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LIQUID CHROMATOGRAPHY MASS SPECTROMETRY IN THE PHARMACEUTICAL INDUSTRY: OBJECTIVES AND NEEDS

F. ERNI
Analytical Research and Development, Pharmaceutical Department, Sandoz Ltd., Basle (Switzerland)

CONTENTS

1. INTRODUCTION

In the past, two-dimensional techniques[1] have proved to be the most powerful tools for solving analytical problems. Amongst all "hyphenated techniques"[1], the combination of separation and spectroscopy is, from a theoretical as well as a practical point of view, the most promising for providing information. The great success of GC–MS in the past and the increasingly important role of high-performance liquid chromatography (HPLC) raises the question about the future of LC–MS coupling in routine laboratory practice. Recent advances in this technique give hope that LC–MS is developing from a research toy to a sophisticated analytical tool. It is the aim of this paper to compare the possibilities of the most advanced approaches of on-line LC–MS coupling, namely the transport interface and the direct liquid inlet (DLI), with the needs of typical applications in the pharmaceutical industry[3,4].

2. ROLE OF LC–MS IN THE ANALYSIS OF PHARMACEUTICALS

Table I gives a list of five different areas of application of LC–MS in the pharmaceutical industry. The reasons for an important role of LC–MS in the future seem to be very similar to those that have led to the success of HPLC over the past few years, *viz.* (i) the ability to handle thermally and chemically labile compounds, (ii) the ability to handle compounds of low volatility, (iii) simple sample preparation and (iv) speed of analysis (chromatography and sample preparation time).

Like GC and HPLC, the future role of LC–MS will be in competition with that of GC–MS. Compounds of low molecular weight which have good thermal stability and adequate volatility will stay a domain of GC–MS application. However, as was

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TABLE I

APPLICATIONS OF LC–MS IN THE PHARMACEUTICAL INDUSTRY

By-products	identification
	quantification of traces
Degradation products	of the drug substance
	in the dosage form
	quantification of special compound
Bio-assays	plasma
	urine
	other biological materials
Metabolism	identification
	stable isotopes
Screening	

the case for HPLC in the past, the future of LC–MS will be with compounds of high molecular weight which have critical thermal and chemical stability and low volatility. Even if this statement sounds trivial, many applications of LC–MS shown in the past have treated samples which would have been much more suitable for GC–MS than for LC–MS. The importance of LC–MS compared to GC–MS is in the expansion of applications to new classes of compounds which are not accessible to GC–MS. This means that before using LC–MS, simple chromatographic methods such as GC and HPLC or the established technique of GC–MS would be tried. The priorities of the use of different techniques is therefore GC > HPLC > GC–MS > LC–MS.

From Table I it can be seen that the primary goals of LC–MS are (i) obtaining structural information, (ii) identification, (iii) selective detection, and (iv) sensitive detection. From Table I it also becomes evident that the needs will be for *qualitative* and *quantitative* analysis.

3. REQUIREMENTS AND PRESENT LIMITATIONS

Looking at LC–MS as an analytical system, one may view the coupling from the LC side, therefore treating the MS as an LC detector, or from the MS side, treating the LC as a system for preparation and introduction of samples. Table II shows that the aims and requirements of the two views appear different. It is obvious that there is still a long way to achieving an ideal LC–MS interface. In view of the strong competition of GC–MS, it seems especially important that thermally labile compounds and compounds with low volatility can be handled with the LC–MS system. It appears from experiments with alkaloids, glycosides and peptides that decomposition on the transport interface is a critical point. Experiments have shown that the mass spectra vary very much with the concentration on the belt. In some cases it was impossible to obtain the interesting higher masses (including the molecular ion) at low sample concentrations whereas at high concentrations the mass spectra appear complete and include abundant molecular ions. It is therefore very dangerous to test an LC–MS interface by simulating LC conditions, *e.g.* by spotting on a belt or by injecting directly into the mass spectrometer without making use of an LC column. This concentration effect not only causes problems in the structural elucidation of components occurring in low abundance but may even lead to artefacts and makes quantification very complicated because of the lack of a simple means of calibration.

