Journal of Chromatography, 474 (1989) 5–19
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 424

LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

THE NEED FOR A MULTIDIMENSIONAL APPROACH

J. VAN DER GREEF

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

and

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

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

SUMMARY

Combined liquid chromatography–mass spectrometry is now used routinely in both qualitative and quantitative analysis. Therefore, it is important to consider the integration of this method in the whole analytical procedure. Attention has to be paid to other topics than interface technology. This paper deals with some of those aspects, especially with those important in target compound analysis. Strategies are outlined and discussed in the tuning of the selectivity of the method, in improving the compatibility of the liquid chromatography, the interface and mass spectrometry, in enhancing the detectability, and with respect to pre- and post-column derivatization techniques.

INTRODUCTION

The successful development of various interface methods for coupling liquid chromatography with mass spectrometry (LC–MS) has made this method an important analytical tool in many laboratories. However, if a method reaches the stage of being used in daily practice for both qualitative and quantitative analysis it becomes very important to achieve an integration within the whole analytical procedure. In particular, for trace analysis each method consists of an interplay between the four basic building blocks: sample pretreatment, separation, detection and data handling.

LC–MS research has been focused strongly on the interface technology. The input has come mainly from the MS side and not from the chromatography side, despite some excellent and stimulating reviews on the chromatographic side of LC–MS[1,2]. This situation is clearly illustrated by the LC–MS presentations in 1988 on the two major annual meetings in both areas, *i.e.* the American Society for Mass Spectrometry (ASMS)[3] and the HPLC 88 meeting[4]. Approximately 13% and 1% of

0021-9673/89/$03.50

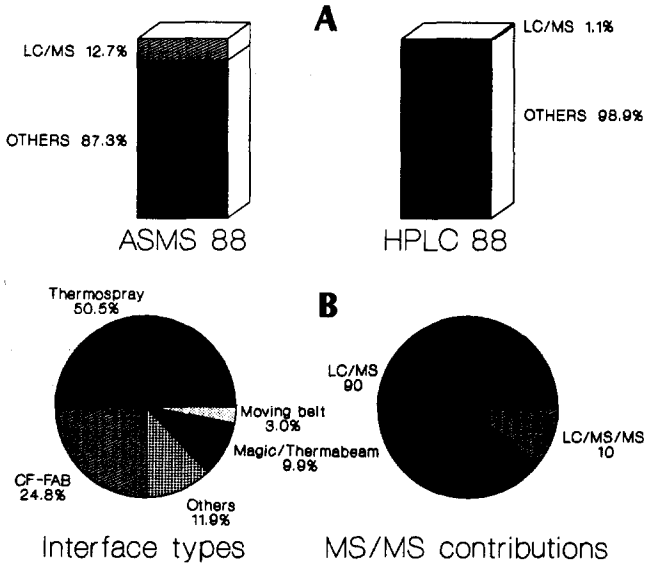

Fig. 1. Summary of presentations on LC–MS at the ASMS 88 and HPLC 88 meetings (A) and the contributions on different interfaces and MS–MS at the ASMS 88 meeting (B).

the presentations at the ASMS and HPLC 88 meetings, respectively, were on LC–MS, as shown in Fig. 1A. This somewhat unbalanced situation is an enormous challenge and, because of the impressive results of today's LC–MS, despite this indicated weakness, it also holds out promise for the near future. An increase in the availability of lower cost instrumentation will certainly be helpful for many chromatographers to overcome the threshold of applying MS detection.

The optimum conditions for coupling chromatography with mass spectrometry are strongly dependent on the type of interface used, since each interface is characterized by quite different values for parameters such as flow-rate and mobile phase composition. The most widely applied interfaces, as indicated in Fig. 1B, are thermospray (TSP), continuous-flow fast atom bombardment (CF-FAB), and particle beam approaches (MAGIC, Thermabeam); some others, such as the moving belt and direct liquid introduction (DLI) methods and the very promising electrospray and the related ionspray techniques, are also used by several groups[5]. The different characteristics can be illustrated by the preference for flow-rates of *ca.* 1.5 ml/min for TSP and of *ca.* 5–10 μl/min for CF-FAB, while the optimum mobile phase for TSP and CF-FAB is more compatible with reversed-phase chromatography than in general for the moving-belt interface.

The use of soft ionization methods in LC–MS has laid more emphasis on the generation of structural information. Tandem mass spectrometry (MS–MS) is an attractive approach, although identification is not always straightforward. The same is true for the fragmentation induced in TSP by variation of the repeller voltage or by applying a discharge electrode[6]. Electron impact (EI) remains of interest in this respect, and the moving belt and the particle beam approaches offer this attractive possibility. In the interfacing of supercritical fluid chromatography with mass

spectrometry (SFC–MS) structurally informative EI spectra can be generated by capillary SFC via direct coupling[7] or by packed-column SFC with a moving belt[8] or via charge exchange using CO_2 in the direct coupling[9]. About 10% of all LC–MS presentations at the ASMS meeting were on LC–MS–MS, with a strong accent however on the increase of selectivity in target compound analysis using selected reaction monitoring.

In this paper three important aspects of LC–MS are discussed, with emphasis on target compound analysis and special attention to the chromatographic side: the tuning of the overall selectivity, the improvement of the compatibility and the enhancement of the detectability. Furthermore, attention is paid to the importance of chemistry, such as derivatization procedures, in both pre- and post-column modes.

SELECTIVITY TUNING

One of the attractive features of the mass spectrometer as a detector is the possibility of using either the universal mode (scanning conditions) for identification purposes or the selective mode (single or multiple ion detection) for target compound analysis. Numerous examples have been presented to illustrate this, but the lack of sufficient selectivity has also been shown in complex problems or in cases of trace analysis. Fig. 2 schematically illustrates several ways to increase the selectivity, such as by applying LC–MS–MS or by using coupled column chromatography (CCC) combined with MS or MS–MS.

