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Short communication

Procedure for the quantification of rider peaks

Simon Jurt[a], Martin Schär[a], Veronika R. Meyer[b,*]

[a]*Department of Chemistry, University of Applied Sciences, Pestalozzistrasse 20, CH-3400 Burgdorf, Switzerland*
[b]*EMPA St. Gallen, Swiss Federal Laboratories for Materials Testing and Research, Lerchenfeldstrasse 5, CH-9014 St. Gallen, Switzerland*

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Abstract

Rider peaks are small peaks which are not well resolved from a large and asymmetrical neighbour but sit on its trailing side. The usual case is a large, tailed peak which is eluted just in front of the small peak, although the opposite situation can also occur (a small peak in front of a large peak with fronting). The common integration techniques, i.e. separating the peaks by vertical drop or by a tangent and determining area or height, give erroneous results. We propose a method for their quantification with low error. It is necessary to set up a ''two-dimensional'' calibration by varying both concentrations, i.e. of the large peak and of the rider. This leads to a series of linear equations which describe the rider size, as found by the integrator, as a function of the size of the large peak. The *y*-axis intercepts *i* of these equations show a linear relationship with the concentration *x* of the rider analyte, whereas the slopes *s* follow a quadratic relationship. These equations can be used to solve the equation $y = s(x) \cdot z + i(x)$ for *x* (*y* and *z* are the integrated peak size of the rider and the large peak, respectively). The procedure was tested with computer-generated peak pairs as well as with HPLC separations of 2,3-dimethylaniline (large tailing peak) and 2,3-dimethylphenol (symmetrical rider peak).

Keywords: Rider peaks; Overlapping peaks

1. Introduction

Poorly resolved peaks need special attention for their quantification. Depending on resolution, peak size ratio, and asymmetry, the determination of peak area or height can be highly erroneous [1]. The errors become large when one of the peaks is much smaller than the other [2]. However, there is one exception: if the peaks are tailed, which is the common case, if the small one is eluted in front of the large one, and if the analysis is based on peak heights, then the error in quantification is zero or very small [3].

In many cases (the exception is the separation of isomers) the problem can be solved by using a mass spectrometer as the detector, but this is not yet a standard method because it is more complex and expensive than, for example, UV detection. Various proposals for the quantification of poorly resolved peaks have been published: differential signal detection [4], generic algorithms [5], or the iterative solution of equations which describe the response surface [6]. All these approaches have a broad field

*Corresponding author. Tel.: +41-71-274-7787; fax: +41-71-274-7788.

E-mail address: veronika.meyer@empa.ch (V.R. Meyer).

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of application but they need special equipment or sophisticated mathematical techniques.

Our proposal is rather simple and has no special requirements for detection or evaluation. It works well for the case where the sizes of both the large and the small peak vary, which is what the analyst faces in reality. The approach needs a greater effort for calibration than is necessary for well-resolved peaks, but the mathematics is restricted to the solution of a quadratic equation. Pure standards of both the compounds involved are necessary.

2. Experimental

All investigated chromatograms (artificial and real) were of the following type: a large, tailing peak is followed by a small, symmetrical peak sitting on the tail of the large peak. The integrator separated the peaks by either a vertical drop or a tangent and determined their area or height.

2.1. Simulations

Artificial chromatograms were simulated with the software CalPeak (Allied Data Scientific, Houghton le Spring, Tyne and Wear, UK), which allows the generation of symmetrical and tailed peaks of any size ratio and resolution. These artificial chromatograms can then be processed by any integrator. We used a HP 3395 model (Hewlett-Packard, Palo Alto, CA, USA). The asymmetry of the peaks was 7.4 and 1.0, respectively (determined as b/a at 5% peak height). The peak area ratio varied between 8.3:1 and 60:1.

2.2. Real chromatograms

The real peak pair was 2,3-dimethylaniline (tailed peak; here called ''aniline''; puriss., Fluka, Buchs, Switzerland) and 2,3-dimethylphenol (symmetrical peak; here called ''phenol''; purum, Fluka). A typical high-performance liquid chromatographic separation is shown in Fig. 1, which was obtained under the following conditions:

- Stationary phase: Nucleosil 100-5 C_{18} HD, 5 μm (Macherey-Nagel, Oensingen, Switzerland).
- Column: 250 mm×4.6 mm I.D.

