The Correct Measurement and Interpretation of Evaluation Factors in Chromatography: The Real Plate Number, the Separation Number, the Dosage Quality. Part 1: Gas Chromatography

R. E. Kaiser

Institute for Chromatography, P.O. Box 1308, D-6702 Bad Dürkheim, W. Germany

Summary

The correct evaluation of separation columns, capillary columns, their interconnections and the dosage technique is important in gas chromatography as well as in liquid chromatographic methods.

The conventional, theoretical plate number or plate height and their corresponding effective values (effective plate number or effective plate height) represent the combined action of column quality, interconnections and dosage, rather than their individual contributions to the overall performance of the separation system. Since these chromatographic criteria are dependent on the nature of the sample and the methods, a directed improvement or selective evaluation is not possible. A new evaluation system and method of measurement with the following characteristics is proposed:

- 1. Substance independent, i. e., independent of the chromatographic position.*
- 2. Real evaluation of a separation column.*
- 3. Individual, quantitative evaluation and measurement of factors which have a negative influence on the overall performance of a separation system, such as dosage, interconnections and detector.*
- 4. A meaningful correlation between the basic factors, which determine the separation capability and the separation power.*

The real plate number n_{real}

the real plate height h_{real}

the dosage and interconnection quality Q_s

the separation number TZ_{real}

the separation power number TZ_t

and the independent optimization of these factors, with respect to a maximum separation allow a correct, comparative evaluation of entirely different chromatographic separation systems. Such an evaluation requires three to eight data pairs, consisting of (correctly measured) peak width and retention data and a statistical regression calculation or inter- and extrapolating by plotting. The measurements and calculations can be carried out within 5 minutes. Highly conclusive evaluation factors (which must be statistically secured by additional calculations)

are obtained. The basic evaluation procedures will be explained, and examples will be given for the method of measurement, the calculation and plotting techniques. (programs for the Hewlett-Packard calculator HP-67 or 97 is provided.) Applications will be discussed for conventionally packed columns, extremely short or long micropacked columns, and thin film capillaries. Part 2 deals with adaptation of the method to liquid column chromatography, and part 3 discusses the application of the method to high performance and normal layer chromatography as well as a comparison of the methods.

It is often claimed, that the theoretical plate number is "theoretically" dependent on k and that the air peak (in GC) cannot be used to evaluate the quality of a chromatogram. This is an interpretation error or a misunderstanding of the method of measurement and will be discussed in depth, thus the basis of the new method can be critically compared and examined with the conventional evaluation method. The derivations are designed in such a way, that newcomers in the field, will be able to apply the method.

*The theoretical background of this paper is discussed in *Chromatographia* 1976 and 1977.*

Measurement and evaluation of the real separation capability and the real separation power in isocratic elution chromatography.

Part 1: Gas Chromatography

In the following, a measuring and evaluation technique will be described which is:

- Simple
- Accessible by a few measurements
- Reliable and conclusive method to evaluate three basic criteria in chromatography:

1. How good is a separation system?
2. Is it utilized fully?
3. How good is it optimized with respect to separation capability and separation power?

The new measuring and evaluation system can be applied to:

- A) Gas chromatography (without any restrictions)
- B) Liquid-elution chromatography (problems may arise in the determination of the substance-dependent hold-up volume, or hold-up time.)
- C) Thin layer chromatography (here, mistakes are easily made if the specified measuring devices are not used.)

In the author's laboratory, the new measuring and evaluation method has led to definite improvements of analytical procedures and (surprisingly) of instruments used in practical chromatography. Impregnation procedures, separation systems, dosage devices, interconnections and installation techniques could be improved. For the first time, by means of a few analytical data, it was possible to obtain conclusive information on the effectiveness of a certain method of operation (i. e., the use and utilization of a separation system by an operator) and on the optimization of a given separation system, using statistically secured, substance independent conditions.

Now it is possible to optimize a gas chromatograph with respect to maximum separation capability or maximum separation power, where the combined action of separation and dosage can be evaluated separately. The author feels, that the results are so convincing, that the conventional measuring and evaluation system which uses the theoretical and effective plate number and other derived terms can be disregarded. The new method eliminates the discrepancy between theory and practice, which was the reason (especially in capillary gas chromatography) for the introduction of the term effective plate number.

A simple demonstration will show that the theoretical plate number, the theoretical plate height, and their corresponding "effective" values are substance-dependent only because the starting width of the peaks was not considered in the measurement, a fact, which was pointed out already in 1959 [1]. Therefore, the known dependence of the plate number n and n_{eff} from the retention data, i. e., from the substance, is only a misunderstanding of the measuring technique and not the theory. Conclusions, such as n or n_{eff} depend on the test substance because of theoretical reasons are therefore simply wrong. Both numbers rather depend on the way the test substance is dosed and the column is connected, that means on a factor completely independent of the separation system. Another drawback of the conventional evaluation numbers is the fact that they are obtained from one substance, although they are aimed to evaluate the separation quality. However, a separation requires at least two substances.

The term which replaces $n_{\text{theoretical}}$ and $n_{\text{effective}}$ is n_{real} . It is independent of the nature of the substance, of the dosage technique by which the substance was applied and connection details. The influence of the dosage technique and interconnection technique on the separation capability can be determined separately and quantitatively as Q_s .

Q_s is a factor whose numerical value is always lower than 1. Multiplied by 100, it expresses the degree of efficient utilization (in percentage) of a column or capillary, therefore the term "utilization factor" could also be applied to Q_s . An examination of the possibilities to optimize Q_s (Q_s approaches 1, i. e., 100 % of the available separation capability is used) reveal quickly the factors which control it (e. g., Q_s is strongly dependent on the particular gas flow.) Q_s can assume values between 0.95 and 0.98. This has an tremendous influence on the operational method, tools, connecting and installation technique.

The new method does not require any mathematical skill. The reader should be familiar with some of the rules of the Euclidean geometry (the law of similar triangles etc.). It is also helpful if the reader has some experience in the general evaluation of data.

More than one measurement should be the basis of any decision, average values and repeated measurements of the standard deviation are mandatory. Data, with a linear relation should be evaluated by means of linear regression, (the method of the least root mean square) instead of a ruler and pencil. Furthermore a mean standard deviation calculation of slope and interception point should always be applied to the regression line or better to say: this is a must. Linear regression can be carried out with programed pocket calculators, as part of a daily routine.

Although it is not necessary to maintain the concept of plate number and plate height in isothermal, isocratic chromatography, because the dosed peak continuously broadens through material exchange between the mobile phase and stationary phase, as well as by diffusion in the phases, we will continue to use the term "plate" (or plate number). Since this process can be associated with a numerical value, the expression "real plate number" (n_{real}) was chosen.

The quotient of column length L and real plate number is expressed as real plate height, $h_{\text{real}} = L/n_{\text{real}}$ (mm), although the concept of plate height or height equivalent to a theoretical plate (HETP) might be inappropriate [2].

The term "real" was selected because n_{real} expresses and measures the real peak broadening, free from contributions of the dosage and substance transfer, i. e., the actual peak width as a result of the separation itself. Since processes, which have a negative influence on the separation system can now be isolated, the number of plates is a realistic and therefore a real value. This is illustrated in Fig. 1, where the conventional plate number is compared to its real value.

