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OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY WITH 30–40 μm I.D. COLUMNS

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SUMMARY

The effect of external column volume on apparent column efficiency is discussed using the peak width expressed in volume units. Although the effect is very large for non-retained solutes, it decreases sharply for retained solutes. Columns with a high k' value and a high theoretical plate number (*e.g.*, 160,000), were demonstrated. Split sample introduction and the method of calculating the thickness of the stationary phase layer are also discussed.

INTRODUCTION

The potential of capillary columns in liquid chromatography (LC) has been demonstrated by numerous workers[1–9], and two different methods are now available: (1) open-tubular microcapillary LC (OTLC)[1–6] and (2) packed microcapillary LC[7–9]. In the latter method, as silica gel or alumina supports are placed in the capillary glass tubing, the paths taken by the mobile phase are complex and tortuous. However, in open-tubular columns, these paths are simple and straight. Hence there are intrinsic demands to use narrower capillary columns in order to achieve high-performance OTLC. Recently, Knox and Gilbert[10] discussed straight OTLC with a view to achieving kinetic optimization, and showed that a theoretical plate number (N) of 10^6 could be obtained in 2 h if a 14 m × 10 μm capillary is used and the detector volume is within 0.0005 μl.

In this paper we consider a capillary of 30–40 μm bore in terms of the limitations of current technology, and show optimal chromatograms that can be obtained in OTLC.

EXPERIMENTAL

An FLC-A-700 pump (Japan Spectroscopic Co., Hachioji-shi, Japan) was used in the constant-pressure mode. Split injection was used, partially modified from previous work[7]. For convenience in discussing split sample introduction problems, a schematic diagram of the apparatus is shown in Fig. 1. Ishizuka Glass Co. (Nagoya, Japan) kindly supplied soda-lime glass tubing of 6 mm O.D. and 0.4–0.3 mm I.D.

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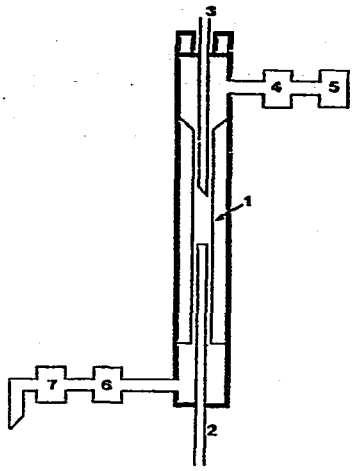

Fig. 1. Schematic diagram of splitter. 1 = Stainless-steel tube, 1.7 or 0.8 mm I.D.; 2 = capillary column; 3 = needle of micro-syringe; 4 = 6-way valve with long handle; 5 = pump operated in constant pressure mode; 6 = stop valve; 7 = fine metering valve with Vernier handle.

The glass was drawn out with a Shimazu GDM-1 drawing machine; the coil diameter was 11 cm. A home-made flow cell and detector were used as in previous work[2–5].

Preparation of capillary columns

As stationary phases β,β'-oxydipropionitrile (OPN) and 1,2,3-tris(2-cyanoethoxy)propane (TCP) were used. The procedure was almost same as in previous work[3] except for the following. The glass capillary tubing was pre-treated with 1 *N* sodium hydroxide solution for 1 day at 52°C or for 2 days at room temperature. The concentration of OPN in dichloromethane varied from 10 to 70% (v/v) and that of TCP in dichloromethane was 20% (v/v). These solutions were used as plugs for dynamic coating. After dynamic coating, the capillary was dried at room temperature for 1 h, and then kept at 52°C under a flow of dry nitrogen until the capillary became whitish.

