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Evaluation of separation quality with a novel threshold criterion[☆]

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Abstract

A novel threshold criterion based on the degree of peak overlap is proposed. It can be used for the evaluation of the degree of separation for simple or complex mixtures. The criterion considers quality of separation for relevant peaks only. Non-symmetrical peak shapes, different peak-height ratios and the influence of other peaks on the separation are also considered. The proposed criterion was tested on overlapped peaks of complex chromatograms obtained by capillary gas chromatography.

1. Introduction

The analysis of complex mixtures cannot be successful without using a powerful separation technique (*e.g.*, gas, liquid or supercritical chromatography or capillary electrophoresis). There are problems, however, with the measurement of separation quality. The conventional approach, which relies on the intuition of the chromatographer as the only means of obtaining good separation, should be changed to a more objective method.

In general, the aim of the development of a separation method is to obtain the required separation of all sample components of interest in a reasonable analysis time [1]. This means that each peak of interest corresponds to a single component, no significant overlap between peaks takes place and the cost of separation is as low as

possible. It is important that the measurement of the quality of a separation is independent of the technique or conditions used to obtain the separation. Several aspects should be considered in the evaluation of separation quality. First, the required separation should be considered. Second, the other factors such as the total analysis time and consumption of mobile phase are also very important. Considering these requirements, both the required separation and the cost of separation should be included in a criterion expressing the quality of separation.

There are several different methods in chromatography and electrophoresis that are used to describe the degree of separation of the components of a mixture, but usually they do not adequately consider peak asymmetry and the cost of separation. Any criteria that have to judge separation quality should condense the information from all of the chromatogram into a single number. The main problem with such evaluation is to define a criterion that adequately quantifies the peak overlap. The chromatograph-

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ic resolution $R_{i,j}$ [2], Kaiser's criterion P [3], separation factor S [4], Trennzahl TZ [5] and selectivity factor α are commonly used to describe the separation quality of two peaks. The elementary criteria already mentioned consider neither the influence of other peaks on the separation nor, except for Kaiser's criterion, the peaks-height ratio and peak shapes. The fraction overlap parameter [6] is more suitable to describe peak overlap, but it considers two neighbouring peaks only.

The aims of the chromatographer vary according to the analytical problem encountered and the technique chosen to solve it. One, several preferred or all components of the recorded signal can be of interest [7]. The resolution of all the peaks composing the mixture is, however, only a rare situation in practice. The question to be answered in each instance is what the chromatographer thinks is a good (or at least acceptable) separation. This varies widely for qualitative or quantitative analysis or preparative chromatography. Therefore, specific goals of a particular problem should be built in into the criteria proposed.

The aim of this paper is to define a threshold criterion that considers peak asymmetry, peak-height ratio and influence of other peaks on the separation. The value of the threshold criterion is affected only by the separation quality of relevant peaks. The cost of separation is considered for evaluation of equal separation quality obtained under different conditions.

2. Theoretical

The separation of one component from the other solutes in a mixture can be calculated in different ways. The most advantageous is the calculation of the overlapped area of the peak of interest with adjacent peaks in a chromatogram. Information about overlapped and total peak areas gives an outline of the separation quality of the peak investigated.

If there are m peaks expected on the chromatogram, the total overlapped area O_k of peak k can be defined as

$$O_k = O_{1,k} + \dots + O_{k-1,k} + O_{k+1,k} + \dots + O_{m,k}$$

where $O_{i,k}$ is a particular overlap of peak k with peak i in area units. The degree of overlap DO_k of peak k with m adjacent peaks can be defined as the ratio of overlapped area O_k to the total peak area A_k (see Fig. 1):

$$DO_k = \frac{O_k}{A_k} \cdot 100\%$$

The DO_k value expresses the overlapped area of peak k in pure peak k area units. O_k represents an addition of all contributions, and therefore the DO_k value lies in the $(0; \infty)$ interval. In a comparison of a procedure based on peak overlap with those using the chromatographic resolution factor $R_{i,j}$, a significant increase in the degree of overlap, DO_A , is shown in Fig. 2 for peak A with increasing peak-height ratio h_B/h_A , while $R_{A,B} = 1$ in all instances. In the Fig. 3, the dependence of DO_A on the peak-height ratio h_B/h_A for two simulated Gaussian peaks ($R_{A,B} = 1$) is presented. The course of the curve depends, however, both on the value of the resolution factor and on the peak shapes.