LC–MS–MS has been extensively demonstrated and thus will not be discussed here. However, it is important to point out that high selectivities have been demonstrated, although this does not mean that the method is guaranteed to be robust. Often severe contamination of the mass spectrometer will influence even the short-term stability of the procedure, and in those cases the overall selectivity has to be readdressed by improving the sample pretreatment and the chromatography.

Hardly any work has been published on LC–LC–MS and LC–LC–MS–MS, despite the fact that CCC has become a routine technique in many bioanalytical laboratories[10]. Edholm *et al.*[11] have investigated the effect of using CCC in LC–MS to increase the overall selectivity for the determination of terbutaline enantiomers in plasma. In this study the LC–MS results, using a β-cyclodextrin column, were

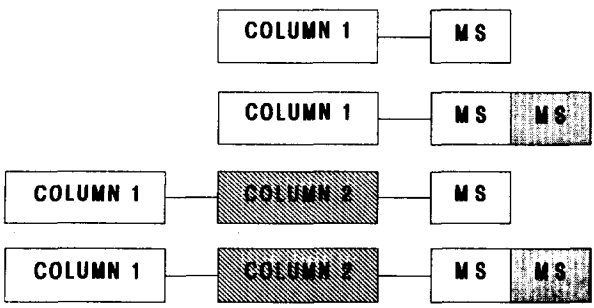

Fig. 2. Schematic diagrams showing different ways of increasing the LC–MS selectivity by applying coupled column chromatography, tandem mass spectrometry or a combination of both.

promising but the limit of determination was mainly set by a rather high background. Using a CCC system in an off-line mode, the selectivity was improved, but the main benefit appeared to be the improvement in the stability of the method.

In principle, one has to distinguish the different components in the background if the selecivity is to be improved. If the sample gives the main contribution to the background, CCC is of great help, but if the background comes predominantly from the LC system itself (*e.g.* mobile phase constituents or impurities) tandem MS is the obvious choice. An important aspect is the fact, that minimum sample loss takes place in the CCC approach, whereas in MS–MS the reduction in the signal is considerable, typically a factor of ten, but depending on the fragmentation pathway selected for monitoring. Thus, in that case the benefit is only due to the strong decrease in the noise yielding an increase in the signal-to-noise ratio[12]. In practical situations a combination of both contributions is encountered, and by removing one of them the other becomes the limiting factor so both have to be dealt with when the aim is to measure low levels. However, not only MS–MS but also high resolution MS, or a combination of the two can be attractive, especially when the analyte under investigation can be resolved at medium resolution, as is often the case with halogen-containing compounds. Effective reduction of the background has been demonstrated in measuring bromazapam and clopentixol in human plasma[13,14] and for the analysis of diuron in *post-mortem* body fluids with LC–MS using the moving-belt interface[15].

The power of CCC–MS–MS has been demonstrated by Edlund and Henion[16] for the determination of dianabol and metabolites in equine urine using an atmospheric ionization (API) source combined with either a heated pneumatic nebulizer or an ionspray interface. Comparison of LC–UV, LC–LC–UV, LC–LC–MS and LC–LC–MS–MS illustrated clearly the effects described above, while in this case the coupled column system appeared to be mandatory to obtain an effective separation of the isomers.

These results reflect the importance of carefully tuning the different building blocks of the overall system to construct a balanced procedure. A consequence of the high selectivity and the rather low contamination of the system is the possibility of increasing the speed of the method considerably, which becomes important if extensive series have to be analysed.

COMPATIBILITY IMPROVEMENT

Although the developed LC–MS interfaces have quite different characteristics they have one thing in common: the incompatibility with mobile phases containing high concentrations of non-volatile additives. On-line extraction has been developed to overcome this problem, as well as to switch from highly aqueous phases to organic phases, which are more compatible with moving-belt interfaces[17–19] and the DLI interfaces using gas nebulization[20]. For the latter also a miniaturized membrane separator has been used for microbore LC–MS[21].

A strategy has been reported based on the use of mixed-mode columns[22]. With a mixed-mode hydrophobic ion-exchange column, compounds that are normally chromatographed by ion-pair LC could be separated and detected by TSP LC–MS. A mobile phase consisting of ammonium trifluoroacetate (1 *M*, pH 2.5) and methanol (75:25) gave good results but, in order to avoid clogging, the flow-rate was limited to 0.5 ml/min.

As outlined in the previous selectivity considerations, the overall selectivity can be very high in CCC–MS–MS. Since this is not always necessary in its extreme form, we started to investigate whether in such cases CCC systems can be developed with additional features to improve the overall performance. Two factors were optimized in one setup: compatibility improvement and detectability enhancement. Simple CCC systems were studied with the configuration shown in Fig. 3. The precolumn configuration has been used extensively for trace enrichment in LC and has been shown[23] to be effective in DLI LC–MS as well, but it is of course interface-independent. The setup is also an elegant way to meet the requirements for injecting aqueous samples in SFC[24] and SFC–MS systems[25], which is important in bioanalysis. The other system, which consisted of a post-column (referred to as a trapping column) following the analytical column, also provides various attractive characteristics. The combination of various precolumns in such a system has been suggested for DLI LC–MS by McKellop *et al.*[26].