TABLE II

AIMS AND REQUIREMENTS FOR (a) MS AS LC DETECTOR AND (b) LC AS MS SAMPLE-INTRODUCTION AND SAMPLE-PREPARATION SYSTEM

Aims	*Requirements*
(a) MS as LC detector	
Selectivity	No restrictions to LC conditions
Sensitivity	Must handle chemically and thermally
Structural confirmation	labile compounds
Structural information	Must handle low volatility compounds
Reproducibility	Mass range ⩾ 1000
(b) LC as MS sample-introduction and sample-preparation system	
Selectivity of separation	No restrictions to MS conditions
Sample clean-up	"Clean" input
Sample concentration device	
Speed	

In routine laboratory work, separations with reversed-phase systems account for more than 90% of all HPLC work. It is therefore obvious that the LC–MS interfaces must be judged by their suitability for reversed-phase mobile phases containing high amounts of water. Present LC–MS systems with transport as well as with direct-inlet interfaces need solvent splitting at flow-rates of *ca.* 2 ml/min for reversed-phase HPLC to cope with mobile phases of up to 95% water. Micro-HPLC may prove to be an excellent solution to this problem in the future.

In the past few years some promising successes in obtaining mass spectra from compounds of low volatility using LC–MS interfaces have been reported[2–4,12,13]. However a lot more work must be done in this field. It should be a challenge for instrument manufacturers to go further in this direction because each increase in the range of applications to less volatile and bigger molecules increases the potential market of LC–MS instruments. There is definitely a great need for LC–MS analysis of molecules with masses over 1000.

4. FLOW-SWITCHING TECHNIQUE

Compared to the requirements of MS, LC is far from being a "clean technique". For biological samples especially, the matrices of the mixtures often cause problems for the clean environment of the mass spectrometer. In many cases, time-consuming clean-up procedures are necessary and even then pollution of the ion source is of major concern. A polluted ion source interferes markedly with trace determinations whereas optimal instrument performance is necessary for adequate sensitivity. Therefore unnecessary excessive amounts of non-volatile material should not be introduced into the mass spectrometer. This is true for the transport interface as well as for the direct liquid inlet. It is especially important if the mixture contains large amounts of inorganic salts (*e.g.* in urine) or organic components which decompose to non-volatile materials in the ion source (*e.g.* sugar in pharmaceutical preparations). To overcome these problems the application of a flow-switching technique was investigated[2,4]. The basic principle of this technique is to bypass the LC–MS interface for parts of the chromatogram while at the same time injecting a steady

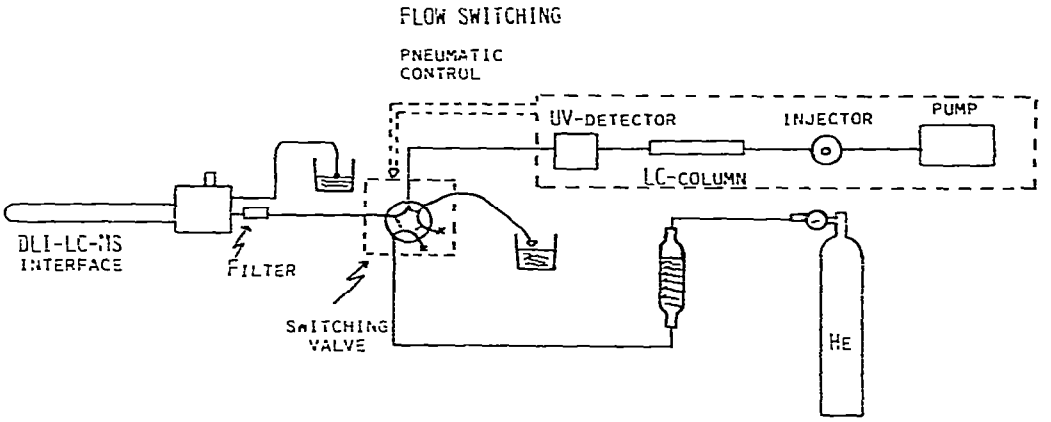

Fig. 1. Schematic drawing of the flow-switching instrument. High-performance liquid chromatograph with gradient-elution capabilities and variable-wavelength UV–visible detector, Model HP 1084 B (Hewlett-Packard, Palo Alto, CA, U.S.A.). Switching valve: Rheodyne Model 7010 A fitted with sample introduction valve (Rheodyne, Berkeley, CA, U.S.A.). Model 5985 B mass spectrometer with LC–MS interface (Hewlett-Packard).

stream of pure mobile phase into the chemical ionization (CI) source. When that portion of the chromatogram which is of interest is eluted, the chromatograph is switched on-line to the LC–MS interface. With this technique the components present in high concentrations which are incompatible with LC–MS do not enter the mass spectrometer and sensitive detection of the interesting components under optimal conditions with a clean ion source is possible. The flow diagram of such a flow-switching device is shown in Fig. 1. The mobile phase from the LC column, after passing an optional UV-visible LC detector, comes to a low dead-volume switching valve, which is preferably pneumatically activated with the possibility of automatic operation. A normal six-port injection valve can be used for this purpose (Fig. 2).

