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Review

The state of the art in thin-layer chromatography–mass spectrometry: a critical appraisal

I.D. Wilson

Department of Safety of Medicines, Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

Abstract

Thin-layer chromatography–mass spectrometry (TLC–MS) is a readily implemented technique that, in its simplest form, puts few demands on either chromatography or spectrometry. Nevertheless, compared to the situation with high performance liquid chromatography, it is much less highly developed. Currently, the bulk of the practical applications of TLC–MS are directed towards the use of fast atom, or ion bombardment. Recent developments, however, include the use of matrix assisted laser desorption ionisation (MALDI), surface assisted laser desorption (SALDI) and the development of a TLC–electrospray interface. Here, the state of the art of TLC–MS is described and future trends identified.

Keywords: Mass spectrometry; Interfaces, TLC–MS; Detection, TLC; Reviews

Contents

1. Introduction

The combination of thin-layer, or planar, chromatography (TLC) with mass spectrometry (MS) is readily achieved and has many benefits not the least of which is the of the modest cost of performing such work. In addition, the ability of the TLC plate to effectively act as a storage device for the separation, enabling chromatography and spectrometry to be performed at locations distant from each other in both time and space, provides a degree of flexibility not present in directly coupled systems. In addition, when TLC is used in 'screening mode' many samples can be expected to be negative and not require further analysis. As an off-line technique planar chromatography therefore enables many samples to be run rapidly, and in parallel, in a screen with subsequent spectroscopy performed only on an 'as

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required' basis. As an obvious example, TLC remains a popular method for screening urine samples for drugs of abuse and many standardised solvent systems have been developed for this purpose (e.g. see Ref. [1]). In such screens confirmation of the identity of the compound(s) of interest is usually undertaken using another method e.g. GC–MS. Very often this requires complex sample manipulation, such as extraction and derivatisation, before confirmation is possible. However, if all that is required is confirmation of identity, the use of mass spectrometric (MS) techniques directly on the material present in the TLC spot/band represents a practical alternative (see below).

Whilst not at the centre of research into the coupling of chromatography with mass spectrometry, nevertheless, the whole range of modern ionisation techniques have, at one time or another, been used for TLC–MS. The potential range of compound types to which TLC–MS might be applied is also very wide. Indeed the published applications of TLC and MS over the last few decades have encompassed a very wide range of analyte types including drugs and metabolites (antibiotics, alkaloids, benzodiazepines, diuretics, phenothiazines, neostigmine, pyridostigmine, anti-inflammatory and antipyretic compounds etc.), lipids, bile acids, peptides, porphyrins, oligosaccarides, polysaccharides, glycosides, dyes, steroids, nucleic acids, surfactants and toxins. The use of TLC–MS has been the subject of a number of comprehensive reviews in recent years and the reader is directed to these articles for details of this early work and for an in depth treatment of the development of the field [2–4]. Here recent developments and applications will be described together with some speculations as to future prospects.

2. Practicalities of TLC–MS

There are essentially two modes of TLC–MS which can be broadly categorised as either manual or instrumental, although, as it will be seen the divide between the two is somewhat arbitrary. In the case of the manual methods the relevant zone or spot of the TLC plate (or stationary phase) containing the analyte is taken and removed from the plate thereby destroying the chromatographic integrity of the separation. Once removed from the plate the material present in the spot can then either be recovered by solvent extraction, and the resulting solution injected into the ion source of the mass spectrometer, or the analyte can be introduced into the mass spectrometer still adsorbed onto the stationary phase (often having been mulled in some way with a matrix for e.g. fast atom bombardment or liquid secondary ion (fast ion bombardment) mass spectrometry (FAB-MS, LSI-MS). Thus, in the earliest method employing FAB-MS the spot was cut out intact from an aluminium backed TLC plate and attached to the probe of the MS using double sided adhesive tape [5]. In contrast, with 'instrumental' methods the plate, or a large portion of it, is left undisturbed and placed on a suitable stage (fixed or movable) inside the mass spectrometer where, depending upon the ionisation technique used, it can be 'scanned' or the spots of analyte 'imaged'. With some of these techniques, such as when motorised probes are used, it is possible to reconstruct a chromatogram based on either single ion monitoring or the total ion current. One such design for a motorised probe is shown in Fig. 1 [6]. Such interfaces, which enable a strip of the TLC plate to be slowly moved through the ion source, are available from a limited number of manufacturers. To date no devices have been prepared that enable the whole of a plate to be 'scanned' in the way that might be performed with a scanning densitometer. Such a device is probably not outside the realms of possibility, however, as several groups have demonstrated 3-dimensional imaging of portions of a plate (see Refs. [2,8,10]).

3. Ionisation techniques

Ionisation in TLC–MS has been achieved by a variety of methods. However, the use of ionisation techniques such as electron impact (EI) for TLC stationary phases, such as silica gel, has generally been limited to relatively non-polar and thermally stable molecules, for which it works very well. Similarly, for less adsorptive materials, such as polyamide, EI provides good results However, for