It is obvious that the theoretical and effective plate number are substance-dependent variables. This is due to the fact, that the width of the starting peak — which has no relation to separation process — was included in the calculation. Fig. 1 also demonstrates that a quantitative evaluation of the width of the starting peak is always possible, and it is unimportant whether or not a starting peak actually exists in a chromatogram. It is logical that a substance cannot be eluted before the hold-up time t_m . Therefore an evaluation of the peak width is meaningful

only at or behind the chromatographic position t_m . This can be done by extrapolation of 4 to 8 other peaks, which possess a measurable net retention time. Here it is insignificant whether the first peak actually elutes near t_m or is broadened by "overloading" of the column

Fig. 1

- (1.1) Peak width at half peak height versus gross retention time t_{ms} . The theoretical plate number n is different for each peak and is listed for peak 3 only.
  - (1.2) same as (1.1), the effective plate number is different for each peak and is listed for peak 3 only.
  - (1.3) same as (1.1), the real plate number is the same for each peak. It can be measured at any location in the chromatogram, after the completion of the regression plotting (straight line between peak width values 1–4 as shown) and determination of the starting peak width b_0 .
- In contrary to Fig. 1.1 and Fig. 1.2, the time axis is divided in multiples of the hold-up time t_m , which is equivalent to a k -scale. At $k = 30$, e.g., $30 + 1\ t_m$ -units were passed.
- The determination of the b_0 -value is demonstrated even more explicitly in Fig. 2.

(solvent peak). Most important is b_0 , the value of the starting peak width, extrapolated to $k = 0$ at exactly t_m . The numerical value of b_0 can be obtained by plotting (as shown in Fig. 1) or by a regression calculation. After many discussions with colleagues, it became obvious, that the particular form in which the dynamic-chromatographic processes are presented in this article, may be unfamiliar to the reader or could be easily misunderstood. Therefore it seems to be helpful to explain the details in Fig. 1, which are the basis of the new chromatographic evaluation, but on the other side, have the disadvantage to freeze certain experimental conditions: e.g., it can not be concluded from the figure, that the slope of the line – peak width versus retention time – is strongly dependent on the gas flow (in gas chromatography) and that the starting width b_0 is dynamically variable. At very low gas flows, b_0 is large, it decreases strongly with increasing gas flow and approaches a smallest value.

This behavior allows the following conclusions:

The starting peak width, which is important for further evaluations is a measure for the real width of the sample applied via the dosage system (expressed as volume), for the interconnections and separation system. The starting width expresses also the time dependent broadening

$$\frac{t_{s3}}{b_3} \neq \frac{t_{s5}}{b_5}; \quad \frac{t_{ms3}}{b_3} \neq \frac{t_{ms5}}{b_5}$$

$$\star \text{ but } \frac{t_{s3}}{b_3 - b_0} = \frac{t_{s5}}{b_5 - b_0} = \frac{t_{s4}}{b_4 - b_0} = \frac{t_{s6}}{b_6 - b_0} = \dots$$

Fig. 2

- Determination and meaning of b_0 . If the average values of the peak widths are reduced by b_0 , the ratio of all net retention times to the – now corrected – b -values is constant and independent of their position on the time axis. This is the basis of the real plate number n_{real} .

Fig. 3

- The evaluation of this capillary chromatogram is critical, because of the very small peaks (peak 1 has a half height width of only 0.5 mm). The evaluation shows, that the linear relation between peak width at half peak height and retention time is still valid at $k = 60$, i. e., net retention time = $60 \times$ hold-up time. Correct b_0 and b_{10} -values depend much on an accurate plotting. The plotted interpolation line must correspond to the mathematically correct linear regression line, where the sum of the mean square deviations of the b_0 -values is a minimum (calculation is far better than the eyeball method).

(outside of the separation system), caused by the volume and the flow characteristics of the detector. Naturally, these are time dependent values, and by no means a linear expansion. Therefore b_0 is not related to the actual width of the air peak, as it would be observed, when a certain volume of air is dosed. However, under the certain experimental conditions, it may happen, that the starting peak width b_0 and the air peak width are identical.

b_0 is not the result of one measurement with one single substance, it is determined from the broadening of all peaks by plotting or preferentially by extrapolating to the "zeronet retention time", which is the abscissa-value at $k = 0$ (Fig. 1).

Therefore, the b_0 -value is highly reliable. Its precision increases with the number of peak width values ($b_{0.5}$ -values of all peaks with $k > 0$) which can be connected by a line. In case it is a straight line, the precision of b_0 can be calculated and expressed as mean standard deviation ($\pm s_{b_0}$) for the intercept of the straight line with the origin (a detailed discussion will follow.) Thus, the b_0 -value is the basis for the unambiguous determination of other fundamental chromatographic evaluation factors. Fig. 1 represents genuine, experimental data which was obtained from a 3.2 m packed column at an extremely high gas flow (inlet pressure: 16 bar). A similar diagram is shown in Fig. 3, a 20 m long capillary was used, operated under standard conditions. The data was obtained

through measurements over an extremely wide range of different pressure and flow conditions, which go far beyond the scope of a standard chromatographic analysis. In all the cases (extreme and standard chromatographic conditions) a linear increase of $b_{0.5}$ with respect to time was observed. Therefore the ratio of net retention time (or the ratio of $k = t_s/t_m$) to the peak width minus the starting peak width b_0 , is a constant (constant for a defined gas flow, at a certain temperature, on a particular column). This fact allows important conclusions, thus the axis-values in Fig. 1–3 will be explained in detail. **x-Axis:** two different units were selected: k -units or multiples of t_m (hold-up time) and the time units for t_{ms} respectively t_s in minutes. The value t_{ms} represents the gross retention time, or the analysis time, which is the sum of the retention time of the substance in the mobile phase and stationary phase. This gross value is the actually measured value. However, only the retention is important for the separation, i. e., the difference between gross retention time t_{ms} and the system dependent gas hold-up time t_m . The latter can be characterized as the retention time in the mobile phase. In GC t_m is approximately the same for all substances (this is not true in liquid chromatography). Most useful is the ratio of net retention time to hold-up time which is usually addressed as k' (English literature) or k (German literature). It is calculated according to the equation: $k = t_s/t_m$.

The k -value is of fundamental importance in the evaluation of a chromatogram. However, errors are made easily in the determination. Incorrect t_m -values, cause equally wrong k -values but the n_{real} -value is not influenced, which is another advantage of the new evaluation factors. An incorrect value for k has a wide ranging effect on b_0 , which in turn has consequences for all the other derived chromatographic characteristics. It is possible to measure k correctly.

y-Axis: the peak width at half peak height is plotted at a scale, enlarged by a factor of 10–50. The data in Fig. 3 were obtained from a 20 m glass capillary column. The increase of the peak width is strongly linear over wide k -range.

In discussions and seminars, this fact was often questioned. However, systematic examination of very many chromatograms from all areas of chromatography by means of linear regression (with the aid of pocket computers) resulted in quantitative data which confirmed this finding: in many experimental cases, the increase of the peak width is strongly linear with respect to the time.

A measure for the linear relation is the regression coefficient r^2 . If it assumes a value of 1.000, all measuring points are located on a straight line. A value of 0.9999 would indicate an extremely good linear relation between peak width and time. Unfortunately the application of linear regression requires a certain amount of calculation, which is time consuming, unless a pocket calculator is available that can produce the absolutely necessary values of r^2 , $\pm s_a$ and $\pm s_b$. Even if pocket computers are not available, linear regression is indispensable for the evaluation of e. g., calibration curves.

The increase of $b_{0.5}$ with respect to k (or t_s) is linear if:

- a) the column is not overloaded,
- b) substances are selected with a symmetrical elution pattern,
- c) the analysis is carried out isothermal at medium temperature,

i. e., at a temperature range near the boiling point of substances with a k -value of approximately 10. Some substances display a significant broader width $b_{0.5}$, than one might expect from their k -values. For example, chair or boat conformation of cyclohexane derivatives have a substantial influence on the correct relation of peak width and the location of the peak in the chromatogram. This fact is important for a qualitative analysis and will be discussed in part 3.

