Journal of Chromatography, 271 (1983) 13–25
Chromatographic Reviews
Elsevier Science Publishers B V , Amsterdam – Printed in The Netherlands

CHROM 15,476

HOW TO INTERFACE A CHROMATOGRAPHIC COLUMN TO A MASS SPECTROMETER

G GUIOCHON* and P J ARPINO
Laboratoire de Chimie Analytique Physique, Ecole Polytechnique, Route de Saclay, 91120 Palaiseau (France)

SUMMARY

A chromatographic column is a separation and a dilution unit. Normally it would be expected that each component of a mixture will elute separated from the others, each of them being about one order of magnitude more dilute in the mobile phase than it was in the original mixture. Complete separation is not always possible, especially with unknowns, with which liquid chromatography–mass spectrometry is mainly concerned. In gradient elution, the composition of the mobile phase is continuously changing, but the dilution is reduced. Some times the compounds eluted are more concentrated than they were in the original sample Typical chromatographic zones are a few seconds to a few minutes wide and contain from 1 mg to less than 1 fg. The chromatographer wants to know the identity of all compounds eluted from the column and seeks very low detection limits (10–100 fg for most compounds would be excellent), reasonably good quantitative results (10%) and a wide range of linearity (up to 100 μg). He also desires some information regarding overlapping peaks and requires the mass spectrometer to accept the column effluent with a very simple, trouble-free interface that will have a very small transit time, so as to contribute as little as possible to the remixing of zones separated by the column With the present equipment the main problems are probably the sensitivity and linear dynamic range

INTRODUCTION

The problems of interfacing a liquid chromatograph to a mass spectrometer (LC–MS) stem from the relative incompatibility between the dilute solution eluted from the chromatographic column and the low-pressure gas plasma inside the source of the mass spectrometer, which makes this coupling much more difficult than that between a gas chromatograph and a mass spectrometer, which is now quite conventional[1–3]. These problems have often been discussed, but most reviews have so far focused on the interface itself, whereas here we want to consider the main constraints introduced by the chromatographic process and the minimum requirements that any interface should meet to have a chance of being competitive and to suggest the main compromises acceptable from the chromatographic point of view.

Chromatography is a separation process; the components of the sample are

0378-4355/83/$03 90

eluted as bands having a profile more or less similar to a Gaussian curve, provided that the column is not overloaded, and which are disengaged from each other with a certain degree of resolution. In chromatography the resolution is the ratio of the distance between band maxima and the half-sum of the bandwidth at their base (*i.e.*, four times the standard deviation of the Gaussian curve).

As this process occurs in spontaneous, *i.e.*, irreversible, conditions from a thermodynamic point of view, the decrease in entropy arising from this separation is more than compensated for by an increase in entropy due to dilution in the mobile phase Accordingly, elution chromatography is also a dilution process. Possible exceptions may occur with temperature programming [in gas chromatography (GC)] or in gradient elution [in liquid chromatography (LC)], provided that the gradient is steep, the retention of the corresponding compound is very large under the initial conditions and a large enough sample is injected, so the entire cross-section of the column is loaded with sample[4]. These conditions are relatively rare and are encountered mainly in the analysis of trace impurities in a simple matrix, such as in air or water pollution analysis[4]

The main problems to be encountered will thus be the compatibility of flow-rates, the resolution and the contribution of the mass spectrometer to band broadening and the detection limits and the dynamic linear range

NATURE AND FLOW VELOCITY OF THE MOBILE PHASE

The number of solvents currently used in liquid chromatography is relatively small (Table I), although in most instances mixtures of these solvents are used and a large number of possible additives can be incorportated. From our point of view, only

TABLE I

GASEOUS VOLUME AND VAPORIZATION ENTHALPIES OF COMMON LC SOLVENTS

V_g = Volume of vapour at boiling point (1 atm) or at 20 C (P^0) obtained by vapourization of 1 ml of liquid solvent, T_b = boiling point ΔH = vaporization enthalpy

