INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION
COMMISSION ON MICROCHEMICAL TECHNIQUES AND TRACE ANALYSIS*

ANALYTICAL TECHNIQUES FOR TRACE ORGANIC COMPOUNDS—VI

APPLICATION OF LIQUID
CHROMATOGRAPHY–MASS SPECTROMETRY

(Technical Report)

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Analytical techniques for trace organic compounds—VI: Application of liquid chromatography–mass spectrometry (Technical Report)

<u>Abstract</u> - The development of combinations of liquid chromatography with mass spectroemtry in an on line fashion has come to a stage where such combinations become part of routine procedures for the analysis of a broad variety of samples. Since several technically very different interfaces have been devised in recent years, the major types are briefly discussed, even though it becomes more and more clear that only a few will survive. The most outstanding device appears to be the atmospheric pressure sources with electrospray and chemical ionization, since it covers many areas of chemical analysis in terms of polarity, lability and molecular weights. The attempt has been made not only to give a picture of the techniques, but to indicate by means of a few examples, how useful the different devices are and where they are at their limits. Furthermore, the criteria are indicated which may be applied to make a choice on the basis of analytical requirements and technical and financial possibilities.

1 INTRODUCTION

The separation power of modern chromatography combined with the selectivity and very low detection limits of modern mass spectrometers enables us to analyze very complex samples with a high degree of confidence. A previous IUPAC report(1) has dealt with the combination of gas chromatography and mass spectrometry, which has been extensively used in trace organic analysis, and in this report we present a look at LC/MS and its applications to trace organic analysis. In recent years LC/MS has made a transition from a developmental stage to a more mature level where the technique is now being applied on a regular basis to many problems. Recently some of the various types of LC/MS interfaces are being used on a routine basis. Although LC/MS still needs further improvements, this technique has clearly reached a point where it now makes very valuable and unique contributions to organic trace analysis. In a book recently published a great number of references has been compiled and a discussion of some of the techniques is given, which indicates clearly the growing importance of this approach(2).

The various forms of separations in the liquid phase, such as high performance liquid chromatography (HPLC), ion chromatography (IC), and capillary zone electrophoresis (CZE), are well known for their analytical power in separating complex samples. However, one of the weak points of LC technology has been the lack of a suitable universal detector which could also achieve low detection limits. In past years, several attempts have been made to harness the power of mass spectrometry to provide the desired universal detector with low detection limits for liquid chromatography, especially for HPLC. The difficulties in interfacing a liquid effluent with the high vacuum system of a mass spectrometer are rather obvious.

Although the combination of mass spectrometry with gas chromatography is still most important and successful(3) (probably about 80% of all mass spectrometers are GC/MS instruments), LC/MS is rapidly increasing in importance. A number of analyses either require derivatization steps for gas chromatography or are poorly compatible with the technique due to problems of poor volatility, high polarity, or thermal lability of the analyte. While derivatization may be very useful even in LC/MS, it is desirable to avoid it if possible, because the level of systematic errors - dilution, unknown loss of analyte - may be increased, and background problems may reduce the gain in sensitivity.

In this paper we will discuss the most important and useful of the currently available LC/MS interfaces and indicate the strengths and weaknesses of each (in a recent book, a different, more detailed approach has been used(4)). Also, some examples of applications will be given to illustrate the types of analyses for which LC/MS has proved to be a powerful technique in organic trace analysis. In the following discussion, we have divided the LC/MS interfaces into three different groups: the first group comprises methods with sputter techniques such as fast atom bombardment (FAB), also known as liquid secondary ion mass spectrometry (LSIMS), or laser desorption; the second group contains techniques with separate aerosol and ion generation; and the third group containing thermospray and electrospray, both techniques in which aerosol and ion generation occur essentially in the same step.

Requirements of Chromatography and Electrophoresis

Before we start the discussion of the interfaces, a short look at the principal requirements of modern liquid chromatography and capillary electrophoretic techniques may shed some light on the difficulties in interfacing them to mass spectrometry:

High Performance Liquid Chromatography (HPLC):
In HPLC, the most important method today, we have to consider the reversed phase eluents H_2O, buffer solutions, methanol, other alcohols and acetonitrile with flow rates ranging from 1-2 ml/min or, for micro HPLC, 2-100 µL/min; and for the normal phases, a broad range of organic solvents with similar flow rates are used. For ion chromatography the need for strong salts and buffers imposes additional complications on the construction of the interfaces.

Supercritical Fluid Chromatography (SFC):
Supercritical fluid chromatography is increasingly used in separations and normally, CO_2, sometimes with modifiers is used as eluent; for capillary SFC, the flow rate is 1 µl/min, which translates into about 1 ml gas/min; the construction of an interface is therefore not a technical problem. For packed columns the flow is in the range of about 10 ml/min and a jet separator is mandatory.

High Performance Electrophoresis:
In high performance capillary electrophoresis (CE), the situation is different. The flow - if there is any - is usually well below 1 µL, since only the electro-osmotic flow (EOF) can be used as driving force. Therefore, only capillary zone electrophoresis (CZE) and isotachophoresis (ITP) with EOF can be interfaced to an MS(5). In addition, the sometimes high concentrations of buffers necessary - particularly in ITP - can create further problems depending on the MS technique employed.

Requirements of Mass Spectrometry

From the discussion above it is evident that conditions for optimum chromatography are often very different. When the requirements of the mass spectrometer are considered along with the various types of MS instruments and techniques, the complexity increases significantly.

Ion generation
Although the analyst may wish to have a free choice of ionizing methods such as electron impact (EI), chemical ionization (CI) or negative chemical ionization as well as the desorption methods FAB/LSIMS or laser desorption, the interface will severely restrict the choice. Some interfaces make use of one particular method, or else the ion generation is an integrated part of the technique (e.g. continuous flow FAB, thermospray, electrospray).

