Journal of Pharmaceutical & Biomedical Analysis
Vol. 2, No. 2, pp. 233–240, 1984
Printed in Great Britain

0731–7085/84 $3.00 + 0.00
Pergamon Press Ltd.

Optimization of detectors for modern liquid chromatography*

H. POPPE

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

Abstract: The development of modern liquid chromatographic methods depends strongly on the development of suitable measuring systems for the separated compounds. This is the case as generally an on-line coupling of separation and detection is preferred because of ease of operation, reliability and more efficient data handling. Unfortunately, the best conditions for the separation are often not optimal from the detection point of view. Conflicts arise in various respects and compromises have to be made. Depending on the complexity, cost and performance of each, either the chromatographic separation, or the detection process, has to be adapted, the one to the other.

A number of contemporary developments in the techniques of liquid chromatography tend to sharpen these conflicts. Further reduction in particle size, reduction of the column diameter, introduction of multicolumn operation and the exploration of open tubular liquid chromatography can be mentioned in this respect. From this point of view a broad discussion of the role of detection techniques in the development and performance of liquid chromatographic analytical procedures is given.

Keywords: *Detection; miniaturization; detection limit; microbore HPLC; open-tubular liquid chromatography; capillary chromatography.*

Introduction

The main properties of detectors for liquid chromatography (LC) are: (1) the selectivity of detection; (2) the contribution to peak width; (3) the detection limit.

Apart from these there are, of course, a number of other more or less important properties of the devices, such as ease of operation, compatibility with different mobile phases and compatibility with automatic data reduction techniques. However, in the present review these finer details are of necessity neglected and the three above-mentioned topics discussed in greater depth below, in the context of contemporary developments in LC.

* Presented at the Symposium on Liquid Chromatography in the Biomedical Sciences, June 1984, Ronneby, Sweden.

Selectivity

Selectivity of detection has been a topic of major concern for many workers in the field during the last 10 or 15 years. Indeed, it should be granted that the peak capacity of liquid chromatography is so low (compared, for example, to state-of-the-art gas chromatography) that there is in general little hope of carrying out the analysis of the complicated samples analysts usually face, with universal detection and with little pre-separation. The selectivity of the chromatographic process has to be supplemented by selectivity conferred by the sample preparation and by the detection process. In this way, the selectivities of subsequent steps in the analytical procedure are multiplied, with the result that, hopefully, there is a greater degree of certainty concerning the identity of the peaks separated.

Examples of the earlier modes of selective detection are noted as follows:

(i) The use of wavelength selection in UV-absorption measurement, based on monochromator-based instruments developed for LC;

(ii) The use of fluorescence detection, with the later fine-tuning of selectivity afforded by the possibility of selecting excitation and emission wavelengths;

(iii) Electrochemical detection, with the additional possibility of choosing the potential at which amperometric or coulometric monitoring takes place.

A major problem in the use of such selective detection schemes is illustrated in Fig. 1, which is intended to express the following: in many cases one needs selectivity in order to

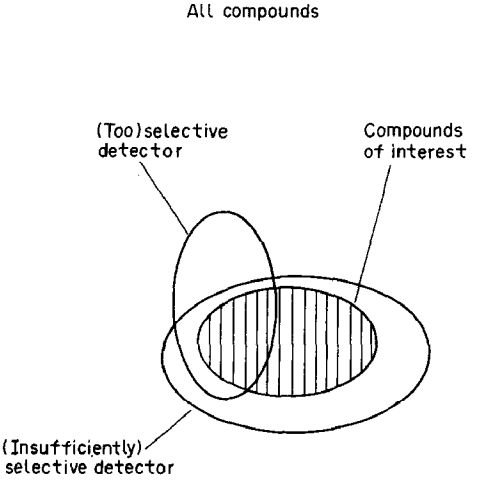

Figure 1
An illustration of the bad match often observed between the selectivities available and the selectivity required. The plane of the figure is conceived to encompass all chemical compounds. The subset of analytical interest is denoted by the hatched area. However, the subset of compounds to which the detector is responsive is not necessarily coincident, and often quite different, from this subset. In many cases foreign compounds will interfere, while others that are of interest do not respond.