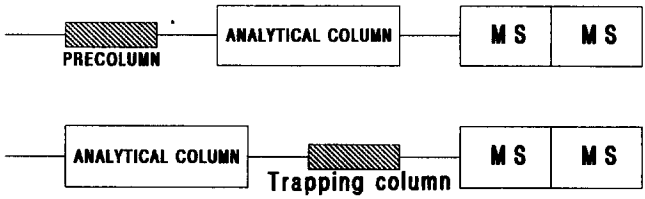

Fig. 3. Schematic diagrams of simplified coupled column systems coupled with tandem mass spectrometry.

Our approach has been to improve the compatibility for LC–MS coupling for different types of interface. The concept is based on "phase-system switching" (PSS)[27]: the experimental setup and the principle[28] are outlined in Fig. 4. After injection into the LC system, chromatography is performed without any restrictions on the mobile phase; for instance, ion-pairing reagents and/or phosphate buffers can be used. Via heart-cutting the analyte is trapped on the trapping column, which can be achieved by selection of the appropriate stationary phase or by changing the mobile phase after the analytical column. In the next step, washing and (often) drying of the trapping column is performed to remove non-volatile materials and, if necessary, the residual water from the washing step. In the last step, desorption of the analyte is effected by the optimum mobile phase for the interface used. Typical mobile phases used are 100% methanol or acetonitrile for the moving belt, 20% methanol in 50 m*M* ammonium acetate for thermospray or glycerol–acetonitrile–water (10:30:60) for CF-FAB. In this way the optimum mobile phase and flow-rate can be used for both the LC and the MS parts. The combination with MS–MS is needed because in most cases the desorbed background might interfere at lower levels. The system has been used for the analysis of mitomycin C[28] with a moving belt using a 50 m*M* phosphate buffer as mobile phase in the LC system. Furthermore, the concept can be extended for two analytes, using two trapping columns, as demonstrated for the determination of metoprolol enantiomers by TSP LC–MS[29].

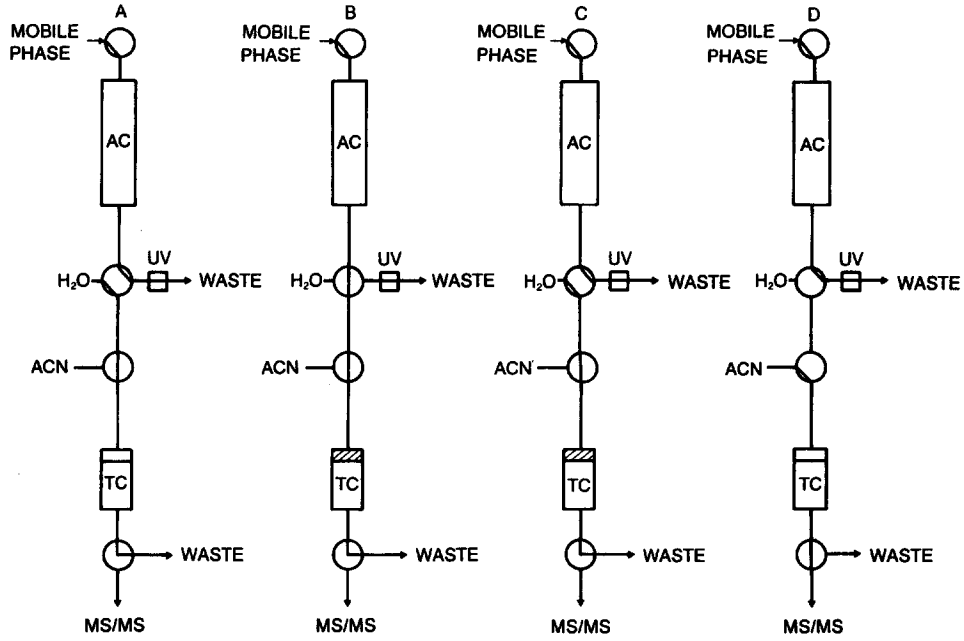

Fig. 4. Principle of the "phase-system switching" approach. After (A) injection of the sample, (B) trapping is achieved and (C) after washing (D) desorption and MS–MS analysis are performed.

DETECTIBILITY ENHANCEMENT

The PSS concept is also very attractive in improving the detection and determination limits, since an important aspect not mentioned so far is the peak compression achieved via the trapping procedure[28]. For the experimental setup in Fig. 4, an estimation of the conditions for obtaining peak compression can be made by considering that the mass spectrometer is a mass-flow-sensitive detector, which means that the signal $U(t)$ is proportional to the mass-flow, dm/dt:

$$U(t) = \tau \, dm/dt \tag{1}$$

where τ is a proportionality factor, usually called the transfer factor. The transfer factor is the sensitivity of the detector, as it is the slope of the line correlating the signal and the mass introduced. The mass-flow can be written as:

$$dm/dt = C(t)F \tag{2}$$

where $C(t)$ is the concentration of the analyte after the chromatography, and F the flow-rate of the mobile phase. The concentration at the peak maximum at the end of the column can be written as:

$$C_m = \frac{4m\sqrt{N}}{\sqrt{2\pi}\,\pi d_c^2 L\,\varepsilon\,(1 + k')} \tag{3}$$

where m is the injected amount, N the plate number, d_c the column diameter, L the column length, ε the column porosity and k' the capacity ratio.

