Journal of Chromatography, 554 (1991) 3–26
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2220

Strategies in developing interfaces for coupling liquid chromatography and mass spectrometry

W. M. A. NIESSEN* and U. R. TJADEN

Division of Analytical Chemistry, Center for Bio-Pharmaceutical Sciences, P.O. Box 9502, 2300 RA Leiden (Netherlands)

and

J. VAN DER GREEF

Division of Analytical Chemistry, Center for Bio-Pharmaceutical Sciences, P.O. Box 9502, 2300 RA Leiden and TNO–CIVO Institutes, P.O. Box 360, 3700 AJ Zeist (Netherlands)

ABSTRACT

Considerable progress has been made over the last 20 years in coupling liquid chromatography and mass spectrometry. A large number of interfaces have been developed and, although only a few have found widespread application, the developments in interfacing are worth reviewing. The developments in interfacing are categorized along three lines: efforts to introduce 1 ml/min of an aqueous mobile phase into the mass spectrometer vacuum system, efforts to achieve analyte enrichment in the interface and efforts to combine interfacing and liquid-based soft ionization techniques. These lines are explored, with special attention being given to the most successful interfaces. Some similarities between the various interfaces are revealed.

INTRODUCTION

The on-line combination of liquid chromatography and mass spectrometry (LC–MS) has been under investigation for about 20 years. The first paper on LC–MS came from the Russian group of Tal'roze *et al.* [1] in 1972. The development of LC–MS has continued via various landmarks and led to a vast number of papers on successful applications that have appeared during the last few years and to several successful and commercially available LC–MS interfaces. The subject has been extensively reviewed [2–6]. This paper can also be considered as a review on LC–MS, but attention is especially focused on the developments in interface technology. In that respect, it is more or less a free interpretation of the history of LC–MS.

In developing on-line LC–MS three fundamental compatibility problems had to be solved, *viz.*, the amount of solvent eluting from the LC column, the composition of the LC mobile phase and the nature of the analytes. Interfaces have been developed to be placed between the LC column outlet and the mass analyser to solve the incompatibility between the LC solvent and the MS high vacuum. Over 25 different interface approaches have been described (see Table I). In the development of these

0021-9673/91/$03.50

TABLE I

HISTORICAL OVERVIEW OF LC–MS INTERFACES

Important features of the various interfaces, *i.e.*, nebulization, analyte enrichment and liquid-based ionization, are indicated.

Year	Type	Nebulization	Enrichment	Liquid-based ionization
1972	Stopped flow		+	
1972	Capillary inlet			
1974	Moving belt	±	+	
1974	Pneumatic nebulizer in atmospheric-pressure source	+		
1975	Solvent vapour separator membrane		+	
1976	Liquid ion evaporation	+		+
1978	Crossed beam	+		
1978	Jet separator		+	
1979	Vacuum nebulizer	+	+	
1980	Direct liquid introduction	+		±
1981	Conical tip	+		
1981	Continuous sample preconcentrator	+	+	
1982	Rotating disk		+	
1983	Helium	+		
1983	Thermospray	+		+
1984	Monodisperse aerosol generator	+	+	
1985	Electrospray	+		+
1985	Frit FAB			+
1986	Continuous-flow FAB			+
1986	Particle beam	+	+	
1986	Heated nebulizer	+		±
1987	Thermabeam	+	+	
1987	Gas nebulized	+		
1987	Ionspray	+		+
1988	Atmospheric-pressure spray	+		+
1989	Frit-EI/CI			

LC–MS interfaces over the past 20 years three development lines can be distinguished, which are concerned with efforts (i) to introduce 1 ml/min of an aqueous mobile phase into the MS vacuum system, (ii) to achieve analyte enrichment in the interface and (iii) to develop liquid-based ionization techniques. These lines are explored and discussed below. As a result, similarities in different LC–MS interfaces are recognized and the possibilities and limitations of the various LC–MS interfaces become clearer and can more readily be explained.

AN INTERFACE FOR LC–MS

The most important feature of an LC–MS interface is to provide a means for the transition of analyte molecules from the liquid state to the high vacuum of the mass spectrometer. During this transition as much analyte as possible must be transferred, while the mobile phase constituents can be partially or completely removed. In

this respect, the LC–MS interface can be characterized by the transfer efficiency and the enrichment factor. The transfer efficiency Y is defined as

$$Y = (Q_{MS}/Q_{LC}) \cdot 100\% \quad (1)$$

where Q_{LC} and Q_{MS} are the amount of analyte eluting from LC column and the amount introduced into the mass spectrometer, respectively. Note that the amount of analyte introduced into the MS system is difficult to define unambigiously, because with some interfaces the analyte is transferred as neutral molecules that are subsequently ionized, whereas with other interfaces ionization is an integral part of the transfer step and the ions are preferentially extracted from the interface.

The enrichment factor E is the ratio of the analyte concentration in the MS flow and that in the LC flow, defined as

$$E = (Q_{MS}/F_{MS})/(Q_{LC}/F_{LC}) \quad (2)$$

where F_{LC} and F_{MS} are the flow-rate from the LC column and the flow-rate introduced into the mass spectrometer, respectively. Again, F_{MS} is sometimes difficult to define, because of the features of the interface. For some interfaces these aspects are discussed in more detail below.

The mass spectrometer is a mass-flow-sensitive detector, which means that the response R is directly proportional to the mass flow, dm/dt:

$$R = \tau(dm/dt) \quad (3)$$

where τ is the response factor. As the mass flow is the change of mass per unit time, eqn. 3 can be written as

$$R = \tau\, C(t)\, F_{MS} \quad (4)$$

where $C(t)$ is the analyte concentration profile *e.g.*, a nearly Gaussian peak from the LC column, and

$$F_{MS} = F_{LC}\, S \quad (5)$$

where S is the splitting ratio. It is important to appreciate some of the features of this equation. As under normal LC–MS conditions F_{MS} is constant, the response is directly proportional to the analyte concentration:

$$R = \tau'\, C(t) \quad (6)$$

where τ' is the response factor multiplied by the constant factor $F_{LC}S$. This conclusion seems obvious, but the linearity in eqn. 6 can be wrongly interpreted in the sense that the mass spectrometer under certain conditions act as a concentration-sensitive device. LC–MS experiments in which the flow-rate F_{MS} is varied cannot readily be

performed. With most interfaces the range of possible flow-rate variation is narrow and, more important, a change in F_{MS} results in a change in the pressure conditions in the source, which in turn influences the ionization efficiency, the ion extraction efficiency and via the pressure in the analyser the ion transmission, *i.e.*, not only the flow-rate varies but also the response factor changes.

The extraction of ions from the source may complicate the evaluation of the influence of the flow-rate and the amount injected on the response. With various ionization techniques operative in atmospheric-pressure ion sources, for instance, the ions produced are preferentially sampled into the mass analyser. However, the ion extraction efficiency is not directly proportional to the concentration of ions in the source over a wide range, which may be due to space charging and possibly some other effects. As is discussed in more detail below, the ion sampling system of atmospheric-pressure ion sources act as an analyte-enrichment interface.

In real-life analytical applications it is generally the analyte concentration in the sample that is the key factor. In selecting the separation conditions for a particular problem to be solved with a particular LC–MS interface, optimum tuning of flow-rate, column diameter and separation efficiency is needed in order to achieve the highest mass flow to the mass spectrometer.

INTRODUCTION OF 1 ml/min OF LIQUID INTO A VACUUM

In this section the various attempts to develop an LC–MS interface that allows the direct introduction of 1 ml/min of an aqueous mobile phase into the MS vacuum system are reviewed.

Obviously, the most straightforward way of coupling LC and MS is by inserting the column outlet capillary directly into the MS vacuum system. This approach has been theoretically and experimentally explored by Tal'roze and co-workers [1,7,8]. In order to cope with the limited pumping capacity of a conventional MS vacuum system, only part of the column effluent could be introduced (typically 1%, *i.e.*, the maximum solvent load to the mass spectrometer is 10 μl/min). As a consequence, for this interface no analyte enrichment takes place ($E = 1$) and the maximum transfer efficiency is 1%, as Q_{MS} can be written as

$$Q_{MS} = S\,Q_{LC} \tag{7}$$

($S = 0.01$ for the capillary inlet). The effective transfer efficiency is analyte dependent (see below).

The outlet of the capillary is heated in order to provide heat for the evaporation of the solvent. This interface is called a capillary inlet interface (see Fig. 1), although the confusing term "direct liquid introduction" is also often used. There is no LC–MS interface that is theoretically so well understood as the capillary inlet. This is a result of the work of Tal'roze and co-workers [1,7,8], Arpino and co-workers [9,10] and Bruins and Drenth [11]. Prototypes of the capillary inlet interface have been described by at least fourteen different groups, but no commercial capillary inlet interface is available. The capillary inlet interfaces have been used in combination with various LC column types, *i.e.*, open bed, conventional packed, microbore, packed microcapillary and open-tubular columns.

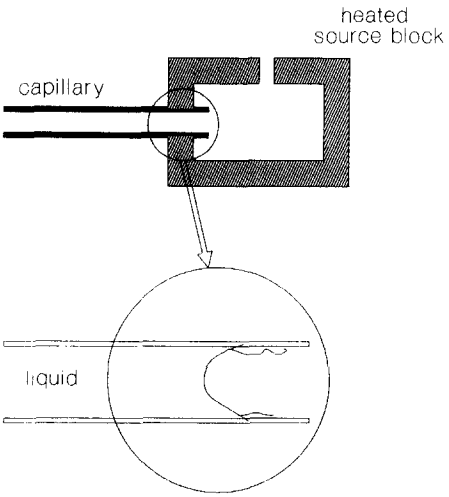

Fig. 1. Schematic diagram of the capillary inlet interface. The enlargement shows the general problem of the capillary inlet interface in the analysis of less volatile analytes.