Increasing polarity differences between sample and stationary phase (in GC), usually result in a non-linearity, i. e., a stronger variation of the data is observed in the linear regression. This will be discussed more detailed in part 3.

It is often questioned how the peak broadening should be measured: by means of $b_{0.5}$ (the width at half peak height) or at a different peak height?

In practice, b is always measured at half peak height. This is the most economical way and leads to correct values. If the peak shape would resemble a Gaussian curve (only

after a questionable correction of the base of the peak), the peak width would be calculated

at 88.2 % of the peak height ($b = 1 \sigma$) (σ , the standard deviation is one of the three basic characteristics of Gaussian curves).

at 60.7 % of the peak height ($b = 2 \sigma$)

instead at 50 % of the peak height ($b = 2.354 \sigma$) $\sqrt{8 \ln 2} \cdot \sigma$
or

at 13.4 % of the peak height ($b = 4 \sigma$).

Since the square of the width, measured at half peak height, is divided by 5.55 ($8 \ln 2$) all the requirements are fulfilled: those of the theory, practice and logic. The large number of data and the representation in Fig. 1 underline the significance of the first new evaluation factor, as part of a new class of gas chromatographic values.

A. The real plate number n_{real}

$$n_{\text{real}} = \left(\frac{\text{any } t_s}{\text{any corresponding } b_{0.5} - b_0} \right)^2 \times 8 \ln 2$$

In view of the other evaluation factors, it seems appropriate to select the mean, interpolated value for the peak width of a substance which elutes at $k = 10$. Since the peak width is independent of the nature of the substance and its position in the chromatogram, we can formulate:

$$n_{\text{real}} = \left(\frac{t_s(k=10)}{b_{10} - b_0} \right)^2 \cdot 8 \ln 2$$

$t_{s(10)}$ can be replaced by the actual time value. The substance at $k = 10$ is eluted with a net retention time $t_s = 10 \times t_m$, because $t_s/t_m = 10$ and therefore $t_{s(10)} = 10 \times t_m$. The final equation for the real number of plates is expressed as:

$$n_{\text{real}} = \left(\frac{10 \cdot t_m}{b_{10} - b_0} \right)^2 \cdot 8 \ln 2 \quad \text{or} \quad n_{\text{real}} = \left(\frac{t_m}{a} \right)^2 \cdot 8 \ln 2 \quad (1)$$

as $b_{10} = 10 \cdot a + b_0$ in $b_{0.5} = a \cdot k + b_0$.

This expression implies that:

1. the t_m -determination must be correct, because all the other evaluation factors depend on it.
2. the b_{10} -value is the result of a regression calculation or plotting; $b_{0.5} = a \cdot k + b_0$;

In terms of the measuring technique, the real number of plates is determined, completely independent of the substance, i. e., it is the result of a genuine measurement consisting of an

average value	(root mean square)
standard deviation	(s_a , see below)
number of measurements	(= number of evaluated peaks).

Only under these conditions, is an unambiguous evaluation of the quality of a column or capillary possible. This should be understood as criticism on the conventional evaluation factors $n_{\text{effective}}$ and $n_{\text{theoretical}}$.

Furthermore, n_{real} contains another term, which can be calculated without difficulty: the mean value obtained from the straight line: $b_{0.5} = a \cdot k + b_0$. This is another

example that the constant is substance independent, influenced only by the temperature, and the p_i/p_0 ratio as well as the inlet pressure p_i of the column.

Evaluation of the separation capability

n_{real} describes the quality of a column, independent of the substance and free of any negative influence by the connection and dosage technique. It will be demonstrated, that the separation capability, which is determined by the quality of the column, is diminished by the fact that the dosage requires a certain time, as well as by the installation technique, connections and detector volume.

The evaluation of a separation requires at least two substances. The uncorrected and therefore real peak width will be the basis for the evaluation of the separation between the two substances. It would be advantageous to have some sort of regulation on the choice of the substances used for the evaluation of the separation.

I would like to propose the selection of a substance at $k = 0$ and at $k = 10$. The choice of such a section in the chromatogram may appear arbitrary. However, in part 3, the exact relation will be derived, which shows that peaks of all sections of the chromatogram can be used to measure the separation capability.

However, if one agrees to select the chromatographic range of $k = 0$ to $k = 10$ as evaluation basis, the following arguments are valid:

- any separation begins at $k = 0$
- all rapid separations are in the range $k = 0$ up to $k = 10$
- most of the routine GC is done in this range
- it represents a decimal system
- the selection of hypothetical substances at $k = 0$ and $k = 10$ implies that the separation capability is determined in the same way as the number of plates: practically neutral, substance-independent and therefore generally applicable. The calculation uses the separation distance ($10 t_m$ -units) and the corresponding peak width (the peak width at $k = 0$ and $k = 10$).

The number of peaks, which can be accommodated within these $10 t_m$ -units will provide a meaningful evaluation number. The number of base-to-base separated peaks will be called "separation number-real" (TZ_{real}). This value represents the separation capability of a column (or capillary) in an accessible and unambiguous form.

B. The evaluation factor for the separation capability: the separation number TZ_{10} is an approximation to TZ_{real}

In the first approximation, the separation number can be expressed as:

$$TZ_{10} = \frac{10 \cdot t_m}{b_{10} + b_0} - 1 \quad (2)$$

The data, necessary for this calculation, are identical with those used for the determination of the real number of plates.

TZ_{10} corresponds to the number of peaks which at least can be completely separated between $k = 0$ and $k = 10$.

A complete separation means: a 4.7-sigma-separation for two adjacent peaks, with an 1.5 % overlapping at the base of the peaks. This degree of overlapping, does not have any influence on the correct determination of the position or the area of a peak, therefore it is appropriate for qualitative and quantitative chromatographic analyses. In a 4.7-sigma-separation, the peak maxima are separated by 4.7 times the distance of the half peak width at 60.7 % of the peak height.

The 4.7-sigma-separation corresponds to a resolution of 1.0, i. e., a baseline separation of two symmetrical peaks of equal size. If the column is overloaded or the main peak contains some minor components, which also need to be separated, a 4.7-sigma-separation is not sufficient. In these cases, the general expressions for all evaluation factors cannot be applied. It should be emphasized once more, that equation (2) is only an approximation, which yields a smaller number of peaks than actually could be accommodated effectively between $k = 0$ and $k = 10$. The exact relation can be expressed as:

$$TZ_{\text{real}} = \frac{\log \frac{10a + b_0}{b_0} - 1}{\log \frac{\sqrt{\frac{n_{\text{real}}}{8 \ln 2}} + 1}{\sqrt{\frac{n_{\text{real}}}{8 \ln 2}} - 1}} - 1 = \frac{\log \frac{10a + b_0}{b_0} - 1}{\log \frac{t_m + a}{t_m - a}} - 1 \geq TZ_{10}$$

TZ_{10} approaches the exact value with increasing quality of the column. Therefore, TZ_{10} never assumes values higher than the actual number. Besides listing the number of completely separated peaks between $k = 0$ and $k = 10$, the separation number records also the necessary time for the separation. This allows a convenient evaluation of the separation power of a column or capillary; TZ_{10} -peaks require a separation time of $(10 + 1) \times t_m$ seconds or minutes.

C. Evaluation number for the separation power of a chromatographic system: the Separation power number TZt

The separation time for TZ_{10} -peaks is exactly $(10 + 1) \times t_m$ therefore:

$$TZt = \frac{TZ_{10}}{11 t_m}, \text{ more precise: } \frac{TZ_{\text{real}}}{11 t_m} \quad (3)$$

TZt is measured in peaks per minute or second. However, there are certain objections against this value. In every chromatographic technique the separation power declines from second to second. Effects, which have a negative influence on the separation, increase with analysis time and counteract the separation power which is invariably determined by the choice of the stationary phase.

