Journal of Chromatography, 354 (1986) 185–202
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 282

APPRAISAL OF NARROW-BORE (1 mm I.D.) HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COLUMNS WITH VIEW TO THE REQUIREMENTS OF ROUTINE DRUG ANALYSIS

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(First received August 5th, 1985; revised manuscript received October 16th, 1985)

SUMMARY

The potential advantages of using 1-mm bore high-performance liquid chromatography columns to replace conventional 4–5-mm bore columns for routine drug analysis have been considered in the light of various claims and counter-claims in the scientific literature. Experimental work has been conducted to measure the changes in mass sensitivity when using both UV and electrochemical detection. Further work has investigated the coupling of these narrow-bore columns in order to achieve high plate counts with particular reference to the influence of peak asymmetry. Finally the linking of the narrow-bore columns to a mass spectrometer with a moving-belt interface has been examined. All results are discussed with reference to the needs of the routine analyst.

INTRODUCTION

High-performance liquid chromatography (HPLC) has found widespread application in the field of drug analysis over the last decade where most routine work is now conducted with columns of 4–5-mm bore. Recently there has been much general interest in the use of columns with reduced internal diameters and we are currently considering the potential advantages of using such columns in forensic toxicology. In a previous paper[1] one of us has described a practical narrow-bore HPLC system using 300 × 1 mm I.D. columns constructed from glass-lined stainless-steel tubing with unique zero dead-volume end assemblies. The columns, both silica and octadecyl-silica (ODS-silica), were packed in the laboratory at less than 10 000 p.s.i. and operated with a commercial pump, injector and ultraviolet (UV) detector. The study clearly demonstrated that the performance of 1-mm bore columns is very satisfactory for routine work, the packing of the columns is relatively straightforward and reproducible, and the equipment is as robust and reliable as that used with conventional columns. Nevertheless, the question remains as to whether such narrow-bore columns offer any significant advantages.

Before considering the potential advantages of narrow-bore columns over con-

ventional HPLC columns it is important to acknowledge that there are also disadvantages. Conventional HPLC equipment is generally unsuitable for operating narrow-bore columns and often requires extensive modifications by the analyst. Many instrument manufacturers are now starting to offer commercial equipment suitable for such columns but the replacement of existing hardware clearly represents a large capital investment. Flow-rates are reduced in proportion to the column cross-sectional area and hence the pumps used with 1-mm bore columns must be capable of flow-rates ranging from about 10 to 200 μl/min. Similarly peak volumes are considerably reduced and hence injectors and detector flow-cells must be of low volume to minimise extra column band broadening. The small injection volumes may also be a disadvantage from the viewpoint of sample preparation as samples may require extra concentration before analysis. Another significant disadvantage is the fact that narrow-bore columns are more difficult to pack than conventional columns. Our own technique of packing 1-mm bore columns can achieve reduced plate heights of about 3.5 which compares with typical values of 2–3 for well packed conventional columns[1].

There have been many claims published in the literature concerning the advantages of narrow-bore over conventional HPLC columns, but several questions remain relating to their application in routine analysis. Reductions in the quantities of HPLC packing materials and the volumes of eluent used have obvious economic advantages (*e.g.* a change from 5 mm to 1 mm I.D. leads to 96% savings). It is also claimed that the reduction in the peak volumes eluting from narrow-bore columns, resulting in less dilution of the injected sample, facilitates lower detection limits[2,3]. This is sometimes referred to as an increase in mass sensitivity. Some authors have highlighted the ease with which narrow-bore columns can be coupled together in series to achieve high plate counts in order to solve difficult separation problems[4,5] while others have suggested that narrow-bore columns provide an effective method of achieving high-speed HPLC separations[6–8]. Also the reduced internal diameters facilitate more effective temperature control within the HPLC column making the use of temperature programming feasible[9]. One further advantage claimed for narrow-bore HPLC is the possibility of using new detection principles arising from the reduced eluent flow-rates[10,11].

A close examination of the advantages claimed for narrow-bore columns indicates three features which would be expected to enhance the capabilities of any laboratory engaged in the routine analysis of drugs. These are the increase in mass sensitivity, the decrease in analysis time and the coupling of columns to achieve high resolution. In view of these potential advantages it is perhaps surprising that few laboratories are presently using narrow-bore HPLC columns for routine analytical work. Thus, the present paper considers these claims with particular reference to the types of problem encountered in forensic toxicology. In addition the coupling of the 1-mm bore columns with a mass spectrometer has been examined using a moving-belt interface. Although much research is underway on the development of new types of detector compatible with the low eluent flow-rates of narrow-bore columns, the mass spectrometer is probably the only new "detector" which is currently commercially available and which is likely to find application in drug analysis in the near future.

The concept of using narrow-bore columns to achieve high-speed separations in HPLC has recently been reviewed by Hartwick and Dezaro[12]. They clearly dem-

onstrate that narrow-bore packed columns offer no theoretical advantages, as the equations derived for optimising separation speeds do not include any terms relating to the column internal diameter. High-speed separations in HPLC are achieved through the use of short columns, small particles, low eluent viscosities, high pressures and high linear flow-rates. However, the authors do point out that narrow-bore columns do perhaps offer practical advantages in this field. Firstly, the fact that narrow-bore columns can be operated at high linear velocities with relatively low volume flow-rates is an important economic factor. Secondly, they point out that the optimisation of column length is an important approach to developing high-speed separations and narrow-bore columns are more easily cut and coupled to achieve this. In view of this thorough examination of narrow-bore columns for achieving high-speed separations no experimental work on this topic has been conducted in the present study. It is clear that narrow-bore columns do not offer a unique method of achieving rapid HPLC separations in drug analysis.