analyse a particular group of compounds, such as polyaromatic hydrocarbons in the environment, aflatoxins in food products, a drug and its metabolites in body fluids, etc. The ideal selective detector would be responsive to the particular group at hand, and completely blind to other compounds. However, the particular physical processes used for detection often display a selectivity that only approximately replicates the selectivity needed. For example, not all aflatoxins fluoresce under given conditions of excitation and emission, whereas many other components also respond under these conditions. This situation limits the applicability of selective detection in many cases.

This situation has been considerably improved by the introduction of chemical reactions as the source of the required selectivity. Because of the enormous range and versatility of chemical manipulations, the selectivity of detection can be tailored to the

particular analytical task at hand, using either pre- or post-column reaction techniques, as illustrated by the work of Huber, Deelder, Frei and Lawrence [1–4]. However, modesty requires one to admit that even in 1975, when most of the earlier work on this topic was being carried out, there was nothing new under the sun: biochemists had used this principle for decades in the analysis of amino acids with ninhydrin, while the reaction principle had been applied on an enormous scale in thin-layer chromatography. Without the application of this technique the analysis of amino acids would have been virtually impossible in those days and the analytical utility of thin-layer chromatography would have been very constrained indeed. It has already been indicated that the LC-pundits have still to admit that the analytical utility of their 'high-performance' method, when faced with the complexity of real samples, is disappointingly small when used without any additional sorting of the compounds by means of special pre-treatment and detection techniques.

The instrumental approach to obtain increased selectivity has reached a new stage in recent years with the introduction of, and increased research efforts in, two spectrometric methods and their coupling with LC, viz. mass spectrometry (MS) and UV spectrometry. In the former case the main impetus for the increased activity is the need for identification or structure elucidation methods in LC. However, it is reasonable to expect that the technique, once developed to a suitable state of technical perfection and reliability, will be used on a large scale for sensitive monitoring of concentration as well, as happened in gas chromatography (GC), where single ion monitoring rapidly became a powerful tool in trace analysis. This then is the next step in the search for an ideal selective detector, because the detector can be fine-tuned by choosing the mass monitored.

It is useful to consider rapid-scanning UV-spectrometry carried out 'on the fly', from the same standpoint. Until recently the instrumental limitations made it practically impossible to do anything other than monitor at a single wavelength, apart from pioneering work in a few research laboratories [5, 6]. The introduction of commercially available instruments with parallel-access optoelectronic detectors [7], of which the photodiode array is the best known, has made it possible to collect spectra of the column effluent several times a second. With suitable data-handling equipment all the techniques known in GC–MS can be applied to the two-dimensional intensity map of absorbance as a function of wavelength and time. Furthermore, there are various similarities in the analytical possibilities available, such as analyte identification, peak purity checks, the use of an internal standard, deconvolution of fused peaks and selective wavelength monitoring [8]. However, this should not obscure some important differences:

(a) The 'resolving power' in the wavelength domain is much smaller than in the mass axis in mass spectrometry; this is not due to instrumental limitations, but arises mainly because the presence of the solvent leads to very broad UV-absorption bands with a much lower information content than would be expected for the isolated molecule in the gas phase.

(b) Contrary to the situation in mass spectrometry, the separated analytes are still in the medium of the separation (the mobile phase) when UV spectrometry is used. Consequently, and also because of the solvent phenomena mentioned above, the UV spectra observed depend on the particular chromatographic system used, so that the possibilities for identification on the basis of UV spectra are further diminished. Structure elucidation work comparable to that carried out in GC–MS is virtually precluded by this state of affairs.

