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## Note

### Gradient elution system for high-performance liquid chromatography with narrow-bore packed columns

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Several gradient elution systems have been reported which are compatible with narrow-bore packed columns (I.D. less than 0.5 mm), including stepwise<sup>1,2</sup> and continuous gradient elution<sup>3,4</sup>. Flow splitting allows the use of an ordinary liquid chromatographic pump at the cost of solvent economy<sup>1,4</sup>, because a very slow flow-rate (1–10  $\mu\text{l}/\text{min}$ ) is employed for narrow-bore columns. Takeuchi and Ishii<sup>3</sup> have reported a simple one-chamber gradient system. The system seems unsuitable for a very efficient column such as a 1-m column packed with 3- $\mu\text{m}$  packing<sup>5–7</sup>, because of its limited pressure and it is time-consuming for routine use since the column conditioning has to be carried out off-line.

We have developed a simple gradient system based on one-chamber mixing and valve switching which can be operated under pressures greater than 300  $\text{kg}/\text{cm}^2$  and produces a good reproducibility. It is suggested that the system can easily be changed to a stepwise gradient mode and automated for routine use.

#### EXPERIMENTAL

Fig. 1 shows the gradient system, which comprises a three-way valve and two six-way valves. A loop (2 m  $\times$  1/16 in. O.D.  $\times$  1 mm I.D.) is connected to the first six-way valve (3), and a mixing chamber to the second one (5). The details of the

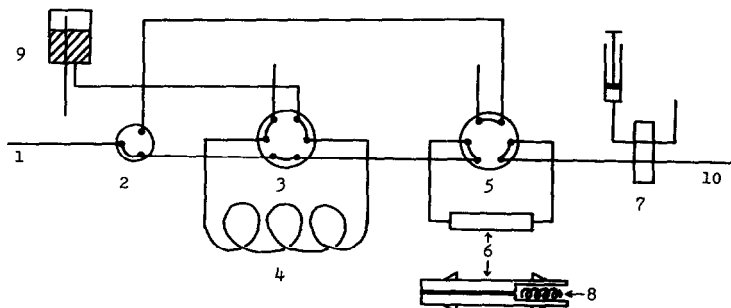


Fig. 1. Schematic diagram of the gradient elution system: 1, from LC pump; 2, three-way valve; 3,5, six-way valves; 4, loop for final solvent; 6, mixing chamber (1/4 in. O.D., 13  $\times$  4 mm I.D.); 7, micro valve injector; 8, steel coil; 9, reservoir for final solvent; 10, to column.

mixing chamber are also shown in Fig. 1. An induction coil was used to stir the solution. Connections between the valves and injector were made with stainless-steel tubing (1/16 in. O.D.  $\times$  0.1 mm I.D.) to minimize the dead volume, using Swagelok-type fittings. Therefore, the entire system can withstand a pressure of more than 300 kg/cm<sup>2</sup>.

A Twinkle liquid chromatograph pump (Jasco, Tokyo, Japan) was operated in a constant-pressure mode. Sample injection was carried out using a valve injector (sample volume 0.1  $\mu$ l). A Jasco UVIDEC-100-III UV spectrophotometer with a modified flow cell (volume 0.04  $\mu$ l) was used as a detector. The column was a fused-silica capillary (50 cm  $\times$  0.2 mm I.D.) packed with Develosil ODS-5 (Nomura Kagaku, Seto, Japan) and connected to the injector using a polyimide ferrule.

Gradient elution using the system was carried out as follows. First the initial solvent was delivered to the column through the mixing chamber as shown in Fig. 1. After sample injection and isocratic analysis for a selected period of time, the Valve 3 was switched to a gradient position. In this case, the final solvent was employed only to push the initial solvent out of the loop. When separation was complete, the valve 5 was switched and the column washed with the final solvent. Then, the loop and mixing chamber were filled with the final and initial solvents, respectively. After all the valves had been reset to the initial positions, column conditioning was carried out until the baseline became constant.

## RESULTS AND DISCUSSION

Fig. 2 shows the separation of polycyclic aromatic hydrocarbons using

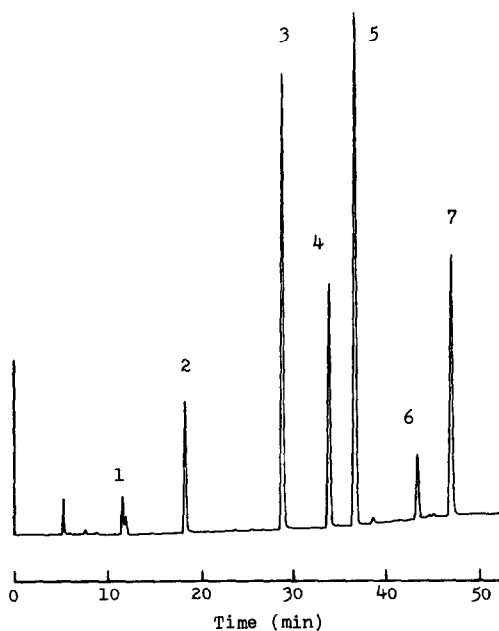


Fig. 2. Separation of polycyclic aromatic hydrocarbons. Column: 50 cm  $\times$  0.2 mm I.D. Develosil ODS-5. Initial solvent: acetonitrile-water (60:40). Final solvent: acetonitrile. Pressure: 200 kg/cm<sup>2</sup>. Detection wavelength: 254 nm. Peaks: 1 = benzene; 2 = naphthalene; 3 = anthracene; 4 = pyrene; 5 = triphenylene; 6 = benzo[fluoranthene]; 7 = benzo[a]pyrene.