RESULTS AND DISCUSSION

Effect of external column volume on column efficiency

Although zone spreading in the column and its external regions was discussed in general by Giddings[11], we have attempted to derive equations that include easy practical parameters for the determination of external column effects. The height equivalent to a theoretical plate (H) is expressed as follows

$$H = \sigma^2 L^{-1} \tag{1}$$

where σ and L are standard deviation and column length, respectively. For an open-tubular capillary column, H becomes

$$H = \underbrace{(6R^2 - 16R + 11)r^2u(24D_m)^{-1}}_{H_m} + \underbrace{2/3R(1 - R)d_f^2uD_s^{-1}}_{H_s} \tag{2}$$

R is the ratio of the zone velocity to the velocity of the mobile phase, and is equal to $(1 + k')^{-1}$, where k' is the capacity factor; r, d_f, D_m and D_s are column radius, film thickness of the stationary phase, and diffusion coefficient in the mobile phase (m) or stationary phase (s), respectively. As in GC, it is generally found that the mobile phase term, H_m, is substantially larger than the stationary phase term, H_s, so to a first approximation H_s can be assumed to be negligible in LC[10]:

$$H = H_m \tag{3}$$

The expression of peak width in volume units, W_v, is introduced:

$$W_v = 4\sigma\pi r^2 \tag{4}$$

Using eqns. 1 and 3, W_v becomes

$$\begin{aligned} W_v^2 &= 16L(\pi r^2)^2 H_m \\ &= \left(\frac{0.212Q}{D_m}\right) S^2 L (6R^2 - 16R + 11) \end{aligned} \tag{5}$$

where Q is the volume flow-rate and S is the cross-sectional area of the column. Eqn. 5 is the expression for a retained solute in the column. For a non-retained solute, R becomes unity, the first approximation disappears and eqn. 6 reduces to

$$W_v^2 = \left(\frac{0.212Q}{D_m}\right) S^2 L \tag{6}$$

Eqn. 6 corresponds to Quano's equation[12], and this is applicable to the external column regions (ext), such as the injection, detection and connection parts, because R is generally unity in these parts.

The addition rule $\tau^2 = \Sigma\tau_i^2$, where τ is the standard deviation of a zone in time units and τ_i is the local variance, is valid. As $\sigma = Ru\tau$, then $W_v = 4\tau RQ$. Hence the addition rule for W_v becomes

$$W_v^2 = R^2 \Sigma \left(\frac{W_i}{R_i}\right)^2 \tag{7}$$

Then the apparent H for the column system, which includes the contributions of the column and the external column parts, becomes

$$\begin{aligned} H &= W_v^2 (16L\pi^2 r^4)^{-1} \\ &= R^2 (16L\pi^2 r^4)^{-1} \Sigma \left(\frac{W_i}{R_i}\right)^2 \end{aligned} \tag{8}$$

where R and L are overall values.

As W_v^2 is proportional to H, it is easy to estimate the contribution of each value, namely $(W_i/R_i)^2$, to H. The sum of the contributions of injection (inj), column (c), connection (con) and detector (d) to W_v is expressed as follows:

$$W_v^2 = R^2 \left(W_{inj}^2 + \frac{W_c^2}{R_c^2} + W_{con}^2 + W_d^2\right) \tag{9}$$

As the contribution of injection to W_v^2 is not always expressed by eqn. 6, to a second approximation this term is neglected for simplicity. By using eqns. 5, 6 and 7, W_v^2 becomes

$$W_v^2 = \left(\frac{0.212QR^2}{D_m}\right)(f_c + f_{ext}) \tag{10}$$

where f_c and f_{ext} are as follows:

$$f_c = S_c^2 L_c (6R_c^2 - 16R + 11) R_c^{-2}$$

$$f_{ext} = (\Sigma S_{con,t}^2 L_{con,t}) + S_d^2 L_d$$

For the estimation of the effect of the external column volume on the apparent column efficiency, we can calculate the relative contributions using eqn. 10 without using the diffusion coefficient. f_c and f_{ext} are calculated from two geometrical factors, namely column cross-sectional area and column length, and R_c, the value of which might be equal to R if the time in which the solute remains in the external column region is small compared with the time in the column.

