

CHROMSYMP. 760

## SOLVENT PROPERTIES AND THEIR EFFECTS ON GRADIENT ELUTION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

### II. TEMPERATURE GRADIENTS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS

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#### SUMMARY

The effects of radial and axial temperature gradients in a high-performance liquid chromatographic column are demonstrated. Irregular peak shape is shown to be partially attributable to temperature gradients in the column. A model for temperature gradient shape is developed. The frictional contribution is explicitly ignored in this preliminary work. The temperature gradient thus modelled is used as an operator on the standard free energy of elution to model thermal distortion of an hypothetical Gaussian peak. The composition of the mobile phase, the magnitude and sign of the difference in temperature between the column wall and the center at the column inlet, the flow-rate, and column efficiency are taken into account.

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#### INTRODUCTION

In high-performance liquid chromatography (HPLC), if the mobile phase temperature is not the same as that of the column wall and packing, radial and axial temperature gradients will occur in the column. The presence of temperature gradients in a supposedly isothermal system can cause skewing or even splitting of peaks. The phenomenon has been observed by Gates and Weber<sup>1</sup> and is demonstrated in Figs. 1-4.

The separation shown in Fig. 1 was improved by controlling the temperature (raising it slightly above ambient). Further improvement in separation speed was obtained by raising the separation temperature to 50°C, but the peaks became distorted. Thermostatting the pre-column at 50°C pre-heated the mobile phase and pro-

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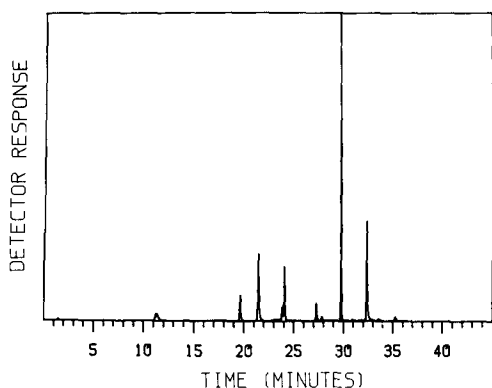


Fig. 1. Temperature induced distortion of peaks. Chromatograph: Beckman 332 (two Model 110A pumps, Model 420 controller). Column: Ultrasphere ODS, 250 mm  $\times$  4.6 mm I.D. Pre-column: Ultrapack ODS (5  $\mu$ m) 70 mm  $\times$  4.6 mm I.D. Mobile phase: 0.2 M phosphate buffer at pH 2.1-acetonitrile; non-linear gradient from 1 to 39% acetonitrile. Flow-rate: 1.5 ml/min. Detection: UV absorbance at 280 nm. Column temperature: ambient (approximately 22°C); no pre-column.

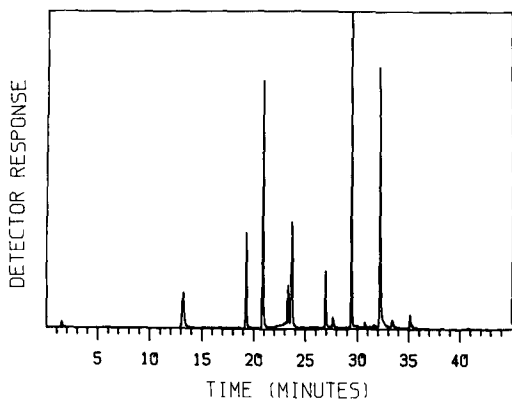


Fig. 2. Temperature induced distortion of peaks. Column temperature: 25°C; pre-column temperature: ambient (about 22°C). Other conditions as in Fig. 1.

