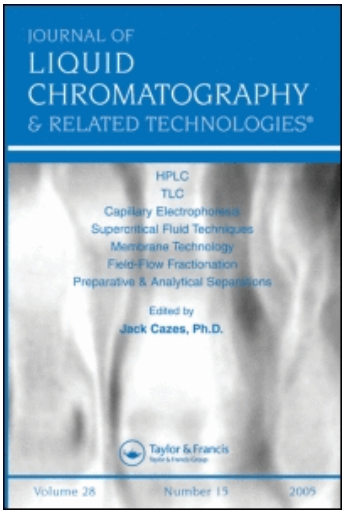

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DETERMINATION OF ZERO RETENTION TIMES (t_o) BY TEMPERATURE DEPENDENT REVERSED PHASE HIGH PERFOMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

For a given reversed phase HPLC system the zero retention time (t_o) can be determined by measuring brutto retention times at different temperatures. For calculation of t_o value temperature intervals must be equidistant in $^{\circ}$ 1/T. If at least two compounds are separated on the column having almost the same sorption enthalpies a more simple intersecting point method can be practised. In this case only two temperature points are necessary for graphic evaluation. The presented methods for determination of t_o values are proved for noradrenaline and adrenaline under selected experimental conditions using aqueous solutions of alkaline perchlorates as mobile phases. Thus t_o values can be determined with an accuracy and a precision of less than $\pm$ 3 %.

INTRODUCTION

For a given separation problem a HPLC system can be described by a set of direct and derived chromatographic values which depend on various parameters like column length and diameter, particle type and size, flowrate, composition and pH of the mobile phase, temperature, etc. and of course, on the compounds to be separated. The most controversively discussed point is the zero retention time (t_o), which is necessary for calculation of mass distribution coefficient (k'), relative retention (α), resolution (R) and effective number of plates (N).

The latter values are important for identifications, selectivity and polarity control, consequently for rating and optimization of the whole chromatographic system (for general review see references cited by Berendsen et al.(1)).

The t_o value is defined as retention time of the compounds in the mobile phase, and should thus be temperature independent. Quite a few experimental methods are known, by which t_o can be evaluated.

Practised methods are:

- Injection of slightly modified mobile phase and measuring with a RI detector,
- injection of O_2 saturated mobile phase and measuring O_2 absorbance with an UV detector,
- doping of the mobile phase with a fluorophore (chininesulfate), injection of undoped mobile phase, and measuring the drop in fluorescence,
- injection of dissolved small molecules - so called "non-retarded" substances - and measuring by various detection methods.

Following recently published papers (1,2), these methods do not allow the determination of the exact t_o value because of its dependency on the porous structure of the stationary phase. Thus, most of the merely experimental determined t_o values are too high, which lead to smaller k' values. In order to overcome this problem a mathematical treatment of experimental data of homologous compounds is recommended. Up to now, except time consumption, this method seems to be the best procedure for the determination of t_o values. One disadvantage of this method is due to the fact, that for most of the practical separation problems simple homologous series do not exist. Consequently, there is still an urgent need for an universal method, which would allow to determine correct t_o values.

OBJECTIVES

Facing this aim, an investigation was started with the assumption, that chromatographic processes are strongly dependent on temperature whereas t_o is not. This led to a first work hypothesis: With increasing temperature the brutto retention time (t_R) should decrease approaching the real t_o value . Thus, by mathematical treatment of temperature dependent t_R values the calculation of t_o should be possible. A first series of temperature dependent t_R measurements for noradrenaline (NA) and adrenaline (A) (chromatographic conditions are described below) resulted in experimental curves (Fig. 1), which showed a monotone

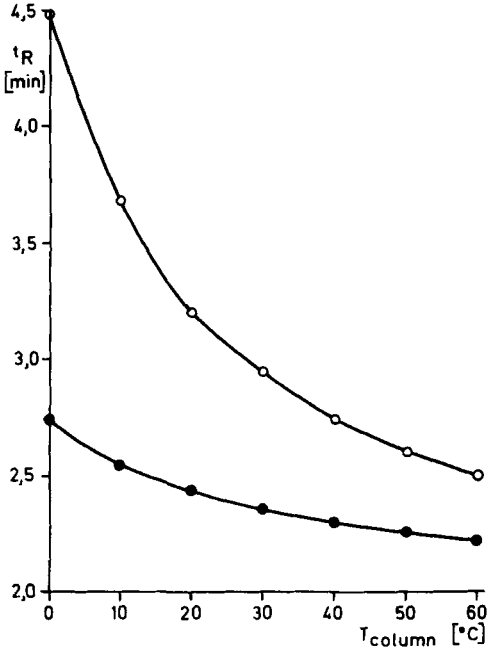

Figure 1: Influence of temperature on brutto retention times for noradrenaline (●) and adrenaline (o) (column: nucleosil 10-C_{18}; mobile phase: 0.1 M $HClO_4$)

