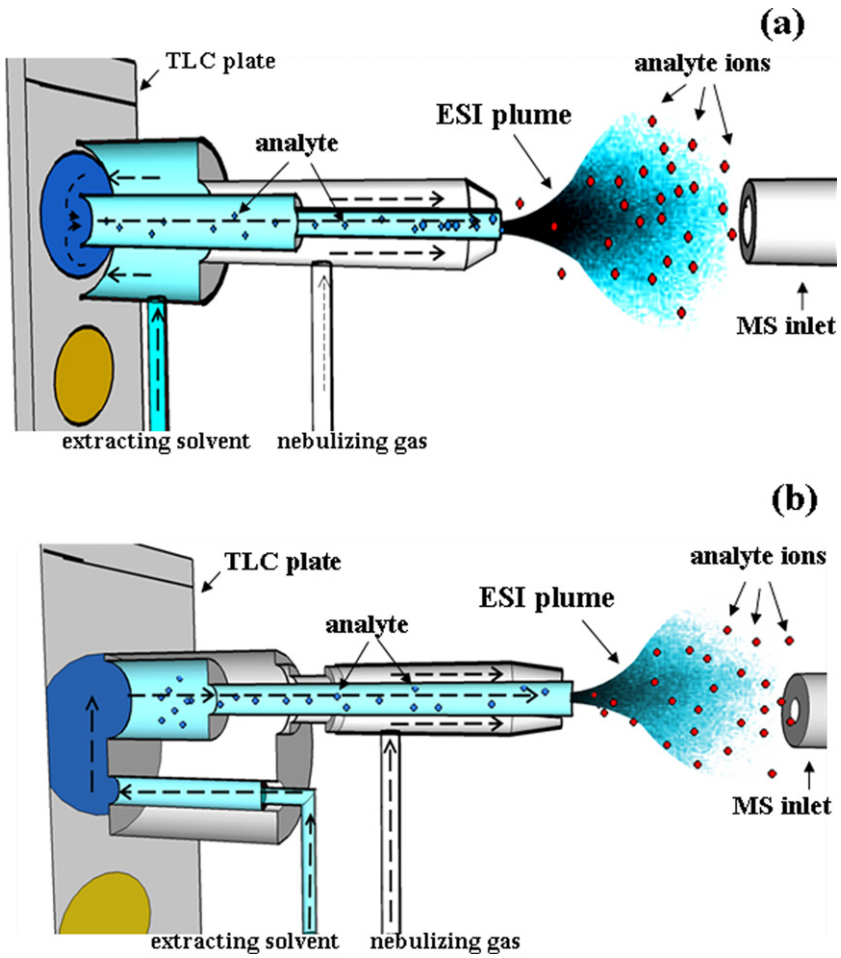

Fig. 5. Schematic representations of ESI-based ambient TLC–MS using (a) a coaxial tube sampling probe and (b) a two-parallel-capillaries device for liquid extraction and ionization.

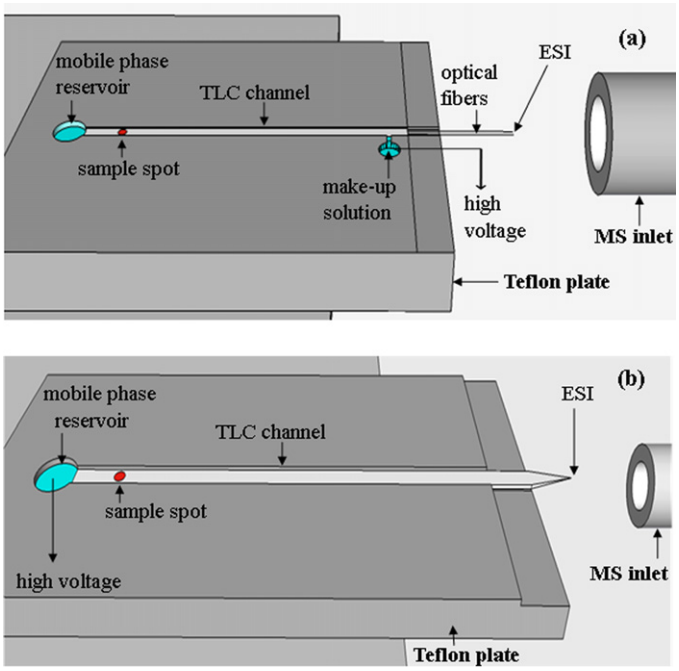

Fig. 6. Schematic representations of continuously eluting devices that directly link TLC with ESI/MS. (a) Two bound optical fibers are inserted into the C_{18} gel bed at the other end of the channel. (b) A small aluminum TLC strip with a sharpened end.

Instead of using a coaxial tube, two parallel capillaries can also be employed for surface sampling in an alternative design [56,69–73]. Fig. 5b displays a schematic representation of this sampling device. A ring-shaped cutting edge positioned on the tip of the plunger is used to define the extract area. Two parallel capillaries are used to deliver the extract solution into and away from the plate surface. The extract solution is pumped using a commercial LC system and delivered to the plate surface through the inlet capillary. The chemical compounds such as vitamins and caffeine separated on the plate surface are extracted by an organic solution; the analyte-containing solution is filtered through a frit and transferred to the electrospray ionization source through the outlet capillary. This surface sampling device was also connected with inductively coupled plasma (ICP) to extract and characterize inorganic species on the plate surface [74]. Because the sampling area is defined by the ring-shaped cutting edge, the resolution of this plunger is limited by the diameter of the ring.

3.1.2. ESI-based ambient TLC–MS using continuously eluting devices

In addition to liquid extraction devices, interfaces that directly link TLC with electrospray ionization source have been developed. In these systems, analytes are continuously eluted out of the plate and delivered to the electrospray ionization source for further analysis [75,76]. To successfully generate electrospray ionization from the TLC plate, some modifications on the configuration of the TLC plate are necessary. There are at least two ways to modify the edge of the TLC plate for ESI/MS analysis (Fig. 6) [75]. In the first, a small channel cut on a Teflon plate is packed with C_{18} gel particles. One end of the channel is connected to a mobile-phase reservoir, two bound optical fibers are inserted into the C_{18} gel bed at the other end of the channel, and a makeup solution reservoir is made and connected to the optical fibers. The high voltage required for electrospray ionization at the tip of the optical fibers is applied to the makeup solution reservoir by inserting a Pt wire into it. The high voltage is conducted by the solution to the tips of the optical fibers. As the analytes continuously elute out of the TLC channel, they are carried by the mobile phase, flowing through the grooves created by the two bound optical fibers and to the tip where electrospray ionization occurs (Fig. 6a).

