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LIQUID CHROMATOGRAPHY WITH A DYNAMIC TEMPERATURE GRADIENT

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SUMMARY

The theoretical principles of chromatography using a dynamic temperature gradient are described in terms of aspects of liquid-solid systems. Along the chromatographic column, an oven moves in the direction of flow of the mobile phase and thus creates a temperature gradient on the column, the temperature of which decreases in the direction of flow of the mobile phase. Each component of the mixture under analysis has a characteristic temperature in the gradient at which it is eluted from the column.

The use of the dynamic temperature gradient has some advantages similar to those for the technique using a concentration gradient of the mobile phase (reduction in the time of analysis, linearization of the sorption isotherms, etc.). However, some additional advantages were found that can be used particularly in high-efficiency liquid chromatography. The composition of the mobile phase does not change and therefore the response of the detector can be used for the quantitative evaluation of the chromatogram. The column has the same adsorption properties after the analysis as before the application of the dynamic temperature gradient. An increase in the number of effective plates per second and in the peak capacity of the column were found, simultaneous with a decrease in the capacity ratio. The method can also be used for the enrichment of samples containing trace amounts of admixtures.

INTRODUCTION

The gradient technique has been used in liquid chromatography for several years and has proved to be particularly useful for separations of complex mixtures in liquid-solid systems and in ion-exchange chromatography. Its main use consists in applications of concentration gradients of the mobile phase¹. A gradient technique is used with the advantage if, owing to a large capacity ratio, broadened zones are obtained and, as a result of this, the sensitivity of the detection decreases, and in those instances when it is necessary to avoid the irreversible adsorption of the solute on the column or to reduce the time necessary for the analysis. The capacity ratio cannot only be decreased by means of the gradient technique but can also be controlled in such a way that the separation efficiency may be increased. Concentration gradients

of the mobile phase, particularly those in liquid–solid systems, change the properties of the stationary phase irreversibly in many instances. This disadvantage is especially important in high-efficiency liquid chromatography, when the preparation of a good column is usually time consuming and the material used is expensive.

The use of temperature gradients^{2–4} has, therefore, been developed recently. The fact that the adsorption properties of the stationary phase change simultaneously with the adsorption properties of the mobile phase leads to a more complicated situation than in systems in which the adsorption properties of the mobile phase can be neglected. On the other hand, if the systems and gradients are selected well, the capacity ratio, k , can be controlled, *e.g.*, by the use of a negative temperature programme^{2,5}.

The method involving the use of a dynamic temperature gradient^{6–8} is used in gas–solid systems, and some of its advantages can also be utilized in liquid–solid systems. The method consists in the application of a variable temperature gradient along the column so that the temperature decreases in the direction of flow of the mobile phase, and the temperature gradient is created on the column by an oven which also moves in the direction of flow of the mobile phase. The chromatographic profile thus obtained can be divided into two parts, the first consisting of the components that leave the column before the front of the gradient reaches the end of the column and the second consisting of the components that are desorbed from the column at a characteristic temperature, *i.e.*, in a certain small range of the temperature gradient on the column.

The dynamic temperature gradient therefore speeds up the analysis, permits the capacity ratio to be regulated and decreases the height equivalent to a theoretical plate (increases the peak capacity). The compounds that have non-linear isotherms of the Langmuir type in the isothermal process give symmetrical curves if the dynamic temperature gradient is applied. The method described above can also be used for the enrichment of samples that contain trace amounts of admixtures.