In microcapillary LC experiments, the relative value of f_{ext}/f_c should be considered very carefully. In this work we used a 30–40 μm capillary column and connections almost identical with those in previous work[2] from the column end to the detector. Thus, the column end was first connected to a stainless-steel tube of I.D. 0.13 mm and length 10 mm, then a PTFE tube of I.D. 0.07 mm and length 53 mm and finally a quartz tube of I.D. 0.17 mm and length 5 mm. The last component was a cell in which the UV light spot was *ca.* 2 mm in length. Under these conditions, the value of f_{ext} is calculated to be $5.11 \cdot 10^{-3}$ mm[5]. The ratio of f_{ext}/f_c is shown in Table I for different column sizes and different k' values. The ratio depends on the column I.D., length and R. A longer capillary makes the ratio smaller, and an increase in k' reduces the ratio very sharply. The temporary design of the connections and detector is not adequate for experiments with a non-retained solute, especially with a 30 μm I.D. column, but this design does not have much effect on the apparent column efficiency for a retained solute, especially a solute with k' greater than 1.

TABLE I

CALCULATION OF THE EFFECT OF EXTERNAL COLUMN VOLUME* ON COLUMN EFFICIENCY

Column I.D. (μm)	*Length (m)*	*k'*	f_{ext}/f_c
50	5.1	0	0.26
40	20	0	0.16
30	20	0	0.51
30	30	0	0.34
30	30	1	0.02
30	30	3	0.003

* For the design of this part, see text.

Sample introduction

In capillary LC there are currently two methods of sample introduction, namely with[7] and without[2,9,13] sample splitting. Although direct introduction techniques, such as the "micro-feeder method"[2] and the "short stainless-steel capillary method"[9,13], in which the sample solution is introduced and then placed on a PTFE adaptor fixed on a capillary column head, have become important in the separation of micro-samples, split sample introduction is simpler if the amount of sample available is of the order of milligrams. With direct sample introduction about 0.01–0.3 μl of sample solution is handled whereas with split sample introduction about 1–10 μl are used, so that in the former instance one should carry out the procedure very carefully.

The conditions of split sample introduction were examined. After an adequate pressure in the pump, which was operated in the constant pressure mode, had been set for a chromatographic run, the fine metering valve was used to adjust the splitting ratio by observing the discard flow-rate, by counting the number of droplets per second. This flow-rate is the key to setting the splitting ratio, which has a range from several hundredths to some thousandths. Then the flow of eluent was stopped by using the six-way valve (as a stop valve). The sample solution (1–10 μl) was introduced above the capillary column head by using a micro-syringe, the needle end of which should be placed between 2 and 8 mm from the capillary column head. It is essential that no air bubbles enter region 1 in Fig. 1, because they would cause broadening of the sample band or would give false results owing to the mixing action of the air bubbles to the sample. After splitting the sample and cleaning up region 1, the discard flow is stopped by operating valve 6.

Typical examples obtained by split injection are shown in Fig. 2. Chromatogram A was obtained by the sample introduction without the discard flow and B was obtained with the normal procedure. In A and B, bare capillary tubing was used, but in C, D and E coated columns were used. It is desirable that sample should be introduced in as narrow a band as possible. In other words, a sharp solvent peak should be observed. This is dependent on both the splitting ratio and the geometrical design of region 1 in Fig. 1. C and D in Fig. 2 show examples of the latter effect. The diameters of region 1 in Fig. 1 were 1.7 mm (C) and 0.8 mm (D); a smaller tube I.D. is more favourable. E shows a typical example of "double injection". After a sharp

