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Note

Ultra-micro high-performance liquid chromatography

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At present, several types of columns are available for high-performance liquid chromatography (HPLC), *viz.*: (1) ordinary HPLC columns; (2) micro-HPLC columns[1]; (3) open-tubular microcapillary columns[2–7]; (4) packed microcapillary columns[8,9]; and (5) packed microbore columns[10,11].

Columns of types 3, 4 and 5 have higher efficiencies in terms of the number of theoretical plates than HPLC or micro-HPLC. For example, Scott and Kucera[10] produced a 10-m microbore column by connecting in series columns (1 m × 1 mm I.D.) packed with Partisil 20 (particle diameter 20 μm) for exclusion chromatography and achieved 250,000 theoretical plates at the dead volume (retention time 4.6 h).

Micro columns (10–20 cm × 0.25–0.5 mm I.D.) are used in micro-HPLC[1], which is advantageous in that the amount of sample required, and the consumption of mobile phase and expensive packings, are greatly decreased in comparison with ordinary HPLC. High performance in this technique is also achieved without wall effects or extra column effects because of the development and improvement of detection, conjunction and injection systems.

The flow-rate of mobile phase used in micro-HPLC does not exceed *ca.* 10 μl/min, which makes feasible the direct coupling of liquid chromatography (LC) to mass spectrometry (MS)[12,13] by using an appropriate jet separator or nebulizer. A flow-rate of less than 1 μl/min would be more advantageous for the direct coupling of LC and MS, but such a low flow-rate would greatly increase the analysis time. Micro columns (which have a much smaller internal volume than micro-HPLC columns) are needed in order to overcome this problem.

We have examined ultra-micro packed columns (3–20 cm × 0.1–0.15 mm I.D.) for use in LC, and in this paper we shall discuss, *e.g.*, the injection volume, the amount of sample injected, the column material and the column length, all factors that affect column efficiency.

EXPERIMENTAL

The apparatus used in this work was almost identical with that previously described for use in open-tubular microcapillary LC[4,5,7]. The home-made flow cell 2 mm × 0.18 mm I.D.) was mounted in a Jasco UVIDEC-100 UV detector (Japan

Spectroscopic Co., Hachioji-shi, Japan) and was connected to the exit of the column as shown in Fig. 1. The total volume of the connection tubes was *ca.* 0.2 μl, and a microfeeder and a 100-μl air-tight syringe were used for the pumping system, with which mobile phase could be supplied to the column at a reasonably low flow-rate (0.14–3.3 μl/min).

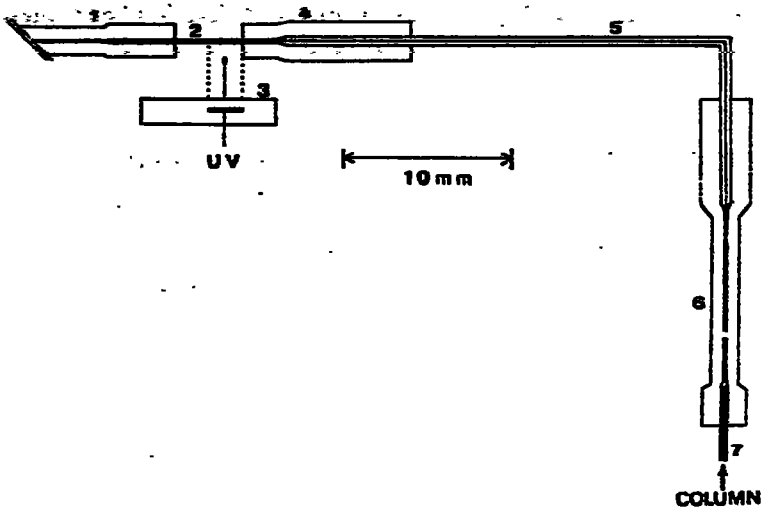

Fig. 1. Schematic diagram of detection system. 1 = PTFE tube (0.15 mm I.D., 2 mm O.D.); 2 = quartz (0.18 mm I.D., 0.33 mm O.D., 10 mm long); 3 = slit (0.2 mm × 2 mm); 4 = PTFE tube (0.15 mm I.D., 2 mm O.D.); 5 = glass capillary (0.03 mm I.D., 0.6 mm O.D., 46 mm long); 6 = PTFE tube (0.1 mm I.D., *ca.* 1.3 mm O.D., 14 mm long); 7 = stainless-steel tube (0.13 mm I.D., 0.13 mm O.D., 5 mm long).