THEORETICAL ASPECTS OF MASS SENSITIVITY

The term "mass sensitivity" is widely used in discussions concerning narrow-bore columns and it is used in the present paper. In the current context it is really synonymus with the limit of detection (LOD) concept in chromatography. The LOD for an HPLC system is defined as that weight of a given analyte, injected on to the column, which gives a peak detectable with reasonable certainty above the baseline noise. It is normal practice to set a limiting peak height of 2 or 3 times the noise level. It is important to appreciate that the LOD is a function of the complete analytical system taking into account the dilution of the sample in the HPLC column before reaching the detector. Furthermore, the term must not be confused with the concept of minimum detectability (MD) which is a property of the detector alone and is defined for a concentration sensitive device, as the minimum concentration of analyte to give a signal discernible above the noise, generally twice the peak to peak noise. The MD has been called the minimum detectable concentration of the detector by some authors. The close similarity between the definitions for LOD and MD has led to much confusion in the chromatographic literature and the clarification of these concepts has been discussed recently[13].

It can be shown that the maximum concentration (C_{max}) of a solute at the apex of a peak eluting from an HPLC column is given by the following equation[14]:

$$C_{max} = m(N/2\pi)^{\frac{1}{2}}/V_R \tag{1}$$

where m is the mass of solute injected, N is the column plate count and V_R is the retention volume of the solute. This equation assumes a gaussian peak profile. It is possible to express the retention volume in terms of the column dimensions

$$V_R = \varepsilon_{tot} D^2 L\, \pi(1 + k')/4 \tag{2}$$

where ε_{tot} is the total porosity of the column, D is the column internal diameter,

L is the column length and k' is the solute capacity factor. Combination of eqns. 1 and 2 results in

$$C_{max} = m(8N/\pi^3)^{\frac{1}{2}}/D^2\varepsilon_{tot}L(1 + k') \quad (3)$$

Examination of eqn. 3 reveals that in an ideal situation, when all other parameters remain constant, changing the column diameter from 5 to 1 mm gives a 25-fold increase in the peak concentration of the solute reaching the detector. Assuming that the MD of the detector remains constant this represents the increase in mass sensitivity claimed for narrow-bore systems. However, it is necessary to consider whether this potential increase can be realised in practice with particular reference to the assumption concerning the MD of the detector.

The practical problems of achieving the theoretical increases in mass sensitivity are well illustrated with UV detection where most modern detectors are equipped with a range of exchangeable flow-cells having different volumes for different applications. The confusion over this topic in the published literature has mainly arisen because authors have not always differentiated between the various types of experiment which can be conducted to compare mass sensitivity. The present section attempts to clarify this situation. Fig. 1 illustrates the types of experiment which can be conducted. As before, the columns being compared are considered to be identical in every respect (L, ε_{tot} and N) except for the different internal diameters and each is operated at the same linear flow-rate. Thus the injection of a given weight of solute will give a 25-fold difference in concentration at the detector (eqn. 3).

In the first experiment (Fig. 1a) both columns are connected in turn to the same flow-cell (X) in the detector. The selection of a microbore flow-cell (typically < 1 μl) allows both columns to operate without any loss in column efficiency arising

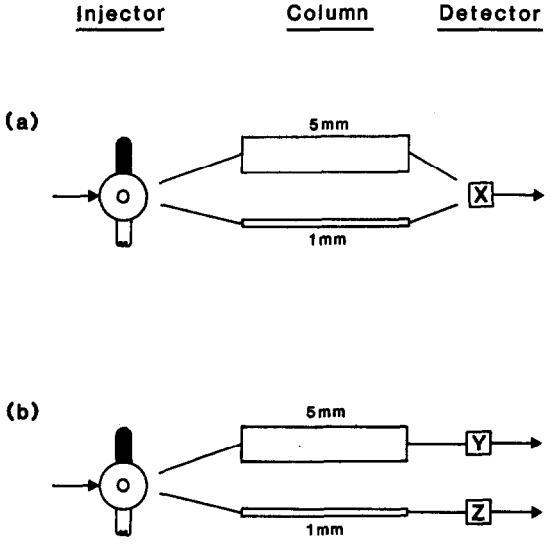

Fig. 1. Schematic diagram showing different types of experiment to compare mass sensitivities of 5- and 1-mm bore columns using a UV detector with exchangeable flow-cells. (a) Comparison using the same flow-cell (X); (b) comparison using different flow-cells (Y and Z).

from extra-column band broadening. Such experiments involve no changes to the detector and unless the difference in volume flow-rate alters the noise level, the MD of the detector will remain the same. Under such conditions the 25-fold increase in mass sensitivity should be observable. However, this experiment uses a flow-cell which is unnecessarily small for the 5 mm bore column placing it at a severe disadvantage in the mass sensitivity comparison. Fig. 1b represents an alternative experiment where each column is used with its own flow-cell of appropriate volume. For example, flow-cells Y and Z may have volumes of 8 and 0.5 μl respectively such that both columns can still be operated without any loss in performance from extra-column band broadening. Under these circumstances it is unlikely that the MD of the detector will remain unchanged since the narrow-bore flow-cell (Z) will inevitably have a shorter optical pathlength giving a reduction in detector response. Changes to the detector noise level may also occur. Thus if flow-cells Y and Z have optical pathlengths of 10 and 1 mm respectively, the potential increase in mass sensitivity is reduced from 25 × to 2.5 × based on the expected detector response. Scott and Kucera[2] have argued that such reductions are compensated by a comparable decrease in the noise level of the detector.

A third type of experiment can be considered in which a single flow-cell is used to compare the mass sensitivities but is selected to have a volume more suitable for 5-mm bore columns (*e.g.* Fig. 1a where X has a volume of 8 μl). In this case the MD of the detector is unlikely to change and any departure from the 25-fold potential increase in mass sensitivity is the result of the peaks from the narrow-bore column experiencing band broadening in the large flow-cell.

EXPERIMENTAL

Materials

Hexane, methanol and acetonitrile were HPLC grades obtained from Rathburn Chemicals (Walkerburn, U.K.). Ammonium perchlorate, 2,6-dinitrotoluene, 3,5-dinitrobenzonitrile and 3,5-dinitrobenzylchloride were obtained from Aldrich (Gillingham, U.K.). All other chemicals were obtained from BDH (Poole, U.K.). Water was distilled in glass in the laboratory. All drugs came from the collection of the Central Research Establishment, Home Office Forensic Science Service.