Chromatography	*Solvent*	ρ (g cm^3)	V_g at 25 C	V_g at T_b	T_b(C)	P^0 (mbar)	ΔH(cal/g)
Normal-phase	*n*-Pentane	0 626	407	220	36	512	87
	n-Heptane	0 684	3630	208	98 4	46	87
	Benzene	0 879	2900	326	80 1	95	104
	Toluene	0 867	8200	296	111	28	99
	Methylene chloride	1 335	870	404	40	436	80 5
	Chloroform	1 492	1550	342	61 2	197	62
Reversed-phase	Water	1 0	56 10^3	1700	100	23.8	583
	Acetonitrile	0 786	5270	554	80	84	204
	Ethanol	0 789		493	78 5	na	
	Methanol	0 791		685	65	na	
	Propanol-2	0 785		380	82	na	na
	Tetrahydrofuran	0.888		341	64	na	na
	Dioxane	1 034		360	101	na	na

non-volatile additives need be considered. These are mainly salts, buffers used to adjust the pH of the mobile phase or ions used in ion-pair chromatography, tetraalkylammonium, alkylsulphates or alkylbenzenesulphonates, with Cl^- or Na^+ as counter ions[5]

Whatever the LC–MS interface selected, the use of a non-volatile buffer seems prone to generate considerable troubles The liquid–liquid extraction scheme studied by Karger *et al.*[6] is a possible solution, although its contribution to band broadening is significant even with 4 mm I.D. columns[7]. Ammonium acetate, trifluoroacetate, formate and chloride can be used as buffers to some extent and a number of volatile acids or bases are available (Table II), but it is nearly impossible to do without the traditional compounds used in ion-pair chromatography. In view of the increasing importance of this method in biochemical analysis, some investigation of this problem is clearly necessary. A solution could come from the use of organic ions that would decompose rapidly in the gas phase As pre-formed ions seem to be transferred easily through some interfaces[8–10] from the introduced solution to the source, the ideal solution would be for the mass spectrometrist to use the conventional LC ion-pair reagents in the source to perform some useful reaction

TABLE II

pK_a VALUES OF SOME VOLATILE ACIDS AND BASES

Compound	pK_a	*Compound*	pK_a
Ammonia	9 25	Acetic acid	4 75
Aniline	4 58	Benzoic acid	4 2
Diethylamine	11	Chloroacetic acid	2 85
Diisobutylamine	10 7	Cyanoacetic acid	2 45
Hydrazine	8 5	Dichloroacetic acid	1 48
Hydroxylamine	6	Formic acid	3 75
Pyridine	5 3	Phenol	9 9
Quinoline	4 8	Picric acid	0.40
Hydrocyanic acid	9 3	Thioacetic acid	3 33
Hydrogen sulphide	7 0		

Although gradient elution is much talked about in liquid chromatography, it is more rarely used In many instances it does not provide a significant reduction in analysis time compared with isocratic elution[11] When it does, a step gradient or a small number of successive analyses carried out under different conditions can replace it, so it is not really necessary that an LC–MS interface can accommodate a rapid change in solvent composition, which with most interfaces would certainly be the source of serious difficulties.

The flow-rate of solvent through a chromatographic column is proportional to the product of the column cross-section and the flow velocity This velocity is chosen as an optimum compromise permitting the achievement of a reasonable efficiency and an acceptable analysis time[12] The column diameter depends on the technology available, as both very narrow and very large columns are difficult to pack and operate At present, columns between 1 and 10 mm I.D are available with comparable performances The choice is a matter of convenience: if one wants to use a large sample or to reduce the importance of equipment contributions to band broad-

ening, a wide column is preferred, whereas when a low flow-rate is desired or only a small sample is available, a narrow column is preferred, although the importance of equipment contributions to band broadening may then be a source of major difficulties

The column efficiency is independent of the volumn cross-section over a reasonable range, provided that a good packing technique is used[13]. It depends only on v, the reduced velocity of the solvent:

$$v = \frac{u\, d_p}{D_m} \tag{1}$$

where u is the actual solvent velocity, d_p the average particle size and D_m the diffusion coefficient of the solute analyzed in the mobile phase.

For well packed columns, the column efficiency is usually a maximum for a value v_0 of the reduced velocity around 3. It decreases only slowly with increasing velocity[12] The decrease in efficiency is approximately 10% at twice the optimum velocity, $2v_0$, 25% at $4v_0$ and 50% at $10v_0$. Because of this slow trend, most analysts tend to operate the columns at relatively high values of the reduced velocity, *e.g.*, around 8 Larger values are precluded in most practical cases because the inlet pressure increases in proportion to the velocity and pressures much higher than 100 atm are avoided, and because a rapid analysis reduces the actual time bandwidth and makes accurate recording of chromatograms and peak integration impossible when the bandwidth becomes smaller than about 10 sec without the use of a microcomputer. Accordingly, the flow-rate, F, across the column is

