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Metrics of separation performance in chromatography. Part 1. Definitions and application to static analyses

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ABSTRACT

Earlier introduced metrics of separation performance are described in a systematic way. After providing the definitions of the metrics suitable for a broad variety of applications, the study focuses on static analyses (isothermal GC, isocratic LC, etc.) and their general separation performance. Statistically expected number of resolved (adequately separated) single-component peaks is treated as the ultimate metric of general separation performance of chromatographic analysis. That number depends on the peak capacity of the analysis and the number of components in a test mixture. The peak capacity, in turn, depends on the separation capacity of a column and the lowest separation required by the data-analysis system for resolving poorly separated peaks. The separation capacity is a special case of a broader metric of the separation measure which is a function of column efficiency, solute separability, and the level of the solute interaction with a column stationary phase. The formulae for theoretical prediction of all these metrics for arbitrary pairs of peaks in static analyses are derived. To provide a better insight into the basic metrics of the separation performance, additional metrics such as the solute discrimination (relative difference in solute velocities), utilization of separability (solute discrimination per unit of their separability), specific separation (the separation per unit of separability), and others are defined and found for static analyses.

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1. Introduction

Performance metrics are necessary for quantitative evaluation of the results of chromatographic analyses, and for optimization of chromatographic analyses and systems.

This report is intended as the first installment in a series on performance metrics in chromatography. The report provides an updated review of the earlier introduced metrics [1–8] that have been successfully applied to performance evaluations and optimization of several separation techniques [1–3,8–12].

The goals for this report are to

1. outline a system of mutually compatible metrics of separation performance;
2. compile the definitions of the metrics in one place;
3. provide interpretations of the metrics, illustrate their mutual relations and application to evaluation of the separation performance of static [13] analyses (isothermal GC, isocratic LC, etc.).

Future installments are intended for applications of the metrics to evaluation of the separation performance of temperature-programmed GC and gradient-elution LC.

The subject of the separation performance includes *specific separation performance* and *general separation performance*. The former is concerned with the separation of particular solutes in particular analyses. The latter addresses such issues as the number of peaks that a given analysis or a given separation technique can *resolve* (adequately separate), the number of peaks that can be resolved during a particular time, during a particular event such as a heating ramp in GC or a gradient time in LC, etc.

The metrics reviewed here are suitable for evaluation of specific and general separation performance. However, only the application of the metrics to the **general separation performance** is discussed below. Additional constraints to the applications of the metrics are described below in the **bold face** type.

An outline of this report was presented in a lecture at the 34th International Symposium on Capillary Chromatography (Riva del Garda, Italy, 2010).

Table 1
Changes in some terms and notations.

Original term (symbol) [references]	New term (symbol) [references]
Separation power ($\mathcal{P}$) [8,9]	Efficiency (E) [10,13]
Intrinsic efficiency (E) [8]	Utilization of separability (U_g) [this report]
Interaction level (λ) [5,8]	Immobility, affinity to stationary phase (ω) [this report]

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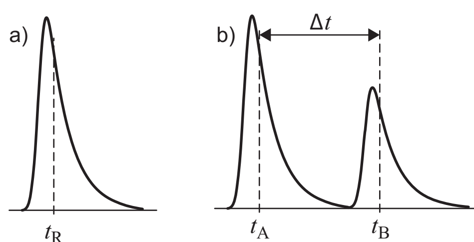

Fig. 1. (a) An asymmetric peak with centroid at t_R. (b) The difference, $\Delta t = t_B - t_A$, in retention times (i.e. in centroids) of two equally wide asymmetric peaks.

During the use of the earlier proposed terminology, it became clear that several terms originally assigned to the proposed metrics were not intuitive. Later, these terms were replaced with more intuitive ones. The most sufficient changes are compiled in Table 1.

2. Theory

2.1. Metrics of separation performance

2.1.1. Peak retention time and width

Retention times and widths of chromatographic peaks are the most basic building blocks of the metrics of separation performance.

Throughout this report, the centroid (the first mathematical moment) of a peak is treated as the peak's retention time (t_R) [13]. The centroid of a symmetric peak coincides with the time-coordinate of its apex. However, this might not be the case for asymmetric peaks as shown in Fig. 1(a).

Also throughout this report, the width of a peak is identified with its standard deviation (σ) (i.e. with the square root of the peak's variance) [13].

2.1.2. Separation measure

Separation (Δs) of two equally wide peaks A and B can be described as [4][1]

$$\Delta s = \frac{\Delta t}{\sigma}, \quad \Delta t = t_B - t_A \tag{1}$$

where t_A and t_B are retention times of the peaks (Fig. 1(b)) and σ is their width. When the peak width (σ) is a function of time then the separation can be defined as [4]

$$\Delta s = \int_{t_A}^{t_B} ds = \int_{t_A}^{t_B} \frac{dt}{\sigma} \tag{2}$$

In the case of a fixed peak width, Eq. (2) converges to Eq. (1).

Generally, quantities t_A and t_B in Eq. (2) do not have to be retention times of two peaks. They can also be arbitrary time markers on the time-axis of a chromatogram. In that case, quantity Δs in Eq. (2) can be viewed as the *separation capacity* [4] of the time interval (t_A, t_B). Both concepts defined in Eq. (2) – the separation of two peaks having retention times t_A and t_B as well as the separation capacity of the time interval (t_A, t_B) – can be collectively called as the *separation measure* of a time interval (t_A, t_B) [4]. Speaking of the separation measure of a time interval, it might be convenient to view it as a union of adjacent non-overlapping σ-long subintervals or σ-slots. According to Eq. (2),

the separation measure of a time interval is the number of adjacent non-overlapping σ-slots in it.

To switch back and forth between the peak separation aspect of the separation measure and its peak capacity aspect, the peak retention times (t_R) and the arbitrary time variable (t) of a chromatographic analysis are treated below as **interchangeable quantities**.

A natural extension of a concept of the separation capacity of an arbitrary time interval is the *running separation capacity*

$$s = s(t) = \int_{t_M}^{t} \frac{dt}{\sigma} \tag{3}$$

of an analysis (or of a chromatogram). This is the separation capacity of the analysis from its *hold-up time* (t_M) – the retention time of the earliest possible peak – up to an arbitrary time t. The separation capacity (s_c) of the entire analysis (entire chromatogram) up to the retention time ($t_{R,last}$) of the last peak can be found as

$$s_c = s(t_{R,last}) = \int_{t_M}^{t_{R,last}} \frac{dt}{\sigma} \tag{4}$$

Eq. (3) can be viewed as a transformation of the *separation space* of a chromatographic analysis (or of a chromatogram) from t-domain into s-domain. In s-domain, the widths of all peaks are equal to 1, the definitions of a column separation performance are generally simpler than they are in t-domain. Thus, the definition (Eq. (2)) of the separation capacity (Δs) of an arbitrary time interval can be replaced with the equivalent definition,

$$\Delta s = s_B - s_A \tag{5}$$

of the separation capacity (Δs) of an arbitrary interval (s_A, s_B) in the s-axis of s-domain.

A plot mode allowing to display a chromatogram in s-domain, Fig. 2, was implemented in several earlier commercial integrators (HP 3393A, HP 3396A, Hewlett-Packard Co., Palo Alto, CA) and was utilized in several research projects [14,15].

2.1.3. Peak capacity

A column separation capacity (s_c) describes a column separation performance in terms of a number of σ-slots in a chromatogram. However, the separation capacity does not describe the number of peaks that a chromatographic analysis can *resolve*, i.e. identify and quantify. That number does depends on the column separation capacity, but it also depends on the ability of the data-analysis system to resolve poorly separated peaks. This property can be quantified by the lowest separation ($\Delta s_{\min}$) required by a given data-analysis system for resolving two peaks. It is typically assumed that 6σ separation ($\Delta s_{\min} = 6$) is necessary for resolving two peaks by a simple data-analysis system. For more powerful data-analysis based on peak deconvolution, $\Delta s_{\min} = 1$ or even smaller $\Delta s_{\min}$ might be sufficient for resolving two peaks [17–19].