Stainless steel, PTFE and Pyrex glass were selected as column materials. Stainless-steel tubing of I.D. 0.13 mm and O.D. 0.31 mm was purchased from Hakko Shoji Co. (Tokyo, Japan), and PTFE tubes (0.15 mm I.D. and 2 mm O.D., or 0.50 mm I.D. and 1.5 mm O.D.) were purchased from Nishio Industry Co (Tokyo, Japan). Pyrex glass capillary tubing (0.12–0.13 mm I.D. and *ca.* 0.7 mm O.D.) was drawn with a Shimadzu GDM-1 drawing machine (Shimadzu Seisakusho, Kyoto, Japan). As column packing, silica ODS SC-01 (particle diameter 5 μm; Japan Spectroscopic Co.) was generally employed in order to investigate the column efficiency.

Packing procedure

The packing technique employed in this work was almost the same in earlier work[1]. PTFE tubes (0.15 mm or 0.25 mm I.D. and 2 mm O.D.) were connected to both ends of the Pyrex glass or stainless-steel tubing, and quartz wool (less than 0.5 mm long) was packed tightly in the PTFE tube at one end of the column to prevent leakage of the packing material. The packing was suspended in acetonitrile and dispersed by ultrasonic vibration for a few minutes. The slurry was then fed into the PTFE tube and was passed into the column by injecting acetonitrile from an air-tight syringe (250 μl).

RESULTS AND DISCUSSION

The column dimensions used in this technique, which we call ultra-micro-HPLC are much smaller than those currently used in LC; the internal volume of a capillary tube 10 cm long and 0.1 mm I.D. is only 0.8 μl. The various column dimensions and flow-rates of mobile phase used in this and in other forms of LC are shown in Table I. The size of column employed in ultra-micro-HPLC is about one-tenth of that used in micro-HPLC and one-thousandth of that used in HPLC.

TABLE I

COLUNN DIMENSIONS AND FLOW-RATES IN LIQUID CHROMATOGRAPHY

*Category**	*Chromatographic column*			*Flow-rate, µl/min*
	I.D., mm	*Length, cm*	*Internal volume, µl*	
HPLC	2–4	10–50	300–6000	1000
Micro-HPLC	0.25–0.5	10–20	5–40	8
OMCLC	0.03–0.06	300–1000	5–30	2
PMC	0.05–0.2	1000–6000	20–2000	2
PMB	1	1000	800	30
Ultr-micro-HPLC	0.1–0.15	3–20	0.2–3	0.5

* OMCLC = Open-tubular microcapillary liquid chromatographic column; PMC = packed microcapillary column; PMB = packed microbore column.

The dimensions and performance of several of the columns examined in this paper are shown in Table II. The sample size (*ca.* 0.02 μl) was so chosen because larger samples caused an increase in the height equivalent to a theoretical plate (HETP), as described later. Table II shows that the column material can seriously affect the column efficiency; thus, Pyrex glass columns gave higher efficiency than did those of stainless steel or PTFE. The HETP values for stainless-steel or PTFE columns are somewhat larger than those for Pyrex glass columns.

TABLE II

DIMENSIONS AND EFFICIENCIES OF COLUMNS EXAMINED

Each column was packed with Silica ODS SC-01 (particle diameter, 5 μm). The sample was pyrene.

Column			*Composition of mobile phase (acetonitrile–water)*	*Linear velocity (cm/min)*	*k' value*	*HETP (mm)*
I.D. (mm)	*Length (cm)*	*Material*				
0.13	10.0	Stainless steel	60:40	4.5	6.5	0.13
0.13	10.0	Stainless steel	70:30	4.2	4.7	0.090
0.15	11.2	PTFE	70:30	4.6	5.8	0.12
0.15	10.2	PTFE	70:30	4.3	6.0	0.064
0.12	10.0	Pyrex	70:30	4.8	4.4	0.034
0.12	10.6	Pyrex	70:30	5.3	4.8	0.034
0.12	5.0	Pyrex	60:40	4.3	6.2	0.025
0.12	3.0	Pyrex	50:50	4.3	11.1	0.024
0.50	9.8	PTFE	70:30	4.7	6.2	0.037

Curves of HETP *versus* linear velocity are shown in Fig. 2; the dependence of HETP on linear velocity for the Pyrex glass column is much smaller than that for a stainless-steel or PTFE column. This may be caused by the wall effect, as, unlike that of Pyrex glass tubing, the inner wall of commercially available PTFE tubing is rough (microscopic observation). Therefore, the void between the inner wall and the packing in a PTFE tube is irregular and this may lead to peak broadening. It can be presumed that stainless-steel tubing also has a rough inner wall. On the other hand, Pyrex glass tubing has a smooth inner wall and can be used to prepare highly efficient columns giving better reproducibility than others.

