Micro-Flow Gradient High Performance Liquid Chromatography Using the Glass-Lined Stainless Steel Microcolumn

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We have been studying glass-lined stainless steel microcolumns in our laboratory.[1-4] Former microcolumn studies by other research groups were almost exclusively related to packed silica capillary columns. The studies of the packed silica columns in seventies and eighties were reviewed by two independent authors.[5,6] While the packed silica capillary columns have been studied by many workers up to date, the packed glass-lined stainless steel microcolumns have been exclusively initiated, developed, and improved in our laboratory since a few years ago. The merit of glass-lined stainless steel tubing over silica capillaries is its strength and easiness of handling. The packed silica capillary columns demand extreme care to make and use since they are fragile.

In isocratic elution, we have so far achieved numbers of theoretical plates of 20,000 for 0.5 mm I.D. columns (30 cm length)[4], and 10,000 for columns of 0.3 mm I.D.[3] In this study, we have successfully constructed a micro-flow gradient HPLC system using the glass-lined stainless steel microcolumns and have obtained good separation results for a few test solute mixtures.

Experimental

Two Shimadzu (Tokyo, Japan) 10AD pumps, an Isco (Lincoln, USA) CV4 capillary window detector, a Valco (Houston, USA) CI4W0.05 injector with a 50nL injection loop, and a Younglin (Seoul, Korea) D520 computing integrator were combined to construct the micro-flow gradient liquid chromatography system. We minimized extracolumn void volume by directly connecting the column to the injector, putting a silica capillary tubing with a sintered silica frit between the column outlet and the detector, and using a very small sample loop (50 nL)[3]. A simple mixer for gradient elution was prepared using a tee union and a 1/16 inch I.D. stainless steel frit. The tubing from each pump was connected to the one of the side ports of the union, and the frit was placed into the central port of the union, upon which the outlet tubing was fitted. The column was prepared as described before using the Alltech (Deerfield, USA) slurry packer.[4] The Adsorbosphere C18 stationary phase (5 μm) from Alltech was used as the packing material.

Methanol, acetonitrile, and water were of HPLC grade and obtained from Fisher (Pittsburg, USA) and used without further purification. All the chemicals for the test solutes except for N,N-diethyl-p-nitroaniline were purchased from Aldrich (Milwaukee, USA) and used as received. N,N-Diethyl-p-nitroaniline were kindly donated from professor Peter W.Carr, University of Minnesota, USA.

Results and Discussion

We first examined the column efficiency in the isocratic elution mode using an eluent composed of 99.5% methanol and 0.5% acetic acid. The chromatogram of the test solutes, p-nitroaniline, N,N-diethyl-*p*-nitroaniline, and propylbenzene at the optimum flow rate (3 μL/ min) is shown in Figure 1, and the numbers of theoretical plates are estimated 15,000-20,000.

In gradient elution, 10 μL/min was chosen as the total flow rate. The lowest flow rate of the Shimadzu pump is 1 μL/min. The first sample we tested was a polyaromatic hydrocarbon (PAH) mixture composed of naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]pyrene, and benzo[ghi]perylene. The solvent A was 0.1% trifluoroacetic acid (TFA) in acetonitrile, and B was 0.1% TFA in water. The initial mobile phase composition was set 50% A+ 50% B. The composition was linearly moved to 70% A + 30% B in 20 min, and finally to 100% A at 40 min. The chromatogram of the

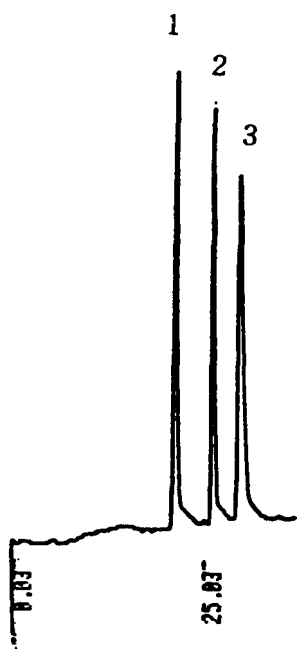

Figure 1. The chromatogram of *p*-nitroaniline, N,N-diethyl-*p*-nitroaniline, and propylbenzene eluted in the eluent composed of 99.5% methanol and 0.5% acetic acid and observed at 254 nm. The flow rate was 3 μL/min. 1: *p*-nitroaniline, 2: N,N-diethyl-*p*-nitroaniline, 3: propylbenzeze.

