Journal of Chromatography, 198 (1980) 229–234
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,055

RELATIVE RETENTION EXPRESSIONS IN CHROMATOGRAPHY

L. S. ETTRE
The Perkin-Elmer Corporation, Norwalk, CT 06856 (U.S.A.)
(First received April 24th, 1980; revised manuscript received June 19th, 1980)

SUMMARY

Computers and data systems usually calculate relative retention as the ratio of two retention times and not adjusted retention times as it should be if theoretical relationships are considered. The purpose of this paper is to demonstrate that such relative retention data can directly be related to the true relative retention (separation factor) and to basic chromatographic relationships.

INTRODUCTION

The basis of separation and identification in chromatography is the relative retention called also the relative volatility or separation factor (α). It is expressed as the ratio of the distribution constants (partition coefficients) of two solutes (sample components)*:

$$\alpha = K_i/K_j \tag{1}$$

Due to the basic chromatographic relationships the ratio of the capacity factors (capacity ratios; k) or the adjusted retention times (t'_R) or volumes (V'_R) may be substituted for the distribution constants:

$$\alpha = k_i/k_j = t'_{Ri}/t'_{Rj} = V'_{Ri}/V'_{Rj} \tag{2}$$

The relative retention (separation factor) is a thermodynamic function which may be related to a number of other physico-chemical values, *e.g.*, to solute chemical potentials, the mole fractions of two solutes in the vapor and liquid phases at equilibrium or to the activity coefficients at infinite dilution and the saturation pressures of the solutes[1].

In the above equations subscripts i and j may have different meanings. In identification i usually refers to the solute of interest and j to the standard while when investigating the selectivity of a stationary phase and the separation of two solutes, i and j refer to them with the assumption that $k_i > k_j$.

As seen in eqn. 2, t'_R, the adjusted retention time is used in the calculation of

* For the meaning of the symbols see the listing at the end of the paper.

0021-9673/80/0000–0000/$02.25

relative retention. The reason for this is that we only consider the selective retardation of the solutes by the stationary phase and this is expressed by this value. The holdup time while contributing to the overall retention of the solutes by the column, is not related to the specific interactions between solutes and the stationary phase; the holdup time is only a function of column length and mobile phase velocity, regardless of the stationary phase and the particular solutes.

Due to the fact that the relative retention (α) is related only to the selective retardation of the solutes by the stationary phase, its value is not related to a given column or instrument; it should remain the same if the same two solutes are analyzed on any instrument and column, assuming that the same stationary phase (and, in liquid chromatography: mobile phase) is used at the same temperature.

This brief discussion makes it clear that, from the theoretical point of view, relative retention calculation must be based on the adjusted retention times.

In spite of this, however, practically all modern chromatography data systems calculate the "relative retention time" (RRT) as

$$\mathrm{RRT} = t_{Ri}/t_{Rj} = V_{Ri}/V_{Rj} \tag{3}$$

in other words, using the retention times (volumes) and not the adjusted retention times (volumes). These values cannot be generalized from one instrument or column to another, even if prepared with the same stationary phase and operated at the same temperature. On the other hand, in a given laboratory and a given system (*i.e.*, instrument and column) such data can be used equally well for identification, by building up data collections through the analysis of standards, particularly by utilizing the speed and automation of present-day data systems. In other words, in spite of the fact that the RRT values do not conform to the chromatographic theory, they are useful in a given laboratory.

It is generally not known that the RRT values can be related to the true relative retention values. Investigation of these relationships is the subject of this paper.

In the discussion below, the symbol α will be used for the true relative retention (separation factor) and the symbol α^* for the RRT, *i.e.*, the ratio of the two retention times. The two solutes will be characterized by the subscripts 1 and 2 assuming that $t_{R2} > t_{R1}$ and that t'_{R2} (t_{R2}) is in the numerator.

RELATIONSHIP BETWEEN α AND α^*

As seen the true relative retention may be expressed as the ratio of the two adjusted retention times or capacity factors:

$$\alpha = t'_{R2}/t'_{R1} = k_2/k_1 \tag{4}$$

On the other hand, α^* is equal to the ratio of the two retention times:

$$\alpha^* = t_{R2}/t_{R1} \tag{5}$$

In order to express α^* as a function of the capacity factors, we first write the retention times as the sum of the adjusted retention times and the hold-up time, and then divide both the numerator and the denominator by the holdup time:

$$\alpha^* = \frac{t'_{R2} + t_M}{t'_{R1} + t_M} = \frac{(t'_{R2} + t_M)/t_M}{(t'_{R1} + t_M)/t_M} = \frac{k_2 + 1}{k_1 + 1} \tag{6}$$

Comparing eqn. 6 with eqn. 4 it is clear that if k_1 and $k_2 \to \infty$, then $\alpha^* \to \alpha$; on the other hand, at low values of the capacity factor, $\alpha^* \neq \alpha$, and we may add that $\alpha^* < \alpha$. This is clear from Fig. 1 which plots α^* against the capacity factor of the second peak (k_2) for four values of the true relative retention (α); for the calculation of α^*, eqn. 8 was used.