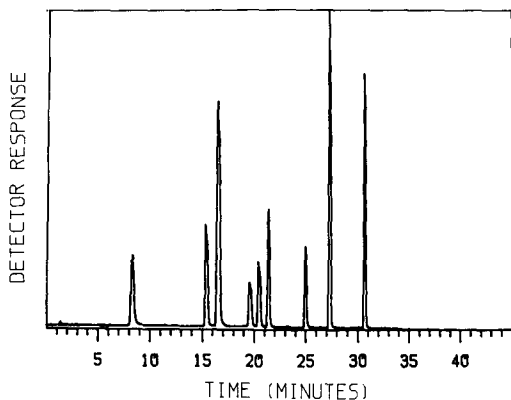


Fig. 3. Temperature induced distortion of peaks. Column temperature: 50°C; pre-column temperature: ambient (about 22°C). Other conditions as in Fig. 1.

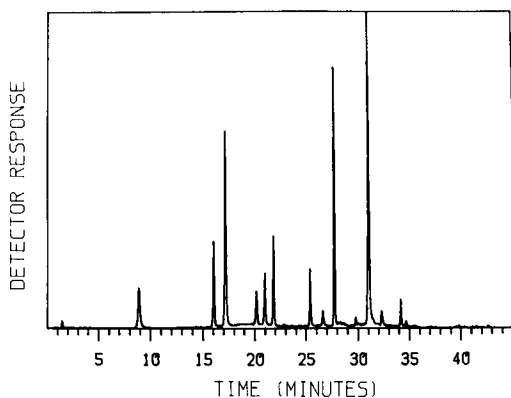


Fig. 4. Temperature induced distortion of peaks. Column and pre-column temperature: 50°C. Other conditions as in Fig. 1.

duced slightly longer retention times. Under this last set of conditions, the peaks were not distorted by axial temperature gradients.

#### MODEL OF TEMPERATURE GRADIENTS IN AN HPLC COLUMN

The magnitude of a temperature gradient in an analytical column can be modelled from knowledge of the column inlet temperature (the mobile phase inlet temperature), the column wall temperature (the temperature at which the column is thermostatted), the thermal conductivity of the mobile phase and the mean linear velocity of the mobile phase.

The radial temperature gradient can be calculated from the thermal conductivity of the mobile phase by calculating the time necessary to conduct sufficient heat from the wall to the center of the column to raise the temperature at the center of the column to that of the wall. Fourier's Law<sup>2</sup> provides:

$$dQ/dt = -TC \cdot A \cdot (dT/dr)$$

where  $dQ/dt$  is the time rate of heat flow from the wall to the center of the column,  $A$  is the area at right angles to the heat flow (the interior surface area of the column, since all thermal conductivity in which we have any interest will take place here).  $TC$  is the thermal conductivity of the mobile phase, and  $dT/dr$  is the temperature difference per unit radius of the column.

The mobile phase will have only a short residence time in any one length of the column. The residence time in a 1 mm axial segment can be calculated by dividing the column length by the mean linear velocity of the mobile phase:

$$t_{seg} = L_c/\mu$$

where  $t_{seg}$  is the segment residence time.  $L_c$  is the column length in mm, and  $\mu$  is the mobile phase mean linear velocity in mm/s. Consequently, the heating process begun in the first axial segment of the column will have to be continued in the second and

subsequent axial segments until the temperature at the column center is the same as that of the wall. Specifically neglecting frictional heating (it will be treated in another paper), we apply the Fourier equation to concentric radial cylindrical segments of the column.

Integrating the Fourier equation over 1 s, the heat which can be conducted into a 0.5 mm thick, 1.0 mm long segment of the column (the packing is assumed to have a negligible thermal conductivity) is given by:

$$Q = -TC \cdot A \cdot (T_{inlet} - T_{wall})/r$$

where  $T_{inlet}$  is the temperature of the mobile phase as it enters the segment,  $T_{wall}$  is the temperature at the exterior boundary of the segment,  $A$  is the exterior wall area of the segment, and  $r$  is the distance from the exterior boundary of the segment to the center of the column. The inlet temperature of the second and all subsequent axial segments must be calculated by dividing the segmental heat input,  $Q$ , by the segmental heat capacity. Segmental heat capacity is calculated by:

$$HC_s = \varepsilon \cdot \pi \cdot (r_o^2 - r_i^2) \cdot V_{formula} \cdot HC(X)$$

where  $HC_s$  is the heat capacity of a segment,  $\varepsilon$  is the void fraction (*i.e.* the fraction of each segment occupied by mobile phase, rather than packing),  $r_o$  is the exterior radius of the segment,  $r_i$  is the interior radius of the segment,  $L_s$  is the length of the segment,  $V_{formula}$  is the formula volume of the mobile phase and  $HC(X)$  is the formula heat capacity of the mixture at composition  $X$ . The void fraction,  $\varepsilon$ , is a function of the ratio of the particle size to the tubing diameter, and can usually be read from a graph or table<sup>3</sup>.