A combination of both formulae gives a rough estimate of the attainable peak compression, defined as G:

$$G = \frac{(dm/dt)_{TC}}{(dm/dt)_{AC}} = \frac{C_{m,TC} \cdot F_{TC}}{C_{m,AC} \cdot F_{AC}} \tag{4}$$

where F_{TC} is the desorption flow-rate through the trapping column, F_{AC} is the flow-rate through the analytical column, and $C_{m,TC}$ and $C_{m,AC}$ are the maximum concentrations of the peak at the ends of the trapping and the analytical columns, respectively. An accurate estimation of $C_{m,TC}$ is hampered by the fact that desorption from the trapping column is generally performed in the backflush mode, in contrast to what is shown in the schematic diagram in Fig. 4. However, it is clear from this last equation that the design characteristics of the trapping column are very important. A compromise between miniaturization of the trapping column and external peak-broadening have to be realized. The capacity ratio of the analyte on the trapping column, k'_{TC}, is the most important parameter. Trapping is possible if:

$$x\sigma_{v,AC} < (1 + k'_{TC})V_{0,TC} \tag{5}$$

in which, $\sigma_{v,AC}$ is the peak standard deviation of the analytical column and $V_{0,TC}$ is the void volume of the trapping column. The factor x determines the total trapping volume: in order to trap a complete chromatographic peak, x must be no less than 6. Rearrangment leads to the following formula, by which the necessary capacity ratio of the analyte on the trapping column can be estimated:

$$k'_{TC} > \frac{xd_{AC}^2 L_{AC}(1 + k'_{AC})}{d_{TC}^2 L_{TC}\sqrt{N_{AC}}} - 1 \tag{6}$$

where d_{AC} and L_{AC}, and d_{TC} and L_{TC} are the I.D. and length of the analytical column and the trapping column, respectively. Equal column porosities are assumed. Aspects with respect to the optimization of the dimensions of analytical and trapping columns, *e.g.* the use of a miniaturized trapping column in line with a conventional LC column, will be discussed in detail elsewhere.

Considerable improvements in detection limits are also possible in CF-FAB, since the flow-rate adjustment by PSS avoids splitting and combines a conventional LC system with a high loadability with an on-line miniaturization to meet the interface requirements of a 5–10 μl/min flow-rate[30]. Direct coupling of miniaturized LC systems with CF-FAB also reduces the split ratio, or even avoids the splitting, but in practical applications this does not mean an improvement in concentration detection limits since the injection volume is correspondingly decreased, as outlined in Table I. Only if the sample size is the limiting factor does miniaturization give significant improvement. Also, if a given mass is injected into miniaturized systems, the increase in the concentration of the analyte is balanced by the reduced flow-rate, as pointed out before[2].

TABLE I

SOME TYPICAL VALUES IMPORTANT IN COUPLING DIFFERENT FORMS OF HPLC WITH CONTINUOUS-FLOW FAB

	Injection volume (μl)	*Flow-rate (μl/min)*	*Split ratio*
Conventional HPLC	100	1000	1:200
Microbore HPLC	5	50	1:10
Packed fused-silica micro-LC	0.5	5	No split
Open tubular HPLC	0.001	0.01	Make-up flow

In LC concentration-sensitive detectors are generally used, therefore most of the effort has gone into those compression techniques that result in an increased concentration of the analyte in the detector. For MS detection the effect of the flow-rate is also worth considering.

Several approaches are possible: *e.g.* the technique of belt-speed programming[31] using the moving-belt interface. The basic concept is extremely simple. The analyte is deposited on to the belt at a low belt speed and desorbed from the belt not at the same belt speed, as is usually done, but at an increased belt speed. This results in a higher mass-flow given by:

$$dm/dt = m'V_2/V_1 \quad (7)$$

where m' is the mass-flow without belt-speed programming, and V_1 and V_2 are the belt speeds for depositing and desorption, respectively. An example of the belt-speed programming approach is given in Fig. 5, comparing peaks obtained with either a constant belt speed or by means of belt-speed programming from 1.6 to 3.6 cm/s after

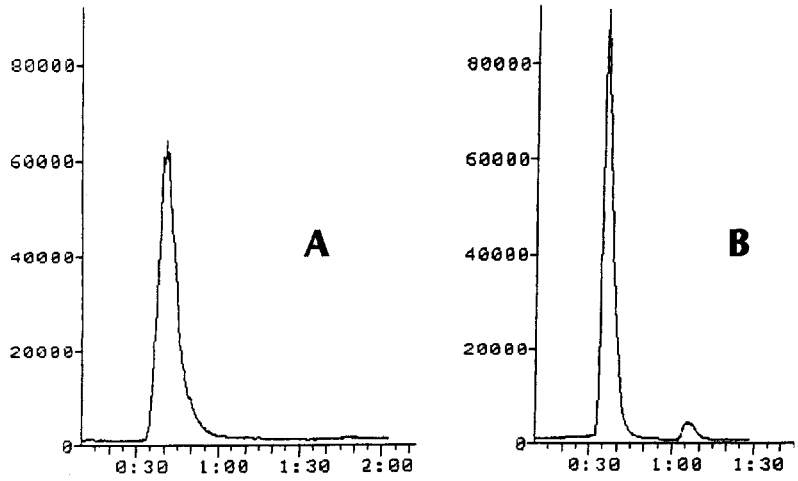

Fig. 5. Belt-speed programming with diuron, with (A) the normal situation with a constant belt speed, and (B) the peak compression achieved by increasing the belt speed after deposition on the belt.

deposition. The increased mass-flow of a factor of *ca.* 2 is reflected by an corresponding increase in peak height and decrease in peak width. A limitation is the reduced maximum flow-rate for deposition as a consequence of the lower applied belt speed. This restricts the attainable benefit from the approach in conventional LC systems, but this is not the case in SFC–MS using the moving-belt interface, as has been demonstrated[31], or if for sample size requirements miniaturized LC systems are used, for instance packed fused-silica columns in combination with the moving belt, as demonstrated by Barefoot and Reiser[32]. Furthermore, the temperature of the sample evaporator must be set at a low value, suitable for the low belt speed, but too low for the high belt speed[31].