Mass Spectrometric Data Acquisition
The solution of an analytical problem may require a) full mass spectra (for library search, structure information), b) raw data of a small mass range (e.g. for the determination of isotope patterns or just molecular weights) c) mass chromatograms of selected ions (for the quantitation of target compounds with lowest detection limits) MS/MS experiments with collisionally activated decompositions (CAD),

such as daughter ion, parent ion and neutral loss scans, may be necessary to get structural information, when the soft ionization methods are used.

The Mass Spectrometer

It is clear that some of the mass analyzers are suitable for the techniques mentioned above, others not. Quadrupoles are the most flexible and affordable analyzers, especially in triple stage instruments. Sector field instruments have some technical problems with LC/MS interfaces, although they provide advantages with respect to resolution, accuracy and mass range. The revival of the time of flight spectrometers has opened up a new area in terms of sensitivity, but interfacing to chromatography is not straight forward. The same holds true for ion traps and ion cyclotron resonance (Fourier transform) MS, although the ion trap may prove to be the mass spectrometric choice for the future in LC/MS.

Taking all these aspects together it is evident that the LC/MS problem has many solutions, dictated by the chromatography, the mass spectrometry / the mass spectrometer and, of course, the problems one has to solve.

2   INTERFACES USING DESORPTION TECHNIQUES

As indicated already, the first chapter in the discussion of the techniques is devoted to interfaces comprising sputter techniques. Aside from the methods discussed here, the coupling of thin layer chromatography (TLC) with SIMS(6), FAB(7) and laser desorption(8) should be mentioned, which has recently given surprisingly good results and holds promise for the future. The method will not be reviewed here in any detail, since it is not an LC/MS technique in a strict sense (for a recent review see(9)).

The Moving Belt Interface

The moving belt system, invented by W. McFadden on the basis of Scott's experiments using a moving wire(10) as one of the first LC/MS interfaces, was in the beginning not used in conjunction with sputter techniques, but rather with thermal desorption of the analyte from the surface of the belt either by direct or indirect heating(11); for a review see(12).

The principle of the technique is shown in Fig. 1: The effluent of the LC is directed onto the surface of a belt made from pure nickel or polyimide. It runs through a series of vacuum stages into the ion source of the mass spectrometer. Infrared heaters and washing steps have been included to minimize the memory effects and to clean the belt(13). Considerable effort has been directed towards the optimization of the
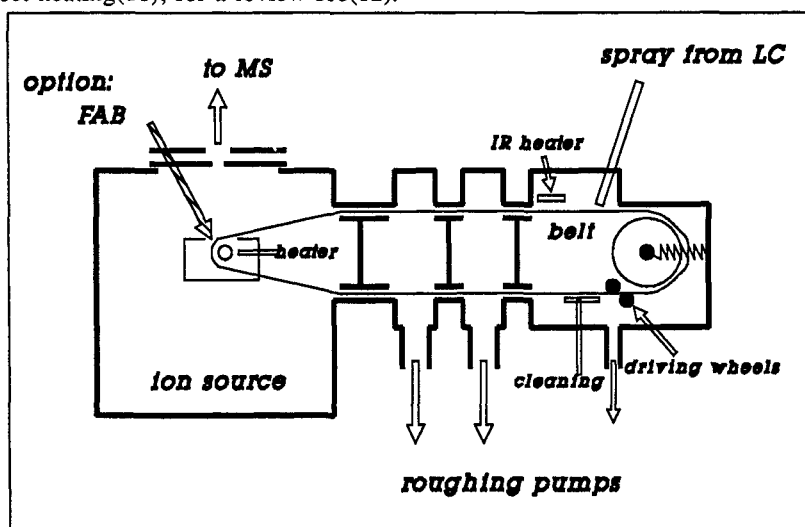
Fig. 1: *The moving belt interface*

spraying process, since the integrity of the chromatography is highly dependent on the efficiency and the precision of the transfer; thermally assisted spraying(14), thermospray(15) and frit systems(16) with contact to the belt have been developed to cover the surface of the belt smoothly and evenly. The interface has an unusual advantage among the LC/MS interfaces in that it achieves the complete separation of chromatography and mass spectrometry, therefore permitting the independent

optimization of chromatographic and mass spectrometric conditions. HPLC using reversed and normal phases, in normal and micro versions and supercritical fluid chromatography have been used with EI, CI, SIMS(17), FAB(18) and laser desorption(19,15).

However, severe background problems and, at least when used with thermal desorption, the limitations due to vaporization in addition to the elaborate technical requirements (including further vacuum systems, maintenance of the belt and consequently the rather high costs) have limited the number of devices used in routine analysis.

The Continuous Flow FAB Interface

Continuous flow FAB (CFFAB), sometimes called on-line FAB, was developed by Caprioli(20), and the closely related frit FAB technique by Ito(21). It appears as a logical extension of the wide spread FAB MS.