$$F = \frac{v D_m}{d_p}\ \varepsilon\pi\ \frac{d_c^2}{4} \tag{2}$$

where ε is the porosity of the packing, usually around 0.7–0.8 (ref 5), and d_c is the column diameter We can rewrite eqn 2, using reasonable values for the constants ($v = 8$, $\varepsilon = 0.75$), as

$$F \approx 4.7\ \frac{D_m}{d_p}\ d_c^2 \tag{3}$$

The recent trend towards the use of small particles (7, then 5, now 3 μm) results in an increase in the flow velocity in proportion to the inverse of the particle size, but it is important to realize that the analysis of high-molecular-weight compounds and the use of viscous eluents (necessary for the separation of many large peptides which lose bioactivity in water–methanol or water–acetonitrile mixtures but not in water–isopropanol mixtures) also result in a marked decrease in the flow velocity The diffusion coefficient can be estimated from the Wilke and Chang[14] equation.

$$D_m = 7.4\ 10^{-10}\ \frac{T\sqrt{\varphi M}}{\eta_1 V_2^{0.6}} \tag{4}$$

where T is the temperature (K), η_1 the viscosity of the solvent, M its molecular

weight, φ an association constant (2.4 for water, 1.7 for methanol and 1 for non-associated solvents) and V_2 the molar volume of the solute Eqn. 4 is only approximate.

In methanol–water mixtures light compounds such as phenol and polymethylphenols, often used to calibrate columns and measure their efficiency, have diffusion coefficients around $1 \cdot 10^{-5}$ cm^2/sec, whereas a compound with a molecular weight of 2000 has a diffusion coefficient between $1 \cdot 10^{-6}$ and $2 \cdot 10^{-6}$ cm^2/sec. To achieve the same efficiency, the flow velocity should be reduced by a factor of about 6. A compromise involving the use of a larger velocity to achieve a shorter analysis time and some loss of efficiency will usually be found[12]. Some numerical data are given in Table III.

TABLE III

TYPICAL FLOW-RATES IN LC COLUMNS

d_p (μm)	D_m (cm^2/sec)	d_c (mm)	F (μl/min)* eqn. 3	u (cm/sec)
5	$1 \cdot 10^{-5}$	4	900	0.16
		2	225	
		1	56	
	$5 \cdot 10^{-6}$	4	450	0.08
		2	112	
		1	28	
	$2 \cdot 10^{-6}$	4	180	0.032
		2	45	
		1	11	
3	$5 \cdot 10^{-6}$	4	750	0.13
		2	188	
		1	47	

* $v = 8$

There is enough flexibility in the parameters of LC columns to optimize separately the flow velocity for maximum efficiency, or for any separation/analysis time compromise, and the volume flow-rate to accomodate the MS requirements. The remaining constraint depends on whether the sample size available is very small, in which event the column used must be narrow. This is often the case in clinical analysis.

RESOLUTION AND EQUIPMENT CONTRIBUTION

The separation of the components of an unknown mixture is a difficult operation The remixing of the bands at the column exit should be carefully limited. It may occur as a result of axial diffusion or convexive mixing or simply because of the parabolic flow profile in empty tubes used for connections.

Although the volume of the ionization source of the MS is extremely large compared with the cell volume of any other LC detector, and the diffusion coefficients under reduced pressure are very large, the residence time in the source is very short and this, more than the volume, is the critical parameter controlling zone remixing. As is well demonstrated in GC–MS, the ion source itself contributes negligibly to

band broadening It is essential, however, that the volume of the connecting tubes be kept to the minimum, that if the effluent is nebulized there is no turbulence inside the droplet cloud to mix them and that if the effluent is placed on a belt prior to solvent vaporization it does not flow on that belt. Convective mixing may be promoted in the last instance by too rapid a vaporization, leading to various forms of instability of the liquid film.

It does not seem too difficult to design and build direct liquid interfaces whose contribution to band broadening is negligible[7,15]. With belts this contribution is also small with the condition that vaporization of the solvent proceeds smoothly, but liquid–liquid extraction is very difficult to miniaturize[7] A more important contribution arises from the response time

The mere direct comparison of chromatograms obtained for a given mixture on the same column, using MS and a conventional chromatographic detector, shows considerable decrease in resolution for the MS trace. This is because usually only one spectrum is recorded every few seconds and mass chromatograms are recalculated from these stored spectra.