decrease of t_R with increasing temperature and which, at least visually, approached the same limit value for both compounds. This result indicated, that the above stated hypothesis was possibly found to be correct. Such a method would be advantageous for direct determination of t_o with a particular chromatographic system under real experimental conditions. The systematic investigation was performed with catecholamines (NA and A), as in connection with occupational physiology studies a quantitative analysis of these compounds became necessary (3).

EXPERIMENTAL

Apparatus: Waters model M 6000A was used as high pressure pump connected to a Rheodyne model 7010 injection valve with 100 µl sample loop. The columns were packed with nucleosil 10-C_{18}, 5-C_{18} and 10-C_8 (Macherey + Nagel) and column dimensions were SS 250/6.4/2.9, SS 200/6.0/4.0 and SS 250/6.0/4.0 resp.. For thermostatic control a Kryostat (Desaga Frigostat) was used and detection was performed with an Aminco-Bowman-Spectrophotofluorometer (8 µl flow through cuvette) connected to a Corning Recorder 840.

Reagents: All chemicals were of analytical grade. Aqueous solutions of $HClO_4$, $LiClO_4$, $NaClO_4$, $KClO_4$ and $KClO_3$ with concentrations of 0.0025, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.5 M were used as mobile phases.

Procedure: The most important requirements for temperature dependent measurements are definite and constant temperatures of the chromatographic system during the experiment. In order to fulfil this prerequisite, the temperature gradient of the mobile phase along its pathway from injection valve to end of column was investigated for different isolation materials and segments using four thermocouples at a time. Thus, it could be shown, that at flow rates of 1 ml/min thermostatic control of at least 20 cm of the stainless steel capillary in front of the column is sufficient if the temperature of the column itself is regulated. In practice 30 cm of the capillary together with the column are incapsuled in a brass tube (i.d.2.4 cm) which is well isolated and

through which the circulating liquid (isopropanole/water:1/1) is pumped (14 l · min $^{-1}$). The experiments were run at temperatures of 0, 10, 20 up to 60^{o} C at a flowrate of 1 ml/min for the mobile phase. The pressure in the system was dependent on temperature and type of RP stationary phase and ranged between 400 psi for 10-C_8 at 60^{o} C and 3800 psi for 5-C_{18} at 0^{o} C. Concentrations of NA and A were 100 ng/ 100 µl and 50 ng/100 µl resp. and detection was performed fluorimetrically (excitation 275 nm/emission 317 nm). For better evaluation of the chromatograms the recorder was set to a velocity of 10 cm · min^{-1}.

MATHEMATICAL MODEL

A common equation of the Arrhenius type is:

$$k' = k_o \cdot e^{-\frac{\Delta(\Delta H)}{RT}} \qquad (1)$$

where k' : mass distribution coefficient
k_o : proportionality constant
$\Delta(\Delta H)$: difference in the sorption enthalpies of eluent and compound for sorption at the stationary phase (4)
R : universal gas constant
T : absolute temperature

but $$k' = \frac{t_R - t_o}{t_o} \qquad (2).$$

Substituting k' from eq. (1) by eq. (2) one gets

$$t_R = k_o \cdot t_o \cdot e^{-\frac{\Delta(\Delta H)}{RT}} + t_o \qquad (3).$$

Equation 3 indicates that at infinite temperature $t_R = k_o \cdot t_o + t_o$, which means, that with increasing temperatures t_R approaches t_o if k_o is negligibly smaller than t_o. For this case the stated work hypothesis is correct.

Under the assumption, that k_o and Δ (ΔH) are temperature independent, which at least is justified within the experimental T interval, the zero retention time can be calculated (5):

$$t_o = \frac{t_R^2 (T_1) - t_R(T_2) \cdot t_R(T_3)}{2t_R(T_1) - t_R(T_2) - t_R(T_3)} \qquad (4).$$

The $t_R(T)$ values are experimentally determined brutto retention timesat different temperatures, with the condition, that T_1, T_2 and T_3 are chosen such, that the $^1/T$ values become equidistant (e.g. 0^o C, 20^o C, 43^o C).

