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Modified resolution factor for asymmetrical peaks in chromatographic separation

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Abstract

The quality of separation is measured by resolution factor (R_s) between adjacent peaks. The current United States Pharmacopeia (USP) method for R_s calculation assumes symmetrical peak shapes. In practice, perfectly symmetrical peaks are rarely encountered. The goal of this study was to correct the inaccuracy due to peak asymmetry by using a new formula for R_s calculation. Peak tailing factor was incorporated into the formula for the calculation of R_s to correct for the peak asymmetry. The resulting modified formula was compared with USP formula using simulated peaks and actual peaks. Through mathematical derivation, the modified R_s was expressed as: $R = [2(t_2 + W_2/2(1 - 1/TF_2) - t_1 - W_1/2(1 - 1/TF_1))]/W_1 + W_2$ where t_1 and t_2 are the retention times of two peaks, W_1 and W_2 are peak widths at baseline, TF_1 and TF_2 are USP tailing factors. All parameters used in the formula are available from commercial data analysis software. Comparisons of modified R_s to USP R_s showed that the modified R_s provided a more accurate measure of peak separation at the baseline level.

Keywords: Tailing factor; HPLC; Pharmaceutical analysis

1. Introduction

Chromatographic systems separate mixture of compounds into individual peaks. The quality of separation is measured by resolution factor (R_s) between adjacent peaks. R_s is widely used as a system suitability criterion in chromatographic analysis. Baseline separation, normally with an R_s greater than 1.5, allows accurate integration of individual peaks and their quantitation.

The most widely used method for R_s calculation is the tangent method, which is adopted by the United States Pharmacopeia (USP) [1,2], as shown in Eq. (1):

$$R_s = \frac{(t_2 - t_1)}{W_2/2 + W_1/2} \quad (1)$$

where t_2 and t_1 are the retention times of the two components, and W_2 and W_1 are the corresponding widths at the bases of the peaks obtained by

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extrapolating the relatively straight sides of the peaks to the baseline (Fig. 1).

One major assumption underlying the USP method is that peaks are symmetrical in peak shape. However, most chromatographic peaks are not perfectly symmetrical. Tailing factor was thus introduced to estimate the degree of peak asymmetry. USP defines the tailing factor as:

$$\mathrm{TF} = \frac{A + B}{2A} \tag{2}$$

where A and B measured at 5% peak height as illustrated in Fig. 2 [1].

Peak asymmetry is commonly observed in various modes of chromatographic separations. Reversed phase high performance liquid chromatography (HPLC) is currently the most widely used method for pharmaceutical analysis and other analytical separations [2]. Despite the emergence of improved packing materials for HPLC columns, peak tailing (or fronting) is still commonly observed.

Despite the importance of asymmetrical peaks in chromatographic separation, the USP method for R_s calculation (with the assumption of symmetrical peak shapes) is still widely used to evaluate separation quality. The purpose of this report is to develop a new calculation method that will provide more accurate estimation of separation quality for both symmetrical and asymmetrical peaks.

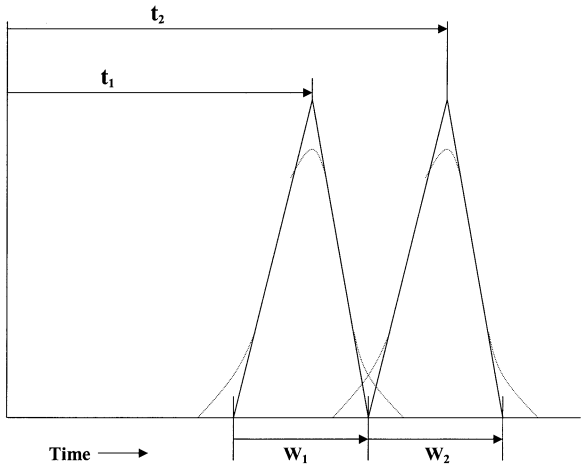

Fig. 1. Illustration for USP resolution factor calculation method (Eq. (1)).