Columns

Narrow-bore HPLC columns (300 × 1 mm I.D.) were constructed from 1/8 in. O.D. glass-lined stainless-steel tubing as in the previous study[1] although 1/4-in. inverted end-fittings (Part No. 204, HETP, Macclesfield, U.K.) were used on some columns instead of the traditional type. The packing procedure for both silica and ODS-silica involving balanced density slurries in tetrabromoethane–methanol (4:1, w/w) has been described previously[1].

Conventional HPLC columns were 160 × 5 mm I.D. (Shandon Southern Products, Runcorn, U.K.) packed using familiar slurry procedures. Silica was packed by dispersal of the packing material in methanol and pressurising with methanol, while ODS-silica was slurried in isopropanol and pressurised with hexane.

Chromatography

Five different HPLC systems were used during the present study all using 5-μm packing materials. An eluent of hexane–methanol (99.5:0.5, v/v) on Hypersil-silica (Shandon Southern Products) was used for the separation of a test mixture of pentane, toluene, nitrobenzene, acetophenone, 2,6-dinitrotoluene, 1,3-dinitrobenzene, 2,6-dinitrobenzyl chloride and 2,6-dinitrobenzonitrile as previously described[1]. Hypersil-ODS (Shandon Southern Products) has been used with an eluent of methanol–water (60:40, v/v) for a test mixture of phenol, *p*-cresol, 2,5-dimethylphenol, anisole and phenetole[1], and with an eluent of aqueous sulphuric acid (0.02 *N*)–methanol–acetonitrile (5:8:9, v/v/v) for the characterisation of cannabis resin. This latter eluent represents a slight modification of a literature procedure by Baker *et al.*[15]. The same reversed-phase packing material has also been used for the chromatography of codeine using an eluent of methanol–aqueous sodium dihydrogen phosphate (0.1 *M*) (30:70, v/v). Several basic drugs have been chromatographed on Spherisorb-silica S5W (Phase Separations, Queensferry, U.K.) using an eluent prepared by dissolving ammonium perchlorate in methanol (0.01 *M*) and then adding a 0.1 *M* solution of sodium hydroxide in methanol (1 ml per l) giving a pH of 6.7[16].

Equipment

Chromatography was performed with a Waters M6000A pump modified by the manufacturers to give a flow-rate range from 0.001 to 9.9 ml/min. Injections were made with a Rheodyne 7410 microbore injection valve fitted with a loop filler port (Model 70-11) and equipped with a range of internal sample loops (0.5–5 μl). Chromatograms were recorded with a Linear Model 500 chart recorder having a pen response of <0.5 s full scale.

Three variable wavelength UV detectors have been used for the studies on mass sensitivity each being equipped with exchangeable flow-cells supplied by the manufacturers. These were the Jasco Uvidec-100-III (Lea Scientific, Milton Keynes, U.K.) with 0.3- and 8-μl flow-cells, the Spectroflow 773 (Kratos Analytical Instruments, Urmston, U.K.) with 0.5-, 8- and 12-μl flow-cells and the LKB2151 (LKB Instruments, Croydon, U.K.) with 0.8- and 10-μl flow-cells. The Jasco detector with the 0.3-μl flow-cell was used for all other experiments with the 1 mm bore columns.

Electrochemical detection was performed with a wall-jet amperometric flow-cell constructed in the laboratory[17]. The flow-cell had an active cell volume of about 0.3 μl with a glassy carbon working electrode (3.1-mm diameter), a stainless-steel auxiliary electrode and a silver–silver chloride reference electrode. The inlet connections of the flow-cell were specially designed to minimise band broadening for narrow-bore HPLC. The flow-cell was operated using a LC4 electronic control unit (Bioanalytical Systems, West Lafayette, U.S.A.). A working potential of +1.2 V *vs.* silver–silver chloride reference was used for the detection of diphenoxylate.

The mass spectrometer used for interfacing experiments was a VG MM 16F magnetic sector instrument operated with a VG2250 Data System (VG Analytical, Altrincham, U.K.). A VG moving belt interface was used having an endless polyimide belt which passes through a narrow channel into the source housing of the mass spectrometer where a small nose heater vapourises the sample. All work reported in this paper has involved electron impact (EI) spectra with an accelerating voltage of 4 kV, 70 eV electron energy, 100 μA trap current and source temperatures from 190

to 230°C. The effluent from the narrow-bore HPLC columns was passed through a short length of 1/16 in. PTFE tubing (5 cm × 0.006 in. I.D.) which was connected by a 1/16-in. zero-dead volume union to a piece of stainless-steel capillary tubing (3.9 cm × 0.006 in. I.D.). The outlet end of this tube had been filed to give a sharp point and was positioned just above the surface of the moving belt.

RESULTS AND DISCUSSION

Mass sensitivity

Theoretical considerations concerning the design of experiments to compare the mass sensitivities of narrow-bore and conventional bore HPLC systems have been presented above. Although a change from 5- to 1-mm bore columns has the potential of achieving a 25-fold increase in mass sensitivity, severe practical problems are predicted and the present section describes experimental work to explore these limitations. The theoretical discussions have considered two columns which are identical in every respect except for having different internal diameters. Our experience with packing 1-mm bore columns[1] has indicated that they are more difficult to pack than conventional columns confirming other observations in the literature. The plate counts achieved with these columns are typically 50–60% of what might be expected for 5-mm bore columns of identical length while the total porosity values are similar to those observed for conventional columns. It is apparent that practical experiments to compare mass sensitivities can only be set up with two columns which have other differences besides their internal diameters. In the present work a narrow-bore column (300 × 1 mm I.D.) and a conventional bore column (160 × 5 mm I.D.) have been compared, the difference in length being selected to give similar plate counts. The columns were packed with 5-μm silica and operated with an eluent of methanol containing ammonium perchlorate (0.01 *M*) at pH 6.7[16]. Diphenoxylate and protriptyline were selected as the test compounds having capacity factors of 0.2 and 1.7 respectively on this system.

