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EFFECT OF MOBILE PHASE PRE-HEATING ON HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMN PERFORMANCE: A NEW TYPE OF COLUMN THERMOSTAT

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SUMMARY

The use of conventional air-bath thermostats as a means of controlling column temperature in high-performance liquid chromatography is limited to a relatively narrow temperature range if full column efficiency is to be maintained. Particularly at high air-bath temperatures, large axial temperature gradients in the column occur, which are detrimental to the column performance. This was demonstrated by both temperature measurements in the column and determination of column performance.

The design of a new type of column thermostat, utilizing a highly efficient, small-volume mobile phase pre-heater, is described. Suitable adjustment of the heat transfer in the mobile phase heater and air heater result in nearly identical temperatures of the mobile phase when entering the column and of the air flowing around the column. Thus axial and radial temperature gradients in the column are minimized, as temperature measurements in the column bed indicate, leading to a marked improvement of column performance at elevated temperatures.

INTRODUCTION

Column temperature has become increasingly accepted as a separation parameter in high-performance liquid chromatography (HPLC). Consequently, the means of maintaining a constant column temperature is now also considered to be an important aspect of HPLC instrument design.

The influence of temperature on the capacity factor (k') can be significant; *e.g.*, in reversed-phase HPLC changes in k' of 2.5–3% per degree of temperature change can be commonly observed. As the magnitude of this effect is also dependent on the substances to be separated, column temperature can be an important means of optimizing separation selectivity. Chmielowiec and Sawatzky¹ published retention data for polynuclear aromatic hydrocarbons (PAHs) showing reversal of the elution order in some instances when the temperature was changed by 10–55°C.

The influence of temperature on column efficiency (plate height) is mainly determined by the relationship between temperature and the diffusion coefficient of the solutes in the mobile phase, and between temperature and the solute mass transfer

in the mobile and stationary phases. According to Melander and Horváth², the plate height in reversed-phase HPLC may decrease by 30% if the column temperature is increased from 15 to 72°C.

A secondary effect of working at elevated column temperature is a reduction in column pressure due to a decrease in mobile phase viscosity. This may become of interest if large plate numbers are required, and the upper pressure limit of the pumping system limits the column length.

In many commercial HPLC instruments, a constant column temperature is achieved by putting the column into a temperature-controlled "air-bath". It is to be expected that insufficient heat transfer from air to the column wall and through the column wall into the mobile phase will lead to considerable axial and radial temperature gradients at temperatures different from ambient if the mobile phase enters the column at ambient temperature. For example, heating water at 4 ml/min from 20 to 80°C would require a heat transfer of 17 J/sec. As heat transfer from air to the column wall and to the mobile phase will depend on various parameters, such as air flow (velocity, direction) and column walls (surface, thickness, material), comparability of analytical results between laboratories and between different types of instrument would be difficult for data measured at elevated column temperatures. Also, it is expected that axial and radial temperature gradients in the column would impair the column efficiency and peak profiles^{3,4}.

It was the aim of this work (a) to develop a concept for HPLC column thermostating that would ensure well defined temperatures and an even temperature distribution in the column bed, with particular attention to safety aspects, and (b) to provide some comparative data on temperature distribution in the column and on separation efficiency for classical air thermostating *versus* the suggested concept.

DESIGN CONSIDERATIONS FOR AN HPLC COLUMN THERMOSTAT

A useful solution for HPLC column thermostating should meet the following requirements:

(a) axial and radial temperature gradients in the column bed should not exceed 1–2°C (influence on column efficiency and symmetry);

(b) variation of the column flow-rate in the range 0–5 ml/min must not result in temperature changes in the column bed of more than 1–2°C (influence on k');

(c) injection of large sample volumes must not cause (temporary) temperature instabilities in the column bed of more than 1–2°C; and

(d) no part in a column thermostat must exceed a temperature of 180°C (the spontaneous ignition temperature of diethyl ether is 186°C and that of *n*-heptane is 247°C).

Pre-heating of the mobile phase before it enters the column, in order to avoid too large temperature gradients in the column bed, has been suggested previously^{4,5}. However, the volume of such "pre-heater columns" necessitates their use upstream of the sample injector. Hence a decrease in the temperature of the mobile phase may result from the injection device, which, as it is not desirable to heat parts of the injector because this could impair reliability, is usually at room temperature. Injection of large sample volumes would result in a temporary disturbance of the temperature equilibrium in the column.