The second method uses a small aluminum TLC strip with a sharpened end; the gel bed at the sharpened end is scrapped off beforehand; the other end of the TLC strip is connected to a mobile phase reservoir. The high voltage required to generate electrospray ionization at the sharpened end is conducted through the solution through a Pt wire inserted into the mobile phase reservoir. The analytes separated on the TLC strips are continuously eluted to the sharpened end and electrospray ionization is induced by applying a high voltage at the tip of the sharpened end. These analyte eluting devices overcome the dynamic difficulties of sample transportation between the stationary phase of the TLC plate and the liquid phase introduced into the ESI system (Fig. 6b).

3.1.3. ESI-based ambient TLC–MS using laser irradiation for sampling and ESI for post-ionization

Electrospray-assisted laser desorption ionization (ELDI) is an ambient ionization technique developed to characterize gas, liquid, and solid samples [77–79]. An ELDI source consists of a sample plate, a pulsed laser beam, and an electrosprayer without a nebulizing gas stream. Some accessories, such as a charge-coupled device (CCD) and XYZ stage can be added to monitor the ion source and move the sample plate for molecular imaging. The analyte on a solid sample surface is sampled by irradiating the surface with a pulsed laser beam. The analytes subsequently enter an ESI plume, located immediately above the laser spot, for ionization. The ionization mechanisms of ELDI are similar to that in a fused-droplet electrospray ionization (FD-ESI) source [80,81]. The analyte molecules or the neutral mists containing analyte molecules are conducted into a solvent electrospray plume, where the analytes are ionized through either (1) fusing analyte with the charged solvent droplets following by electrospray ionization from the analyte containing droplets, or (2) ion–molecule reactions with charged solvent ions (including protons, hydronium ions, protonated solvent ions, and cluster ions) in the plume [82–86].

Because a large amount of energy can be brought to a solid sample surface by the pulsed laser irradiation, ELDI-MS is suitable for characterizing chemical compounds distributed on solid surfaces, such as TLC plates [63]. Fig. 7a displays a schematic representation of an ELDI-MS system used for the analysis of analytes on the surface of a TLC plate. The analytes adsorbed on the gel surface of the TLC plate are desorbed by pulsed UV- or IR-laser irradiation; the desorbed analyte molecules subsequently enter a methanol/water electrospray plume, where they are post-ionized. The matrix solution commonly used in MALDI and FAB to assist desorption and ionization is not necessary in TLC/ELDI/MS, thereby avoiding any decrease in resolution due to lateral diffusion of the analytes on the TLC plate when applying the matrix solution or any interference originating from matrix ions in the lower-mass regions of the mass spectra. TLC/ELDI/MS has been successfully used to characterize dyes, amines, and extracts of drug tablets [63]. In addition, a TLC/ELDI/MS system featuring plate dealing, conveying, and collecting systems has been developed for high throughput TLC analysis [87]. Furthermore, with the use of an XYZ stage for precise movement of the TLC plate and a highly focused pulse laser for sampling, high spatial resolution imaging of analytes on a TLC plate has been demonstrated (Fig. 7b).

Laser-induced acoustic desorption/electrospray ionization mass spectrometry (LIAD–ESI/MS) is a recently developed type of ambient ionization mass spectrometry [88]. Although the LIAD–ESI source, which contains a sample plate, pulsed laser, and electrosprayer, is nearly identical to that of ELDI, the use of the laser beam in LIAD–ESI is different from that in ELDI. For LIAD–ESI, the rear side of a metal foil bearing a pre-deposited sample solution is irradiated