This long time between two successive mass spectra stored during a chromatographic analysis stems from two reasons, both of which have now become obsolete First, the cost of a computer memory was large: the typical 3–5 sec interval is a compromise between measurement frequency, memory size and the time during which spectra corresponding to one analysis can be stored. The recent development of cheap, 10–60 Mbyte disks and of rapid microprocessors has made possible both on-line data reduction and large-scale storage Second, magnetic instruments cannot be scanned very rapidly, because of the important self-induction of magnetic coils. Thus a significant time is required to scan the spectrum by exponential decay of the current and then to restore the magnetic field to the starting conditions. The fastest scanning speed was about 1–2 sec per mass decase, and still is on many instruments used for LC–MS coupling: this means that it is difficult to store more than one spectrum every 4 5 sec. Advanced magnet technology has now made it possible to record one mass spectrum every 1 sec (for one mass decade; faster speeds are possible for narrower ranges)

Increasingly often quadrupole instruments are being used, however, and these can be scanned much faster. It is possible and useful, however, to spend a longer time on each mass and to jump from mass to mass unit, assuming the analyst knows the exact masses of the ions (within *ca.* 0 1 dalton) and the decimal position is the same for all the ions he is looking for Current value acquisition for a few milliseconds on each mass requires about 1 sec to scan a range of 500–1000 daltons. On the other hand, a negligible time is necessary for restarting Thus magnetic and quadrupole instruments offer comparable performances from the scanning time point of view The quadrupole permits shorter scan times, however, if necessary by reducing the time spent on each mass, with a correlative decrease in the signal-to-noise ratio. It would be possible to store one spectrum every 0 1 sec, which is the requirement for the accurate analysis of a typical LC band as discussed below

Finally, magnetic instruments offer a relatively wide scan range, exceeding several thousand daltons on many current instruments, while mass spectrometers with a capability considerably exceeding 10,000 daltons are under development Although obvious problems of scan range frequency and sensitivity have to be solved,

these instruments will be timely for the generation of LC–MS systems devoted to protein and polynucleotide analysis On the other hand, it does not seem that the mass range available to quadrupole instruments will significantly exceed 2000 in the near future, barring a possible breakthrough in the design of high-frequency, high-voltage power supplies. The time, t, during which ions of a given mass are collected with a magnetic instrument (continuous scan with exponential decay) is

$$t = \frac{t_{10}}{R \ln 10} \approx 0.43 \frac{t_{10}}{R} \tag{5}$$

where t_{10} is the scan time for one mass decade and R the resolution (MS definition). For $t_{10} = 1$ sec and $R = 1000$, this time is 0.43 msec, while it can be 2 6 times longer with a quadrupole scanning masses of 100–1000 in 1 sec with the same resolution. This advantage in terms of sensitivity may not be very significant, apart from offsetting the discrimination of the quadrupole against ions of larger masses.

It is important to increase the frequency of data acquisition above 1 Hz, especially for the early peaks of the chromatogram, because it has been shown that in order to observe a decrease in column efficiency smaller than 10% (and hence a decrease in band resolution smaller than 5%) due to data acquisition speed, it is necessary to have a detector with a time constant smaller than one fifth of the time standard deviation of the peak. This standard deviation, σ, is related to the analysis time, t_R and the column efficiency, N, by the conventional equation

$$\sigma = \frac{t_R}{\sqrt{N}} \tag{6}$$

Most LC analyses are now carried out using 10–25 cm long columns with an efficiency between 1 10^4 and $2.5 \cdot 10^4$ plates With a velocity of 0 05 cm/sec, the elution time of the first components is between 200 and 600 sec, corresponding to standard deviations between 2 and 4 sec As can be seen in Table III, this is a low velocity and most often the first component has a width of 1 sec. Less drastic specifications can be accepted for the first compounds, which are rarely the most interesting, but for most compounds the peak width will be between 4 and 20 sec Consequently, it seems necessary to store more than one spectrum every 1 sec, preferably one every 0 1–0 5 sec. Otherwise, resolution is lost, and quantitative analysis and identification made more difficult because cross-contamination between the spectra of separated compounds is created by the data system (Fig 1)* A considerable loss of valuable information results. At a distance from peak maximum of 2 standard deviations (Fig. 2), the signal height is about 15% of the maximum, so in many instances the signal is still large enough to be used If two compounds of similar concentration are separated by 0.1 standard deviation (resolution 0 025), the ratio of their relative concentrations on the tailing and leading edges of their common peak, at a distance from the maximum of $+2\sigma$ and -2σ, will be 0.80 and 1 20, respectively, which should result in an observable difference between the two mass spectra. At a distance of $\pm\sigma$ these ratios are still 0.90 and 1 11, respectively. On the other hand, to obtain a complete separation of these two compounds ($R = 1$) a column 1600 times longer should be used, as the