This work was therefore concentrated on investigating the possibilities of designing a small-volume heat exchanger (volume less than $2 \mu\text{l}$) which could be used between the injector and the column. Heat transfer from steel capillary walls into liquid flowing through the capillary at flow-rates typical of analytical HPLC (up to 5 ml/min) was systematically studied for capillary inner diameters from 0.10 to 0.25 mm. In comparing the results of temperature measurements with those of heat transfer calculations, it was found that for I.D. < 0.20 mm heat transfer equations for turbulent flow had to be used to match the experimental results. This was surprising because, according to the Reynolds numbers at the flow-rates applied, laminar flow profiles were expected. These results are interpreted such that at least in short capillary tubes (less than 20 cm) of I.D. < 0.20 mm radial mass transfer similar to that in turbulent flow profiles occurs at even small Reynolds numbers. Fig. 1 shows temperature variations in water flowing through a capillary tube of I.D. 0.13 mm, calculated with equations valid for turbulent flow. Frictional heat was neglected in these calculations, as its influence is very small. Temperature measurements in the water at the outlet of the capillary matched the end-point of the plots (75 and 47°C) within $\pm 2^\circ\text{C}$. The temperature of the (external) wall surface of the capillary tube was maintained by sand casting it into an aluminium block, together with a steel-sheathed heating element with a large contact area.

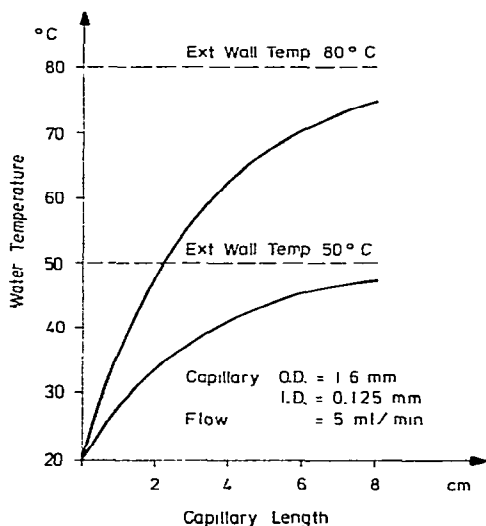


Fig. 1. Calculated temperature increases over the length of a 0.13 mm I.D. capillary for water at a flow-rate of 5 ml/min.

The next task was to ensure near identical temperatures of the column environment (and thus of the column walls) and of the mobile phase liquid at the outlet of the pre-heater capillary. This was achieved by designing the capillary heating block to function simultaneously as the air heater of an air thermostat (column oven). Hence its surface was shaped such that recirculated air flowing across it at a given velocity and surface temperature would be heated (or cooled) enough just to replace its heat loss through the oven walls, and that air temperature and the mobile phase temperature (when entering the column) would be near identical. Fig. 2 illustrates this for

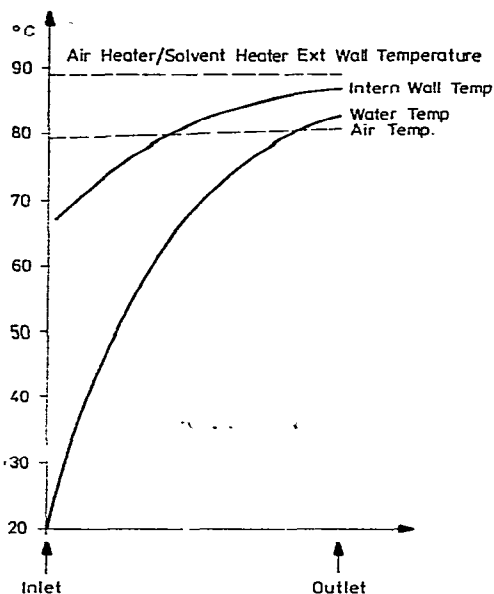


Fig. 2. Calculated temperature profiles over a longitudinal view of the combined solvent and air heater. Water temperature and capillary internal wall surface temperature over capillary length calculated for a flow-rate of 5 ml/min.

certain operating conditions. The surface temperature of this combined solvent-air heater is measured and controlled by a suitable device.