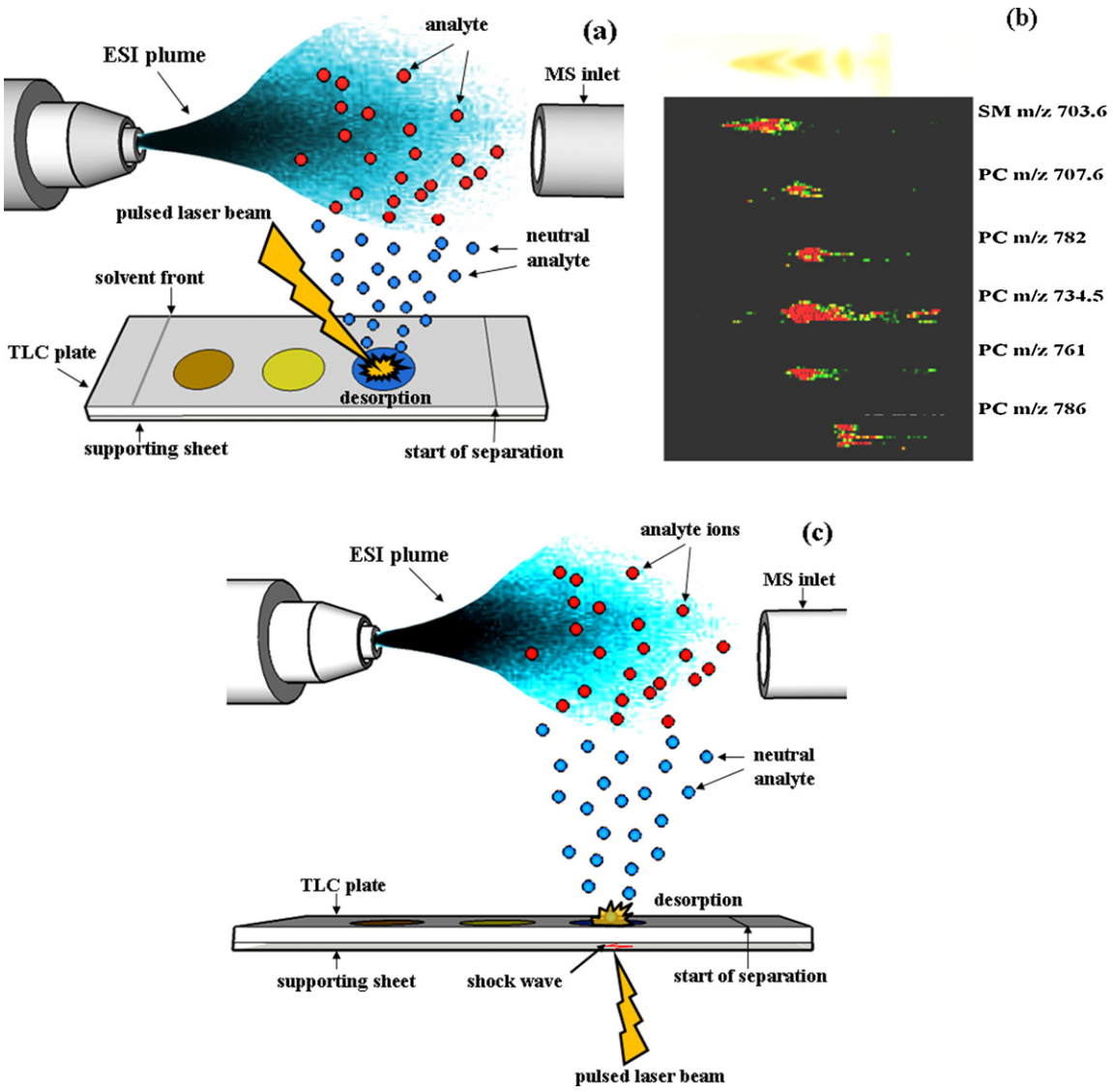

Fig. 7. (a) Schematic representation of an ELDI-MS system for the analysis of chemical compounds on the surface of a TLC plate. (b) Photograph and molecular images of a separated lipid mixture (PC, phosphatidylcholine; SM, sphingomyelin) on a silica gel TLC plate. (c) Schematic representation of an LIAD–ESI/MS system for the analysis of chemical compounds on the surface of a TLC plate.

with a pulsed laser beam. Analyte molecules or fine droplets are generated through the actions of acoustic and shock waves induced by the pulsed laser irradiation. The ionization mechanisms in the LIAD–ESI source are similar to those in FD-ESI and ELDI. The desorbed analyte molecules or fine droplets through LIAD are then post-ionized in the solvent electrospray plume.

In a recent report, LIAD–ESI/MS was successfully interfaced with reversed-phase C_{18} and normal-phase silica TLC [64]. To efficiently generate and transfer acoustic and shock waves to desorb the analyte-containing TLC gels, a glass slide was attached to the rear of the TLC plate and the gap between the glass slide and the TLC plate was filled with a viscous solution (glycerol or polyethylene glycol). Fig. 7c displays a schematic representation of this TLC/LIAD–ESI/MS system. Although the source configurations of ELDI and LIAD–ESI systems are nearly identical, the expansion of the shock wave propagated from rear side of the metal film to the TLC plate surface means that LIAD usually provides lower spatial resolution for sampling than does ELDI. In addition, higher laser energy is usually required in LIAD–ESI to efficiently desorb the analyte from the TLC plate. LIAD–ESI/MS has been used to characterize drugs, dyes, and rosemary essential oil separated on reversed-phase C_{18} and normal-phase silica TLC plate [64].

3.1.4. ESI-based ambient TLC–MS using charged or neutral droplets for sampling and ionization

Desorption electrospray ionization (DESI) is an ambient ionization technique capable of sampling/ionizing the analyte on a solid surface by impacting the sample surface with pneumatically charged solvent droplets [89–92]. Although the ionization mechanisms of DESI remain unclear, droplet–pickup mechanisms have been suggested as the most probable processes. In these processes, after impacting by charged solvent droplets, a thin film containing sprayed solvent is formed on the solid surface such that the analytes distributed on the solid surface are contained (or dissolved) within the thin film; the splash droplets containing analyte molecules are generated by the following droplet impact; electrospray ionization processes then proceed from the splash droplets to generate analyte ions. The mass spectra provided by DESI are similar to those obtained using ESI. DESI has been used to characterize analytes on various solid surfaces, including glass, wood, tissue sections, fruit,

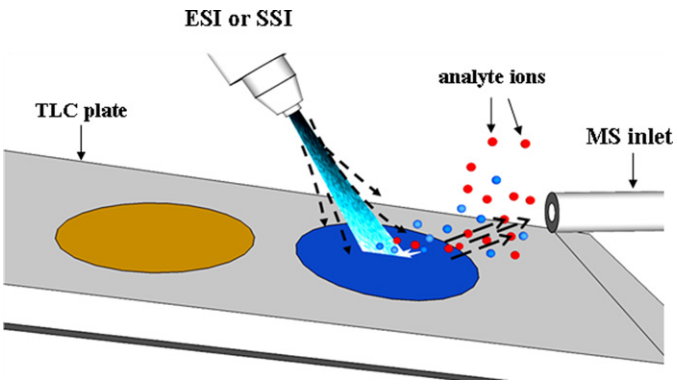

Fig. 8. Schematic representation of DESI and EASI systems for the analysis of chemical compounds on the surface of a TLC plate.

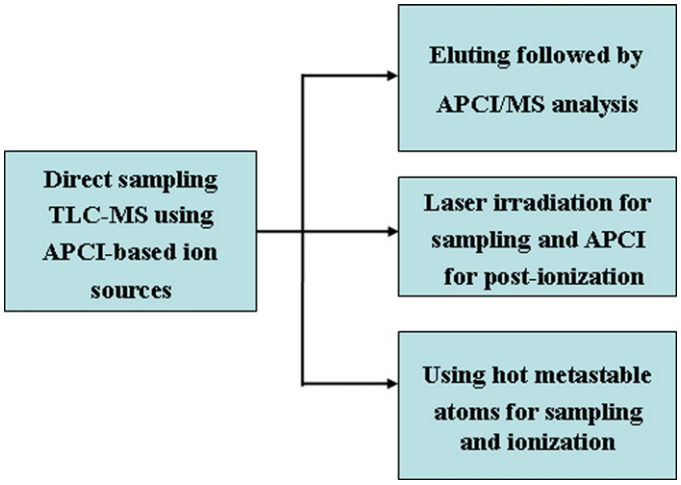

Fig. 9. Flow chart of direct sampling TLC–MS methods using APCI-based ambient mass spectrometry to characterize chemical compounds on the surfaces of TLC plates.

Teflon, and TLC plates [61,89–91]. Several mixtures, including peptides, dyes, and lipids, have been successfully characterized using TLC/DESI/MS [61,92–94].

Fig. 8 displays a schematic representation of a DESI system used for the analysis of compounds on the surface of a TLC plate. A jet of charged solvent droplets, generated through electrospray ionization and accelerated by a N_2 stream, is directed to the plate surface at an optimal angle to sample and ionize the analytes from the plate surface. The analyte ions are subsequently analyzed after entering the mass spectrometer. Molecular images of analytes distributed on a TLC plate surface after two dimension separation have also been obtained using a DESI/MS system equipped with a motor stage for fine movement [95–98].