THEORETICAL

The chromatographic procedure using the dynamic temperature gradient is shown in Fig. 1. On the chromatographic column, with a total length, L , a temperature gradient is established over the column section, l . At the column inlet, *i.e.*, in the warmer part of the gradient, a temperature $T \rightarrow \infty$ is assumed, which practically means that such a temperature is reached that the sorption can be neglected of the component adsorbed most strongly from the chromatographic mixture at lower temperatures, *i.e.*, its retention volume $V_{Rl} = V_M$ (V_M is the dead volume of the column). At the cold end of the gradient, the temperature tends to the isothermal column temperature, $T = T_I$, at which the column section of length $L-l$ operates at the beginning of the experiment. The mobile phase flows through the column at a rate u ($\text{cm} \cdot \text{sec}^{-1}$) and the temperature gradient moves along the column at a rate w ($\text{cm} \cdot \text{sec}^{-1}$). The rate of movement of the temperature gradient is assumed to be sufficiently low (and the column diameter sufficiently small) that temperature equilibrium is established at each point of the column, *i.e.*, the temperature gradient keeps its character with respect to its length, l , and temperature, T . Two groups of substances

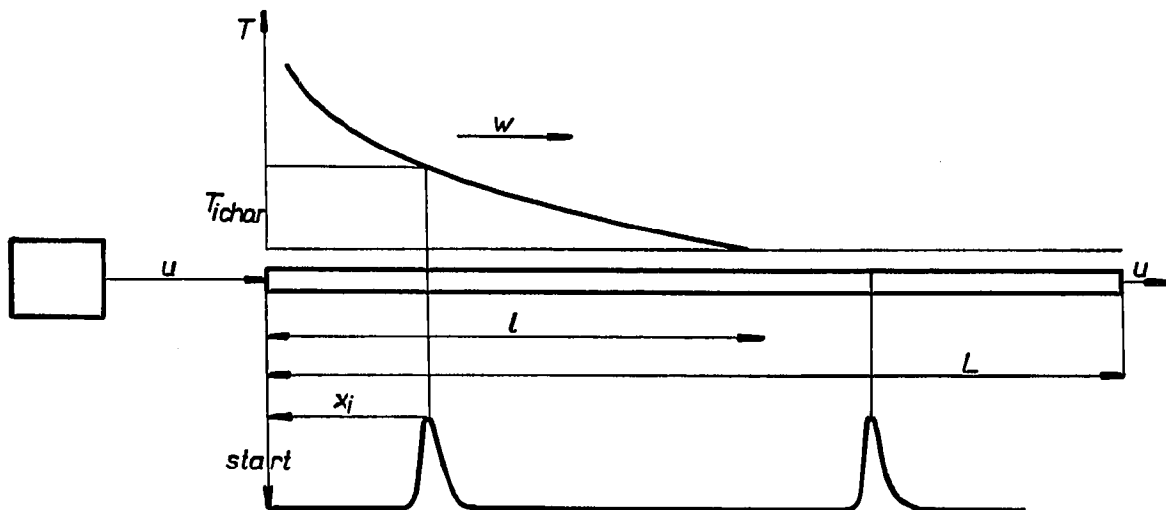


Fig. 1. Experimental arrangement using the dynamic temperature gradient.

exist under these circumstances in the mixture during the chromatographic process. The first group consists of components that proceed through the column more rapidly in comparison with the rate of movement of the temperature gradient, and their capacity ratio is represented by k_I . The other group consists of components whose translational speed in the column is identical with w . The capacity ratio of these components is represented by k_G , and $k_G > k_I$. The translational speed of the component i in the column is identical with the rate of movement of the gradient if there is a characteristic temperature, $T_{i \text{ char}}$, for which $t_{Ri} = L/w$. If a hyperbolic temperature distribution is assumed in the gradient, then the component i occurs in the gradient at a distance x_i from its origin, and

$$x_i = C/T_{i \text{ char}} \quad (1)$$

where C is a constant. The retention volume of the component being desorbed in the temperature gradient, V_{Ri} , is given by

$$V_{Ri} = V_M \cdot \frac{u}{w} \left(1 + \frac{l - x_i}{L} \right) \quad (2)$$

and the capacity ratio of the component i , k_i , is

$$k_i = \frac{V_{Ri} - V_M}{V_M} = \frac{u}{w} \cdot \frac{l - x_i}{L} + \frac{u}{w} - 1 \quad (3)$$