The measurement of the separation power is therefore only possible if time and chromatographic distances are defined. It is proposed here, to use a separation distance of $10 t_m$ -units and a separation time of $(10 + 1) \times t_m$.

Such a definition is advantageous, not only for a comparison of methods, but also for the optimization of a

chromatographic analysis with respect to the separation power. It is important to know that an optimized separation capability (maximum separation number TZ_{10}) does not mean a maximum separation power (number of separated peak per time).

An optimum, determined by means of the above equations represents the technically possible maximum and is therefore real.

The adjustment of temperature and gas flow to a TZ_t -optimum (in isothermal and isobar gas chromatography) guarantees the maximum separation power which can be obtained from a system with a given column, installation method, dosage technique, interconnections and detector. If the separation power is insufficient, the instrumental conditions have to be changed, e. g., the dosage system and/or interconnection system or different conditions or a different column.

The influence of factors outside of the column, can be measured quantitatively. The large contribution of these effects to the overall performance of a separation system is surprising. Therefore, a selective determination of these factors, which is the first step toward their elimination, is extremely valuable for the chromatographer. This is the key for the successful application of systems with a high separation power such as micro packed columns, short columns, thin film capillaries or thin layer capillaries.

In the following, these basic factors will be derived and combined with the earlier mentioned evaluation number.

D. Combination of the evaluation factors n_{real} and TZ_{10}

The equations for both numbers are solved for $10 t_m$

$$10 t_m = \sqrt{n_{\text{real}}} \cdot \frac{1}{\sqrt{8 \ln 2}} \cdot (b_{10} - b_0)$$

$$10 t_m = (TZ_{10} + 1) \cdot (b_{10} + b_0).$$

thus:

$$TZ_{10} + 1 = \sqrt{n_{\text{real}}} \cdot \frac{1}{\sqrt{8 \ln 2}} \cdot \frac{(b_{10} - b_0)}{(b_{10} + b_0)}$$

or

$$TZ_{10} = \frac{b_{10} - b_0}{b_{10} + b_0} \cdot \sqrt{\frac{n_{\text{real}}}{8 \ln 2}} - 1. \quad (4)$$

n_{real} can also be formulated as L/h_{real} . This expresses the influence of column length (capillary length) and quality of the column packing (stationary phase coated on the capillary) on the separation.

However, the separation is also dependent on the value of the expression $\frac{b_{10} - b_0}{b_{10} + b_0}$. This factor diminishes the separation capability, if it becomes smaller than 1.000. This is always the case when b_0 is larger than zero. It is obvious that b_0 cannot be zero, and it might be interesting to examine the separation capability when the b_0 -values and 1 %, 5 %, 20 % or 50 % of b_{10} . These values are summarized in Table 1, the strong influence of the expression $\frac{b_{10} - b_0}{b_{10} + b_0}$ is characterized as utilization factor Q_s .

It accounts for all effects outside of the column, which have a negative influence on the separation to Q_s .

Table 1. The influence of Q_s on the separation capability.

$b_0 =$	$Q_s =$	decrease of separation capability =
1 % of b_{10}	0.98	2 %
2 % of b_{10}	0.96	4 %
5 % of b_{10}	0.90	10 %
10 % of b_{10}	0.82	18 %
20 % of b_{10}	0.67	33 %
50 % of b_{10}	0.33	66 %

If equation (4) is rearranged, Q_s can be formulated as the deficiency factor, (expressed in percentage) which diminishes the separation capability of a good column or capillary. Q_s -values of 0.98 or higher (the system operates with 98 % of the possible separation capability) are preferable. However, the use of capillaries results often in Q_s -values between 0.8 and 0.5, which is equivalent to a 20–50 % loss of separation capability. Both Q_s and b_0 should be understood as negative dynamic evaluation factors. It is important to know that the Q_s optimum does not coincide with the maximum of separation capability, separation power and the real number of plates.

Q_s can be improved, if the installation, dosage and transfer technique is improved. The Q_s -value is not dependent on the quality of the column (although it is calculated from the b_{10} -value). For example, Q_s can be increased if the sampling is done under non-isothermal conditions. This is the reason Grob's [3] dosage technique in capillary-gas chromatography – often misquoted as splitless injection – is so successful.

Q_s is therefore an important evaluation factor used to improve instrumental conditions, if the full separation power of a column or capillary – measured as n_{real} or h_{real} – is to be obtained.

A final formulation of the previous discussed evaluation factors is given below.

$$TZ_{10} = Q_s \cdot \sqrt{\frac{n_{\text{real}}}{8 \ln 2}} - 1$$

$$TZ_{10} = Q_s \cdot \sqrt{\frac{L}{h_{\text{real}} \cdot 8 \ln 2}} - 1$$

$$TZ_t = \frac{0.91}{b_{10} + b_0} - \frac{0.091}{t_m} = \frac{0.91}{10 \cdot a} - \frac{0.091}{t_m} \quad (5)$$

$$TZ_t = \frac{1}{11 t_m} \left(Q_s \cdot \sqrt{\frac{n_{\text{real}}}{8 \ln 2}} - 1 \right)$$

The correct calculation of t_m , b_0 , b_{10} or a is crucial for the application of these evaluation factors.

Furthermore it is necessary to know the length of the separation system L (in mm), the inlet pressure p_i or the mobile phase flow F_0 , measured at the end of the column. t_m , b_0 and b_{10} or a are calculated from k and from the measured values of t_{m_s} and $b_{0.5}$. Errors in this measurement will strongly effect Q_s and consequently TZ_{10} or TZ_{real} and TZ_t . In the following, the acquisition and processing of data will be discussed in detail.

Experimental procedure

• Requirements

1. The chromatographic analysis is carried out under isothermal and isobar conditions.
2. Chart speed, recorder and detector response are adjusted in such a way, that there is no alteration of the peak position and peak shape. The precision of the measurement is at least 0.5 seconds, 0.1 seconds can be achieved. It is desirable, that at least four different peak widths are measured with a precision of 15 % rel. or better,
3. The column is not overloaded.
4. At least 5–7 symmetric peaks are obtained.
5. A minimum of 4–8 peaks are suitable for evaluation.
6. All requirements to measure or calculate the hold-up time t_m are met.

The impression may exist that these requirements impose some restrictions on the application of the above discussed evaluation method. However, a meaningful and correct evaluation is only possible on the basis of correct data.

A recorder, with a response time of 250 milliseconds and a chart speed of 1 mm/s should be used for these measurements. Magnifying glasses are used for the correct determination of the peak width (e. g. from the left side of the pen trace to the left side of the pen trace or vice versa).

A test mixture, which fulfills the above mentioned conditions 3–6 can be easily found.

The reliability of this evaluation method, which allows optimum operation of a separation system, justifies expenses such as a good separation column, a recorder with above mentioned characteristics and the analysis time.

• Instructions for measurement and calculation

- The exact position of the injection has to be marked on the chart paper; the chart speed is set to 60 mm/min. The motor is turned on, at the moment of dosage. Both hands are needed, or the help of an assistant.
- After the chromatogram is obtained, list the data in form of a table:

t_{ms1}	$b_{0.5(1)}$ (precision: 1 %)	t_{ms} (mm)	$b_{0.5}$ (mm)
t_{ms2}	$b_{0.5(2)}$	47.3	0.44
t_{ms3}	$b_{0.5(3)}$	52.6	0.50
		90.7	1.00!!
		92.7	0.95
		103	1.1
		135.3	1.45
		236	2.3
t_{ms8}	$b_{0.5(8)}$	553	5.55

chart paper speed. 100 mm = 10 min.