Peak capacity (Δn) of an arbitrary time interval (t_A, t_B) is the number of adjacent $\Delta s_{\min}$-long intervals in its separation measure (Δs), i.e. [4]

$$\Delta n = \frac{\Delta s}{\Delta s_{\min}} = \frac{1}{\Delta s_{\min}} \int_{t_A}^{t_B} \frac{dt}{\sigma} = n_B - n_A \tag{6}$$

where

$$n = n(t) = \frac{s}{\Delta s_{\min}} = \frac{1}{\Delta s_{\min}} \int_{t_M}^{t} \frac{dt}{\sigma} \tag{7}$$

is the *running peak capacity*. The peak capacity (n_c) of the entire analysis is

$$n_c = n(t_{R,last}) = \frac{s_c}{\Delta s_{\min}} \tag{8}$$

The peak capacity (n_c) defined in Eq. (8) is conceptually identical (although differently worded) to the peak capacity employed

[1] Previously [4,9,10], this metric was denoted as S.

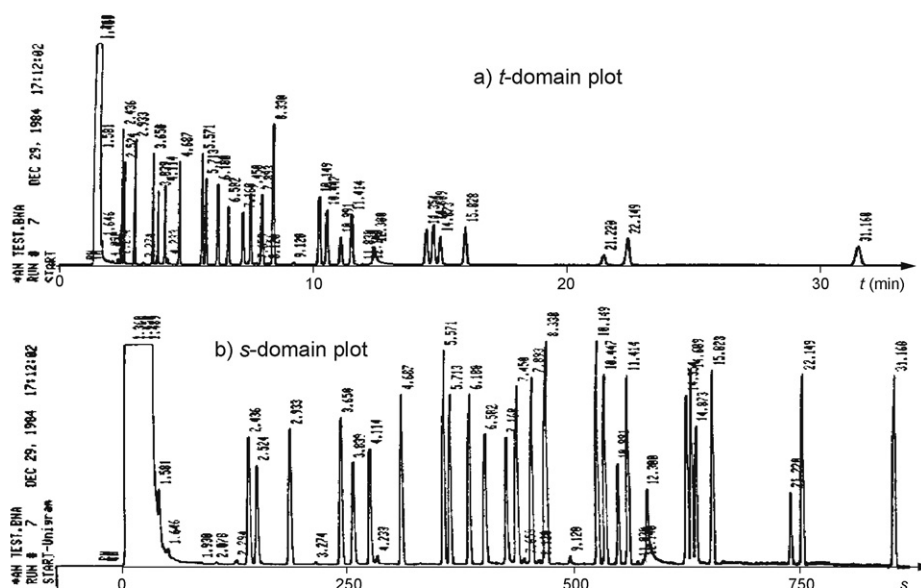

Fig. 2. (reconstructed from Ref. [16]) A chromatogram of the same static GC analysis presented in (a) t-domain and in (b) s-domain. The plot mode (known as a unigram plot) leading to (b) was available in several earlier commercial integrators from Hewlett–Packard Co., Palo Alto, CA. In the unigram plot mode, the speed of a plot paper advancement was inversely proportional to expected peak width (σ) while the pen deflection across the paper was proportional to the product σy of σ and detector output (y). All non-overloaded peaks in (b) appear with the same widths. The height of each peak is proportional to its area. The peaks in (b) are more evenly distributed than they are distributed in (a). It is also evident from (b) that, for example, the area of the last peak is approximately equal to the area of the peak at 5.571 min. This is not evident from (a).

by Davis and Giddings in their studies of overlap statistics in chromatography [1–3]. Eq. (8) shows that

two factors affect the peak capacity (n_c) of a chromatographic analysis:
One of them is the peak capacity (s_c) of the analysis, i.e. the total number of adjacent σ-slots in its chromatogram.
Equally important is the lowest separation (Δs_{min}) required by the data-analysis system for resolving two peaks.

Similar observations are valid for the peak capacity (Δn) of an arbitrary time interval. A ($s_{\min}\sigma$)-long segment in t-domain and $\Delta s_{\min}$-long segment in s-domain can be called as the *resolution slot* in a respective domain.

2.1.4. Number of resolved single-component peaks

The number of peaks that can be resolved in an arbitrary segment of a chromatogram can be equal to its peak capacity (Δn), but only under the following unrealistic conditions:

1. there are exactly Δn peaks in the segment;
2. the distance between any two neighboring peaks is exactly one resolution slot.

Realistically, however, the number of the single-component peaks that can be resolved in a segment is much smaller than Δn. Nevertheless, metric Δn is an important benchmark that allows one to estimate a realistic number of resolved peaks.

Davis and Giddings have shown that, if the peak distribution in a chromatogram is *statistically uniform* (the probability of a peak within an arbitrary segment of a chromatogram does not depend on the location of the segment in a separation space of a chromatogram) then the statistically expected number (Δp_{sing}) of resolved single-component peaks in an arbitrary segment of the chromatogram can be found as [1,2]

$$\Delta p_{\mathrm{sing}} = \Delta m e^{-2\alpha} = \Delta n \alpha e^{-2\alpha}, \quad \alpha = \frac{\Delta m}{\Delta n} \tag{9}$$

where Δm is the number of solutes represented in the segment and α is the *saturation* of the segment. In the case of statistically non-uniform peak distribution, α could be a function of a running peak capacity (n). Summation of quantities Δp_{sing} over the net peak capacity (n_c) of a chromatogram yields the net number ($p_{sing,c}$) of single-component peaks in a chromatogram with statistically non-uniform peak distribution [3].

The expected number (Δp_{sing}) of the single-component peaks that can be resolved in a given segment of a chromatogram depends on its saturation (α). Quantity Δp_{sing} has a maximum (Fig. 3)

$$\Delta p_{sing,\max} = \frac{\Delta n}{2e} \approx 0.184 \Delta n \tag{10}$$

at $\alpha = \alpha_{opt}$ and, therefore, at $\Delta m = \Delta m_{opt}$ where

$$\alpha_{opt} = 0.5, \quad \Delta m_{opt} = 0.5 \Delta n \tag{11}$$

This suggests that

The largest statistically expected number ($\Delta p_{sing,max}$) of resolved single component peaks in a segment of a chromatogram with statistically uniform peak distribution is equal to about 18.4% of the segment peak capacity (Δn). This result takes place when the number (Δm_{opt}) of components represented in the segment is half of Δn.

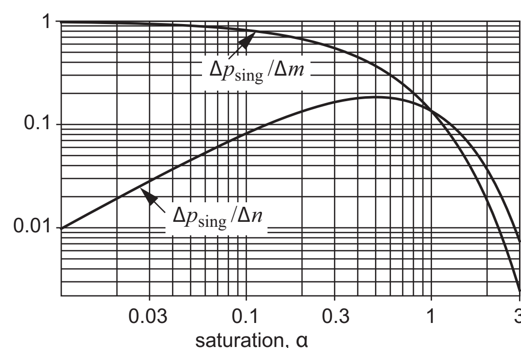

Fig. 3. Expected number (Δp_{sing}) of resolved single-component peaks vs. saturation (α), Eq. (9).

Schure has demonstrated that under more favorable statistics of random peak distribution, $\Delta p_{sing,\max}$ can be significantly larger than 18% of Δn [12]. However, only **statistically uniform** peak distribution is assumed below.

2.2. Factors affecting the separation in static analysis

Static analysis (like isothermal isobaric GC, isocratic isothermal isobaric LC, etc.) is a one whose conditions do not change with time. Conversely, *dynamic* analysis (like temperature-programmed GC, gradient-elution LC, etc.) is a one whose conditions change in time (typically according to a pre-determined time-program) [13]. Only the **static** analyses are considered below.

2.2.1. Column efficiency

In static analyses, the width (σ) of a peak relates to its retention time (t_R) as [13]

$$\sigma = \frac{t_R}{E} \tag{12}$$

where E is a column *efficiency* defined as [10][2]

$$E = \sqrt{N} \tag{13}$$

where N is the column *plate number*.