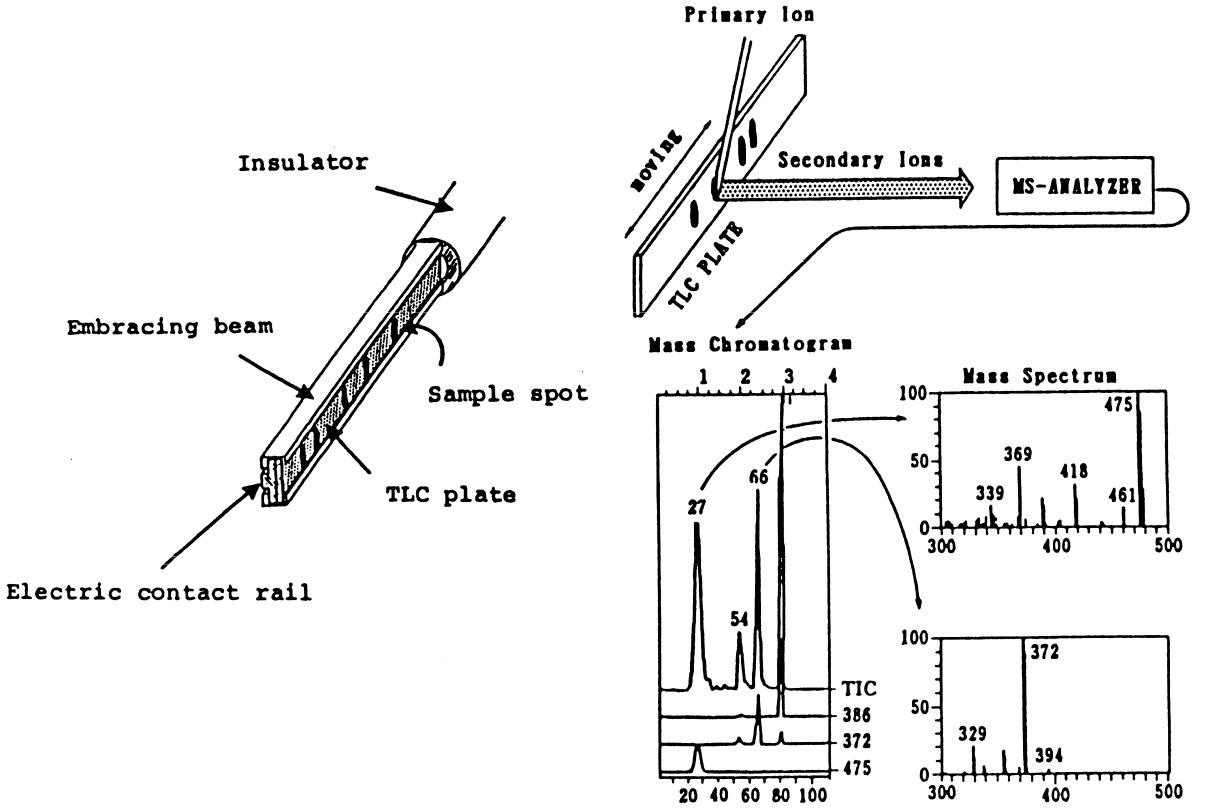

Fig. 1. A schematic representation of a simple probe for scanning segments of TLC plates in order to obtain mass spectra (from Ref. [6]).

polar and/or thermally unstable substances ionisation techniques such as FAB, LSI or laser desorption (LD) (most recently matrix-assisted laser desorption/ionisation (MALDI)) and surface-assisted laser desorption/ionisation (SALDI) can be more appropriate.

3.1. FAB-MS and LSI-MS

In the case of FAB-MS or LSI-MS it is necessary to apply an appropriate matrix to the stationary phase. This is usually glycerol but *m*-nitrobenzyl alcohol (*m*-NBA) and a variety of other compounds have been used for this. The purpose of the matrix is two-fold in that it is used to induce ionisation and promote the transfer of the analyte to the surface phase of the plate. The addition of small amounts of methanol and other modifiers such as trifluoroacetic acid (TFA) can also assist in ensuring efficient ionisation of analytes in the presence of chromatographic media. When analytes are present in small amounts or in a diffuse spot it can be difficult to obtain good quality FAB spectra. The total surface area of a highly porous adsorbent such as silica gel from a 6–8 mm diameter TLC spot is large with the consequence that the concentration of the analyte per mm^2 will be relatively low. Thus, in trace analysis, even where there is sufficient material for FAB-MS, there may be difficulties in obtaining spectra when attempting to perform tandem mass spectrometry (MS–MS) as there may be insufficient ions to enable CID (collision induced dissociation) to be performed. To counter this several techniques have been developed to concentrate the analytes in the spot into a smaller area. We have found for many of our applications that the method described by Suzuki et al. has worked well [7]. This technique involves a trapezoidal shape being scribed around the spot or

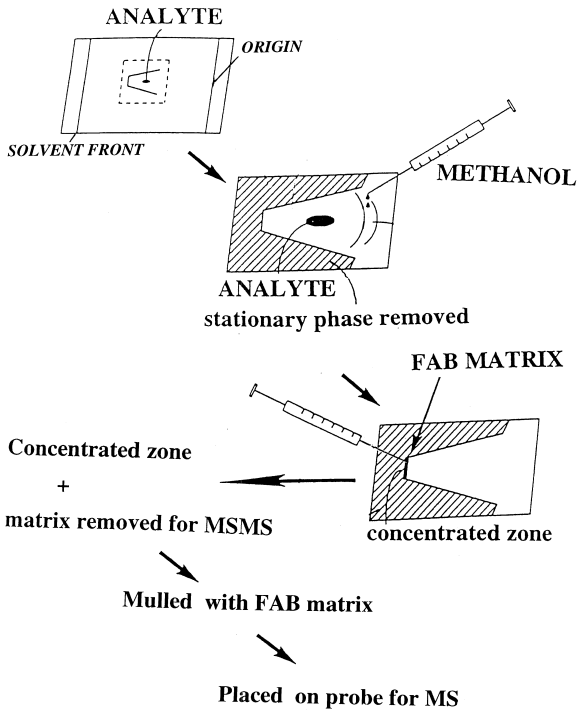

Fig. 2. A schematic diagram showing the preparation of a TLC plate for sample preconcentration prior to MS (based on Oka et al. Ref. [7])

band of interest followed by the application of a strong solvent, such as methanol, to the open base (see Fig. 2). This causes the analyte to migrate to the tip of the trapezoid which for UV absorbing or coloured compounds is readily monitored. The analyte is now concentrated in the top 0.5–1 mm of the stationary phase, which is relatively easy to treat with the FAB matrix and then remove for subsequent MS.

3.2. LD-MS, MALDI-MS and SALDI-MS

Laser desorption has been used by a number of groups and spectra for a range of compounds types have been successfully obtained with sensitivity in the pg/ng spot range (reviewed in Refs. [2,3]). As indicated above the main advantage of LD is that no liquid matrix or extraction solvent is needed in order to obtain spectra. This enable spots or bands on the TLC plate to be examined and imaged without loss of spatial resolution. However, with UV-based LD poor reproducibility and significant fragmentation have been cited as disadvantages of the technique [8]. A partial solution to this, which reduces the problems associated with excessive fragmentation, is the use of IR laser desorption followed by multiphoton ionisation (MUPI). In the case of TLC–MS this has been described for four analytes, two dipeptides (GlnTrp and GlyTyr) and pentagastrin and gramicidin D, which were used as model compounds [9]. Thus, MUPI with time of flight (TOF) from silica gel and cellulose were described in a procedure, whereby, following development, the plate (ca. 10 cm in length by 2 cm wide) was segmented into 4×20 mm strips each of which were then glued to the probe tip for analysis. The sequential analysis of each of these strips thereby enabled a rough, low resolution chromatogram to be reconstructed from the resulting mass spectra. The experiments were performed using a Bruker-Franzen Analytick TOF-1 mass spectrometer with a MUPI ion source with the CO_2 laser beam focused into a 0.5 mm diameter beam desorbing the sample directly in front of an expanding gas jet. Laser ionisation was then performed using a frequency doubled dye laser collimated to produce a laser beam of 1–2 mm diameter with a power density in the ionisation region in the order of 10^5–10^6 W cm^{-2}. Molecular ions for each of the dipeptides were obtained on either silica gel or cellulose, but the results were much better on the latter material. The result for silica gel, which showed also showed a fair amount of fragmentation, was improved by adding some (unspecified) solvent to the silica prior to desorption. On the basis of the studies undertaken here, limits of detection using this non-optimised chromatographic separation of 250 pg and 1.2 ng on cellulose and silica gel, respectively, were estimated for GlnTrp and 10 and 35 ng for GlyTyr. Spectra were also illustrated for pentagastrin and gramicidin D at the 10 μg/spot level.