The technique has the advantage of technical simplicity, although the details are not straightforward, especially when used as a routine technique on a day to day basis. Therefore, special attention has been paid to the target and several versions have been made. The stability of the total ion current can be a problem, although the frit FAB versions have proven to be very reliable; the introduction of a wick of filter paper improved the situation considerably.
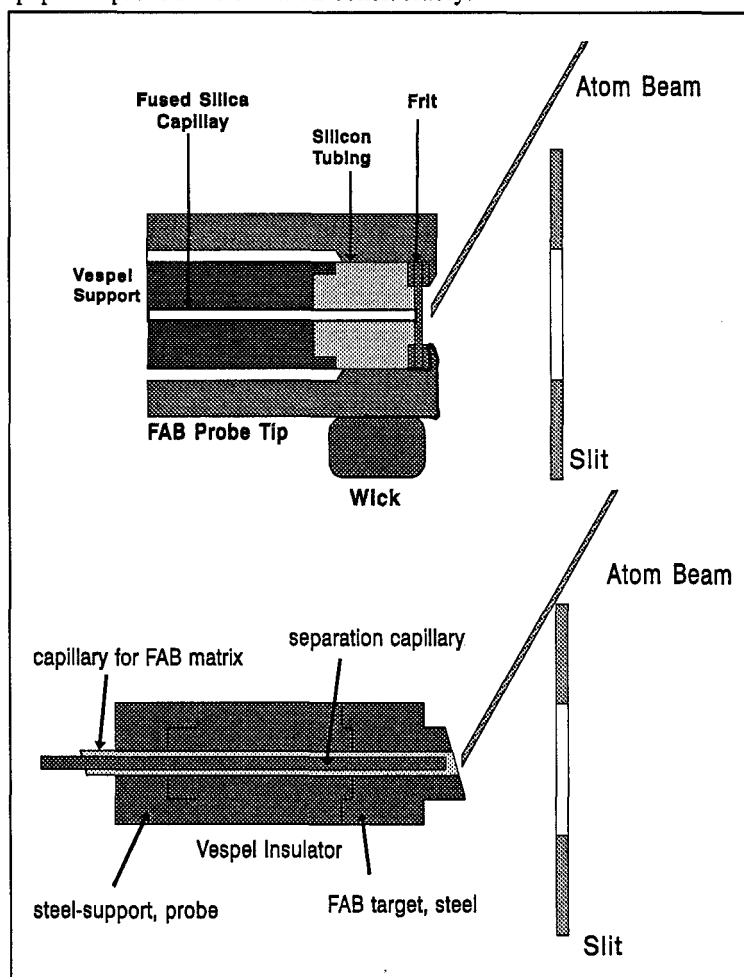

Fig. 2: *Two common designs for CFFAB targets: a frit as surface (top) and the coaxial construction for low flow rates*

The sensitivity can be enhanced by adding fast pumping to the source by a cold trap and/or

turbomolecular pumps. It is necessary to add typically 1-5% glycerol to the eluent reaching the target, which can be done in a post column setup(22) or by concentric tubing(23); the main limitation is that only micro HPLC can be used with flow rates up to 15 μL/min. Recently, capillary zone electrophoresis has been interfaced with success(24,25), although serious timing problems due to very narrow peaks from the electrophoretic separation have to be handled, and it seems that spectrometers with simultaneous detectors compare very favorably(26) to scanning spectrometers.

3   INTERFACES USING AEROSOLS AND *ADDITIONAL* ION GENERATION

A second principal method for interfacing liquid chromatography and mass spectrometry is to produce an aerosol from the column eluate, allow the droplets to decrease in size by evaporation until the dissolved analyte remains and then generate ions. Several methods have been employed to produce an aerosol of as small as possible sized droplets either in vacuum or under atmospheric pressure. In vacuum, the fine droplets tend to aggregate and the heat transfer has to be optimized to minimize this effect.

The Direct Liquid Introduction (DLI) Interface

The direct liquid introduction, proposed by Tal'roze in 1968(27) and again by Baldwin and McLafferty in 1973(28), was probably the first LC/MS interface to be used in modern organic mass spectrometry. A flow of typically 10-20 μl/min is allowed to enter the vacuum chamber of the spectrometer without any separation of eluent and analyte. This can be achieved by using a micro HPLC(29), a narrow capillary or a diaphragm with a typically 5 μm hole, splitting 1-4% from the mainstream(30); the latter approach was used in a first commercial version(31). The construction is relatively simple and the interface could be used with any common ion sources without changing the pumping systems(32); the main
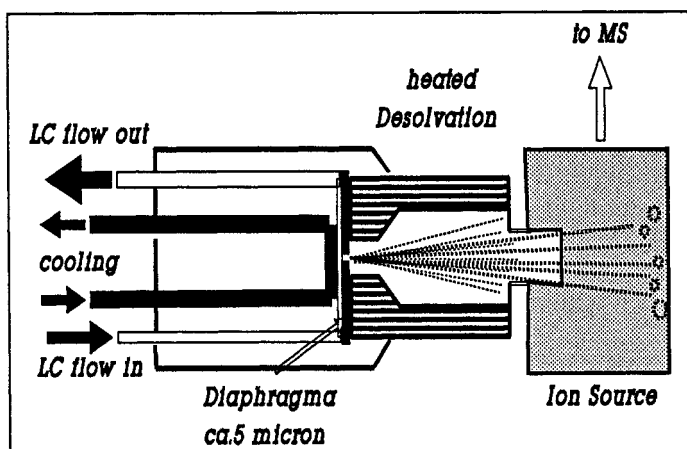

Fig. 3: *The direct liquid introduction interface*

disadvantage can be seen in the inflexibility with respect to the ionizing method (CI only using the eluent as reactant gas) and limitations in compounds amenable to analysis, which were not substantially different from those possible by GC/MS. In addition, only volatile buffers can be used. Although this interface demonstrated the feasibility of LC/MS in organic analysis and several attempts have been made to improve the performance by adding makeup gas streams to the liquid(33,34), the disadvantages were so problematic that today it is rarely in use.