The difference in above-mentioned approaches can be more clearly illustrated for the peak doublet and triplet in Fig. 4a and b, respectively. The chromatographic resolution factors are equal ($R_{A,B} = R_{B,C} = 1$), but the degrees of overlap DO_B are different in Fig. 4a and b. The overlapped area reflects the influence of all peaks on the separation quality of the investigated peak.

The separation quality of peak k describes via DO_k eliminates problems connected with non-symmetrical peak shapes, different height ratios of overlapped peaks and influences of other

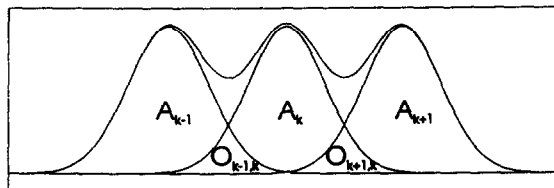


Fig. 1. Peak areas for degree of overlap calculation.

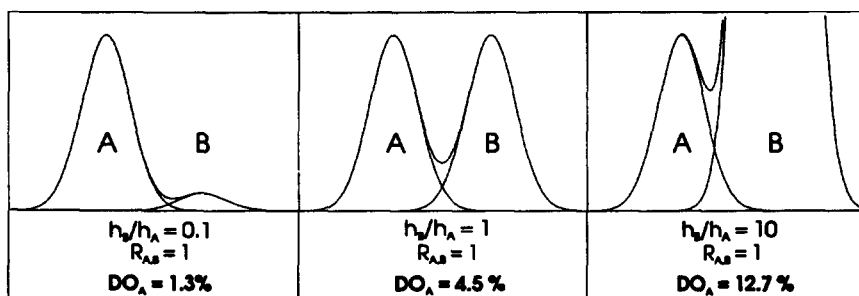


Fig. 2. Comparison of the degree of overlap DO_k approach with the chromatographic resolution factor $R_{i,j}$ method.

peaks on the separation. By setting a threshold value P_k (e.g., 5%) for each relevant peak k , below which the degree of overlap DO_k is acceptable, a term I_k can be defined:

$$I_k = 1 \text{ if } DO_k \leq P_k$$

$$I_k = 0 \text{ if } DO_k > P_k$$

The value of P_k is a numerical expression of the required separation of peak k and therefore the choice of P_k depends on the required peak purity. When the relevant peaks are separated better than the required P_k value, $I_k = 1$, and if the separation is worse, then $I_k = 0$. Based on

this, a primary part of the proposed threshold criterion can be defined as

$$F_1 = \sum_{k=1}^N I_k$$

where N is the number of peaks of interest. The primary part of the criterion can be a whole number from the interval $(<0; N>)$ and is affected by the quality of the relevant peak

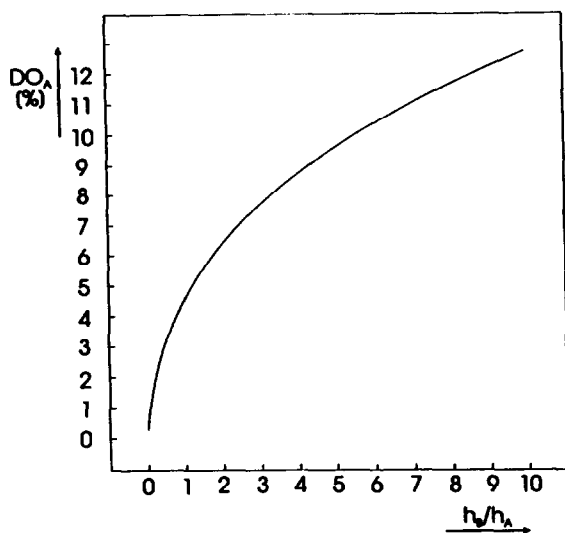


Fig. 3. Dependence of DO_A on peak-height ratio h_b/h_A for two Gaussian peaks ($R_{A,B} = 1$).