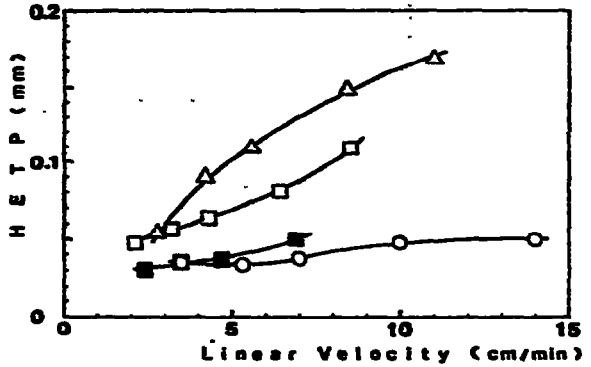

Fig. 2. Relationship between linear velocity and HETP. Columns: stainless steel, 0.13 mm I.D. × 10.0 cm (△); PTFE, 0.15 mm I.D. × 10.2 cm (□); Pyrex glass, 0.12 mm I.D. × 10.6 cm (○); PTFE, 0.50 mm I.D. × 9.8 cm (■). Mobile phase: acetonitrile–water (70:30). Sample: pyrene.

The curve of HETP *versus* linear velocity for a micro-HPLC column is also shown in Fig. 2. The resolution of such a Pyrex glass column is high, and its efficiency compares well with that of an micro-HPLC column, despite its small column volume.

The separation of some aromatic hydrocarbons on a Pyrex glass column (10.6 cm × 0.12 mm I.D.) and on a micro-HPLC column (9.8 cm × 0.50 mm I.D.) is illustrated in Fig. 3. The six components are separated in *ca.* 10 min on the ultra-micro column, and the sensitivity in ultra-micro-HPLC is apparently some 10 times better than in micro-HPLC owing to the decreased peak broadening.

Unfortunately, the operating flow-rate cannot be attained instantaneously, owing to the stopped-flow injection method used, so that the retention volume must be corrected (this problem may be overcome in future by using a flow-injection method).

The separation of the aromatic compounds on short columns is shown in Fig. 4 (the samples and flow-rate are the same as in Fig. 3A). Both 5-cm and 3-cm columns gave high resolution if the appropriate composition of mobile phase was used, *i.e.*, acetonitrile–water (60:40, in the former instance, and 50:50 in the latter). The HETP values of these short columns are smaller than those of a 10-cm column (see Table II), which indicates that extra column effects are negligible with these short columns. In addition, good separation was obtained with a column length of 20 cm.

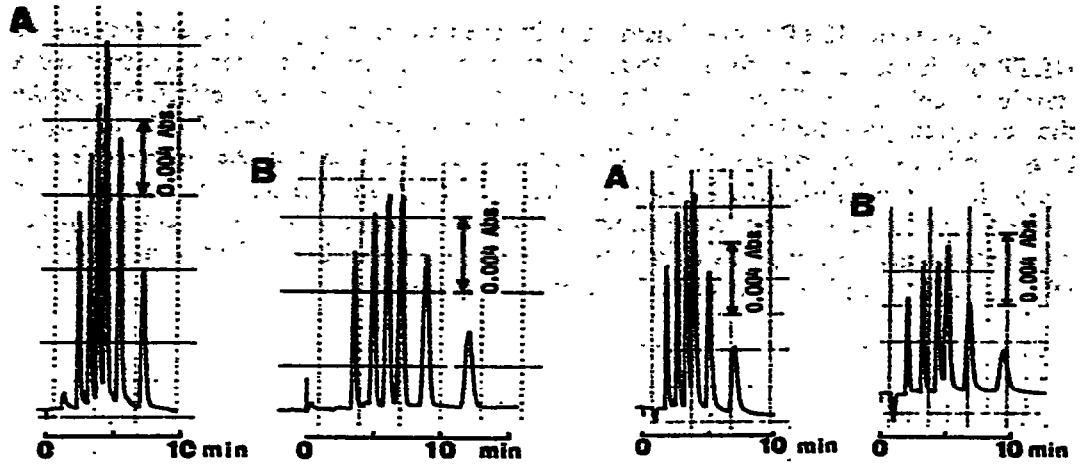

Fig. 3. Separation of aromatic hydrocarbons. Columns: Pyrex glass, 0.12 mm I.D. × 10.6 cm (A), and PTFE 0.50 mm I.D. × 9.8 cm (B). Mobile phase as in Fig. 2; flow-rate, 0.83 μl/min (A); 8.3 μl/min (B). Sample: 0.96% of benzene, 0.090% of naphthalene, 0.018% of biphenyl, 0.028% of fluorene, 0.0028% of anthracene and 0.019% of pyrene (eluted in that order); sample size, 0.02 μl (A) and 0.18 μl (B).