Table I shows the results obtained from experiments comparing the mass sensitivities of 1- and 5-mm bore columns using UV detection. In all experiments volume flow-rates of 24 and 600 μl/min were used for the narrow-bore and conventional columns respectively, representing identical linear flow-rates. The work has involved three commercial UV detectors each having a range of exchangeable flow-cells. No experiments have been conducted in which the two columns were compared using a "microbore" flow-cell (Fig. 1a, X having a volume of < 1 μl) since it has already been pointed out that such comparisons are predicted to show a large increase in mass sensitivity for the narrow-bore column but that this is a gross misrepresentation of the true situation as a conventional column would not normally be operated with such a small flow-cell.

The first 3 experiments shown in Table I correspond to the illustration in Fig. 1b where different flow-cells (Y and Z) are selected for each column to be as large as possible without causing extra-column band broadening. Each of these experiments was conducted with a different detector and in each case the smallest flow-cell available was selected for the 1-mm bore column (ranging from 0.3 to 0.8 μl) while flow-cells ranging from 8 to 12 μl were selected for the 5-mm bore column. It can be seen that the optical pathlengths of these flow-cells varied considerably. Operating

TABLE I

COMPARISON OF UV DETECTOR NOISE AND RESPONSE FOR INJECTIONS OF DIPHENOXYLATE AND PROTRIPTYLINE ON 1- AND 5-mm BORE HPLC COLUMNS

Each experiment involved a comparison of two columns (300 × 1 mm I.D. and 160 × 5 mm I.D.) packed with 5-μm silica (Spherisorb S5W) operated at the same linear flow-rates (*i.e.* 24 and 600 μl/min respectively). Eluent: methanol containing ammonium perchlorate (0.01 *M*) pH 6.7. Injection: diphenoxylate (1 ng) and protriptyline (0.2 ng) in methanol (2 μl).

Expt. No.	*Detector**	*Flow cell (1 mm column)*		*Flow-cell (5 mm column)*		*Detector noise (A.U. × 10^5)*		*Relative peak height response (1 mm/5 mm)*	
		Volume (μl)	*Optical pathlength (mm)*	*Volume (μl)*	*Optical pathlength (mm)*	*1 mm column*	*4 mm column*	*Diphenoxylate***	*Protriptyline***
1	A	0.8	3	10	10	3.2	2.4	2.28	2.41
2	B	0.5	1	12	8	1.2	1.2	0.99	★★★
3	C	0.3	0.3	8	10	1.3	1.6	0.13	0.09
4	B	8	10	8	10	★★★	★★★	5.07	7.47

* A = LKB2151, B = Spectroflow 773, C = Uvidec-100-III.

** Diphenoxylate and protriptyline had capacity factors (k') of 0.2 and 1.7 respectively.

*** Data not available.

the detectors at high sensitivity ranges with an appropriate eluent flow-rate enabled the short term noise levels to be measured. The results are included in Table I where it can be seen that the change of flow-cell had little effect on the noise level observed with any of the three detectors. These results differ from those reported by Scott and Kucera[2], using home-made flow-cells in a commercial detector, where the noise level was found to decrease in proportion to the optical pathlength. For example, with Detector C where the optical pathlengths of the flow-cells differ by a factor of 33 the measured noise levels were 1.3×10^5 and 1.6×10^5 AU for the 1 and 5 mm columns respectively.

The relative peak height responses (1 mm/5 mm) for injections of the drugs on to the two columns with detectors A, B and C are shown in the right hand columns of Table I (expts. 1–3). As the noise levels were relatively constant these figures are good approximations for the relative mass sensitivities. It can be seen that the narrow-bore column only showed an increase in mass sensitivity in expt. 1. The columns showed similar mass sensitivities in expt. 2 while the 5-mm bore column showed the advantage in expt. 3 by a factor of about 10. These results reflect the increasing difference between the optical pathlengths of the two flow-cells selected for each experiment with detectors A–C.

The average increase in mass sensitivity observed in the best case (expt. 1) of about 2.35 is a long way from the potential 25-fold advantage in changing from 5- to 1-mm bore columns. The use of a 1-mm bore column with approximately double the length of the 5-mm bore column in order to produce similar plate counts is predicted to reduce this factor to about 12.5 (see eqn. 3). Furthermore, the optical pathlengths of the flow-cells in expt. 1 (3 and 10 mm) differ by a factor of 3.3 which further reduces the factor to about 3.7. Consequently, the experimental result is not unreasonable in view of the approximate nature of the calculations and the unknown influence of extra-column band broadening.

The final results in Table I (expt. 4) involve a different type of experiment where both columns were tested using a single 8-μl flow-cell in detector B. In this case the narrow-bore column showed increases in mass sensitivity of 5.07 and 7.47 for diphenoxylate and protriptyline respectively. As before it can be predicted that the drug concentrations at the column exits should differ by a factor of about 12.5 but the peaks from the narrow-bore column will experience band broadening in the relatively large flow-cell such that the observed increase in mass sensitivity will be less than this figure. Once again the experimental results are reasonable and the different values for the two drugs can be explained since diphenoxylate ($k' = 0.2$) had a shorter elution time than protriptyline ($k' = 1.7$) and hence, having a smaller peak volume, experienced greater band broadening.

The operation of narrow-bore columns with conventional flow-cells provides the most effective method of using these columns to achieve significant increases in mass sensitivity but does so at the cost of sacrificing column efficiency. The practical value of such approaches depends on the type of analytical problems to be tackled. A method involving the separation of the analyte from a complex mixture of matrix peaks may not be viable with any significant loss in plate count while other methods involving a small number of well resolved components may tolerate considerable losses in the chromatographic resolution.

The practical problems of achieving large increases in mass sensitivity with

TABLE II

ELECTROCHEMICAL DETECTOR NOISE AND RESPONSE FOR THE INJECTION OF DIPHENOXYLATE ON 1- AND 5-mm BORE HPLC COLUMNS

Wall-jet electrochemical flow-cell (0.3 μl) with a glassy-carbon working electrode (3.1 mm) operated at +1.2 V *vs.* silver–silver chloride reference electrode. Packing material: Spherisorb S5W, 5 μm. Eluent: ammonium perchlorate (0.01 *M*) pH 6.7. Injection of diphenoxylate: 2 μl containing 100 ng. The drug had a *k'* value of 0.2 on the HPLC system.

