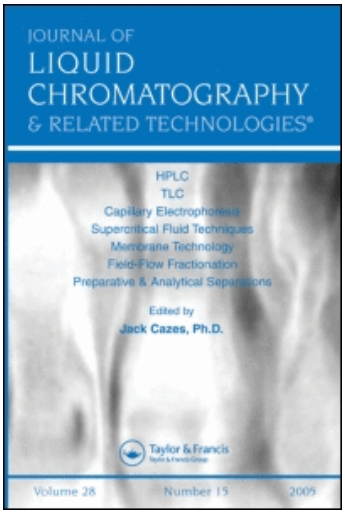

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MOBILE PHASE FLOW PROGRAMMING IN LIQUID CHROMATOGRAPHY USING SHORT NARROW BORE COLUMNS

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ABSTRACT

Application of mobile phase flow programming to effect fast liquid chromatographic analysis of multi-components sample in short narrowbore columns was investigated. It was found that the generally accepted limited power of flow programming can, nevertheless, be enhanced by the right configuration of operating parameters, such as column I.D. and length, particle size of packing material, mobile phase, detector cell volume, etc. Average reproducibility of flow programming in the separation of five aromatic compounds were 0.18 % in retention time and 6.0 % in peak area. Initial flow rate was restored within 1 minute, all of which are favourable for reliable and fast analysis in routine HPLC laboratory.

INTRODUCTION

In the chromatographic separation of complicated samples containing components with great difference in capacity factor, chromatographers usually employ mobile phase gradient elution or temperature-programming (especially

in GC). Flow programming is often considered limited and has rarely been used. Among the limited number of publications on flow programming, most are on its application in GC with few in HPLC.[1-4]

Narrow bore columns (2.0 mm I.D) are columns whose diameter are between that of microbore (<1.0 mm I.D.) and conventional columns (4-5 mm I.D.). With special size, narrow bore columns have some combined characteristics possessed by microbore and conventional columns. Narrowbore-LC can be easily implemented on conventional equipment with little modification, while maintaining some advantages possessed by micro-LC, such as reduction in solvent and stationary phase consumption, and higher mass sensitivity.[5-9]

This paper reports the application of flow programming in LC using a 10 cm x 2.0 mm I.D. narrowbore column. We discuss the use of the appropriate combination of column length and I.D., particle size of packing material, mobile phase, and flow cell volume in order to achieve fast separation of some aromatic compounds.

EXPERIMENTAL

Instrumentation and Reagents

Chromatography was carried out with a JASCO (Tokyo, Japan) Model PU980 pump, a Model 7825 Rheodyne (Cotati, CA, USA) injection valve equipped with 5-μL loop, and a programmable Model 786A UV absorbance detector (Applied Biosystems, Foster City, CA, USA), connected to a Shimadzu (Tokyo, Japan) Model CR-6A integrator. A prepacked (C_{18}) short narrowbore column (100 mm x 2.0 mm I.D.) (Upchurch, WA, USA) was used.

Alkylphenols were purchased from Fluka (Buchs, Switzerland)). Benzene, toluene, ethylbenzene, acenaphthene, dibenz[*a,h*]anthracene were obtained from Sigma (St. Louis, MO, USA). LC-grade methanol, acetonitrile, and water from a MilliQ purification system (Millipore, Bedlford, MA, USA) were employed.

RESULTS AND DISCUSSION

The successful application of flow programming for faster analysis requires the appropriate configuration of the LC system. It is known that

solvent gradient elution can be easily implemented in conventional columns (e.g. 25 cm x 4.6 mm I.D.), but much more difficult in microbore columns (I.D. < 1 mm). On the other hand, temperature programming can be easily performed in microbore columns (small heat capacity), but more difficult in conventional columns (large heat capacity). With regards to flow programming, it can be more easily and effectively implemented in short narrowbore columns (e.g. 100 mm x 2.0 mm I.D.).