If a stainless-steel tube of suitable dimensions is also die cast into the aluminum block (parallel to the heating element) for circulating cooling fluid, temperature control of the solvent and the column can be extended to ambient and sub-ambient temperatures.

EXPERIMENTAL

Apparatus

Liquid Chromatograph. A Hewlett-Packard 1081B with an automatic injector and UV detector, connected to a 3356 data system, was used.

Columns. A 200×4.6 mm RP-8 ($10 \mu\text{m}$) column was used for temperature measurements in the column bed, and a 100×4.6 mm I.D. ODS Hypersil ($5 \mu\text{m}$) column for chromatographic tests, both supplied by Hewlett-Packard (Waldbronn, G.F.R.).

Temperature sensors. Philips Thermocoax 2ABAC 025 steel-sheathed thermocouples of 0.25 mm O.D. were used for temperature measurements in the column, and a Heraeus WS011 surface resistance thermometer for surface temperature measurements.

Materials

Solvents. Water was used for temperature measurements and acetonitrile-water (70:30) for chromatographic tests.

Samples. Aniline, benzo(a)anthracene and 1,2,5,6-dibenzanthracene were dissolved in acetonitrile-water (70:30).

Methods

For temperature measurements in the column bed two steel-sheathed thermocouples, of 0.25 mm O.D., were inserted 1.5 cm deep into the centre of either column end by means of T-junctions with PTFE compression seals. A third sensor was fixed to the outer surface of the column wall to measure the air temperature in the immediate column environment.

Comparative measurements of column performance in an air thermostat (without any solvent pre-heater) *versus* the new solvent + column thermostat (as described in previous section) were performed at room temperature and at temperature settings of 40, 50, 60, 70 and 80°C and at mobile phase velocities of 0.13–0.26 and 0.52 cm/sec. In order to avoid the influence of slow column degradation (a *ca.* 25% decrease in plate number was observed over the measurement duration of 2 weeks) on comparative results, measurements were carried out in the sequence given in Table I. Equilibration times of 1 h and 20 min were allowed after each change of temperature setting and of flow setting, respectively.

Retention times (t_R), capacity factors (k'), theoretical plate numbers (N) and symmetry numbers (S) were determined as follows:

t_R = time at peak maximum – time at injection;

$k' = \frac{t_R - t_0}{t_0}$; $t_0 = \frac{V_0}{\text{flow-rate}}$; $V_0 = \epsilon_t \cdot V_{\text{column}}$ (ϵ_t obtained from Rozing⁶, who measured it according to ref. 7.

$$N = 4 \left(\frac{t_R}{W_{0.607}} \right)^2 ; W_{0.607} = \text{peak width at 60.7\% of peak height};$$

$$S = \frac{W_{b0.1}}{W_{f0.1}} ; W_{b0.1} = \text{width of back part of peak}; W_{f0.1} = \text{width of front part}$$

of peak at 10% of peak height.

Each measurement was repeated five times and the average value and relative standard deviation were calculated. Typical values for the relative standard deviation were 0.5% in retention time measurements, 1% in plate number measurements and 4% in symmetry number measurements.

RESULTS AND DISCUSSION

Temperature measurements in the column

As expected, large temperature differences are measured between the inlet, outlet (1.5 cm deep from either end of the column bed) and wall environment of an air-thermostatted column operated at elevated temperatures and flow-rates (Fig. 3). Surprisingly, considerable temperature changes were measured at the column outlet when the flow-rate was changed. Both effects result in axial and radial temperature gradients. When the same column and temperature sensor arrangement is installed in the solvent + column thermostat, the measured temperature differences in the column are reduced to 1.5°C or less (Fig. 4). This confirms that the new combined

TABLE I
MEASUREMENT SEQUENCE

Temperature (°C)	40	50	60	70	80	40	Room (23)
Flow-rate (cm/sec)	0.13/0.26/0.52	0.13/0.26/0.52	0.13/0.26/0.52	0.13/0.26/0.52	0.13/0.26/0.52	0.13/0.26/0.52	0.13/0.26/0.52
Air thermostat	●	●	●	●	●	●	●
Solvent + column	●	●	●	●	●	●	●
thermostat	●	●	●	●	●	●	●

