CHROMSYMP. 1664

DIRECT COUPLING OF CAPILLARY LIQUID CHROMATOGRAPHY WITH CONVENTIONAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

TOYOHIDE TAKEUCHI*

Research Centre for Resource and Energy Conservation, Nagoya University, Chikusa-ku, Nagoya 464 (Japan)

and

MASASHI ASAI, HIROKI HARAGUCHI and DAIDO ISHII

Department of Applied Chemistry, School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464 (Japan)

SUMMARY

A capillary column liquid chromatograph was combined with a conventional liquid chromatograph and the system was evaluated by using aromatic hydrocarbons as test analytes. A capillary fused-silica column of 0.35 mm I.D. was employed in the former chromatograph. The combination of normal-phase and reversed-phase separations was possible because the first dimension involved separation with the capillary column, which resulted in very small peak volumes. A whole sample band from the former chromatograph could be transferred to the sample loop of the latter. The system was applied to the normal- and reversed-phase separation of aromatic hydrocarbons in a fuel for body warmers (benzine).

INTRODUCTION

There are inherent limits to improvements of the selectivity in liquid chromatography (LC). Successive column chromatography is one of the best means of increasing the separation efficiency. Giddings[1] defined the peak capacity, φ, as a measure of the separation efficiency:

$$\varphi = 1 + \frac{\sqrt{N}}{m} \ln (1 + k')$$ (1)

where N is the theoretical plate number, k' is the capacity factor of the last-eluted peak of interest and m is the number of standard deviations, σ, that are taken as equalling the peak width.

Freeman[2] has shon that if two or moe different chromatographic modes are coupled, the total peak capcity of a multi-dimensional separation, φ_T, approaches

$$\varphi_T = \prod_{i=1}^{n} \varphi_i$$ (2)

where φ_i are the peak capacities of each mode and n is the number of modes. Coupled chromatographic systems offer much greater peak capacities than single-column systems, but the addition of band broadening is a critical factor in the design of an interface. In order to combine two conventional LC systems, the heart-cutting method has been proposed, in which a portion of a primary band is transferred into the secondary system[3,4].

Capillary LC systems facilitate direct combination with conventional LC systems, because the peak volumes of the former systems are so small that a whole primary band can be quantitatively transferred to the secondary systems. Floyd[5] has recently published a paper on "two-dimensional" LC–LC, in which microbore LC systems with 1 mm I.D. columns and conventional LC systems are employed as the primary and secondary systems, respectively.

The attractive features and requirements for coupled LC–LC and LC–gas chromatographic separations have been described[6]. A coupled chromatographic system, capillary LC–conventional LC, was used in this work, and the system assembly was evaluated by using aromatic hydrocarbons at test analytes.

EXPERIMENTAL

Apparatus

The coupled system was composed of a capillary liquid chromatograph and a conventional high-performance liquid chromatograph. The former chromatograph was assembled from a Microfeeder (Azumadenki Kogyo, Tokyo, Japan), equipped with an MS-GAN 050 gas-tight syringe (0.5 ml) (Ito, Fuji, Japan) as a pump, an ML-525 micro valve injector (0.02 μl) (JASCO, Tokyo, Japan), a laboratory-packed fused-silica column and a UVIDEC-100 UV spectrophotometer (JASCO) with a modified flow cell. A schematic diagram of the modified flow cell is shown in Fig. 1. Stainless-steel tubing of 0.05 mm I.D. $\times$ 0.30 mm O.D. (Nomura Chemical, Seto, Japan) was glued in fused-silica tubing of 0.32 mm I.D. with an epoxy-resin adhesive. The detection volume was *ca.* 0.1 μl. The capillary fused-silica columns were prepared according to previous work[7].
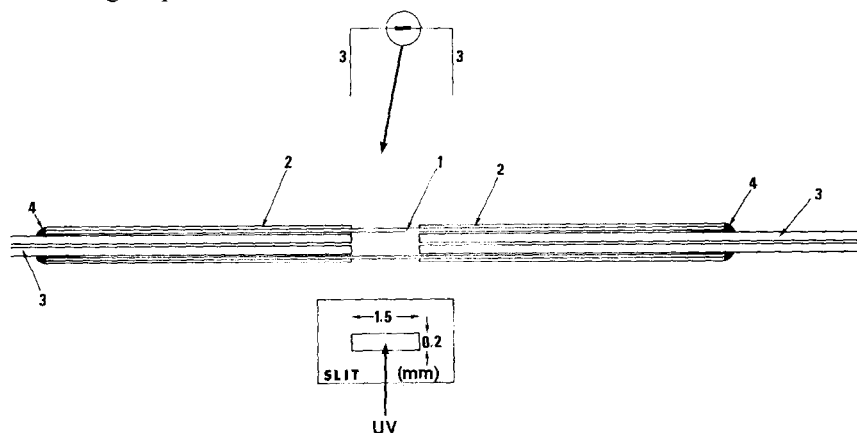