During the elution of components which are not of interest or which may pollute the mass spectrometer at higher concentrations, the switching valve is on position "bypass" and the LC–MS interface is fed with pure solvent to maintain the

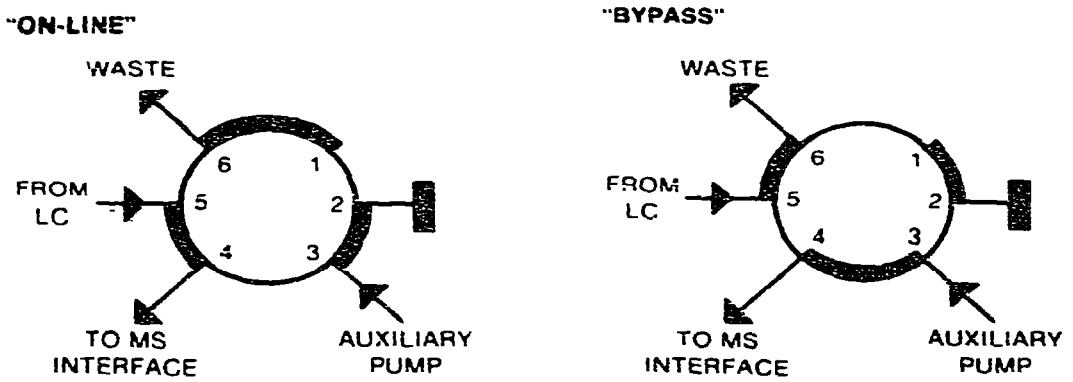

Fig. 2. The six-port injection valve as a low dead-volume flow-switching valve.

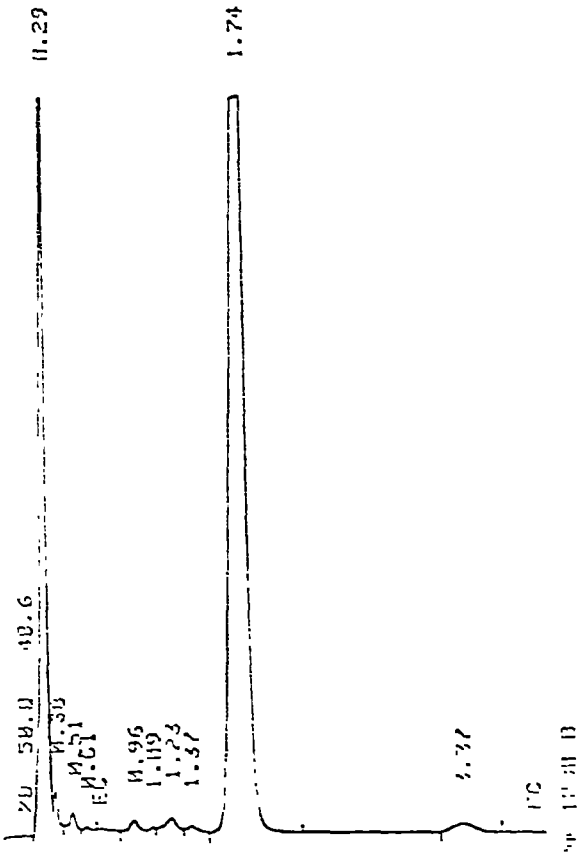

Fig. 3. Liquid chromatogram of an extract of a Parlodel tablet. Sample preparation: 1 tablet in 2 ml mobile phase for 10 min in an ultrasonic bath, clear solution injected after centrifugation. Column: RPS, 10 μm, 10 × 0.32 cm. Mobile phase: acetonitrile–0.01 *N* ammonium formate (1:1). Flow-rate: 2 ml min. Injection volume: 10 μl.

dynamic steady state conditions in the CI ion source. Before elution of the interesting parts of the chromatogram, the switching valve is turned to "on-line" (Fig. 2) and the eluate enters the conditioned and clean ion source. A simple inexpensive stainless-steel cylinder filled with the desired solvent mixture and pressurized with helium can be used as an auxiliary pump for the delivery of clean solvent during the bypass cycle.

4.1. Application of LC–MS with flow switching to pharmaceutical preparations

Fig. 3 shows the liquid chromatogram obtained from the analysis of a tablet of Parlodel which contains the brominated ergot alkaloid bromocriptine as an active ingredient. It is not recommended to inject all the components of a crude tablet extract into the mass spectrometer. In particular the excipients which elute without retention may seriously affect the sensitivity of the following LC–MS analysis. With the flow-switch-ing technique these excipients can easily be bypassed. The interesting part of the LC chromatogram can then be analysed with the DLI–LC–MS technique and good mass spectra are obtained (Fig. 4).