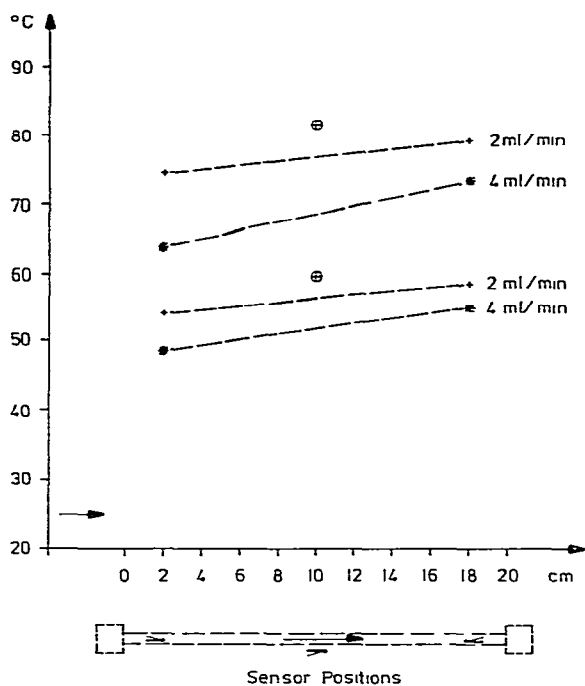


Fig. 3. Temperatures measured in the column bed, 1.5 cm deep from the column inlet and outlet, and in air close to the column wall. Column operated by an air thermostat heating only the column wall. Column: 200×4.6 mm I.D. RP-8 ($10 \mu\text{m}$). Solvent: water.

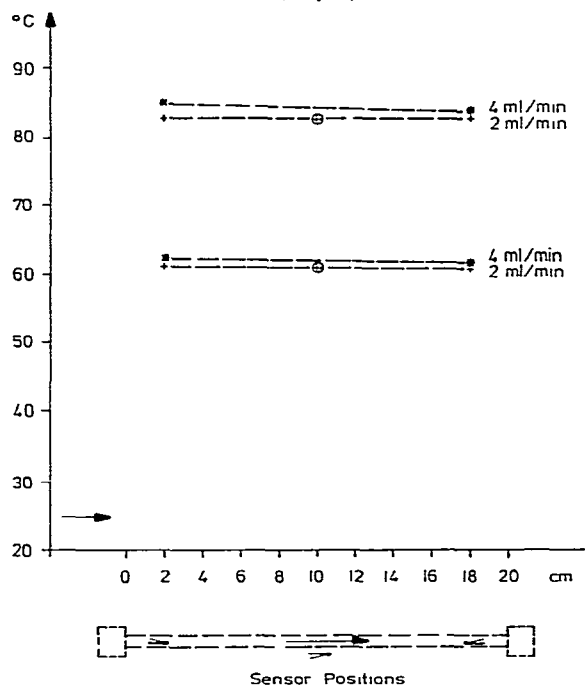


Fig. 4. Temperature measured in the column bed, 1.5 cm deep from the column inlet and outlet, and in air close to the column wall. Column operated by the combined solvent + column thermostat.

solvent + column heater behaves as predicted from the calculated temperature plots presented in Figs. 1 and 2.

Chromatographic tests

After temperature measurements had confirmed the substantial influence of the method of column heating on the temperature distribution in the column bed, the relevance for chromatography was investigated. Column efficiency, peak symmetry and k' were compared for both methods of column thermostating, as described under Experimental.

Fig. 5 shows percentage ratios of plate numbers under otherwise identical conditions in the air thermostat, relative to the combined solvent + column thermostat. Whereas at room temperature identical plate numbers are measured, at 40°C and 0.5 cm/sec a decrease in column efficiency of 30% in the air thermostat is observed, increasing to a 90% decrease at 80°C and 0.5 cm/sec (examples of chromatograms obtained in the two thermostats under these conditions are given in Fig. 8). This means that "incorrect" column thermostating can result in up to a 10-fold reduction of column efficiency.