Another approach in increasing the mass-flow by flow-rate programming is possible for miniaturized LC systems. With those systems a post-column increase of the flow-rate will result in an increased mass-flow. Flow-rate programming in the usual way is rather limited with respect to the attainable flow-rate range, therefore the possibilities of analyte trapping were studied, not on a precolumn as before in the PSS approach but in a storage loop that can be switched into a high flow-rate system as used for the TSP analysis. The general setup for such an experiment is given in Fig. 6. The results of a preliminary experiment are given in Fig. 7. A peak for metoprolol is produced at a flow-rate of 10 μl/min, which is a typical value for packed fused-silica columns of 0.32 mm I.D. After storage the analyte is measured by TSP LC–MS at a flow-rate of 1.5 ml/min. The increase in mass-flow is now given by:

$$dm/dt = m'F_2/F_1 \tag{8}$$

where m' is the mass-flow without flow-rate programming, and F_1 and F_2 are the flow-rates in the micro-LC and the TSP systems, respectively. Theoretically, a peak

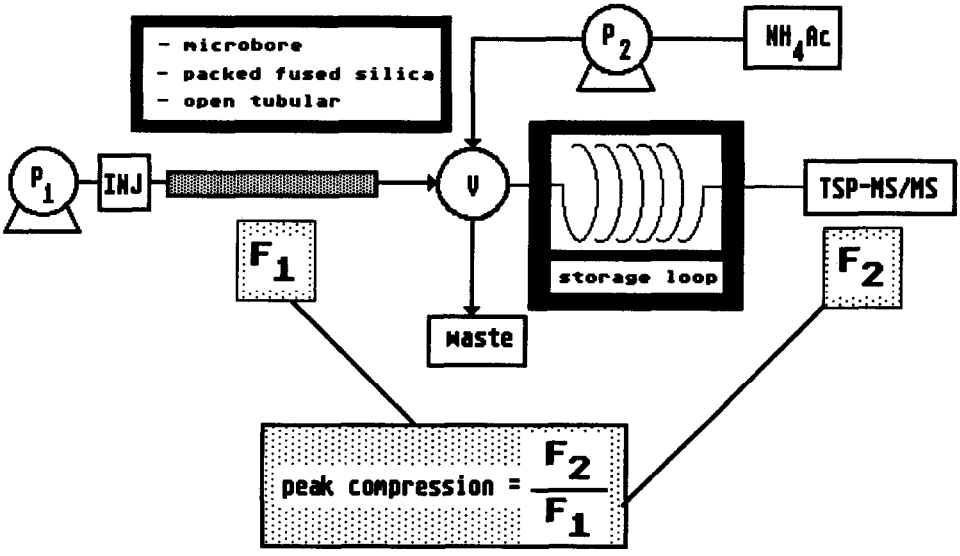

Fig. 6. Schematic diagram of mass-flow programming by trapping of an analyte from a miniaturized LC system via a storage loop and analysis by TSP LC–MS using a high flow-rate.

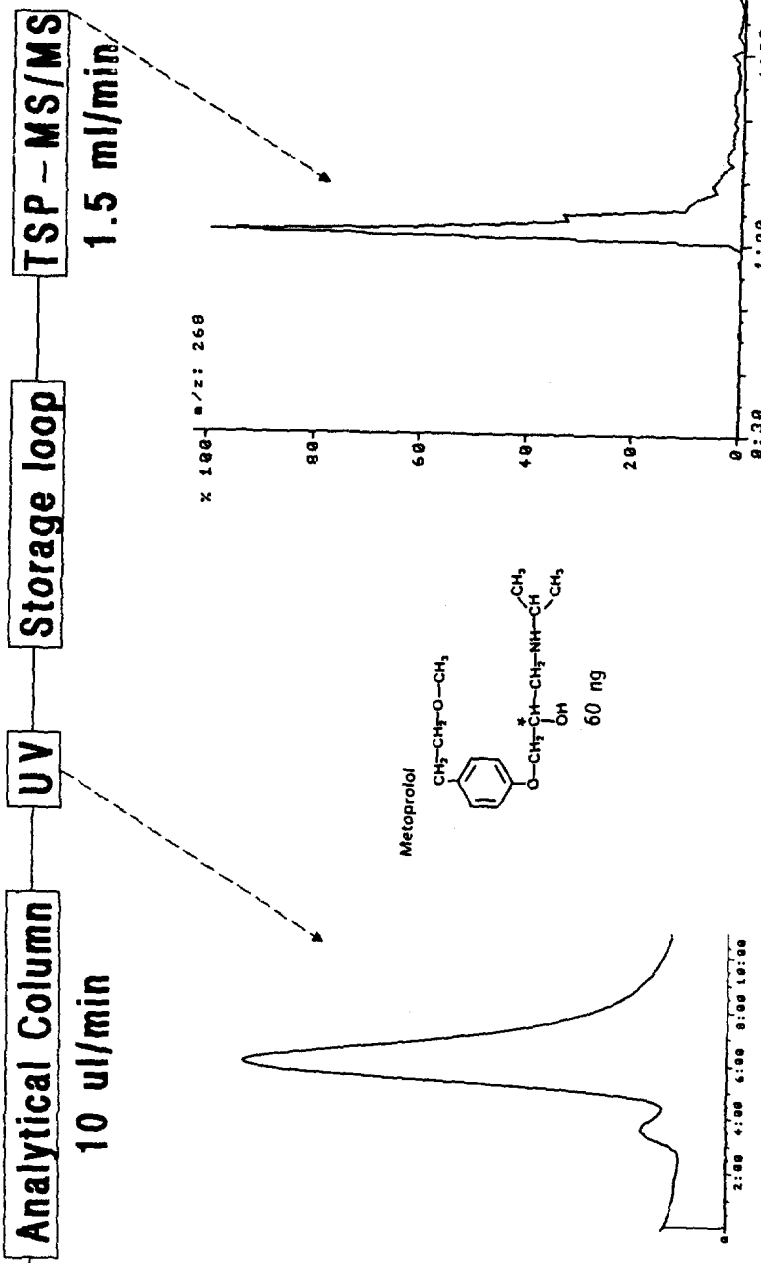

Fig. 7. Mass-flow programming experiment by trapping of metoprolol in a storage loop at a low flow-rate and analysis at a high flow-rate using TSP LC–MS. Peak compression of *ca.* 40-fold is achieved. A schematic diagram of the experimental setup is in Fig. 6.