Notwithstanding these limitations, the combination of LC with UV-spectrometry produces an enormous amount of information on a sample, information which could only have been obtained with relatively large experimental effort when using a single-channel detector. As such it appears to be a competitor of multicolumn chromatography. Further work on data handling and optimal extraction of relevant analytical information from the two-dimensional data set will play a key role in the exploitation of this technique [5, 8].

In conclusion, it can be stated that the aspect of selectivity is one of large and continuing interest for liquid chromatographic analysis. Pre- and post-column chemical reactions will continue to find more and more applications, while newer multidimensional techniques such as mass spectrometry and rapid-scanning UV-spectrometry with multiwavelength detection are, from the application viewpoint, still in their infancy.

Contribution to peak width, detection limit and miniaturization

A number of contemporary developments in column liquid chromatography have a strong influence on the requirements to be met by detectors in terms of peak-broadening effects. The reduction of particle size to obtain higher speed and resolution, the use of shorter columns for faster analysis, the decrease of column diameter in order to reduce dilution in the column, and, as an extreme case, the introduction of capillary columns, have all contributed to a drastic reduction in the scale on which chromatography takes place.

All these developments can be described as 'miniaturization' and invariably lead to a requirement for miniaturized detector volumes. However, before going into the physical and technical details of these detectors, it is necessary to discuss the objectives of this miniaturization process, as the requirements of these new miniaturized systems depend to a large extent on the expectations one has about the analytical performance in terms of selectivity, resolving power, detection limit etc.

The use of small diameter columns by Scott and Kucera [9, 10] was the first example of deliberate miniaturization. The authors of this pioneering work were convinced that this approach, using the particles of the same diameter as in conventional systems, offered many advantages, some of which, in retrospect, may appear to have been exaggerated. In the following paragraphs the relative performance of these systems will be treated from the theoretical point of view.

The speed of a separation, expressed as the retention time of the unretained component, and the resolving power, expressed as the plate number N, are related through equations which depend on the particular column geometry chosen (e.g. packed or open columns, on the boundary conditions or constraints put on the optimization of the particular separation technique (such as maximum pressure, minimum particle size and minimum detector volume), and on a series of physical constants. The problem has been covered exhaustively in two papers by Knox and coworkers [11, 12], for packed-column and open-tubular chromatography respectively. Their results indicate the following:

For packed columns the trade-off between speed and resolving power is independent of the column diameter, so that the capability of a fat column in this respect is matched by that of a thin column, and vice versa. This conclusion is contingent on the requirement that the values of reduced plate height ($h = H/d_p$) and the permeability factor ($\phi = K_o/d_p^2$) should be the same for all column diameters. So far there are no experimental indications that significant differences occur for these parameters in the range of diameters of possible analytical interest. It follows that the choice of column diameter

can and should be made on the basis of other factors, such as the available sample size, the amount of mobile phase available per analysis and the properties of the detection system employed. The most convincing argument for miniaturized systems is based on the smaller dilution of samples. This leads to lower detection limits for cases where only a limited amount of sample can be made available for the LC experiment, either because the original sample is limited in size, or because the pre-treatment steps cannot be carried out on a larger scale. Such cases will be described as microanalysis.

The advantage of miniaturization is limited to these cases. According to Huber *et al.* [13] and Karger *et al.* [14] the dilution can be reduced to a factor of about 3 on any column, if sufficient sample is available.

Other arguments in favour of small-bore columns, such as the reduction in solvent cost and the ease with which high plate numbers are obtained, require only a limited degree of miniaturization. The solvent cost is already reduced to 25% of conventional levels when using 2 mm i.d. columns.

Open-tubular chromatography [15], or other forms of open-channel chromatography [16, 17], do provide higher speeds and higher plate numbers. Experimentally this has been demonstrated already by Tyssen *et al.* [15]. It follows from the earlier analysis [12] that the most significant constraint in the optimization of open tubular chromatography is the volume peak broadening, expressed for example as the volume standard deviation σ_{Vd}, of the detector. If this is larger than about 10 nl, there would be little advantage in the use of open-tubular columns relative to packed columns. On the other hand, if it should prove possible to design detectors with a σ_{Vd} value of *ca* 1 nl, the prospects for high-resolution liquid chromatography would improve considerably, while plate numbers of 10^5–10^6 would then be obtained within reasonable analysis times.