Thus the temperature of a segment is given by:

$$T_{seg} = Q/HC_s + T_{inlet}$$

and this temperature can be used to calculate the heat flux into subsequent radial and axial segments. The calculations are simplified by assuming no mixing between adjacent radial segments. The temperature of a segment has a limit, however, of the wall temperature, assuming that no frictional heating takes place. The results of typical calculations are shown in Fig. 5. The slope of the temperature gradient is more dependent on the flow-rate than on the composition. The length of column required to established thermal equilibrium can be more than half the length of a 3- $\mu$ m column (typically 75 mm).

In the light of the foregoing, it is no surprise that the shape of a peak is influenced by temperature gradients. If we assume that the efficiency of the column remains constant over a time band of approximately 2 min, it is possible to calculate the effect of various temperature gradients on the shape of a hypothetical peak. We postulate that this hypothetical peak will have Gaussian shape, and we accept the conventional definition of column efficiency:

$$n = (t'_R/\sigma)^2$$

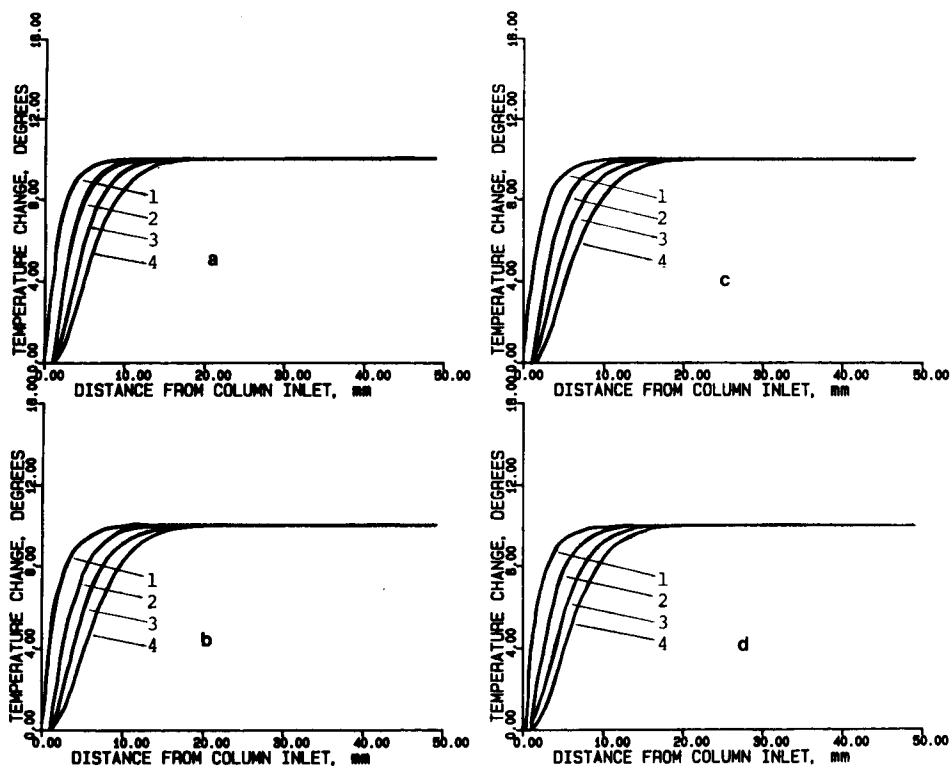


Fig. 5. Model of radial and axial temperature gradients in a 4.6 mm I.D. column. Mobile phase compositions: (a) water; (b) acetonitrile-water (25:75, v/v); (c) acetonitrile-water (50:50, v/v); (d) acetonitrile-water (75:25, v/v). Curve 1: 0.5 mm from column wall; curve 2: 1.0 mm from column wall; curve 3: 1.5 mm from column wall; curve 4: 2.0 mm from column wall. Flow-rate: 1.0 ml/min.  $T_{wall} - T_{inlet} = 10.0^{\circ}\text{C}$ .