2. Experimental and results

2.1. Derivation of tailing factor modified R_s

The USP method for R_s calculation simplifies the Gaussian peak shape (smooth curvature) to a triangular shape (straight lines) (Fig. 1). This simplification is necessary for the ease of calculation. Moreover, the USP method also assumes that peaks are perfectly symmetrical. For a symmetrical triangle peak, the retention time represents the middle point on the baseline. The separation of two peaks is represented by the difference of their baseline middle points. Based on the assumption of symmetrical peaks, the baseline middle points correspond to the peak retention times. Therefore, the difference in retention times ($t_2 - t_1$) is used instead of the difference in the baseline middle points in the R_s calculation.

However, in practice, perfectly symmetrical peaks are rarely encountered [3]. For asymmetrical peaks, peak retention time does not coincide with the middle point on the baseline. Therefore, the difference between the retention times (i.e. $t_2 - t_1$) no longer represents the separation of the two peaks. To accurately represent the separation of two peaks, the peak retention time needs to be corrected to represent the middle point on the baseline.

As shown in Fig. 2, by neglecting peak dispersion and simplifying peak shape as a asymmetrical triangle, the USP tailing factor can be approximated by Eq. (3):

$$\mathrm{TF} = \frac{A + B}{2A} = \frac{A' + B'}{2A'} = \frac{W}{2A'} \tag{3}$$

where A and B are measured at 5% of peak height, A' and B' are measured at baseline and W is the peak width at baseline ($W = A' + B'$).

Rearranging Eq. (3), we obtain Eq. (4):

$$A' = \frac{W}{2\mathrm{TF}} \tag{4}$$

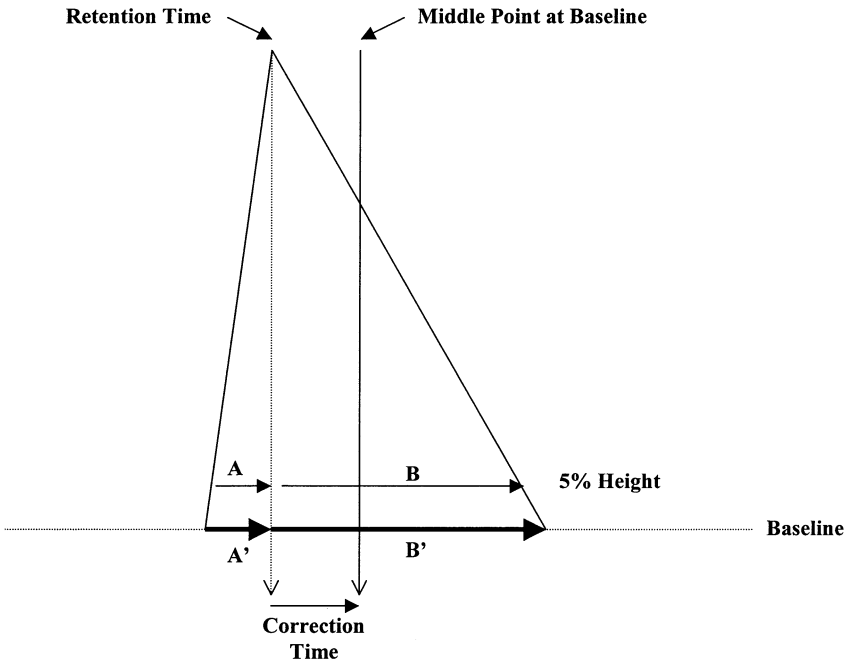

Fig. 2. Illustration for USP tailing factor calculation method and retention time correction based on USP tailing factor (Eqs. (2)–(6)).

The correction time between the peak retention time and the baseline middle point is described by the following equation, incorporating Eq. (4):

$$\text{Correction time} = \frac{B' - A'}{2} = \frac{W - 2A'}{2}$$
$$= \frac{W - W/TF}{2} = \frac{W}{2}\left(1 - \frac{1}{\text{TF}}\right) \quad (5)$$

The tailing factor modified retention time, which corresponds to the baseline middle point, is obtained in Eq. (6):

$$T_{\text{corrected}} = t + \frac{W}{2}\left(1 - \frac{1}{\text{TF}}\right) \quad (6)$$