Quantity N and, therefore, E significantly depend on a column dimensions and on the mobile phase flow rate. These quantities can also be different for different solutes eluting with different retention times. As a result, E can be a function of time. However, the change in E with time is typically minor [13]. It is assumed in all forthcoming discussions that, for a given column, ***E* is independent of time**.

Sometimes (and this is true for my own earlier publications), the plate number (N) is treated as a parameter directly related to a concept of column *separation efficiency* [20,21]. This might suggest that a column efficiency (E) can be interpreted as the column "separation efficiency". However, this interpretation can be misleading. Indeed, the concepts of the plate height, the plate number (N) and, therefore, the column efficiency (E) defined in Eq. (13) are meaningful even in the case of an *inert tube* having no stationary phase and incapable of separation. Furthermore, the largest value of E is higher for an inert tube than it is for a capillary column made from the same tube [13,22]. Because the tube's higher efficiency (E) comes together with its inability to separate, it seems to be misleading to call parameter E as the "separation efficiency". If E is the efficiency of a particular aspect of a column performance then it is the efficiency of the delivery of the solutes to the column outlet as the sharp peaks. Thus, the larger is E, the sharper are the peaks eluting at a given time.

Metrics of separation performance (separation capacity, peak capacity, number of resolved peaks, etc.) are proportional to E, i.e. to $\sqrt{N}$. A conventional approach of dealing with metric N rather than with E gives an exaggerated impression of the spread of the separation performances available from the realistic columns. It also gives an exaggerated impression of the effects of column and method parameters on the column separation performance.

2.2.2. Solute-column interactions

Different solutes might be differently distributed between stationary an mobile phases of a column. This is the root cause of separation in chromatography. The distribution of each particular solute in a given column is governed by the solute *distribution constant* [2]

$$K_c = \frac{C_{stat}}{C_{mob}} \tag{14}$$

where C_{mob} and C_{stat} are the concentrations of the solute in mobile and stationary phases, respectively. The thermodynamics of the distribution can be described as [2]

$$K_c = e^g, \quad g = \frac{G}{\mathcal{R}T} \tag{15}$$

where $\mathcal{R} = 8.31447$ J/(K mol) is the *molar gas constant*, G is *Gibbs free energy* of a solute transport from the stationary to the mobile phase, and T is the temperature. Quantity g can be viewed as *dimensionless Gibbs free energy* [8].

Distribution of a solute material between the mobile and the stationary phases in a particular column can be expressed in one of the following ways [5,13]:

$$k = \frac{a_{stat}}{a_{mob}}, \quad \mu = \frac{a_{mob}}{a}, \quad \omega = \frac{a_{stat}}{a} \tag{16}$$

where $a = a_{mob} + a_{stat}$, a_{mob} and a_{stat} are, respectively, the total amount of a solute and its amounts in mobile and stationary phases.

Metric μ was introduced in 1944 [23]. Since then, it was denoted by several symbols and was known under several terms [5,13]. Some of them, like the *retardation factor* [24,25] and the *retention ratio* [2,26,27], are counterintuitive. They assign the largest values of the retardation factor and the retention ratio to the fastest moving (least retarded, least retained) solutes. Conversely, they assign the lowest values of the retardation factor and the retention ratio to the slowest moving (most retarded, most retained) solutes. More recently proposed term *solute mobility* (*mobility factor*) [5,13] is justified by the fact that the net velocity, v, of a solute migration along a column is proportional to μ, i.e.

$$v = \mu u \tag{17}$$

where u is the mobile phase velocity.

It follows directly from their definitions in Eq. (16) that quantities k, μ and ω relate to each other as

$$\mu = 1 - \omega = \frac{1}{1+k}, \quad \omega = 1 - \mu = \frac{k}{1+k}, \quad k = \frac{1-\mu}{\mu} = \frac{\omega}{1-\omega} \tag{18}$$

and are bound by the conditions

$$0 \le \mu \le 1, \quad 0 \le \omega \le 1, \quad 0 \le k \le \infty \tag{19}$$

The formulae describing relations between μ and ω show that these quantities are complimentary to each other. Thus, if $\mu = 0$ then $\omega = 1$. Conversely, if $\mu = 1$ then $\omega = 0$. This justifies the interpretation of quantity ω as a solute *immobility* – a normalized measure of its *interaction* with the stationary phase (its *affinity* to the stationary phase).

Due to the one-to-one relations (Eq. (18)) between metrics k, μ and ω, any one of them is sufficient for all evaluations. However, a need for the simplicity of theoretical results and their interpretations dictates preferential choice of one of these metrics over the others depending on the type of the evaluations. Thus, due to Eq. (17), metric μ offers the simplest relation of a solute velocity (v) to the mobile phase velocity (u). Metric ω most directly affects the solute separation (see below).

Retention factor (k) most directly relates to a solute distribution constant (K_c). It follows from Eqs. (14)–(16) that, in the case of a **liquid stationary phase** – the most frequently used one and the only one considered in forthcoming illustrations,

$$k = \frac{K_c}{\beta} = \frac{e^g}{\beta} \tag{20}$$

[2] Earlier [8,9], this metric was called as the *separation power* ($\mathcal{P}$).

where

$$\beta = \frac{\text{mobile phase volume}}{\text{stationary phase volume}} \quad (21)$$

is the *phase ratio*. For **capillary columns**, $\beta = 1/(4\varphi)$ where

$$\varphi = \frac{d_f}{d_c} \quad (22)$$

is *dimensionless film thickness* [13] in a column with internal diameter d_c and stationary phase film thickness d_f.

Due to Eq. (17), a peak retention time (t_R) in a L-long column can be found as

$$t_R = \frac{L}{\bar{v}} = \frac{L}{\mu \bar{u}} = \frac{t_M}{\mu} \quad (23)$$

where $\bar{v} = L/t_R$ and $\bar{u} = L/t_M$ are the time-averaged (briefly, *average*) velocities of the solute and the carrier gas [13]. Eqs. (23) and (16) yield:

$$\mu = \frac{t_M}{t_R}, \quad k = \frac{t_R}{t_M} - 1, \quad \omega = 1 - \frac{t_M}{t_R} \quad (24)$$

2.2.3. Separability of two solutes

As mentioned earlier, different distribution of solutes between the stationary and the mobile phase in a column is the root cause of the solute separation. According to Eq. (15), a solute distribution constant (K_c) can be expressed via one parameter – the dimensionless Gibbs free energy (g) of the transport of a solute from stationary to mobile phase. An additive metric (Δg) defined as the difference,

$$\Delta g = g_B - g_A = \ln \frac{K_{c,B}}{K_{c,A}} \quad (25)$$

in dimensionless Gibbs free energies (g_A and g_B) of solutes A and B in the same column under the same conditions can be called as the *separability* of the solutes A and B [8].[3]

Due to Eqs. (20) and (24), the definition in Eq. (25) can be expressed via observable parameter of corresponding peaks as (Fig. 4):

$$\Delta g = \ln \frac{k_B}{k_A} \quad (26)$$

$$\Delta g = \ln \frac{t_{R,B} - t_M}{t_{R,A} - t_M} = \ln \frac{\tau_{R,B} - 1}{\tau_{R,A} - 1} \quad (27)$$

where

$$\tau = \frac{t}{t_M} \quad (28)$$

is *dimensionless* time [13,29] – the time expressed in units of hold-up time.

The metric of separability (Δg) of two solutes is an alternative to the metric

$$\alpha_k = \frac{k_B}{k_A} \quad (29)$$

of the solute *selectivity*. For the reasons discussed in Appendix A and elsewhere [8], metric α_k is not included in the system of metrics proposed in this report.