From the theoretical understanding of the interface, the limitations of the capillary inlet are readily indicated. The position of the liquid–vapour transition depends on the solvent properties, the temperature and the tube inside diameter. For a homogeneously heated capillary, Arpino *et al.* [9] derived an equation to calculate this position in terms of the ratio of the length l of the capillary possessed by the vapour and the length $(L-l)$ of the capillary occupied by the liquid:

$$\frac{l}{L-l} = \frac{M\,\eta_l(P_v^2 - P_s^2)}{2\,\rho\,R\,T\,\eta_g[(P_i - P_v) + 4\,\gamma/d_c]} \tag{8}$$

where L is the length of the capillary, M is the molecular weight of the liquid (in kg/mol), η_l the viscosity of the liquid, P_v the saturated vapour pressure of the liquid, P_s is the ion source pressure, η_g the viscosity of the vapour, P_i the inlet pressure, γ the surface tension of the liquid and d_c is the inside diameter of the capillary. As the vapour pressure of common LC solvents is considerably higher than common ion source pressures, the length l will always be larger than zero, which means that in practical situations the evaporation of the eluent will always take place inside the capillary. The volatile solvent evaporates whereas non-volatile impurities in the liquid stream precipitate at the inner wall of the capillary (see Fig. 1). The most important group of non-volatile "impurities" in the effluent stream is the analytes of interest. As a result, the application of the capillary inlet interface is restricted to fairly volatile analytes that are generally amenable to gas chromatographic (GC)–MS analysis.

The problem with the analyte evaporation can be avoided by the use of nebulization methods: the eluent is dispersed as an aerosol in either an atmospheric- or a reduced-pressure chamber, and the droplets are desolvated as a result of the more efficient evaporation of the solvent from the greatly enlarged surface area of the liquid created by the aerosol. The analyte molecules are transferred essentially unheated to

the gas phase, where they can be subjected to a gas-phase ionization method, *e.g.*, chemical ionization (CI), or where ions are produced by ion evaporation (IEV, see below).

Various nebulization methods have been explored for their potential in LC–MS coupling (see Table II). Ultrasonic nebulizers, where a piezoelectric crystal has been used to impose artificial instabilities on the liquid jet produced at a diaphragm, have been explored by Christensen *et al.* [12] and by Willoughby [13]. No significant improvement relative to conventional DLI (see below) was observed. Electrospray, where a solvent is nebulized as a result of charging the liquid by means of applying a potential difference, typically 3 kV, between the capillary and a counter electrode, appears to be limited to low flow-rates, *i.e.*, below 10 μl/min.

Pneumatic nebulization (PN) is the best-know nebulization technique; it is also used in other analytical techniques, such as atomic absorption and emission spectrometry. For LC–MS, PN has first been used in atmospheric-pressure (AP) ion sources to study AP-CI [14] and AP-IEV [15]. Later, various PN systems in reduced-pressure regions were described, where the nebulization is performed either directly into the ion source [16–18] or an additional pumping stage is applied between the nebulizer and the ion source [19,20]. The latter interface is also called a vacuum nebulizer (see Fig. 2b).

The disintegration of a liquid jet has only been explored in reduced-pressure regions. The use of a disintegrating liquid jet to transfer non-volatile analytes from the liquid to the gas phase was first described by Melera [21] and subsequently further developed by Arpino and co-workers [9,10]. The liquid jet is formed at a diaphragm with a 2–5-μm pinhole. The minimum flow-rate $F_{jet,min}$ at which a stable liquid jet is formed at a diaphragm is given by

$$F_{jet,min} = \frac{\pi d_{jet}^2}{4}\left(\frac{8\gamma}{\rho d_{jet}}\right)^{1/2} = 0.015\, d_{jet}^{3/2} \tag{9}$$

TABLE II

NEBULIZATION TECHNIQUES APPLIED IN LC–MS

Technique	Abbreviation
Disintegration of a liquid jet	DLI
Ultrasonic nebulization	
Pneumatic nebulization	PN
Applied in: medium-pressure ion sources	
atmospheric-pressure ion sources	
vacuum nebulizers	
effluent deposition in moving-belt interfacing	
particle-beam interface	
ion-evaporation system	
ionspray interface	
Electrical atomization	ES
Thermal nebulization	TS
Combinations of these techniques	

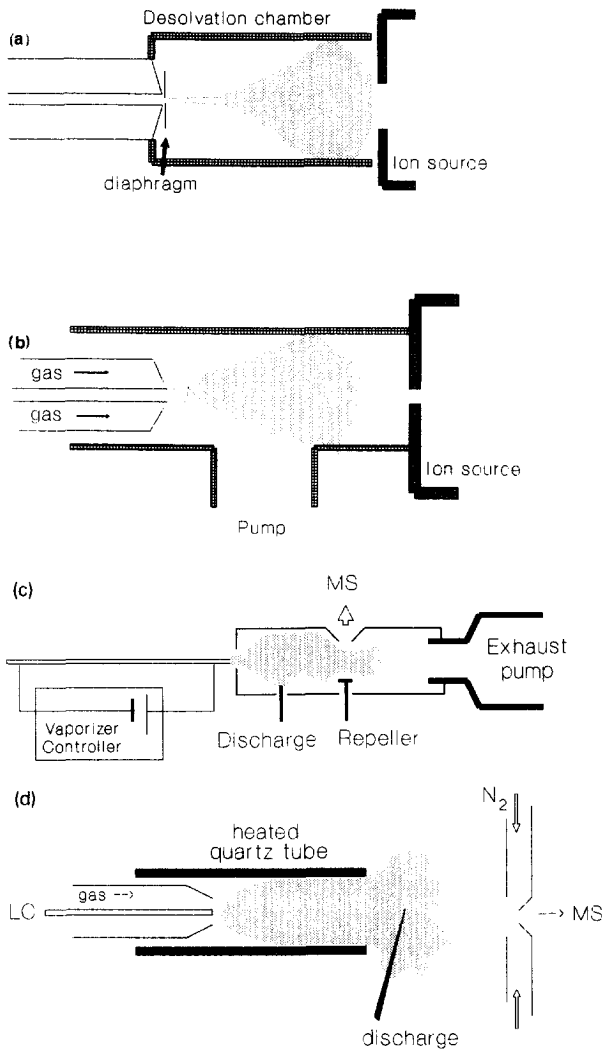

Fig. 2. Various nebulizer interfaces for LC–MS. (b) Vacuum nebulizer: a pneumatic nebulizer with an additional heated pumping stage between the nebulizer and the ion source. (a) Direct liquid introduction: a liquid jet nebulizer with a heated desolvation chamber between the nebulizer and the ion source. (c) Thermospray: a thermal nebulizer with an exhaust pump line containing a liquid nitrogen trap at the ion source opposite to the nebulizer. (d) Heated nebulizer: pneumatic nebulizer with a heated quartz desolvation chamber in an atmospheric pressure ion source, also showing the special ion sampling system with the nitrogen curtain gas.

The right-hand side of eqn. 9 is valid for solvents commonly used in reversed-phase LC. The droplets are typically twice the diaphragm diameter in size [22]. They are subsequently desolvated in a heated desolvation chamber and the gas mixture is mass analyzed using CI with the solvent as the reagent gas. This LC–MS interface is known as the direct liquid introduction (DLI) system (see Fig. 2a); it is commercially available from various manufacturers. Usually, 10–50 μl/min of liquid are introduced,

necessitating the use of a split in combination with conventional LC columns (splitting ratio 0.01–0.05). The most important drawback of the DLI system is frequent clogging of the pinhole [23,24].

It is interesting to note the similarities between the DLI [9,10,23,24] and the PN interface [16–20]. With both systems the nebulization is performed in a reduced-pressure region. In most instances, a heated desolvation chamber is used to achieve heat transfer to the droplets in the low-pressure vapour phase. Analyte ionization is achieved by solvent-mediated CI. It has been demonstrated by Arpino and Beaugrand [10] for DLI, but it also holds for the PN, that the maximum amount of liquid that can be introduced is limited to *ca.* 100 μl/min. This upper limit is not limited by the pumping capacity of the MS vacuum system, as a vacuum system suitable for high liquid flow-rates was used for these experiments [10], but is actually the result of the inefficient heat transfer from the desolvation chamber wall to the droplets, *i.e.*, the limited heat conductance of the low-pressure vapour.

There are two ways to solve the problems with the heat transfer to the droplets, *i.e.*, nebulization in a heated atmospheric pressure region or preheating the liquid prior to the nebulization. Both approaches lead to additional problems. With the former approach the analytes have to be efficiently transferred from an atmospheric pressure region into the high vacuum region (see the next section). With the latter approach both efficient pumping and sufficient heating power must be provided. The high pumping efficiency needed with the latter approach is achieved by directly pumping the ion source by means of a mechanical pump equipped with a liquid nitrogen trap in the exhaust pump line.