Substituting Eq. (6) into the USP R_s calculation formula (Eq. (1)), the tailing factor modified R_s formula is obtained, as shown in Eq. (7):

$$R = \frac{2(t_2 + W_2/2(1 - 1/\text{TF}_2) - t_1 - W_1/2(1 - 1/\text{TF}_1))}{W_2 + W_1} \quad (7)$$

2.2. Comparison of modified R_s with USP R_s using simulated ideal peak profiles

Fig. 3 shows three simulated peak pairs with identical baseline separation but different peak shapes. Peak dispersion was neglected to simplify the comparison. Two symmetrical peaks (Fig. 3A), one tailing peak followed a fronting peak (Fig. 3B), and one fronting peak followed by a tailing peak (Fig. 3C) were simulated. The USP R_s obtained from these simulated peak pairs were 1.0, 0.5 and 1.5, for Fig. 3A–C, respectively. Since all three peak pairs had identical baseline separations, the large variation in USP R_s was solely due to the misrepresentation of baseline middle points by peak retention times.

Table 1 summarized the corrected retention times and corrected resolution factors using the peak profiles simulated in Fig. 3. Using Eq. (7), the corrected R_s for all three peak pairs was 1.0, correctly reflecting the identical separation at baseline. Therefore, using the simulated ideal peak profiles, the tailing factor modified R_s