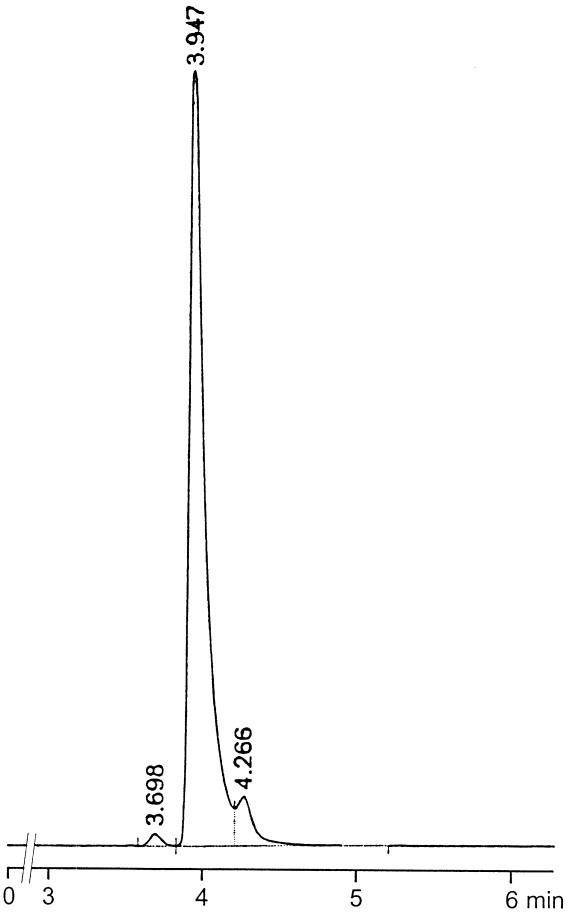

Fig. 1. HPLC separation of a mixture of 1501 mg/l 2,3-dimethylaniline (peak at 3.947 min) and 60.2 mg/l 2,3-dimethylphenol (peak at 4.266 min). Conditions as described in Experimental.

- Mobile phase: water (Millipore)–methanol (Absolute HPLC, Biosolve, Valkenswaard, The Netherlands), 2:8 (v/v), 1 ml/min.
- Instrument: HP 1100 (Hewlett-Packard) with UV detection at 280 nm.
- Integrator: HP 3395 (Hewlett-Packard).
- Sample: ''aniline'' and ''phenol'' were dissolved in the mobile phase in mass ratios ranging from 5:1 (500.3 and 100.3 mg/l) to 62.4:1 (2501.7 and 40.1 mg/l). The injected sample size was 10 μl.

2.3. Quantification procedure

Our procedure was as follows:

- Prepare 20 reference solutions: five different

concentrations of the main compound (approx. 500, 1000, 1500, 2000, and 2500 mg/l), each containing four different concentrations of the minor compound (approx. 40, 60, 80, and 100 mg/l).

- Choose an integration method. The best results were obtained with peak separation by a tangent and the determination of peak heights.
- Perform the HPLC separation and integration of all reference solutions.
- Present the integrated rider size y' as a function of the integrated size of the large peak z'. This gives a series of four more or less parallel lines with five points each. Their slope is positive if the peaks were separated by a vertical drop, whereas it is negative when using a tangent (Fig. 2).
- Present the intercepts i of these four lines as a function of the concentration of the rider peak analyte x'. This gives a straight line with positive slope which can be described by a linear equation of the type $i = f(x') = a_i x' + b_i$ (Fig. 3(bottom)).
- Present the slopes s of these four lines as a function of the concentration of the rider peak analyte x'. This gives a bent line which can be described by a quadratic equation of the type $s = f(x') = a_s x'^2 + b_s x' + c_s$ (Fig. 3(top)).
- Inject the sample with the poorly resolved peak pair and integrate it by the same method as the standards. Determine the sizes of both peaks (y is the size of the rider peak, z is the size of the large peak).
- Solve the equation

$$y = z_s + i = z(a_s x^2 + b_s x + c_s) + (a_i x + b_i)$$

for x, the unknown concentration of the rider peak.

Nomenclature: x' is used for the reference and x for the sample.

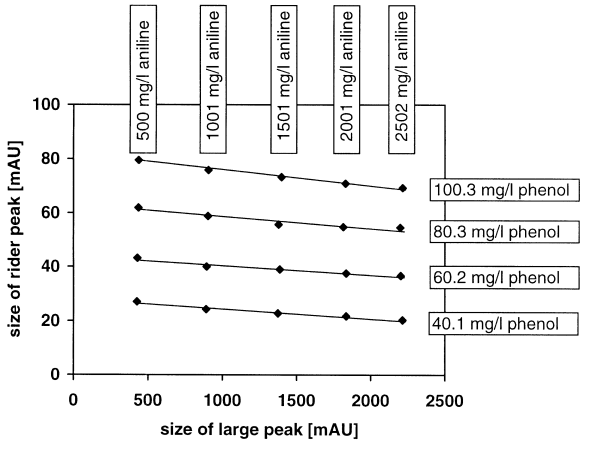

Fig. 2. Peak sizes as found by the integrator. The integration method was peak separation by a tangent and determination of heights.