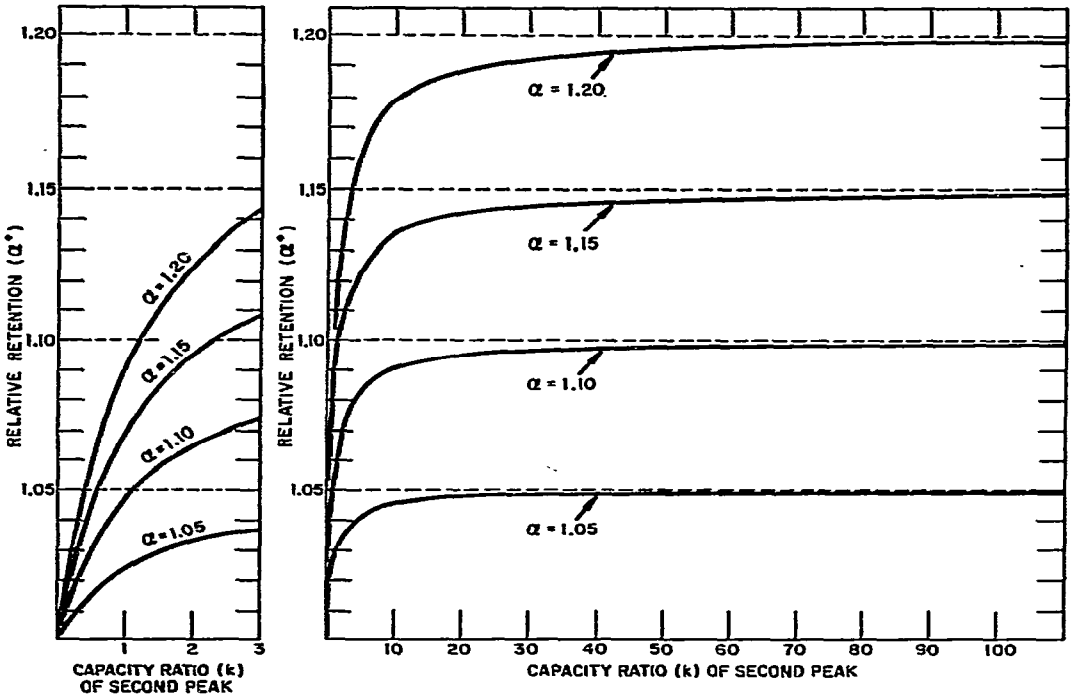

Fig. 1. Plots of α^* against the capacity factor (k) for given true relative retention (α) values.

By substituting eqn. 4 into eqn. 6, α^* can be expressed as a function of α:

$$k_1 = k_2/\alpha \tag{7}$$

$$\alpha^* = \frac{k_2 + 1}{(k_2/\alpha) + 1} = \frac{\alpha(k_2 + 1)}{k_2 + \alpha} \tag{8}$$

Similarly we can deduct that

$$\alpha^* = \frac{\alpha k_1 + 1}{k_1 + 1} \tag{9}$$

In turn, we can express α as a function of α^*:

$$\alpha = \frac{\alpha^* k_2}{(k_2 + 1) - \alpha^*} \tag{10}$$

$$\alpha = \frac{\alpha^*(k_1 + 1) - 1}{k_1} \tag{11}$$

Thus, if k_1 or k_2 are known, α^* can be calculated from α and *vice versa*.

The relationships expressed in eqns. 8–11 underline again the importance of the capacity factor (k) values representing one of the most fundamental terms in chromatography. Since for their calculation knowledge of the holdup time is necessary, it would be very important to have enough information available in each chromatographic run to permit this calculation[2].

THE FUNDAMENTAL RESOLUTION EQUATION AND α^*

The fundamental relationship of chromatography expressing peak resolution (R_s) as a function of the theoretical plate number (n), HETP (h), capacity factor (k), relative retention (α) and column length (L) is as follows[1]:

$$R_s = \frac{\sqrt{n_2}}{4}\left[\frac{\alpha - 1}{\alpha}\frac{k_2}{k_2 + 1}\right] = \frac{1}{4}\sqrt{\frac{L}{h_2}}\left[\frac{\alpha - 1}{\alpha}\frac{k_2}{k_2 + 1}\right] \tag{12}$$

or

$$n_2 = \frac{L}{h_2} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{k_2 + 1}{k_2}\right)^2 \tag{13}$$

In eqns. 12 and 13 the plate number, plate height and capacity factor refer to the second peak ($k_2 > k_1$). Let us see how could α^* be used in these expressions.