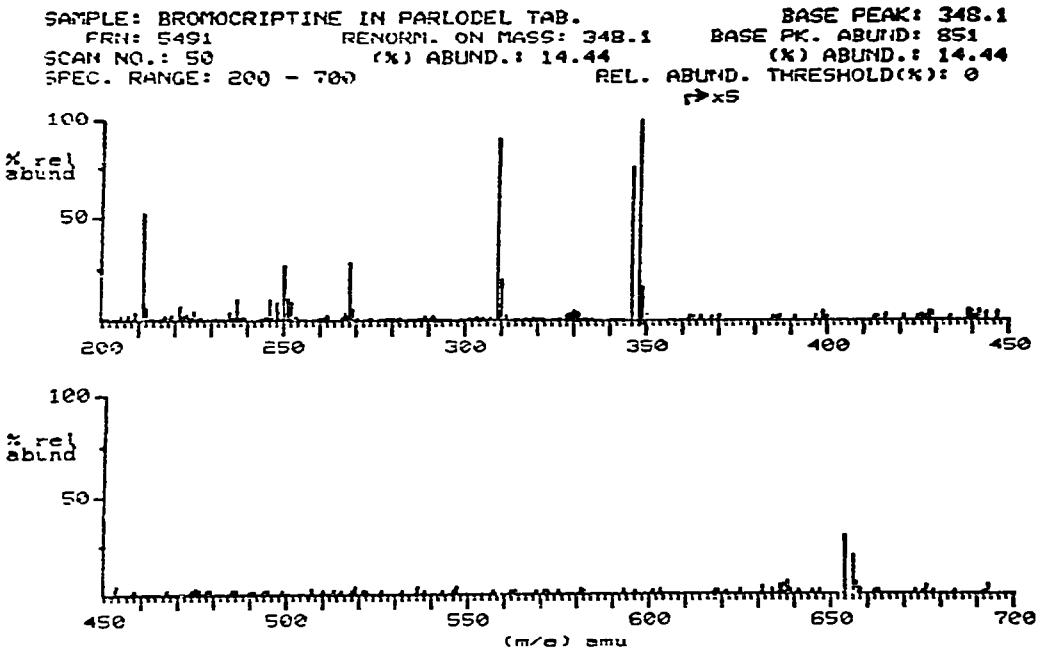

Fig. 4. Positive ion chemical ionisation (PI-CI) mass spectrum of bromocriptine in Parlodel tablet. LC conditions as in Fig. 3. Ionisation gas: mobile phase; source: 350°C at 0.7 Torr; analyser: $1.4 \cdot 10^{-4}$ Torr; scan: 200–700 a.m.u., 100 a.m.u./sec.

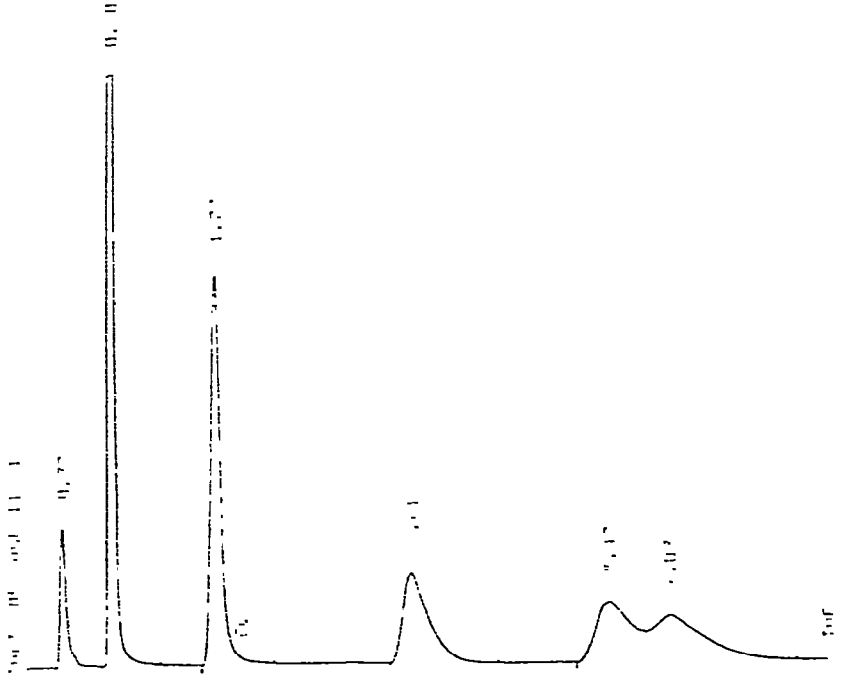

Fig. 5. Liquid chromatogram with UV detection of Hydergine drop solution. Direct injection without further sample preparation. Mobile phase: acetonitrile–aqueous 0.01 *N* ammonium formate (35:65). Other conditions as in Fig. 3.

