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EVALUATION OF CAPILLARY COLUMNS BY SEPARATION NUMBER OR PLATE NUMBER

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SUMMARY

The separation number (Trennzahl: TZ) as a measure of separation efficiency has been criticized because of its dependence on temperature, and its replacement by a measure based on the temperature-independent HETP concept has been suggested. We discuss the temperature dependence of TZ, then we show that this dependence, rather than being a weak point of the TZ concept, is directly related to an essential part (selectivity) of the column quality. The fact that this part is missing from all separation efficiency expressions based on HETP makes such expressions have poor suitability, and possibly be misleading, for direct practical extrapolation. Another consequence, which is against the interests of most users, is that most commercial columns, at least in the past, have been manufactured with relatively thick films. Obviously, in order to obtain comparable TZ values more specifications regarding the method of determination are required than for plate numbers. This drawback is overcome by using a standardized test procedure, an essential part of which may be the use of temperature programming.

CHARACTERISTICS OF THE SEPARATION NUMBER OR TRENNZAHL

There is no common agreement on how to determine the separation efficiency of a capillary column. One of us has been using the separation number, or Trennzahl (TZ), since Kaiser discussed it in his book[1]. Positive experiences with TZ, first measured isothermally and later by a standardized, temperature-programmed procedure[2], led us to study recent criticisms[3,4] of it in some detail, with the result that we now favour the TZ concept even more clearly than before.

Plate numbers (theoretical, effective, real plates) concentrate on peak width, assuming that a column eluting a sharper peak at a given retention time is more efficient. The exact meaning is that it causes less peak broadening. However, "more efficient" is most often, and almost automatically, interpreted as meaning separating more substances within a given mixture.

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The Trennzahl indicates the resolution of two neighbouring homologues differing by one methylene group. Resolution obviously depends on peak width and also on the distance between the two peaks, commonly reported as relative retention, α.

Consequently, the fundamental question is whether or not relative retention should be included in the efficiency concept. The controversy stems from conflicting answers to this question. Relative retention is essential in practical application work where resolution is required. In contrast, relative retention is of no interest when the homogeneity of the liquid film, *i.e.*, the coating quality has to be tested, as this latter purpose calls for a peak broadening measurement exclusively. The plate concept has the advantage of being almost independent of column temperature and of the test compound producing the peak to be measured.

As TZ values are calculated on the basis of peak width and relative retention, they depend on factors that affect α in addition to the factors that influence plate numbers. This fact has recently been discussed by Rooney and Hartigan[4]. To compare columns on the basis of TZ, some additional information or standardization is required in order to account for this dependence[5]. Jennings and Yabumoto[3] re-emphasized the dependence of TZ on column temperature. They consider the temperature dependence to be due to temperature-dependent relative retention. They did not consider the question of whether the TZ value determined with constant test compounds is temperature dependent, or whether the observed decrease in TZ with increasing temperature is caused by the increasing molecular weight of the alkanes used with increasing temperature. As the relative molecular weight difference between two neighbouring alkanes decreases with increasing molecular weight, it is tempting to think that TZ values could depend on the test compounds used.

The dependence of TZ on factors such as column temperature (and/or test substances?) caused the authors of recent papers[3,4] to question the TZ concept as a basis for column quality evaluation and even to term it "less 'safe' than other column efficiency criteria". N_{eff} was recommended as a "safer" value. In disagreement with this statement (which was probably influenced by incorrect translation), we emphasize as before[6] that TZ provides more reliable measurement of separation efficiency and is of direct practical utility.

The "more reliable measurement" is exemplified by repeated determinations ($n = 6$) of TZ and N_{eff} as carried out to obtain the data in Table III. The relative standard deviation of TZ values was 1.4%, compared with 9% for N_{eff} values. Thus "safer" means more reproducible. In addition, the determination of N_{eff} is less practical because more stringent analytical conditions, particularly temperature control, are required. It is obvious that comparability of TZ values requires the specification of column temperature for isothermal determination. In contrast to N_{eff}, a strong point of TZ is its compatibility with programmed column temperatures. This offers an application of TZ[2] which, in addition to assuring comparability, provides a maximum of practical information (see the last section).

DEPENDENCE OF SEPARATION NUMBER ON TEST COMPOUNDS OR ON TEMPERATURE?