Sufficient heat must be transferred to the liquid to achieve almost complete evaporation after the nebulization; evaporation inside the capillary should be avoided because of the risk of precipitation of non-volatiles (*cf.*, Fig. 1). Experiments performed by Arpino and Beaugrand [10] with preheating the liquid prior to liquid jet formation by means of a heated liquid recirculating through the DLI probe were not successful; insufficient heat was transferred in this way. A more successful approach resulted from experiments with the rapid evaporation of the column effluents as is achieved in thermospray [25]. To provide sufficient heating power the use of lasers and hydrogen flames was explored, but it later appeared that indirect or direct electrical heating of a narrow-bore (typically 0.15 mm I.D.) column outlet capillary also resulted in sufficiently rapid vaporization of the column effluent [25,26]. With the thermospray (TS) interface over 1 ml/min of an aqueous mobile phase can directly be introduced into the MS vacuum system without solvent evaporation problems due to heat-transfer limitations.

In the TS interface (see Fig. 2c) heat is transferred directly from the vaporizer capillary to the liquid. As a result, the liquid starts to evaporate inside the vaporizer, resulting in disruption of the liquid phase by the formation of vapour bubbles that rapidly expand owing to the high temperature. In this way the risk of analyte precipitation is greatly reduced because the liquid is efficiently kept away from the heated surface after the onset of the evaporation. A stream of vapour and superheated liquid droplets is produced, which traverses the ion source to the exhaust pump. Ions that are produced in the source may be sampled by a sampling cone and mass analysed. In practice, the amount of heat transferred to the liquid is sufficient for complete evaporation, but the kinetics of the vaporization are sufficiently slow that part of the liquid

emerges from the vaporizer as small droplets, which may act as carriers for the non-volatile analytes (see below). However, as these droplets consist of superheated liquid, problems may arise with the decomposition of thermolabile compounds, especially with those which readily undergo thermal solvolysis, *e.g.*, with compounds that contain a glycoside binding. As an example, the TS mass spectrum of a 5-methoxypodophyllotoxin-4-β-D-glycoside (mol.wt. 606) in Fig. 3b shows no protonated molecule, but an intense fragment ion resulting from the loss of the sugar group. Many other examples of thermal degradation effects can be found in literature.

Nevertheless, the TS interface has become the most widely used LC–MS interface [27,28]. Over 300 papers have appeared and the number of papers on TS actually exceeds the number of papers devoted to all other LC–MS interfaces.

Two interfaces used in an atmospheric-pressure ion source are closely related to TS, *viz.*, the heated nebulizer [29,30] and the atmospheric-pressure spray (APS) interface [31,32]. The heated nebulizer is a pneumatic nebulizer equipped with a heated quartz desolvation chamber; it is used in combination with a discharge electrode for AP-CI (see Fig. 2d). The APS interface consists of an indirectly electrically heated vaporizer; it is used with a discharge electrode for AP-CI and also in the buffer

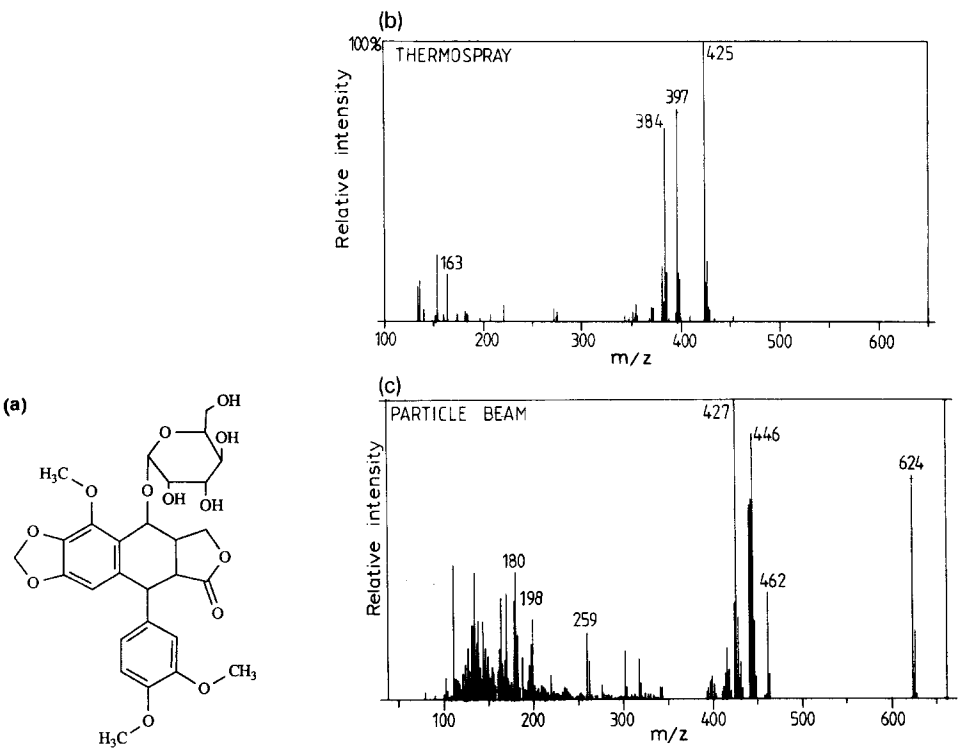

Fig. 3. (a) Structure of a 5-methoxypodophyllotoxin-4-β-D-glycoside (mol.wt. 606) and mass spectra obtained with (b) a thermospray and (c) a particle beam interface. Conditions: Finnigan MAT (San José, CA, USA) TSQ-70 instrument, equipped with either a Finnigan MAT thermospray interface or a Hewlett-Packard Model 59980A particle-beam interface (column bypass mode). Solvent, methanol–water (40:60) at 1.2 ml/min (thermospray) or 0.5 ml/min (particle beam). Thermospray in discharge-on mode (1 kV), vaporizer temperature 90°C, block temperature 230°C. Particle beam in ammonia CI mode, desolvation chamber 50°C, source temperature 250°C.

TABLE III

MAXIMUM ALLOWABLE FLOW-RATES OF SOME LC–MS INTERFACES

Interface	Flow-rate (μl/min)	Interface	Flow-rate (μl/min)
Capillary inlet	10	Ionspray	200
Direct liquid introduction	50	Particle beam	500
Pneumatic nebulizers in medium-pressure ion sources	50	Thermospray	2000
		Heated nebulizer (APCI)	2000
Electrospray	10	Atmospheric-pressure spray	2000

ionization mode (see below). Obviously, the TS interface itself can also be used in combination with a discharge electrode for AP-CI.

The TS interface, and the related heated nebulizer and the APS interfaces, are the end of the first line of development: how to introduce 1 ml/min of aqueous solvents into the MS vacuum system (see Table III).

ANALYTE ENRICHMENT: REMOVAL OF SOLVENT PRIOR TO THE MS VACUUM

The incompatibility problem with respect to the flow-rate, as discussed in the previous section, was also met in coupling packed GC columns with a mass spectrometer [33,34]. The interfaces developed for GC–MS, such as the Watson–Biemann fritted glass or effusion interface, the membrane interface and the jet separator, are based on gas-phase analyte enrichment. In the interface the ratio of the amounts of analyte and carrier gas are improved by selective removal of the carrier gas (*cf.*, eqn. 2). Typically 30–70% of the amount of analyte can be transferred from the GC column to the mass spectrometer with an enrichment factor of 10–20 [33,34].

Various analyte enrichment interfaces have also been developed for LC–MS, *e.g.*, the moving belt interface (MBI), the particle beam interface (PBI) and vacuum nebulizers (see Fig. 2b). The PBI resembles a jet separator for GC–MS. Very high enrichment factors have been achieved with the MBI and PBI, as essentially all the solvent is removed prior to introduction of the analytes into the mass spectrometer; the vacuum nebulizers are less efficient. However, the transfer efficiency of these interfaces is limited. The analyte-enrichment approach seems to be the most versatile means of LC–MS coupling, especially from the MS point of view. It makes LC and MS virtually independent and opens up the possibility of obtaining both CI and EI spectra of the analytes.

The approaches to analyte enrichment in MBI and PBI are widely different, although similar problems are met. With the MBI (see Fig. 4a) the column effluent is deposited on a continuously moving endless belt, from which the solvent is evaporated prior to the flash desorption of the analytes into the ion source [35,36]. The deposition of the eluent is most efficiently performed by means of either pneumatic or thermospray nebulization. The desorption of the analytes from the belt is mostly done by flash evaporation, although desorption by fast atom bombardment (FAB) directly from the belt has also been described [37,38]. After the flash evaporation, analyte ionization is performed by either EI or CI. The transfer efficiency in the flash