To compensate for the perceived problems of LD, several groups have examined the use of MALDI/SALDI, but unlike LD these techniques do involve the use of a matrix in order to obtain spectra. In the original MALDI studies the matrix was applied to the analyte on the plate (cellulose or silica gel) following the application of an ‘extraction’ solvent employed to bring the analyte molecule to the surface of the plate [8]. Once the matrix had

crystallised it was possible to obtain mass spectra via MALDI using a time of flight (TOF) instrument and also to image spots directly on the plate. Various MALDI matrices were investigated with the best detection limits achieved with those based on ferulic acid/fucose or sinapinic acid, whilst the best crystal homogeneity and surface coverage was obtained using 2-(4-hydroxyphenylazo)benzoic acid. In this and subsequent studies the plates were pre-developed with a methanol–water solution to reduce the occurrence of sodium and potassium adducts. The TLC–MALDI-TOF technique then enabled the detection of various bradykinin derivatives, angiotensin I–III and enkephalin derivatives with an absolute detection limit of between 2 and 4 ng [8]. The effect of ninhydrin, used as a visualisation reagent, on spectral quality was also investigated.

Subsequently this protocol has been adapted in such a way as to avoid excessive analyte spreading [10,11]. The new technique involves the preparation of a layer 0.003 mm thick of the matrix (α-cyano-4-hydroxycinnamic acid (α-CHCA) and alpha-L-fucose (3:1)) on a stainless steel plate [10]. This layer of matrix, roughly 50×50 mm, was then transferred onto the developed TLC plate that had been pre-wetted with the 'extraction' solvent, which was a methanol–water (1:1) solution, via gentle pressure (4–8 kg/mm^2). The authors emphasised the critical importance of the extraction solvent, which serves to faciltate the transfer of the matrix layer, the extraction of the analyte and its subsequent incorporation into the matrix structure. The whole process is illustrated in Fig. 3. Three types of silica gel TLC plate were studied with either no binder, an organic binder or gypsum, and whilst the detection limits were similar on all three the organic binder did produce more of a background. This protocol produced minimal analyte broadening and was applied to compounds such as rhodamine B, guinea green B, bradykinin and angiotensin II. Detection was possible with sub nanogram quantities of the analytes representing a ten-fold improvement on the previous-

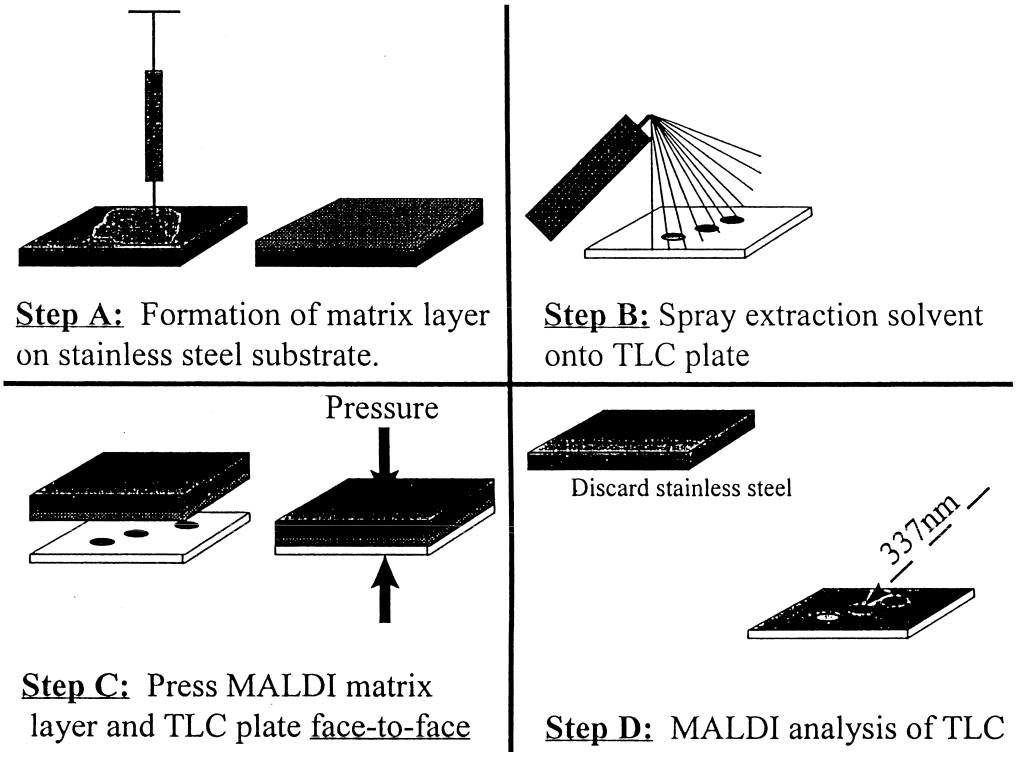

Fig. 3. Preparation of a TLC plate for MALDI (from Ref. [11] with permission).

ly reported methodology (detection limits of 200 pg for angiotensin II and 50 pg for rhodamine B were claimed), and imaging of the spots was achieved with a spatial resolution of 250–500 μm. On the basis of these studies it was felt that the ultimate spatial resolution would be in the region of 50 μm.

This technique has also been employed for the quantitative analysis of cocaine from both silica gel and reversed-phase plates [12]. Cocaine-D_3 was used as an internal standard and quantification over the range 0–16 ng was demonstrated with good precision. A detection limit of 60 pg was estimated for both types of plate.

More recently this protocol has been subject to further study and optimisation [11], where the influence of factors such as pressure, analyte R_F in the extraction solvent and extraction time on extraction efficiency and sensitivity were examined for analytes on silica gel. In these studies the optimum extraction time was found to be approximately 2 min. The optimum extraction system was concluded to be a combination of an extraction solvent, which if used as a chromatographic eluent would give an R_F of between 0.4 and 0.6, and a pressure of 4 kg/mm. The amount of pressure that could be applied being limited by the mechanical stability of the plate. Somewhat against their expectations they found that signal intensity did not increase linearly with extraction efficiency due to lateral spreading of the spot decreasing the surface concentration. The estimated upper extraction efficiency (using 4 kg/cm and an extraction solvent of $R_F=0.60$) was 22%.