2.2.4. Discrimination of two solutes

Because two solutes are differently distributed between the stationary and the mobile phases, they migrate through the column with different mobilities (μ) and, as follows from Eq. (17), with different velocities (v). The difference (Δv) in the solute velocities causes the difference (Δt_R) in their retention times. These observations suggest that, while the separability (Δg) of two solutes is the underlying cause of their separation, more immediate cause of the separation is the difference ($\Delta\mu$) in their mobilities. Due to Eq. (17), Δt_R for solutes A and B can be expressed as

$$\Delta t_R = \left(\frac{t_{R,B}}{t_{R,A}} - 1\right) t_{R,A} = \left(\frac{v_A}{v_B} - 1\right) t_{R,A} = \frac{\mu_A - \mu_B}{\mu_B} t_{R,A} \quad (30)$$

An interesting case is the one where the value of the relative difference in mobilities of two closely spaced solutes is small, i.e. $|(\mu_A - \mu_B)/\mu_B| \ll 1$. In this case, $\mu_B \approx \mu_A$, $t_{R,B} \approx t_{R,A}$ and Eq. (30) can be expressed as

$$\Delta t_R = \frac{\Delta\mu}{\mu} t_R = \Delta\delta t_R, \quad \Delta\mu = \mu_A - \mu_B \quad (31)$$

where the relative difference,

$$\Delta\delta = \frac{\Delta\mu}{\mu} \quad (32)$$

in mobilities of two solutes can be viewed as the solute *discrimination* in the column [30]. As stated earlier, the separability (Δg) of two solutes is the root cause of their retention time difference while the solute discrimination ($\Delta\delta$) is the immediate cause of that difference. The dependence of $\Delta\delta$ on Δg can be expressed via the derivative,

$$U_g = \frac{d\delta}{dg} = \lim_{\Delta g \to 0} \frac{\Delta\delta}{\Delta g} \quad (33)$$

which can be interpreted as the *utilization of separability*. Due to Eqs. (26), (32) and (18), U_g can be found as

$$U_g = \lim_{\Delta k \to 0} \left(\frac{\Delta\mu}{\mu} \frac{1}{\ln(k_B/k_A)}\right) = \frac{\Delta k}{1+k} \frac{k}{\Delta k} = \frac{k}{1+k} = \omega \quad (34)$$

In other words,

The utilization of separability (U_g) of two closely spaced solutes is equal to their immobility (ω)

The immobility (ω) of a solute is its fraction in the stationary phase. When that fraction is mall (say, much smaller than 50%) for each solute (as a result, for example, of too thin stationary phase film) then the utilization of their separability is small. As a result, even relatively large solute separability can cause only a relatively small difference in their velocities, and eventually, a relatively small difference in the solute retention times. Conversely, when the stationary phase fraction of each solute is large (say, close to 100%) then nearly 100% of their separability is utilized. As a result, the discrimination of two closely spaced solutes (the relative difference in their velocities) is nearly equal to the solute separability.

2.3. General separation performance of static analysis

Earlier defined separation performance metrics are suitable for evaluation of specific and general separation performance in many separation techniques. Following is an illustration of the use of the metrics for evaluation of general separation performance of static analysis.

Substitution of Eq. (12) in Eq. (3) and accounting for Eq. (28) yields:

$$s = \int_{t_M}^{t} \frac{E\, dt}{t} = E \ln \frac{t}{t_M} = E \ln \tau \quad (35)$$

Let's call a range (x, ex) of some variable x as an *exponential* one. Such are the intervals $(1, e)$, (e, e^2), etc. in the τ-axis of Fig. 5.

[3] In his 1958 review of GC terms and definitions [28], Golay used this metric (parameter ε in Eq. (6)) without giving it a specific name.

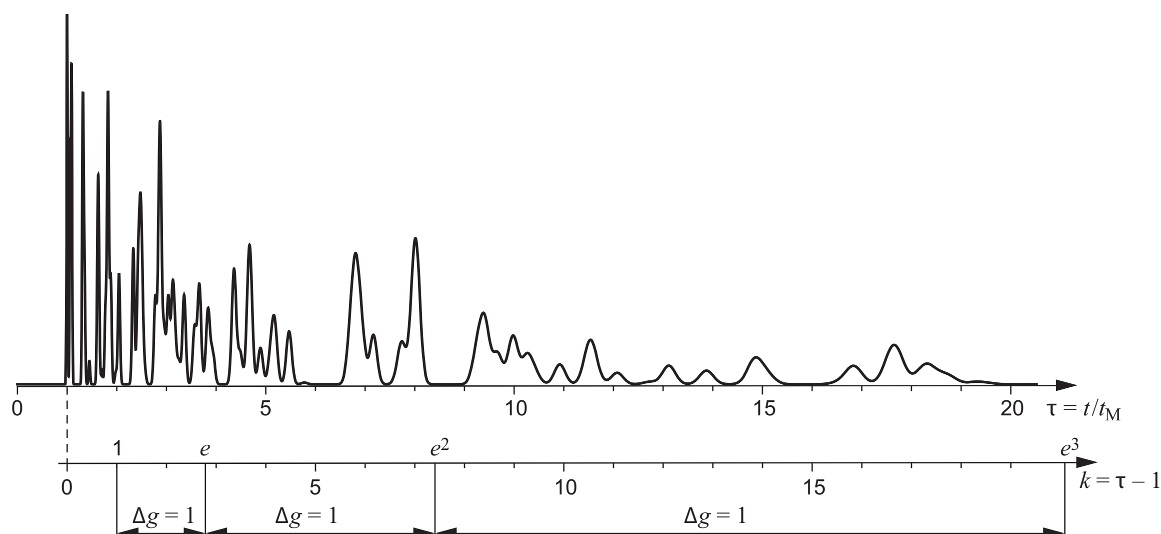

Fig. 4. Dimensionless time (τ, Eq. (28)), retention factor (k) and separability (Δg, Eqs. (25) and (27)) in static analysis.

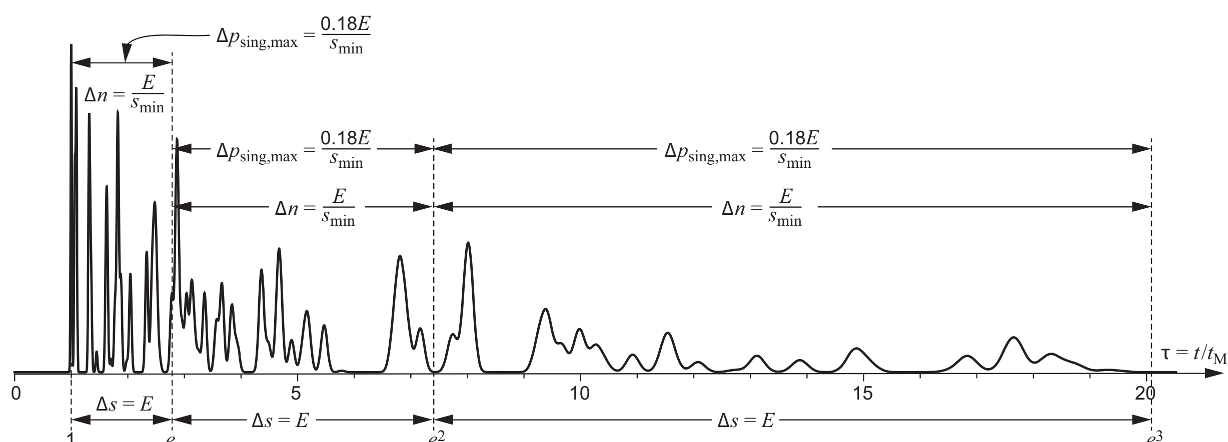

Fig. 5. Separation performance metrics in a chromatogram of static analysis. According to Eq. (35), separation capacity (Δs) of each exponential intervals (τ, $e\tau$) is equal to column efficiency (E). Peak capacity (Δn, Eq. (7)) and the largest statistically expected number ($\Delta p_{sing,max}$, Eq. (10)) of resolved single-component peaks are also the same in each exponential interval.