At the same time, the condition must be kept that $u > w$. The maximum capacity ratio that can be reached under the isothermal regime, $k_{I \text{ max.}}$, can be found for $x \rightarrow l$, i.e., for $T_{\text{char}} \rightarrow T_I$:

$$k_{I \text{ max.}} = \frac{u}{w} - 1 \quad (4)$$

The maximum capacity ratio of the components eluted in the gradient can be found for $x_i \rightarrow 0$ and is

$$k_{G \max.} = \frac{u}{w} \left(1 + \frac{l}{L}\right) - 1 \quad (5)$$

The dependence of the retention volumes, V_{Ri} , on the reciprocal value of the temperature was used for the calculation of the heat of adsorption. In order to derive the relationship expressing the dependence of the characteristic temperature on the values significant in the chromatographic method using the dynamic temperature gradient, the relationship was modified into the following form:

$$\ln k = \frac{Q}{RT} + \ln A \quad (6)$$

where the heat of adsorption, Q , is considered to be constant (linear dependence of $\ln V_R$ on $1/T$) in the temperature range applied to the temperature gradient used, A is a constant, R is the gas constant and T is the absolute temperature. By using eqns. 3 and 6, the relationship for the characteristic temperature is obtained in the form

$$T_{\text{char}} = \frac{Q}{R \ln A / \left[\frac{u}{w} \left(1 + \frac{l-x}{L}\right) - 1 \right]} \quad (7)$$

The broadening of the chromatographic peak eluted in the temperature gradient was characterized with the aid of the expression for the peak capacity⁹, n . The following well known relationship was used:

$$n = 1 + (N^\pm/m) \ln (V_n/V_1) \quad (8)$$

where N is the number of theoretical plates, m is a constant ($m = 4$ for a peak resolution of 4σ), V_n is the retention volume of the most retained component and V_1 is the retention volume of the less retained component. From eqn. 2 and conditions for eqns. 4 and 5, the following expression is obtained for the components eluted in the temperature gradient:

$$V_n = V_M \cdot \frac{u}{w} (1 + l/L) \quad (9)$$

or

$$V_1 = V_M \cdot \frac{u}{w} \quad (10)$$

By substituting $N = L/H$ and combining eqns. 8, 9 and 10, the following expression is obtained for the peak capacity, n_G , of the components eluted in the temperature gradient:

$$n_G = 1 + \frac{1}{m} \left(\frac{L}{H}\right)^\pm \ln \left(1 + \frac{l}{L}\right) \quad (11)$$

Similarly, for the components eluted in the isothermal regime before the temperature gradient (assuming that $V_{1I} = V_M$ and $V_{nI} = V_1$):

$$n_I = 1 + \frac{1}{m} \left(\frac{L}{H} \right)^{\frac{1}{2}} \ln \frac{u}{w} \quad (12)$$

The total peak capacity, n , is then the sum of both the contributions n_G and n_I :

$$n = n_G + n_I \quad (13)$$

EXPERIMENTAL

Sorbent

Silica gel CH (Lachema, Brno, Czechoslovakia) with a specific surface area of $395 \text{ m}^2 \cdot \text{g}^{-1}$ was used for the analytical and stabilization columns. It was dried prior to use for 4 h in a drying oven at 150°C . The fractions with grain sizes 50–63 and 71–100 μm were used for the preparation of the analytical and stabilization columns, respectively. After packing both columns, the silica gel was finally dried at 150°C in a stream of nitrogen.

Mobile phase

n-Heptane was used as the mobile phase and, prior to use, it was purified for the admixtures of aromatic hydrocarbons and water¹⁰ by pouring it through a column of neutral alumina (Reanal, Budapest, Hungary). For purifying 3 l of *n*-heptane, 600 g of alumina were used. Prior to use, the *n*-heptane was dried on molecular sieve 4A.

Solutes

Benzene for UV spectrometry, analytical grade naphthalene and zonally pure, fluoranthene were obtained from Lachema, Brno, Czechoslovakia, and anisole and analytical grade nitrobenzene from Chemapol, Prague, Czechoslovakia.