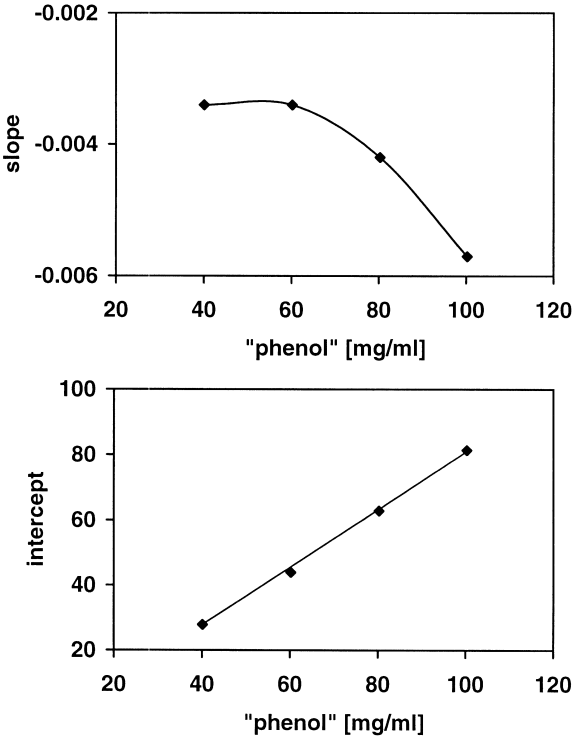

Fig. 3. Slopes and intercepts of the four lines shown in Fig. 2 as a function of the concentration of the small peak.

3. An example

As already mentioned, the method yielding the most accurate results was peak separation by a tangent and the evaluation of peak heights. The 20 reference solutions had ‘‘aniline’’ concentrations of 500.3, 1000.7, 1501.0, 2001.4, and 2501.7 mg/l and ‘‘phenol’’ concentrations of 40.1, 60.2, 80.3, and 100.3 mg/l.

Fig. 2 shows the heights of the rider peak as a function of the heights of the large peaks. The slopes of the four lines are −0.0034, −0.0034, −0.0042, and −0.0057. Their intercepts are 27.80, 43.95, 62.87, and 81.43. The correlation coefficients are $r = 0.9826$, 0.9713, 0.9501, and 0.9935.

The slopes s and intercepts i are presented as a function of the ''phenol'' concentration x' in Fig. 3. The relationships can be described by:

$$s = -9.3 \cdot 10^{-7} x'^2 + 9.28 \cdot 10^{-5} x' - 5.614 \cdot 10^{-3} \quad (r = 0.99995)$$

$$i = 0.896x' - 8.909 \quad (r = 0.9994)$$

Now the equation

$$y = z(a_s x^2 + b_s x + c_s) + (a_i x + b_i)$$

must be solved for x. As an example, the following data are used:

- $y = 39.0$ mAU (the experimental height of the ''phenol'' peak of a sample solution);
- $z = 1386$ mAU (the experimental height of the ''aniline'' peak of the same solution);
- $a_s = -9.3 \cdot 10^{-7}$ (the first factor in the equation for s above);
- $b_s = 9.28 \cdot 10^{-5}$ (the second factor);
- $c_s = -5.614 \cdot 10^{-3}$ (the third factor);
- $a_i = 0.896$ (the first factor in the equation for i above);
- $b_i = -8.909$ (the second factor).

This gives a quadratic equation:

$$y = z(a_s x^2 + b_s x + c_s) + (a_i x + b_i)$$

$$39.0 = 1386(-9.3 \cdot 10^{-7} x^2 + 9.28 \cdot 10^{-5} x - 5.614 \cdot 10^{-3}) + 0.896x - 8.909$$

$$1.29 \cdot 10^{-3} x^2 - 1.025x + 55.7 = 0$$

Its negative root yields $x = 58.7$ mg/l, which is 97.5% of the true value of 60.2 mg/l.

4. Accuracy

In all investigated cases the tangent method gave more accurate results than peak separation by a vertical drop. Also, the determination of peak heights was superior to peak areas. The artificial chromatograms gave almost perfect results with this approach. The deviation of the experimental peak heights from the true heights was less than 1% over the whole range covered by the standard chromatograms (usually 0.1%, but some results had a deviation of 0.2% and one of 0.7%). With the vertical drop/peak area approach, some deviations reached 11%.

For real separations the deviations of the tangent/peak height method were slightly larger than with artificial peaks, however they were always lower than 4% within the range covered by the standard solutions. This is remarkably low when compared with the data shown in Ref. [1]. When the peaks were separated by a vertical drop the inaccuracy was in the 9% range in some cases of small ''phenol'' peaks (40 mg/l).

5. Conclusions

Our procedure needs 20 calibration points (or more), but its mathematical treatment is straightforward and needs no special knowledge or software other than how to solve quadratic equations. Its accuracy depends on peak resolution, tailing, and peak size ratio, but a bias of less than 4% can be expected for chromatograms such as those shown in Fig. 1.

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