compression of 1500/10 = 150 should be possible in this case. A factor of *ca.* 40 is measured, which is considerable but still much less than theoretically achievable. This can be explained by the fact that the dimensions of the various system components and the appropriate time windows for switching had not yet been optimized in this preliminary experiment. Nevertheless, such a compression factor could be obtained in a straightforward way. This approach is of course only of value if miniaturized LC systems are needed as outlined above.

DERIVATIZATION IN LC–MS

The role of chemistry in LC–MS has been small and is generally neglected, since often as a primary goal of LC–MS coupling it is formulated that analysis can be performed without the need to perform the kind of chemical modifications which are often necessary in gas chromatography (GC)–MS[33]. However, some of the interfaces strongly rely on chemistry in their LC–MS operation, such as DLI, TSP and CF-FAB, and it is clear that the detection can be improved considerably if the chemistry is tuned properly. This has been pointed out by Vouros *et al.*[34]. In particular, derivatization procedures are of importance and will be discussed, although other forms of incorporating chemical interactions, electrochemistry or biochemistry, are attractive. For instance, mobile phase additives can enhance detection in DLI in the negative ion CI mode[35–37], the use of a TSP interface as a flow reactor[38], electrochemistry in combination with TSP–MS[39] or TSP–MS–MS[40] or the elegant approach of combining LC columns and various columns containing immobilized enzymes for rapid protein sequencing[41,42].

In LC, derivatization is widely used to improve the detection properties. The scarce attention to this method in LC–MS can be partly explained again by the low input from the chromatographic side in this area. Furthermore, the combination of derivatization and detection makes other (selective) detection methods, *e.g.* fluorescence, competitive with LC–MS. The incorporation of selective derivatization reactions is in general only applicable in target compound analysis.

In pre-column derivatization the chemistry influences the chromatographic, the interface and the MS detection properties, whereas in post-column mode only the latter two are affected, with the additional problem of the high concentrations of derivatization reagent in the mobile phase. To improve the MS detection the approaches well known in GC–MS can be used, such as increasing the molecular weight to enhance the selectivity, volatility enhancement, the introduction of electrophoric groups for negative ion chemical ionization, and increasing the stability of the molecular-weight-related ions.

Pre-column derivatization

Several papers have appeared describing the analysis of derivatized compounds but without a clear strategy for LC–MS analysis, so they will not be discussed.

Silylation has been applied by Quilliam and Yaraskavitch[43] for the analysis of fatty acids for similar reasons as in GC–MS, namely to increase the volatility and to broaden the range of compounds to be analysed by the moving-belt interface. The reduced polarity also allows the choice of less polar mobile phases for the chromatography, which is advantageous for this interface type. *tert.*-Butyldimethyl-

silyl derivatives were preferred for stability reasons, and provided both molecular weight and structure information in the EI mode. Anthryl esters of fatty acids were used in another study[44] using a non-aqueous reversed-phase system.

Separation of enantiomeric amphetamines and detection with a moving-belt interface combined with EI has been performed[45] after derivatization with N-(trifluoroacetyl)-1-propyl chloride to form the diastereoisomers, which could be separated by a chiral column. Derivatization was applied to achieve the separation but the label might be attractive to enhance to detection in negative ion chemical ionization (CI) mode as well.

Amino acids, peptides and oligopeptides have been studied by several approaches. Yu *et al.*[46] used a moving belt to study the behaviour of N-acetyl-N,O,S-permethylated derivatives of oligopeptides. CI with isobutane as the reagent gas yielded sequence information, while the chromatographic integrity of the gradient system was maintained by using a heated-gas nebulizer for effluent deposition on the belt. DLI LC–MS for N-acetyl-O-methylester derivatives of oligopeptides were presented by Arpino and McLafferty[47]. Studies on derivatized amino acids have been published for LC–MS using atmospheric pressure ionization[48], DLI[49], the moving belt and TSP[50]. For the latter two a comparative study revealed that underivatized amino acids decompose to some extent at low concentrations on the moving-belt system and to a lesser extent in thermospray, although one compound could not be detected. Phenylthiohydantoin-amino acids gave satisfactory results on the belt system.

Derivatization of carbonyl compounds with 2,4-dinitrophenylhydrazine yields informative spectra in positive and negative ion CI in combination with the moving belt[51]. Aldehydes and ketones can be distinguished in the negative ion mode, and low-molecular-weight compounds became sufficiently involatile to be analysed by LC–MS.

The negative ion behaviour of pentafluorobenzyl (PFB) esters of hydroxylated metabolites of docosahexanoic acid in TSP[52] with the auxiliary filament on was used to confirm the molecular weight observed in the positive ion mode. An abundant $[M-PFB]^-$ ion, as normally observed under electron-capture conditions, was obtained for various metabolites present in a rat brain incubate.