where  $\sigma$  is the standard deviation of the peak and  $t'_R$  is the adjusted retention time of the peak. We then model the effect of temperature change on retention time by postulating a 2.5-min void time ( $t_m$ ) and calculating the standard free energy change of elution:

$$\Delta G = -RT \ln k$$

where  $R$  is the ideal gas constant,  $T$  is the thermodynamic temperature of the column,  $\Delta G$  is the standard free energy change of elution and  $k$  is the capacity factor:

$$k = (t_R - t_m)/t_m$$

We first calculate  $\Delta G$  for the isothermal condition at the column inlet temperature. Re-arranging and combining the above equations, we obtain:

$$t_R = [\exp(\Delta G/-RT)] \cdot t_m + t_m$$

Then  $t_R$  and  $\sigma$  are used as the input for the Gauss function, first solving for  $\sigma$ :

$$\sigma = t'_R/n^{1/2}$$

and then solving for  $y$  (response) as a function of  $t$  (time):

$$y(t) = \{1/[\sigma \cdot (2 \cdot \pi)^{1/2}]\} \cdot \exp\{[(t - t_R)/\sigma]^2/(-2)\}$$

The above scheme was implemented as a Fortran 77 program, plotting the results on an  $X$ - $Y$  plotter. The fate of the hypothetical peak depends on the shape of the temperature gradient and the efficiency of the column, as Figs. 6, 7 and 8 show.

## CONCLUSION

The insertion of terms for the heat capacity of the packing, for frictional heating and for the flow-rate gradient induced by the temperature gradient further complicates the issue. The latter two effects are the subjects of an improved model de-