It follows that there are basically two modes of detector miniaturization: the one concerns micro- or small-bore chromatography, while the other concerns open-tubular chromatography. The former mode is likely to reach its limit when the volume standard deviations are reduced to values between 1 and perhaps 0.1 μl; at that point further reduction, at least as judged from present experiences and insight, would hardly be meaningful. Indeed, at that stage of miniaturization the band-spreading phenomena on the injection side will probably predominate.

Apart from the design of the injector, there is the intrinsic peak width of the injected sample plug itself, corresponding to a standard deviation σ_V equal to $V_{inj}/\sqrt{12}$. The manipulation of samples smaller than 1 μl in the steps preceding LC appears questionable. Although the situation would be improved with the application of on-column or pre-column concentration methods [18, 19] in microbore columns, the limit set by the mass loadability of the column would still preclude the injection of a 10 μl aliquot of most samples on, for instance, a 0.1 mm column. In this respect it should be noted that, since the primary application of miniaturized systems is in microanalysis, a virtually quantitative transfer of sample is necessary to exploit the system.

The limit of 0.1 to 1 μl in σ_{Vd} for microbore column miniaturization suggests that reasonable success can be obtained by suitable adaptation of present detection devices, such as UV absorption and fluorescence systems, for which the values of σ_{Vd} are of the order of 5–10 μl. Indeed various workers have adopted this approach, followed closely by the instrument manufacturers, who are producing UV cells, for example, which are significantly smaller than 10 μl. Still, in much of the published material devoted to this approach, the loss in concentration sensitivity due to the reduction in optical path-length is perhaps inadvertently concealed.

An analysis of the factors which determine signal and noise in UV detectors [20], for example, reveals that, if parameters such as the light source intensity are equal, the detection limit in terms of concentration in the cell, C_{id}, is inversely proportional to the square root of the cell volume. Cell volume is also the ultimate factor which determines the value of the volume standard deviation, when proper design has eliminated any significant contribution from tubing and electronics [21]. The detection limit expressed as a mass, Q_i, is the product of the minimum volume standard deviation and the concentration detection limit [21]. This leads to the following consequences if the volume scale of the chromatographic process is reduced by a given factor, e.g. 100:

(i) the mass detection limit for microanalysis falls by a factor of 10;

(ii) the concentration detection limit, relevant for all but the microanalytical cases, rises by a factor of 10.

This conclusion indicates that there it would be advantageous to redesign the UV-cell; the result should be better than that obtained by simply using make-up flow at the end of the column. In that case there would be no improvement at all in the mass detection limit of the detector, Q_{id}. However, there is a price to be paid; the redesigned system would be less suitable for the more usual case where sufficient sample is available. This latter fact does not seem to be universally appreciated.

Such trade-off situations are more or less clear, depending on the measurement principle involved. Especially noteworthy is the fact that electrochemical detection generously allows scaling down, even to the nanolitre scale, as has been elegantly shown by Manz and Simon [22] and by Krejčí and Slaiš [23].

New Routes Towards Miniaturized Detection

The situation with respect to detection for open-channel chromatography is considerably less favourable, as here the σ_{Vd} values should be 3–4 orders of magnitude smaller than those observed with present equipment. Jorgenson suggested that 'Miniaturization of conventional LC detectors is probably not the answer, . . .' [24] and the author fully agrees with this.