Column	*Flow-rate (μl/min)*	*Detector* noise (nA)*	*Relative peak height response (1 mm/5 mm)*
300 × 1 mm I.D.	24	0.25	2.5–3.1
160 × 5 mm I.D.	600	0.5	

* Noise measurements were made with the 5-mm bore column operated at two flow-rates (24 and 600 μl/min) to avoid disturbing the electrochemical flow-cell between measurements.

narrow-bore columns using UV detection arise from the reductions in optical pathlengths which occur in "microbore" flow-cells designed to avoid extra-column band broadening. The miniaturisation of an electrochemical detector does not suffer from this type of problem and flow-cells with nano-litre volumes have been described (*e.g.* ref. 18). It might be expected that such detectors would be ideal for achieving the potential increases in mass sensitivity of narrow-bore columns. Table II shows the results of experiments to compare the 1- and 5-mm bore columns already considered with UV detection. A wall-jet flow-cell was used having a volume of about 0.3 μl[17] and this was expected to give little band broadening with either column. It can be seen that the eluent flow-rate had a significant effect on detector noise with the narrow-bore flow-rate (24 μl/min) giving half the noise level seen with the 5 mm bore flow-rate (600 μl/min). Experiments to determine the relative peak height response (1 mm/5 mm) for 100-ng injections of diphenoxylate gave values between 2.5 and 3.1 which translates to an overall increase in mass sensitivity for the 1-mm bore column of 5–6 when the change in noise level is also considered.

Once again the observed increase in mass sensitivity of narrow-bore columns with electrochemical detection is relatively small. In this case the problem arises from the volume flow-rates used with the two columns. Theoretical work[19] indicates that the signal from a wall-jet amperometric flow-cell is directly proportional to $V^{3/4}$, where V is the volume flow-rate. The situation is little better with a thin-layer amperometric flow-cell where the signal is directly proportional to $U^{1/2}$, where U is the linear fluid velocity within the flow-cell. In both cases the signal is also directly proportional to the concentration of the electroactive solute. The term relating to flow-rate markedly reduces the expected response from the narrow-bore column and accounts for the relatively low increase in mass sensitivity.

It is clear that the practical increases in mass sensitivity which can be achieved with 1-mm bore columns using both UV and electrochemical detection are not as large as might be expected from some claims in the literature. Whether the relatively small increases demonstrated in the present work are sufficient incentive to convert existing HPLC methods over to narrow-bore columns will depend on the nature of the analytical problem. Moreover it is important to identify those areas in routine

drug analysis where an increase in mass sensitivity in HPLC could provide significant advantages. Where samples are large and/or re-sampling is easy an increase in mass sensitivity is of little consequence. Furthermore, the use of conventional bore columns rather than narrow-bore columns should be encouraged since the handling of large sample volumes should improve the precision of a quantitative analysis. Some aspects of the work performed in forensic toxicology fall into this category *e.g.* the examination of body fluids and tissues following an autopsy. In contrast, where samples are small and re-sampling is difficult or impossible, an increase in mass sensitivity would allow smaller aliquots to be taken for each analysis. A good example of this type of problem in forensic toxicology is the blood samples presented for examination following road traffic offences where the volumes are often less than 5-ml and multiple analyses are required to screen for unknown drugs. In such cases the relatively small increases in mass sensitivity may still prove to be valuable.

Column coupling

The production of high plate counts in HPLC through the use of long columns of conventional bore (> 30 cm, 4–5 mm I.D.) is not practical as such columns are very difficult to pack. Many workers have coupled conventional columns in an attempt to increase plate counts but this has often not been completely successful with the overall plate count being less than the sum of the plate counts of the individual columns (*e.g.* ref. 4). Nevertheless the literature does contain examples where such column concatenation has achieved additive plate counts[20–22]. At the present time it is not clear why different authors have experienced different results but discussions have centred on the method of coupling (butting columns end to end or using narrow capillary tubing) as well as on the intrinsic properties of the columns to be coupled. It has been argued that additive plate counts can only be achieved with columns showing good peak symmetries[23] and more recently that the columns require special packing techniques[24]. Despite the difficulties experienced with conventional columns, the coupling of small bore columns (≤2 mm I.D.) appears to be much more successful and additive plate counts have been reported by several workers[4,5,25–27].

Experiments have been conducted with the present 1 mm bore columns coupled together with short lengths (5 cm) of narrow capillary tubing (0.006 in. I.D.). It has been found that the coupling of two or three columns has consistently given additive plate counts when making measurements using the peak width at half-height method. Furthermore, the influence of peak asymmetry on the additivity of plate counts[23] has also been tested. Three narrow-bore silica columns were selected with greater than average asymmetry factors ranging from 1.33 to 1.43 when operated with the standard test mixture. Nevertheless, on coupling these three columns, the overall plate count was found to be additive (Table III). A further experiment was conducted in which a large peak asymmetry was deliberately induced by the use of codeine as the test compound and the selection of a methanol–phosphate buffer eluent with an ODS-silica column. It is well known that many basic drugs show poor peak shapes on ODS-silica unless amine additives are present in the mobile phase (*e.g.* ref. 28). In this second experiment (Table IV) codeine showed peak asymmetries of 3.42 and 2.85 on the two separate ODS-silica columns yet additive plate counts were still observed when the columns were coupled. It is interesting that the coupled columns showed a further deterioration of the codeine peak shape in this case.

TABLE III

PLATE COUNTS AND ASYMMETRY FACTORS FOR THE COUPLING OF THREE SILICA COLUMNS

Columns (A, B and C): each 300 × 1 mm I.D. packed with Hypersil-silica (5 μm). Eluent: hexane–methanol (99.5:0.5, v/v). Flow-rate: 25 μl/min. Detection: 235 nm. Solute: 2,6-Dinitrotoluene (k' = 1.23). Injection: 0.5 μl of eluent containing 100 ng of solute.