The Particle Beam Interface

In the particle beam interface (called MAGIC by its developers Willoughby and Browner(35), Monodiperse Aerosol Generator Interface for Chromatography), an aerosol is produced under atmospheric pressure and the droplets allowed to dry in a heated expansion chamber. The dried particles, embedded in an additional gas flow, are separated from the sol-vent molecules in a momentum separator prior to reaching the conventional ion source. Here, the particles are vaporized upon impact and the analyte can be ionized by a rather wide choice of techniques, ranging from EI to FAB(36). Electron impact spectra rather similar to common EI spectra from direct probe or GC/MS can be obtained, even allowing library searches. The efficiency of the technique depends largely on the aerosol generation, since uniformly sized droplets result in particles of a narrow size distribution, which can be handled more easily by the separator; several manufactorers have designed different

technical versions of that interface type. The main problems of the technique are the unsatisfactory detection limits (ng range), the wide variations in sensitivity for rather similar compounds and the difficulties in obtaining linear responses over a wider range of concentrations, resulting in severe complications in quantitative determinations(37). In addition, components eluting at the same time interfere rather strongly with unpredictable effects. The technique holds great promise, but many problems have still to be solved.
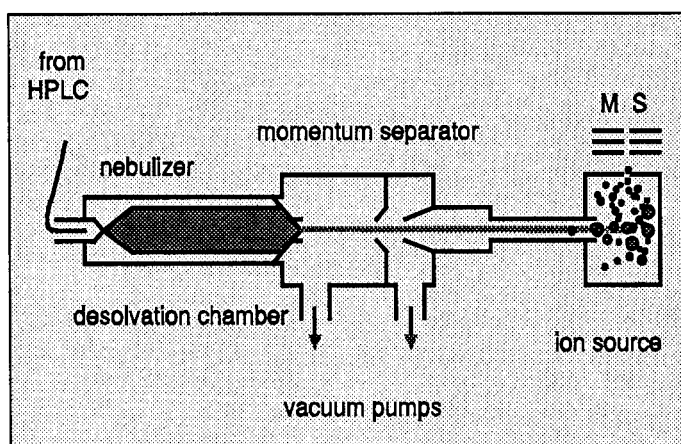

Fig.4: *The Particle Beam Interface*

The Supercritical Fluid Chromatography Interface

From a technical point of view the SFC interface is a direct introduction system with a careful temperature control to the very end of the capillary(38). The end of the capillary is a frit or a diaphragm keeping the phase supercritical until it leaves the capillary into the ion source; otherwise the dissolved analyte would clog the end of the capillary. The nature of this frit or diaphragm may be of importance for particular applications (see Fig.5). Since the gas load can be rather high, usually chemical ionization is used with capillary SFC; when packed columns are used, momentum separators can reduce the pressure in the ion source and electron impact becomes possible(39). Limitations of the method with respect to classes of compounds are mainly due to SFC itself, since in most cases CI is an appropriate ionizing technique; both, scope and limitatins have been discussed in a recent review(40).
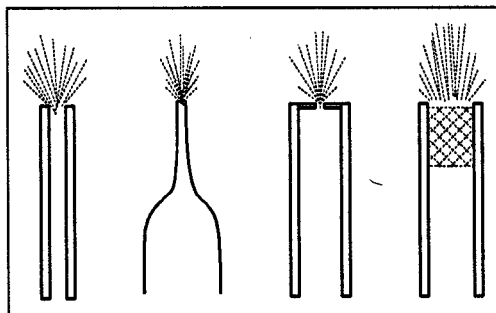
Fig. 5: *SFC restrictor types*

Chemical Ionization at Atmospheric Pressure

Atmospheric Pressure Ionization is one of the earliest techniques and its potential for an LC/MS interface has already been described in 1960 by Horning and coworkers(41). But almost 20 years passed before the technique was transformed into a mature method, which holds promise for several important applications(42,43). The technical realization is shown in Fig. 6. A stable spray is generated by heating an aerosol from the liquid effluent of the LC with a sheathflow of gas at atmospheric pressure(44). Ions are made by a
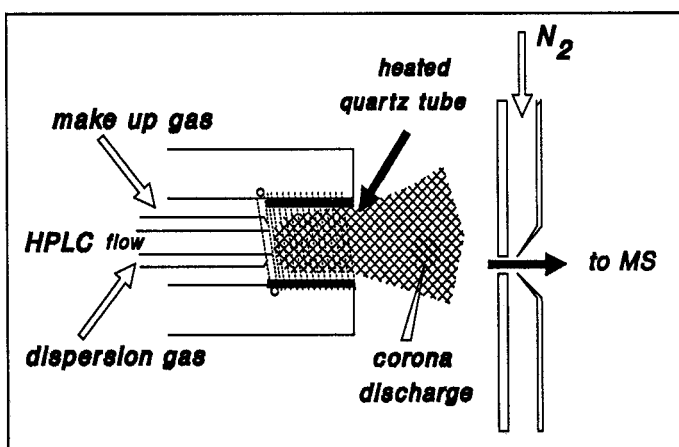
Fig. 6: *Atmospheric Pressure Chemical Ionization*

corona discharge in the spray, forming a chemical ionization plasma. The ions are extracted into the mass spectrometer by means of a skimmer system with a curtain of drying gas to reduce the background cluster ions from the solvent.

This principle is modified to some extent by the different manufacturers of instruments. In general, basic compounds with high proton affinity can be detected with lowest detection limits(45), but in some cases a strong temperature dependence was observed(46). All the API techniques have been reviewed repeatedly, e.g. see(47,48).

4 INTERFACES USING AEROSOLS *INCORPORATING* ION GENERATION

In this section, two important LC/MS techniques, thermospray and electrospray, will be described. Both techniques have the common feature that the generation of ions is an integrated part of the interface, which therefore dictates the ionization mode. But the success of both techniques depends largely on this fact, because the processes of ion generation, although greatly different, are rather unique. Mass spectrometric information has been obtained for compounds which were previously intractable to MS analysis. Both techniques have been discussed in noumerous reviews; for thermospray, see, for example, refs.(2,49,50) and for electrospray refs.(51,52).