Fig. 1. Schematic diagram of the modified flow cell. 1 = Fused-silica tubing of 0.32 mm I.D.; 2 = polyimide resin; 3 = stainless-steel tubing of 0.05 mm I.D. $\times$ 0.30 mm O.D.; 4 = epoxy-resin adhesive.

The conventional chromatograph was composed of an 880-PU pump (JASCO), a laboratory-modified loop injector, a 100 or 250 × 4.6 mm I.D. column and a UVIDEC-100V UV spectrophotometer (JASCO). The micro LC and the conventional LC instruments were interfaced by small-bore (50–70 μm I.D.) fused-silica or stainless-steel tubing having a dead volume of < 1 μl. The effluent from the capillary LC system was introduced into the loop tubing of the valve injector of the conventional LC system. The valve was manually operated, taking account of the time lag due to the dead volume of the interface. The loop injector of the secondary system was prepared from a Model 7000 switching valve (Rheodyne, Cotati, CA, U.S.A.) and a stainless-steel loop with various volumes.

Aminopropylsilica, TSKgel NH$_2$-60 (5 μm) (TOSOH, Tokyo, Japan), Develosil ODS-3K (3 μm) (Nomura Chemical) and Develosil ODS-5 (5 μm) (Nomura Chemical) were employed as packing materials. The capillary LC columns were prepared from fused-silica tubing of 0.35 mm I.D. (Gaskuro Kogyo, Tokyo, Japan) in the laboratory. The conventional columns were supplied by Nomura Chemical.

Reagents

All the reagents were of analytical-reagent grade, except for HPLC-grade distilled water. The reagents were supplied by Wako (Osaka, Japan) or Tokyo Chemical Industry (Tokyo, Japan) and employed without any treatment.
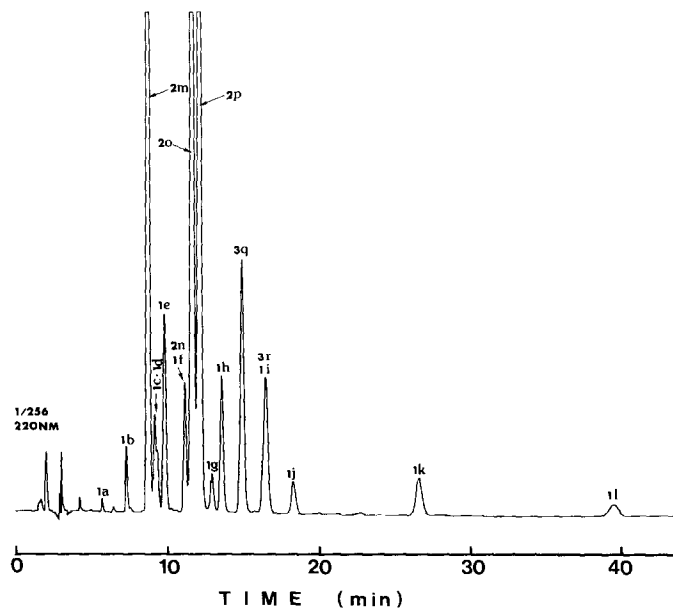