Firstly, a short narrowbore column with plate numbers around 4000 is efficient enough for many routine analyses whose requirements for efficiency are usually within the range of several hundred to several thousand plates. Using a long standard column with much higher efficiency is unnecessary and is sometimes a "waste" of column efficiency, analysis time, and solvent consumption. Secondly, a short narrowbore column has lower resistance which makes it possible for flow programming to be used across a wider range of flow rates and allows for a more rapid change in flow rate. Thirdly, a much lower volumetric flow rate (about 5 times lower than that in a conventional column) is required to generate high linear velocity in a narrowbore column. A high programming rate can be more easily achieved with great reduction in solvent consumption. Further, low volumetric flow rates (often less than 0.8 mL/min) cause less damage to pumps, extending their lifespans, and reducing mechanical trouble. At above the optimal flow rate, the HETP curve for a narrowbore column is flatter than for a conventional column, allowing for increasing flow rate without much compromising separation, especially for late-eluting bands.[9] In addition, a narrowbore column is compatible with most conventional HPLC equipment, unlike a microbore column putting stringent demands on many parts in HPLC.

Particle sizes of 3μm, 5μm and 10μm of LC packing materials are commonly available. Short columns packed with 3-μm particles provide greater resolving power per column length. The column can be run at higher flow rates with little reduction in the number of theoretical plates.[10] The 3-μm column, however, is not without disadvantages. It is often run at much higher back pressure, which is caused by fines present in the particle size distribution. Usually 0.5-μm porosity column inlet frits are used; these can plug easily and cause excessive back pressure, leading to short column lifetimes. In addition, the column often exhibits >3 - 3.5 d_p plate height, greater than 2 - 2.5 d_p plate height typical of well-made commercial columns packed with 5-μm particles. Finally, the sharp, low volume peaks are often degraded because of extra-column effects.[10] A short narrowbore column packed with 10-μm particles often exhibits inadequate efficiency for some routine separations. Further, the plate height increases more rapidly with

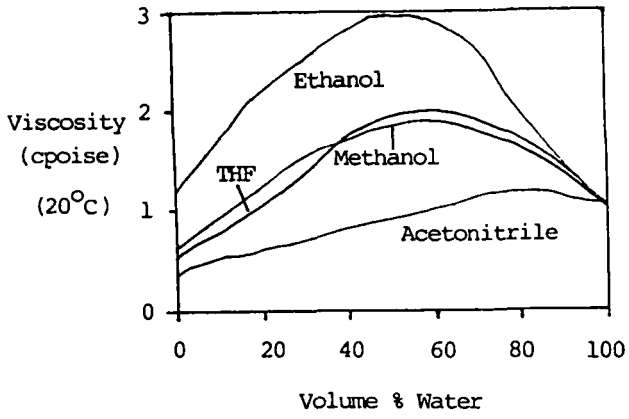

Figure 1. Viscosity curves of water:organic solvent mixture

velocity, which makes the column inappropriate for high speed analysis, although it is a preferred choice in the view of low flow resistance. In the present work, 5 μm was chosen as the size of packing material. This represents a good compromise when considering column efficiency, back pressure, and extra-column dispersion effects.

Emphasis in the following was placed on choosing an appropriate solvent system and testing the effects of detector cell volume on flow programming.

In mobile phase flow programming, the programming range is often limited by the back pressure that the pump, injector and column can handle. For routine operation, the back pressure should be less than 197 atm (2900 psi). The viscosity of the eluent has a great effect on the system back pressure. Acetonitrile has the lowest viscosity among the four most popular organic solvents (the other three being methanol, ethanol, and tetrahydrofuran), used in reversed-phase LC. Further, the viscosity of an acetonitrile-water eluent varies only slightly within the whole range of different acetonitrile-water ratios with