Fig. 5 shows the LC chromatogram[5] of Hydergine drop solution which contains the hydrogenated ergot alkaloid codergocrine mesylate. Fig. 6 shows the reconstructed ion chromatogram for three typical masses. The chromatogram demonstrates clearly the high selectivity of the LC–MS determination and its ability to solve complicated separation problems by means of selective detection. The ions at *m/e* 270 and the UV trace in Fig. 5 both show overlapping peaks for dihydroergocristine and dihydroergocriptine. The more selective ions at *m/e* 211 and 245, which originate from peptide fragments typical for each of the two compounds, give single peaks with no interference.

4.2. Applications to biological samples

In the analysis of urine spiked with lysergic acid *N,N*-diethylamide (LSD) (Figs. 7 and 8), the high selectivity and sensitivity of the LC–MS detection is again shown. Fig. 9 illustrates that in the urine blank no interference is detectable. The detection limit of LSD in urine is *ca.* 200 pg for single-ion monitoring (SIM).

Because of the high selectivity of the detection and the absence of interferences, on-line concentration techniques[6–8] can be used so that much larger sample volumes (up to several millilitres) can be used. This is especially effective when combined with column-switching techniques[9,10].

All the examples demonstrate clearly that with the flow-switching technique sample preparation prior to LC–MS analysis can be minimal. This is not only time efficient but also minimizes the formation of artefacts during the sample-treatment steps.

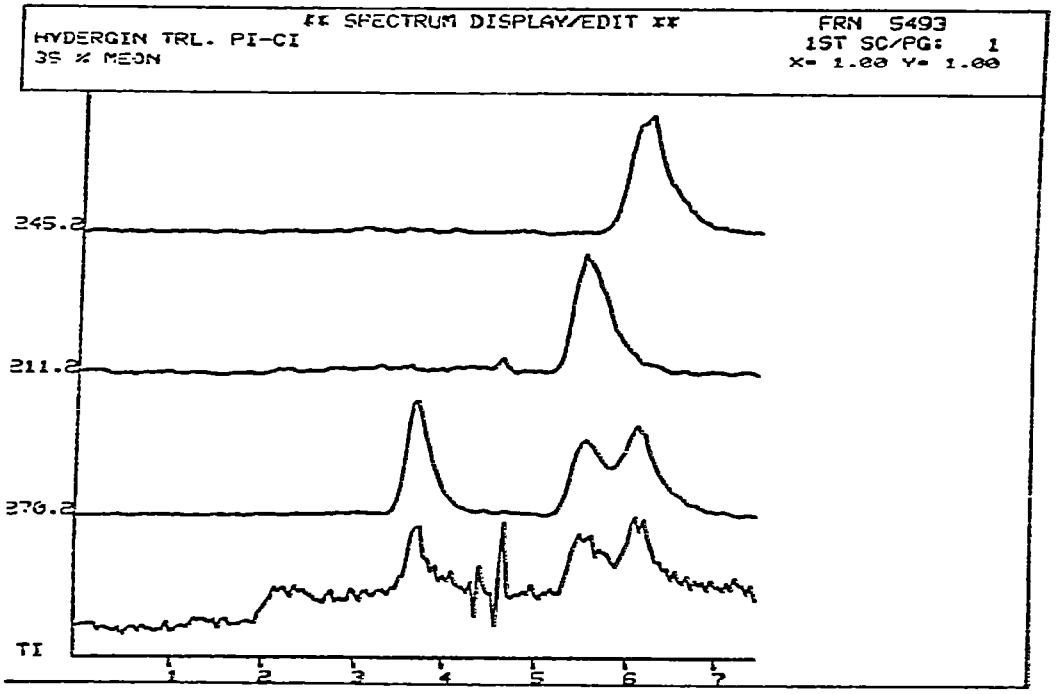

Fig. 6. Reconstructed ion chromatogram of the LC–MS analysis of Hydergine drop solution.