Column(s)	*Plate count (N)*	*Asymmetry factor (A_s)*
A	13 140	1.33
B	13 156	1.43
C	13 413	1.34
A + B + C	40 459	1.34

TABLE IV

PLATE COUNTS AND ASYMMETRY FACTORS FOR THE COUPLING OF TWO ODS-SILICA COLUMNS

Columns (X and Y): each 300 × 1 mm I.D. packed with ODS-Hypersil (5 μm). Eluent: methanol–aqueous sodium dihydrogen phosphate (0.1 *M*) (30:70, v/v). Flow-rate: 50 μl/min. Detection: 284 nm. Solute: codeine (k' = 0.58). Injection 0.5 μl of eluent containing 2.2 μg of solute.

Column(s)	*Plate count (N)*	*Asymmetry factor (A_s)*
X	3209	3.42
Y	2766	2.85
X + Y	5982	4.31

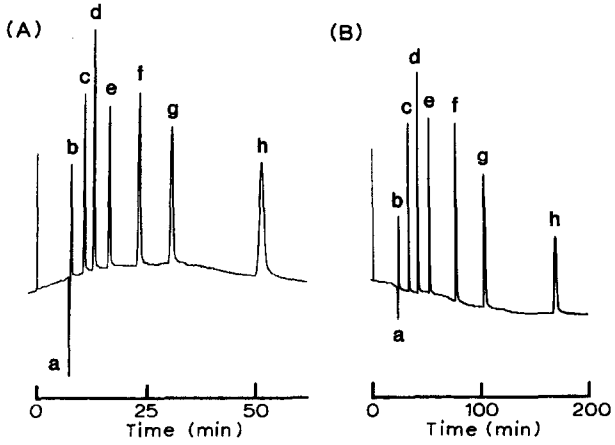

Fig. 2. Chromatograms of a test mixture on silica narrow-bore columns (300 × 1 mm I.D.). (A) Single column; (B) three columns coupled in series using capillary tubing (5 cm × 0.006 in. I.D.). Packing material: Hypersil-silica, 5 μm. Eluent: hexane–methanol (99.5:0.5, v/v). Flow-rate: 25 μl/min. Detection: 235 nm. Injection volume: 0.5 μl. Peaks: a = pentane; b = toluene; c = nitrobenzene; d = acetophenone; e = 2,6-dinitrotoluene; f = 1,3-dinitrobenzene; g = 2,6-dinitrobenzyl chloride; h = 2,6-dinitrobenzonitrile.

In our experience the coupling of 1-mm bore columns is a practical and reliable method of achieving high plate counts and hence improving the resolution between compounds in HPLC. This is demonstrated in Fig. 2 for the separation of the standard test mixture on three silica columns. Nevertheless it must always be borne in mind that the increase in column efficiency is achieved at the expense of longer analysis times (*cf.* Fig. 2A and B). In the context of routine drug analysis it is debatable under what circumstances such extended analysis times might be tolerated to achieve the advantages of better resolution.

For the analysis of drugs in complex matrices, often of biological origin, it is common to encounter a situation where the compound of interest is not resolved from the other components in the sample. Although it might be possible to achieve base-line resolution by modification to the eluent or to the sample preparation pro-

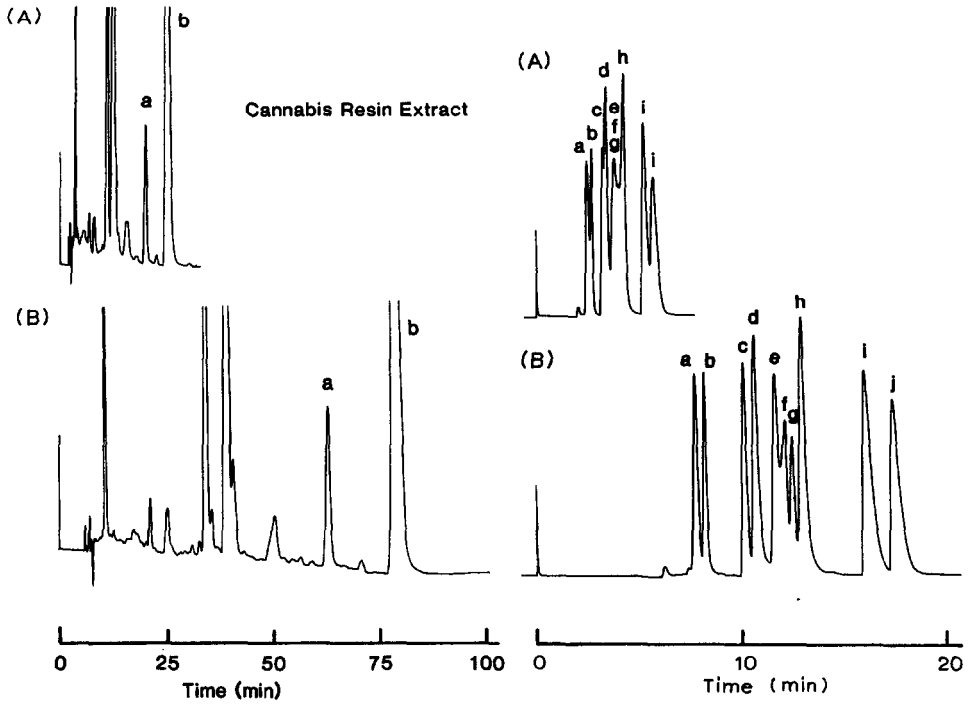

Fig. 3. Chromatograms of a cannabis resin extract on ODS-silica narrow-bore columns (300 × 1 mm I.D.). (A) Single column; (B) three columns coupled in series. Packing material: ODS-Hypersil, 5 μm. Eluent: sulphuric acid (0.02 *N*)–methanol–acetonitrile (5:8:9, v/v/v). Flow-rate: 70-μl/min. Operating pressures: 1250 and 4900 p.s.i. for one and three columns respectively. Detection: 220 nm. Resin extracted with methanol–chloroform (4:1, v/v) with injections of 2 μl. Peaks: a = cannabinol (CBN); b = delta-9-tetrahydrocannabinol (THC).