According to the definition of dimensionless time (τ) in Eq. (28), an exponential interval in τ axis corresponds to the exponential interval in t-axis. It follows from Eq. (35) that (Fig. 5)

the separation capacity (Δs) of an exponential interval in time-axis of a static analysis is equal to column efficiency (E)

This observation can be used as an alternative (to Eq. (13)) definition of a column efficiency:

Column efficiency (E) is the separation capacity (Δs) of an exponential interval in the separation space of a static analysis. In other words, column efficiency (E) is the number of σ-slots in an exponential interval of the separation space of a static analysis

It is interesting to compare Figs. 4 and 5. In the former, all exponential intervals in k-axis have the same separability ($\Delta g = 1$) while in the latter, all exponential intervals in τ-axis have the same separation capacity ($\Delta s = E$), and other important separation performance metrics.

As mentioned earlier, Eq. (3) can be viewed as transformation of t-domain into s-domain. Eq. (35) describes that transformation in static analysis in more specific terms. The inverse transformation of s-domain into t-domain of static analysis is

$$t = t(s) = t_M e^{s/E}$$

To find the separation (Δs), of two arbitrary peaks, let's assume that t_0 is the retention time of a reference peak and t is the retention time of the other peak (quantities t_0 and t can also be, respectively, a reference and an arbitrary markers in the time-axis). Solving together Eqs. (5), (35), (18) and (26), one can express Δs as a function [8],

$$\Delta s = s - s_0 = E \ln(1 + (e^{\Delta g} - 1)\omega_0) \quad (36)$$

of the separability (Δg) of two peaks, the immobility (ω_0) of the reference peak, and the column efficiency (E).

To get additional insight into Eq. (36), consider the derivative

$$S = \frac{ds}{dg} = \lim_{\Delta g \to 0} \frac{\Delta s}{\Delta g} \quad (37)$$

which describes the separation (Δs) per unit of separability (Δg), and can be called as the *specific separation measure* (*specific sep-*

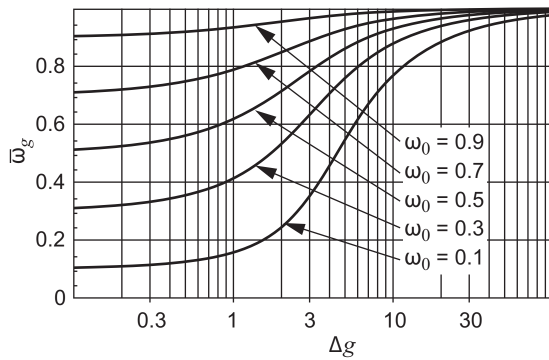

Fig. 6. Average immobility ($\bar{\omega}_g$, Eq. (42)) of the solutes having immobilities between the reference immobility $\omega_0 = \omega(g_0)$ and arbitrary immobility $\omega(g_0 + \Delta g)$ in a static analysis. If the separability span (Δg) is relatively small (say, $|\Delta g| < 0.3$) then $\bar{\omega}_g \approx \omega_0$. When Δg increases, $\bar{\omega}_g$ eventually approaches unity, i.e. $\bar{\omega}_g \to 1$.

aration, *specific separation capacity*) [8].[4] It follows from Eq. (36) that

$$S = E\omega \tag{38}$$

suggesting that the separation (Δs) of two *low-separability* solutes (the ones with $|\Delta g| \ll 1$) can be expressed as the product [8,28]

$$\Delta s = S\Delta g = E\omega\Delta g, \quad (\text{when} \quad |\Delta g \ll 1|) \tag{39}$$

where ω is the solute immobility which is nearly the same for both low-separability solutes.

Following a guidance of Eq. (39), one can come up with a structurally similar expression

$$\Delta s = E\bar{\omega}_g \Delta g \tag{40}$$

for an *arbitrary* pair of peaks where

$$\bar{\omega}_g = \frac{1}{\Delta g}\int_{g_0}^{g_0+\Delta g} \omega\, dg \tag{41}$$

is the *separability-averaged immobility* which, due to Eqs. (18) and (15) can be expressed as (Fig. 6):

$$\bar{\omega}_g = \frac{1}{\Delta g}\int_{g_0}^{g_0+\Delta g} \frac{e^g\ dg}{1+e^g} = \frac{\ln(1+(e^{\Delta g}-1)\omega_0)}{\Delta g} \tag{42}$$

thus confirming identity of Eqs. (36) and (40) for arbitrary Δg.

In static analysis, the separation of two solutes is equal to the product of their separability (Δg), separability-averaged immobility ($\bar{\omega}_g$), and column efficiency (E).

It is not necessary, but convenient to assume that the reference eluite is the earliest one. In that case, Δg and Δs are positive quantities, and if ω_0 is close to unity then Eqs. (42) and (40) yield:

$$\bar{\omega}_g = 1, \quad \Delta s = E\Delta g, \quad (\text{when} \quad \omega_0 \approx 1) \tag{43}$$

To view Eqs. (36) and (40) as well as their special cases from a single cause-and-effect perspective, one can notice that, as follows from Eq. (34), the immobility (ω) in Eq. (39) represents the utilization of separability of the closely space solutes. Extending the concept of utilization of separability to arbitrary solutes, one can express Eqs. (36) and (40) as

$$\Delta s = EU_g\Delta g \tag{44}$$

[4] Previously denoted as Δs [8].

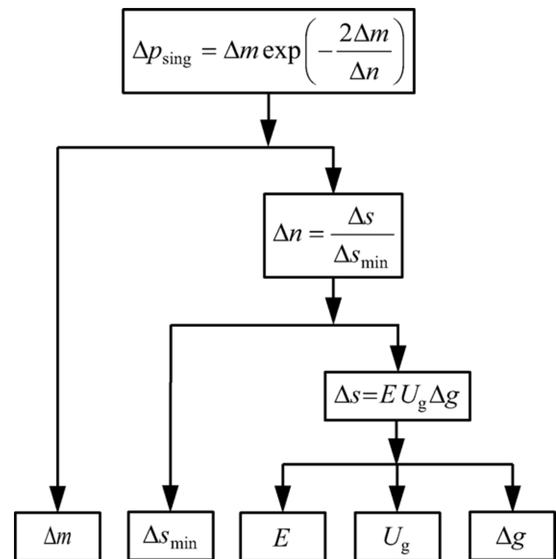

Fig. 7. Block-diagram of a top-down view of a system of metrics of separation performance in chromatography.

where

$$U_g = \bar{\omega}_g = \begin{cases} \Delta g^{-1}\ln(1+(e^{\Delta g}-1)\omega_0), & \text{all cases} \\ \omega, & |\Delta g| \ll 1 \\ 1, & \omega_0 \to 1 \end{cases} \tag{45}$$

Eq. (44) shows that

The separation of two solutes is proportional to three factors: separability (Δg) of the solutes, utilization (U_g) of the separability, and column efficiency (E).

3. Discussion

3.1. Overview of the system of metrics

Relationships between the earlier defined metrics is illustrated in the block-diagram of Fig. 7. Its components represent the following groups of metrics and parameters. Metrics of separation performance: Δp_{sing}, Δn and Δs. Sample/solute parameters: Δm, Δg and U_g. Column parameters: E, Δg and U_g (parameters U_g and Δg depend on the solutes and on the column). Parameter of data-analysis system: $\Delta s_{\min}$. Metric Δp_{sing} (the expected number of resolved peaks within a given interval of the separation space) can be viewed as the ultimate metric of the separation performance. On the bottom of the diagram are those parameters (operational metrics) of the sample and the system that affect Δp_{sing}. In the middle of the diagram are the metrics of the separation performance (Δs and Δn) that affect the metric Δp_{sing} and can be viewed as being subordinate to Δp_{sing}. Similar relationships exist between metric $p_{sing,c}$ (the expected number of resolved peaks in the entire chromatogram), its subordinate metrics n_c and s_c, as well as the system and sample parameters, E, $\Delta s_{\min}$, m, Δg and U_g.