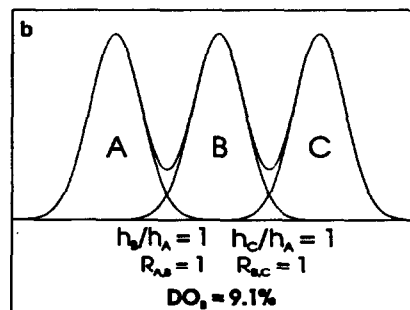
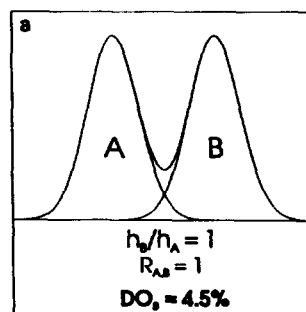


Fig. 4. Degree of overlap of peak B (DO_B) calculated for (a) one and (b) two adjacent peaks while $R_{A,B} = R_{B,C} = 1$.

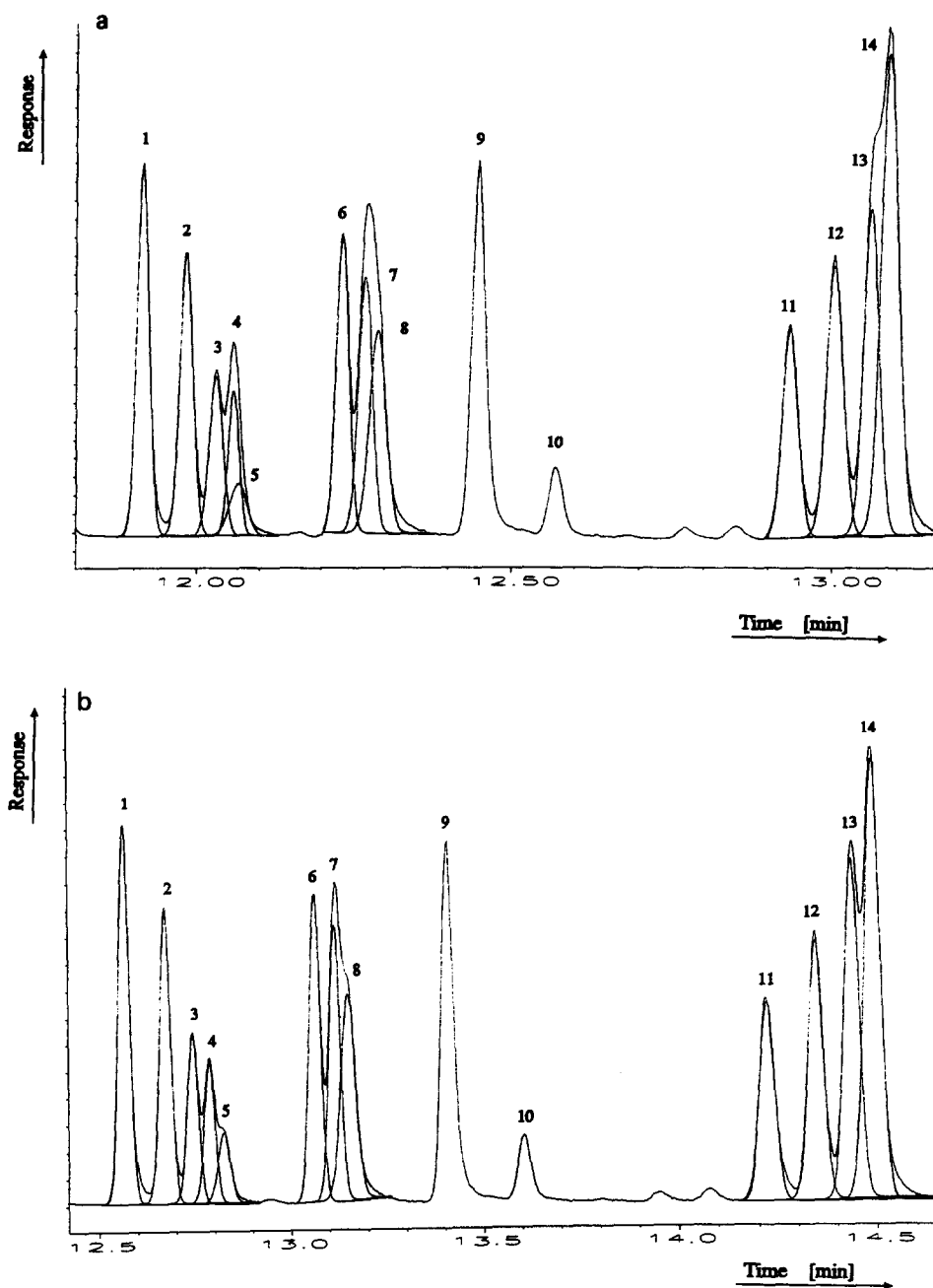


Fig. 5. Two chromatograms of the test mixture recorded using slightly different temperature programmes. The pure peaks are also depicted.

separation only. The separation of irrelevant components does not affect the F_1 value. With the choice of P_k we can fine tune the criterion with regard to the separation goals.