Fig. 4. Separation of ten basic drugs on silica narrow-bore columns (300 × 1 mm I.D.). (A) Single column; (B) three columns coupled in series. Packing material: Spherisorb S5W, 5 μm. Eluent: ammonium perchlorate (0.01 *M*) in methanol adjusted to pH 6.7 with sodium hydroxide. Flow-rate: 80 μl/min. Operating pressures: 800 and 2750 p.s.i. for one and three columns respectively. Detection: 254 nm. Drugs dissolved in methanol, 2 μl injection. Peaks: a = diphenoxylate; b = caffeine; c = methysergide; d = lysergamide; e = anileridine; f = amphetamine; g = tyramine; h = practolol; i = protriptyline; j = desipramine.

cedure, or even by the selection of a new column packing material or the use of a more specific detector, in some circumstances it may be more convenient to use the same HPLC system with coupled columns of higher efficiency. In a similar way column coupling may prove to be useful for the comparison of illicit drugs of biological origin (*e.g.* cannabis, opium) where the chromatogram shows a complex pattern of largely uncharacterised components. Such patterns can be very useful for intelligence purposes[15]. Fig. 3 shows how three coupled ODS-silica columns can provide a more detailed "fingerprint" of a cannabis resin extract by the separation of many more components.

At the present time one of the important roles of HPLC in forensic toxicology is to provide a tentative identification of unknown drugs from retention measurements. In this respect many HPLC systems have been developed for the separation of drugs within a given class (*e.g.* barbiturates[29], amphetamines and narcotic analgesics[30], local anaesthetics[31]). Such systems depend on the number of compounds which can be resolved across the chromatographic range and this can be considerably increased by the use of columns with higher efficiency. It is in this area that coupled narrow-bore columns might find further applications. Fig. 4 shows the separation of 10 basic drugs on Spherisorb S5W using an eluent system recommended for basic drug screening[16]. It is clear that it is possible to differentiate between many more compounds using the coupled columns. Furthermore it is particularly worth noting that all 10 drugs elute with k' values of less than 2, a region in an HPLC chromatogram where resolution is generally poor.

The mass spectrometer as a detector

Several interfaces are now commercially available for the on-line combination of HPLC with mass spectrometry (MS) including moving-belt, direct liquid inlet (DLI) and thermospray devices. Recent reviews have described the current progress in this area[32,33]. In the present study only a moving-belt interface on a magnetic sector instrument operated in the electron-impact (EI) mode has been examined. This interface involves the deposition of the effluent from the HPLC column on to a moving polyimide belt which passes through a narrow channel into the source region of the mass spectrometer where it is heated to effect volatilisation.

As a detector for HPLC the mass spectrometer has enormous potential for drug analysis. It can be operated as a universal detector by continuous scanning over a wide mass range or as a very selective detector operated in the multiple ion detection (MID) mode. In theory the continuous scanning mode should offer the greatest flexibility since through computer reconstruction methods it should be possible to obtain chromatograms using the total ion current (TIC) or at any specific mass ion as well as obtaining a complete mass spectrum for any separated component. However, experience with the belt interface has shown that there are numerous background ions originating from the belt itself which limit the value of the scanning mode when analytes have significant ions at low masses[34]. Useful mass spectra with ions below m/z 150 are often difficult to obtain, even with the application of computer background subtraction methods, except when drug concentrations are very high. Furthermore, the TIC detection mode often gives noisy chromatograms and hence is rather insensitive for drug detection. In contrast, the detection limits for many drugs using the MID mode are in the low ng range[34].

The HPLC–MS belt interface relies upon the efficient evaporation of solvents from the eluent before the analyte on the belt reaches the source region of the mass spectrometer. Initial experiments using conventional HPLC columns encountered considerable problems associated with the use of flow-rates $\geqslant 1$ ml/min and the use of eluents with a high water content. Satisfactory results could only be obtained when a powerful infra-red heater was positioned over the belt in the inlet chamber immediately after the effluent was deposited on the belt. Furthermore the inlet chamber had to be maintained under vacuum. The intense source of heat was responsible for the thermal decomposition of several sensitive drugs and for a considerable reduction in the useful life of the moving belt[34].

The low flow-rates used with narrow-bore HPLC columns have obvious advantages for use with belt interfaces in HPLC–MS and several workers have now explored this approach[35–39]. Previous HPLC–MS studies in our laboratory have used 0.5 mm I.D. columns constructed from thick-walled PTFE tubing, but these columns showed very poor plate counts. Their replacement with the present 1 mm bore columns has greatly improved the chromatographic performance and reliability of the HPLC–MS equipment. With the use of flow-rates $\leqslant 100$ μl/min it has been possible to operate the interface with the infrared heater switched off and with the inlet chamber at atmospheric pressure while the use of eluents containing a high proportion of water is now feasible.

Experiments have been conducted to examine the chromatographic performance of the 1 mm bore HPLC columns using the mass spectrometer as a detector. The plumbing was arranged such that the column effluent passed through *ca.* 9 cm of capillary tubing (0.006 in. I.D.) before deposition on the belt. An ODS-silica column was used with an eluent of 60% methanol and injecting a test mixture containing phenol, *p*-cresol, 2,5-dimethylphenol, anisole and phenetole. As the molecular weights of these compounds are relatively small, ranging from 94 to 122, the MID detection mode was necessary to facilitate sensitive detection. Fig. 5 shows the chromatograms obtained for the 5 compounds at three detection ions (m/z 94, 108 and 122) and clearly demonstrates the facility of the mass spectrometer to act as a very selective detector for HPLC. By the superposition of the appropriate peaks from Fig. 5 on to a single time axis (Fig. 6) it is possible to appreciate the excellent chromatographic resolution which can be achieved with the present equipment. Furthermore, comparison of Fig. 6 with the test chromatograms using UV detection shown in the previous paper[1] indicates that the loss in chromatographic performance with the mass spectrometer as a detector is very small. This agrees with the work of Games *et al.*[40] who have concluded that the moving belt interface on a mass spectrometer has a low time constant and a low effective dead volume.