Fig. 2. Reversed-phase separation of an artificial mixture of aromatic hydrocarbons. Column, Develosil ODS (250 × 4.6 mm I.D.); mobile phase, acetonitrile–water (70:30); flow-rate, 1.0 ml/min; sample, *ca.* 0.01% each. 1a = Benzene; 1b = toluene; 1c = *o*-xylene; 1d = *m*- and *p*-xylene; 1e = ethylbenzene; 1f = cumene; 1g = mesitylene; 1h = *n*-propylbenzene; 1i = *sec.*-butylbenzene; 1j = *n*-butylbenzene; 1k = *n*-amylbenzene; 1l = n-hexylbenzene; 2m = naphthalene; 2n = biphenyl; 2o = 1-methylnaphthalene; 2p = 2-methylnaphthalene; 3q = phenanthrene; 3r = anthracene. Sample volume, 12 μl; detection, 220 nm.

RESULTS AND DISCUSSION

The peak volume depends on the column dimensions and efficiency and also on the capacity factors of the analytes when isocratic elution is applied. The peak volumes observed in capillary LC with 100 or 250 × 0.25 or 0.35 mm I.D. columns are ca. 10 μl or less. This volume is smaller than the acceptable injection volumes for ordinary columns in conventional LC (ca. 100 μl). This situation facilitates direct combination of the capillary and conventional LC systems[6]. The sample-loop volume of the secondary system must at least be larger than the peak volumes observed in the primary system, because the sample band increases in volume when passing through the interface capillary and the sample loop itself. The loop employed 25 cm × ca. 0.25 mm I.D. allowed quantitative injection into the secondary system of up to 10 μl at a flow-rate of 4.2 μl/min.

The two-dimensional separation system was evaluated by using aromatic hydrocarbons as test analytes. The reversed-phase separation of an artificial mixture of aromatic hydrocarbons is demonstrated in Fig. 2. Several peaks overlapped and could not be separated under the operating conditions used. Real samples will give
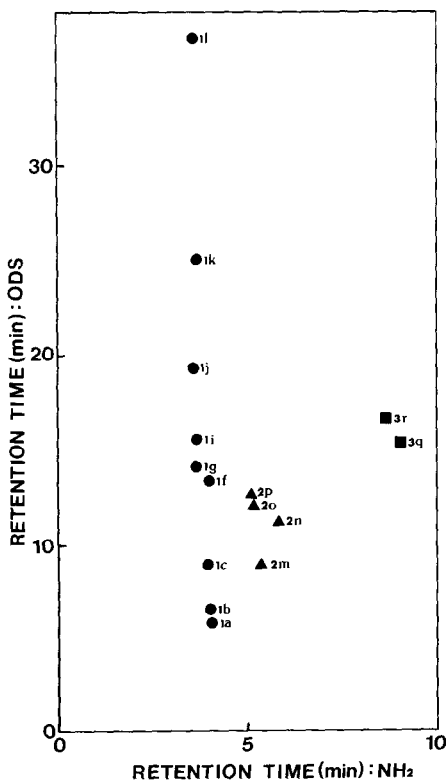

Fig. 3. Retention times on the ODS column versus those on the NH$_2$ column. Columns, Develosil ODS-3K (100 × 0.35 mm I.D.); TSKgel NH$_2$-60 (150 × 0.35 mm I.D.); mobile phases, acetonitrile–water (70:30) for the ODS colomn, hexane for the NH$_2$ column; flow-rate, 4.2 μl/min; sample as in Fig. 2.

much more complicated chromatograms. In such a case, the coupled-column system will improve the separation efficiency and simplify the chromatogram. For example, aromatic hydrocarbons can be separated on the basis of differences in aromatic moieties in the normal-phase mode and then separated on the basis of differences in aliphatic moieties in the reversed-phase mode.