Experience with HPLC in the present form shows that the prospect of an analytical method can only be good if the method is capable of trace analysis in the μg/l range. It follows from earlier work [13] that with sensible optimization of the injection volume a dilution factor of about three can be maintained. As a result the detection limit for the analyte in the injected sample is related to the concentration detection limit of the detection device used. The favourable concentration detection limit offered by the UV detector has made HPLC the method of choice for many analyses. Earlier and even contemporary attempts to use less sensitive detectors have not led to any success. It can therefore be safely concluded that the prospects for open-channel liquid chromatography depend not only on the feasibility of nanolitre volume detection, but likewise on the possibilities to realize detection limits in the μg/l range with small volume cells. As noted by B. L. Karger (personal communication), the requirement for low detection limits is even stronger in realistic open-channel chromatography; furthermore the complex samples to be analysed by this high-resolution technique will inevitably contain solutes with a wide range of concentrations.

A simple multiplication of volume (of the cell, if any) and concentration (in the μg/l range as noted above) clearly indicates that such detection schemes should be capable of detecting 10^{-15} g of analyte, corresponding to some 10^6 molecules. Although such levels

may look esoteric, there is no reason for pessimism. Working with 3-μm particles of remarkable size uniformity on a routine basis, or the routine measurement of picogram amounts of catecholamines in a plasma matrix with electrochemical techniques, would certainly have seemed an esoteric prospect for workers in the field less than 15 years ago.

As far as can be seen at present, a number of techniques are available which have the potential of such performance, and which have a reasonably large field of application.

The first of these is the application of lasers in UV and fluorescence detection. The limited possibilities for extreme miniaturization of the optical methods with conventional radiation sources has been mentioned already. The laser allows much more radiation to be concentrated into a small volume, because of the very small solid angle within which the radiation leaves the source. With this high local radiation intensity the chance for every molecule to be excited, and thus to contribute to the signal, is considerably enhanced.

Numerous workers have been active in this field, with the same or similar objectives. At present the work in fluorescence detection is experimentally the least cumbersome. As early as 1977 the detection of femtogram amounts was reported by Diebold and Zare [25]. Since then considerable progress in the direct application of lasers has been made (cf. [26, 27]).

Detection by UV absorption requires more sophisticated techniques as the direct measurement of transmission does not provide low detection limits, *inter alia* because of the poor stability of lasers compared to conventional light sources. Much attention has been given to the exploitation of thermal lens effects. Because of the high laser intensity, the sample liquid is heated when absorption occurs, and this effect can be measured optically via the local change in refractive index [28–31]. In a recent paper Carter and Harris [32] reported the measurement of 6 μg/l of iodine (a weak absorber) in a volume of 30 nl. It appears, therefore, that with suitable instrumental sophistication the results are rapidly improving.

The prospects of electrochemical detection for nanolitre samples, unfortunately a method of considerably smaller scope than UV absorption, have been mentioned already [22, 23].

A final word would be devoted to mass spectrometry. The absolute detection limit of this technique, when applied, for example, in single ion monitoring in GC, are certainly impressive. Taking into account the enormous physical and chemical degrees of freedom for further optimization, especially with respect to the still very low ionization efficiencies, the possibilities of MS for detection in open-channel chromatography should be extensively examined.

Conclusion

In the present discussion an effort has been made to compile an inventory of the most important developments in HPLC detection, viewed from the point of view of long-term developments in the field. As a result of this the emphasis given to various points has of necessity to reflect the author's subjective opinion.

Contemporary developments in HPLC detection include the further refinement of selective detection by instrumental and chemical means within the framework of conventional HPLC on the one hand, and the trend towards miniaturization on the other. Within the latter development a clear distinction should be made between the work for microbore columns, requiring volume standard deviations of the order of a few

tenths of a microlitre, and the work for open-tubular chromatography, where nanolitre standard deviations are needed. In the former case an adaptation of existing devices may lead to success; in the latter case, however, unconventional approaches will have to be taken.