Fig. 4. Separation of aromatic hydrocarbons on short columns. Columns: Pyrex glass, 0.12 mm I.D. × 5.0 cm (A) and 0.12 mm I.D. × 3 cm (B). Mobile phase: acetonitrile–water (60:40) (A) and (50:50) (B). Flow-rate and samples as in Fig. 3A; sample size, 0.01 μl.

The effect of the size of sample injected on the column efficiency was examined for samples from 0.02 to 0.1 μl; the injection method for such a small volume was almost the same as that previously described[1]. The results are shown in Fig. 5. For the column of I.D. 0.12 mm and length 10 cm, the injection volume should be as small as possible; the effect of injection volume is smaller for the longer-retained sample.

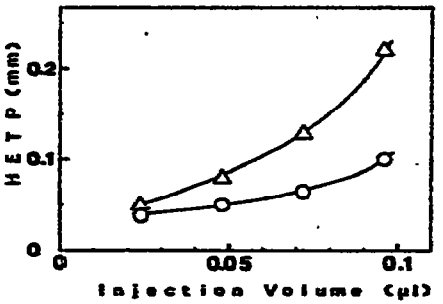

Fig. 5. Effect of injection volume on HETP. Column: Pyrex glass, 0.12 mm I.D. × 10.0 cm. Mobile phase: acetonitrile–water (60:40); flow-rate, 0.83 μl/min. Sample: 3.4 ng of biphenyl (△) and 3.6 ng of pyrene (○) ($k' = 3.7$ and 8.8, respectively).

Fig. 6 shows the relationship between the amount of sample injected and the peak height on a Pyrex glass column of 10.0 cm × 0.12 mm I.D. The relationship was linear over a wide range of amounts (0–200, 0–20 and 0–30 ng for naphthalene,

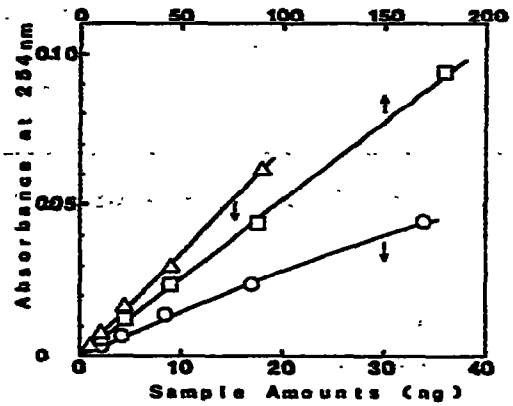

Fig. 6. Relationship between sample amount and peak height. Column: Pyrex glass, 0.12 mm I.D. × 10.6 cm. Mobile phase: acetonitrile–water (70:30); flow-rate, 0.56 μl/min. Sample: naphthalene (□), biphenyl (△) and pyrene (○) (k' = 1.5, 2.0 and 4.6, respectively).

biphenyl and pyrene, respectively), and no deterioration, such as skew of peak shape due to overloading, was observed in these ranges.

Ultra-micro-HPLC columns of I.D. 0.1–0.15 mm and length 3–20 cm gave good resolution, without undue column effect or wall effect. The internal volume of such a column is less than 1 μl, and only 10 μl of mobile phase is required to separate six aromatic compounds. This work suggests that ultra-micro-HPLC offers a valid method for analysing trace amounts of samples and for the direct coupling of LC and MS. The direct coupling of ultra-micro-HPLC and MS is currently being investigated.

REFERENCES

1 D. Ishii, K. Asai, K. Hibi, T. Jonokuchi and M. Nagaya, *J. Chromatogr.*, 144 (1977) 157.
2 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 632.
3 G. Nota, G. Marino, V. Bucnocore and A. Ballio, *J. Chromatogr.*, 46 (1970) 103.
4 K. Hibi, D. Ishii, I. Fujishima, T. Takeuchi and T. Nakanishi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 21.
5 T. Tsuda, K. Hibi, T. Nakanishi, T. Takeuchi and D. Ishii, *J. Chromatogr.*, 158 (1978) 227.
6 R. Tijssen, *13th Int. Symp. Advances in Chromatography, St. Louis, Mo., October 16–19, 1978.*
7 K. Hibi, T. Tsuda, T. Takeuchi, T. Nakanishi and D. Ishii, *J. Chromatogr.*, 175 (1979) 105.
8 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 271.
9 Y. Hirata, M. Novotny, T. Tsuda and D. Ishii, *Anal. Chem.*, 51 (1979) 1807.
10 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 125 (1976) 251.
11 R. P. W. Scott, *Analyst (London)*, 103 (1978) 37.
12 T. Takeuchi, Y. Hirata and Y. Okumura, *Anal. Chem.*, 50 (1978) 659.
13 S. Tsuge, Y. Hirata and T. Takeuchi, *Anal. Chem.*, 51 (1979) 166.