Fig. 3 illustrates relationships between retention times of the analytes on the ODS column in the reversed-phase mode and those on the NH_2 column in the normal-phase mode. In the normal-phase separation mode the analytes are separated on the basis of differences in the number and size of their aromatic ring moieties, while both aromatic and aliphatic moieties significantly affect the retention times in the reversed-phase mode.

Fig. 4 demonstrates the normal-phase separation of nineteen aromatic hydrocarbons on the NH_2 column. The first peak contains thirteen monocyclic aromatic hydrocarbons, the second contains three bicyclic aromatic hydrocarbons, the third contains biphenyl and the fourth contains two tricyclic aromatic hydrocarbons. The volume of the last peak is 5.3 μl.

The fraction containing the first peak in Fig. 4 was transferred to the secondary separation system and subjected to reversed-phase separation, as shown in Fig. 5. The detection wavelength was 210 nm. All monocyclic aromatic hydrocarbons examined, except xylenes, were well separated.

Fig. 6 demonstrates the reversed-phase separation of the components in the fraction containing the second and third peaks. Fig. 7 demonstrates the reversed-phase separation of the components in the fraction containing the fourth peak. Fig. 6 was obtained with detection at 220 nm and Fig. 7 at 250 nm. In order to carry out the reversed-phase separation of the components present in each fraction, a normal-phase separation was carried out prior to each reversed-phase separation.
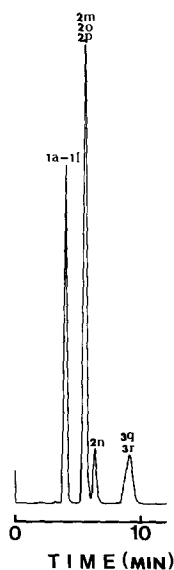

Fig. 4. Normal-phase separation of an artificial mixture of aromatic hydrocarbons. Column, TSKgel NH_2-60 (150 × 0.35 mm I.D.); mobile phase, hexane; flow-rate, 4.2 μl/min; sample as in Fig. 2, except concentration (*ca.* 0.1% each); detection, 220 nm.
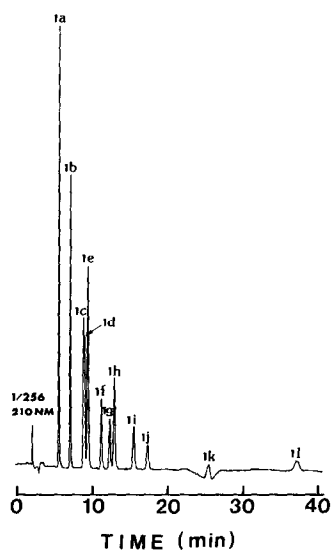
Fig. 5. Reversed-phase separation of a fraction of monocyclic aromatic hydrocarbons. Column, Develosil ODS (250 × 4.6 mm I.D.); mobile phase, acetonitrile–water (70:30); flow-rate, 1.0 ml/min; detection, 210 nm.

The coupled system was applied to the analysis of aromatic hydrocarbons in a fuel for body warmers (benzine). The results are shown in Fig. 8. A few peaks were observed in the normal-phase separation. Fig. 8 also shows the reversed-phase
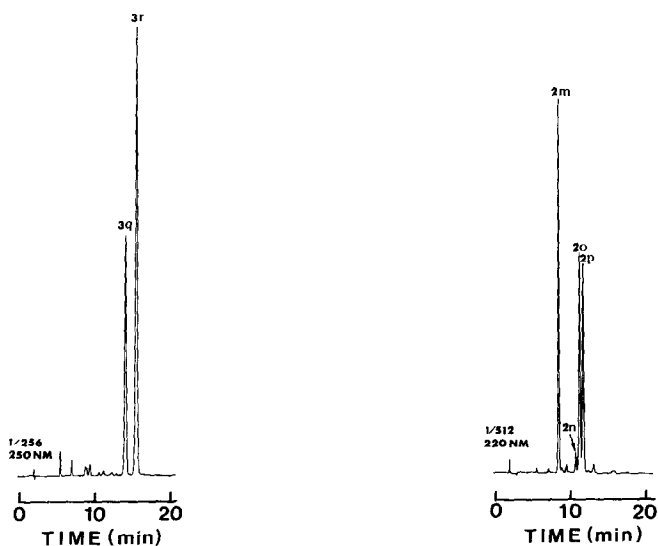

Fig. 6. Reversed-phase separation of a fraction of bicyclic aromatic hydrocarbons and biphenyl. Detection, 220 nm; other operating conditions as in Fig. 5.