Thus, the error of t_o is determined by the precision of the experimental t_R values. In order to minimize this error, temperature intervals should be as wide as possible, and temperature should be exact and constant. The temperature interval, naturally given by the chromatographic system and the separation problem, ranges practically between 0^o C and 80^o C.

Using the above described procedure t_o can be determined with one component only. In practice, however, one has to deal mostly with multicomponent systems. For such cases a more simple method for the determination of t_o can be practised, if given conditions are fulfilled.

Starting with a more common equation for mass distribution coefficients

$$k'_A = a \cdot g_A(T) \text{ and } k'_B = b \cdot g_B(T) \qquad (5)$$

A,B : components A and B
a,b : temperature independent factors

and with the condition

$$g_A(T) = g_B(T) \text{ for all experimental temperatures}$$

follows

$$t_o = \frac{t_{R,A}(T_1) \cdot t_{R,B}(T_2) - t_{R,B}(T_1) \cdot t_{R,A}(T_2)}{t_{R,A}(T_1) + t_{R,B}(T_2) - t_{R,A}(T_2) - t_{R,B}(T_1)} \qquad (6)$$

$t_{R,A(B)}(T)$: brutto retention time for component A(B) at temperature T.

In this case the temperatures must not necessarily be equidistant in $^1/T$.

The identity of $g_A(T)$ and $g_B(T)$ means practically, that the sorption enthalpies of the two components are equal or at least very similar, which is the case for many chemically related substances. Hereof it results automatically, that the selctivity coefficient α is temperature independent.

For practical determination of t_o the following procedure is recommended:

1. measurement of $t_{R,A}$ and $t_{R,B}$ at two different temperatures (T_1, T_2)
2. plotting of $t_{R,A}$ and $t_{R,B}$ for both temperatures at arbitrary positions of the abzisses X_1 and X_2 respectively
3. Y value of point of intersection of the two straight lines for T_1 and T_2 is identical with the exact t_o value.

Again, in order to minimize the error of t_o the range between T_1 and T_2 should be as wide as possible.

RESULTS AND DISCUSSION

For proving the mathematical model quite numerous experiments were performed at various temperatures with different types and concentrations of the mobile phase and for three different RP materials. As the chosen compounds NA and A are chemically related, the intersecting point method was used for the calculation of zero retention times. An example for graphic evaluation of t_o is shown in Fig. 2.

In the following tables (1,2) mean t_o values are listed which were calculated from experimental $t_R(T)$ values using

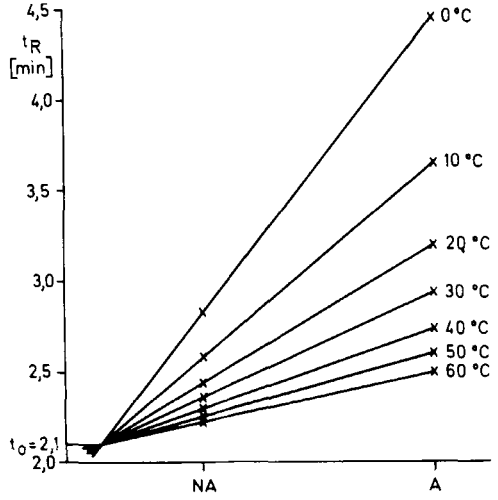

Figure 2: Graphic evaluation of t_o via temperature dependent t_R values of noradrenaline and adrenaline
(column: nucleosil 10-C_{18}; mobile phase: 0.1 M $HClO_4$)

equation 6. In order to keep the influence of errors of single t_R values small, the mean t_o values were calculated from intersecting points of at least 5 temperature pairs which were obtained by cyclic permutation of different experimentally given temperatures.

Tables 1 and 2 demonstrate, that the zero retention times of the systems under investigation are neither dependent on the mobile phase nor on its concentration; standard deviation is about $\pm$ 3 % rel.. A comparison of these t_o values with those calculated from equation 4 showed the results in excellent agreement (Tab. 3,4).