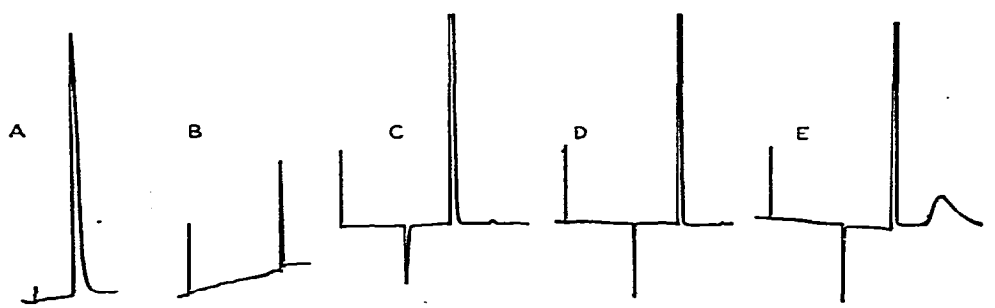

Fig. 2. Chromatograms obtained with split sample introduction. (B) Obtained by operating with normal procedure and (A) without discard flow; (C) was obtained by using 1.7 mm I.D. tube and (D) 0.8 mm I.D. tube at 1 in Fig. 1; (E) is a typical example of "double injection".

solute peak had appeared, it was followed by a second broad peak due to the same solute. This effect occurred when the discard flow was stopped too fast to clean up region 1 in Fig. 1. If the correct procedure is followed, split sample introduction is very easy and the reproducibility is good.

Calculation of thickness of stationary phase film

Knox and Gilbert[10] calculated the thickness of an ODS stationary phase film in a 60 μm I.D. capillary column that we had used in previous work[2]. They estimated the thickness of the ODS film by using H values given by eqn. 2 and experimental values, and obtained a value of 17 μm. This value might be over-estimated for a 60 μm I.D. capillary column, because the residual "space" for the mobile phase is only 16 μm. Here the thickness of the stationary phase film, d_f, is calculated by using the difference between two H values for retained solutes with k' values greater than 1. We must be careful to treat the apparent H value, because it always includes a contribution from the external column parts. From the discussion of the effect of the external column volume on the apparent column efficiency, there is no addition rule, such as $H = H_c + H_{ext}$. However, if k' for a certain solute is greater than one, f_{ext} becomes negligibly small compared to f_c, as shown in Table I. Thus H reduces to H_c in this instance. Using eqn. 2, ΔH becomes

$$\Delta H = (H_{m,1} - H_{m,2}) + (H_{s,1} - H_{s,2}) \tag{11}$$

where subscripts 1 and 2 represent different solutes. The thickness of the stationary phase film was calculated using the following column. A 22.5 m × 33 μm I.D. capillary column was coated with OPN by the dynamic coating method using 20% OPN–dichloromethane solution, at a coating rate of *ca.* 0.8 cm/sec. *n*-Hexane saturated with OPN was used as the mobile phase. Aniline ($k' = 1.07$) and β-naphthylamine ($k' = 2.11$) were used as solutes. The diffusion coefficients D_m and D_s were derived mainly from Othermer's equation[14,15] by using physical data such as viscosity, boiling point, heat of vaporization and molar volume. The mobile phase was assumed to be pure *n*-hexane in the calculation of D_m. The calculated diffusion coefficients at 20°C are as follows: aniline in *n*-hexane, $2.61 \cdot 10^{-5}$; β-naphthylamine in *n*-hexane, $2.07 \cdot 10^{-5}$; aniline in OPN, $8.91 \cdot 10^{-7}$; and β-naphthylamine in OPN, $7.07 \cdot 10^{-7}$ cm^2/sec.