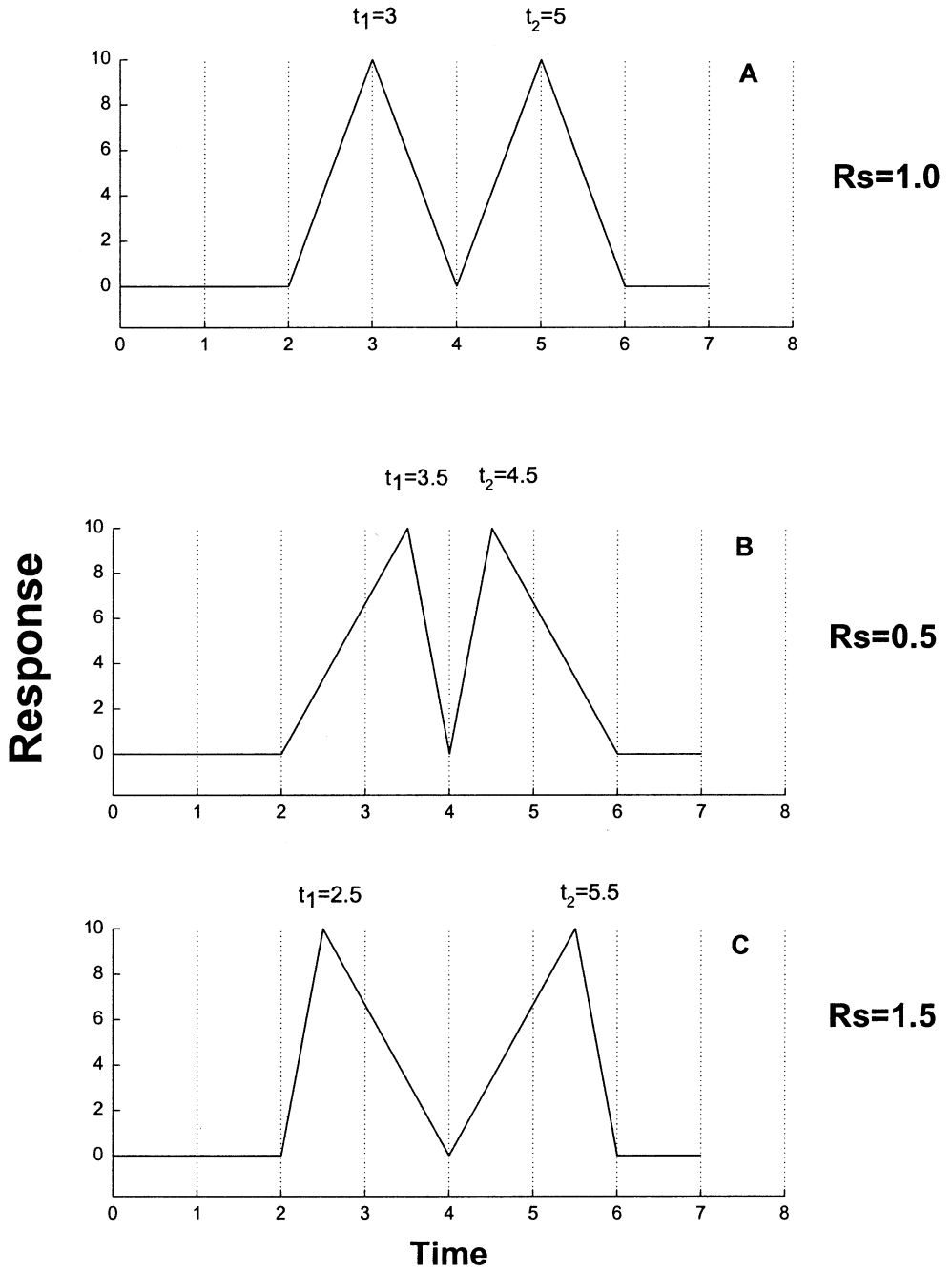

Fig. 3. Simulated peak pairs assuming ideal triangular peak shapes; (A) two symmetrical peaks, (B) a fronting peak and a tailing peak, (C) a tailing peak and a fronting peak. The resolution factors (R_s) shown are calculated according to USP method.

Table 1
Corrected retention times and resolution factors using the values provided in Fig. 3

	t_1	t_2	W_1	W_2	R_s (USP)	TF_1	TF_2	t_1 corrected	t_2 corrected	R_s modified
A	3	5	2	2	1.0	1	1	3	5	1.0
B	3.5	4.5	2	2	0.5	0.67	2	3	5	1.0
C	2.5	5.5	2	2	1.5	2	0.67	3	5	1.0

t_1 and t_2, retention times of peak 1 and 2; W_1 and W_2, baseline widths of peak 1 and 2; TF_1 and TF_2, tailing factors for peak 1 and 2; t_1 corrected and t_2 corrected, tailing factor corrected retention times for peak 1 and 2; R_s (USP), resolution factor according to USP tangent method; R_s modified, resolution factor modified by tailing factors.

provided better estimation of separation quality than the USP R_s.

2.3. Comparison of modified R_s with USP R_s using a real-life chromatogram

A real-life example was provided in Fig. 4. Only the three peaks of interest were shown in the representative chromatogram. Three closely eluting peaks had retention times of 45.4, 47.0 and 49.2 min, respectively. Peak B showed moderate tailing (USP tailing factor of 1.86). The top panel shows the full-scale chromatogram whereas the bottom panel shows the expanded baseline view. From the expanded view, it was evident that the separation of peak A from peak B was completely baseline to baseline, whereas the separation of peak B from peak C suffered from a slight overlap (i.e. a valley point). The chromatographic data was analyzed by computer software (CHEMSTATION, Agilent Technologies, Palo Alto, CA, USA) and the resulting resolution factors and tailing factors were summarized in Table 2. Using the USP specified tangent method, the R_s between the partially separated peaks (i.e. peak B and peak C) was 2.026, whereas the R_s between the completely separated peaks (i.e. peak A and peak B) was 1.654. This misrepresentation of separation quality by the USP R_s was mainly due to the tailing of peak B.

Table 2 also summarized the corrected retention times and resolution factors using the method described in this report. The corrected R_s between peaks A/B and peaks B/C were 1.85 and 1.79, respectively. These corrected numbers were better approximations of the separation quality than the USP R_s method.

2.4. Comparison of modified R_s with other alternative R_s calculation methods using the real-life chromatogram

Table 2 also summarized the resolution factors calculated by the software using three other methods (i.e. half-width, five sigma, and statistical methods). The equations used for these methods are:

Half-width method:

$$R_s = \frac{(2.35/2)(T_2 - T_1)}{W_{50(2)} + W_{50(1)}} \quad (8)$$

Five sigma method:

$$R_s = \frac{2.5(T_2 - T_1)}{W_{4.4(2)} + W_{4.4(1)}} \quad (9)$$

Statistical method:

$$R_s = \frac{M_{1(2)} - M_{1(1)}}{W_{s(2)} + W_{s(1)}} \quad (10)$$

where $M_{1(x)}$, mean retention time for peak x (1st statistical moment) (min); $W_{50(x)}$, width at 50% height for peak x (min); $W_{4.4(x)}$, width at 4.4% height for peak x (min); $W_{s(x)}$, width derived from statistical moments $= (M2)^{1/2}$ for peak x (min).