Easy ambient sonic-spray ionization (EASI), also known as desorption sonic spray ionization, is another spray sampling/ionizing technique that has an experimental setup and operating manner similar to that of DESI [99]. In EASI, a sonic spray is used to generate the pneumatic droplets, but a high voltage is not applied to the spray solution; it has been suggested that the imbalance in the distribution of charges in the droplets induces the generation of charged droplets. EASI/MS has also been interfaced with TLC for the on-spot characterization of analytes on the surface of the TLC plate; drug tables, a mixture of semi-polar compounds, and biodiesels separated on TLC plates have all been characterized using TLC/EASI/MS (Fig. 8) [100,101].

Although on-line TLC–MS has been performed using the ESI-based ion sources described above, the requirement of a high analyte polarity for electrospray analysis has meant that these techniques are limited to the characterization of mid- to high-polarity compounds. No ion signal would be obtained for low-polarity or nonpolar compounds because of their low solubilities in the electrospray solution and/or their low proton affinity in the electrospray plume. Other mass spectrometric ion sources capable of ionizing low-polarity and nonpolar compounds must be used as TLC–MS interfaces if they are to be characterized successfully.

3.2. Ambient TLC–MS using APCI-based ion sources

The driving force for moving the mobile phase in TLC is capillary action between the gel particles; this mechanism is different from that involved in HPLC, where the mobile phase is driven by high pressure pumping. Large analytes, like proteins, have difficulty being carried through and efficiently separated by TLC. Therefore, since its development, TLC has most often been a chromatographic method dedicated to the separation of small molecules. Because APCI/MS is a sensitive technique for the characterization of small molecules having polarities ranging from nonpolar to highly polar, interfacing TLC with APCI/MS has the advantage of allowing the analyses of a wide range in polarity of small chemical compounds. Similar to the case of ESI-based ambient TLC–MS systems, several types of APCI and interfaces have been employed to connect TLC with mass spectrometry, including (a) continuously eluting the analyte from TLC plate following by atmospheric pressure chemical ionization from the eluted solution; (b) sampling the analyte on the plate first and then post-ionizing the analyte molecules in the APCI region; (c) sampling/ionizing the analyte directly on the plate surface using ions or metastable inert atoms (Fig. 9).

3.2.1. APCI-based ambient TLC–MS using analyte eluting device

Rotation planar chromatography (RPC) separates analytes on a rotating circular plate in which the mobile phase is driven by centrifugal force, rather than capillary forces. In general, the sample solution is applied near the center of a rotating circular plate, followed by pumping the developing solvent to the plate for separation. Analytes deposited on the plates are separated in a ring shape and then eluted from the edge of the plate. A fraction of the eluted solution is usually collected for further analysis. Coupling commercially available RPC with APCI to separate and characterize a mixture of dye standards has been performed by applying a tube to link the outlet of the eluted sample solution with the inlet of the APCI source [99]. The RPC–APCI/MS device has been applied to characterize a mixture of three dyes – Solvent Green 3, Solvent Blue 35, and Fat Red 7B [102].

3.2.2. APCI-based ambient TLC–MS using laser irradiation for sampling and APCI for post-ionization

Focused laser beam is used in vacuum-based and ambient ionization techniques, such as LDI, MALDI, and ELDI, to desorb and/or ionize the analytes. Similar to the set-up in an ELDI source, LD–APCI uses a laser beam to desorb the analyte from the sample plate; the analyte molecules are then ionized through reactions with metastable atoms or charged ion species in the ionization region, generated by applying a high voltage (+5.3 to +8.1 kV) to a metal electrode for corona discharging [103,104].

Although continuous and pulsed lasers can both be used for solid desorption, the combination of TLC and LD–APCI has been applied successfully only when coupling a diode laser with a corona discharge-based APCI source to the on-line characterization of chemical compounds separated on the surface of a TLC plate (Fig. 10). The separated compounds were thermally desorbed by the continuous-wave diode laser. The desorbed analyte molecules were transported to the APCI region through a glass tube; a gas

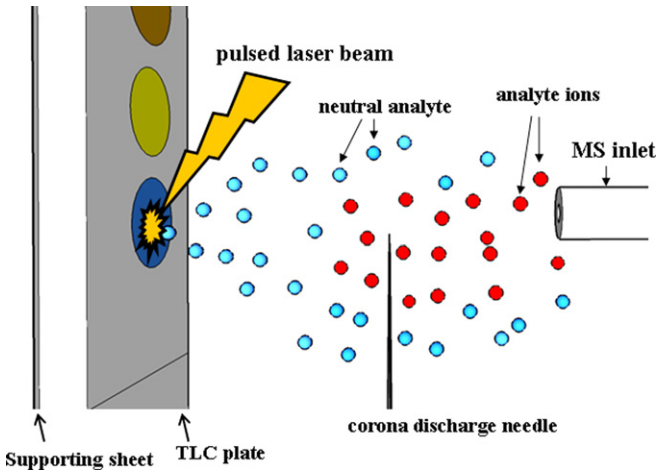

Fig. 10. Schematic representation of an LD–APCI system for the analysis of chemical compounds on the surface of a TLC plate.

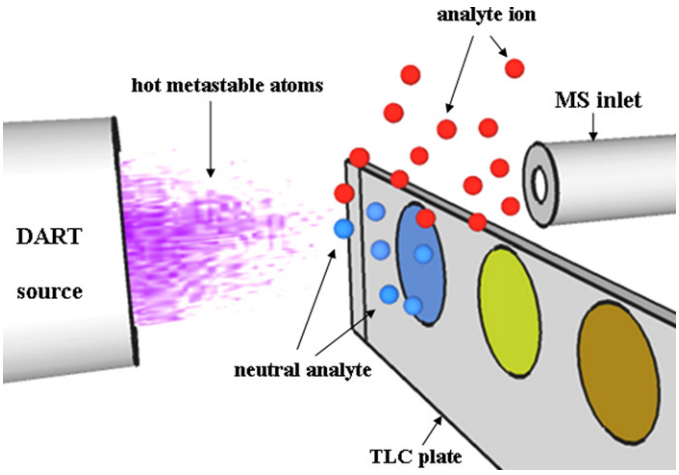

Fig. 11. Schematic representation of a DART system for the analysis of chemical compounds on the surface of a TLC plate.

jet pump was used to suck the desorbed compounds into the glass tube coiled with a heating wire. Phospholipids, lecithin, and sphingomyelin separated on a silica TLC plate surface were successfully characterized using this TLC/LD–APCI/MS system [105].