* See "Note added in proof" on p 25

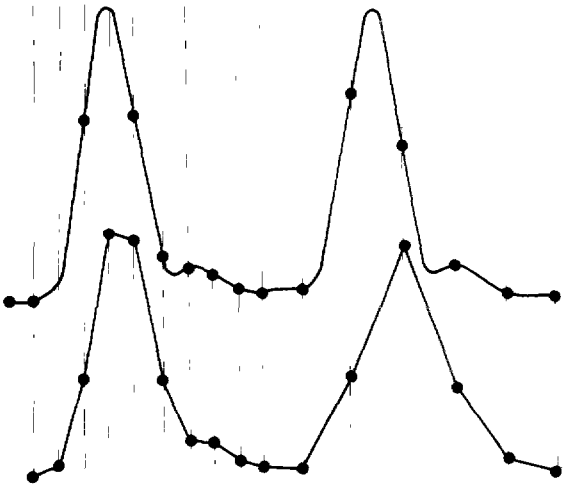

Fig 1 Upper trace concentration profile at column outlet; two successive identical injections Lower trace reconstructed mass fragmentogram derived from mass spectra recorded at a frequency of one per standard deviation (left) and one per 2 standard deviations (right)

resolution increases only as the square root of the column length To observe significant band broadening by coelution with an authentic compound, the bandwidth at half-height must increase by at least 10%, which requires a resolution of about 0.40 between two bands of equal size, 16 times more than the resolution at which a significant difference between the mass spectra of the two wings can be observed.

This illustrates the kind of sensitivity at which the purity of a band could be checked, at least if the signal-to-noise ratio is large enough, provided that spectra can

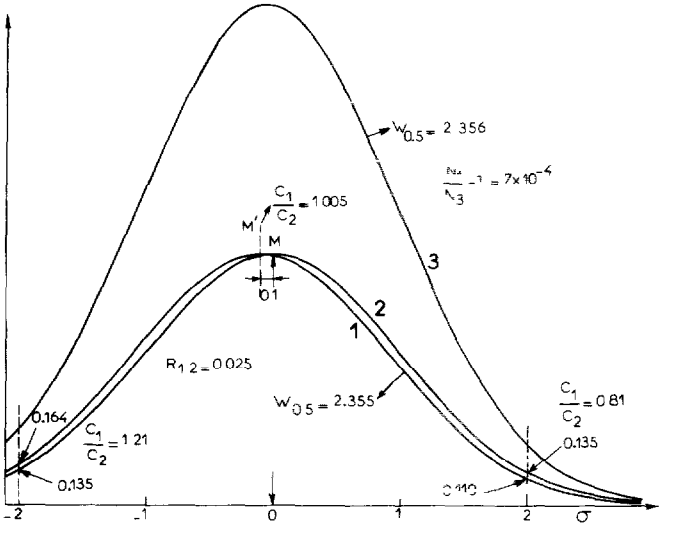

Fig 2 Concentration profiles of two solutes (1 and 2) at column exit. Resolution 0 025 The chromatogram recorded is profile 3, slightly wider than profiles 1 and 2 which are identical but shifted by 0 1 × σ

be recorded with a small enough time constant. Admittedly, one data point per standard deviation would be sufficient for this application