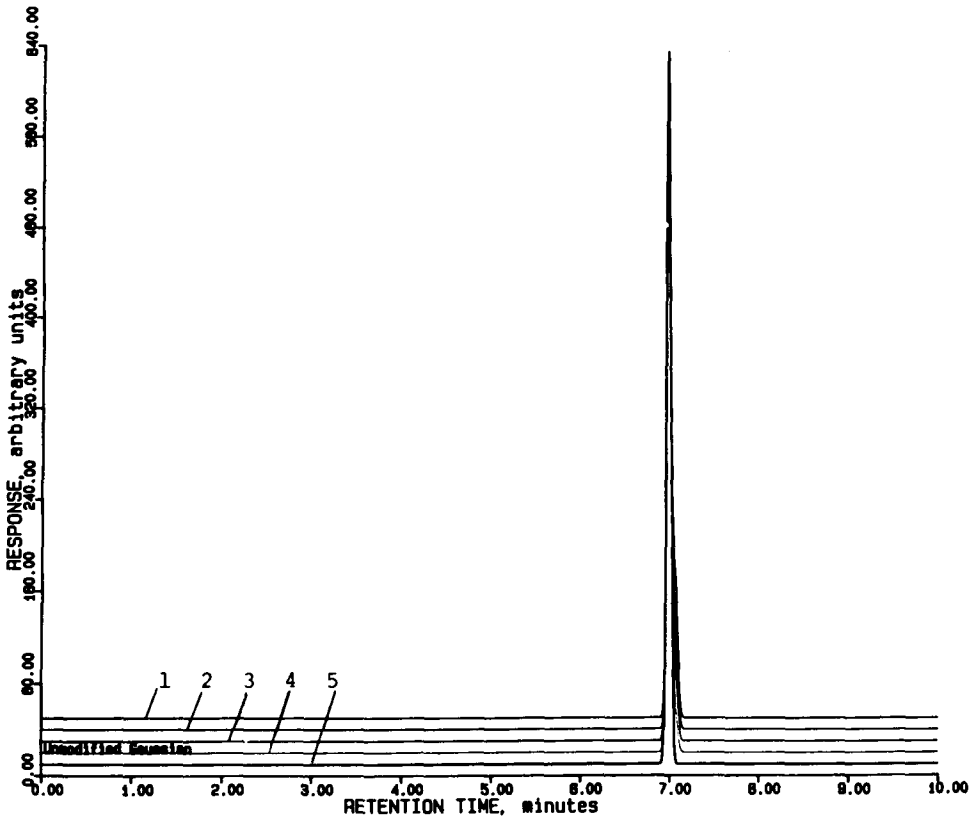


Fig. 6. Model of the influence of radial and axial temperature gradients on peak shape. Column efficiency:  $n = 12\,500$ . Curve 1: 0.5 mm from column wall; curve 2: 1.0 mm from column wall; curve 3: 1.5 mm from column wall; curve 4: 2.0 mm from column wall; curve 5: unmodified Gaussian. Column dimensions: 250 mm  $\times$  4.6 mm I.D. Flow-rate: 1.0 ml/min.  $T_{\text{wall}} - T_{\text{inter}} = 10.0^\circ\text{C}$ .

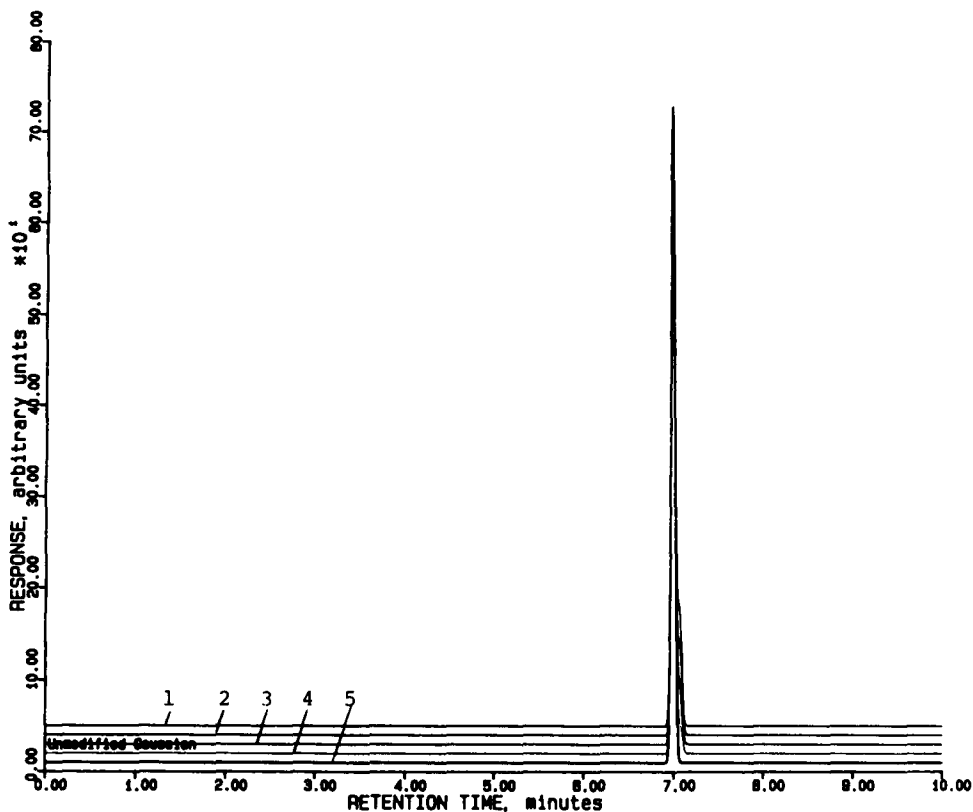


Fig. 7. Model of the influence of radial and axial temperature gradients on peak shape. Column efficiency:  $n = 16\ 500$ . Other conditions as in Fig. 6.

scribed in another paper<sup>4</sup>. Although the calculations shown here would not be undertaken routinely (preceded as they are by considerable effort to obtain heat capacities and thermal conductivities), they serve to emphasise the need for temperature control, not only for the column, but for the mobile phase before it enters the column. Obviously, better quantitative results will be obtained if the injector is thermostatted as well.