H versus u relationships are shown in Fig. 3. The relationship between ΔH ($H_{\beta\text{-naphthylamine}} - H_{\text{aniline}}$) and linear velocity is first order, as predicted by eqn. 11 and shown in Fig. 4. The four diffusion coefficients given above were put in eqn. 11, and the calculated d_f value is 1.6 μm. This value is calculated under the assumption that the liquid film fixed on the inner glass wall is a complete, uniform layer. However, in practice the layer might be not uniform, so it should include a configuration factor, q, which is assumed to be larger than 1. The actual average thickness of the layer, d_f', might then be $d_f' = d_f q^{-1}$. Hence the film thickness of OPN in this instance would be 1.6 μm or less. This value corresponds well with the film thickness on capillary columns in gas chromatography, which lies in the range 0.2–2 μm[16]. Comparing the calculation for ODS by Knox and Gilbert with the present calculation, there is a great difference in the estimation of D_m/D_s, which is 1.7 in the former instance and 29 in the present work. This is one of the reasons why their calculated

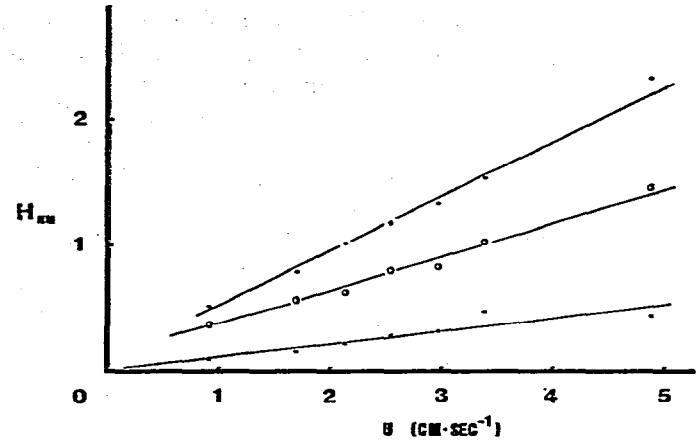

Fig. 3. Plate height *versus* linear velocity for aniline (○), β-naphthylamine (+) and isooctane (■) on OPN column, 22.5 m × 33 μm I.D. Mobile phase: *n*-hexane saturated with OPN; $k' = 1.07$ for aniline and 2.11 for β-naphthylamine; solvent for sample solution, isooctane.

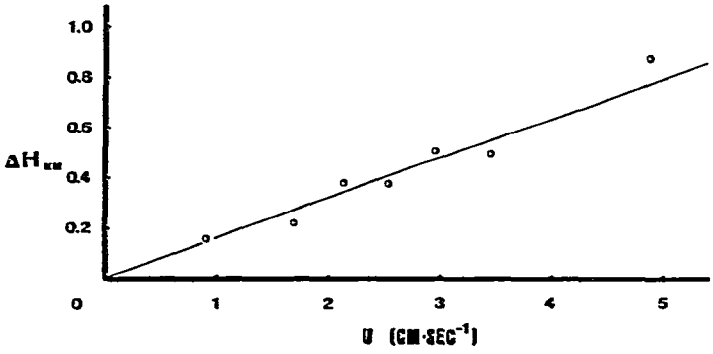

Fig. 4. Difference between H for β-naphthylamine and aniline *versus* linear velocity.

value of a 17 μm film thickness for ODS in a 60 μm I.D. capillary column is far greater than our value. Although a thinner stationary phase film might be better in liquid chromatography, a value of 1.6 μm would be reasonable with the current state of the art.

Capillary column with thick stationary phase

One of the problems in OTLC is that the capacity factor is relatively small: the largest value that we have obtained in previous work[2–5] is *ca.* 3. It is necessary to have a value of 10 for the analysis of complex mixtures. This purpose can be achieved in two ways: either by increasing the thickness of the stationary phase film or by increasing the surface area of the inner glass wall, for example making whiskers and/or treating with sodium hydroxide solution. The latter method at present does not generally give columns with larger k' values. In the former method, the drawback

is that H_s increases in proportion to the square of d_f. Therefore OPN was selected because its D_s value is comparatively large owing to its low viscosity. Capillary tubes of length 11 m and I.D. 40 μm were coated by the dynamic coating method using OPN–dichloromethane solutions with OPN concentrations varying from 10 to 70% (v/v).