DETECTION LIMITS

It is usual to consider two classes of chromatographic detectors, those which respond to changes in concentration of solutes in the eluent, such as optical detectors (UV photometers, refractive index detectors, etc.), and those which respond to changes in the mass flow of the solute, such as destructive detectors[16]. The mass spectrometer belongs to the latter class. The main properties are as follows (i) if the eluent stream is switched off the signal falls to zero exponentially, (ii) if the eluent is diluted in a stream of scavenger, the signal is unchanged, as the solute mass flow rate remains constant; (iii) if the velocity of eluent through the column increases, the maximum peak height increases constantly; and (iv) the peak area remains independent of the flow-rate. These properties assume that within the range of solvent velocities considered, the response factor, *i.e.*, in this instance the ionization efficiency, remains independent of the eluent flow-rate. In GC–MS this seems to be reasonable assumption over a sufficiently large range to be practical. In LC–MS this is more questionable. In interfaces where the solvent is eliminated before the solute enters the ionization source, the response factor remains constant provided that the solvent flow-rate does not overload the interface. When the whole column effluent, or a constant aliquot of it, is injected into the source, the density of vapour in the source will be a function of the flow-rate whose changes may affect the response. In such a case the total flow-rate of solvent and/or reagent gas or vapour to the source must be optimized separately. For this reason, the use of ammonia or another chemical ionization reagent in the DLI has also the advantage of eliminating the influence of flow-rate oscillations due to pulsations of the pump[17]. It also makes the response factor independent of solvent flow-rate, within some limits, and permits the use of properties (ii) above, which is interesting when using very narrow bore packed columns or capillary columns, and (iv), which is important because it provides for good quantitative results.

The detection limit of a chromatographic detector is defined as the mass of compound that generates a signal equal to twice the noise. This definition can be extended straightforwardly to the mass spectrometer working with single ion monitoring, as the signal obtained is identical with a classical chromatogram. We note in passing that in such a mode hardly any problem arises because of too large a time constant, even with magnetic instruments. In good conditions the detection limit is of the order of a few picograms, unless the corresponding compound has an unusually large ionization yield, as happens, for example, to haloaromatics in electron-capture ionization and negative-ion detection, a case in which the detection limit can be several orders of magnitude smaller[18].

The chromatographer is always surprised by the low ionization yield of the mass spectrometer, E, the number of ions collected on the MS detector per molecule introduced into the source. For this reason, it is worth reviewing briefly the various sources of losses[19].

(i) To detect a signal on a given mass and calculate the coordinates of its maximum, *i.e.*, the corresponding molecular weight, we need about 100 ions at the

detector entrance slit; practically all ions reaching this slit are detected

(ii) The object and image slits are rectangular. Because of the scanning the convolution product of these two slits, assumed to be identical, is a triangle and we require 200 ions to enter the analyser Losses in the analyser are assumed to be negligible.

(iii) The extraction yield of ions from the source to the analyser across ion optics (focusing of ions) is about 10%. We need to make 2000 ions in the source

(iv) To obtain a spectrum useful for identification purposes, the previous figure must be applied to ions accounting only for small peaks in the mass spectrum. Peaks that are 10% of the base peak should be detectable as described in (i) above. We need to make at least $2 \cdot 10^4$ molecular ions during the time when the corresponding mass is scanned.

(v) The ionization yield varies widely with the ionization method used and the particular compound being analysed. Although it can be close to 1 for electron capture by haloaromatics, it can also be as low as 10^{-4} for electron impact Assuming an average value of 10^{-3} means that $2 \cdot 10^7$ molecules should be present during the scan.

(vi) The scan of one mass lasts about 1 msec The introduction of sample molecules into the source must proceed at a speed of 2 10^{10} molecules/sec.

The maximum concentration of the Gaussian band of a solute of retention volume V_R and efficiency N is

$$C_M = \frac{m\sqrt{N}}{V_R\sqrt{2\pi}} \tag{7}$$

where m is the sample mass. If the column capacity factor is k' and the liquid cross-section of the column is s, we have

$$C_M = \frac{m}{s} \cdot \frac{\sqrt{N}}{L(1 + k')\sqrt{2\pi}} \tag{8}$$

where L and u are the column length and the solvent velocity, respectively The mass flow-rate of sample to the MS source is then the product $C_M F$, where $F(= Su)$ is the solvent flow-rate. With a splitting ratio r, the mass flow-rate of sample to the source is

$$\frac{dm}{dt} = C_M Fr = \frac{m u r \sqrt{N}}{L(1 + k')\sqrt{2\pi}} \tag{9}$$

Comparing eqn. 9 with the condition (vi) above, we must have

$$\frac{m u r \sqrt{N}}{ML(1 + k')\sqrt{2\pi}} N' = 2 \ 10^{10} \tag{10}$$

where M is the molecular weight of the solute and N' is Avogadro's number. With L = 15 cm, $N = 1.5 \ 10^4$ plates, $u = 0.05$ cm/sec, $r = 1$, $M = 500$ and $k' = 1$, we have $m = 2 \cdot 10^{-10} = 0.2$ ng This is in agreement with the specifications of modern

instruments[20], which give a detection limit of 100 pg of methyl stearate (M = 298, hence m = 120 pg), although the specifications may not have been calculated with the rather favourable chromatographic conditions selected above narrow peaks with small retention give large maximum concentration.