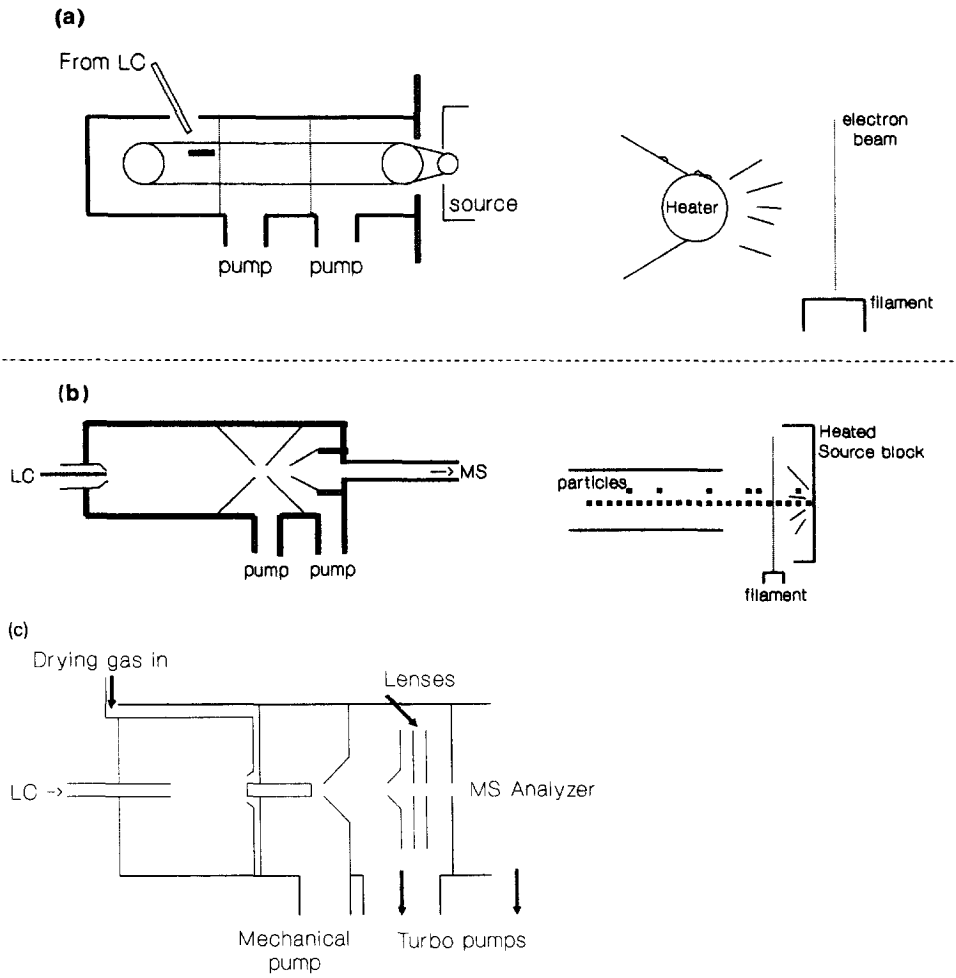

Fig. 4. Schematic diagram of some analyte-enrichment interfaces for LC–MS: (a) moving belt interface; (b) particle beam interface; (c) one-stage differentially pumped system for electrospray.

evaporation step in the ion source is determined by the analyte volatility and by interaction between the analyte and the belt surface [35,36,39]. Volatility-enhancing derivatization procedures, such as ion-pair formation or methylation [40], have successfully been applied to improve the detectability of less volatile analytes.

In the PBI (see Fig. 4b) the LC column effluent is nebulized by means of pneumatic or thermospray nebulization [41,42]. Droplet desolvation takes place in a slightly heated desolvation chamber which essentially is at atmospheric pressure. Helium is used for the nebulization, as it provides a high thermal conductivity. The non-volatile molecules nucleate to form sub-micrometer particles, which are subsequently separated from the solvent molecules in a two-stage momentum separator. The particles are transferred to the ion source, where they collide with the heated surface of the source and are volatilized. Subsequently, the analyte molecules are

ionized by EI or CI. Similarly to the situation in the MBI, the transition of the analyte from the liquid or solid state to the gas phase is thermally induced in the PBI. The potential of volatility-enhancing derivatization procedures to improve analyte detectability in PBI LC–MS has recently been explored [43].

From the description above, some corresponding features of the MBI and the PBI can be deduced. With both interfaces the nebulization takes place at atmospheric pressure. The solvent is subsequently removed completely prior to the introduction of the analyte molecules into the MS ion source. Although the heat transfer in the atmospheric-pressure desolvation chamber is improved relative to the medium-pressure chambers as used in vacuum nebulizers and DLI, the maximum flow-rate of aqueous solvents is still limited to *ca.* 0.5 ml/min, unless preheating of the solvent is applied, *i.e.*, unless thermospray nebulization is performed. The transition of the analyte molecules to the gas phase is thermally induced. As a consequence, the applicability range is limited to fairly volatile thermally stable compounds, although the short duration of the heating in the flash evaporation from the belt surface or at the ion source surface allows the analysis of compounds that are not amenable to GC analysis. As an example, the ammonia CI mass spectrum of a 5-methoxypodophyllotoxin-4-β-D-glycoside (mol.wt. 606) is given in Fig. 3c. With the PBI no thermal hydrolysis takes place; an intact ammoniated molecule is observed for this compound in addition to structure-informative fragment peaks. However, some of the fragments might be due to thermally induced reactions.

The universal or thermospray EI interface, recently developed by Vestec [44], is closely related to the PBI. It consists of a thermospray nebulizer, a spray chamber to remove the large droplets from the aerosol, a countercurrent gas diffusion membrane separator to remove most of the solvent molecules and a two-stage momentum separator (see Fig. 5). It thus combines various analyte enrichment approaches employed in GC–MS coupling.

An important aspect that should be addressed in relation to the MBI and PBI, but also to some other interfaces, *e.g.*, interfaces used in atmospheric-pressure sources, is the transition from an atmospheric-pressure chamber to the high-vacuum region. Three different approaches have been used. In the PBI and the MBI a two-stage differentially pumped system equipped with mechanical pumps is used. With the MBI the belt traverses a series of two narrow slits in the baffles between these pumping stages (Fig. 4a), whereas with the PBI molecular beam technology is applied in an arrangement with a nozzle and two skimmers (Fig. 4b). Several one-stage differentially pumped systems with a nozzle-skimmer system (see Fig. 4c) have also been described, *e.g.*, for electrospray interfaces [45,46]. Such a system can also be considered as a gas-phase analyte-enrichment system. In the third approach the ionization of the analytes also takes place in the atmospheric-pressure source. The ions are extracted from the source into the high-vacuum mass analyzer through a small sampling aperture (typically 25 μm I.D.) (see Fig. 2d) [15,29,47]. The analyzer region is efficiently pumped by means of a cryogenic pump [29]. In order to prevent clogging or contamination of the ion sampling aperture, and to provide additional droplet desolvation, a countercurrent flowing nitrogen curtain gas is applied around the sampling aperture (see Fig. 2d) [47]. The latter is also applied in the electrospray-type systems (*cf.* Fig. 4c).

In conclusion, conventional analyte-enrichment interfaces, *i.e.*, MBI and PBI,

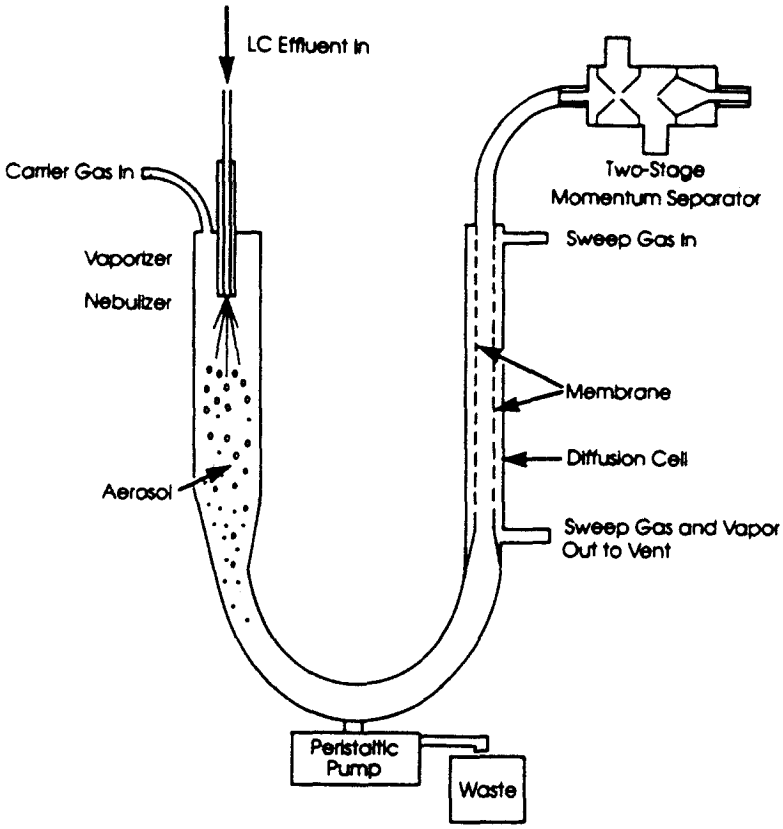

Fig. 5. Schematic diagram of the Vestec thermospray EI interface (reprinted with permission from Vestec, Houston, TX [44]).

are useful in providing EI and solvent-independent CI spectra for a limited range of compounds; the limitations are due to the necessity for volatilization of the analyte. The application range of MBI and PBI can be increased by applying other desorption techniques, as demonstrated by the experiments with FAB desorption in combination with MBI [37,38] and with PBI [48,49]. However, PBI and MBI with FAB ionization have not yet found widespread application.

WHY THE SOLVENT SHOULD NOT BE REMOVED IN LC–MS

In previous sections it was concluded that nebulization is an important step in the transfer of analytes and in the transition from the liquid phase to the gas phase. The early research on LC–MS was based on the assumption that ionization must follow the vaporization of the intact neutral molecules. By studying various liquid-phase ionization techniques, which at that time (1982) were mostly still in an early stage of development, Arpino and Guiochon [50] recognized the importance of the liquid in transferring the analytes and even assisting in the ionization. The ability of the DLI interface to transfer labile compounds to the gas phase, where they can be

ionized in ion–molecule reactions with the solvent as reagent gas (solvent-mediated CI), is believed to be strongly supported by the presence of the mobile phase [51]. The analyte molecules, preferentially present in the droplets, are softly desolvated and subjected to a desorption CI type of process [52]. In their review, entitled "Why the solvent should not be removed in LC–MS interfacing methods?" [50], Arpino and Guiochon concluded that preformed ions in solution can be transferred to the gas phase directly and mass analysed when sufficient energy is supplied to the liquid, *e.g.*, by means of particle bombardment (FAB, plasma desorption), laser radiation or electric fields. Vestal [53] later came to similar conclusions.