Instrumentation and procedure for measurements

A modified S 200 liquid chromatograph (Siemens, Karlsruhe, G.F.R.) was used for the measurements with the dynamic temperature gradient. A sampler with a needle guide and a stainless-steel column, 47 or 46 cm long and I.D. 2 mm, were used in place of the original sampler and column. A stabilization pre-column (50 cm long, I.D. 6 mm) packed with silica gel was inserted before the sampler and used for final drying of the mobile phase. A UV (254 nm) analyzer (Development Workshop, Czechoslovak Academy of Sciences, Prague, Czechoslovakia), in which the original cell was replaced with a quartz capillary with a volume of 27.2 μl and an optical path of 1 mm, was used for the detection of the components of the mixture under analysis. Isothermal measurements and the measurements with programmed temperature were carried out in the S 200 liquid chromatograph using the same columns as for the measurements with the dynamic temperature gradient.

The dynamic temperature gradient was established by means of an electrical oven that moved along the columns. One or two heating elements used for electrical soldering, fed from a regulating transformer, were used as the oven. The course of

the temperature in the dynamic gradient was measured by means of a double copper-Constantan thermocouple. One thermocouple joint was soldered on the column and the other was maintained at 0°C in an ice-water bath. An EZ 4 recorder (Laboratory Instruments, Prague, Czechoslovakia) was used for recording the voltage. The course of the temperature measured is shown in Fig. 2.

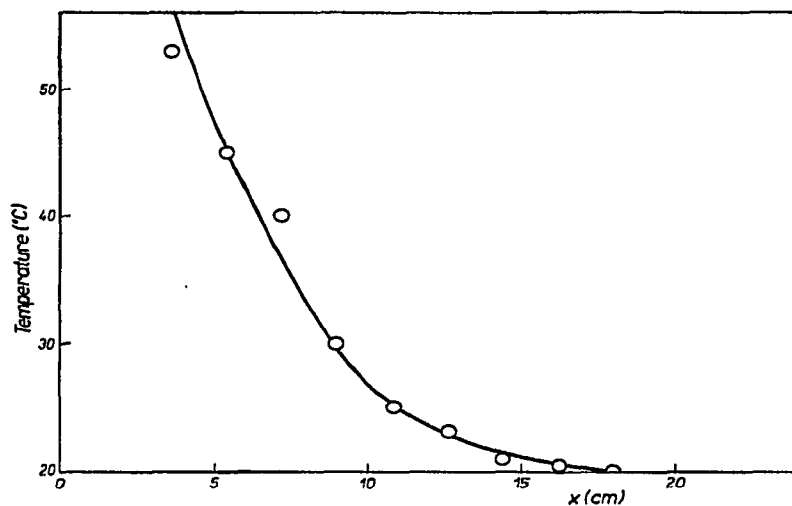


Fig. 2. Temperature course in the dynamic gradient. Column length $L = 46$ cm, I.D. = 2 mm; packing, silica gel CH, 50–63 μm . Mobile phase: *n*-heptane. $u = 0.91$ cm·sec⁻¹; $w = 0.077$ cm·sec⁻¹.

Experiments with the dynamic temperature gradient were carried out in two systems. In the first case, the mobile phase which was not purified to remove aromatic hydrocarbons and the 47 cm long column packed with silica gel were used, and the temperature gradient was established with one heating element. The mixture that was sampled contained benzene, naphthalene, fluoranthene and nitrobenzene. In the second case, the mobile phase purified to remove aromatic hydrocarbons and the 46 cm long column were used and the temperature gradient was established by means of two heating elements. The mixture that was sampled contained benzene, anisole and nitrobenzene. The measurements were taken in the same manner in both instances. The mixture under analysis was led into the column and the heating and the oven translation were switched on simultaneously.

Isothermal runs at several temperatures and experiments with programmed temperature were carried out for both systems. The samples were always injected with a Chrominject injection syringe.