Do TZ values depend only on the pair of homologues being separated, only on column temperature, or on both? Using the data of Rooney[7] (Table I) it is evident

TABLE I

EXPERIMENTAL DATA FROM ROONEY[7]

Temperature (°C)	*n-Alkane test compounds*	*TZ measured*[*]
155	C_{16}/C_{17}	48.5
80	C_{11}/C_{12}	68.2

[*] $TZ_{11/12}$ (calculated) $= \frac{TZ_{16/17} \cdot M.W._{17}}{M.W._{12}} = \frac{48.5 \cdot 240}{170} = 68.3.$

that the TZ value measured is inversely proportional to the molecular weight of the alkane homologues. We have produced corresponding data involving greater differences (Table II). TZ for one temperature can be calculated from the other (measured) TZ by multiplication by the ratio of the molecular weights of the test compounds (which should be eluted with a similar partition ratio; in our case $k > 10$). These results suggest that TZ decreases with increasing molecular weight of the test compounds. One is even tempted to think that a column might "see" the same difference (one methylene unit) between two small homologues much better than between two large ones.

TABLE II

DEPENDENCE OF TZ VALUES ON TEST COMPOUNDS

Column 20 m × 0.30 mm I.D., 1.9-μm film of OV-73.

Temperature (°C)	*n-Alkane test compounds*	*TZ measured*[*]
250	C_{25}/C_{26}	12.5
33	C_7/C_8	38.5

[*] $TZ_{25/26}$ (calculated) $= \frac{TZ_{7/8} \cdot M.W._{8}}{M.W._{26}} = \frac{38.5 \cdot 114}{366} = 12.0.$

It may at first be unexpected that the second hypothesis, dependence of TZ on temperature but not on test compounds, is empirically supported also. If the same mixture of homologues (C_{15}/C_{16}) is eluted at different temperatures but with a constant partition ratio from two columns that differ only in film thickness (Table III), identical TZ values should be expected based on the first hypothesis (dependence only on test compounds). Very different TZ values are observed, however. Running the thin-film column at 150°C with the test compounds C_{23}/C_{24} produces a similar TZ value at the same temperature, but with different test compounds. Here, a valuable use of N_{eff} as a measure of coating quality can be made. The thick-film column ($N_{eff} = 66{,}000$) has a more homogeneous film (a difference to be expected from column manufacturing

TABLE III

DEPENDENCE OF TZ VALUES ON COLUMN TEMPERATURE

Both columns 15 m × 0.30 mm I.D., with different film thicknesses of OV-73.

Film thickness (μm)	*Temperature (°C)*	*n-Alkane test compounds*	*α*	*TZ*	$N_{eff.}$
1.90	150	C_{15}/C_{16}	1.63	24.7	66,000
0.04	75	C_{15}/C_{16}	2.07	34.0	56,000
0.04	150	C_{23}/C_{24}	1.60	22.5	57,000

experience). We can correct the lower TZ (22.5) for the poorer coating quality of the column by multiplication by the square root of the ratio of N_{eff} values. The corrected TZ is 24.3, which agrees reasonably well with TZ = 24.7 obtained with the thick-film column.

These results suggest that TZ (or α) depends on column temperature exclusively. However, recalling the first hypothesis, TZ for the thin-film column at 150°C can be calculated, in the same way as shown in Tables I and II, from the value obtained at 75°C. The calculated TZ value is 22.7, compared with the measured value of 22.5.

Ettre[5] mentioned that the TZ values measured for C_5/C_6 primary alkanols are very similar to those for C_{12}/C_{13} *n*-alkanes on the same Carbowax 20M column at the same temperature. We obtained a similar result on a Carbowax 400 column (Table IV). The TZ value of the primary C_3/C_4 alkanols is compared, at the same column temperature, with that of the C_{12}/C_{13} *n*-alkanes. The alkane solutes are insufficiently compatible with the strongly polar stationary phase so that some additional peak broadening of *n*-alkanes, affecting both TZ and N_{eff}, is observed, whereas the relative retention (α) remains identical. When the *n*-alkane TZ is corrected for the reduced plate number, it becomes 43.5. Thus, the much greater relative difference in the molecular weights and volumes of the alkanols does not result in an increased relative retention and resolution.