The concentration of OPN in the plug has a linear first-order relationship with the k' value of dimethylphenols with an intercept of zero. In the column obtained by using 70% OPN–dichloromethane, the k' value obtained was 16, which is the highest attained in OTLC. At 20% OPN the k' value was nearly twice that at 10% OPN, and at 70% OPN the plug was viscous. Hence it is preferable to use a 20–50% OPN concentration. An example is shown in Fig. 5, in which the chromatographic pattern is nearly identical with that obtained by using a normal packed column of 4.6 mm I.D. The retention time of the solvent peak is reasonably short compared with the retention times of the components of interest.

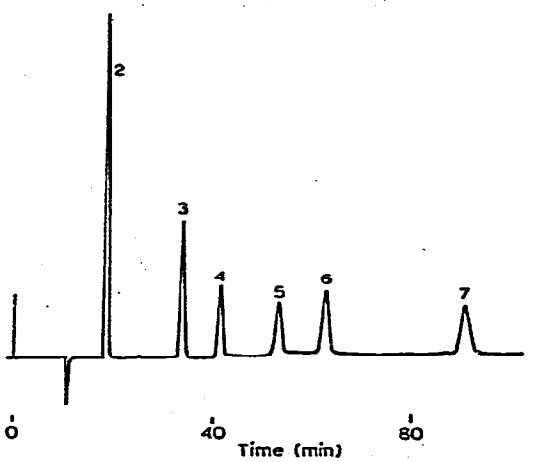

Fig. 5. Chromatograms of dimethylphenols and *m*-cresol. Column: 11 m × 40 μm I.D. coated with OPN; mobile phase, *n*-hexane saturated with OPN; inlet pressure (P_i), 10 atm; UV detection, 280 nm. Peaks: 1 = isooctane; 2 = 2,6-; 3 = 2,4-; 4 = 2,3-; 5 = 3,5-; 6 = 3,4-dimethylphenol; 7 = *m*-cresol.

Chromatograms obtained with columns of 30–40 μm I.D.

Three typical chromatograms are shown in Figs. 6–8. Although a narrower capillary column is preferable, there are technical problems in treating those of I.D. less than 30 μm, related mainly to the detector volume and connecting parts. The current design of the external column system is acceptable for a capillary column of I.D. 30 μm and length 30 m when the k' value of the solute is greater than 1, as shown in Table I.

The linear velocities in Figs. 6, 7 and 8 were 9, 1.9 and 3.6 mm/sec, respectively, which are 3–15 times greater than the value of 0.6 mm/sec, which would be obtained by applying a flow-rate of 0.5 ml/min to a column of I.D. 4.6 mm and length 25 cm.

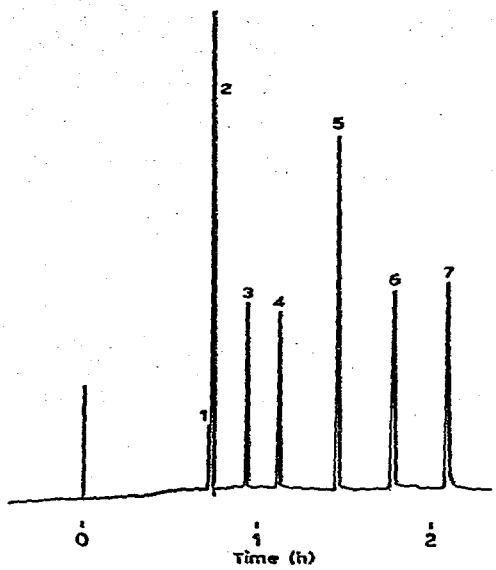

Fig. 6. Chromatogram of aromatic amines. Column: 23 m × 33 μm I.D., coated with OPN; mobile phase, as in Fig. 5; P_i, 20 atm; UV detection, 235 nm. Peaks: 1 = isooctane; 2 = N,N-dimethylaniline; 3 = N-phenyl-α-; 4 = N-phenyl-β-naphthylamine; 5 = aniline; 6 = α-; 7 = β-naphthylamine. *N* for the last peak is 85,000.