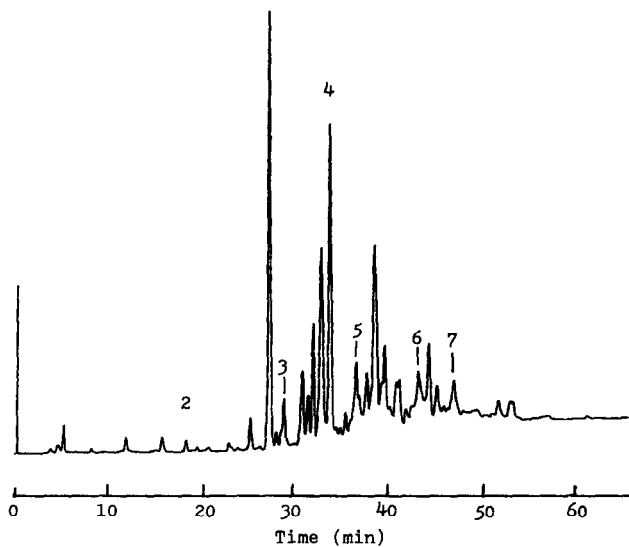


Fig. 3. Separation of an extract from diesel engine particulates. Conditions and peak identities as in Fig. 2.

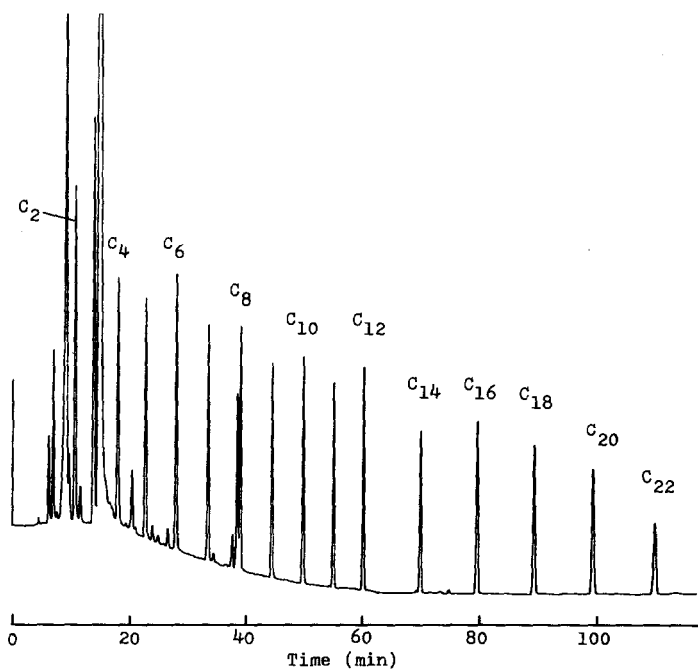


Fig. 4. Separation of *p*-nitrobenzyl esters of fatty acids ( $C_2$ - $C_{22}$ ). Initial solvent acetonitrile-water (60:40). Final solvent: acetonitrile-tetrahydrofuran (85:15). Other conditions as in Fig. 2.

acetonitrile–water (60:40) and pure acetonitrile as the initial and final solvents, respectively. The column was operated at 200 kg/cm<sup>2</sup>. The reproducibility obtained from four runs was as follows: coefficients of variation, <1.8% for retention time and 2.6% for peak area. This implies that mixing of the solvent is carried out effectively.

Fig. 3 shows the separation of an extract from diesel engine particulates. The conditions were as in Fig. 2. Several peaks were identified by comparing their retention times with those in Fig. 2.

*p*-Nitrobenzyl esters of fatty acids have a very wide range of capacity factors under isocratic conditions<sup>8</sup>. Fig. 4 shows the separation of a mixture of these esters using acetonitrile–water (60:40) and acetonitrile–tetrahydrofuran (85:15). All the peaks are almost equally spaced and eluted as narrow bands, and the C<sub>8</sub> derivative is well resolved from impurities. When pure acetonitrile was used as the final solvent, slowly eluted peaks were broad and the C<sub>8</sub> derivative overlapped with impurities. Thus, any solvent can be used as the final solvent to change the strength or selectivity of the mobile phase, providing it is miscible with the initial solvent.

The operation of this system is simple and reproducible. If necessary, it can readily be automated. The system can be changed to a stepwise gradient mode only by replacing the mixing chamber with a loop.

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