3.2. Practical measurement of the separation

While providing a conceptually simple definition of the separation measure (Δs), Eq. (2) is inconvenient for practical applications. Practical measurement of quantity Δs for two peaks having substantially different widths is described elsewhere [4] where it is also shown that, if the difference between the widths (σ_A and σ_B) of peaks A and B in static analysis is smaller than a factor of two

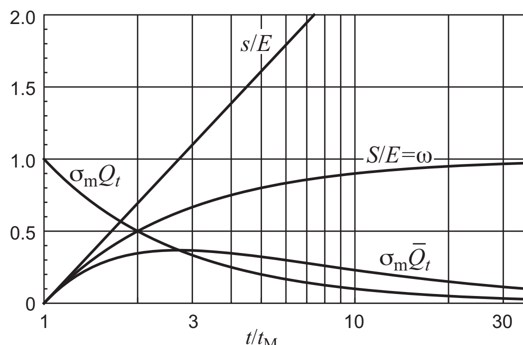

Fig. 8. Performance metrics s, S, Q_t and $\bar{Q}_t$ defined in Eqs. (3), (37), (49) and (51), and evaluated for a static analysis in Eqs. (35), (38), (50) and (52).

then, with the better than 5% accuracy, Δs can be approximated as [4]

$$\Delta s \approx \frac{\Delta t}{\bar{\sigma}}, \quad \bar{\sigma} = \frac{\sigma_A + \sigma_B}{2} \quad \left(\text{if } \frac{1}{2} \le \frac{\sigma_B}{\sigma_A} \le 2\right) \tag{46}$$

Recall that the resolutions (R_s) of *Gaussian peaks* can be described as [2,26,27,29–32]

$$R_s = \frac{\Delta t}{4\bar{\sigma}}, \quad \bar{\sigma} = \frac{\sigma_A + \sigma_B}{2} \quad \text{(Gaussian peaks)} \tag{47}$$

Comparison of Eqs. (46) and (47) suggests that

$$\Delta s \approx 4R_s \quad \left(\text{Gaussian peaks}, \quad \frac{1}{2} \le \frac{\sigma_B}{\sigma_A} \le 2\right) \tag{48}$$

For the reasons discussed in Appendix A and elsewhere [4], the resolution (R_s) is not included in the system of metrics summarized in Fig. 7. On the other hand, the resolution is widely used for the measurement of the degree of the peak separation in practical applications. Eq. (48) suggests that, for Gaussian peaks having equal or moderately different widths, the difference between the separation measure (Δs) and the resolution (R_s) is primarily only in the scale. This suggest that, in practical applications, there is a smooth transition from conventionally used resolution to theoretically self-consistent and meaningful separation measure. It should be recognized, however, that

- Eq. (46) is not the definition of Δs, but only an approximation suitable for the peaks having equal or moderately different widths.
- Eq. (47), on the other hand, is the definition of R_s (and the source of the shortcomings of R_s described in Appendix A).
- Eq. (46) (as is the basic definition in Eq. (2)) is suitable for the peaks of *any shape*.
- On the other hand, Eq. (47) and approximation in Eq. (48) are valid for Gaussian peaks. For non-Gaussian peaks, the very definition of the resolution is unclear.

It should be also emphasized that Eqs. (36) and (40) are suitable for theoretical prediction of the separation measure of the peaks having arbitrary separability. In that regard, the scope of Eqs. (36) and (40) is much broader than the scope of Appendix A's Eqs. (56) and (58) describing only the resolution of the low-separability peaks [31].

3.3. Separation rates

Running separation capacity (s) of a static analysis is a logarithmic function (Eq. (35), Fig. 8) of the analysis time. Addition of each exponential time interval to the analysis time increments s by the value of a column efficiency (E) (Fig. 5). The temporal rate,

$$Q_t = \frac{ds}{dt} \tag{49}$$

of increase of the number of σ-slots during analysis can be used as a metric of the speed of analysis. Due to Eqs. (35) and (28), the speed of a static analysis can be found as (Fig. 8)

$$Q_t = \frac{E}{t} = \frac{1}{\sigma_m \tau}, \quad \sigma_m = \frac{t_M}{E} \tag{50}$$

where σ_m is the *unretained peak width*. The speed of analysis (Q_t) – the number of σ-slots generated per unit of time (t) – declines with time. This is not surprising because the peak width – the duration of one σ-slot – is proportional to t.

The speed of analysis (Q_t) defined in Eq. (49) is the instant speed at time t. One can also speak of the *average speed of analysis*,

$$\bar{Q}_t = \frac{s}{t} \tag{51}$$

Substitution of Eqs. (35) and (28) in this formula yields (Fig. 8):

$$\bar{Q}_t = \frac{E}{t} \ln \frac{t}{t_M} = \frac{\ln \tau}{\sigma_m \tau} \tag{52}$$

The speed of analysis is the rate of generating the σ-slots per unit of time. One can consider another rate. A chromatographic analysis can be viewed as a process of transforming the separability of the solutes into the separation of the corresponding peaks. From that perspective, the larger is the separation per a given separability the better is the separation performance of the analysis. This aspect of the separation performance can be expressed via the

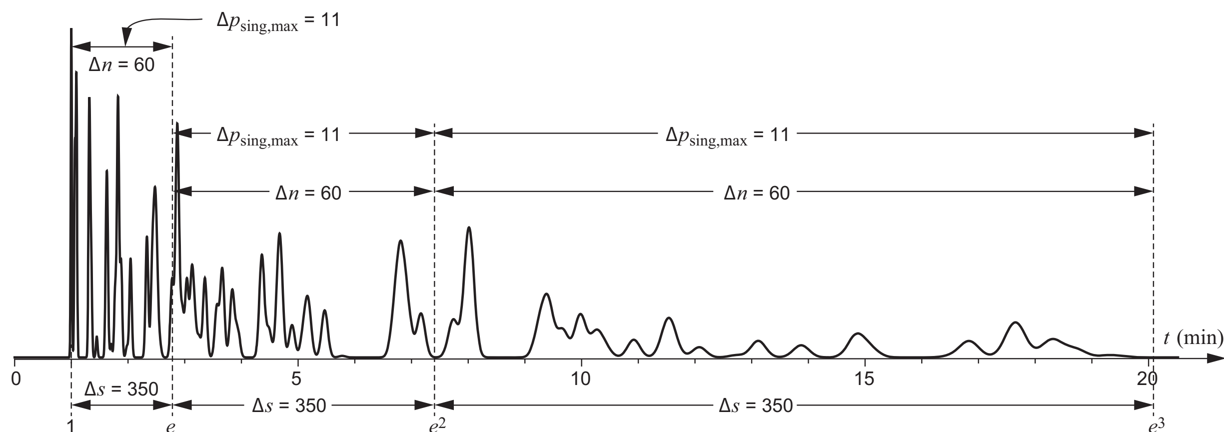

Fig. 9. Numerical values of several separation performance metrics of static analysis with conventional column. Operational parameters of the analysis are described in the main text.

specific separation (S, Eq. (37)) describing the separation per unit of separability.

Substitution of Eqs. (18) and (24) in Eq. (38) yields:

$$S = E\left(1 - \frac{t_M}{t_R}\right) = E\frac{\tau - 1}{\tau} \quad (53)$$

indicating that the slightly retained peaks appearing at or shortly after the hold up time (t_M) have nearly zero specific separation (Fig. 8). As a result, relatively high separability (Δg) is required for the separation of these peak. This phenomenon has a simple explanation. The peaks are slightly retained because the fractions (ω) of their material residing in the stationary phase are small. According to Eq. (34), this results in inefficient transformation (U_g, Eq. (33)) of the separability of these solutes into their discrimination (the relative difference, $\Delta\delta$, Eq. (32), in their mobilities) and, as follows from Eq. (38), in low separation per unit of separability. The solutes eluting later in the analysis do so because larger fractions (ω) of their material reside in the stationary phase. As a result, relatively smaller separability of the solutes is required for obtaining their sufficient discrimination and separation. Eventually, somewhere after $7t_M$ or so, ω reaches its nearly 100% plateau causing nearly 100% utilization (U_g) of separability and elevating S to near its plateau level of E (Fig. 8).

3.4. Numerical example

Consider a static GC–MS analysis with conventional column having $L = 30$ m and $d_c = 0.25$ mm and operating under the following conditions. Carrier gas: helium at speed-optimized flow rate of 2 mL/min [13,33] and vacuum at the column outlet. Let's assume that at least 6σ-separation ($\Delta s_{\min} = 6$) is required for resolving two peaks.