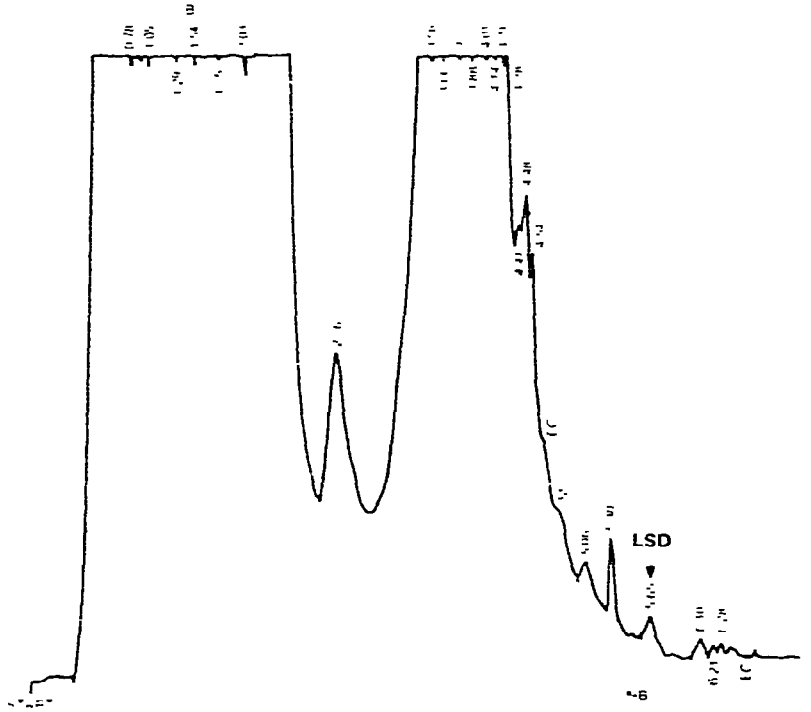

Fig. 7. Liquid chromatogram with UV detection of urine spiked with 2 ng LSD. Mobile phase: acetonitrile–aqueous 0.01 *N* ammonium formate (10:90). Gradient: start after 2 min, 10–90 % in 3 min, heart cut to LC–MS interface by flow switching from 4.7 to 6.3 min. Other LC conditions as in Fig. 3.

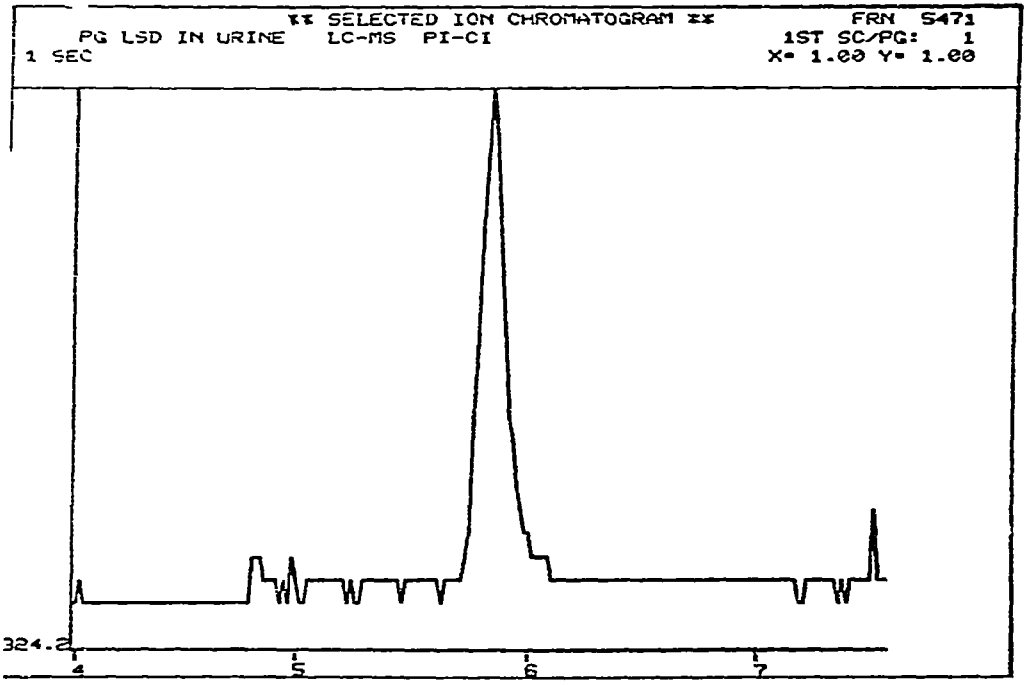

Fig. 8. Single-ion monitoring (SIM) of 2 ng of LSD in urine (*cf.* UV trace of Fig. 7).