- Measure or calculate t_m according to appendix 1:
Example, $t_m = 8.5$ (calculation of t_m with HP-65, HP-97 according to appendix 3)
- Calculate k 1 to k 8, using the equation:
 $k = (t_{ms} - t_m)/t_m$. Example: $k = (553 - 8.5)/8.5 = 64.06$

- Plot $b_{0.5}$ versus k according to Fig. 3 or carry out a linear regression calculation.
- Calculate or measure b_0 (for the calculation use program HP-97 in appendix 2), the measurement of b_0 is shown in Fig. 2, use a large scale for the $b_{0.5}$ -values.)
- Calculate or measure b_{10} (same as above, Fig. 2 respectively appendix 2), calculation of a is dense and $b_{10} = 10 \cdot a + b_0$.
- Express the length of the column in millimeters, example: $L = 20\,000$ mm.

Example: from previous calculation we know:

$$t_m = 0.50 \text{ mm} = 0.85 \text{ min.}$$

$$b_0 = 0.0975 \text{ mm,}$$

$$b_{10} = 0.9477 \text{ mm}$$

- Calculate n_{real} , h_{real} , TZ_{10} , TZt , Q_s
 $n_{\text{real}} = (8.5 \cdot 10 : (0.94776 - 0.0975))^2 \cdot 8 \ln 2 = 55\,366$
 $h_{\text{real}} = 20\,000 : 55\,366 = 0.36 \text{ mm}$
 $TZ_{10} = ((8.5 \cdot 10) : (0.94776 + 0.0975)) - 1 = 80.32$
 $TZt = 80.32 : 11 \cdot 8.5 \cdot 0.1 = 8.6 \text{ Peaks/min}$
 $Q_s = (0.94776 - 0.0975) : (0.94776 + 0.0975) = 0.813$

• Evaluation and control of the data

$$\text{Requirement: } TZ_{10} = Q_s \cdot \sqrt{\frac{n_{\text{real}}}{8 \ln 2}} - 1$$

$$\text{Control: } 80.32 = 0.813 \cdot \sqrt{\frac{55366}{8 \ln 2}} - 1 = 81.345 - 1 = 80.35$$

• Evaluation of the data

$Q_s = 0.813$ Only 81 % of the available separation capability (55366 plates) are used. The examined separation system consists of a 0.33 mm glass-thin film capillary by H and G. Jaeggi, mobile phase: hydrogen, stationary phase: squalane, length: 20.0 m. With a plate height of $h_{\text{real}} = 0.36$ mm the examined capillary is excellent. This value is 91.7 %

$$\left(\eta = \frac{0.33}{0.36} \cdot 100 = 91.7 \% \right) \text{ of the theoretically possible plate height.}$$

$TZ : 80.3$ peaks between $k = 0$ and $k = 10$. This result is very good, considering the high separation capability of over 55 000 real plates and the still acceptable dosage quality $Q_s = 0.81$. Because of the short hold-up time, the separation power is remarkable.

$TZt : 8.6$ An average value of 8.6 peaks per minute can be considered excellent.) Such an evaluation is possible only if enough data are available for a comparison especially when an attempt is made to find the maximum separation capability and the different maximum of the separation power.

Reliability of the evaluation method

How reliable are the evaluation factors? This question can only be answered by means of statistics.

The standard evaluation factors, if applied correctly, represent the mean standard deviation for the slope of the straight line, obtained from the $b_{0.5}$ and k -values. The mean standard deviation for b_0 and consequently the precision of Q_s can be calculated. Now it is possible to determine whether it was correct to discard inconsistent data, e.g. the peak width $b_{0.5} = 1.00$ of the peak at $t_{ms} = 90.7$. Although the plot in Fig. 3 shows peak 3 much out of the line, the statistic calculation reveals that the deviation is not significant (a more detailed discussion is found in part 3).

Data for the linear regression

Initial data: (according to appendix 2)

No.	$b_{0.5}$	t_{ms}	t_s	k	
1	0.44	47.3	38.8	4.56	n-Decane
2	0.50	52.6	55.1	5.188	5-Nonanone
3	1.00!!	90.7	82.2	9.671	2-Propylcyclohexanol
4	0.95	92.7	84.2	9.91	2-Propylcyclohexanol
5	1.1	103	94.5	11.12	n-Undecane
6	1.45	135.3	126.8	14.92	Naphthalene
7	2.3	236	227.5	26.76	n-Dodecane
8	5.55	553	544.5	65.06	n-Tridecane

The following data was obtained through linear regression:

peak number	$b_{0.5}$ measured	$b_{0.5}$ with peak 3	average without peak 3	regression with peak 3	coefficient without peak 3
1	0.44	0.499	0.485	1.000	1.000
2	0.50	0.55	0.5386	0.9998	
3	1.00	0.932	0.919	0.9893	0.9999999
4	0.95	0.95	0.94	0.9925	0.9985
5	1.1	1.05	1.04	0.9952	0.9993
6	1.45	1.38	1.37	0.9899	0.992
7	2.3	2.38	2.37	0.9987	0.99899
8	5.55	5.54	5.54		

If the peak 3 is included in the calculation, the b_0 and b_{10} values are 0.1121 and 0.9599, respectively, i.e., $Q_s = 0.813$. Without peak 3: $b_0 = 0.0975$ and $b_{10} = 0.9478$. The mean standard deviation for the slope of the regression line (including peak 3) is $s_a = \pm 0.0021$, which is $\pm 1.4\%$ of its value. The mean standard deviation for b_0 (without peak 3) is $s_{b_0} = \pm 0.0332$ which represents $\pm 29\%$ of its value. If peak 3 is discarded from the calculation, $s_a = \pm 0.022$ ($\pm 1.4\%$ of its value) and $s_{b_0} = \pm 0.0319$ ($\pm 32\%$ of its value).

These statistical calculations demonstrate that more data is necessary, before any conclusion on peak 3 can be reached (e.g., the peak width of peak 3 is significantly wrong) or compound 3 behaves like a run a way and may be excluded. However, according to the regression coefficient r^2 , the linearity is slightly better without peak 3. In any case, n_{real} is obtained relatively accurate

($\pm 1.5\%$ of its value) where b_0 is recorded with only $\pm 30\%$ of its value. This fact is consistently observed in linear regression: the slope can be measured accurate, however, the axis values are spread. The data, which was used in this particular case, is especially critical, because a peak width of approximately 3 mm has to be measured with a precision of 0.1 mm. The chart speed was too low, furthermore, this was an evaluation of a capillary column. An evaluation by means of n_{eff} would hardly be possible in such a case.

Comparison of the results with the separation number 100 (TZ_{100})

The separation number TZ_{100} lists the number of peaks which can be separated within a retention index range of 100 units. In contrast to TZ_{10} which can be determined only once in a separation system, TZ_{100} can be measured several times in a long chromatogram. TZ_{100} is characterized by a number, which refers to the carbon-atom range for which it was measured. The exact formula for TZ_{100} depends on the polarity of the separation system, as shown in the following equation:

$$TZ_{100,real} = \frac{\log \frac{a \cdot k_2 + b_0}{a \cdot k_1 + b_0}}{\log \frac{\sqrt{\frac{n_{real}}{8 \ln 2}} + 1}{\sqrt{\frac{n_{real}}{8 \ln 2}} - 1}} - 1$$

where k_2, k_1 represents the k -data of the two substances to be separated. TZ_{100} , an approximation to the real $TZ_{100,real}$ -formulae can be calculated easily, because the linear regression allows an accurate determination of the peak widths of n-alkanes. In the above example, the following peaks represent n-alkanes:

Peak 1 n-decane $b_{0.5}$ average: 0.485
 5 n-undecane $b_{0.5}$ average: 1.04
 7 n-dodecane $b_{0.5}$ average: 2.37
 8 n-tridecane $b_{0.5}$ average: 5.54

and the approximation formulae $TZ_{100} = \frac{\Delta t_{21}}{b_2 + b_1} - 1$,

where $\Delta t_{21} = t_{alkane_1} - t_{alkane_2}$ and b_2, b_1 are the respective peak width of the alkanes 2 and 1 used.