From this it may be concluded that solvent removal prior to ionization in the MS should not be complete, otherwise the liquid–gas transition must be thermally induced, limiting the applicability range as discussed for the PBI and the MBI in the previous section. For preformed ions in solution, *i.e.*, ionic compounds such as quaternary ammonium salts and compounds that readily undergo protolysis at acidic or basic functional groups, the nebulization may even result in additional liquid-based ionization techniques, such as FAB or IEV. The investigation and implementation of liquid-based ionization techniques in LC–MS is the third line of development; it is explored in more detail below.

LIQUID-BASED IONIZATION TECHNIQUES

Continuous-flow fast atom bombardment (CF-FAB) [54–56] is an implementation of FAB in LC–MS. The FAB matrix liquid, *e.g.*, glycerol, is added pre- or post-column to the LC mobile phase. Part of the eluent (5–15 μl/min) flows to the FAB target which is either a frit [54] or a modified FAB target [55]. As the liquid is bombarded at the target close to the outlet capillary, the actual mobile phase mixture acts as the FAB matrix solution. Subsequently, the evaporation of the volatile mobile phase constituents is achieved and the excess of glycerol is collected on a wick of compressed paper [57]. As a result of the dynamic nature and the composition of the FAB matrix at the CF-FAB target, various advantages over conventional FAB are observed, *e.g.*, improved detection limits, partly attributed to the reduction of matrix cluster ion intensities, and reduced ion suppression effects for mixtures of hydrophilic and hydrophobic peptides [56,58]. The CF-FAB technique appears to be limited with respect to the mass range of compounds that can be analysed. Whereas with conventional FAB mass spectra for peptides up to a molecular weight of 24 000 have been reported, the highest mass reported in CF-FAB is 5730 for bovine insulin with a low signal-to-noise ratio; in most reports compounds up to a molecular weight of *ca.* 2000 are analysed [56]. A clear explanation for this is lacking, but it most likely is a matrix related effect.

Other liquid-based ionization techniques are based on nebulization techniques, especially thermospray and electrospray. There is considerable debate on the mechanism of these techniques. The discussion here is mainly focused on TS.

The thermospray technique is not only a liquid introduction and nebulization technique, but also as a soft ionization technique [25,29]. This soft ionization method, called thermospray buffer ionization, is operative without a primary source of electrons when a volatile buffer, *e.g.*, ammonium acetate, is present in solution. The ionization mechanism is primarily explained by Vestal and co-workers [25,27,53] in

terms of ion evaporation (IEV) [15,60], while they suggest that some gas-phase ion–molecule reactions (CI) might also be involved [27]. Besides TS buffer ionization two other ionization modes can be applied in TS, *i.e.*, filament-on and discharge-on. Both modes resemble solvent-mediated CI. The ionization in APS buffer ionization appears to be similar to that in TS buffer ionization [32]. With the heated nebulizer [29,30] only discharge-on experiments have been reported, although buffer ionization is probably also possible. The possibility of buffer ionization in combination with a heated nebulizer interface was actually demonstrated at this symposium [61].

Various results in the literature shed more light on the TS ionization mechanism. First, it has been shown by various workers [62–65] that gas-phase ion–molecule reactions, *i.e.*, solvent-mediated CI, play an important role in the TS buffer ionization mechanism. For instance, the reagent gas composition in TS buffer ionization can be explained from equilibrium constants for gas-phase reactions [62]. A simple experiment to distinguish between ion evaporation of preformed ions and gas-phase ion–molecule reactions was described by Bursey *et al.* [64]: the relative acid–base order of aniline and ammonia reverses on going from the liquid to the gas phase. Aniline is not protonated by ammonia in the liquid phase, but it is in the gas phase, demonstrating the importance of gas-phase ion–molecule reactions in TS. The influence of the repeller potential on solvent and analyte mass spectra in TS buffer ionization is also most readily explained by ion–molecule reactions [65]. In conclusion, solvent-mediated CI is an important mechanism of analyte ionization in TS, not only in filament-on and discharge-on modes, but also in the TS buffer ionization mode. Ammonium ions and especially ammonium solvent cluster ions, produced as a result of the TS nebulization, form the CI reagent gas in the solvent-mediated CI mode operative in positive-ion TS buffer ionization [65], whereas in the negative ion mode the acetate-related ions play a major role [63].

The IEV mechanism was proposed by Iribarne and Thomson [15,60]. During the nebulization process charged droplets are produced owing to statistical fluctuations in the distribution of the positive and negative ions over the droplets on disruption of the liquid. Subsequent solvent evaporation results in an increasing electrical field at the droplet surface, finally permitting the emission of ions from the droplets, *i.e.*, field-induced ion evaporation [15,60]. This process is assumed to be similar to the field-induced desolvation mechanisms known from field desorption (FD) [66]. The IEV mechanism, and especially whether it is active in TS, has been questioned by Röllgen and co-workers [67,68]. Considering the ammonium acetate concentration commonly used in TS buffer ionization (0.1 mol/l), desolvation of the droplets will result in solid ammonium acetate particles with possibly included analyte molecules, instead of highly charged liquid droplets. The particles rapidly decompose owing to the low decomposition and volatilization temperature of ammonium acetate. Moreover, the actual electric fields at which ion evaporation can take place can be calculated to be much higher [68]. The removal of a solvated ion from the droplet surface under field stress would most likely generate a sequence of events in which a number of charges are removed from the droplet. Such a sequence is a typical electrohydrodynamic instability event, which takes place at the Rayleigh instability limit. The alternative mechanism suggested by Röllgen and co-workers [67,68] is based on soft desolvation of the ions by solvent evaporation from small charged droplets produced by either electrohydrodynamic or mechanical instabilities, or both.

Despite the questions with regard he actual ionization mechanism, the abbreviation IEV is used here to describe the observation of ions from nebulized solutions containing preformed ions, *i.e.*, irrespective of whether these ions are produced by ion evaporation as described by Iribarne and Thomson [15,60], by soft desolvation as described by Röllgen and co-workers [67,68] or by any other yet unknown mechanism.

Reviewing the literature on TS, it appears that in the majority of applications under commonly used solvent conditions, *i.e.*, solvents containing 0.1 mol/l ammonium acetate, the ionization can be readily explained from gas-phase ion–molecule reactions, *i.e.*, CI. The IEV mechanism appears to have only a minor contribution to the TS ion production under these conditions. However, when the ammonium acetate concentration in the mobile phase is greatly reduced, IEV may become important. This view is supported by the comparative study of Voyksner [69] on nucleotides analysed with and without ammonium acetate. Compounds that are ionic in solution, such as sulphate conjugates [70] and sulphonated azo dyes [71,72], are preferentially analysed with a low concentration or even without ammonium acetate present in solution. For instance, the total ion current observed for the disulphonated azo dye Direct Red 81 (mol.wt. 675, disodium salt) first increases with increasing ammonium acetate concentration to a maximum at *ca.* 10^{-3} mol/l, but on further increase in the ammonium acetate concentration it decreases dramatically (see Fig. 6). With increasing ammonium acetate concentration, exceeding 10^{-3} mol/l, the intensity of the ion at m/z 630, $[M - 2Na + H]^+$, which is assumed to be produced by a gas-phase reneutralization reaction, also increases [71]. This means that the ammonium acetate concentration for IEV must be lower than that commonly applied in TS, *i.e.*, 10^{-3} instead of 0.1 mol/l. However, unlike other methods discussed below, some electrolyte in the solution appears to be necessary. An extensive set of experiments can be formulated based on these observations, *e.g.*, related to the influence of ionic strength, nature and charge of the ionic additive. It also appears to be important for IEV to tune the nebulization in such a way that smaller droplets are produced [72]; this aspect is addressed in more detail below.

The IEV ionization mechanism is also assumed to play a major role in two other liquid-based ionization techniques, *viz.*, electrospray and ionspray. The devel-

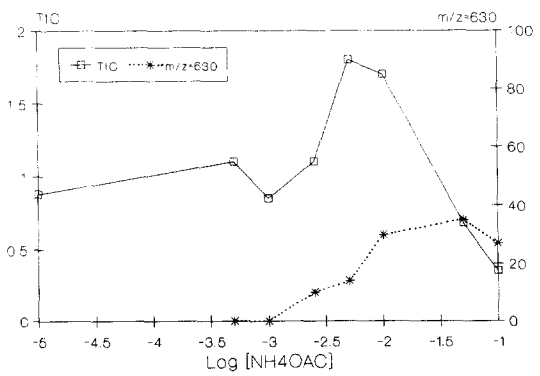

Fig. 6. Influence of the ammonium acetate concentration on the total ion current (TIC) and the ion intensity at m/z 630, $[M - 2Na + H]^+$, of the disulphonated azo dye Direct Red 81. Data for the plot obtained from ref. 71. TIC-axis: counts; m/z = 630 axis: relative abundance.