RESULTS AND DISCUSSION

According to eqn. 2, the retention volume, V_{Ri} , of the component i that is desorbed from the column of length L , in a temperature gradient of length l , is dependent on the ratio of the rates of movement of the mobile phase, u , and the temperature gradient, w , and on the distance of this component from the origin of the tempera-

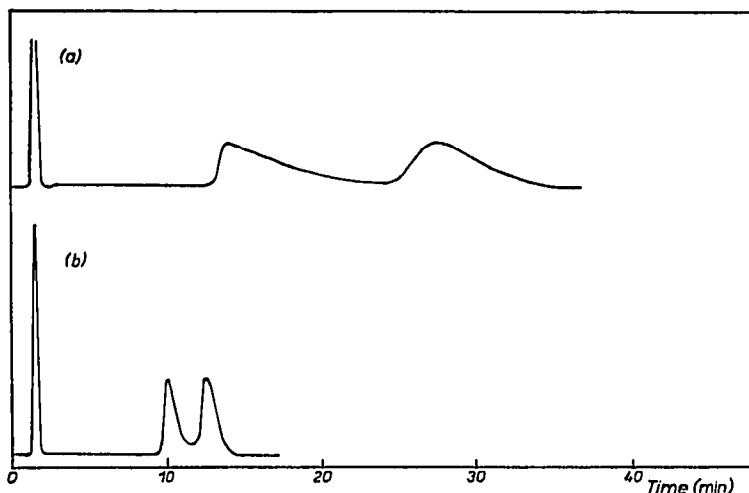


Fig. 3. Chromatograms of benzene, anisole and nitrobenzene mixture. Column length $L = 46$ cm, I.D. = 2 mm; packing, silica gel CH, 50–63 μ m. Mobile phase: *n*-heptane. $u = 0.91$ cm \cdot sec $^{-1}$. (a) isothermal run; $t = 20^\circ\text{C}$. (b) run with dynamic temperature gradient; $w = 0.077$ cm \cdot sec $^{-1}$.

ture gradient, x_i , which is determined by the course of the temperature in the gradient. If a certain retention volume was to be obtained for the component i , the values of two of these parameters could be selected while the third parameter had to be determined either experimentally or by calculation.

The use of chromatography with a dynamic temperature gradient for the reduction in the capacity ratio, k , for anisole and nitrobenzene is obvious from Fig. 3 and Table I. At the same time, the values of k calculated from the experimental values of the mobile phase velocity, u , temperature gradient translation speed, w , and column length, L , according to eqn. 3 are also given. The agreement between the experimental and calculated values is considered to be acceptable as the length of the temperature gradient, l , which is not identical with the size of the oven used, could not be deter-

TABLE I
RESULTS OF MEASUREMENTS OF ISOTHERMAL RUNS AND RUNS WITH DYNAMIC TEMPERATURE GRADIENTS

Component	Isothermal					Dynamic temperature gradient					
	V_R (ml)	H (mm)	N_{eff}	N_{eff}/sec	k	V_R (ml)	H (mm)	N_{eff}	N_{eff}/sec	k	k_{calc}
System I ($t = 25^\circ\text{C}$ or $w = 0.0795$ cm/sec)											
Benzene	1.77	1.59	55.3	0.601	0.77	1.75	1.55	55.3	0.608	0.75	
Naphthalene	2.44	1.94	84.8	0.668	1.44	2.38	2.03	78.0	0.629	1.38	
Fluoranthene	4.22	2.49	109.8	0.499	3.22	4.15	2.35	114.8	0.531	3.15	
Nitrobenzene	17.55	1.74	240.1	0.262	16.55	10.27	0.245	1563.1	2.922	9.27	10.4
System II ($t = 20^\circ\text{C}$ or $w = 0.077$ cm/sec)											
Benzene	1.64	2.05	36.3	0.425	0.67	1.60	2.23	33.0	0.396	0.63	
Anisole	16.92	6.92	63.0	0.073	16.3	11.65	0.68	585.0	0.980	10.8	12.9
Nitrobenzene	31.00	1.92	230.6	0.143	30.8	14.65	0.40	1028.6	1.371	13.9	15.1

mined with sufficient precision. The metal column distributes heat so that the gradient front moves in front of the oven. Also, a radial temperature gradient is established on the column. The temperature could not be measured directly in the column packing with respect to the dimensions of the column used and the column efficiency which was required.