The need to thermostat the solvent reservoirs of the chromatograph is also clear. Because the thermal coefficients of expansion for water and acetonitrile are different, a change in the temperature of the solvents over time will produce a change in the polarity of an isocratic mixture, even though both solvents are at the same temperature. Mole fraction composition of the mobile phase is the proper term to use for purposes of discussing "polarity" or "solvent strength". The effect of changes in the temperatures of the supplied solvents upon mobile phase composition is shown in Table I.

Thermal distortion of chromatographic peaks has been demonstrated in a model which deals explicitly with wall temperature and mobile phase inlet temperature. The thermal conductivity of the mobile phase (computed in Part I<sup>5</sup>) and the thermal conductivity of the packing are taken into account in the model, in which

TABLE I  
VOLUMETRIC AND MOLE FRACTION COMPOSITION AT SEVERAL TEMPERATURES

Acetonitrile (mole fraction)	278.15 K			288.15 K			298.15 K			308.15 K		
	Formula density 278.15 K	Aceto- nitrile (%, v/v)	Change from 278.15 K (%)	Formula density 288.15 K	Aceto- nitrile (%, v/v)	Change from 278.15 K (%)	Formula density 298.15 K	Aceto- nitrile (%, v/v)	Change from 278.15 K (%)	Formula density 308.15 K	Aceto- nitrile (%, v/v)	Change from 278.15 K (%)
0	1.0000	0	0	0.9991	0	0	0.9971	0	0	0.9941	0	0
0.05	0.9881	13.07	1.05	0.9842	13.21	1.05	0.9779	13.35	2.12	0.9744	13.48	3.13
0.10	0.9725	24.10	0.91	0.9673	24.32	0.91	0.9586	24.54	1.84	0.9544	24.76	2.72
0.15	0.9557	33.52	0.80	0.9498	33.79	0.80	0.9399	34.06	1.61	0.9350	34.32	2.37
0.20	0.9390	41.67	0.70	0.9327	41.96	0.70	0.9223	42.26	1.41	0.9167	42.54	2.08
0.25	0.9232	48.79	0.61	0.9166	49.09	0.61	0.9062	49.39	1.24	0.8997	49.67	1.82
0.30	0.9087	55.05	0.54	0.9018	55.35	0.54	0.8915	55.65	1.08	0.8842	55.93	1.59
0.35	0.8957	60.61	0.47	0.8883	60.90	0.47	0.8782	61.19	0.95	0.8702	61.46	1.39
0.40	0.8841	65.58	0.41	0.8672	65.85	0.41	0.8662	66.12	0.83	0.8575	66.38	1.22
0.45	0.8737	70.04	0.36	0.8653	70.30	0.36	0.8552	70.55	0.72	0.8460	70.78	1.06
0.50	0.8642	74.08	0.31	0.8553	74.31	0.31	0.8451	74.54	0.62	0.8355	74.75	0.91
0.55	0.8553	77.74	0.27	0.8641	77.95	0.27	0.8357	78.16	0.53	0.8259	78.35	0.78
0.60	0.8471	81.08	0.23	0.8376	81.27	0.23	0.8270	81.45	0.45	0.8170	81.62	0.66
0.65	0.8392	84.15	0.19	0.8296	84.30	0.19	0.8187	84.46	0.38	0.8087	84.61	0.56
0.70	0.8318	86.96	0.16	0.8220	87.09	0.16	0.8111	87.23	0.31	0.8009	87.36	0.46
0.75	0.8248	89.55	0.12	0.8150	89.67	0.12	0.8040	89.78	0.25	0.7937	89.88	0.37
0.80	0.8184	91.96	0.10	0.8085	92.04	0.10	0.7975	92.13	0.19	0.7870	92.21	0.28
0.85	0.8125	94.18	0.07	0.8026	94.25	0.07	0.9716	94.31	0.14	0.7808	94.38	0.20
0.90	0.8074	96.26	0.04	0.7972	96.30	0.04	0.7863	96.34	0.09	0.7752	96.38	0.13
0.95	0.8026	98.19	0.02	0.7923	98.21	0.02	0.7813	98.23	0.04	0.7702	98.25	0.06
1.00	0.7980	100.00	0.00	0.7878	100.00	0.00	0.7767	100.00	0	0.7656	100.00	0