At optimal flow rate, the column efficiency can be estimated as $E \approx \sqrt{L/d_c} \approx 350$. The hold-up time ($t_M$) in this analysis is close to 1 min (the exact value depends on the column temperature). Let assume that $t_M = 1$ min. For these parameters, the numerical values for the metrics in Fig. 5 are shown in Fig. 9.

Suppose that the analysis lasts for 20 min. According to Fig. 9, the separation performance metrics of the entire analysis are: $s_c = 1050$ (there are 1050 σ-slots in the entire chromatogram), $n_c = 180$ (peak capacity of the entire chromatogram is 180), $p_{sing,c,\max} = 33$ (the analysis is expected to resolve about 33 peaks, but only in a test-mixture consisting of about 525 component).

In order to increase these metrics by 1/3 ($s_c = 1400$, $n_c = 240$, $p_{sing,c,\max} = 44$), the analysis should be about 2.72 times longer, i.e. about 55 min. Replacing helium as a carrier gas with hydrogen reduces the analysis time by about 35% (from 55 min to 36 min) without affecting the separation performance.

The separation performance of the analysis can be substantially improved by using more powerful data analysis based on the peak deconvolution techniques [17–19]. Suppose that this reduces the lowest required separation from $\Delta s_{\min} = 6$ to $\Delta s_{\min} = 1$. At $\Delta s_{\min} = 1$, the peak capacity is equal to the separation capacity. Therefore, the peak capacity (Δn) of each exponential time-interval becomes 350, and the net peak capacity (n_c) of the four-exponential-interval analysis becomes 1400. Quantities $\Delta p_{sing,\max}$ and $p_{sing,c,\max}$ become 64 and 258, respectively. This significant improvement highlights two important factors:

1. The ability of a data-analysis system to resolve poorly separated solutes significantly affects the peak capacity of the analysis and the number of the peaks that the analysis can resolve.
2. Speaking of peak capacity of an analysis, it is important to specify the lowest required peak separation ($\Delta s_{\min}$) on which the value of the peak capacity is based. Comparison of the peak capacities of two analyses utilizing different data-analyses systems with unspecified $\Delta s_{\min}$ can be misleading.

4. Conclusion

Metrics of separation performance like the separation of two peaks and the peak capacity of a time-interval in a chromatogram (collectively called as the separation measure), the peak capacity, and the statistically expected number of resolved peaks are defined. Also defined are the accompanying metrics like the peak separability and discrimination, a column efficiency, the utilization of separability, the specific separation and others. The metrics comprise a system of mutually compatible metrics. The values of all metrics for arbitrarily distant peaks and arbitrary time-intervals in static analyses (isothermal GC, isocratic LC, etc.) are found. Mutual relations of several metrics to one another and to other historically known metrics are discussed.

Such well-known metrics as the peak resolution, the selectivity, and the effective plane number are not included in the proposed system of metrics. The reasons for that are addressed in Appendix A.

Conventions and Nomenclature

Subscripts

c	parameter describes the complete (entire) analysis
R	parameter is measured at a peak retention time (a solute elution time)

Symbols

d_c	internal diameter of column tubing
d_f	stationary phase film thickness
E	column efficiency, Eq. (13)
G	Gibbs free energy of solute transport from stationary phase, Eq. (15)
g	dimensionless Gibbs free energy, Eq. (15)
Δg	separability of two solutes, Eq. (25)
k	retention factor, Eq. (16)
K_c	distribution constant, Eq. (15)
L	column length
Δm	number of solutes eluting within a time-interval
N	plate number
n	running peak capacity, Eq. (7)
n_c	peak capacity of entire analysis, Eq. (8)
Δn	peak capacity of a time-interval, Eq. (6)
Δp_{sing}	statistically expected number of resolved single-component peaks within a time-interval
Q_t	speed of analysis, Eq. (49)
$\bar{Q}_t$	average speed of analysis, Eq. (51)
$\mathcal{R}$	molar gas constant, $\mathcal{R} = 8.31447$ J/(K mol)
S	specific separation measure, Eq. (37)
s	running separation capacity, Eq. (3)
s_c	separation capacity of the entire analysis, Eq. (4)
Δs	separation measure (separation of two peaks, separation capacity of time-interval), Eq. (2)
Δs_{min}	lowest separation required for resolving two peaks
U_g	utilization of separability, Eq. (33)
t	time
t_M	hold-up time
t_R	retention time
$t_{R,last}$	retention time of the last peak
u	mobile phase velocity
v	solute velocity
α	saturation, Eq. (9)

α_k selectivity, Eq. (56)
$\Delta\delta$ solute discrimination, Eq. (32)
μ solute mobility, Eq. (16)
σ peak width (its standard deviation)
σ_m unretained width of a peak, Eq. (50)
τ dimensionless time, Eq. (28)
φ dimensionless film thickness, Eq. (22)
ω solute immobility, Eq. (16)
ω_0 immobility of a reference solute
$\bar{\omega}_g$ separability-averaged immobility, Eq. (41)

Appendix A. Resolution, selectivity, effective plate number

A.1. Resolution

Metric of the peak *resolution* [24,31] (R_s) is not included in the system of metrics (Fig. 7) described in the main text. In many respects, the metric of peak resolution is similar to the metric (Δs) of their separation. In fact, as shown in Eq. (48), for Gaussian peaks having no more than moderately different widths, the difference in R_s and Δs is primarily only in the scaling.

Historically, the resolution was essential for the development of art of chromatography, and it continues to play a vital role in current practice of chromatography. Unfortunately, due to its substantial deficiencies [4,34,35], the use of the resolution in theoretical studies is limited.

The resolution (R_s) of peaks A and B is defined as [24]

$$R_s = \frac{t_{R,B} - t_{R,A}}{\bar{w}_b}, \quad \bar{w}_b = \frac{w_{b,A} + w_{b,B}}{2} \tag{54}$$

where w_b is the *base width* of a peak [24]. The roots of this definition go back to the celebrated van Deemter et al. [36] 1956 paper where it was suggested that "a simple case [of separation of two peaks] is obtained when the tangents of the elution curves of the subsequent solutes just touch at the base of the chromatogram". The definition in Eq. (54) has been proposed by Phillips in 1958 [34]. The same year, the metric has been formally recommended by the leading authorities in chromatography [37] "as one which might be useful for the time being". Right from its introduction, the metric was met with skepticism as being "unnecessarily cumbersome" [34] and "difficult to use in most cases" [35].

Following are some deficiencies of R_s described elsewhere in more details [4].

The base width (w_b) of a peak in Eq. (54) might be difficult to measure [38] and, for non-Gaussian peaks, difficult to theoretically predict from experimental conditions.

To avoid using the base width (w_b) in Eq. (54), some workers define resolution as [2,26,27,32,39]

$$R_s = \frac{t_{R,B} - t_{R,A}}{4\bar{\sigma}}, \quad \bar{\sigma} = \frac{\sigma_A + \sigma_B}{2} \tag{55}$$

This definition is based on the fact that, for Gaussian peaks, $w_b = 4\sigma$. For non-Gaussian peaks, however, it is very likely that $w_b \neq 4\sigma$. Thus, for exponential peaks, $w_b = \sigma$ [13]. In all cases where $w_b \neq 4\sigma$, Eqs. (54) and (55) could yield substantially different results. The quotient 4 in the denominator of Eq. (55) becomes an arbitrary scaling factor that has no physical justification.

Eqs. (54) and (55) define R_s as a *non-additive* metric. Thus, for consecutive peaks A, B and C having different widths, $R_{s,AB} + R_{s,BC} \neq R_{s,AC}$.