The Thermospray Interface/Ion Source

Thermospray, pioneered in 1980 by Vestal and coworkers(53,54) is to date the most widely used LC/MS technique for routine applications in the chemical industry, although electrospray is moving in very fast. The technique is sketched in Fig. 7. The effluent of an LC containing a volatile buffer is rapidly heated in a steel capillary either by direct resistance heating(55,56) or by indirect heating using a cartridge system(57).

A very fine aerosol is formed in this process with a droplet size smaller than 1 μm. Some of the droplets are charged and may form ions; whether this is due to a process analogous to ion evaporation(58,59), by desolvation of the droplets to particles(60) or to processes similar to chemical ionization is still uncertain,

Fig. 7: *Thermospray Ion Source*

and a definitive theory is still to be written(61). The parameters have been studied very careful by Vestal and coworkers. The ions are sampled into the mass spectrometer by means of a cone reaching into the center of the inner chamber of the ion source, but the major part of the solvent is rapidly pumped away. The source has to be very tight to assure the pressure outside being a few pa.

It is possible to include a Townsend discharge or a filament to add plasma chemical ionization. With such an approach, it is not necessary to add buffer to the solvent; thus the sensitivity, the selectivity and the fragmentation behavior is changed. In a few cases a repeller electrode was used to increase the sensitivity.

A major advantage of thermospray is the ease to adapt an already developed method in liquid chromatography for LC/MS, since the normal flow rates and some of the most common buffers can be used.
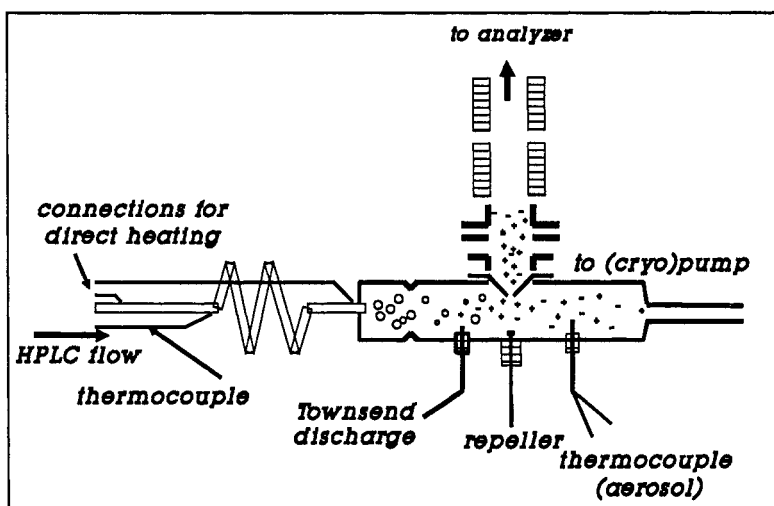
The Electrospray Interface

Electrospray is most promising in the biosciences to measure molecular weights of proteins, nucleic acids and similar compounds with molecular weights over 100,000 Daltons; therefore, much the attention is currently focused on this technique. The difference to thermospray is that the spray is formed by charging the aerosol to such an extent that the droplets explode by coulomb repulsion into smaller droplets, which eventually can be dried to yield highly charged molecules.

The setup is as follows(62): A capillary, connected to a micro HPLC (or a CZE capillary) with a flow of a few μL/min, is held at high voltage in atmospheric pressure to generate the spray. Sometimes this spraying process is supported by means of a make up flow of an organic solvent and, in a third layer, a gas. This version - called "ionspray" - allows the use of higher flow rates (up to 500 μL/min). The spray is dried further either using a heated gas curtain or a heated capillary and finally, the ions are extracted into the mass spectrometer by skimmer devices as explained for API/CI. (Fig. 8: Desolvation is performed using a gas curtain (A), a steel capillary (B) on acceleration potential or a glas capillary (C) with opposite potentials at both ends and gas curtain; the third version allows the ESI capillary to be on 3-4 kV above ground irrespective the acceleration voltage. The second pumping stage (B,C) is necessary for sector field type instruments. 1- sprayer, 2- desolvation, 3,4- skimmer, 5- to focusing elements, 6- to roughing pump, 7- to tubomolecular pump) The main advantage is that due to the multiple charging (up to 100 charges have been observed) molecules with very high molecular weights can be detected at low m/z values allowing the use of rather inexpensive quadrupole instruments. In addition, the combination with capillary electrophoresis appears to be very powerful(63-66), although this interface is not fully developed yet.
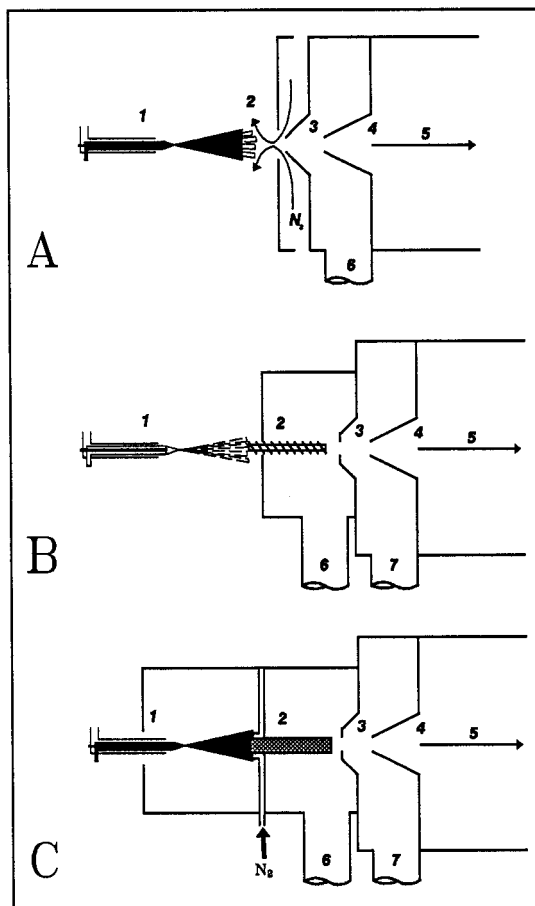
Fig.8: *Three different designs for ESI sources; for explanations see text*