Fig. 7. Reversed-phase separation of a fraction of tricyclic aromatic hydrocarbons. Detection, 250 nm; other operating conditions as in Fig. 5.
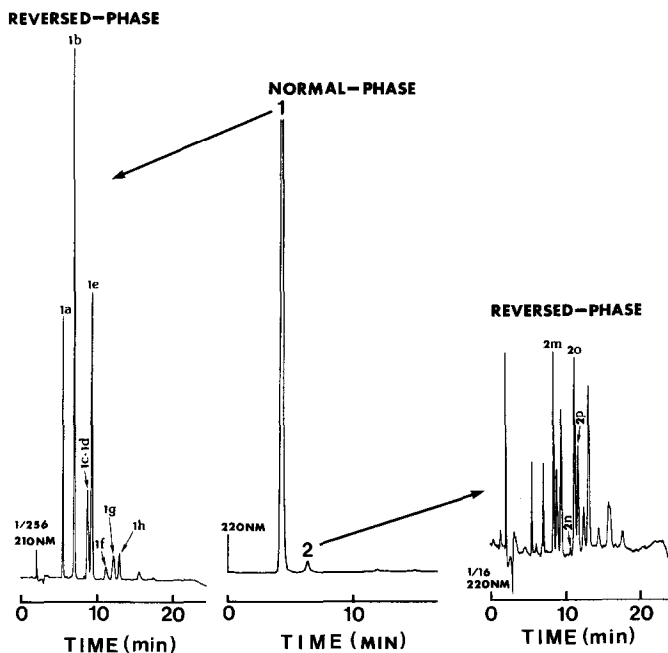
Fig. 8. Coupled-column chromatography of aromatic hydrocarbons in a fuel for body warmers (benzine). Operating conditions of capillary system: column, TSKgel NH$_2$-60 (150 × 0.35 mm I.D.); mobile phase, hexane; flow-rate, 4.2 μl/min; sample, 0.02 μl of 10% benzine; detection, 220 nm. Operating conditions of conventional system: column, Develosil ODS (250 × 4.6 mm I.D.); mobile phase, acetonitrile–water (70:30); flow-rate, 1.0 ml/min; detection, 210 nm for monocyclic aromatic hydrocarbons fraction and 220 nm for bicyclic aromatic hydrocarbons fraction.

separation of the components in the fraction containing the first or second peak in the normal-phase separation. Several components were identified on the basis of their retention times. The use of a multi-channel UV detector will give more reliable qualitative information.

In addition to the above separation modes, various other modes are available in LC, e.g., ion exchange, ion pair, size exclusion and chirality recognition. There are many possibilities for selecting two different separation modes for coupled-column chromatography. This technique is generally capable of increasing the peak capacities and will be of practical importance for the separation of complex mixtures.

ACKNOWLEDGEMENT

REFERENCES

1 J. C. Giddings, *Anal. Chem.*, 56 (1984) 1258A.
2 D. H. Freeman, *Anal. Chem.*, 53 (1981) 2.
3 E. L. Johnson, R. Gloor and R. E. Majors, *J. Chromatogr.*, 149 (1978) 571.
4 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 571.
5 Th. R. Floyd, *Chromatographia*, 25 (1988) 791.
6 T. V. Raglione, N. Sagliano, Jr., Th. R. Floyd and R. A. Hartwick. *LC · GC*, 4 (1986) 328.
7 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 213 (1981) 25.