The detailed information for these calculation methods was summarized in the software manual. Nonetheless, all these methods gave a higher resolution factor between peaks B/C as compared with peaks A/B, which is contrary to the true separation quality at the baseline.

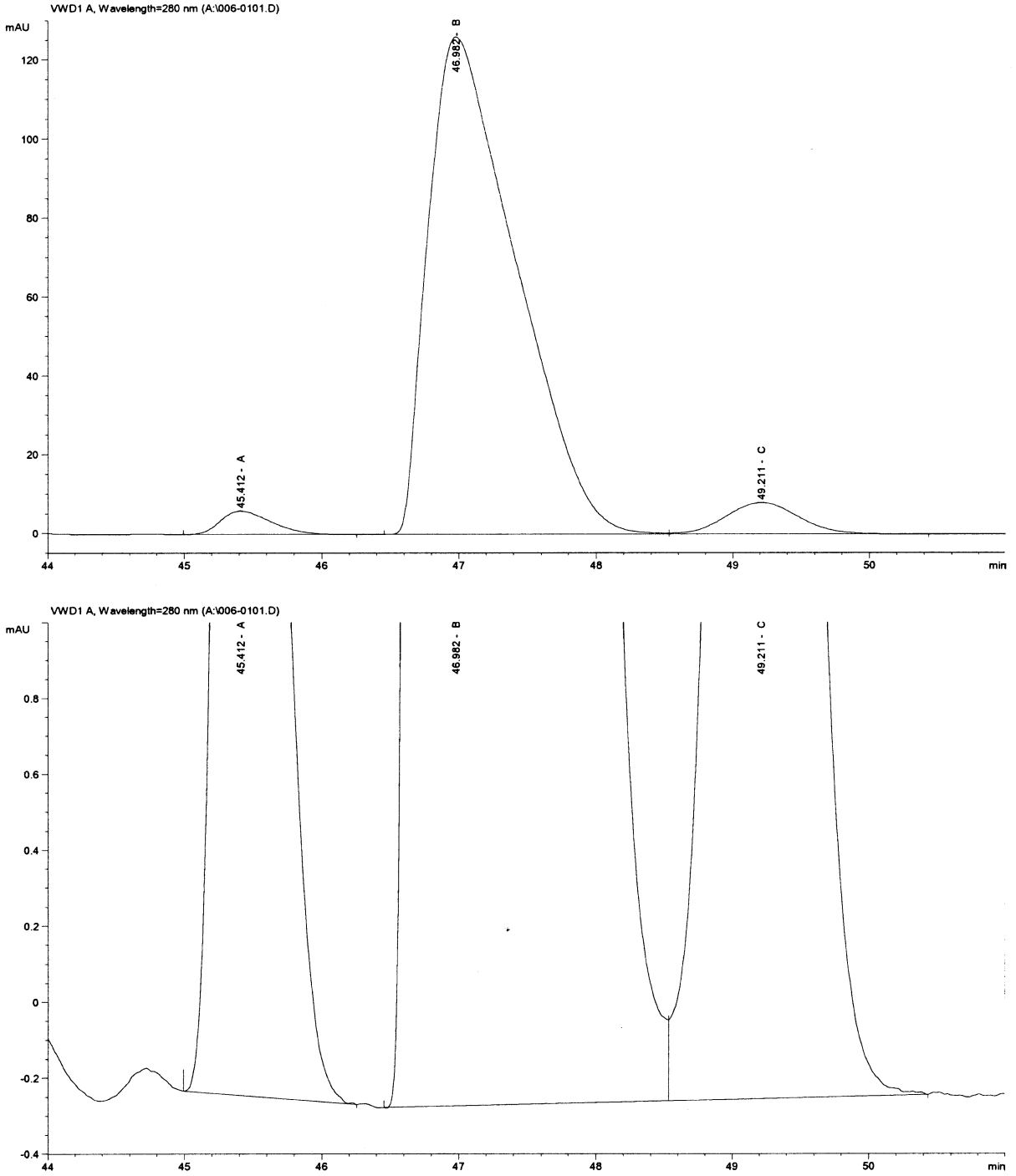

Fig. 4. A real-life chromatogram demonstrating the difference between different resolution factor calculation methods. Top panel: full scale chromatogram between 44 and 60 min. Bottom panel: expanded view of the same chromatogram as in top panel.

Table 2
Comparison of resolution factors calculated using different methods based on chromatographic profile shown in Fig. 4

Peak	Retention time (min)	Baseline width (min)	Tailing factor	Correction time (min)	Retention time Corrected (min)	Resolution factor				
						Tangent (USP)	Tangent (modified)	Half width	Five sigma	Statistical
A	45.4	0.68	1.38	0.094	45.5					
B	47.0	1.22	1.86	0.282	47.3	1.65	1.85	1.66	1.77	1.79
C	49.2	0.98	1.06	0.026	49.2	2.03	1.79	2.03	2.13	1.91

3. Discussion

Resolution factor is one of the most important parameter in chromatographic separation. During method development, chromatographic systems are optimized for maximum resolution between closely eluting peaks. To obtain accurate quantitation results, a baseline separation is preferred.

The USP Tangent R_s method (i.e. Eq. (1)), due to its simplicity and adoption by the regulatory agency, is the most widely used method for resolution calculation. For pharmaceutical analysis, the USP R_s method was used almost exclusively for regulatory compliance. However, as illustrated in Tables 1 and 2, the USP R_s method failed to correlate with the quality of separation where asymmetrical peaks were involved. This error was due to the assumption of symmetrical peak shape by the tangent method.

In pharmaceutical analysis of finished drug products and drug substance, the International Conference on Harmonization (ICH) guideline generally requires that the quantitation of impurity or degradation peaks down to 0.1% of the main peak level. Furthermore, most pharmaceutically active drugs are basic molecules with intermediate to strong basic moieties that ionize at neutral pH. Despite the advent of various improved silica materials and the adoption of low pH mobile phase to suppress silanol ionization, residual silanol activity is