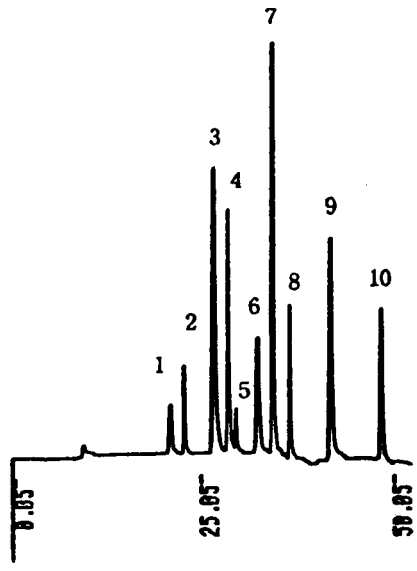

Figure 2. The chromatogram of PAH's by gradient elution observed at 254 nm. The total flow rate was 10 μL/min. The eluent composition was initially 50% A(0.1% TFA in acetonitrile)+50% B (0.1% TFA in water) and was linearly changed to 70% A+30% B in 20 min, then to 100% A in next 20 min. 1: naphthalene, 2: acenaphthylene, 3: fluorene, 4: phenanthrene, 5: anthracene, 6: fluoranthene, 7: pyrene, 8: chrysene, 9: benzo[a]pyrene, and 10: benzo[ghi]perylene.

PAH mixture obtained by such gradient elution is shown in Figure 2. All the PAH's are well separated. We also observed that the chromatogram was reproducible.

The second test sample was a dialkyl phthalate mixture composed of dimethyl, dietyl, dipropyl, dibutyl, diphenyl, dicyclohexyl and dioctyl phthalates. The initial mobile phase composition was 50% A (0.1% TFA in acetonitrile) and 50% B (0.1% TFA in water). The composition was linearly changed to 100% A in 25 min. The chromatogram of the phthalates obtained by the gradient elution is shown in Figure 3. All the phthalates are well separated and nicely distributed over the run time. The retention of each solute was highly reproducible.

Thus, we were able to construct a reliable micro-flow gradient HPLC system using the glass-lined stainless steel

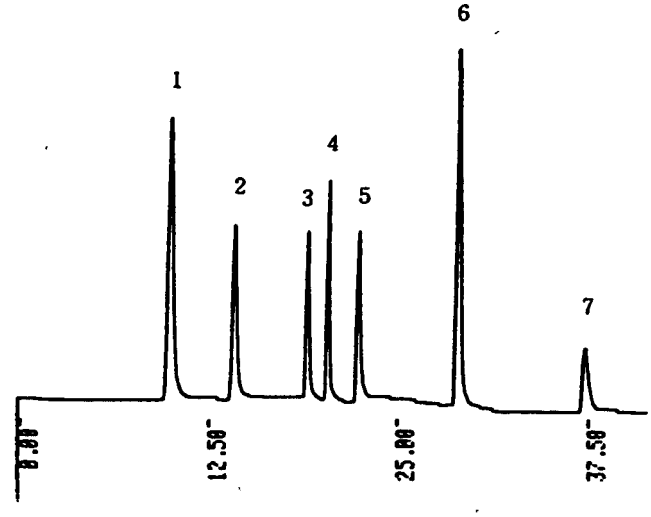

Figure 3. The chromatogram of phthalates by gradient elution observed at 280 nm. The total flow rate was 10 μL/min. The eluent composition was initially 50% A+50% B and was changed to 100% A in 25 min. 1: dimethyl phthalate, 2: diethyl phthalate, 3: dipropyl phthalate, 4: dibutyl phthalate, 5: diphenyl phthalate, 6: dicyclohexyl phthalate, and 7: dioctyl phthalate.

packed microcolumn and two micro-pumps.

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