From eqn. 8 we can express ($\alpha^* - 1$):

$$\alpha^* - 1 = \frac{\alpha(k_2 + 1)}{k_2 + \alpha} - 1 = \frac{(\alpha - 1)k_2}{k_2 + \alpha} \tag{14}$$

Dividing eqn 14. by eqn. 8 we get:

$$\frac{\alpha^* - 1}{\alpha^*} = \frac{(\alpha - 1)k_2}{k_2 + \alpha}\frac{k_2 + \alpha}{\alpha(k_2 + 1)} = \frac{\alpha - 1}{\alpha}\frac{k_2}{k_2 + 1} \tag{15}$$

If we compare eqn. 15 with eqn. 12 it is evident that the right-hand-side of eqn. 15 is equal to the bracketed term in eqn. 12. In other words,

$$R_s = \frac{\sqrt{n_2}}{4}\left(\frac{\alpha^* - 1}{\alpha^*}\right) = \frac{1}{4}\sqrt{\frac{L}{h_2}}\left(\frac{\alpha^* - 1}{\alpha^*}\right) \tag{16}$$

and similarly

$$n_2 = \frac{L}{h_2} = 16R_s^2\left(\frac{\alpha^*}{\alpha^* - 1}\right)^2 \tag{17}$$

In other words, using α^* we can directly obtain the number of theoretical plates needed to achieve a desired resolution (R_s) for a peak pair (or the resolution corresponding to a column with a given length and efficiency), without the need to establish the corresponding capacity factor value.

RELATIONSHIP OF THE PLATE NUMBERS

Eqn. 17 looks similar to the expression describing the number of effective plates (effective plate number, N), a term which can be traced back to Purnell[*3,4] and obtained its name from Desty[5]:

$$N_2 = \frac{L}{H_2} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2 \tag{18}$$

where H is the height equivalent to one *effective* plate (HEEP) or effective plate height. Using the respective symbols of A and A^*:

$$A = \frac{\alpha}{\alpha - 1} \text{ and } A^* = \frac{\alpha^*}{\alpha^* - 1}$$

and dividing eqn. 17 by eqn. 18 we get:

$$n/N = (A^*/A)^2 \tag{19}$$

In other words, the ratio of the two terms in the right-hand-side of the equation gives the ratio of the number of theoretical and effective plates.

NOTE

It should be noted that α^* is equivalent to the "separation factor" of Glueckauf[6]. As pointed out by Tang and Harris[7], confusing it with the real separation factor (*i.e.*, α), particularly in the interpretation of Glueckauf's widely reproduced charts predicting the number of theoretical plates needed to obtain a given purity of products for the separation of species having a given "separation factor" (see *e.g.* Keulemans[8]) led to conflicting and highly misleading conclusions.

LIST OF SYMBOLS[6]

A	symbol for $\alpha/(\alpha - 1)$
A^*	symbol for $\alpha^*/(\alpha^* - 1)$
h	height equivalent to one theoretical plate; theoretical plate height, HETP
H	height equivalent to one effective plate; effective plate height, HEEP
k	capacity factor, capacity ratio
K	distribution constant, partition coefficient
L	column length
n	number of theoretical plates, theoretical plate number
N	number of effective plates, effective plate number
R_s	peak resolution
RRT	see α^*

* In Purnell's original equations the symbols can easily be misinterpreted. Using our present-day symbols[6] his V_R is V_R', V_d is V_M, and V_R' is V_R^o ($V_R^o = jV_R$, the retention volume corrected for gas compressibility).

t_M holdup time: retention time of a solute not retained by the stationary phase
t_R retention time (measured from the instant of sample introduction)
t'_R adjusted retention time; $t'_R = t_R - t_M$
V_M holdup volume: retention volume of a solute not retained by the stationary phase
V_R retention volume (measured from the instant of sample introduction)
V'_R adjusted retention volume; $V'_R = V_R - V_M$
α relative retention, separation factor; $\alpha = t'_{Ri}/t'_{Rj} = V'_{Ri}/V'_{Rj}$
α^* "relative retention time" (RRT); $\alpha^* = t_{Ri}/t_{Rj} = V_{Ri}/V_{Rj}$

REFERENCES

1 B. L. Karger, L. R. Snyder and Cs. Horváth, *An Introduction to Separation Science*, Wiley, New York, 1973.
2 L. S. Ettre, *Chromatographia*, 13 (1980) 73.
3 J. H. Purnell, *J. Chem. Soc. (London)*, (1960) 1268.
4 J. H. Purnell, *Gas Chromatography*, Wiley, New York, 1962, p. 113.
5 D. H. Desty, A. Goldup and W. T. Swanton, in N. Brenner, J. E. Callen and M. D. Weiss (Editors), *Gas Chromatography (1961 Lansing Symposium)*, Academic Press, New York, 1962, pp. 105–135.
6 E. Glueckauf, *Trans. Faraday Soc.*, 51 (1955) 34.
7 S. H. Tang and W. E. Harris, *Anal. Chem.*, 45 (1973) 1978.
8 A. I. M. Keulemans, *Gas Chromatography*, Reinhold, New York, 1957; 2nd ed., 1959, pp. 126–129.
9 L. S. Ettre, *J. Chromatogr.*, 165 (1979) 235.