TLC combined with surface-assisted laser desorption ionisation, TLC–SALDI-MS has also recently been described [13]. For this work the appropriate zone of the developed, plastic backed, silica gel TLC plate was attached to the stainless steel sample plate of the mass spectrometer with double sided adhesive tape and then coated with 2 μm activated carbon particles. This was achieved by applying a suspension of the activated carbon in a water–methanol solution of glycerol and sucrose to the TLC spot followed by glycerol. Mass spectrometry was then performed using a Voyager RP time of flight mass spectrometer. Using this methodology spectra were obtained for a variety of peptides (bradykinin and angiotensin II) and low molecular mass organic compounds (e.g. hydrochorothiazide and prometryn). Currently the technique seems to be less sensitive than MALDI (ca. 25 ng for bradykinin), but background interference is limited. Spectra for bradykinin and angiotensin II obtained by TLC–SALDI are shown in Fig. 4.

However, innovation in TLC–MS has not been limited to the introduction of the modern laser desorption methods. Recently Busch et al. have described a custom built interface, essentially a micro solvent extraction device, that enables electrospray (ES) ionisation to be performed on compounds separated by TLC [14]. In this instance using TLC–ESI-MS a suitable solvent (methanol–water mixtures) is delivered through a capillary tube to a spot (or band) on the developed TLC plate. The solvent diffuses a short distance through the spot taking the analyte into solution before being drawn up by capillary action into a sheath of absorbent material contained in a further tube surrounding the solvent delivery capillary. The analyte is then recovered from the sorbent with an aliquot of solvent for mass spectrometry. The general arrangement is shown in Fig. 5. These authors also described a microcapillary array for use with bands rather than spots. A modified extraction device was also described that

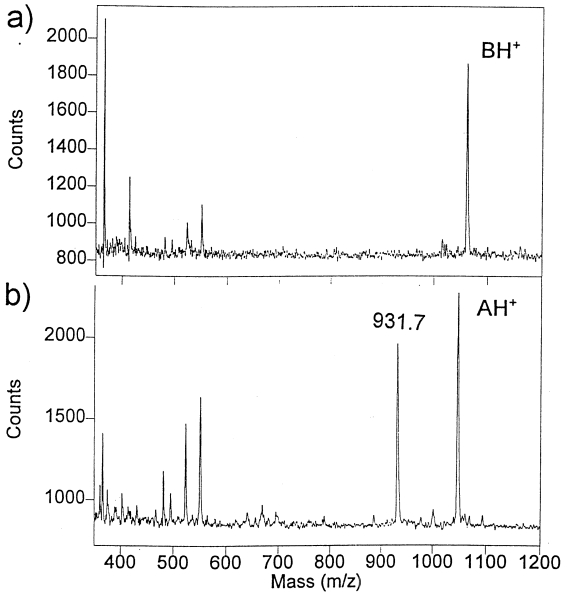

Fig. 4. The TLC–SALDI-MS spectra of (a) bradykinin and (b) angiotensin II (from Ref. [12].

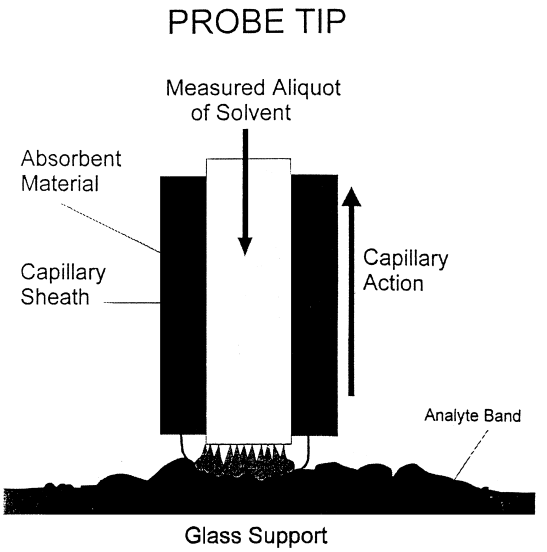

Fig. 5. A stylised diagram of the capillary probe used for extraction, elution and collection of analytes from TLC plates prior to ESI-MS (from Ref. [14]).

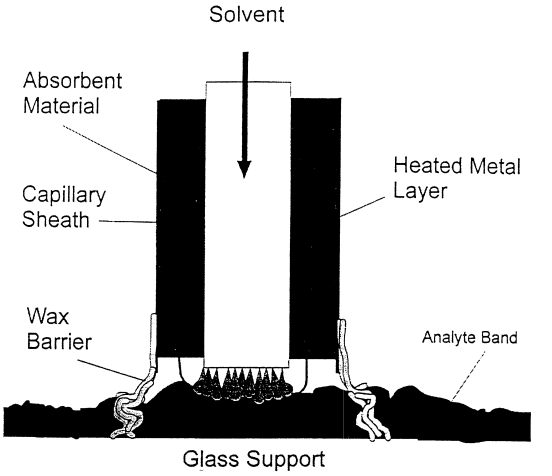

Fig. 6. The modified extraction probe from Fig. 5.

allowed the use of local heating to facilitate the extraction process whilst at the same time minimising the possibility of spot diffusion. In this case an extraction probe with an outer metal sheath that can be slid over the inner capillary. This outer metal sheath was coated with a small amount of wax, which when placed in contact with the surface of a heated TLC plate melted and flowed into the silica gel. However, due to the viscosity of the wax it did not disperse far and following the removal of the heat source it solidified. The extraction solvent was then applied to the still warm TLC plate, as before, with the extraction efficiency consequently improving, whilst the physical barrier provided by the wax directed the flow of solvent into the waiting adsorbent. As the authors point out there is obviously a balance between the type of wax used, the nature of the extracting solvent and the temperatures that can be employed (Fig. 6).

The interface was used to recover ethoxytriphenylphosphonium chloride from a C_{18} bonded TLC plate. In this case the authors applied 0.5 ng to the plate but based on their results estimated that as little as 2 pg of the test analyte could have been detected with ESI-MS. A representative ESI mass spectrum is shown in Fig. 7. In addition MS–MS spectra were also obtained which were essentially indistinguishable from those of the standard. Whilst still at a very early stage this represents an interesting development.

4. Recent applications of TLC–MS

Techniques, such as MALDI, SALDI and the electrospray interface described above, represent recent innovations and are still at an experimental stage. As such they that have not, as yet, spawned much in the way of applications to ‘real’ samples. However, the techniques of TLC–FAB-MS and TLC–LSI-MS (and MS–MS) have been more widely applied. Thus applications of TLC–FAB-MS-MS include the identification of natural products, non-steroidal, anti-inflammatory and antipyretic drugs; drug metabolites such as sulphate, glucuronide and glycine conjugates and drugs of abuse have been described. Applications up to 1996 have been reviewed [4], however, then since then a number of new studies have been performed, described below, which illustrate the capabilities of this type of TLC–MS.