The separation numbers have the following values:

$$TZ_{100} \text{ between } C_{10} \text{ and } C_{11} : TZ_{100} = \frac{103 - 47.3}{1.04 + 0.485} - 1 = 35.5$$

$$TZ_{100} \text{ between } C_{11} \text{ and } C_{12} : TZ_{100} = \frac{236 - 103}{2.37 + 1.04} - 1 = 38.0$$

$$TZ_{100} \text{ between } C_{12} \text{ and } C_{13} : TZ_{100} = \frac{553 - 236}{5.54 + 2.37} - 1 = 39.1$$

Usually TZ_{100} is smaller than half of TZ_{10} .

The separation number 100 requires n-alkanes as a test mixture (or other homologues, which results in somewhat different TZ_{100} -values) whereas TZ_{10} is an universal number, because it is independent of the detector and

Fig. 4

- 1. Maximum of real plate number
- 2. Maximum of the separation number (TZ_{10})
- 3. Maximum of the separation power (TZ_t , at a flow of $12 \text{ cm}^3/\text{min}$ and a pressure of 7 bar, the optimum of the 2.7 m micro packed OV 101 column is not yet reached). Theoretical and effective plate numbers are also listed for the C_7 -hydrocarbon.

Table 2. Data for the optimization of a 2.7 m, micro packed glass column OV-101/silica gel Merck, particle size: 0.07–0.1 mm.

P_i = inlet pressure (in bar N_2),

F = gas flow in cm^3/min , 20°C , 760 torr,

t_m = hold-up time in seconds,

b_0 = starting peak width in seconds (obtained through linear regression, appendix 2)

b_{10} = peak width of the peak at $k = 10$ in seconds, (obtained through linear regression, appendix 2),

n_{real} and the other evaluation factors: see text.

r^2 = regression coefficient from linear regression. Some of the data in this table is presented in Fig. 4 for a better comparison.

P_i (bar N_2)	F (cm^3/min)	t_m (s)	b_0 (s)	b_{10} (s)	n_{real}	h_{real} (mm)	Q_s	TZ_{10}	TZ_t (Peaks/min)	TZ_{100} (C_6/C_7)	$n_{\text{theor.}}$	n_{eff}	r^2
0.8	0.47	238.9	13.006	100.693	4115	0.66	0.77	20.0	0.46	4.8	3280	1820	0.999
1.2	0.74	159.3	6.856	54.548	6182	0.44	0.78	25.0	0.85	6.1	5017	2820	0.999
1.5	0.98	129.9	5.825	40.055	7976	0.34	0.75	27.3	1.15	6.6	5830	3280	0.997
1.8	11.26	111.5	4.701	32.831	8704	0.31	0.84	18.7	1.40	6.9	6440	3590	0.995
2.1	1.58	96.2	3.822	28.431	8462	0.32	0.76	28.8	1.63	7.1	6540	3620	0.999
2.4	1.93	82.3	3.192	23.545	9066	0.30	0.76	29.8	1.97	7.1	7040	3980	0.997
2.7	2.32	76.5	2.673	19.493	11470	0.24	0.76	33.5	2.39	8.3	8730	4820	0.997
3.3	3.19	67.5	2.410	17.129	11662	0.23	0.75	33.6	2.71	8.2	8570	4560	0.997
3.7	3.85	58.6	2.051	14.549	12162	0.22	0.75	34.3	3.19	8.3	8950	4930	0.994
4.0	4.39	49.0	1.717	12.881	11227	0.23	0.75	33.7	3.75	8.1	8950	5180	0.992
5.0	6.32	50.3	1.113	13.25	9541	0.28	0.84	34.0	3.70	9.0	9951	5340	0.999
6.0	8.57	42.3	1.058	11.402	9265	0.29	0.83	32.9	4.25	8.5	9150	4873	0.999
7.0	11.69	34.7	0.787	10.719	6742	0.40	0.86	29.1	4.58	7.6	7550	4062	0.999

the chemical nature of the substance. Nevertheless, TZ_{100} is often used for the evaluation of capillaries, but also depend on b_0 -data, as can be seen in the exact $TZ_{100, \text{real}}$ formula.

It might be mentioned here, that the meaning of TZ_{100} is often misunderstood. TZ_{100} is not related to resolution, because there is no relation between resolution and the 100 retention index units, which are the basis for TZ_{100} .

E. Optimization by means of the new evaluation method

The following conditions are used in the separation:

column: micro packed glass column, 2.7 m long inner diameter: 0.8 mm.

Stationary phase: Methyl-silicon oil OV-101, 4 % wt in CFCl_3 according to the IfC-method: after preheating, filling and homogenizing, the impregnation solvent (CFCl_3 , $B_p = 23^\circ\text{C}$) is removed instantaneously, using vacuum and a temperature of 40°C . After drying, the column is packed under pressure and vacuum by means of an ultravibrator.

Installation method: Separation Cassette IfC.

Instrument: Becker-Packard Typ 409, FiD

Test sample: mostly n-alkanes

Carrier gas: nitrogen

The optimization was carried out isothermally at 80°C , by varying the gas pressure from 0.8–4.2 bar. In the following discussion, the data table will be supplemented by figures. Fig. 4 shows three distinct maxima:

1. The maximum of the real plate number, n_{real} respectively the minimum of the real plate height. Both are dependent on the column filling, temperature and nature of the carrier gas. This maximum is different from the optimum value, obtained by the conventional evaluation method.
2. The maximum of the separation capability is different from the maximum of the real plate number.
3. The maximum of the separation power is completely different from maximum of 1 and 2. The curve in Fig. 4 does not even show the maximum.

The operation of a system at a maximum separation power depends on the particular separation problem (because of a decreased separation capability). However, a minimum of separation capability is always guaranteed by the given selectivity of a column.

Selectivity was not mentioned in this treatise, because only substance independent evaluation factors were discussed and selectivity is strongly dependent on the sample to be separated. A selective column, with good evaluation factors will always be preferred.

It might be interesting to note, that a 2.7 m packed column – optimized correctly – can yield more than 10 000 plates. So far, 16 000 plates/m could be obtained with a micro packed separation cassette (particle size: 0.18–0.20 mm) at a separation number $100\text{ TZ}_{100} = 24$ for C_{11}/C_{12} .

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References

- [1] R. E. Kaiser, Gas-Chromatographie (1959), Monographie, Verlag Geest und Portig, Leipzig 1960. (b_0 in der Trennstufenzahl).
- [2] Many private conversations since 1959, speeches by J. F. K. Huber, Vienna.
- [3] Grob, K., Grob, G., Chromatographia 5, 3 (1972) (direct sample injection).