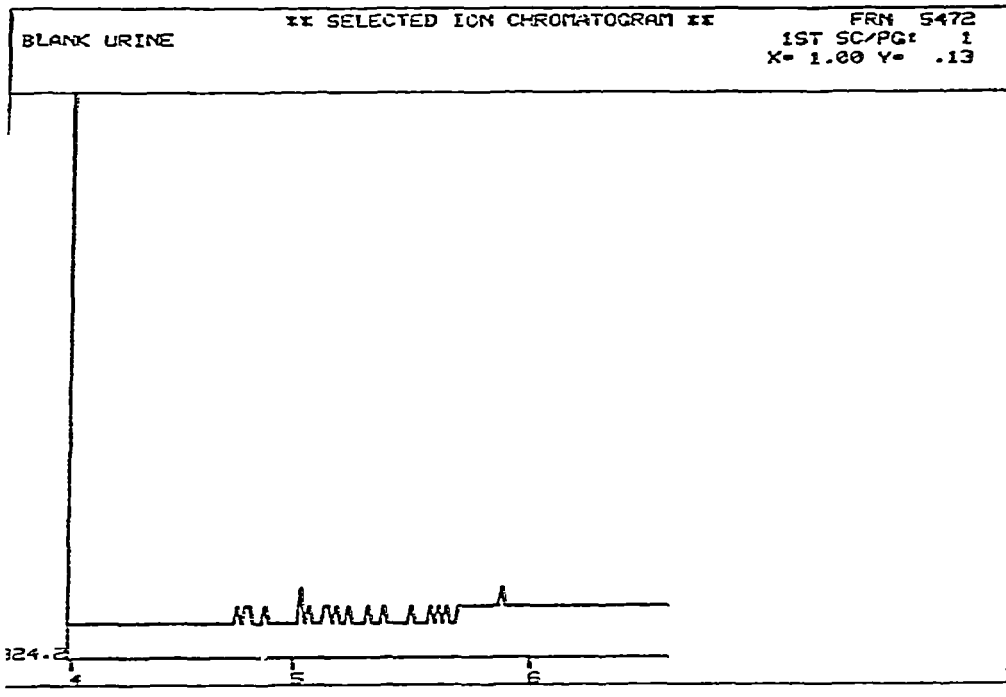

Fig. 9. Urine blank SIM (*cf.* Fig. 8).

5 FLOW INJECTION

The application of sophisticated on-line sample-preparation techniques in LC–MS as described previously makes use of the selectivity of separation of modern HPLC. There are, however, applications where no complicated sample treatment is necessary because of the great selectivity of MS detection. In some cases there is not even any need for separation with LC at all and the LC–MS interface is used for simple sample introduction. The set-up, shown in Fig. 10, is comparable to a flow-injection analysis[11] system with a sophisticated highly sensitive and selective detector.

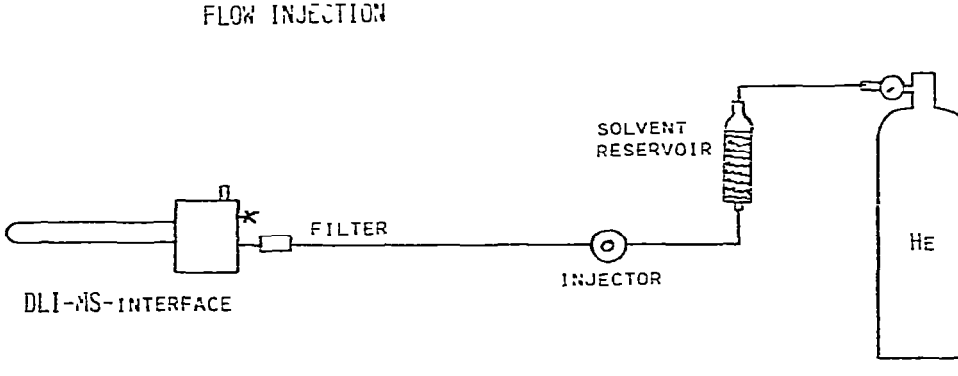

Fig. 10. Schematic drawing of the components of the flow-injection instrument.

Fig. 11 shows the sensitivity of such a system for quantitative analysis of picogram levels of an ergot alkaloid[12]. If necessary, more complex sample-preparation modules can be used for on-line sample extraction from solids[14], for on-line filtering[14], for liquid–liquid distribution and ion-pair extraction[15] and for evaporation to dryness[15] and there are many possibilities for on-line derivatisation[16]. The sample throughput of such methods is up to ten samples per minute. In all the above-mentioned examples the LC–MS coupling stripped from the LC column and expanded with on-line sample preparation techniques make use of the selectivity and sensitivity of modern mass spectrometers. This technique may be of interest to other MS techniques such as MS–MS or Q^3.