TABLE IV

TZ VALUES OF DIFFERENTLY SIZED COMPOUNDS AT IDENTICAL COLUMN CONDITIONS

Column 30 m × 0.29 mm I.D., 0.18 μm film of Carbowax 400.

Compounds	*Temperature (°C)*	α	*TZ*	$N_{eff.}$
C_3/C_4 primary alcohols	31	2.33	44.3	66,000
C_{12}/C_{13} *n*-alkanes	31	2.31	33.9	40,000

Corresponding results were found when TZ values for *n*-alkanes were compared with those for alkyl nitriles (Table V) and alkylbenzenes (Tables VI) at the same temperature, using columns chosen to be strongly selective for the more polar homologous compounds.

TABLE V

TZ VALUES OF ALKYLNITRILES AS COMPARED TO *n*-ALKANES

Column 15 m × 0.30 mm I.D., 1.5 μm film of OV-225.

Compounds	*Temperature (°C)*	α	*TZ*	$N_{eff.}$
C_7/C_8 alkyl nitrilis	55	2.10	30.7	45,000
C_{14}/C_{15} *n*-alkanes	55	2.11	29.6	42,000

TABLE VI

COLUMN EFFICIENCY DETERMINED BY *n*-ALKYL BENZENES AND *n*-ALKANES

Compounds	*Temperature (°C)*	α	*TZ*	$N_{eff.}$
C_5/C_6 alkylbenzenes	60	2.21	36.2	54,000
C_{13}/C_{14} *n*-alkanes	60	2.22	35.9	52,000

This shows that relative retention found for two neighbouring homologues at a given column temperature is not dependent on the nature of the substances. Provided that the compounds are compatible with the stationary phase (producing similar peak broadening effects), TZ depends only on column temperature.

This statement does not contradict the observation that TZ is inversely proportional to the molecular weight of the test compounds. The relative retention of neighbouring homologues depends on the difference in the (free energy of) interaction between the solute and the stationary phase. This difference is, in our case, always caused by a structural difference of one methylene group. The interaction per methylene unit decreases with increasing column temperature, thus causing the relative retention of homologues (*i.e.*, the relative retention of the column) to decrease.

A decrease in (absolute) retention caused by an increase in column temperature can be restored in two ways. The reduced solute–solvent interaction may be matched by an increased partition ratio which, at constant temperature, can be obtained by an increased film thickness. The other way involves restoring the original solute–solvent interaction by increasing the molecular weight of the solute. If, for instance, a temperature increase cuts the solute–solvent interaction to half, the original interaction will be observed with a test compound with double the molecular weight.

Let us apply the same example to relative retention. When the solute–solvent interaction is halved by a temperature increase, the interaction per methylene unit also is halved, which means that the relative retention (and TZ) for a pair of homologues is reduced to half. This occurs when the molecular weight of test compounds is doubled to restore the original retention (the original partition ratio). Thus, relative retention and molecular weight are inversely proportional.

TZ is influenced by temperature only. The reason why TZ (for a constant film thickness) is inversely proportional to molecular weight is that temperature, simultaneously and independently, also influences the choice of test compounds.

EVALUATION OF COLUMN QUALITY BY SEPARATION NUMBER OR BY PLATE NUMBER ?

TZ depends on column temperature because of the temperature dependence of relative retention. TZ is independent of the nature of the test compounds.

The term Trennzahl is directly related to resolution. Resolution is the essential purpose that a column has to serve. As resolution depends on temperature, we can conclude that TZ is the ideal practical measure of column efficiency. Let us reconsider the two OV-73 columns characterized in Table III: if the columns are evaluated on the basis of plate numbers, the thick-film column will be preferred. However, it shows a 30% lower resolution for a given sample than the thin-film column when the partition ratio for a given substance is kept constant. Hence the plate concept does not provide the information required for practical application. The fact that resolution is temperature dependent seems often to be regarded as a detail of secondary importance. From this view point the plate number appears to be the better and more straightforward concept. In contrast, we consider this aspect to be of great importance in practical work. Consequently, the influence of temperature should be included in column evaluation, which is the case when TZ is used.

INFLUENCE OF QUALITY EVALUATION ON COLUMN MANUFACTURE

Among column producers it is a common experience that ideally homogeneous films (yielding high plate numbers) are easier to make with relatively large film thicknesses, whereas thin films are known for their tendency to break up into pools and droplets. A simple consequence of this experience may not be commonly known.