Thus, a recent example of the ‘traditional’ scrape and elute method combined with LSI-MS is provided by studies on mixtures of organic sulphonium salts [15]. In this work the appropriate bands of analyte (approximate area of each band was ca. 1.5 cm^2)

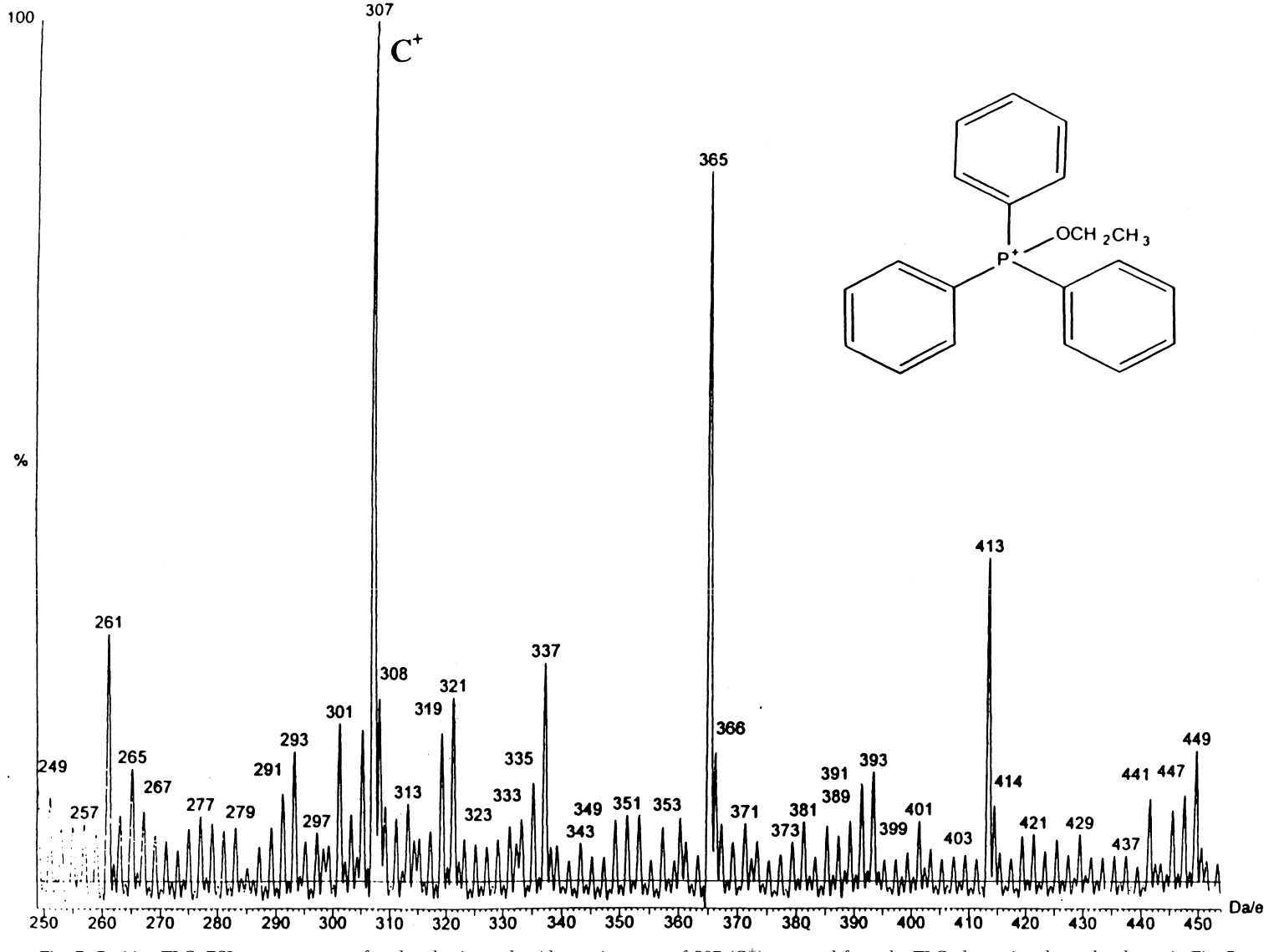

Fig. 7. Positive TLC–ESI mass spectrum of a phosphonium salt with a cation mass of 307 (C^+) extracted from the TLC plate using the probe shown in Fig. 5.

were removed from the plate and the analytes recovered from the silica gel using 1 ml of methanol. The extracts were then concentrated to 500 μl and 5 μl, applied to the probe tip and mixed with *m*-NBA as the matrix, the spectra were then obtained using liquid secondary ion (LSI) mass spectrometry. These LSI spectra were obtained on a VG 70SEQ hybrid mass spectrometer of EBqQ geometry. As well as LSI spectra the authors also obtained electrospray ionisation (ESI) MS data via the direct injection of the concentrated methanol extract into the ion source of a VG-Quattro II instrument. Representative positive ion LSI-MS and ESI-MS spectra are shown for one of these compounds in Fig. 8. In the LSI