Compared with the pulse laser used in ELDI, the energy provided by a diode laser in a LD–APCI source is quite low, thereby restricting the desorption of large analytes from TLC plates. Nevertheless, when applying a graphite suspension to the plate surface (cf. SALDI), the laser energy is efficiently adsorbed by the graphite powder and transferred to the analyte, allowing the thermal desorption of the large analyte molecules [59,105].

3.2.3. APCI-based ambient TLC–MS using hot metastable inert atoms for sampling and ionization

The direct analysis in real time (DART) source uses a stream of excited hot inert gas species (He) to desorb and ionize volatile and semivolatile analytes from solid surfaces [106]. A stream of heated He gas is discharged by passing it through a corona-discharged needle to generate excited-state He gas species (including ions, electrons, and metastable neutral species). After passing the discharged He gas through a filter electrode positioned at the exit of the gas flow, the heated gas stream, containing mostly metastable He atoms, is used to desorb and ionize the analyte. It has been suggested that Penning ionization is the main ionization mechanism in DART: ionized water and its cluster ions (formed through interactions between water molecule in the air and metastable He atoms) react with the analyte molecules to form analyte ions. In DART, the analyte molecules are evaporated through thermal heating; therefore, this technique is limited to the analysis of volatile and semivolatile compounds. DART has been used to analyze many polar and nonpolar small chemical compounds [106–109].

The combination of TLC and DART has recently been reported [62,110–113]. Fig. 11 displays a schematic representation of a TLC–DART/MS system. The TLC plate is cut to a strip through the spots of interest. The edge of the TLC plate is placed in front of the entrance of the mass spectrometer and the separated spots are directed into the excited-state He stream. TLC–DART/MS has been used successfully to separate and characterize isopropyl-9H-thioxanthen-9-one and caffeine [110,113].

4. Direct sampling TLC–MS using atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) mass spectrometry

Except for the fact that it is performed under atmospheric pressure, the instrumental setup for AP-MALDI is similar to that of vacuum MALDI in that a pulsed laser beam is used to irradiate co-crystal spots containing the analyte and the matrix. Because AP-MALDI is operated under ambient conditions, its main advantages are its ability to analyze volatile and semivolatile compounds and its rapid sample switching. In addition, the ionization processes in AP-MALDI might be as soft as, or even softer than, those in vacuum MALDI because the ions generated under ambient conditions are thermally stable; as a result of collisional cooling, less fragmentation of analyte ions occurs [54].

When coupling AP-MALDI with TLC, the size of the TLC plate need not to be restricted by the dimensions of the vacuum chamber and, in addition, the desorbed gel particles do not accumulate in the ionization source, thereby avoiding damage to the ion source [54,114]. The innate advantage of laser irradiation provides AP-MALDI with high spatial resolution for sampling, allowing the acquisition of chromatographic information and molecular images of analytes separated through one- and two-dimensional TLC. Although its operation under atmospheric pressure provides AP-MALDI with several advantageous features relative to vacuum MALDI, it also has several disadvantages as that of vacuum MALDI, including interference from matrix ions in the low mass range and lateral diffusion of analytes along the plate, thereby decreasing the chromatographic resolution.

5. Direct sampling TLC–MS using laser ablation-inductively coupled plasma (LA-ICP) mass spectrometry for the analysis of inorganic compounds

Unlike other desorption/ionization techniques, ICP/MS can provide information regarding the metal composition in a sample. This multi-element characterization technique can be used to determine up to 90% of the elements in the periodic table. LA-ICP/MS is a two-step ionization technique. The desorption/ionization processes of LA-ICP/MS are similar to those operating in ELDI and LD–APCI, except that an ICP source is used for post-ionization of the analytes, which are usually inorganic compounds. When combining LA with ICP/MS, the solid sample is placed in a sealed chamber, a laser beam is used to irradiate the solid surface for surface ablation, the ablated materials are carried by He or Ar gas stream and transmitted through a coaxial glass tube to the plasma source for atomization and ionization. LA-ICP/MS has been applied in a variety of fields to characterize elemental profiles and distributions on solid surfaces. The combination of LA-ICP/MS and TLC has been used to characterize arsenic and chromium species that had been separated on the plate surface [60,115].

6. Detection limit and reproducibility of TLC–MS

The sampling area and the efficiency of extraction/desorption and ionization are the main factors that influence the detection limit of TLC–MS. For example, the use of a pulsed laser or focused ion beam (like MALDI, LD, or SIMS) to desorb the analyte on TLC plate has a small sampling area but high desorption efficiency. Direct sampling with ESI or APCI (like DESI and DART) provides a larger sampling area but lower desorption efficiency than that in laser desorption. Table 1 shows the detection limit of the TLC–MS using different ionization techniques. Among the vacuum-based TLC–MS techniques, MALDI is one of the most popular ionization techniques used for directly sampling and ionizing the analytes on TLC plate surface; the technique provides the detection limit of less than 1 ng for glycosphingolipids [45]. Other vacuum-based desorption/ionization techniques such as SALDI, SIMS, and LDI give the detection limit in the range between 50 pg and 500 ng. Comparing to vacuum-based ionization techniques, more ambient ionization techniques have been developed for direct TLC–MS analysis. These ambient ionization techniques include ESI, APCI, AP-MALDI, DART, ELDI, LIAD/ESI and ICP. Among the ESI-based ambient ionization techniques used in direct TLC–MS analysis, the sampling area of liquid extraction junction (diameter: 2–4 mm) is larger than that in DESI and gives a detection limit of 20 pg which is approximately two orders better than that of DESI [71]. ELDI uses a pulsed laser beam (focused to 100 μm $\times$ 250 μm) for sampling and provides a detection limit of 10^{-6} M when using FD&C red dye as the sample [63]. The LD/ICP gave a detection limit of 3 pg using arsenic species as the analytes [60]. The analyte – isopropyl-9H-thioxanthen-9-one of 1.3 ng was successfully detected from TLC plate by DART which has spatial resolution approximately 3 mm [62,110].

The detecting mass range of TLC–MS is mainly determined by the method used for ionization. For example, EI, CI, DART, and ICP are usually used for the analysis of small and volatile compounds and LDI, SIMS, FAB, MALDI, SALDI, APCI, and ESI are useful for the analysis of large and polar compounds. The repeatability of several ambient TLC–MS techniques has been investigated and reported. The relative standard deviation (RSD) of liquid extraction junction in the analysis of caffeine was $<\pm$3.75%. A stable isotope-labeled internal standard was added into the stock solution and analyzed by TLC–DART that showed a RSD of $\pm$5.4%. For AP-MALDI, the drugs separated on the TLC plates were repeatedly analyzed and gave a RSD of near 22%.