As the capacity ratio, k , of the most strongly adsorbed components decreases, the peak capacity, n , of this system with the dynamic temperature gradient also increases simultaneously. Whereas in the isothermal run the column had a peak capacity $n = 12.5$, this value increased to 13.2 when calculated according to eqns. 11, 12 and 13 for the run with the dynamic temperature gradient. The experimental value was $n = 13.7$ for the procedure with the dynamic temperature gradient. The increase in the peak capacity does not illustrate the reduction in the analysis time sufficiently. The number of effective plates per second, which is also given in Table I, is a more conclusive characteristic. This quantity improves approximately 10 times for nitrobenzene. Similar results were also obtained for the values of the height equivalent to a theoretical plate, H , which also decreased approximately 10 times in the experiments with the dynamic temperature gradient in comparison with the isothermal runs.

The results obtained with the dynamic temperature gradient were compared with those obtained in the experiments with programmed temperature. The results in Table II show that the capacity ratio can be reduced by using programmed temperature, but the values of N_{eff}/sec are substantially worse than in the experiments using the dynamic temperature gradient (*cf.* Table I).

These results show that a substantial increase in the efficiency of the system can be obtained with a simultaneous reduction in the analysis time by a suitable selection of the length of the temperature gradient, l , and of its character (constant C in eqn. 1).

The dependence of the corrected retention volumes, V'_R , on the reciprocal value of the temperature is shown in Fig. 4. The retention volumes decrease as the temperature increases and the dependence of $\log V'_R$ on $1/T$ is linear. The variability of these dependences, especially in liquid-solid systems using a mobile phase moderator², permits the capacity ratio, k , not only to be reduced but also to be controlled within suitable limits.

The heat of adsorption, Q , was calculated for nitrobenzene according to eqn. 6 and on the basis of the results in Fig. 4, and the values obtained were compared with those obtained in the experiments using the dynamic temperature gradient (change in u and w) according to eqn. 6. A value of $6.00 \text{ kcal} \cdot \text{mole}^{-1}$ was obtained in the isothermal runs, and a value of $5.84 \text{ kcal} \cdot \text{mole}^{-1}$ was obtained from the experiments

TABLE II

EXPERIMENT WITH PROGRAMMED TEMPERATURE

Column, 0.58 g of silica gel CH; mobile phase, *n*-heptane, purified to remove aromatic hydrocarbons. Temperature programme: $1.6 \text{ }^\circ\text{C} \cdot \text{min}^{-1}$. $t_0 = 22^\circ\text{C}$.

Component	V_R (ml)	H (mm)	N_{eff}/sec	t_{des} ($^\circ\text{C}$)
Benzene	1.8	2.65	0.362	24.0
Anisole	11.7	5.20	0.122	42.0
Nitrobenzene	17.4	3.32	0.137	48.5