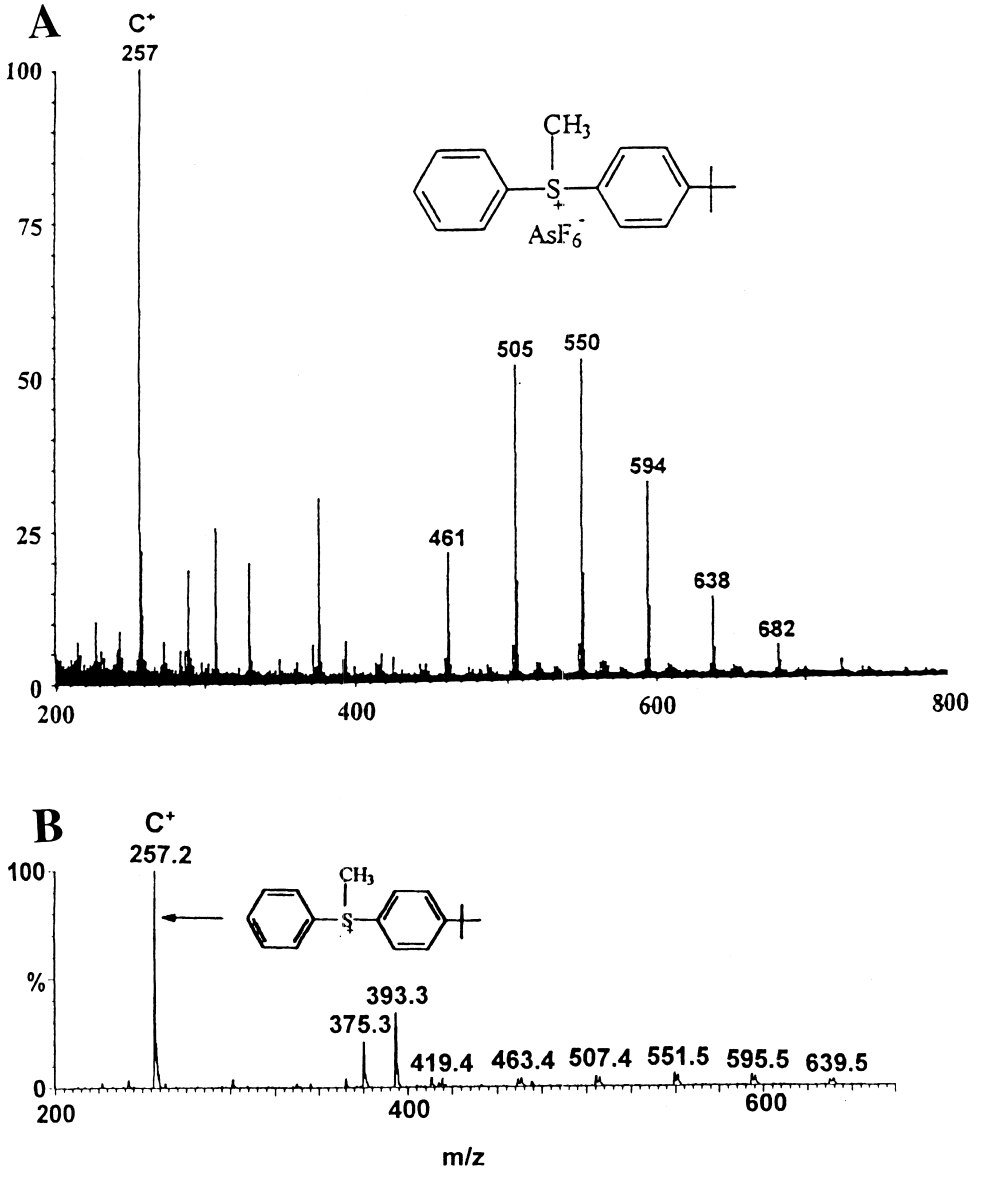

Fig. 8. Spectra obtained from a band on a TLC plate obtained from a mixture of sulphonium salts: (a) the positive LSI mass spectrum and (b) the positive ESI spectrum (from Ref. [15]).

spectrum (Fig. 8a) there are a range of ions in addition to those in the analyte. These correspond to the *m*-NBA matrix and the polymeric binder used to prepare the plates. This illustrates one potential problem faced in TLC–MS, which is that background and matrix peaks can be large and may indeed obscure those of the analyte (especially when the latter is present in low concentration). As will be illustrated below, by using MS–MS techniques such problems can be circumvented. In contrast the ESI-MS data (Fig. 8b) does not suffer to the same extent from background problems and the two ions at m/z 375 and 393, which are present in addition to the ion at m/z 257 for the analyte, are due to related structures present as impurities.

The use of this straightforward scrape and elute TLC–LSI-MS method for the analysis of organic sulphonium salt mixtures is, self evidently, fit for its intended purpose, and there is no doubt that much can be achieved with simple TLC–MS. However, the advantages of using tandem mass spectrometric (MS–MS) techniques are just as valid in the case of TLC as in other areas, enabling compounds to be identified with a much greater degree of certainty than with FAB-MS alone. Recent examples of the use of TLC–MS–MS have concentrated on the identification of drugs (or metabolites) in urine extracts [16–19]. Thus ibuprofen and paracetamol were unequivocally identified in urine samples after normal therapeutic doses following alkaline or enzymic hydrolysis of conjugates to liberate the aglycones, using solid phase extraction (SPE) and TLC separation on Diol-bonded HPTLC plates [16]. In the same study the aspirin metabolite salicylhippuric acid was also identified by FAB-MS–MS using the same Diol-HPTLC system. In this study urine samples were obtained for the period of 0–3 h following the administration of either 500 mg of paracetamol, 600 mg of aspirin or 400 mg of ibuprofen (all normal therapeutic doses). Ester glucuronide conjugates in the ibuprofen metabolite-containing urine were hydrolysed with alkali (1 *M* NaOH, pH 10), whilst sulphate and glucuronide metabolites of paracetamol were hydrolysed with hydrolytic enzymes (*Helix pomatia*). The aspirin metabolite-containing urine was not subjected to any sample pretreatment prior to SPE. Extraction was performed on a C_{18} bonded phase and the resulting methanolic eluates were reduced to dryness under a stream of nitrogen. The concentrated extracts were then redissolved in methanol (100 μl) for application to the TLC plates. TLC was performed on both silica gel and Diol-bonded silica gel HPTLC plates using chloroform–methanol (90:10, v/v). In general the Diol phase gave better band shapes with less tailing for the biological extracts than silica gel and was therefore more useful in this application. However, mass spectrometry was equally satisfactory from either silica or Diol phases.

MS and MS–MS data were acquired with a VG Analytical ZAB–HSQ tandem mass spectrometer of BEqQ geometry. All samples were ionised by FAB using fast xenon atoms at an accelerated potential of 8 kV. MS–MS data were acquired at a collision energy of 10–20 eV. As discussed earlier it is sometimes useful to concentrate the analytes on the plate prior to removal of the phase and the method of Oka et al. [6], was used in this instance. A quantity of the appropriate FAB matrix (glycerol or *m*-NBA+TFA) was then applied to the top 0.5–1 mm of analyte-containing stationary phase in order to moisten it. This zone was then carefully removed and applied to the target of the FAB probe where it was slurried with a further quantity of matrix and 2 μl of 1% TFA in methanol.

In the case of standards, with 1–2 μg of material on the plate, detectable molecular ions were produced. However, the spectra obtained with FAB-MS alone were complex due to ions resulting from matrix adduct ions, together with ions from other components which presumably represent coeluting impurities (e.g. from the solvents used for chromatography) or substances present in the stationary phase (binders etc.). Thus, whilst the M^+ for the analytes was obviously present it was by no means the dominant ion. Although, adequate for the identification of pure standards such data are not convincing in the case of unknowns present in biological fluids. Thus in Fig. 9a, the result of the TLC–FAB-MS of the paracetamol urine shows the region for the $[M+H]^+$ of the analyte (152 amu) in the spectrum. Clearly the $[M+H]^+$ for paracetamol is not prominent, providing a powerful practical argument for using tandem MS techniques. Indeed when FAB-MS–MS was applied to analyse the urine extract for which the FAB-MS spectrum is shown in Fig. 10a