Appendix 1:

Determination or calculation of the hold-up time t_m

- **Meaning:** The hold-up time is of prime importance for the analysis speed. t_m is part of all fundamental relations, it is the same for all substances, as long as the conditions are not changed. t_m is never zero. t_m is a statistically to be calculated means of the "retention time" of substances with mobile phase.
- **Equation:** $t_m = t_{ms} - t_s$
- **Conditions:** Isobaric, isothermal analysis.
- **Experimental procedure:**
  1. Inject a substance which shows no retention under the given conditions. GC-FID: CH_4 on a "hot" column or N_2 respectively H_2 and an extremely high sensitivity of the FiD, which is operated outside of its optimum. GC-ECD: CH_2Cl_2 in N_2 at temperature higher than 150°C . e. g., analyses of pesticides on partition columns. GC-SID: COS in N_2 at temperatures above 150°C on partition columns. LC-UV detector: injection of O_2 in the mobile phase. At a high sensitivity a signal is produced which can be evaluated. As control, He and air may be injected. If air is separated into two peaks, then the second peak is O_2 . t_{ms} of the N_2 -peak represents the hold-up time.
  2. In GC, calculate t_m according to:

$$\frac{\log \frac{t_{ms3} - t_m}{t_{ms1} - t_m}}{\log \frac{t_{ms2} - t_m}{t_{ms1} - t_m}} = \frac{I_3 - I_1}{I_2 - I_1} = \text{const.}$$

t_m is subtracted iteratively from the experimental value of the gross retention time, until the above equation is fulfilled. Three index-values and the three corresponding gross retention times are necessary. The calculation is carried out with a programable pocket calculator (for more information, see Chromatographia 12, pps. 696–698 (1974). Please note: the program contains an error. The command: running number 50 reads 84 R/S instead

of 24 RTN. A detailed description of the mathematical and experimental methods for the determination of the hold-up time is discussed in Chromatographia 2, p. 219 (1969).

• Application

1. The determination of t_m precedes any qualitative evaluation in GC (under constant chromatographic conditions).
2. k is a universal, qualitative "address" in each chromatographic technique and cannot be determined without t_m .
3. t_m is necessary for any correct data transfer:
LC _____ TLC
TLC _____ LC

Important:

Any system errors or random errors in the t_m -determination will change the qualitative data. However, an evaluation based on the retention index in isothermal GC is less sensitive to a slightly incorrect t_m . The measurements should be carried out with a precision of 0.1 seconds.

Appendix 2:

Linear regression with the aid of pocket calculators

- **Equations:** see textbooks for statistical mathematics.
- **Application:** The regression coefficient, the mean standard deviation of the slope and axis values are mandatory for the evaluation. Calculators, which do not allow the immediate determination of r^2 (regression coefficient), s_a and s_{b0} should not be used. The following programs are described in detail. Repeated application will make the chromatographer more familiar with this highly informative mathematics.
This program needs more powerful calculators as HP-65 or Texas Instrument SR-52.
This program allows a very elegant solution of the problem because of total recordings based on one single magnetic card programming.
Users interested in SR-52 or HP-65-programs may consult the German version of this green pages in Chromatographia 9 (1976) 337.

HP 97 (= HP 67) program for linear regression and calculation of $B = r^2$

n_{real} real plate number
 a slope according to $a \cdot k + b_0 = b_{0.5}$ (seconds)
 b_0 intercept according to $a \cdot k + b_0 = b_{0.5}$ (seconds)
 $\pm s_b$ standard deviation of b_0
 $\pm s_a$ standard deviation of a
 α_r regression angle (angle between the two regression lines)
 TZ_{real} real separation number according to the accurate log-formula
 $Q_s = (b_{10} - b_0) : (b_{10} + b_0) (= 10 : 10a + 2b_0)$
 $TZ/t_{real} = TZ_{real} : 11 t_m$

The program calculates any b from any k

Procedure: switch the calculator
ON, NORM, RUN
feed program, Enter t_m in sec STORE 9
Enter t_m in minutes STORE 2

press f a
 Enter $b_{0.5} 1$ Press A (all data in seconds)
 Enter $t_{ms} 1$ Press D (all data in seconds)
 :
 :

Continue until N pairs of data have been entered. Press C, wait. The HP 97 prints the data according to the copy printed. When HP 67 is used all data are stored according to the copy of "REG", see "print out". Prior any next calculation press f a.

When used for linear regression only, please use this steps:

f a
 Enter y_1 Press A Enter x_1 Press B
 Enter y_2 Press A Enter x_2 Press B
 :
 :
 Enter y_N Press A Enter x_N Press B
 Press C Printout = $r^2 = D$
 a
 b_0
 s_{b0}
 s_a
 α_r

Any further details can be read in the calculators program copy.

The program allows to enter any further pair of data and to recalculate the regression data without "clear data", if the additional data group belongs to the total of the data to be handled by regression.

001	*LBLa	21 16 11	057	X ²	53	113	÷	-24	169	÷	-24
002	P⇒S	16-51	058	RCLC	36 13	114	1/X	52	170	LOG	16 32
003	CLRG	16-53	059	÷	-24	115	RCLD	36 14	171	ENT↑	-21
004	CLX	-51	060	1/X	52	116	÷	-24	172	RCLA	36 11
005	P⇒S	16-51	061	STO1	35 01	117	ENT↑	-21	173	1/X	52
006	DSP5	-63 05	062	PRTX	-14	118	1	01	174	√X	54
007	RTN	24	063	$\bar{x}$	16 53	119	RCLD	36 14	175	RCLB	36 12
008	*LBLA	21 11	064	RCL1	36 01	120	-	-45	176	√X	54
009	ENT↑	-21	065	X	-35	121	X	-35	177	X	-35
010	RTN	24	066	CHS	-22	122	TAN ⁻¹	16 43	178	1	01
011	*LBLB	21 12	067	+	-55	123	PRTX	-14	179	+	-55
012	SF2	16 21 02	068	STOD	35 00	124	F2?	16 23 02	180	ENT↑	-21
013	Σ+	56	069	PRTX	-14	125	RTN	24	181	ENT↑	-21
014	STOI	35 46	070	SPC	16-11	126	SPC	16-11	182	2	02
015	RTN	24	071	S	16 54	127	GTOE	22 15	183	-	-45
016	*LBLC	21 13	072	X ²	53	128	*LBLD	21 14	184	÷	-24
017	P⇒S	16-51	073	RCLI	36 46	129	RCL9	36 09	185	LOG	16 32
018	RCL8	36 08	074	1	01	130	-	-45	186	÷	-24
019	RCL4	36 04	075	-	-45	131	RCL9	36 09	187	1	01
020	RCL6	36 06	076	X	-35	132	÷	-24	188	-	-45
021	X	-35	077	ENT↑	-21	133	Σ+	56	189	STO3	35 03
022	RCL9	36 09	078	ENT↑	-21	134	STOI	35 46	190	PRTX	-14
023	÷	-24	079	$\bar{x}$	16 53	135	RTN	24	191	ENT↑	-21
024	-	-45	080	X ²	53	136	*LBLE	21 15	192	RCL2	36 02
025	ENT↑	-21	081	X⇒Y	-41	137	DSP2	-63 02	193	1	01
026	RCL9	36 09	082	R↓	-31	138	2	02	194	1	01
027	1	01	083	÷	-24	139	LN	32	195	X	-35
028	-	-45	084	1/X	52	140	8	08	196	÷	-24
029	÷	-24	085	RCLI	36 46	141	X	-35	197	PRTX	-14
030	STOC	35 13	086	1/X	52	142	STOA	35 11	198	DSP5	-63 05
031	ENT↑	-21	087	+	-55	143	ENT↑	-21	199	RTN	24
032	ENT↑	-21	088	√X	54	144	RCL9	36 09	200	*LBLd	21 16 14
033	P⇒S	16-51	089	RCLC	36 15	145	RCL1	36 01	201	RCL9	36 09
034	S	16 54	090	X	-35	146	÷	-24	202	-	-45
035	X	-35	091	X	-35	147	X ²	53	203	RCL9	36 09
036	÷	-24	092	STO4	35 04	148	X	-35	204	÷	-24
037	X ²	53	093	PRTX	-14	149	STOB	35 12	205	RCL1	36 01
038	STOD	35 14	094	S	16 54	150	PRTX	-14	206	X	-35
039	PRTX	-14	095	X ²	53	151	RCL1	36 01	207	RCL0	36 00
040	SPC	16-11	096	RCLI	36 46	152	ENT↑	-21	208	+	-55
041	RCLI	36 46	097	1	01	153	RCL1	36 01	209	PRTX	-14
042	1	01	098	-	-45	154	-	-62	210	RTN	24
043	-	-45	099	X	-35	155	2	02	211	*LBLc	21 16 15
044	ENT↑	-21	100	√X	54	156	RCL0	36 00	212	DSP4	-63 04
045	RCLI	36 46	101	1/X	52	157	X	-35	213	SPC	16-11
046	2	02	102	RCLC	36 15	158	+	-55	214	RCL5	36 05
047	-	-45	103	X	-35	159	÷	-24	215	PRTX	-14
048	÷	-24	104	X	-35	160	STO8	35 08	216	RCL1	36 01
049	ENT↑	-21	105	STO5	35 05	161	PRTX	-14	217	÷	-24
050	1	01	106	PRTX	-14	162	RCL1	36 01	218	PRTX	-14
051	RCLD	36 14	107	S	16 54	163	1	01	219	RCL4	36 04
052	-	-45	108	X ²	53	164	0	00	220	PRTX	-14
053	X	-35	109	X⇒Y	-41	165	X	-35	221	RCL0	36 00
054	√X	54	110	X ²	53	166	RCL0	36 00	222	÷	-24
055	STOE	35 15	111	+	-55	167	+	-55	223	PRTK	-14
056	S	16 54	112	RCLC	36 13	168	RCL0	36 00	224	RTN	24