Voyksner *et al.*[53] showed that the sensitivity for prostaglandins and thromboxane B_2 in TSP was limited by the low proton affinity of the compounds. Derivatization with diethylaminoethyl chloride (or bromide) increased the proton affinity, which was reflected in a considerable improvement in the detection limit. The positive ion mode was more sensitive than the negative ion mode, but an improvement by a factor of 2–3 was still observed for the latter technique. Detection of a prostaglandin metabolite in plasma at a level of 30 ppb was shown after including the derivatization step.

Derivatization to improve the detectability in CF-FAB seems to be straightforward, since considerable work has been performed for conventional FAB[33]. The derivatization to form charged derivatives or preformed ions, for instance by Girard's P or T, will increase the sensitivity as observed by us for progesterone in FIA in the CF-FAB mode. However, if derivatization is performed in pre-column mode the chromatography for the ionic compounds, will be less compatible with on-line LC–MS.

Post-column derivatization

The extraction device developed for the moving belt enabled Karger and co-workers[17,18] to perform on-line ion-pair extraction and to study the MS behaviour of the ion-pairs formed. The volatilization of ionic compounds using this method has been studied[54,55], and alkylsulphonates and sulphates were studied as counter-ions in both EI and CI modes. The flash evaporation induces thermal degradation, yielding for instance an alkene, sulphuric acid and an amine in the case of sulphate–amine ion-pairs. This ion-pair concept was further evaluated to a derivatization system based on the alkylation of analytes during flash evaporation. In this case trimethylanilinium hydroxide was used as the ion-pairing reagent[34], and a complete conversion of stearic acid into methylstearate was shown. Furthermore, volatile carboxylic acids were analysed by this approach with tetra-*n*-butylammonium hydroxide[56] as well as derivatization of primary amines with carbon disulphide to form isothiocyanates[57].

In TSP, post-column derivatization of prostaglandins was achieved by forming methyl esters[58] with tetra-*n*-butylammonium hydroxide as the reagent and the TSP vaporizer and the ion source as the reaction chamber. The yield of the methyl esters was maximized by setting the optimal temperatures of both the vaporizer and the ion source. A gain in the ion current of a factor of 3–6 has been reported.

CONCLUSIONS

LC–MS extended by multidimensional approaches in both LC and MS opens new research areas, which allow the improvement of the compatibility of the LC and MS parts, the optimal tuning of the overall selectivity and the enhancement of the detectability. These items will become even more important because of the successful development of new interface types that have special characteristics, such as low flow-rate requirements or extremely good sensitivity for ionic compounds, which can be applied successfully only if the chromatographic part is carefully optimized as well.

The role of chemistry is becoming more important since many interfaces are linked with ionization conditions possessing an important chemistry component. Derivatization, although not often applied in LC–MS, can be used successfully but a better understanding of the ionization mechanisms involved will be of great help in this area.

The present state of the art of LC–MS makes the method a powerful tool for an analytical laboratory and, because of the rapid developments in the field, much more can be expected in the near future.

REFERENCES

1 G. Guiochon and P. J. Arpino, *J. Chromatogr.*, 271 (1983) 13.
2 B. L. Karger and P. Vouros, *J. Chromatogr.*, 323 (1985) 13.
3 *The 36th American Society for Mass Spectrometry meeting on Mass Spectrometry and Allied Topics, June 5–10, San Francisco, CA, 1988.*
4 *The 12th International Symposium on Column Liquid Chromatography, Washington, DC, June 20–24, 1988.*
5 T. R. Covey, E. D. Lee, A. P. Bruins and J. D. Henion, *Anal. Chem.*, 58 (1986) 1451A.
6 W. H. McFadden and S. A. Lammert, *J. Chromatogr.*, 385 (1987) 201.
7 R. D. Smith, H. T. Kalinoski and H. R. Udseth, *Mass Spectrom. Rev.*, 6 (1987) 445.
8 A. J. Berry, D. E. Games and J. R. Perkins, *J. Chromatogr.*, 363 (1986) 147.