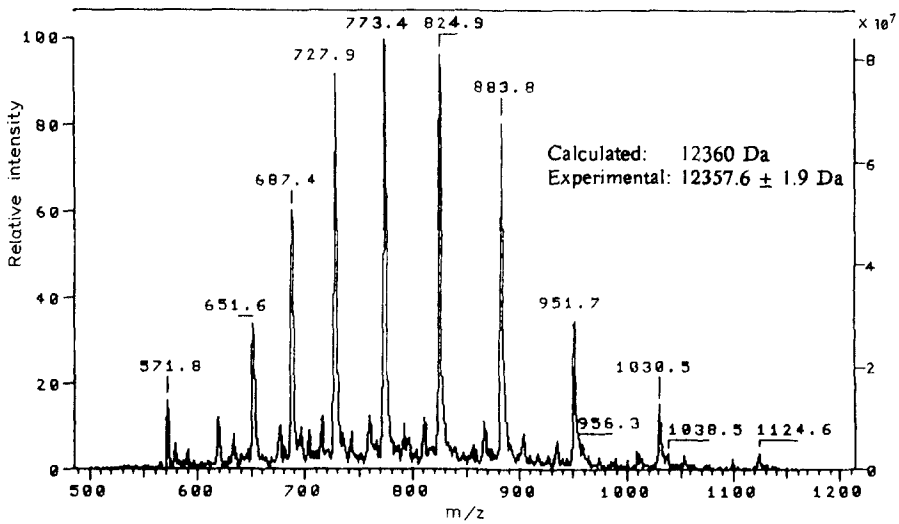

Fig. 7. Typical electrospray spectrum of cytochrome *c*. Conditions: Finnigan MAT TSQ-70 instrument equipped with a Finnigan MAT ESI system. Cytochrome *c* (10 pmol/μl, purchased from Fluka, Buchs, Switzerland) in methanol–water (50:50) at a constant infusion flow-rate of 1 μl/min. The spectrum is the average of 30 scans accumulated in 1 min.

opment of these techniques is based on initial experiments by Dole *et al.* [73] to produce macro-ions by desolvation of the charged droplets obtained in electrospraying dilute solutions of macromolecules, *i.e.*, the same mechanism later proposed by Röllgen and co-workers [67,68] from their experimental evidence and physical understanding of the process. Fenn and co-workers [74–77] continued this electrospray (ES) research, first using small molecules, but subsequently also larger molecules, such as polyethylene glycols and proteins. The results with the proteins, for which envelopes of multiply charged ions in the mass range between *m/z* 500 and 1500 were obtained, introduced a new era in the application of MS in the biological sciences. A typical ES mass spectrum for cytochrome *c* (mol.wt. 12 360), as obtained in the authors' laboratory, is given in Fig. 7. The ES interface operates at 1–5 μl/min. Although an LC–MS interface based on ES has been described [45], the research in ES now appears to be completely focused on the analysis of proteins and other biomacromolecules, which is partly due to the apparent incompatibility of ES with conventional LC in terms of flow-rate.

The ionspray (IS) interface is a modification of the ES concept; pneumatic nebulization is used to assist the electrospray [47]. As a result, the IS interface can accommodate larger solvent loads, *i.e.*, up to 200 μl/min, which more readily allows the coupling with LC, although improved performance is achieved at lower flow-rates. The IS interface can also be used for the analysis of proteins [78]; in these studies generally lower flow-rates, *e.g.*, 10 μl/min, are applied.

The solvent typically used in the analysis of proteins by ES and IS is methanol–water (50:50), often containing some acetic acid. It has recently been demonstrated that with such a solvent it is also possible to obtain multiply charged ions from large proteins in TS [79]. TS apparently is considerably less sensitive than ES and IS;

100–4000 pmol of protein are needed in TS, whereas 1–100 pmol are generally sufficient in ES and IS. However, as 100-μl injections at 1.3 ml/min are performed in TS and 1 μl/min infusion in ES, the sample concentration needed for the spectrum is similar with both techniques. It can be concluded that the overall ionization efficiency in TS is less than that in ES and IS: more analyte molecules are needed in TS than in ES to achieve a comparable signal-to-noise ratio in the spectrum. Unfortunately, the analysis of peptides is frequently limited by the available amount of sample.

From the results briefly indicated above, it can be concluded that the IEV ionization can be operative, irrespective of the nebulization technique. Multiply charged ions from proteins can be produced via electrospray, electrospray-assisted pneumatic nebulization (ionspray) and thermospray. However, it appears that the maximum of the ion envelope shifts to higher m/z values with an increasing solvent load to the source, presumably indicating the growing importance of gas-phase ion–molecule reneutralization reactions. Gas-phase ion–molecule reactions in an ES source have recently been investigated by Ikonomou *et al.* [80]. The data presented at this symposium by Voyksner [81] strongly emphasize the importance of gas-phase ion–molecule processes in ES also.

A similarity between most liquid-based ionization techniques discussed here, *viz.*, ES, IS and CF-FAB, is that they are low-flow-rate techniques. Low-flow-rate interfaces necessitate splitting, either post-column after a conventional LC column or pre-column in terms of a reduced injection volume for a microbore column. Splitting is a serious drawback, as it restricts the achievable detection limits (*cf.* eqn. 4). The larger sample loadability of a microcapillary packed column operated in the gradient elution mode by the application of on-column peak-focusing techniques may obviate these disadvantages. The low-flow-rate techniques can also benefit from high-efficiency separation techniques, such as capillary electrophoresis (CE); the narrow peaks result in improved $C(t)$ values (*cf.*, eqn. 4). On the other hand, the narrow peaks require rapid scanning of the mass spectrometer, *e.g.*, in order to acquire a sufficient number of peaks from the ion envelope of multiply charged protein ions to perform accurate molecular weight determinations in the analysis of unknowns. At present, most of these spectra are acquired, depending on the amount, in times from several seconds to even minutes, which is necessary from ion-statistical arguments to average the statistical distribution of charges over the molecule, and to average the statistical fluctuations in the peak heights which appear to be unavoidable when nebulization techniques are applied for analyte introduction. The application of array detectors, such as the PATRIC array detector [82], shows great promise in this respect, in terms of both sensitivity and acquisition speed, as recently demonstrated by Reinhoud *et al.* [83]. Considerable progress is to be expected in this area in the near future.

NEBULIZATION IN LC–MS

From the previous discussion it appears that two aspects of LC–MS interfacing are especially important: the nebulization and the liquid-based soft ionization methods.

Pneumatic nebulization is most widely used in LC–MS (see Table II); it is also the technique of choice in many other analytical techniques where nebulization is important.

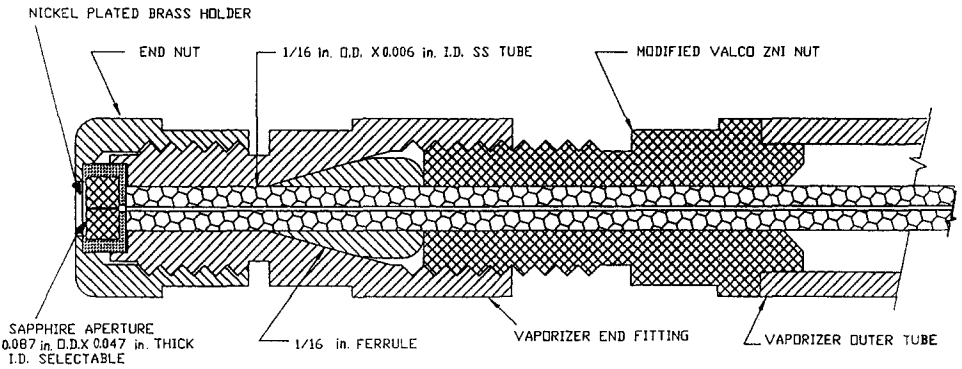

Fig. 8. Schematic diagram of the diaphragm-equipped thermospray interface (reprinted with permission from Vestec, Houston, TX, USA [85]).

In LC–MS the actual nebulization technique applied to produce the droplets is apparently less important. The maximum allowable flow-rate is found to be limited by the transfer of heat needed for the evaporation of the solvent, although with ES the flow-rate appears to be primarily limited by the nebulization process itself. The liquid-based soft ionization methods are operative irrespective of the way in which the droplets are produced, although the droplet size appears to be important, especially in IEV techniques. Droplet size decreases in the order liquid jet, pneumatic, thermospray, electrospray, although with both the liquid jet and the pneumatic nebulization, in principle, smaller droplets are achievable. For liquid jet nebulization the droplet size depends on the diaphragm diameter, but in practice also on the heat transferred to the liquid prior to the nebulization: the evaporation of solvent from heated droplets more readily produces sufficiently small droplets within the time frame available for the ionization. For pneumatic nebulization the droplet size depends on the gas speed relative to the liquid linear velocity, according to the equation given by Nukiyama and Tanasawa [84]. However, decreasing the droplet size with the liquid jet or the pneumatic nebulizers would lead to systems that are more prone to clogging.

An interesting development in the application of nebulization techniques in LC–MS is the exploration of combination techniques, although most possible combinations have not yet been investigated. For instance, the IS interface [47] is a combination of electrospray and pneumatic nebulization; it is a highly successful approach to LC–MS interfacing.