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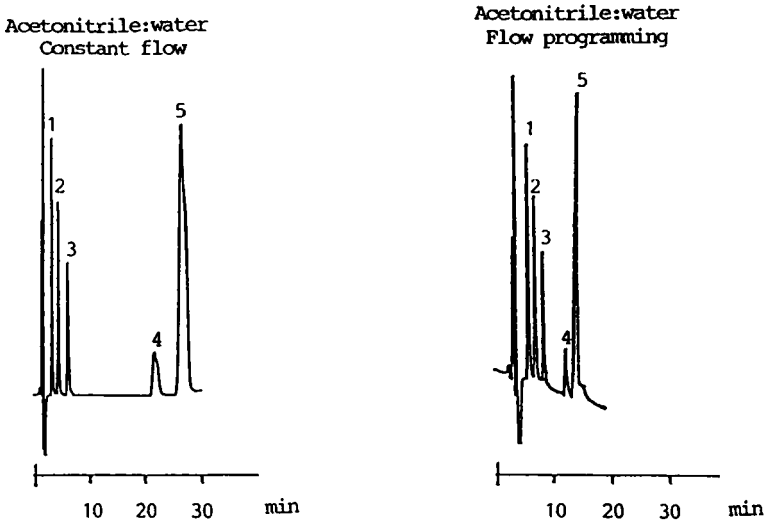
Figure 2. (**right**) Comparison of flow programming LC and constant flow LC. Separation of 5 alkyphenols using two different mobile phases: acetonitrile:water (54%:46%) and methanol:water (68%:32%). Column: 100 mm x 2.0mm I.D.; stationary phase: C_{18} (5- μm particle size). Peaks: 1) *p*-Ethylphenol, 2) *p*-Propylphenol, 3) *p*-Butylphenol, 4) *p*-Heptylphenol , 5) *p*-Octylphenol.

a



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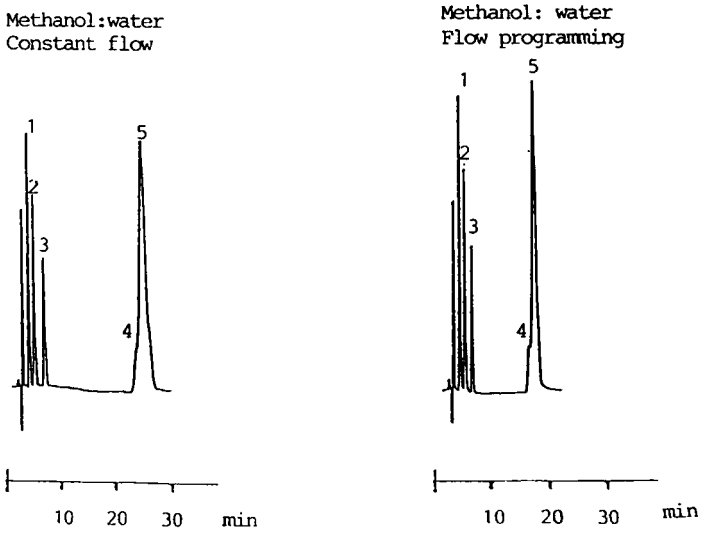

back pressure remaining low (Fig. 1). Hence, mobile phase flow programming can be performed satisfactorily within almost the whole range of mixing ratios without sacrificing selectivity.

Figure 2 shows the chromatograms of 5 alkylphenols obtained by constant flow and flow programming modes, for acetonitrile:water (54%:46%) and methanol:water (68%:32%) eluent mixtures. The two mobile phases □□are similar solvent strengths and selectivities for early eluting peaks. The viscosity of acetonitrile:water is, however, only about half of that of methanol:water. As shown in Fig. 2, flow programming when acetonitrile-water eluent is used can be performed across a wider flow rate range (0.1 - 0.6 mL/min) with pressure still below 200 atm (2940 psi). Flow programming is effective in reducing analysis time by half whilst maintaining the resolution of the three early- eluting peaks. On the other hand, as shown in Fig. 2b, with methanol-water, flow programming can be performed only within a narrow range of 0.1 - 0.3 mL/min when the back pressure is over 200 atm. Analysis time is only slightly reduced compared with the constant flow mode. Further, the last two bands are baseline separated when acetonitrile-water is used, but not when methanol-water is used. In this case, acetonitrile-water mobile phase is preferred to methanol-water not only insofar as the flow programming range is concerned, but also in terms of selectivity.