17A.85000 STO9
 30.00000 ÷
 STO2
 5.82833 ***
 GSBA
 .95000 GSBA
 196.00000 GSBD
 1.00000 GSBA
 217.50000 GSBD
 1.30000 GSBA
 261.00000 GSBD
 1.90000 GSBA
 348.00000 GSBD
 3.20000 GSBA
 524.00000 GSBD
 5.00000 GSBA
 879.00000 GSBD
 11.50000 GSBA
 1591.00000 GSBD

1. Application for Chromatography

Switch ON/NORM/RUN

ENTER program from magnetic card

Enter dead time (sec) STO 9

Divide by 60, STO 2

clear part of memories by pressing f a

Enter peak width 1 (sec); press A

enter non adjusted retention time 1 (sec); press D

enter peak width 2, press A

enter non adjusted retention time 2, press D

continue til last pair of data (minimum: three pairs of data) Press C

Printer of HP-97 prints ten data as a list of results (HP-67 stores the data, see list RCL)

without pressing f a

any further pair of $b_{0,5} - t_{ms}$ - data can be entered.

After "press C" an new list of results is printed (HP-97) or stored (HP-67).

Check r^2 , $\pm s_{b_0}$, $\pm s_a$. Qualified results need acceptable high r^2 -data and acceptable low standard deviations for the slope and the b_0 -value. New sets of data need to clear memories by pressing f(a) and entering t_m (sec) STO 9 t_m (sec): 60, STO 2.

HP-97 print out:

1. r^2	HP-67	RCL	D
2. slope a [sec]		RCL	1
3. b_0 [sec]		RCL	0
4. standard deviation s_{b_0}		RCL	4
5. standard deviation s_a		RCL	5
6. regression angle			
7. number of real plates n_{real}		RCL	B
8. Q_s		RCL	8
9. real separation number TZ_{real} according to the logarithmic formula			

$$TZ_{real} = \frac{\log \frac{b_{10}}{b_0}}{\log \frac{\sqrt{\frac{n_{real}}{8 \ln 2} + 1}}{\sqrt{\frac{n_{real}}{8 \ln 2} - 1}}} - 1$$

RCL 3

10. $TZ_{real} : 11 \cdot t_m$		stored in display

Data

```

1. 0.99909 ***
2. 1.32739 ***
3. 0.63574 ***
4. 0.06312 ***
5. 0.01789 ***
6. 0.02500 ***
7. 96216.97 ***
8. 0.91 ***
9. 202.22 ***
10. 3.15 ***

0.0179 ***
0.0135 ***
0.0631 ***
0.0953 ***

```

PREG

```

0.6357 0
1.3274 1
5.8283 2
202.2182 3
0.0631 4
0.0179 5
0.0000 6
0.0000 7
0.9126 8
174.8500 9
5.5452 A
96216.9711 B
11.2078 C
0.9991 D
0.0330 E
7.0000 I

```

P ≅ S

PREG

```

0.0000 0
0.0000 1
0.0000 2
0.0000 3
15.9711 4
87.1005 5
25.6500 6
183.3325 7
125.7695 8
7.0000 9
5.5452 A
96216.9711 B
11.2078 C
0.9991 D
0.0330 E
7.0000 I

```

HP-67 cannot print out the results, therefore recall commands have to be used as indicated in the list of results

Data example:

$t_m = 174.85$ (mm) paper speed = 30 mm/min

data pairs

$b_{0,5}$ (mm)	t_{ms} (mm)
0.95	196.0
1.0	217.5
1.3	261.0
1.9	348
3.2	524
5.8	879
11.5	1591

results

```

r2 = 0.99909
a = 1.32739
b0 = 0.63574 (mm)
± Sb0 = 0.06312 = 1.4 % of a
± Sa = 0.01789 = 9.9 % of b0
αr = 0.205
nreal = 96, 216.97
Qs = 0.91
TZreal = 202.22 (peaks between k = 0 and k = 10)
TZ/t = 3.15 (peaks/min in this range k = 0 til
k = 10)

```

2. Application for simple linear regression:

ON NORM RUN Enter program

Press f(a)

enter y_1 press A

enter x_1 press B B

enter y_2 press A

enter x_2 press B

Continue til last pair (minimum: three pairs)

Press C

GS6a
 1.00000 GSBA
 2.00000 GSBB
 2.00000 GSBA
 4.00000 GSBB
 4.00000 GSBA
 7.90000 GSBB
 Data
 0.99996 ***
 0.50907 ***
 -0.02536 ***
 0.01680 ***
 0.00321 ***
 0.00092 ***

PREG
 -0.02536 0
 0.50907 1
 0.00000 2
 0.00000 3
 0.01680 4
 0.00321 5
 0.00000 6
 0.00000 7
 0.00000 8
 0.00000 9
 0.00000 A
 0.00000 B
 4.58333 C
 0.99996 D
 0.00891 E
 3.00000 I

P $\Rightarrow$ S
 PREG
 0.00000 0
 0.00000 1
 0.00000 2
 0.00000 3
 13.90000 4
 82.41000 5
 7.00000 6
 21.00000 7
 41.60000 8
 3.00000 9
 0.00000 A
 0.00000 B
 4.58333 C
 0.99996 D
 0.00891 E
 3.00000 I
 P $\Rightarrow$ S

HP-97 prints

r^2
 a
 b_0
 $\pm S_b$
 $\pm S_a$
 α_r

stores data to get results

RCL D
 RCL 1
 RCL 0
 RCL 4
 RCL 5
 stored in display

any new pair of data can be entered and calculation can be continued.

Before a new set of paired data press f(a)

Data example

y	x
1	2
2	4
4	7, 9

results

$y = ax + b_0$
 $y = (0.50907 \pm 0.00321) x - 0.02536 \pm 0.0168$
 $r^2 = 0.99996$
 $\alpha_r = 0.00092$ degree

$$y = (a \pm S_a) \cdot x + (b_0 \pm S_{b_0})$$