In an attempt to produce smaller droplets by TS, McLean and Freas [72] experimented with short, narrow-bore, fused-silica capillary restrictors at the outlet of the TS vaporizers (*cf.*, Arpino and Beaugrand [10]). The use of laser-drilled sapphire diaphragms at the outlet of the TS vaporizer has recently been introduced by Vestec [85] (see Fig. 8). Various aperture diameters can be selected, depending on the flow-rate to be introduced. The flow-rate that must be introduced in a conventional TS vaporizer capillary to achieve stable TS nebulization conditions depends on solvent properties, such as vapour pressure, viscosity and molecular weight, on the capillary diameter and on the temperature. In principle, stable liquid jets can be produced by means of vaporizers with smaller inside diameters, but the risk of clogging the vapor-

TABLE IV

OBJECTIVES IN DEVELOPING ON-LINE LC–MS

A universal detector LC
A free choice of LC solvent composition and flow-rate
Analysis of thermolabile and non-volatile analytes
Avoid analyte derivatization
Low detection limits
Identification of analytes

izer increases. With a vaporizer, with a sapphire diaphragm fitted at the outlet, the clogging is actually more likely to occur at the diaphragm, which can be replaced more readily than the complete vaporizer capillary. Using eqn. 9, it can be shown that for the common diaphragm diameters (25–75 μm I.D.) and flow-rates (1–1.5 ml/min), a stable liquid jet can be produced. The heat transferred in the vaporizer capillary to the liquid, prior to the formation of the liquid jet, ensures rapid solvent evaporation from the droplets produced upon jet disintegration. With the use of smaller diaphragms smaller droplets are produced: the common effect of decreasing droplet size with smaller diaphragms [22] is further enhanced by the more effective evaporation of the solvent from the smaller superheated droplets. As such, the TS vaporizers with a diaphragm can be considered as a combination technique: thermospray and liquid jet formation.

The reduction in the flow-rate in TS is expected to be beneficial for the sensitivity of the TS system. From a comparison of the detection limits for organophosphorus pesticides in DLI [86] and TS [87], this statement can be sustained. Parker *et al.* [86] reported for the DLI that 100 ng injected on-column was needed to detect the major peaks in the spectrum. Barcelo [87] reported an on-column detection limit of 20–50 ng for the TS. In judging these figures it must be emphasized that in DLI a splitting ratio of 0.01 was used, *i.e.*, 1 ng is introduced into the source, whereas in TS 20–50 ng are introduced into the source. It can be concluded that the ionization efficiency and/or the ion extraction efficiency in TS are considerably worse than in DLI. This may be due to the high pressures in the TS source, typically 2000 Pa, relative to the DLI (10 Pa). The transition region from the TS source at 2000 Pa to the analyser region at 10^{-3} Pa seems hardly optimized in this respect. Reduction of the flow-rate from the TS vaporizer would decrease the pressure in the source and could enhance the ion extraction efficiency, although it is unclear whether similar ionization efficiencies can indeed be achieved at the lower flow-rates and source pressures. Experiments performed in this area by Osterman *et al.* [88] are not well documented.

CONCLUSIONS

Some conclusions from the present discussion can be drawn by comparing the research objectives in developing on-line LC–MS, as summarized in Table IV, and the present state-of-the-art in LC–MS.

The coupling of LC and MS has become a successful analytical tool, but has not resulted in a universal detector for LC. Various steps in the LC–MS interfacing

show selectivity or discrimination towards certain analytes, especially the inherent properties of the ionization techniques used.

No free choice of LC solvent composition is possible. Most favourable solvents in LC–MS are reversed-phase LC solvents containing volatile buffer constituents. This limits the LC–MS application of several powerful LC techniques, such as ion-exchange and ion-pair LC, although some impressive results in this area have recently been described [89–92]; these techniques are especially useful for separating the type of analytes for which LC–MS would be most attractive. Pseudo-electro-microchromatography, as demonstrated by Verheij *et al.* [93], appears to be a powerful alternative for the analysis of ionic compounds; the technique is readily compatible with common low-flow-rate LC–MS interfaces. In target-compound analysis, valve-switching techniques, *e.g.*, phase-system switching, can be applied. Solving incompatibilities in this respect has been discussed in a previous paper [94]. In general, the use of non-volatile mobile phase constituents, *e.g.*, phosphate buffers, in routine LC–MS application is prohibited, which is a disadvantage limiting the practical use of LC–MS. This problem is not expected to be solved in a general way in the near future.

Thermolabile and non-volatile analytes can be handled in LC–MS. However, because mostly soft ionization techniques, *i.e.*, (solvent-mediated) CI, FAB and IEV, are applied in LC–MS, the information that can be achieved is limited. For many compounds no EI spectra can be produced. Tandem MS (MS–MS) and some other fragmentation-inducing techniques can be applied, but the spectra obtained are not as readily interpretable as EI spectra. As a result, identification of unknowns is not straightforward.

The desire to avoid analyte derivatization results from GC and GC–MS. However, in LC–MS analyte derivatization can be very useful, especially considering the fact that most ionization techniques used in LC–MS rely strongly on chemistry [94]. More attention should be paid to this aspect.

The detection limits achievable in LC–MS are generally insufficient, especially in the field of bioanalysis and environmental analysis, were the complexity of the sample matrix prevents the effective use of sample preconcentration techniques. Lower, preferably much lower, detection limits are needed in various application areas. This statement is nearly always true for a variety of analytical techniques, but certainly in LC–MS. A more profound study on the causes of the disappointing detection limits would be appropriate, but is beyond the scope of this paper.

The importance of miniaturization in LC has varied with time. Originally, the coupling of conventional LC columns to MS systems was pursued. The necessity for splitting in DLI stimulated the application of microcolumns. The interest decreased again with the introduction of the TS interface, capable of introducing the total effluent from a conventional LC column. However, a revival of the interest in microcolumns resulted from the introduction of low-flow-rate liquid-based soft ionization techniques, such as CF-FAB and ES. Considerable progress in this field can be expected in the near future. The various aspects of interfacing microcolumn separation techniques to mass spectrometry are discussed in more detail elsewhere [95].

LC–MS has acquired a place amongst the various applicable analytical techniques. Real problems are solved with LC–MS, sometimes with great elegance, but still many questions remain unanswered and many goals remain to be achieved. This paper has clarified some important technological aspects in LC–MS interface devel-

opments. It is hoped that it will give a better understanding of LC–MS, and will open up new research directions to improve the performance of LC–MS.

ACKNOWLEDGEMENT

5-Methoxypodophyllotoxin-4-β-D-glycoside was kindly provided by Dr. Harkes, MT–TNO, Zeist, Netherlands.

REFERENCES

1 V. L. Tal'roze, V. E. Skurat, I. G. Gorodetskii and N. B. Zolotai, *Russ. J. Phys. Chem.*, 46 (1972) 456.
2 P. J. Arpino and G. Guiochon, *Anal. Chem.*, 51 (1979) 682A.
3 D. E. Games, *Biomed. Mass Spectrom.*, 8 (1981) 454.
4 T. R. Covey, E. D. Lee, A. P. Bruins and J. D. Henion, *Anal. Chem.*, 58 (1986) 1451A.
5 K. Vekey, D. Edwards and L. F. Zerilli, *J. Chromatogr.*, 488 (1989) 73.
6 K. B. Tomer and C. E. Parker, *J. Chromatogr.*, 492 (1989) 189.
7 V. L. Tal'roze, V. E. Skurat and G. V. Karpov, *Russ. J. Phys. Chem.*, 43 (1969) 241.
8 V. L. Tal'roze, I. G. Gorodetskii, N. B. Zolotai, G. V. Karpov, V. E. Skurat and V. Ya. Maslennikova, *Adv. Mass Spectrom.*, 7 (1978) 858.
9 P. J. Arpino, P. Krien, S. Vajta and G. Devant, *J. Chromatogr.*, 203 (1981) 117.
10 P. J. Arpino and C. Beaugrand, *Int. J. Mass Spectrom. Ion Processes*, 64 (1985) 275.
11 A. P. Bruins and B. F. H. Drenth, *J. Chromatogr.*, 271 (1983) 71.
12 R. G. Christensen, E. White, V. S. Meiselman and H. S. Hertz, *J. Chromatogr.*, 271 (1983) 61.
13 R. C. Willoughby, *Ph.D. Thesis*, Georgia Institute of Technology, 1983.
14 E. C. Horning, D. I. Carroll, I. Dzidic, K. D. Haegele, M. G. Horning and R. N. Stillwell, *J. Chromatogr.*, 99 (1974) 13.
15 J. V. Iribarne and B. A. Thomson, *J. Chem. Phys.*, 64 (1976) 2287.
16 J. A. Apffel, U. A. Th. Brinkman, R. W. Frei and E. I. A. M. Evers, *Anal. Chem.*, 55 (1983) 2280.
17 K. Matsumoto, H. Kojima, K. Yasuda and S. Tsuge, *Org. Mass Spectrom.*, 20 (1985) 243.
18 F. S. Pullen, D. S. Ashton and M. A. Baldwin, *J. Chromatogr.*, 474 (1989) 335.
19 Y. Hirata, T. Takeuchi, S. Tsuge and Y. Yoshida, *Org. Mass Spectrom.*, 14 (1979) 126.
20 S. Tsuge, in M. V. Novotny and D. Ishii (Editors), *Microcolumn Separations*, Elsevier, Amsterdam, 1985, p. 217.
21 A. Melera, *Adv. Mass Spectrom.*, 8 (1980) 1597.
22 R. N. Berglund and B. Y. H. Liu, *Environ. Sci. Technol.*, 7 (1973) 147.
23 W. M. A. Niessen, *Chromatographia*, 21 (1986) 277.
24 W. M. A. Niessen, *Chromatographia*, 21 (1986) 342.
25 C. R. Blakley and M. L. Vestal, *Anal. Chem.*, 55 (1983) 750.
26 M. L. Vestal and G. J. Fergusson, *Anal. Chem.*, 57 (1985) 2373.
27 D. A. Garteiz and M. L. Vestal, *LC Mag.*, 3 (1985) 334.
28 P. J. Arpino, *Mass Spectrom. Rev.*, 9 (1990) 631.
29 *The API Book*, Sciex, Thornhill, Canada, 1989.
30 T. R. Covey, E. D. Lee and J. D. Henion, *Anal. Chem.*, 58 (1986) 2453.
31 M. Sakairi and H. Kambara, *Anal. Chem.*, 60 (1988) 774.
32 M. Sakairi and H. Kambara, *Anal. Chem.*, 61 (1989) 1159.
33 C. F. Simpson, *CRC Crit. Rev. Anal. Chem.*, 3 (1972) 1.
34 G. A. Junk, *Int. J. Mass Spectrom. Ion Phys.*, 8 (1972) 1.
35 J. van der Greef, W. M. A. Niessen and U. R. Tjaden, *Spectra*, 12, No. 1 (1989) 26.
36 P. J. Arpino, *Mass Spectrom. Rev.*, 8 (1989) 35.
37 P. Dobberstein, E. Korte, G. Meyerhoff and R. Pesch, *Int. J. Mass Spectrom. Ion Phys.*, 46 (1983) 185.
38 J. G. Stroh, J. Carter Cook, R. M. Milberg, L. Brayton, T. Kihara, Z. Huang, K. L. Rinehard, Jr., and I. A. S. Lewis, *Anal. Chem.*, 57 (1985) 985.
39 J. van der Greef, A. C. Tas, M. C. ten Noever de Brauw, M. Höhn, G. Meyerhoff and U. Rapp., *J. Chromatogr.*, 323 (1985) 81.
40 P. Vouros, E. P. Lankmayr, M. J. Hayes, B. L. Karger and J. M. McGuire, *J. Chromatogr.*, 251 (1982) 175.