References

[1] J. F. K. Huber, K. M. Jonker and H. Poppe, *Anal. Chem.* **52**, 2–9 (1980).
[2] R. S. Deelder, M. G. F. Kroll, A. J. B. Beeren and J. H. M. van den Berg, *J. Chromatogr.* **149**, 660–682 (1978).
[3] A. H. M. T. Scholten, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.* **54**, 1932–1938 (1982).
[4] J. F. Lawrence, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.* **185**, 473–481 (1979).
[5] M. J. Milano, S. Lam and E. Grushka, *J. Chromatogr.* **125**, 315–326 (1976).
[6] R. E. Dessy, W. G. Nunn, C. A. Titus and W. R. Reynolds, *J. Chromatogr. Sci.* **14**, 195–200 (1976).
[7] Y. Talmi, *Appl. Spectrosc.* **36**, 1–18 (1982).
[8] A. F. Fell, H. P. Scott, R. Gill and A. C. Moffat, *J. Chromatogr.* **282**, 123–140 (1983).
[9] R. P. W. Scott and P. Kucera, *J. Chromatogr. Sci.* **18**, 49–54 (1980).
[10] R. P. W. Scott and P. Kucera, *J. Chromatogr.* **169**, 51–72 (1978).
[11] J. H. Knox and M. Saleem, *J. Chromatogr. Sci.* **7**, 614–622 (1969).
[12] J. H. Knox and M. T. Gilbert, *J. Chromatogr.* **186**, 405–418 (1979).
[13] J. F. K. Huber, J. A. R. J. Hulsman and C. A. M. Meijers, *J. Chromatogr.* **62**, 79–91 (1971).
[14] B. L. Karger, M. Martin and G. Guiochon, *Anal. Chem.* **46**, 1640–1647 (1974).
[15] R. Tyssen, J. P. A. Bleumer, A. L. C. Smit and M. E. van Kreveld, *J. Chromatogr.* **218**, 137–165 (1981).
[16] J. C. Giddings, J. P. Chang, M. N. Myers, J. M. Davis and K. D. Caldwell, *J. Chromatogr.* **255**, 359–379 (1983).
[17] R. F. Meyer, P. B. Champlin and R. A. Hartwick, *J. Chromatogr. Sci.* **21**, 433–438 (1983).
[18] J. Lankelma and H. Poppe, *J. Chromatogr.* **149**, 587–598 (1976).
[19] W. T. Kok, U. A. Th. Brinkman, R. W. Frei, H. B. Hanekamp, F. Nooitgedacht and H. Poppe, *J. Chromatogr.* **237**, 357–369 (1982).
[20] H. Poppe, *Anal. Chim. Acta* **145**, 17–26 (1983).
[21] H. Poppe, *Anal. Chim. Acta* **114**, 59–70 (1980).
[22] A. Manz and W. Simon, *J. Chromatogr. Sci.* **21**, 326–330 (1983).
[23] K. Slaiš and M. Krejčí, *J. Chromatogr.* **235**, 21–29 (1982).
[24] J. W. Jorgenson, *J. Pharm. Biomed. Anal.* **2**, 191–196 (1984).
[25] G. J. Diebold and R. N. Zare, *Science* **196**, 1439–1441 (1977).
[26] S. Folestad, L. Johnson, B. Josefsson and B. Galle, *Anal. Chem.* **54**, 925–929 (1982).
[27] R. B. Green, *Anal. Chem.* **55**, 20A–32A (1983).
[28] C. E. Buffett and M. D. Morris, *Anal. Chem.* **55**, 376–379 (1983).
[29] C. A. Carter and J. M. Harris, *Anal. Chem.* **55**, 1256–1261 (1983).
[30] K. Fujiwara, W. Lei, H. Uchiki, K. I. Tsunoda, K. Fuwa and T. Kobayashi, *Appl. Spectrosc.* **36**, 157–161 (1982).
[31] K. Mori, T. Imasaka and N. Ishibashi, *Anal. Chem.* **54**, 2034–2038 (1982).
[32] C. A. Carter and J. M. Harris, *Anal. Chem.* **56**, 922–925 (1984).

[Received for review 17 July 1984]