The mathematical model was proved by the experiments. By plotting ln k' versus $^1/T$ straight lines were obtained from which k_o values and $\Delta(\Delta H)$ values were calculated. k_o values were found to be in the range of 10^{-4}, which means,

TABLE 1

mean t_o values [min] calculated from eq. 6 for different types and concentrations of the mobile phase (column: nucleosil 10-C_{18}; standard deviation for $n \geq 10$)

conc. [M]	$LiClO_4$	$NaClO_4$	$KClO_4$	$KClO_3$
0.005	2.02 ± 0.06	2.06 ± 0.06	2.03 ± 0.04	2.09 ± 0.07
0.01	2.00 ± 0.05	2.10 ± 0.07	2.05 ± 0.03	2.06 ± 0.06
0.02	2.07 ± 0.05	2.06 ± 0.03		
0.05		2.11 ± 0.03	2.04 ± 0.04	2.07 ± 0.03
0.1	2.06 ± 0.06	2.08 ± 0.06		
0.5	2.05 ± 0.04	2.10 ± 0.05		

TABLE 2

mean t_o values [min] of different column types calculated from eq. 6 for various concentrations of $HClO_4$ as mobile phase (standard deviation for $n \geq 10$)

conc. [M]	10-C_{18}	10-C_8	5-C_{18}
0.0025			2.25 ± 0.05
0.005	2.03 ± 0.02	3.09 ± 0.05	2.26 ± 0.06
0.01	2.05 ± 0.03	3.08 ± 0.05	2.21 ± 0.05
0.02	2.04 ± 0.05	3.08 ± 0.06	2.23 ± 0.06
0.05	2.07 ± 0.02	3.08 ± 0.06	2.20 ± 0.06
0.1	2.09 ± 0.07	3.15 ± 0.07	2.25 ± 0.04
0.5	2.06 ± 0.03	3.12 ± 0.05	

TABLE 3

mean t_o values $[min]^*$ calculated from different temperature or concentration dependent equations for various types of mobile phases (column: nucleosil 10-C_{18}; concentration see table 1)

	$LiClO_4$	$NaClO_4$	$KClO_4$	$KClO_3$
eq.6, T dependent	2.04 ± 0.02	2.09 ± 0.02	2.04 ± 0.02	2.07 ± 0.03
eq.6, c dependent	2.06 ± 0.06	2.08 ± 0.06	2.04 ± 0.04	2.10 ± 0.06
eq.4, T dependent	2.03 ± 0.06	2.11 ± 0.03	2.06 ± 0.04	2.08 ± 0.03

*weighted mean ($n \geq 3$) of the mean ($n \geq 10$)

TABLE 4

mean t_o values $[min]^*$ calculated from different temperature or concentration dependent equations for three column types and $HClO_4$ as mobile phase (concentrations see table 2)

	10-C_{18}	10-C_8	5-C_{18}
eq. 6, T dependent	2.05 ± 0.01	3.10 ± 0.02	2.24 ± 0.02
eq. 6, c dependent	2.06 ± 0.05	3.07 ± 0.06	2.28 ± 0.05
eq. 4, T dependent	2.08 ± 0.03	3.11 ± 0.04	2.26 ± 0.02

*weighted mean ($n \geq 3$) of the mean ($n \geq 10$)

that the stated work hypothesis is correct. $\Delta(\Delta H)$ values were found to be in the range of 15 kJ $\cdot$ mole^{-1} and were almost identical for the two compounds under given experimental conditions (type and concentration of mobile phase, type of stationary phase), which in fact led to temperature independent α values.

As t_R values are also dependent on the concentration of mobile phase, one may think about the possibility of determining the zero retention times by concentration dependent RP HPLC. Using the intersection point method, t_o values were calculated on basis of eq. 6 after replacement of $t_R(T)$ by $t_R(c)$. Tables 3 and 4 show the results in quite good agreement, but standard deviation of these t_o values are obviously larger than those resulting from the temperature dependent measurements. It seems as if the temperature method is less sensitive to little changes in the chromatographic system than the concentration method.

For comparison t_o values were also determined using the experimental methods listed in the introduction. In some cases agreement was quite good but in other cases extremely bad. This confirms the statement, that "classical methods" must not necessarily lead to exact zero retention times.

CONCLUSIONS

For a given chromatographic RP system the zero retention time can be determined by measuring t_R values at different temperatures ($n_T > 2$). The temperature intervals must be equidistant in $^1/T$. If at least two compounds are separated on the column having almost the same sorption enthalpies a more simple intersecting point method can be practised. In this case only two temperature points are necessary for graphic evaluation.

The presented methods for determination of t_o values are, of course only proved for two compounds (NA and A) under selected experimental conditions. Although, further experiments are needed to elucidate the applicability and the

limitations of the methods, we believe, that this procedure can be utilized to very many practical problems. This will have the advantage of working with a given system without any changes of those chromatographic parameters, which might influence the zero retention time.

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