7. Conclusion

TLC is a well-developed simple chromatographic technique that allows the rapid separation of a mixture. The combination of TLC and MS provides a means to characterize the chemical compounds after their chromatographic development. The TLC–MS techniques published in recent years reveal a series of developing trends in this field: (1) rather than using indirect techniques, TLC plates are being analyzed increasingly in a direct manner without tedious pretreatment; (2) rather than using vacuum-based mass spectrometry, ambient mass spectrometry is being applied increasingly to characterize analytes on developed TLC plates; (3) instead of characterizing well-defined spots, TLC plates are being increasingly scanned to obtain chromatographic information and molecular images of the various chemicals distributed on their surfaces.

Because TLC is a chromatographic technique capable of high throughput separation, there is a challenge to develop automatic introduction systems in which several TLC plates are delivered to the TLC–MS system for rapid detection. With the combination of TLC and ambient mass spectrometry, TLC plates can be characterized under atmospheric pressure, making it easier, relative to vacuum-based TLC–MS techniques, to develop TLC–MS systems for continuous detection. We believe that the development of automatic plate introducing systems will be an important issue for the adaptation of high throughput TLC plate analysis using mass spectrometry.

Although ambient mass spectrometry has been used widely to characterize analytes on TLC plates, several problems remain unresolved: (1) an interface or device must be developed to prevent TLC gel particles from entering the mass spectrometer. Several desorption/ionization techniques, including ELDI, LIAD–ESI, DESI, and EASI, result in detached gel particles during surface sampling; the charged droplet stream may then transfer these desorbed gels to the spectrometer. (2) The detection sensitivity of direct TLC–MS analysis must be increased. Because the analytes on the TLC plate are characterized directly by mass spectrometry without extraction or concentration, the detection sensitivity of direct TLC–MS by ambient mass spectrometry is usually lower than that in indirect TLC–MS methods. (3) The separation efficiency of TLC must be improved. Developing an interface to connect TLC with a second separation/detection technique, such as LC/MS, GC/MS, or capillary electrophoresis/MS, would provide a higher efficiency in separation and detection.

In addition, although they have yet to be reported, the direct characterization of analytes on solid surfaces provided by many other ambient ionization techniques, including dielectric barrier desorption ionization (DBDI), desorption atmospheric pressure chemical ionization (DAPCI), and atmospheric solids analysis probe (ASAP), suggest that they are all potential interfaces for connecting TLC with MS [116–118].

References

[1] H. York, W. Funk, W. Fischer, H. Wimmer, Thin-Layer Chromatography, VCH, New York, 1990.
[2] F. Geiss, Fundamentals of Thin Layer Chromatography (Planar Chromatography), Huethig, Heidelberg, 1987.
[3] B. Fried, J. Sherma, Thin-Layer Chromatography, 4th ed., Marcel Dekker, New York, 1999.
[4] A.-M. Siouffi, Sep. Purif. Rev. 34 (2005) 155.
[5] J. Sherma, Anal. Chem. 80 (2008) 4253.
[6] J. Sherma, Anal. Chem. 82 (2010) 4895.
[7] C.F. Poole, J. Chromatogr. A 1000 (2003) 963.
[8] I.D. Wilson, J. Chromatogr. A 856 (1999) 429.
[9] B. Fuchs, R. Süß, A. Nimptsch, J. Schiller, Chromatographia 69 (2009) S95.
[10] S.P. Pasilis, G.J. Van Berkel, J. Chromatogr. A 1217 (2010) 3955.
[11] G. Morlock, W. Schwack, Trends Anal. Chem. 29 (2010) 1157.
[12] C.S. Lee, Y.G. Kim, H.S. Joo, B.G. Kim, J. Mass Spectrom. 39 (2004) 514.
[13] I. Meisen, J. Peter-Katalinić, J. Müthing, Anal. Chem. 76 (2004) 2248.
[14] I. Meisen, A.W. Friedrich, H. Karch, U. Witting, J. Peter-katalinić, J. Müthing, Rapid Commun. Mass Spectrom. 19 (2005) 3659.
[15] P. Ghosh, M.M.K. Reddy, V.B. Ramteke, B.S. Rao, Anal. Chim. Acta 508 (2004) 31.
[16] T. Watanabe, H. Kawasaki, T. Kimoto, R. Arakawa, Rapid Commun. Mass Spectrom. 21 (2007) 787.
[17] A.I. Gusev, A. Proctor, Y.I. Rabinovich, D.M. Hercules, Anal. Chem. 67 (1995) 1805.
[18] A. Conchillo, L. Cercaci, D. Ansorena, M.T. Rodriguez-Estrada, G. Lercker, I. Astiasaran, J. Agric. Food Chem. 53 (2005) 7844.
[19] Q.-T. Liu, J.L. Kinderlerer, J. Chromatogr. A 855 (1999) 617.
[20] Y. Minami, S. Yokoi, M. Setoyama, N. Bando, S. Takeda, Y. Kawai, J. Terao, Lipids 42 (2007) 1055.
[21] Y. Kawai, M. Miyoshi, J.-H. Moon, J. Terao, Anal. Biochem. 360 (2007) 130.
[22] S. Berkov, J. Bastida, M. Nikolova, F. Viladomat, C. Codina, Phytochem. Anal. 19 (2008) 411.
[23] A. Gorchein, Biomed. Chromatogr. 17 (2003) 526.
[24] H. Therisod, V. Labas, M. Caroff, Anal. Chem. 73 (2001) 3804.
[25] H. Ji, N. Sato, W.K. Nonidez, J.W. Mays, Polymer 43 (2002) 7119.
[26] J. Schiller, R. Süß, B. Fuchs, M. Müller, O. Zschörnig, K. Arnold, Chromatographia 57 (2003) S297.
[27] D. Sommerer, R. Süß, S. Hammerschmidt, H. Wirtz, K. Arnold, J. Schiller, J. Pharm. Biomed. Anal. 35 (2004) 199.
[28] M. Shariatgorji, Z. Spacil, G. Maddalo, L.B. Cardenas, L.L. Ilag, Rapid Commun. Mass Spectrom. 23 (2009) 3655.
[29] P. Hoffmann, M. Hulsewig, S. Duvar, H. Ziehr, M. Mormann, J. Peter-Katalinic, A.W. Friedrich, H. Karch, J. Müthing, Rapid Commun. Mass Spectrom. 24 (2010) 2295.

