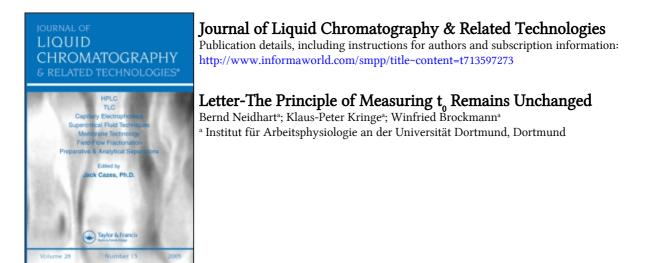
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## LETTER:

THE PRINCIPLE OF MEASURING  $t_{O}$  REMAINS UNCHANGED

## Dear Sir,

We acknowledge the preceeding comment of Grushka <u>et.al.</u> on our paper concerning the determination of  $t_0$  by temperature dependent RP-HPLC (1) which reveals, that the problem of measuring  $t_0$  is still far from being completely solved on a pure theoretical basis. This means that all methods for measuring  $t_0$ , 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  $\Delta$  S 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 <u>et.al.</u> have used by chance the data of two compounds which in the system of Vigh and Varga-Puchony (2), have almost identical  $\Delta$ S values ( $^{1}k_{0} / ^{2}k_{0} = 1.04$ ). In the system noradrenaline - adrenaline which we used, the ratio  $^{1}k_{0} / ^{2}k_{0}$  ranged between ~2.5 and ~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  $T_{1} = \frac{2}{T_{2}} \cdot \frac{T_{3}}{T_{2}}$  from ref. 5 of ref. 1.

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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  $t_0$ , 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  $t_0$  values of the whole chromatographic system, including the dead volumes, which indeed led to "apparent  $t_0$ " 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  $t_0$  values, which should not replace but supply the already known methods.

We agree with Grushka <u>et.al.</u> that the method described in ref. 1 (like all other methods for measuring  $t_0$ ) 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.

> Bernd NEIDHART Klaus-Peter KRINGE Winfried BROCKMANN

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