still a major problem for strongly basic compounds. Due to their dual interaction with the residual silanol groups and the reversed phase bonded material (e.g. C_{18}), basic drugs often show tailing or fronting peaks during HPLC separation at levels above 1 μg per injection in a injection amount dependent manner (unpublished results). Limited by the sensitivity of the UV detector and the requirement to measure 0.1% of degradation product, it is often necessary to inject $\sim$1–10 μg of drug per injection. Consequently, this large injection amount often results in moderately asymmetric peaks. The peak asymmetry is often variable depending on a multitude of factors, including column batch-to-batch variation and column temperature. Therefore, accurate calculation of resolution factors between a large asymmetrical peak and an adjacent impurity or

degradation product peak is of particular interest to pharmaceutical analysis.

The modified resolution factor proposed in this study was derived from tangent method and peak tailing factor, both are adopted by the USP and ICH guidelines. As a result, the tailing factor adjusted R_s could be easily calculated from these two parameters. Comparing with other available resolution methods (i.e. half-width, five sigma, and statistical), the tailing factor modified resolution factor also provided a better correlation with the separation quality as illustrated in Table 2. Half-width method and five-sigma method still assumed peak symmetry. Therefore, their calculation results were similar to that obtained from the USP R_s method than statistical method and the modified method. The statistical method does not assume peak asymmetry. As a matter of fact, the first statistical moment ($M_{1(x)}$) does correspond to the middle point on the baseline [4]. Therefore, the results calculated using the statistical method was the closest to that obtained from the modified method. However, the calculation of the statistical moments is more involved than the modified method and the results are less accurate.

In summary, we have developed and validated a simple and novel method for calculating R_s between asymmetrical peaks with no additional measurement. The modified R_s is superior to currently available methods in comparing separation qualities between asymmetric peaks.

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