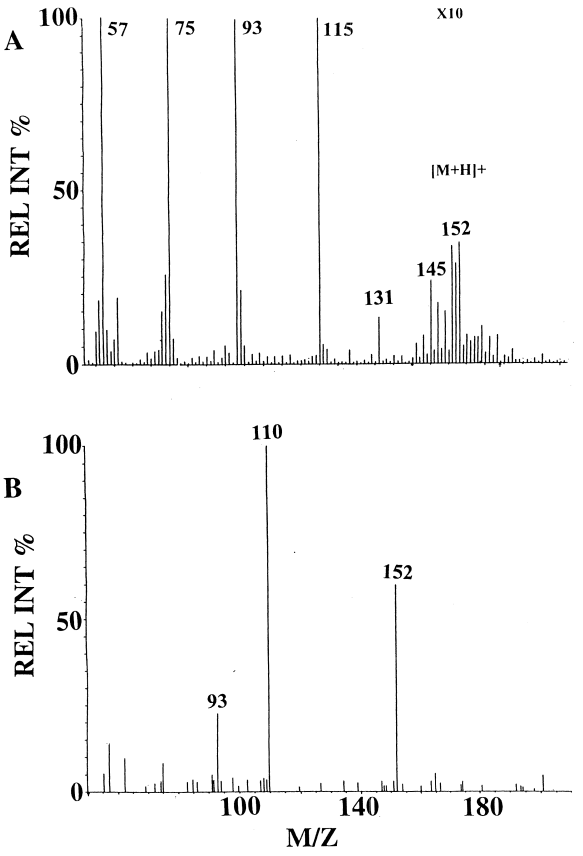

Fig. 9. Spectra obtained for paracetamol following TLC of an urine extract: (a) positive FAB-MS and (b) FAB-MS–MS spectra.

the result was the spectrum shown in Fig. 9b. This spectrum is identical to the reference standard of the drug.

This work has now been extended to drugs of abuse with an application to morphine in urine extracts [17]. In this work urine samples were collected from clients receiving methadone replacement therapy. The samples were screened for opiates using an enzyme immunoassay (EIA). Confirmation was by reversed-phase HPLC with electrochemical detection, HPTLC on silica gel and capillary GC with nitrogen-phosphorous detection. HPLTC–MS–MS was performed on an extract, obtained by SPE using 10×10 cm glass backed silica gel HPTLC plates containing a fluorescent indicator activated for 1 h at 120°C before use. Following sample application, the plates were developed in ethyl acetate–

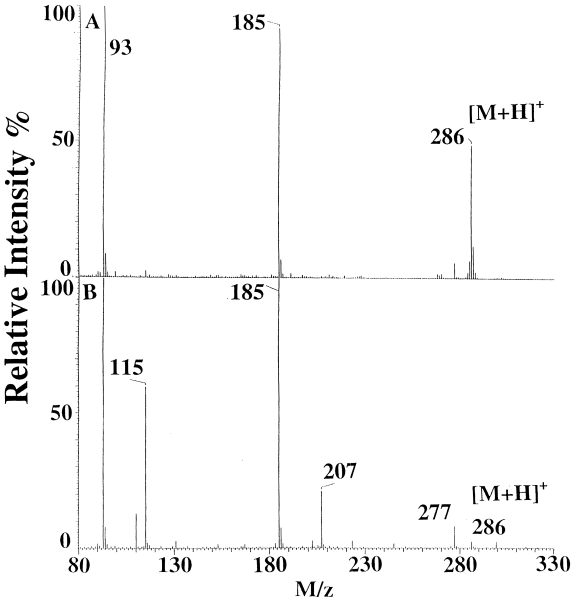

Fig. 10. Spectra obtained for morphine from silcia gel TLC plates: (a) a standard and (b) a sample obtained from TLC of a urine extract.

methanol–concentrated aqueous ammonia (8:1.3:0.4, v/v) for 4 cm, after which the plates were air dried and then developed in ethyl acetate–methanol–concentrated aqueous ammonia (8:0.2:0.05, v/v) for 5 cm. After development the spots were visualised under UV light at 254 nm and MS and MS–MS data were acquired as for the analgesics described above.

HPTLC–FAB-MS of a morphine standard gave the result shown in Fig. 10a. The $[M+H]^+$ ion for morphine was clearly visible in the FAB-MS spectrum at m/z 286, with ions at 93 and 185 amu due to the matrix. Such a result, used together with the R_F from the TLC separation, might be adequate in confirming the identity of a pure standard or a spiked urine. However, the equivalent results seen with the urine extract (Fig. 10b) are much less convincing. Thus, whilst it is possible to detect an ion at 286 amu corresponding to the $[M+H]^+$ of morphine it is weak compared to the ions from the matix components and an unequivocal identification of the analyte by HPTLC–MS alone is not possible for this extract. However, with HPTLC–MS–MS more convincing data can be obtained. Thus HPTLC–FAB-MS–MS for a morphine standard gave the spectrum

shown in Fig. 11a. When the urine sample was interrogated using MS–MS a similar result was obtained (Fig. 11b). Whilst the relative intensities of the various ions are not identical to that of the standard, the overall 'fingerprint' is qualitatively the same, and would provide the basis for a rather more confident identification. This study was the first application of HPTLC–MS–MS without substance elution to the detection and identification of a drug of abuse in urine and shows the possible applications for HPTLC–MS–MS as a rapid and technically undemanding means of confirmation of identity for drugs of abuse.

Further recent examples involving the use of TLC–FAB-MS and MS–MS are provided by two studies [18,19] on the new anxiolytic drug deramciclane ((1*R*,2*S*,4*R*)-(−)*N*,*N*-dimethyl-2[(1,7,7-trimethyl-2-phenylbiciclol-2,2,1-hept-2yl)oxy]ethanamine-2-(E)-butanediote. In the first of these studies radiolabelled metabolites were obtained by in vitro incubations with hepatocytes with sample of the incubation medium and the cell themselves examined. Separation was then achieved using 20×20 cm plastic backed silica gel TLC plates with butanol–acetic acid–water (4:1:1, v/v) as the mobile phase. The metabolites were detected following separation using a Berthold LB 287 Digital Autoradiograph and the appropriate zones were removed from the plate with a standard stationary paper punch, giving an approximately 3 mm diameter sample. This was attached to the circular target area (also 3 mm) of the FAB probe using double-sided adhesive tape. The spots were then treated with a mixture of glycerol–methanol–acetic acid (66:33:1) as the matrix (the methanol was added to reduce the viscosity of the glycerol). FAB-MS and MS–MS were then performed using a VG-ZAB-2SEQ mass spectrometer with ionisation achieved with a beam of 30 kV Cs^+ ions. For MS–MS experiments argon was used as the collision gas. Using this approach the authors were able to obtain spectra for the unchanged parent compound and a number of metabolites including the previously identified *N*-desmethylderamciclane. Detection limits of ca. 100 ng/spot for MS and 200 ng/spot for MS–MS were claimed.