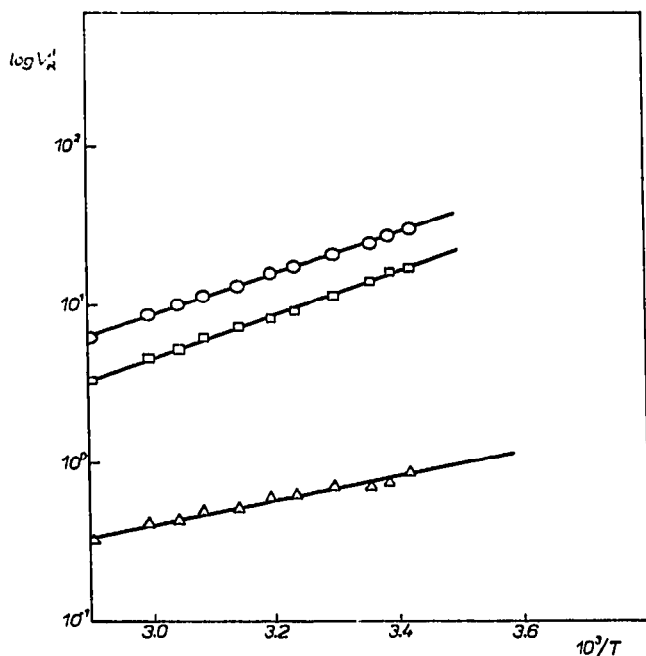


Fig. 4. Dependence of the corrected retention volumes on the reciprocal value of the temperature: Δ , benzene; \square , anisole; \circ , nitrobenzene. Column packing: 0.58 g of silica gel CH, surface area 395 $\text{m}^2 \cdot \text{g}^{-1}$. Mobile phase: *n*-heptane.

using the dynamic temperature gradient. The values of A were $1.0 \cdot 10^{-3}$ and $1.3 \cdot 10^{-3}$, respectively.

The isotherm of anisole is non-linear in this system, the values of the heat of adsorption, Q , and of the constant A depend on the amount that is sampled. The values of Q and A for four amounts of anisole sampled are given in Table III.

The characteristic desorption temperature was calculated according to eqn. 7 with the use of values of the capacity ratio, k , calculated according to eqn. 3. The characteristic temperatures, t_{char} , are 31°C for anisole and 41°C for nitrobenzene. By reading the temperatures on the plot of the dependence of $\log V_R'$ on $1/T$, values of 34°C and 46°C, respectively, were obtained. These differences are explained by the slow establishment of equilibrium in the column and by the existence of a radial temperature gradient.

TABLE III

DEPENDENCE OF THE HEAT OF ADSORPTION, Q , AND THE CONSTANT A ON THE AMOUNT OF ANISOLE SAMPLED

Amount sampled (μg)	Q ($\text{kcal} \cdot \text{mole}^{-1}$)	$A \cdot 10^4$
1.0	7.38	0.76
1.5	6.76	1.9
2.5	6.21	3.8
5.0	5.68	6.8

LIST OF SYMBOLS

A	constant
C	constant
H	height equivalent to a theoretical plate (cm)
L	column length (cm)
N	number of the theoretical plates
N_{eff}	number of the effective plates
Q	heat of adsorption ($\text{cal} \cdot \text{mole}^{-1}$)
R	gas constant ($\text{cal} \cdot \text{deg}^{-1} \cdot \text{mole}^{-1}$)
T	absolute temperature ($^{\circ}\text{K}$)
T_I	column temperature in the isothermal regime ($^{\circ}\text{K}$)
T_i char	characteristic temperature of the component i ($^{\circ}\text{K}$)
V_M	dead volume of the column (ml)
V_{Ri}	retention volume of the component i (ml)
V'_R	corrected retention volume, $V'_R = V_{Ri} - V_M$ (ml)
V_n	retention volume of the most strongly retained component (ml)
V_1	retention volume of the least retained component (ml)
k	capacity ratio
k_G	capacity ratio of the components eluted in the temperature gradient
$k_{G \text{ max.}}$	maximum capacity ratio of the components eluted in the temperature gradient
k_I	capacity ratio of the components eluted in the isothermal regime
$k_{I \text{ max.}}$	maximum capacity ratio of the components eluted in the isothermal regime
l	length of the temperature gradient (cm)
m	constant ($m = 4$)
n	peak capacity of the column
n_G	peak capacity of the column for the components eluted in the temperature gradient
n_I	peak capacity of the column for the components eluted in the isothermal regime
t	temperature ($^{\circ}\text{C}$)
t_{Ri}	retention time of the component i (sec)
u	linear velocity of the mobile phase ($\text{cm} \cdot \text{sec}^{-1}$)
w	linear velocity of the temperature gradient ($\text{cm} \cdot \text{sec}^{-1}$)
x	distance from the origin of the temperature gradient (cm)
σ	standard deviation of the concentration profile of the compound

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