[30] I. Marchi, S. Rudaz, J.-L. Veuthey, Talanta 78 (2009) 1.
[31] A.J. Kubis, K.V. Somayajula, A.G. Sharkey, D.M. Hercules, Anal. Chem. 61 (1989) 2516.
[32] T.T. Chang, J.O. Lay, R.J. Francel, Anal. Chem. 56 (1984) 109.
[33] G.C. DiDonato, K.L. Busch, Anal. Chem. 58 (1986) 3231.
[34] F. Tames, I.D. Watson, W. Morden, I.D. Wilson, J. Chromatogr. B 729 (1999) 341.
[35] A.A. Parent, T.M. Anderson, D.J. Michaelis, G. Jinang, P.B. Savage, M.R. Linford, Appl. Surf. Sci. 252 (2006) 6746.
[36] A. Oriňák, H.F. Arlinghaus, G. Vering, R. Oriňáková, Surf. Interface Anal. 36 (2004) 1122.
[37] A. Oriňák, H.F. Arlinghaus, G. Vering, R. Oriňáková, S. Hellweg, J. Chromatogr. A 1084 (2005) 113.
[38] Y.-C. Chen, J. Shiea, J. Sunner, J. Chromatogr. A 826 (1998) 77.
[39] A.I. Gusev, O.J. Vasseur, A. Proctor, A.G. Sharkey, D.M. Hercules, Anal. Chem. 67 (1995) 4565.
[40] J.T. Mehl, A.I. Gusev, D.M. Hercules, Chromatographia 46 (1997) 358.
[41] L.S. Santos, R. Haddad, N.F. Hoehr, R.A. Pilli, M.N. Eberlin, Anal. Chem. 76 (2004) 2144.
[42] K. Dreisewerd, J. Müthing, A. Rohlfing, I. Meisen, Z. Vukelic, J. Peter-Katalinic, F. Hillenkamp, S. Berkenkamp, Anal. Chem. 77 (2005) 4098.
[43] V.B. Ivleva, L.M. Sapp, P.B. O'Connor, C.E. Costello, J. Am. Soc. Mass Spectrom. 16 (2005) 1552.
[44] V. Panchagnula, A. Mikulskis, L. Song, Y. Wang, M. Wang, T. Knubovets, E. Scrivener, E. Golenko, I.S. Krull, M. Schulz, H.-E. Hauck, W.F. Patton, J. Chromatogr. A 1155 (2007) 112.
[45] U. Distler, M. Huelsewig, J. Souady, K. Dreisewerd, J. Haier, N. Senninger, A.W. Friedrich, H. Karch, F. Hillenkamp, S. Berkenkamp, J. Peter-Katalinic, J. Müthing, Anal. Chem. 80 (2008) 1835.
[46] J. Souady, J. Soltwisch, K. Dreisewerd, J. Haier, J. Peter-Katalinic, J. Müthing, Anal. Chem. 81 (2009) 9481.
[47] I. Meisen, U. Distler, J. Müthing, S. Berkenkamp, K. Dreisewerd, W. Mathys, H. Karch, M. Mormann, Anal. Chem. 81 (2009) 3858.
[48] A. Müsken, J. Souady, K. Dreisewerd, W. Zhang, U. Distler, J. Peter-Katalinić, H. Miller-Podraza, H. Karch, J. Müthing, Rapid Commun. Mass Spectrom. 24 (2010) 1032.
[49] J. Müthing, U. Distler, Mass Spectrom. Rev. 29 (2010) 425.
[50] Y.-C. Chen, Rapid Commun. Mass Spectrom. 13 (1999) 821.
[51] Y.C. Chen, J.Y. Wu, Rapid Commun. Mass Spectrom. 15 (2001) 1899.
[52] J.Y. Wu, Y.C. Chen, J. Mass Spectrom. 37 (2002) 85.
[53] A. Crecelius, M.R. Clench, D.S. Richards, V. Parr, J. Chromatogr. A 958 (2002) 249.
[54] P.K. Salo, H. Salomies, K. Harju, R.A. Ketola, T. Kotiaho, J. Yli-Kauhaluoma, R. Kostiainen, J. Am. Soc. Mass Spectrom. 16 (2005) 906.
[55] G.J. Van Berkel, A.D. Sanchez, J.M.E. Quirke, Anal. Chem. 74 (2002) 6216.
[56] H. Luftmann, Anal. Bioanal. Chem. 378 (2004) 964.
[57] K.G. Asano, M.J. Ford, B.A. Tomkins, G.J. Van Berkel, Rapid Commun. Mass Spectrom. 19 (2005) 2305.
[58] M.Z. Huang, C.H. Yuan, S.C. Cheng, Y.Z. Cho, J. Shiea, Annu. Rev. Anal. Chem. 3 (2010) 43.
[59] S. Peng, N. Ahlmann, K. Kunze, W. Nigge, M. Edler, T. Hoffmann, J. Franzke, Rapid Commun. Mass Spectrom. 18 (2004) 1803.
[60] M. Resano, E.G. Ruiz, V.G. Mihucz, A.M. Moricz, G. Zaray, F. Vanhaecke, J. Anal. At. Spectrom. 22 (2007) 1158.
[61] G.J. Van Berkel, M.J. Ford, M.A. Diebel, Anal. Chem. 77 (2005) 1207.
[62] G. Morlock, W. Schwack, Anal. Bioanal. Chem. 385 (2006) 586.
[63] S.-Y. Lin, M.-Z. Huang, H.-C. Chang, J. Shiea, Anal. Chem. 79 (2007) 8789.
[64] S.-C. Cheng, M.-Z. Huang, J. Shiea, Anal. Chem. 81 (2009) 9274.
[65] M.J. Ford, G.J. Van Berkel, Rapid Commun. Mass Spectrom. 18 (2004) 1303.
[66] M.J. Ford, V. Kertesz, G.J. Van Berkel, J. Mass Spectrom. 40 (2005) 866.
[67] M.J. Ford, M.A. Diebel, B.A. Tomkins, G.J. Van Berkel, Anal. Chem. 77 (2005) 4385.
[68] V. Kertesz, M.J. Ford, G.J. Van Berkel, Anal. Chem. 77 (2005) 7183.
[69] M. Aranda, G. Morlock, J. Chromatogr. A 1131 (2006) 253.
[70] A. Alpmann, G. Morlock, Anal. Bioanal. Chem. 386 (2006) 1543.
[71] U. Jautz, G. Morlock, J. Chromatogr. A 1128 (2006) 244.