5 APPLICATIONS AND EXAMPLES

Generally speaking, LC/MS is always the method of choice when liquid chromatography is an essential step in an analytical procedure anyway, namely for the determination of highly polar, chemically or thermally labile compounds. It is also the best method, when the detector has to be more sensitive, more specific or unknowns have to be identified. An advantage is that, at least for some of the LC/MS techniques, the development of a method needs no compromise for adaptation to mass spectrometry; drawbacks are the added degree of complexity in instrumentation and, in studies requiring precise quantitative work, the need for internal standards. In a book, which appeared recently, applications of LC/MS in several fields are highlighted(67).

In the following discussion the main topics are only highlighted rather than discussed in a comprehensive way, since the literature is so large and growing so rapidly that it is impossible to cover it in detail.

Environmental Applications

The examples from application in environmental analysis have to be very divers, because the matrices and the analytes span over a wide range of physically and chemically different materials. Matrices can be soil, sand or sludge, drinking or waste water and air (gaseous analytes or particulates). The analytes range chemically from nonpolar hydrocarbons to ionic organo metallic species, all requiring an appropriate means of preconcetration, separation and detection. Some of this aspects are covered in reviews(68,69) and the references given in the following dicussion are only meant to demonstrate the possibilities of the various techniques. For one class of compounds - methylcarbamates - it has been shown in a careful comparison(70) that the major techniques perform rather well even for quantitative determinations, although API/CI was the most sensitive technique in this case. The result may be different for another class of compounds.

Pesticides
For the analysis of several herbicides, LC/MS(71) and in particular thermospray LC/MS, appears to be the method of choice(72). Triazine derivatives have been determined(73) (using LC/MS/MS) and their photolysis products also(74). The chlorophenols and the phenoxyalkanoic acids, rather widely used herbicides, can be determined(75-77) as well as the sulfonylurea herbicides using thermospray(78) and CZE/MS with ion spray(79) and organophosphorus compounds(80).
The same holds true for insecticides(81), although the application of SFC/MS for organophosphorous compounds has also been shown(82). Other pesticides have been investigated by means of the DLI interface(32,83), but later the thermospray was introduced successfully(84,85), although postcolumn addition of buffer may be necessary(86). When normal phase chromatography is indispensable, postcolumn extraction into the thermospray interface is feasible(87). Again, organophosphates were determined by thermospray LC/MS(88), and packed column SFC/MS using a thermospray source with filament has been employed(89). API techniques have been used recently for methyl carbamates as already mentioned(70) and for sulfonylureas as well(71)

Polycyclic Aromatic Hydrocarbons
The PAH's were studied using the moving belt interface(90,91), and later SFC/MS was used, since the separation of PAH's is very efficient in SFC(92). A comparison of several approaches used for the determination of PAH's in sediments was published(93).

Organometallic compounds
Organometallic compounds are usually determined either with atomic detection methods such as AAS, giving information about the element only, or, after derivatization, by GC/MS of the fully alkylated species. It has been shown that not only the organo lead species derived from tetraethyllead in gasoline can be identified using thermospray LC/MS(94), but some tin compounds such as fentinacetate (triphenyltin acetate), a fungicide, and marine paint additives such as di- and tributyltin chlorides have been measured with thermospray as well. The latter and some arsenic species were determined at low levels using the API/CI and electrospray technique, although not using LC/MS but flow injection(95,96).
An overview over this field has been given recently in(97).

Technical Products

Since technical products cover a wide field in a chemical sense, most of the techniques have been employed here. In fact this may be the type of application, where the particle beam is most suited: Sensitivity is not always of concern and the spectra can be most useful.
Among the technical products, explosives such as TNT(98) and some of its metabolites(99,100) have been studied using direct liquid introduction or thermospray(101). The second class of compounds investigated by thermospray LC/MS quite extensively is dyes(102-104). In wastewater, azo dyes have been determined(105), and in particular the structure of sulfonated azo dyes has been elucidated(106,107); the benefit of repeller-induced dissociations in thermospray was also reported(108). In a detailed study a combination of several analytical techniques has been employed to analyze the

products of a quaternary ammonium salt used as preservative in cosmetics(109). Very recently it has been shown that even hydrocarbons from oil and coal sources can be analyzed by means of LC/MS(110,111).

Biomedical Applications

There is no doubt that in this field thermospray has got strong competition by the atmospheric pressure techniques, which perform often superior in terms of sensitivity and thermal stress, although the interface itself to the separation techniques are not as mature as in the thermospray case. Electrospray is, particularly for peptides, a technique with very unique features.

Amino Acids
Amino acids were among the first compounds analyzed by LC/MS. A moving belt interface combined with SIMS and laser desorption(19) and thermospray(112) was used, although the amino acids served to examine the interface rather than vice versa. A more detailed study of derivatized and underivatized amino acids was performed later(113) and for sequencing purposes phenylthiohydantoin amino acids(114) and hydrolysates of peptides were analyzed(115). Using the thermospray technique, unusual amino acids have been detected(116).