The cost of analysis is often an important factor in practice. The time needed to realize any separation can play a very important role in routine analysis and if the analysis is too long a worse separation (from the point of view of resolution) is sometimes accepted. Factors affecting the cost of separation (such as the analysis time and consumption of mobile phase) can be included in the proposed criterion as a secondary part. The threshold criterion can be found, as we recently showed [8], from the equation

$$F = \sum_{k=1}^N I_k + \frac{\text{cost}_{\max} - \text{cost}}{\text{cost}_{\max}}$$

The chosen maximum acceptable cost of analysis (cost_{\max}) should be higher than the real cost. The cost of a real analysis is never zero and therefore the ratio $(\text{cost}_{\max} - \text{cost})/(\text{cost}_{\max})$ is always less than 1. The secondary part of this criterion therefore distinguishes between different separations when an equal number of relevant compounds are separated between as required but the separation costs are different. The higher the

criterion value for recorded signal, the better is the separation and the cheaper the analysis. The use of the threshold criterion in computer-assisted optimization procedures will be published separately [9].

3. Verification

The validity of the proposed threshold criterion was verified by an evaluation of the separation quality two example chromatograms obtained by capillary gas chromatography. The chromatograms differ slightly in appearance owing to variations in the temperature programme. The calculations of the primary part of the criterion, corresponding to selected peaks from a fraction of a complex chromatogram, are presented in Fig. 5a and b, is shown in Table 1. For calculation of the degree of peak overlap a deconvolution procedure was used [9]. Fourteen peaks were detected in both fractions of the chromatograms. Twelve of them were selected as a relevant ($N = 12$) and corresponding P_k values were set. Six peaks have a degree of overlap $DO_k > P_k$ and therefore they were not counted as pure enough. Six were acceptably pure ($F_{1A} = F_{1B} = 6$) as for these $DO_k \leq P_k$. The elution

Table 1
Calculation of the primary part of the criterion for the chromatograms in Fig. 5a and b

Peak number (k)	Is peak relevant?	P_k (%)	Fig. 5a		Fig. 5b		
			DO_k (%)	I_k	DO_k (%)	I_k	
1	Yes	5	0.04	1	0.01	1	
2	Yes	5	1.71	1	0.86	1	
3	Yes	5	25.20	0	11.71	0	
4	Yes	10	66.97	0	30.45	0	
5	Yes	10	106.05	0	27.13	0	
6	Yes	10	8.35	1	7.17	1	
7	No	–	44.91	–	30.98	–	
8	Yes	10	41.37	0	29.09	0	
9	No	–	0.00	–	0.00	–	
10	Yes	7	0.00	1	0.00	1	
11	Yes	7	0.54	1	0.11	1	
12	Yes	7	2.56	1	1.54	1	
13	Yes	7	31.90	0	19.81	0	
14	Yes	7	20.48	0	13.68	0	
$N = 12$				$F_1 = 6$		$F_1 = 6$	

times of the last peaks in the chromatograms in Fig. 5a and b were 25 and 42 min, respectively, which correspond to the costs of analyses. For $\text{cost}_{\max} = 60$ min the values $F = 6.58$ for Fig. 5a and $F = 6.30$ for Fig. 5b were found using the data given in Table 1, which means that the separation in Fig. 5a is superior to that in Fig. 5b.

By setting other threshold P_k values ($P_k = 15\%$ for all peaks), other F values were found: $F = 6.58$ for Fig. 5a and $F = 8.30$ for Fig. 5b, from which the separation in Fig. 5b is superior to that in Fig. 5a. Hence the criterion can be tuned by the choice of the threshold values P_k . From the above examples it follows that the objectives of the separation should be well defined in order to make a reliable decision.

4. Acknowledgement

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