For the routine drug analyst the prospect of an HPLC detector with the versatility of a mass spectrometer is very exciting. In particular, the high selectivity of MID promises less arduous sample preparation procedures for the quantification of drugs in body fluids, while continuous scanning offers information on the identity of unknown components. Although continuous scanning is at present rather insensitive there are some problem areas where samples are plentiful and the technique has proved to be particularly useful, *e.g.* the identification of acid/neutral impurities in illicit heroin samples[41]. This last example clearly demonstrates the need for on-line HPLC–MS as the chromatograms are extremely complex such that off-line fraction collection and transfer to a mass spectrometer would be virtually impossible.

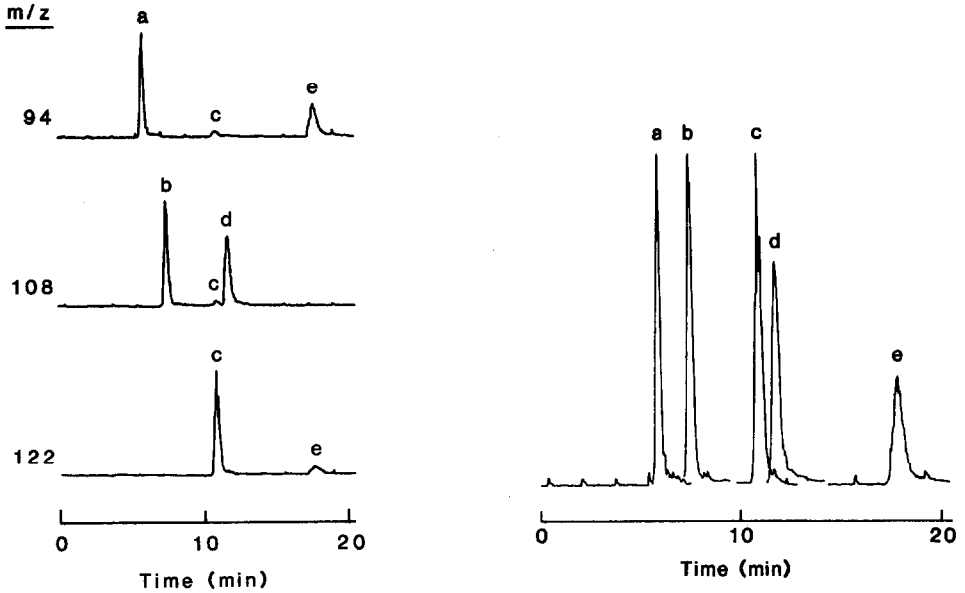

Fig. 5. HPLC–MS of a standard test mixture on an ODS-silica narrow-bore column using multiple ion detection (MID) at *m/z* 94, 108 and 122. Column: Hypersil-ODS, 5 μm (300 × 1 mm I.D.). Eluent: methanol–water (60:40, v/v). Flow-rate: 80 μl/min. Injection volume: 2 μl (test compounds in eluent). Peaks: a = phenol; b = *p*-cresol; c = 2,5-dimethylphenol; d = anisole; e = phenetole.

Fig. 6. Chromatogram of a standard test mixture on an ODS-silica narrow-bore column produced by overlaying the MID traces shown in Fig. 5. Peaks: a = phenol (*m/z* 94); b = *p*-cresol (*m/z* 108); c = 2,5-dimethylphenol (*m/z* 122); d = anisole (*m/z* 108); e = phenetole (*m/z* 94).

CONCLUSION

The present paper has addressed the question as to whether narrow-bore HPLC columns (1 mm I.D.) offer any important advantages over conventional columns for routine drug analysis. Large savings (>95%) on consumables (packing materials and solvents) are certainly significant when considering the running costs of narrow-bore systems. Nevertheless it is questionable whether this alone would be enough to justify the necessary capital investment in replacement pumps, injectors and detectors to accommodate such columns in the short term. Such economic factors might only prove to be important when expensive solvents or packing materials (*e.g.* chiral stationary phases for the analysis of enantiomeric drugs) are used.

The present experimental work has demonstrated some increases in mass sensitivity for the narrow-bore columns over conventional columns using both UV and electrochemical detection. However, it is clear that the increases in mass sensitivity which might be predicted from simple considerations of decreasing column diameter are difficult to achieve in practice. The problems encountered with UV detection are particularly significant as the vast majority of routine analyses use these detectors. Such relatively small increases (5–7.5 in the most favourable circumstances) would probably only prove to be of value in circumstances where the sample size is severely restrictive.

On a more encouraging note the coupling of 1 mm bore columns has proved to be an extremely effective method of producing columns with very high plate counts. Such coupled columns have been shown to be capable of separating complex drug mixtures. Nevertheless it is questionable how many routine drug analyses require more than the 10 000–15 000 plates currently achievable with a single column of conventional bore.

Finally, our experience has confirmed that narrow-bore (1 mm) HPLC columns solve many of the chromatographic problems associated with the use of the moving-belt HPLC–MS interface. In a similar way narrow-bore columns are being used with other types of HPLC–MS interface[32,33]. Research and development into interface design continues at a rapid pace but HPLC–MS is still in its infancy and no single device is yet able to handle all types of compound successfully. Consequently HPLC–MS can not be described as a routine tool at this stage. As narrow-bore columns have been shown to be advantageous with several types of interface they would appear to have a secure future in this area. It is worthy of note that the latest commercial interface, the thermospray[42], can operate with virtually any solvent including water with flow-rates up to at least 2 ml/min and hence is well suited to conventional columns.

Thus 1 mm bore HPLC columns do offer some advantages over the use of conventional columns, but these advantages are perhaps not as great as might be suspected from the vast literature which has been published over the last five years. Furthermore, for the routine drug analyst it may seem difficult to justify the necessary investment to change over to such columns at the present time. Nevertheless practical 1 mm bore systems are easy to set up and reliable in operation, and will always find applications where difficult analytical problems dictate that the properties of such narrow-bore columns are truely significant.

ACKNOWLEDGEMENTS

We wish to thank Mr. J. R. Joyce for operating the mass spectrometer during experiments to investigate the performance of the narrow-bore columns with the moving-belt interface and LKB Instruments for the loan of the 2151 UV detector.

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