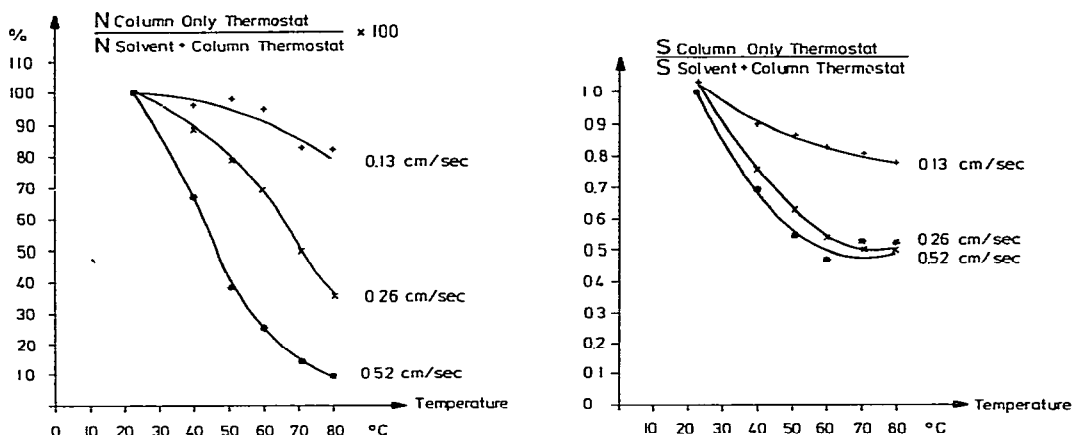


Fig. 5. Percentage ratio of plate numbers showing effects of "column only (air-) thermostat" against "solvent + column thermostat" at various mobile phase flow-rates, plotted against thermostat temperature. Column: 100 × 4.6 mm I.D. ODS Hypersil (5 μm). Solvent: acetonitrile-water (70:30). Solute: 1,2,5,6-dibenzanthracene.

Fig. 6. Ratio of symmetry numbers showing effects of "column only thermostat" against "solvent + column thermostat" at various mobile phase flow-rates, plotted against thermostat temperature. Conditions as in Fig. 5.

Fig. 6 shows the ratios of peak symmetries as observed in both types of thermostats; this plot indicates that under the applied chromatographic conditions, temperature gradients occurring in an air thermostatted column will cause a front-leading tendency of the peaks.

In Fig. 7 k' is plotted against flow-rate at various column temperatures for both thermostating methods. As expected from the results of temperature measurements in the column bed, air thermostating of the column leads to a considerable increase in k' with increasing flow-rate. Actually an "effective mean bed temperature" could be estimated from the k' values in comparison with those measured in the

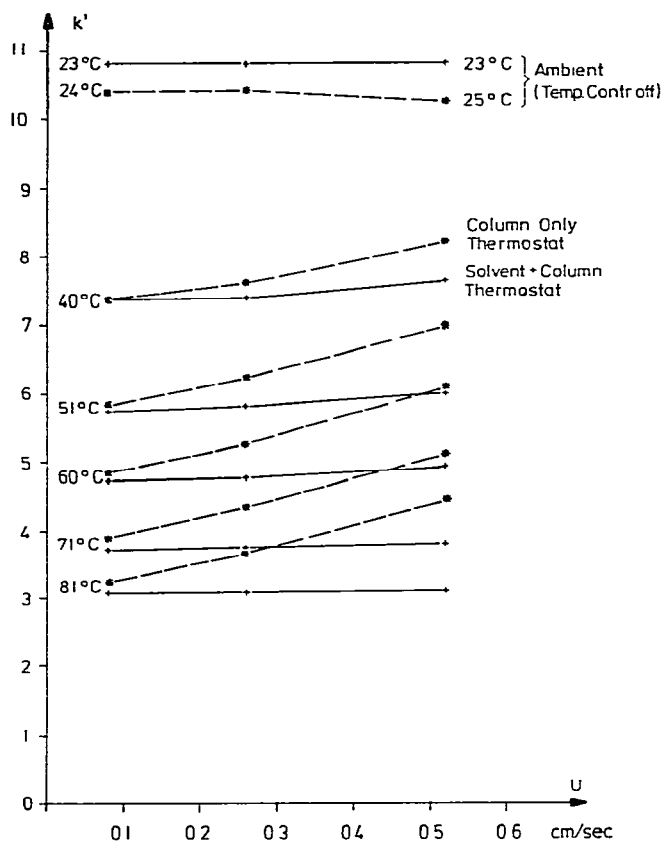


Fig. 7. Comparison of capacity factors showing effects of "column only thermostat" (*---*) against "solvent + column thermostat" (+——+) at various thermostat temperatures, plotted against mobile phase flow-rate. Conditions as in Fig. 5.

combined solvent + column thermostat: *e.g.*, at an air temperature of 81°C and a flow-rate of 0.52 cm/sec, k' in the air thermostat is about 4.45, and this value is reached in the solvent + column thermostat at about 66°C. The conclusion is that under these conditions the "effective mean column bed temperature" in the air thermostat is about 15°C lower than the air temperature around the column. This is consistent with the results of the temperature measurements shown in Fig. 3.