Nucleosides and Nucleotides
What was just said about the amino acids, is true for the nucleic acid constituents too as quite often they served to probe the new interfaces, since they are well known as difficult to analyze materials(11,19,62,117-119). Attempts to develop the DLI technique for the analysis of nucleic acid constituents have been made(120,121) and the method has been used to analyze human urine for nucleosides(122). Enzymic hydrolysates were analyzed using thermospray(123) and with the same technique, adducts of carcinogens(124) to guanosine and alkylation mechanisms(125) were investigated. Modified nucleobases(126) and analogs were determined(127,128), and with continuous flow FAB nucleotides and their metal complexes were studied(129). cyclic nucleotides from plant tissues were studied(130).

Saccharides
The most often used technique for the sugars and oligosaccharides is continuous flow FAB, which appears to be sensitive enough(131,132). In one study on permethylated saccharides, a comparison of normal FAB with CFFAB has been made(133). Upon silylation oligosaccharides are amenable to SFC/MS with good results(134).

Peptides and Proteins
For the structure analysis of proteins it not only necessary to determine the molecular weight, but also to analyze fragments made with enzymatic digests and even the amino acid composition, if necessary. The last was mentioned already, but the determination of molecular weight can be achieved either by electrospray coupled with microHPLC or, even more efficient with capillary electrophoretic techniques. As yet those methods are by no means routine, but the development especially of electrospray appears promising and fast. The first report on thermospray of recombinant eglin c with a molecular weight exceeding 8000 daltons appeared in 1986(135). Later, the potential of electrospray has been demonstrated(64,136,137) and there is no doubt that this technique is very well suited to give the most accurate molecular weight information on proteins, although CFFAB(138) and thermospray(139) have been successfully employed to determine molecular weights of peptides. When it becomes possible to extract the information on peptide sequence from the electrospray LC/MS/MS experiments made with multiply charged ions(140,141), that technique will be a very valuable tool for the protein chemists. This is also true for synthetic peptides, since the test for a correct systhesis and the purity has been the slowest step in the past; with CZE/MS using electrospray this has become a fast control experiment(142).

Bile Acids
In the past, procedures using static FAB methods were developed for the analysis of bile acids, but

with API LC/MS interfaces(143) and with thermospray(144) they have been determined successfully as well.

Steroids
The analysis of body fluids to determine the steroids has several aims; the most important ones are the detection of steroid drugs and the profiling of endogenous steroids. Both can be done quite effectively using LC/MS techniques. Among the drugs, dexamethasone and its metabolites have been determined(145), stilboestrol analogues(146) using thermospray, trenbolone(147) and testosterone esters(148) in tissue and injectables. By LC/MS/MS, boldenone sulfonates and other conjugates(149) were determined.In a quantitative study using isotope dilution, budenoside metabolites(150) were measured using a moving belt interface.
The use of thermospray LC/MS in profiling steroid conjugates in body fluids has been demonstrated successfully(151,152), in particular steroid sulphates(153) can be detected with high sensitivity. In another study, plasmaspray was used to analyze saliva of patients with congenital adrenal hyperplasia(154) for steroid hormones.

Pharmaceuticals

For the determination of compounds of pharmaceutical interest, some studies addressing more general topics(155), the routine requirements in industry(156) and some technical questions concerning the application of moving belt(23) and the direct liquid introduction(29) technique have been published. Special attention has been paid to the separation of optically active drugs(157), a problem of growing interest.
Antibiotics(158) have been studied as well as candidate antimalarials(159) and carnitine and derivatives(160) using thermospray and CFFAB for carnitine in urine(161). In a study on nivalenol(162), a comparison has been made for LC/MS and GC/MS. LC/MS has also been employed to identify bromazepam and other drugs in a case of intoxication(163). Additionally, detromethorphan(164) (with CFFAB) and metoprolol enantiomers(165) in plasma (using thermospray, MS/MS and phase switching) were identified as well as diphenhydramine, doxylamine and carbinoxamine(166) with thermospray. Sulfonamides were determined at very low levels using CZE and electrospray(167).

Drug Metabolites
The analysis of drug metabolites is one of the most important applications for LC/MS, since most of the metabolites are either chemically or thermally labile, and usually the isolation and purification involves some sort of liquid chromatography. Therefore, the development of analytical procedures is straight forward, if a suitable interface and MS technique is available. Depending on the nature of the drug and the metabolite, moving belt and DLI techniques have been used; recently the thermospray and increasingly the API techniques have shown great potential for the analysis of labile and highly polar metabolites. Metabolic profiling using thermospray LC/MS/MS has proven to be a rapid and successful technique for the search for new metabolites(168). As a few examples, studies shall be mentioned concerning the metabolism of ranitine(169,170), betamethasone(171), doxylamine(172,173), 8-methoxypsoralen(174) and almitrine(175) in body fluids and the determination of N-methylformamide-glutathione adducts(176).

Glucuronides
Conjugates of glucuronic acid are candidates for thermospray mass spectrometry due to their high polarity and several studies have been published(177,178). Steroid glucuronides(179) were characterized as well as those from methapyrilene(180) and from several phenolic compounds(181). Using CZE/MS drug glucuronides derived from formamides have been identified(182)

Conjugates
Among the metabolites discussed earlier are a broad range of conjugates, but a few classes should be mentioned here separately. Steroidal conjugates have been studied extensively, normally employing thermospray as mentioned already(150,152,179). Likewise, glutathione and its conjugates(183,184),

especially with methylformamide(176) and monomethylcarbamates(185) were assessed using thermospray, and in an comparison of CFFAB and thermospray, its adduct with chlorambucil was measured(186).