[72] H. Luftmann, M. Aranda, G.E. Morlock, Rapid Commun. Mass Spectrom. 21 (2007) 3772.
[73] M. Aranda, G. Morlock, Rapid Commun. Mass Spectrom. 21 (2007) 1297.
[74] B. Meermann, I. Moller, S. Nowak, H. Luftmann, U. Karst, J. Anal. At. Spectrom. 25 (2010) 1654.
[75] F.L. Hsu, C.H. Chen, C.H. Yuan, J. Shiea, Anal. Chem. 75 (2003) 2493.
[76] W. Chai, C. Leteux, A.M. Lawson, M.S. Stoll, Anal. Chem. 75 (2003) 118.
[77] J. Shiea, M.Z. Huang, H.J. Hsu, C.Y. Lee, C.H. Yuan, I. Beech, J. Sunner, Rapid Commun. Mass Spectrom. 19 (2005) 3701.
[78] M.Z. Huang, H.J. Hsu, J.Y. Lee, J. Jeng, J. Shiea, J. Proteome Res. 5 (2006) 1107.
[79] M.Z. Huang, H.J. Hsu, C.I. Wu, S.Y. Lin, Y.L. Ma, T.L. Cheng, J. Shiea, Rapid Commun. Mass Spectrom. 21 (2007) 1767.
[80] J. Shiea, C.H. Wang, J. Mass Spectrom. 32 (1997) 247.
[81] J. Shiea, D.Y. Chang, C.H. Lin, S.J. Jiang, Anal. Chem. 73 (2001) 4983.
[82] D.Y. Chang, C.C. Lee, J. Shiea, Anal. Chem. 74 (2002) 2465.
[83] I.F. Shieh, C.Y. Lee, J. Shiea, J. Proteome Res. 4 (2005) 606.
[84] C.-Y. Lee, J. Shiea, Anal. Chem. 70 (1998) 2757.
[85] J. Shiea, C.-M. Hong, F.-C. Tsai, Anal. Chem. 72 (2000) 1175.
[86] H.J. Hsu, T.L. Kuo, S.H. Wu, J.N. Oung, J. Shiea, Anal. Chem. 77 (2005) 7744.
[87] J. Shiea, L.J. Wu, M.Z. Huang, Proc. Am. Soc. Mass Spectrom., 58th, Salt Lake City, Utah, TP570, 2010.
[88] S.C. Cheng, T.L. Cheng, H.C. Chang, J. Shiea, Anal. Chem. 81 (2009) 868.
[89] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks, Science 306 (2004) 471.
[90] J.M. Wiseman, S.M. Puolitaival, Z. Takáts, R.G. Cooks, R.M. Caprioli, Angew. Chem. Int. Ed. 44 (2005) 7094.
[91] Z. Takáts, J.M. Wiseman, R.G. Cooks, J. Mass Spectrom. 40 (2005) 1261.
[92] G.J. Van Berkel, B.A. Tomkins, V. Kertesz, Anal. Chem. 79 (2007) 2778.
[93] S.P. Pasilis, V. Kertesz, G.J. Van Berkel, M. Schulz, S. Schorcht, Anal. Bioanal. Chem. 391 (2008) 317.
[94] E.L. Harry, J.C. Reynolds, A.W.T. Bristow, I.D. Wilson, C.S. Creaser, Rapid Commun. Mass Spectrom. 23 (2009) 2597.
[95] J.H. Kennedy, J.M. Wiseman, Rapid Commun. Mass Spectrom. 24 (2010) 1305.
[96] G.J. Van Berkel, V. Kertesz, Anal. Chem. 78 (2006) 4938.
[97] S.P. Pasilis, V. Kertesz, G.J. Van Berkel, M. Schulz, S. Schorcht, J. Mass Spectrom. 43 (2008) 1627.
[98] G. Paglia, D.R. Ifa, C. Wu, G. Corso, R.G. Cooks, Anal. Chem. 82 (2010) 1744.
[99] R. Haddad, R. Sparrapan, M.N. Eberlin, Rapid Commun. Mass Spectrom. 20 (2006) 2901.
[100] R. Haddad, H.M.S. Milagre, R.R. Catharino, M.N. Eberlin, Anal. Chem. 80 (2008) 2744.
[101] L.S. Eberlin, P.V. Abdelnur, A. Passero, G.F. de Sa, R.J. Daroda, V. de Souza, M.N. Eberlin, Analyst 134 (2009) 1652.
[102] G.J. Van Berkel, J.J. Llave, M.F. De Apadoca, M.J. Ford, Anal. Chem. 76 (2004) 479.
[103] J.J. Coon, K.J. McHale, W.W. Harrison, Rapid Commun. Mass Spectrom. 16 (2002) 681.
[104] J.J. Coon, H.A. Steele, P.J. Laipis, W.W. Harrison, J. Mass Spectrom. 37 (2002) 1163.
[105] S. Peng, M. Edler, N. Ahlmann, T. Hoffmann, J. Franzke, Rapid Commun. Mass Spectrom. 19 (2005) 2789.
[106] R.B. Cody, J.A. Laramee, H.D. Durst, Anal. Chem. 77 (2005) 2297.
[107] S.D. Maleknia, T.L. Bell, M.A. Adams, Int. J. Mass. Spectrom. 279 (2009) 126.
[108] S. Yu, E. Crawford, J. Tice, B. Musselman, J.T. Wu, Anal. Chem. 81 (2009) 193.
[109] O.P. Haefliger, N. Jeckelmann, Rapid Commum. Mass Spectrom. 21 (2007) 1361.
[110] G. Morlock, Y. Ueda, J. Chromatogr. A 1143 (2007) 243.
[111] E. Dytkiewitz, G.E. Morlock, J. AOAC Int. 91 (2008) 1237.
[112] A. Alpmann, G. Morlock, J. Sep. Sci. 31 (2008) 71.
[113] N.J. Smith, M.A. Domin, L.T. Scott, Org. Lett. 10 (2008) 3493.
[114] P.K. Salo, S. Vilmunen, H. Salomies, R.A. Ketola, R. Kostiainen, Anal. Chem. 79 (2007) 2101.
[115] J.P. Lafleur, E.D. Salin, Anal. Chem. 80 (2008) 6821.
[116] A. Sonnenfeld, T.M. Tun, L. Zajíčková, K.V. Kozlov, H.-E. Wagner, J.F. Behnke, R. Hippler, Plasma Polym. 6 (2001) 237.
[117] Y. Song, R.G. Cooks, Rapid Commum. Mass Spectrom. 20 (2006) 3130.
[118] C.N. McEwen, R.G. McKay, B.S. Larsen, Anal. Chem. 77 (2005) 7826.