From the results presented here it appears obvious that effective use of the advantages that elevated temperatures would offer in many HPLC analyses can hardly be made in many of the column thermostats that are in use at present. A good column thermostat can save analysis time and improve detection limits, because at higher column temperatures lower plate heights (H) are usually observed and the optimum of the H - u curve shifts to higher mobile phase velocities (band broadening due to axial diffusion increases, but this is more than compensated for by the lower resistance to mass transfer at higher temperatures, while the Eddy diffusion should be little affected by temperature). In the 100-mm ODS Hypersil column, the minimum of the H - u curve was shifted from about 0.1 cm/sec at room temperature to about 0.3 cm/sec at 81°C. Plate heights at 0.5 cm/sec were reduced from about 27 μm at 23°C to about 22 μm at 81°C. These results, however, may be impaired by the influence of extra-column band broadening effects, which are not negligible when this chromato-

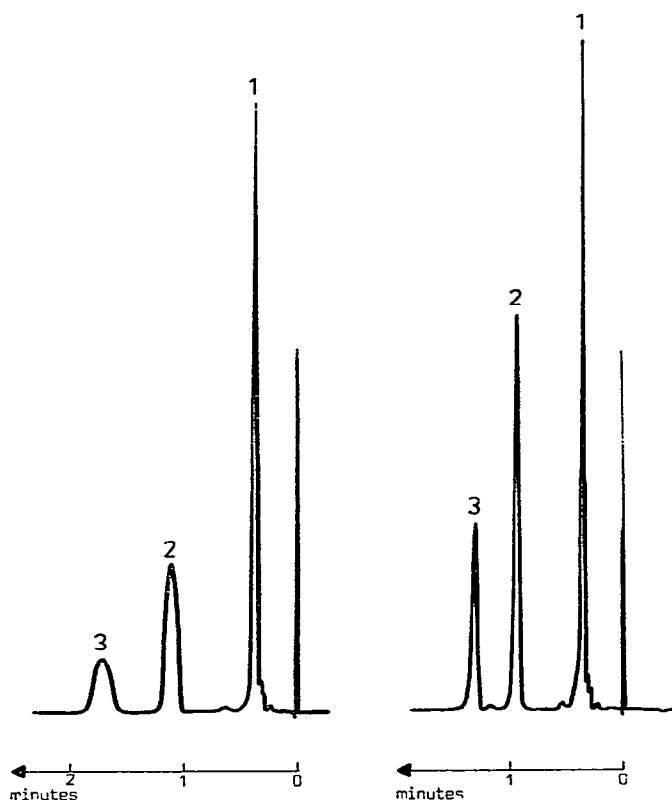


Fig. 8. Examples of chromatograms obtained from "column only thermostat" (left) and from "solvent + column thermostat" (right) under identical chromatographic conditions: Column: 100×4.6 mm I.D. ODS Hypersil ($5 \mu\text{m}$). Solvent: acetonitrile-water (70:30). Flow-rate: 3.8 ml/min. Sample: (1) aniline, (2) benzo(a)anthracene and (3) 1,2,5,6-dibenzanthracene dissolved in mobile phase. Injection volume: $5 \mu\text{l}$. Thermostat setting: 80°C . UV detector (254 nm), 0.128 absorbance unit full-scale. Chart speed: 1 in./min. Effects of column heating method are less marked on early peaks (k' near 0), partly owing to the influence of extra-column band broadening.

graphic equipment is used with small-volume columns packed with $5\text{-}\mu\text{m}$ material. Similar or better improvements of column efficiency with increasing temperature were reported by Herbut and Kowalczyk⁸ for adsorption chromatography. More detailed studies would be necessary to obtain a comprehensive picture of the potential that elevated column temperatures might have in optimizing the analysis time in routine HPLC analysis.

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