Natural Products

From the large number of compounds found in natural sources only the most important shall be discussed here: the lipids, the alkaloids and the fatty acids, all of which were characterized quite successfully with approaches involving LC/MS.

Lipids
The lipids have been characterized depending on their complexity by DLI or moving belt interface for the less polar, and by thermospray when glycerol or phosphate moieties are involved. Sphingoid bases were analyzed using the moving belt interface(187), and the direct liquid introduction interface was employed for parts of complex lipids(188-190). Intact phospholipids need the thermospray approach(191-193).

Alkaloids
Alkaloids have been analyzed rather early in the development of LC/MS, and in an impressive study on ergot alkaloids the potential of the moving belt has been demonstrated(194). Recently, CFFAB(195) and thermospray has been used to study catharanthus alkaloids(196), castanospermine related alkaloids(197) and senecio alkaloids(198).

Fatty Acids
The analysis of fatty acids, in particular hydroxylated or unsaturated fatty acids has always been a challenge for the analyst. Free fatty acids were determined using a DLI technique(199) and monohydroxy acids by thermospray(200). Oxygenated unsaturated acids were characterized using thermospray also(201,202).

6 CONCLUSIONS AND RECOMMENDATIONS

This report has given some brief explanations and summarized the current state of affairs with regard to LC/MS techniques, and has given a number of examples of applications of the various techniques. With the rapidly growing use of the various liquid chromatographic techniques, and with the unique capability of mass spectrometry to provide peak identification, selective detection, and low detection limits, it is very clear that LC/MS will become an even more important factor in organic trace analysis than it already is. Every laboratory working in this field should definitely have some kind of LC/MS equipment.
The best choice for an individual laboratory will be determined by a number of factors, namely
- nature of applications (most important)
- types of mass spectrometers already available
- types of chromatographic equipment to be interfaced
- infrastructure available
- equipment cost (investment)
- equipment complexity (maintainance)
- skill level of the operator

Generally speaking, we would not recommend the DLI or moving belt techniques, whereas particle beam, thermospray, atmospheric pressure chemical ionization, CFFAB, electrospray, and SFC/MS techniques could be recommended in the right circumstances.

We would be of the opinion that **SFC/MS** has a relatively limited set of analyses for which it is optimal (synthetic polymers, aromatic compounds).
The **CFFAB** technique, one of the best accepted techniques, will generally be very useful in the case of rather polar type compounds, and is well suited for many compounds of biological interest as

discussed above.

Thermospray analysis is also generally suited for the analysis of relatively polar or thermally labile compounds, although the mass range which can be achieved may be somewhat more limited; its strong point is the compatibility with usual HPLC practices supporting the integrity of the chromatography.

The **Particle Beam** technique is complementary to thermospray in that it works better for less polar compounds, and the interface is compatible with normal EI sources. There is even commercially available a simultaneous interface for LC/GC/MS without the need of modifying the instrument.

The great attraction for **Electrospray** to date is its ability to analyze samples of significantly higher mass than the other techniques through the multiple charging phenomenon. More recently, it appears that electrospray techniques may also have some interesting possibilities even for lower molecular weight compounds for obtaining low detection limits.

Atmospheric pressure chemical ionization (API/CI) techniques also provide a very powerful tool with their ability to analyze relatively polar compounds and to tolerate buffer systems more easily than most other techniques; very low detection limits are often obtained also. The weak side for the latter two is the need for micro separation techniques such as micro HPLC or capillary electrophoresis, because this requires special method development work, which is very time consuming.

Structural information

A very important factor to consider in the selection of an LC/MS technique is the requirement for structural information for unknown samples. Most of the LC/MS techniques provide most often only molecular weight information, with at best rather limited fragment ions to use for structure identification. One of the strengths of particle beam LC/MS is the ability to obtain EI spectra. Thus if the materials to be analyzed are amenable to particle beam techniques and identification of unknown peaks is required, then particle beam LC/MS is probably the best choice.

However, if the materials to be analyzed are better handled by one of the softer ionization techniques, and structural elucidation is also required, then **LC/MS** combined with **MS/MS** becomes necessary. MS/MS, which has been discussed in another IUPAC report(203), is extremely useful for trace organic analysis and provides an additional dimension of selectivity to the analysis which can be uniquely valuable. If it is not possible to utilize MS/MS analysis, some structural information can also be obtained in the thermospray and electrospray techniques by varying certain voltages yielding fragments due to collisions, which may give them an additional advantage in that case.

In conclusion, we hope to have provided information here to allow the analyst to ask the right questions, when LC/MS appears to be a useful tool. It seems not possible the construct an audit trail to find the only correct answer, since the priority of the mentioned points may be very individual. We have attempted here to ouline the possibilities from an analytical point of view only; all the other factors cannot be generalized.

7 GLOSSARY

AAS	atomic absorption spectrocopy	IC	ion chromatography
API	atmospheric pressure ionization	ITP	isotachophoresis
CAD	(- CID), collision activated dissociation	LC	liquid chromatography
CID	(- CAD), collision induced dissociation	LSIMS	(- FAB), liquid secondary ion mass spectrometry
CFFAB	(- frit FAB), continuous flow FAB		
CI	chemical ionization	MS	mass spectrometry
CZE	capillary zone electrophoresis	MS/MS	(- tandem MS), mass spectrometry of selected ions
EI	electron impact		
EOF	electro-osmotic flow	NCI	negative chemical ionization
FAB	(- LSIMS), fast atom bombardment	PAH	polycyclic aromatic hydrocarbon
GC	gas chromatography	SFC	supercritical fluid chromatography
HPLC	high performance liquid chromatography	SIMS	secondary ion mass spectrometry

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