The sensitivity in this scanning mode is difficult to define as we are not looking for a threshold above which the detector signal corresponds to the elution of a band, but for a more complex set of information "Chemical noise" resulting from column bleeding, minor sample constituents or other sources of eluent pollution contributes significantly to the detection limit, and in some instances makes sample "clean-up" mandatory. The definition of this sensitivity and its measurement are clearly the mass spectrometrist's problem In most instances it does not seem that the detection limit is below 1 ng

If there is no scanning but MS is working in a true single-ion monitoring (SIM) mode, a smaller amount of sample is necessary, with a 1 sec time constant, 1000 times less, around 1 pg. The values are similar for a magnetic instrument or a quadrupole. The only possibility of improving them markedly is to use a very efficient ionization technique, which explains why haloaromatics such as polychlorodioxins can be detected at the femtogram level in GC–MS with negative ions

To be meaningful these figures must be compared with the sample size that can be accomodated by the column. With a 4 mm I.D. column it rarely exceeds a few milligrams In other words, the current state of the art permits in most instances the identification of impurities at the ppm level and their detection at the ppb level, assuming the total effluent would be injected into the ion source With 1 mm I D columns these figures become 16 times larger, *i e* , identification of compounds above 10–20 ppm and a detection limit in SIM above 20 ppb.

These figures must be reduced further by up to one order of magnitude, sometimes more, because the column does not always accept such a large sample The solubility of some compounds in the eluent is very low and the solutions injected must be more dilute than a saturated solution if the equilibrium isotherm corresponding to the chromatographic mechanism used is to be linear Otherwise, the band profile is not Gaussian, but unsymmetrical and broader and the resolution is poor, although sometimes the profile of trace component bands which are well resolved from the main compounds is still acceptable.

For all of the above reasons, it is hoped that mass spectrometrists will find ionization mechanisms permitting a reduction in these detection limits by one or, better, two orders of magnitude, unless specialists in ion optics find a more efficient design for the extraction and focusing of the ions that are formed but escape collection.

If these figures seem demanding, they can be compared with the specifications that we can draft for a mass spectrometer to be coupled to an LC capillary column. It is easy to calculate that in order to be competitive with present packed LC columns, such capillary columns should have an inner diameter smaller than 10 μm[21]. The resolving power of these columns, which will probably be used in some advanced laboratories within a few years, would be tremendous, as are the equipment problems which they produce The maximum sample size is of the order of 10 ng at most (volume flow-rate *ca* 7 10^{-3} μl/min) To detect an impurity at the ppb level, we have to be able to obtain a signal with fewer than 12,000 molecules This is a challenge and

probably requires new approaches, such as the use of cross-collision molecular beams

The linearity is the last important property of the MS detector to be discussed. It is usually represented by the dynamic linear range, which is the ratio of the sample size for which the deviation from a linear response is 10% to the detection limit[16]. A large dynamic linear range is required in chromatography as the concentration of a given compound in a series of samples can vary over several (3–4) orders of magnitude, while the range of concentration investigated during one analysis can vary from almost 1% for the main component (column overloaded) to less than 10^{-10}%. This quality is required from the mass spectrometer especially when it is used in single-ion monitoring. In many instances it permits the quantitative analysis of incompletely resolved or non-resolved compounds The degree of resolution necessary for the use of another, non-selective, detector would be such that the analysis time would be so long and the dilution so great that the analysis would become impossible Alternatively, selective extraction and enrichment must be used, which are tedious, time consuming and increase the risk of sample pollution, alteration and errors. The use of a detector with a dynamic linear range of 100–1000 is still possible if it is sensitive enough, using dilution and an internal standard, although tedious. The narrow dynamic linear range of the mass spectrometer, although smaller than that of other LC detectors, is the last complaint of chromatographers.

CONCLUSION

Up to now most work on LC–MS coupling has been instrumental and has focused on interfaces that permit the transformation of the sample solution into a vapour mixture at a pressure low enough for the proper functioning of more or less conventional ion sources[1–3]. Some work has been done to adapt LC instruments with this aim, but little to modify MS instruments, although advantage has been taken of the recent progress in ionization methods and instrumentation

Now that the feasibility of LC MS coupling has been amply demonstrated, it may be time to develop an LC–MS instrument which would be integrated.