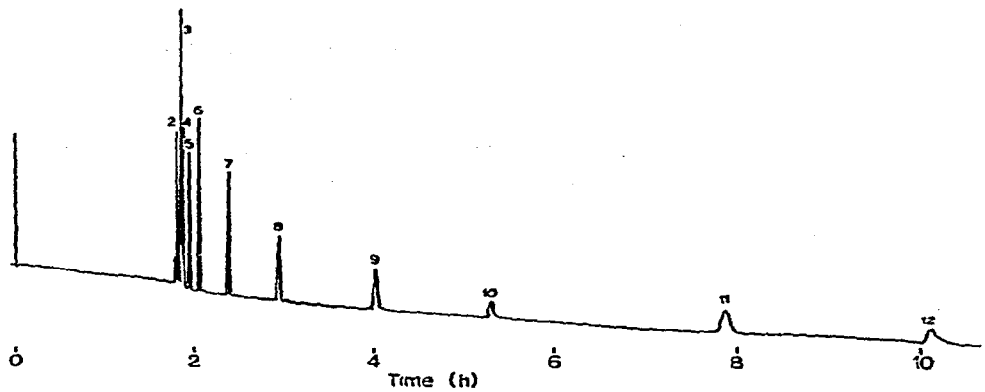

Fig. 7. Chromatogram obtained at an inlet pressure of 1.2 atm. Column: 12.4 m × 42 μm I.D., coated with TCP; mobile phase, *n*-hexane saturated with TCP; UV detection, 235 nm. Peaks: 1 = isooctane; 2 = N,N-diethyl-; 3 = N,N-dimethyl-; 4 = N-butyl-; 5 = N-propyl-; 6 = N-ethyl-; 7 = N-methylaniline; 8–12 = 3–7 in Fig. 6 in the same elution order.

The plate numbers of the last peaks in Figs. 6 and 8 are about 85,000 and 160,000, respectively. These high numbers would indicate the potential ability of OTLC.

The inlet pressure in Fig. 7 was only 1.2 atm, which is extremely low owing to the high permeability of open-tubular microcapillary columns.

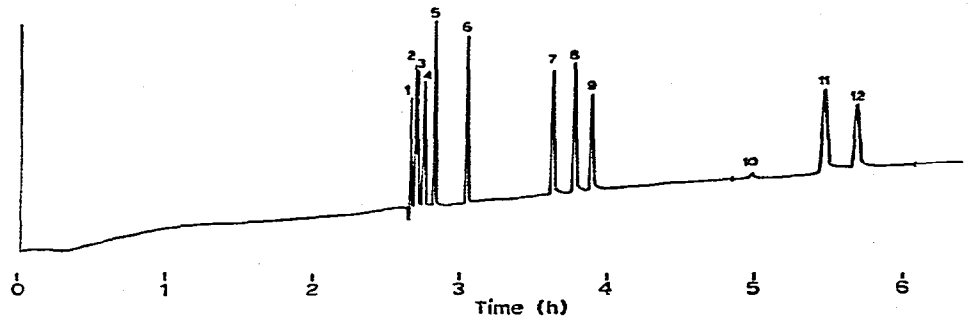

Fig. 8. Chromatogram of substituted anilines. Column: 34.6 m × 28.5 μm I.D., coated with OPN; mobile phase, as in Fig. 5; P_i, 10 atm; UV detection, 235 nm. Peaks: 1–6 = as in Fig. 7; 7 = *o*-; 8 = *p*-; 9 = *m*-toluidine; 10 = unknown; 11 = *m*-chloro-; 12 = *p*-chloroaniline. The last peak has a plate number of 160,000.

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