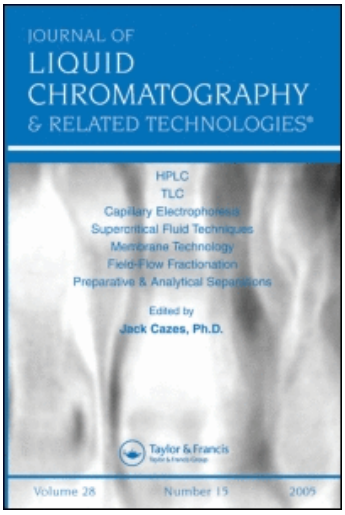

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Letter-The Principle of Measuring t_0 Remains Unchanged

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LETTER:

THE PRINCIPLE OF MEASURING t_o REMAINS UNCHANGED

Dear Sir,

We acknowledge the preceeding comment of Grushka et.al. on our paper concerning the determination of t_o by temperature dependent RP-HPLC (1) which reveals, that the problem of measuring t_o is still far from being completely solved on a pure theoretical basis. This means that all methods for measuring t_o, known so far, are more or less restricted concerning their validity. The limits of validity of each single method are difficult to predict and therefore still discussed controversively (see e.g. ref. 2 of ref.1). At any rate, it must be taken into account that mathematical equations, which have been derived under defined assumptions are only used for data handling if the stated assumptions are fulfilled.

If the ΔS values of the two solutes are very similar, it is obvious that eq.6 of ref. 1 cannot be used as the factors a and b in eq. 5 of ref. 1 are equal, which turns the denominator of eq. 6 to zero. In order to check eq. 6 of ref. 1, Grushka et.al. have used by chance the data of two compounds which in the system of Vigh and Varga-Puchony (2), have almost identical ΔS values (^1k_o / ^2k_o = 1.04). In the system noradrenaline - adrenaline which we used, the ratio ^1k_o / ^2k_o ranged between $\sim$2.5 and $\sim$3.5. Furthermore eq.4 of ref. 1 should only be used if eq. 1 of ref. 1 is valid within a wide temperature range, taking into consideration the condition $T_1 = \frac{2\ T_2 \cdot T_3}{T_2 + T_3}$ from ref. 5 of ref. 1.

The latter condition of equidistant 1/T values was, to our excuse, mixed up in equation 4 of ref. 1. Concerning the precision of measuring t_o , using the intersecting point method of ref. 1,the graphic evaluation of the data is recommended for a better rating of the errors.

Comming from the more practical side of HPLC, we have determined, calculated, and compared t_o values of the whole chromatographic system, including the dead volumes, which indeed led to "apparent t_o" values ($\varepsilon > 0.9$). This fact, however, was of no consequence concerning the aim of the study, which was the development and proof of a new method for the determination of t_o values, which should not replace but supply the already known methods.

We agree with Grushka et.al. that the method described in ref. 1 (like all other methods for measuring t_o) should be used only after careful examination of the chromatographic parameters, and we add, that it should be used only by those who have great practical experience in HPLC.

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