Among other features it is important that this instrument should permit the scanning of mass spectra up to large masses (several thousands), as the elution of large peptides, small proteins, polynucleotide sequences, etc., is now possible, if not always easy, easy adjustment of this mass range and the scanning frequency up to at least 10 Hz, true single-ion monitoring on a number of masses simultaneously, flexible adjustment of the composition of gases and vapours making up to source plasma and an improved ionization yield

The choice of interface is difficult A direct liquid interface must be used, because it permits the ionization of heavy, complex, sensitive molecules, the direct transfer of pre-formed ions and the use of complex reactions leading to ionization. It gives spectra with few characteristic features, however. Often only the quasi-molecular ion and a few aggregate ions with solvent molecules, the composition of which is not easily predictable, are observed[22] With large molecules some fragments are also recorded This does not permit easy identification, assuming that for molecules of that size there is an easy way. Possibly for smaller molecules, in the 100–300 to 500 dalton range, electron impact spectra may provide enough useful information to

warrant the design and use of a multi-interface instrument. Electron impact spectra of large molecules are often too complex to be useful, however.

At any rate the development of digital electronics will permit the design of more flexible, easier to use instruments

NOTE ADDED IN PROOF

In a discussion at the meeting, Dr Henneberg pointed out that we look upon the mass spectrometer as an LC detector and demand from it the same performance as that given by other LC detectors, in spite of its cost and complexity Dr. Henneberg showed that with a frequency of one data point per standard deviation, sufficient information is obtained to decide whether a band is pure or not —the essential use of the mass spectrometer This is true, but then we need another detector to obtain the chromatogram and to see for which bands the purity should be checked. This does not make the design of the interface easier. Perhaps we need to be able to use the MS instrument for performing both tasks and to choose a compromise for each analysis between the scanning rate (*i.e.*, response time) and the signal-to-noise ratio.

REFERENCES

1 P J Arpino and G Guiochon, *Anal Chem.*, 51 (1979) 682A
2 D E Games, *Biomed Mass Spectrom*, 8 (1981) 454
3 W H McFadden, *J Chromatogr Sci*, 17 (1979) 2
4 W Golkiewicz, C E Werkhoven-Goewie, L A Th Brinkman, R W Frei and H Colin, *J Chromatogr Sci*, in press
5 L R Snyder and J J Kirkland *Modern Practice of Liquid Chromatography* Wiley, New York, 2nd ed , 1979
6 B L Karger, D P Kirby, P Vouros, R L Foltz and B Hidy, *Anal. Chem*, 51 (1979) 2324
7 D P Kirby P Vouros, B L Karger, B Hidy and B Petersen, *J Chromatogr* , 203 (1981) 139
8 B A Thomson and J V Triborne, *J Chem Phys*, 71 (1979) 4451
9 P J Arpino and G Guiochon, *J Chromatogr* , 251 (1982) 153
10 C R Blakley, J L Carmody and M L Vestal, *Anal Chem*, 52 (1980) 1636
11 H Colin, G Guiochon and J C Diez-Masa, *Anal Chem* 53 (1981) 146
12 G Guiochon, in Cs Horváth (Editor). *HPLC —Advances and Perspectives*, Vol. 2, Wiley, New York, 1980 p 1
13 R Bonmati and G Guiochon, *Perfum Flavorist* 3 No 5 (1978) 17
14 C R Wilke and P Chang, *AIChE J*, 1 (1955) 264
15 R D Smith and A L Johnson, *Anal Chem* 53 (1981) 739
16 G Guiochon and H Colin, in J Albaiges (Editor), *Proceedings of the 2nd International Congress on Techniques in Environmental Chemistry, Barcelona, Spain, Nov. 1981*. Pergamon Press, Oxford, 1982, pp 169–176
17 P J Arpino, P Krien, S Vajta and G Devant *J Chromatogr* , 203 (1981) 117
18 F. W Karasek and F. I Onuska, *Anal Chem*, 54 (1982) 309A.
19 B J Millard, *Quantitative Mass Spectrometry* Heyden, London, 1978
20 *Technical Literature for the 4000 Series GC MS Systems*, Finnigan Corp , Sunnyvale, CA, U S A , 1982
21 G Guiochon *Anal Chem* , 53 (1981) 1318
22 M Dedieu C Jum, P J Arpino, J P Bounine and G Guiochon, *J. Chromatogr* , 251 (1981) 203