Commercial manufacturers supplying tested columns with an indication of plate numbers naturally tend to sell thick-film columns. Most customers ignore the fact that such columns must be used at relatively high temperatures, with a consequently reduced resolution. Rooney and Hartigan[4] state that TZ can be manipulated (by running tests at low temperatures). We feel that column quality expressions in terms of plate numbers can be manipulated by producing thick films, *i.e.*, by suppressing in the quality expression the second criterion of resolution, which is relative retention.

On the other hand, column producers guaranteeing TZ values of their columns tend to sell thin-film columns, which show higher resolution because they are run at lower temperatures.

The user may be misled by plate numbers (film thickness is not commonly indicated in addition to plate numbers); he can hardly be misled by TZ values. No wonder that commercial columns sold with an indication of TZ are among the columns with highest reputation.

HOW TO MAKE FULL USE OF THE SEPARATION NUMBER CONCEPT

Jennings and Yabumoto[3], strongly supported by Rooney and Hartigan[4], state that "for comparative purposes, it is unfortunate that no restrictions relative to temperature, partition ratio or flow-rate are placed on the determination of separation numbers". Of course, we agree with the importance given to these details. The authors of both papers, however, do not mention that their demand has been fully considered in our testing procedure[2]. It may be useful to summarize (for the complete background see ref. 2) how the dependence of TZ on temperature has been incorporated into the procedure.

TZ is based on the relative retention and peak widths of two neighbouring homologues. Relative retention depends on column temperature, which may, in turn, depend on the selection of homologues. Peak width is influenced by flow conditions in terms of retention (partition ratio) and mobile phase flow-rate.

One of the former two parameters can be freely selected, and will then dictate the second one (to produce a standard retention). The latter two parameters can be optimized. It is reasonable to standardize them near to their optimal values.

As pointed out by Jennings and Yabumoto[3], TZ increases with increasing retention to reach a flat plateau which declines slowly with further increase in retention. A maximal TZ (when all other parameters are kept constant) is assured when the first compound of a homologous pair is eluted with a partition ratio of at least 10. Like other users of TZ, we select this experimental fact as part of our standardization.

The average gas velocity yielding a maximal TZ is greater than $\bar{u}_{opt}$ because, up to a certain deviation from $\bar{u}_{opt}$, relative peak broadening is more than compensated for by the simultaneous increase in relative retention which is caused by the reduced temperature. Our empirical standard flow-rate is 50 cm/sec for hydrogen and 25 cm/sec for helium (for columns with inner diameters not deviating significantly from 0.3 mm).

While there is clear experimental evidence in favour of both above-mentioned standards, standardization of column temperature or test compounds is more arbitrary. Some users work with a constant pair of test compounds and vary the column temperature to obtain a standard retention. Others prefer a fixed column temperature and vary the test compounds. The frequent TZ determinations carried out with partial or no standardization are the probable source of the justified criticism directed against the practice rather than against the nature of TZ. Technically, neither option, standardization of test compounds or of column temperature, presents problems. However, as information about the resolution of given substances on different columns is more interesting than information about the resolution of varying substances at a given column temperature, the former standardization is the more useful.

The solution to the problem is to run constant test compounds with temperature programming. The test compounds are of sufficiently low volatility to be cold-trapped in the column inlet at room temperature. In the course of the temperature-programmed run they automatically select their specific temperature range for migration, and this is shifted to higher temperatures by an increase in film thickness. Thus, the decrease in resolution (and TZ) due to an increase in column temperature, which is typical for thick-film columns, is automatically included in the test result. Instead of suffering from the dependence of elution temperature on film thickness, the test provides a non-destructive determination of film thickness, as the elution temperature of a given compound from a given stationary phase under standardized chromatographic conditions depends only on film thickness (or, more exactly, on the phase ratio, β). The essential point is that no pre-selection of column temperature is required. The last point holds only when the mobile phase flow-rate and temperature programme are carefully standardized, as they influence the elution temperatures in a temperature-programmed run.

Consequently, temperature programming is one of the essential features of the test procedure. TZ is the only separation efficiency concept that is compatible with temperature programming.

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