6. CONCLUSIONS

LC–MS will play an important role in the analysis of drugs in the near future. The main field of application will be thermally labile compounds, and compounds with low volatility and with high molecular weights, all of which are not generally suitable for GC–MS analysis. The presented data demonstrate that LC–MS coupling combined with a flow-switching technique can be used for the analysis of mixtures containing large amounts of components which would be detrimental to the LC–MS technique. The possibility of bypassing the mass spectrometer while maintaining steady-state conditions in the ion source provides excellent sensitivity and good reproducibility for quantitation. The technique can easily be automated and ensures the

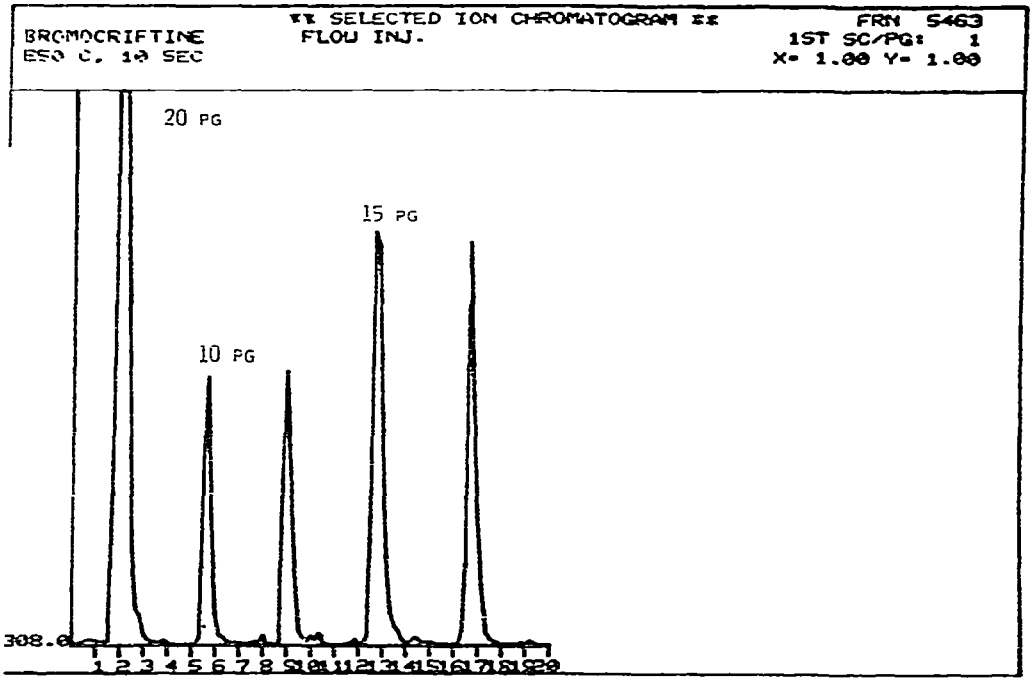

Fig. 11. SIM of a flow-injection analysis of 20, 10 and 15 pg samples of bromocriptine with negative-ion chemical ionisation. Ion-source temperature 300°C.

analysis of complex mixtures without complicated preparation of samples.

With flow-injection techniques the LC–MS interface is used as a sample-introduction system with many possibilities for sample preparation, sample cleanup and chemical derivatisation. These methods may be of interest as well for MS–MS and Q^3 techniques.

7. SUMMARY

The role of liquid chromatography–mass spectrometry (LC–MS) in the analysis of drugs is discussed. The main fields of application are thermally labile compounds, compounds with low volatility and compounds with rather high molecular weights, all of which are not generally suitable for analysis by combined gas chromatography–mass spectrometry. The objectives, needs, limitations and abilities of LC–MS for the analysis of by-products, degradation products, traces of drug substances for pharmacokinetic studies and metabolites in complex matrices are presented. The LC–MS coupling is discussed as a sophisticated LC detector for sensitive and selective quantitative determinations or as an on-line sample-introduction system for the mass spectrometer to obtain structural information for identification or structural elucidation. LC–MS combined with a flow-switching technique can be used for the analysis of mixtures containing large amounts of components which otherwise would be detrimental to the LC–MS technique. With flow-injection techniques the LC–MS interface is used as a sample-introduction system with possibilities for sample preparation, sample clean-up and chemical derivatization.

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