One consequence of non-additive character of R_s makes it unsuitable for the measurement of quality of separation of the peaks having very different widths. Consider a static analysis where all peaks are Gaussian and, therefore, Eqs. (54) and (55) yield equal results. A running peak capacity (n_b) calculated from Eq. (7) for $\Delta s_{\min} = 4$ is the number of w_b-wide segments between t_M and an arbitrary time t. As shown in Fig. 10, the resolution of any peak in static analysis and the unretained peak can never be larger that $E/2$ ($R_s < E/2$) which is much smaller than potential number (n_b) of w_b-wide segments between t_M and t. The fact that R_s might not represent the number of w_b-wide segments between two peaks questions the very meaning of metric R_s. It appears that Eqs. (54) and (55) are just mathematical formulae with no transparent physical meaning.

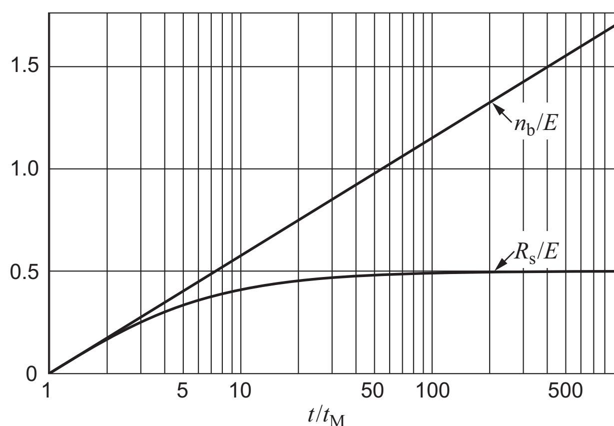

Fig. 10. Resolution (R_s) of a peak eluting at time t and unretained peak vs. the number (n_b, Eq. (7) for $\Delta s_{min} = 4$) of w_b-wide segments between t_M and t. The value of R_s does not get larger than $E/2$ regardless of the distance between the two peaks. A noticeable departure of R_s from n_b starts at $t > 3t_M$ or so and grows with t. *Conditions*: Gaussian peaks in static analysis.

Many formulae for R_s of two Gaussian peaks in static analysis are known from the literature [31]. The ones that are suitable for the peaks having substantially different widths are rather complex and difficult to interpret. However, in view of Fig. 10, there is even more fundamental issue with the formulae than their mathematical complexity. If the value of the resolution of two arbitrary peaks in a given analysis cannot exceed a certain number regardless of the distance between the peaks then what does the very concept of the resolution mean? Without a clear answer to this question, only a limited use of the metric of the resolution is possible.

For closely spaced peaks A and B, the resolution defined in Eq. (55) can be found as [31]

$$R_s = \frac{\sqrt{N}k(\alpha_k - 1)}{4(1+k)} \quad (\text{when} \quad |\alpha_k - 1| \ll 0) \tag{56}$$

where α_k is the *selectivity* [31] of solutes A and B defined in Eq. (29). It follows from Eqs. (26) and (29) that

$$\alpha_k = e^{\Delta g} \tag{57}$$

Substitution of this formula in Eq. (56) and use of notations adopted in the main text yields:

$$R_s = \frac{E\omega\Delta g}{4} \quad (\text{when} \quad |\Delta g| \ll 1) \tag{58}$$

Comparison of this formula with Eq. (39) indicates that (see also Eq. (48)):

$$R_s = \frac{\Delta s}{4} \quad (\text{when} \quad |\Delta g| \ll 1) \tag{59}$$

A.2. Selectivity

Like the resolution, the selectivity (α_k) that appears in Eq. (56) is also not included in the system of metrics in Fig. 7. Because there is one-to-one relationship, Eq. (57), between α_k and separability (Δg) either of these two metrics of the difference in the interaction of two solutes with stationary phase is sufficient for finding all parameters

that depend on that difference. However, there are the differences between α_k and Δg that speak decisively in favor of Δg [8]. Some of them are mentioned below.

Unlike the separability, the selectivity is not an additive metric (for consecutive solutes A, B and C, $\alpha_{k,AB}+\alpha_{k,BC} \neq \alpha_{k,AC}$). It can also be counterintuitive. Thus, it is a little odd that R_s in Eq. (56) is proportional to $\alpha_k - 1$. For comparison, the separation (Δs) in Eq. (39) and R_s in Eq. (58) are proportional to Δg. As a result Eqs. (39) and (58) are more transparent than Eq. (56). Several cases can additionally highlight this point. It follows from Eqs. (29) and (57) that the case where $\alpha_k = 0$ represents a large difference in the interactions of two solutes with the column. In a typical analysis, these solutes almost certainly can be resolved. For two unseparable solutes ($\Delta g = 0$) identically interacting with a column, $\alpha_k = 1$. Two solutes with slightly larger α_k (say, $\alpha_k = 1.02$) can typically be easily resolved.

The non-additive nature of α_k also complicates the extension of α_k to dynamic conditions (temperature-programmed GC, gradient elution LC, etc.) while Δg is very suitable for that extension [8]. The additive nature of separability was the basis for defining such concepts as utilization of separability (U_g) and specific separation (S) in Eqs. (33) and (37). It is also interesting to mention that, as early as in 1958 [28], Golay expressed the relative separation (a metric proportional to the resolution) via $\ln \alpha_k$ (the separability, in this report) rather than via α_k.

A.3. Effective plate number

Quantity N in Eq. (13) defining a column efficiency (E) is the column plate number. Also widely known in chromatography is the *effective plate number* [24,25,40–42],

$$N_{eff} = \frac{Nk^2}{(1+k)^2} \tag{60}$$

introduced by Desty et al. and similar to the earlier proposed concepts of *resolving power* [28] and *separation factor* [43]. In the notations of this report, quantity N_{eff} can be expressed as

$$N_{eff} = (E\omega)^2 \tag{61}$$

Special attention to quantity N_{eff} is typically based on the fact that Eq. (56) for the resolution (R_s) of two low-separability solutes (the ones with $|\Delta g| \ll 1$ and, therefore, with $|\alpha_k - 1| \ll 0$) can be expressed as

$$R_s = \frac{\sqrt{N_{eff}}(\alpha_k - 1)}{4} \quad (\text{when} \quad |\alpha_k - 1| \ll 0) \tag{62}$$

suggesting that N_{eff} completely defines the resolution of two solutes having a given selectivity (α_k) (a given separability, Δg, in terms of this text).

Sometimes, it is suggested that, because N_{eff} completely defines R_s at a given α_k, the plate number (N) should be abandoned in favor of N_{eff}. Along with that, the metric of a column efficiency (E) would be abandoned as well. It should be noticed, however, that, as follows from Eq. (61), parameter N_{eff} is a combination of two *independent* parameters, E and ω. It is true that the use of parameter $\sqrt{N_{eff}}$ in Eq. (62) eliminates the need for parameters E and ω in order to find R_s. It is also true that the product $E\omega$ in Eq. (39) for the peak separation (Δs) can be replaced with quantity $\sqrt{N_{eff}}$. Generally, however, parameters E (column efficiency) and ω (solute immobility) have their own *independent* effects on the peak separation. These two independent effects cannot be expressed via a single parameter N_{eff} in expressions for R_s [31] and Δs (Eq. (36)) of an *arbitrary* peak-pair in static analysis. This is also the case for dynamic analyses [8].

As shown in the main text, the product $E\omega$ defining N_{eff} in Eq. (61), has a certain significance in chromatographic theory. According to Eq. (38), the specific separation (S) in static analysis (the separation per unit of separability in static analysis) is equal to $E\omega$. However, a certain significance of metrics N_{eff} and S does not mean that they can replace metrics N and E. Indeed, being essentially a column parameters, metrics N and E uniquely serve as the solid anchors in evaluations of a column separation performance. Thus, all metrics of a column separation performance considered in this report and beyond [8] are proportional to metric E. On the other hand, metrics N_{eff} and S are solute-dependent variables. Depending on a solute retention, the value of N_{eff} can change from zero to N. Similarly, the value of S can change from zero to E. In addition to that, a significance of quantities N_{eff} and S outside of the formulae for the separation of low-separability solutes in static analyses is unknown. One can conclude that a solute-dependent variable N_{eff} is not a suitable replacement for a basic column parameter N. Similarly, a solute-dependent variable S is not a suitable replacement for a basic column parameter E.

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