9 D. E. Games, A. J. Berry, I. M. Mylchreest, J. R. Perkins and S. Pleasance, *Eur. Chrom. News*, 1 (1987) 10.
10 L.-E. Edholm and L. E. Ogren, in I. Wainer (Editor), *Liquid Chromatography in Pharmaceutical Development*, Aster, Springfield, OR, 1986, p. 345.
11 L.-E. Edholm, C. Lindberg, J. Paulson and A. Walhagen, *J. Chromatogr.*, 424 (1988) 61.
12 J. V. Johnson and R. A. Yost, *Anal. Chem.*, 57 (1985) 759A.
13 A. C. Tas, J. van der Greef, M. C. ten Noever de Brauw, T. A. Plomp, R. A. A. Maes, M. Hoehn and U. Rapp, *J. Anal. Toxicol.*, 10 (1986) 46.
14 J. van der Greef, A. C. Tas, M. C. ten Noever de Brauw, M. Hohn, G. Meyerhoff and U. Rapp, *Adv. Mass Spectrom.*, 10 (1986) 587.
15 E. R. Verhey, G. F. LaVos, W. van de Pol, W. M. A. Niessen and J. van der Greef, *J. Anal. Toxicol.*, 13 (1989) 8.
16 P. O. Edlund and J. Henion, presented at the *36th ASMS Conference on Mass Spectrometry and Allied Topics, June 5–10, San Francisco, CA*, p. 1095.
17 B. L. Karger, D. P. Kirby, P. Vouros, R. L. Foltz and B. Hidy, *Anal. Chem.*, 51 (1979) 2324.
18 B. L. Karger, D. P. Kirby and P. Vouros, *J. Chromatogr. Sci.*, 18 (1980) 111.
19 D. P. Kirby, P. Vouros, B. L. Karger, B. Hidy and B. Petersen, *J. Chromatogr.*, 203 (1981) 139.
20 J. A. Apffel, U. A. Th. Brinkman, R. W. Frei and E. A. I. M. Evers, *Anal. Chem.*, 55 (1983) 2280.
21 J. A. Apffel, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.*, 312 (1984) 153.
22 J. R. Lloyd, M. L. Cotter, D. Ohori and A. R. Oyler, *Anal. Chem.*, 59 (1987) 2534.
23 F. A. Maris, R. B. Geerdink, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 323 (1985) 113.
24 W. M. A. Niessen, P. J. M. Bergers, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, 454 (1988) 243.
25 W. M. A. Niessen, M. A. G. de Kraa, E. R. Verhey, P. J. M. Bergers, G. F. LaVos, U. R. Tjaden and J. van der Greef, *Rapid Commun. Mass Spectrom.*, 3 (1989) 1.
26 K. McKellop, G. Hansen and F. Hatch, presented at the *33rd ASMS Meeting, San Diego, CA, May 26–31, 1985*, p. 765.
27 J. van der Greef, W. M. A. Niessen and U. R. Tjaden, *J. Pharm. Biomed. Anal.*, 6 (1988) 565.
28 E. R. Verhey, H. J. E. M. Reeuwijk, G. F. LaVos, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *Biomed. Environ. Mass Spectrom.*, 16 (1988) 393.
29 A. Walhagen, L.-E. Edholm, C. E. M. Heeremans, R. A. M. van der Hoeven, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, 474 (1989) 257.
30 P. Kokkonen, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, 474 (1989) 59.
31 E. R. Verhey, G. F. LaVos, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, 474 (1989) 275.
32 A. C. Barefoot and R. W. Reiser, *J. Chromatogr.*, 398 (1987) 217.
33 R. J. Anderegg, *Mass Spectrom. Rev.*, 7 (1988) 395.
34 P. Vouros, E. P. Lankmayr, M. J. Hayes, B. L. Karger and J. M. McGuire, *J. Chromatogr.*, 251 (1982) 175.
35 C. E. Parker, K. Yamaguchi, D. J. Harvan, R. W. Smith and J. R. Hass, *J. Chromatogr.*, 319 (1985) 273.
36 R. D. Geerdink, F. A. Maris, G. J. de Jong, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 394 (1987) 51.
37 D. Barceló, F. A. Maris, R. B. Geerdink, R. W. Frei, G. J. de Jong and U. A. Th. Brinkman, *J. Chromatogr.*, 394 (1987) 65.
38 M. M. Siegel, R. K. Isensee and D. J. Beck, *Anal. Chem.*, 59 (1987) 989.
39 G. Hambitzer and J. Heitbaum, *Anal. Chem.*, 58 (1986) 1067.
40 K. J. Volk, M. S. Lee, R. A. Yost and A. Brajter-Toth, *Anal. Chem.*, 60 (1988) 722.
41 H. Y. Kim, D. Pilosof, D. F. Dyckes and M. L. Vestal, *J. Am. Chem. Soc.*, 106 (1984) 7304.
42 K. Stachowiak, C. Wilder, M. L. Vestal and D. F. Dyckes, *J. Am. Chem. Soc.*, 110 (1988) 1758.
43 M. A. Quilliam and J. M. Yaraskavitch, *J. Liq. Chromatogr.*, 8 (1985) 449.
44 J. D. Baty, R. G. Willis and R. Tavendale, *Biomed. Mass Spectrom.*, 12 (1985) 565.
45 S. M. Hayes, R. H. Lin, W.-S. Tsang, M. G. Legendre, R. J. Berni, D. J. Pillion, S. Barnes and M. H. Cho, *J. Chromatogr.*, 398 (1987) 239.
46 T. J. Yu, H. A. Schwartz, S. A. Cohen, P. Vouros and B. L. Karger, *J. Chromatogr.*, 301 (1984) 425.
47 P. J. Arpino and F. W. McLafferty, in F. C. Nachod, J. J. Ackermann and E. W. Randall (Editors), *Determination of Organic Structures by Physical Methods*, Academic Press, New York, 1976, Vol. 6, p. 1.
48 E. C. Horning, D. I. Caroll, I. Dzidic, K. D. Haegele, M. G. Horning and R. N. Stillwell, *J. Chromatogr.*, 99 (1974) 13.

49 D. J. Dixon, *Analusis*, 10 (1982) 343.
50 D. E. Games and E. D. Ramsey, *J. Chromatogr.*, 323 (1985) 67.
51 K. L. Olsen and S. J. Swarin, *J. Chromatogr.*, 333 (1985) 337.
52 J. A. Yergey, H.-Y. Kim and N. Salem, Jr., *Anal. Chem.*, 58 (1986) 1344.
53 R. D. Voyksner, E. D. Bush and D. Brent, *Biomed. Environ. Mass Spectrom.*; 14 (1987) 523.
54 D. P. Kirby, P. Vouros and B. L. Karger, *Science (Washington, DC)*, 209 (1980) 495.
55 D. P. Kirby, P. Vouros, B. L. Karger, B. Hidy and B. Petersen, *J. Chromatogr.*, 203 (1981) 139.
56 C.-P. Tsai, A. Sahil, J. M. McGuire, B. L. Karger and P. Vouros, *Anal. Chem.*, 58 (1986) 2.
57 P. Vouros, A. Sahil, C.-P. Tsai and B. L. Karger, presented at the *33rd ASMS Meeting on Mass Spectrometry and Allied Topics, San Diego, CA, May 26–31, 1985*, p. 379.
58 R. D. Voyksner and E. D. Bush, *Biomed. Environ. Mass Spectrom.*, 14 (1987) 213.