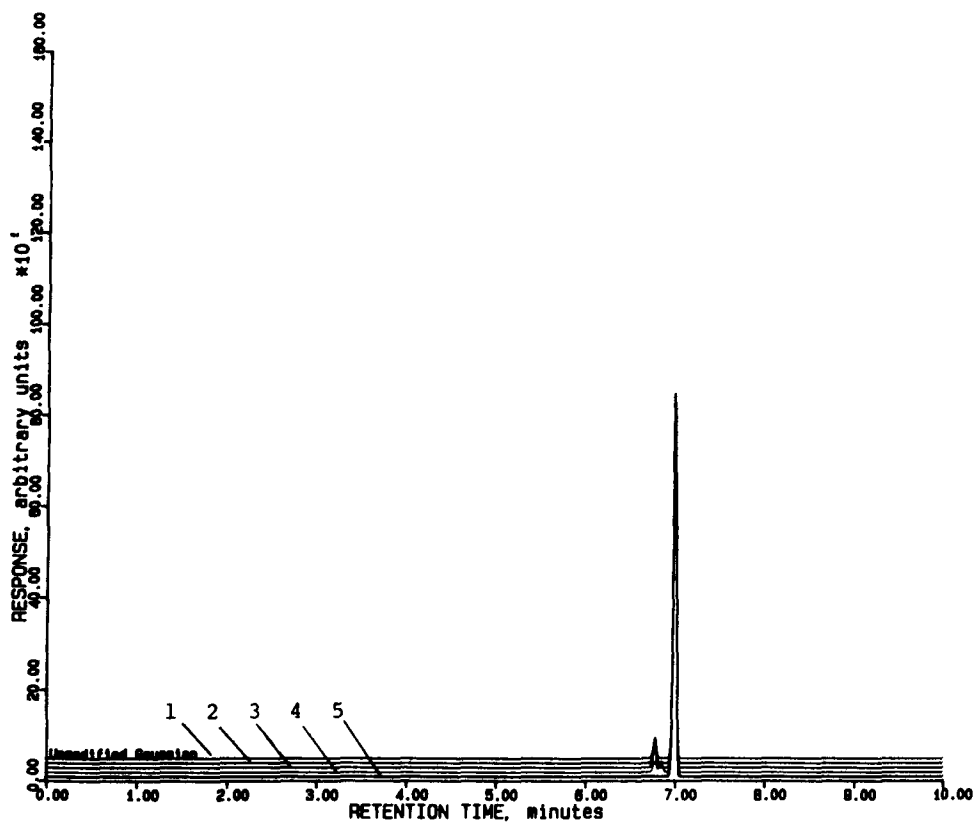


Fig. 8. Model of the influence of radial and axial temperature gradients on peak shape. Column efficiency:  $n = 22\,500$ .  $T_{\text{wall}} - T_{\text{inlet}} = -25.0^\circ\text{C}$ . Other conditions as in Fig. 6.

the column is divided into a series of concentric cylinders (radially). Each of the radial cylinders is divided axially into convenient short lengths. After the calculation of the temperature in each segment of each cylinder, each temperature is used as a variable in the equation for standard free energy change of elution, from which the adjusted retention time of a hypothetical Gaussian band was calculated. The formula for a Gaussian distribution is then applied, using  $t_R$  for the median of the distribution. The standard deviation of the distribution was calculated from the Gaussian column efficiency formula. Both leading-edge and trailing-edge distortion can be partially explained using this model.

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