In the miniaturized HPLC, the achievement of theoretical limit of speed is limited by the extra-column dispersion which comes primarily from the injector, detector, and connecting tube. The external column band broadening becomes very critical, especially for early elution peaks with low k' values, when column internal diameter, length and particle size are decreased.[9] Hence, appropriate choice of detector cell volume, injection volume, and connecting tube is necessary in order to obtain the maximum performance from short narrow-bore column with flow programming. In this work, we investigated the effect of detector cell volume.

Figure 3 shows the chromatograms of five aromatic compounds. As can be seen, early-eluting peaks are unresolved using constant flow mode with a detector cell volume of 12 μL (Fig. 3a). Improvement in the resolution of these coincident peaks, and reduction in analysis time were obtained by using flow programming together with a smaller-volume flow cell (2.4 μL) (Fig. 3b).

In flow programming (Fig. 3b), the initial flow rate was restored and stabilized within 1 minute, which was much shorter than that required in solvent gradient elution and temperature programming operations. Taking this

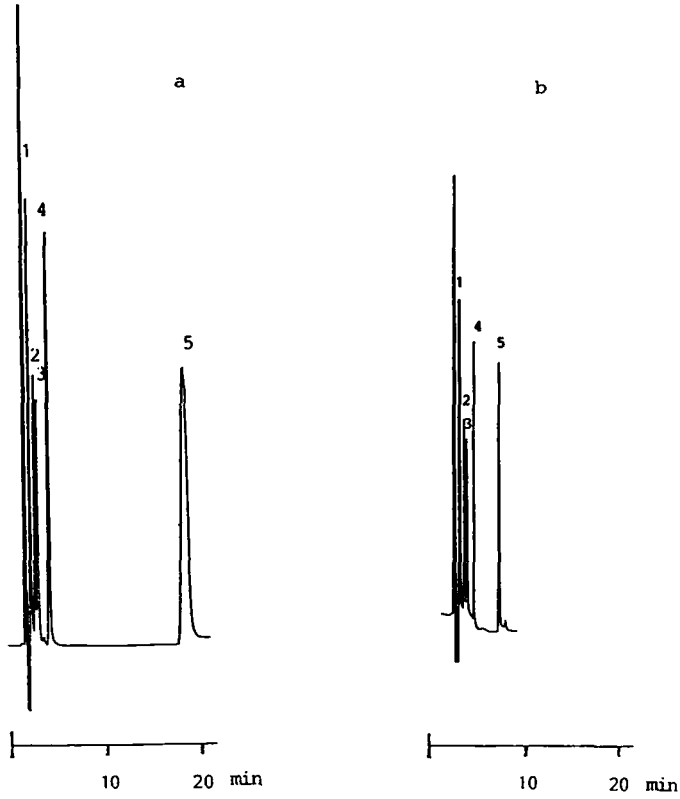

Figure 3. Effect of detector cell volume on flow programming LC. Conditions: (a) 12-μL cell with constant flow at 0.2 mL/min (b) 2-μL cell with flow programming: initial 0.20 mL/min, then increasing to 0.40 mL/min after 4 min., then further increase to 0.8 mL/min after 6 min. Peaks: 1) Benzene, 2) Toluene, 3) Ethyl benzene, 4) Acenaphthene, 5) Dibenz[*a,h,*]-anthracene.

into consideration, the total analysis time is in some cases even shorter than those required for solvent gradient and/or temperature programming.

The retention time and concentration reproducibilities for 12 consecutive flow-programmed runs were calculated, and showed an average relative standard deviation of 0.18 in retention time and 6.0 % in peak area, which are favourable for reliable routine analysis.

CONCLUSION

Compared with conventional or microbore columns, short narrowbore columns with flow programming provide a more practical configuration for fast analysis of multi-component samples in a routine HPLC lab. The power of flow rate programming, though still limited, can be expanded through the appropriate combination of some operating parameters, including column length, I.D, particle size of packings, solvent type, and detector cell volume. In routine analysis where the sample is not very complicated, flow programming with short narrowbore column can be used as an alternative to temperature programming and solvent gradient elution while providing simplicity in operation, better reproducibility of chromatographic behaviour, and even shorter overall analysis times in some cases.

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