41 R. C. Willoughby and R. F. Browner, *Anal. Chem.*, 56 (1984) 2626.
42 R. D. Voyksner, C. S. Smith and P. C. Knox, *Biomed. Environ. Mass Spectrom.*, 19 (1990) 523.
43 A. P. Tinke, R. A. M. van der Hoeven, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, 554 (1991) 119.
44 M. L. Vestal, D. Winn, C. H. Vestal and J. G. Wilkes, in M. A. Brown (Editor), *Liquid Chromatography/Mass Spectrometry. Applications in Agricultural, Pharmaceutical and Environmental Chemistry (ACS Symposium Series*, Vol. 420), American Chemical Society, Washington, DC, 1990, p. 215.
45 C. M. Whitehouse, R. N. Dreyer, M. Yamashita and J. B. Fenn, *Anal. Chem.*, 57 (1985) 675.
46 R. D. Smith, J. A. Olivares, N. T. Nguyen and H. R. Udseth, *Anal. Chem.*, 60 (1988) 1948.
47 A. P. Bruins, T. R. Covey and J. D. Henion, *Anal. Chem.*, 59 (1987) 2642.
48 J. D. Kirk and R. F. Browner, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 355.
49 P. E. Sanders, *Rapid Commun. Mass Spectrom.*, 4 (1990) 123.
50 P. J. Arpino and G. Guiochon, *J. Chromatogr.*, 251 (1982) 153.
51 M. Dedieu, C. Juin, P. J. Arpino and G. Guiochon, *Anal. Chem.*, 54 (1982) 2372.
52 M. A. Baldwin and F. W. McLafferty, *Org. Mass Spectrom.*, 7 (1973) 1353.
53 M. L. Vestal, *Mass Spectrom. Rev.*, 2 (1983) 447.
54 Y. Ito, T. Takeuchi, D. Ishii and M. Goto, *J. Chromatogr.*, 346 (1985) 161.
55 R. M. Caprioli, T. Fan and J. S. Cottrell, *Anal. Chem.*, 58 (1986) 2949.
56 R. M. Caprioli, *Anal. Chem.*, 62 (1990) 477A.
57 R. M. Caprioli, paper presented at the *36th ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 1988, p. 729.*
58 R. M. Caprioli, W. T. Moore and T. Fan, *Rapid Commun. Mass Spectrom.*, 1 (1987) 15.
59 C. R. Blakley, J. J. Carmody and M. L. Vestal, *J. Am. Chem. Soc.*, 102 (1980) 5931.
60 B. A. Thomson and J. V. Iribarne, *J. Chem. Phys.*, 71 (1979) 4451.
61 M. S. Bolgar, B. M. Warrack and G. C. DiDonato, presented at the *7th Montreux Symposium on LC–MS, Montreux, October 31–November 2, 1990.*
62 A. J. Alexander and P. Kebarle, *Anal. Chem.*, 58 (1986) 471.
63 C. E. Parker, R. W. Smith, S. J. Gaskell and M. M. Bursey, *Anal. Chem.*, 58 (1986) 1661.
64 M. M. Bursey, C. E. Parker, R. W. Smith and S. J. Gaskell, *Anal. Chem.*, 57 (1985) 2597.
65 C. E. M. Heeremans, R. A. M. van der Hoeven, W. M. A. Niessen, J. van der Greef and N. M. M. Nibbering, *Org. Mass Spectrom.*, 26 (1991) 519.
66 U. Giessmann and F. W. Röllgen, *Int. J. Mass Spectrom. Ion Phys.*, 38 (1981) 267.
67 G. Schmelzeisen-Redeker, L. Bütfering and F. W. Röllgen, *Int. J. Mass Spectrom. Ion Processes*, 90 (1989) 139.
68 F. W. Röllgen, H. Nehring and U. Giessmann, in A. Hedin, B. U. R. Sundqvist and A. Benninghoven (Editors), *Ion Formation from Organic Solids (IFOS V)*, Wiley, New York, 1989, p. 155.
69 R. D. Voyksner, *Org. Mass Spectrom.*, 22 (1987) 513.
70 D. Watson, G. W. Taylor and S. Murray, *Biomed. Mass Spectrom.*, 12 (1985) 610.
71 D. A. Flory, M. A. McLean, M. L. Vestal and L. D. Betowski, *Rapid Commun. Mass Spectrom.*, 1 (1987) 48.
72 M. A. McLean and R. B. Freas, *Anal. Chem.*, 61 (1989) 2054.
73 M. Dole, R. L. Hines, L. L. Mack, R. C. Mobley, L. D. Ferguson and M. B. Alice, *J. Chem. Phys.*, 49 (1968) 2240.
74 M. Yamashita and J. B. Fenn, *J. Phys. Chem.*, 88 (1984) 4451.
75 S. F. Wong, C. K. Meng and J. B. Fenn, *J. Phys. Chem.*, 92 (1988) 546.
76 C. K. Meng, M. Mann and J. B. Fenn, *Anal. Chem.*, 61 (1989) 1702.
77 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Mass Spectrom. Rev.*, 9 (1990) 37.
78 T. R. Covey, R. F. Bonner, B. I. Shushan and J. D. Henion, *Rapid Commun. Mass Spectrom.*, 2 (1988) 249.
79 K. Straub and K. Chan, *Rapid Commun. Mass Spectrom.*, 4 (1990) 267.
80 M. G. Ikonomou, A. T. Blades and P. Kebarle, *Anal. Chem.*, 62 (1990) 957.
81 R. D. Voyksner, presented at the *7th Montreux Symposium on LC–MS, Montreux, October 31–November 2, 1990.*
82 C. Brunnee, R. Pesch and E. Schröder, *Rapid Commun. Mass Spectrom.*, 4 (1990) 173.
83 N. J. Reinhoud, E. Schröder, U. R. Tjaden, W. M. A. Niessen, M. C. ten Noever de Brauw and J. van der Greef, *J. Chromatogr.*, 516 (1990) 147.

84 S. Nukiyama and Y. Tanasawa, *Trans. Soc. Mech. Eng. Jpn.*, 5 (1939) 68.
85 *Vestec Diaphragm Interface*, Vestec, Houston, TX, 1990.
86 C. E. Parker, C. A. Haney and J. R. Hass, *J. Chromatogr.*, 237 (1982) 233.
87 D. Barcelo, *Biomed. Environ. Mass Spectrom.*, 17 (1988) 363.
88 R. M. Osterman, C. R. Blakley, G. J. Fergusson and M. L. Vestal, in *Proceedings of the 35th ASMS Conference on Mass Spectrometry and Allied Topics, May 24–29, 1987, Denver, CO*, ASMS, East Lansing, MI, 1987, p. 364.
89 R. C. Simpson, C. C. Fenselau, M. R. Hardy, R. R. Townsend, Y. C. Lee and R. J. Cotter, *Anal. Chem.*, 62 (1990) 248.
90 J. J. Conboy, J. D. Henion, M. W. Martin and J. W. Zweigenbaum, *Anal. Chem.*, 62 (1990) 800.
91 K. W. M. Siu, R. Guevremont, J. C. Y. Le Blanc, G. J. Gardner and S. S. Berman, *J. Chromatogr.*, 554 (1991) 27.
92 R. E. A. Escott, P. G. McDowell and N. P. Porter, *J. Chromatogr.*, 554 (1991) 281.
93 E. R. Verheij, U. R. Tjaden, W. M. A. Niessen and J. van der Greef, *J. Chromatogr.*, 554 (1991) 339.
94 J. van der Greef, W. M. A. Niessen and U. R. Tjaden, *J. Chromatogr.*, 474 (1989) 5.
95 W. M. A. Niessen, U. R. Tjaden and J. van der Greef, in preparation.