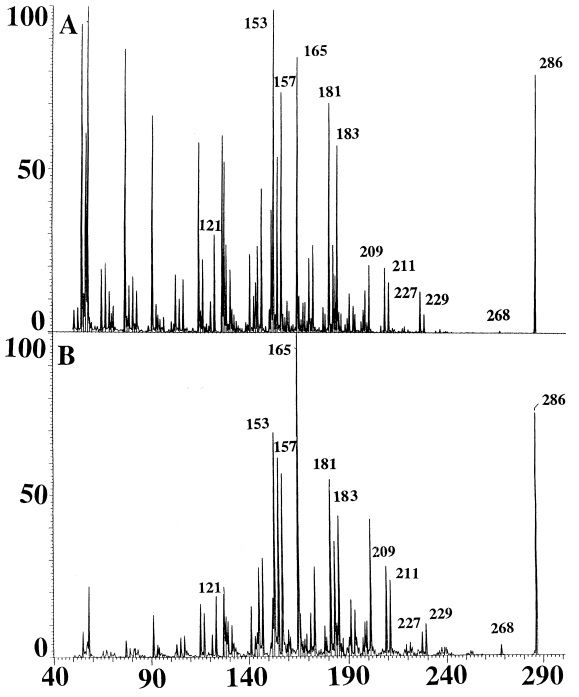

Fig. 11. MS–MS spectra of the morphine samples shown in Fig. 10: (a) MS–MS of the standard and (b) MS–MS of the urine extract.

In the second study [19], samples of blood plasma and urine were obtained from male beagle dogs to which the radiolabelled (either ^3H or ^{14}C) deramciclane had been orally administered at a dose of 10 mg/kg. Urine and plasma samples were concentrated and partially purified by solid-phase extraction and then analysed using overpressured-layer chromatography (OPLC) on silica gel. Metabolites were either eluted from the plate using an on-line system and fractions collected, or following development, the radioactive bands were located, as in the previous study, using a digital autoradiograph. Selected fractions obtained by on-line collection were also subjected to a further OPLC separation. The authors were able to obtain TLC–MS and MS–MS data on some of the metabolites and make some deductions about the metabolism of the compound in vivo. The MS–MS data for the example described here (a hydroxylated metabolite) were obtained on spots containing ca. 5–10 μg of material.

5. Further possibilities for the coupling of TLC with spectroscopy

Although this review is concerned with the combination of TLC with MS there are other spectroscopic

techniques which can be used in combination with this form of chromatography (reviewed in Ref. [3]), both in the 'scrape and elute' mode and in situ. If hyphenation is taken as meaning combining a chromatographic separation with a spectroscopy then it should be remembered that with a modern scanning spectrodensitometer that it is very easy to obtain good quality UV spectra from spots or bands on TLC plates. In addition it is possible to obtain FT-IR, Raman and surface enhanced resonance Raman (SERRS) spectra (the latter with very high sensitivity) [20]. There has also been a demonstration of the possibility of obtaining ^{1}H NMR spectra from analytes via 'magic angle' NMR [21]. Clearly it should be possible, given the non-destructive nature of many of these techniques, to use them to complement the information provided by TLC–MS by performing multiple experiments on the same spot or band. This would be analogous to the experiments currently appearing in the literature concerned with multiple hyphenated liquid chromatographic systems where HPLC–NMR–MS [22] or HPLC–NMR–IR [23] systems have been demonstrated.

6. Future developments in TLC–MS

Given the pre-eminence of HPLC and the enormous technical advances that have occurred in the area of HPLC–MS, there can be little doubt that this technology will be the dominant hyphenated technique for the first part of the 21st century. Equally, there seems little doubt that no matter what advances occur in the hyphenation of planar chromatographic techniques they will remain essentially a niche application compared to HPLC-based methods. However, this is not to say that TLC–MS and related techniques cannot perform a valuable service for the analyst. Thus, TLC–MS can be applied to a very wide range of analytes (at least as wide as HPLC-based methods) and to samples that might well be considered quite unsuitable for other separation techniques. The advantages of TLC-based methods outlined at the beginning of this review are those which distinguish planar techniques from column-based methodologies and these will not change.

What then are the barriers to the more widespread use of TLC–MS? One of the reasons that TLC–MS is unlikely to become more widely used is the general lack of commercially available interfaces suitable for the most popular mass spectrometers. This represents a real problem for TLC–MS as, in the absence of suitable interfaces, any possibility of automation and high throughput will remain out of reach. The ease of implementation of the simple 'manual' methods will not compensate for an inability to make them less tedious when faced with large numbers of samples which will be much more easily handled by column-based methods. However, given the low regard in which planar chromatographic techniques are generally held, it is an opinion that is unlikely to change in the near future and the prospect of manufacturers investing in the development of commercial interfaces looks bleak. It will therefore be necessary where no suitable interface exists for those wishing to use them to construct them themselves and this will of necessity restrict the use of such techniques to a few groups.

Of course it is possible that technical developments in other areas will result in the production of the required technology. Thus, it is possible to envisage that demands led by the current interest in proteomics and the consequent need to identify proteins separated by 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE) may result in a mass spectrometer designed to scan gels. Such a development might well make TLC-based methods of great interest to groups engaged in drug discovery using high throughput screening, combinatorial chemistry or other such techniques. The technical challenges in producing such a device are by no means trivial, but if successful such a device should be readily adapted to TLC. The use of MS with planar electrophoresis has been reviewed [24].

Are there any other methods for linking planar separations with mass spectrometry that might provide a way forward?. One area that remains underexploited in this respect is in the linking of forced flow planar chromatographic (FFPC) methods (see Poole [25]) particularly over pressure planar liquid chromatography (OPLC)-based methods to mass spectrometry. Given the numerous well developed HPLC–MS interfaces that are currently available the hyphenation of FFPC is simply a matter of 'plumbing' and should cause no real technical difficulties. Presumably the lack of publications in this area reflects the relatively small number of users of FFPC. Similarly it should be possible to link 'electri-

cally driven' separations to mass spectrometer interfaces designed for use with capillary electrophoresis/electrochromatography. Research in the area of electrically driven planar chromatography [26] is, however, still in its infancy compared to the column-based techniques and it may be several years before this possibility can be fully investigated.

In conclusion, TLC–MS has a long history and has considerable potential as a problem solving technique. The coupling of planar chromatography to mass spectrometry is readily achieved at a range of levels of sophistication from the very simple 'scrape and elute' approach to specialised interfaces. However, no matter what the mechanism for the transfer of the separation achieved on the plate to the mass spectrometer the resulting mass spectra can be obtained with high quality and good sensitivity. Based on the literature examples the range of analytes on which the TLC–MS approach can be used seems to be at least as wide as that encompassed by HPLC–MS, but the lack of suitable sample introduction methods currently limits the application of the approach.

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