Improved Quantitation Protocols in Ion Chromatography

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ABSTRACT

In chromatography, high resolution indicates a good separation but does not automatically translate into good quantitation. An inadequate quantitation can easily lead to erroneous results and therefore compromise the analysis result. We will discuss the many ways to improve quantitation protocols in ion chromatography.

INTRODUCTION

Suppressed ion chromatography (IC) is an essential tool for anion and cation analysis, for a variety of samples from drinking water to biological samples. Today with highly efficient columns and optimal suppressors, the quantitation limits are continuously being pushed lower. In addition to separation, quantitation is an integral part of an efficient analysis. In this poster we present the benefits of weighted calibration, data interpolation, evaluate the different types of quantitation and elaborate on impurity quantitation.

MATERIALS AND METHODS

Sample Preparation

Standard solutions or solution mixtures were used for analysis.

Test Method

IC data were generated with a Thermo Scientific/Dionex IC-25 system with an isocratic pump or a Thermo Scientific™ Dionex™ ICS-5000 HPIC system with a gradient pump. Other components included a Thermo Scientific™ Dionex™ EG40 Electrolytic KOH Eluent Generator, injection volume of 10 µL (2 µL for Fig 8), 2 mm bore Thermo Scientific™ Dionex™ IonPac™ AG20/AS20 guard and separation column, LC30 temperature controlled oven (30 ºC), Thermo Scientific™ Dionex™ ASRS™ Ultra II Anion Self-Regenerating Suppressor in external water mode, and a conductivity detector, which were integrated into the system.

Data Analysis

Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS), Microsoft Excel.

Percent Error is a Better Indicator of a Good Fit

Standard linear regression based quantitation (R^2) fundamentally minimizes absolute errors and therefore increases the relative errors at the low end.

The accuracy and reliability of any quantitation protocol is perhaps best judged by the degree of mismatch (error) between the actual value and that predicted by the calibration equation.

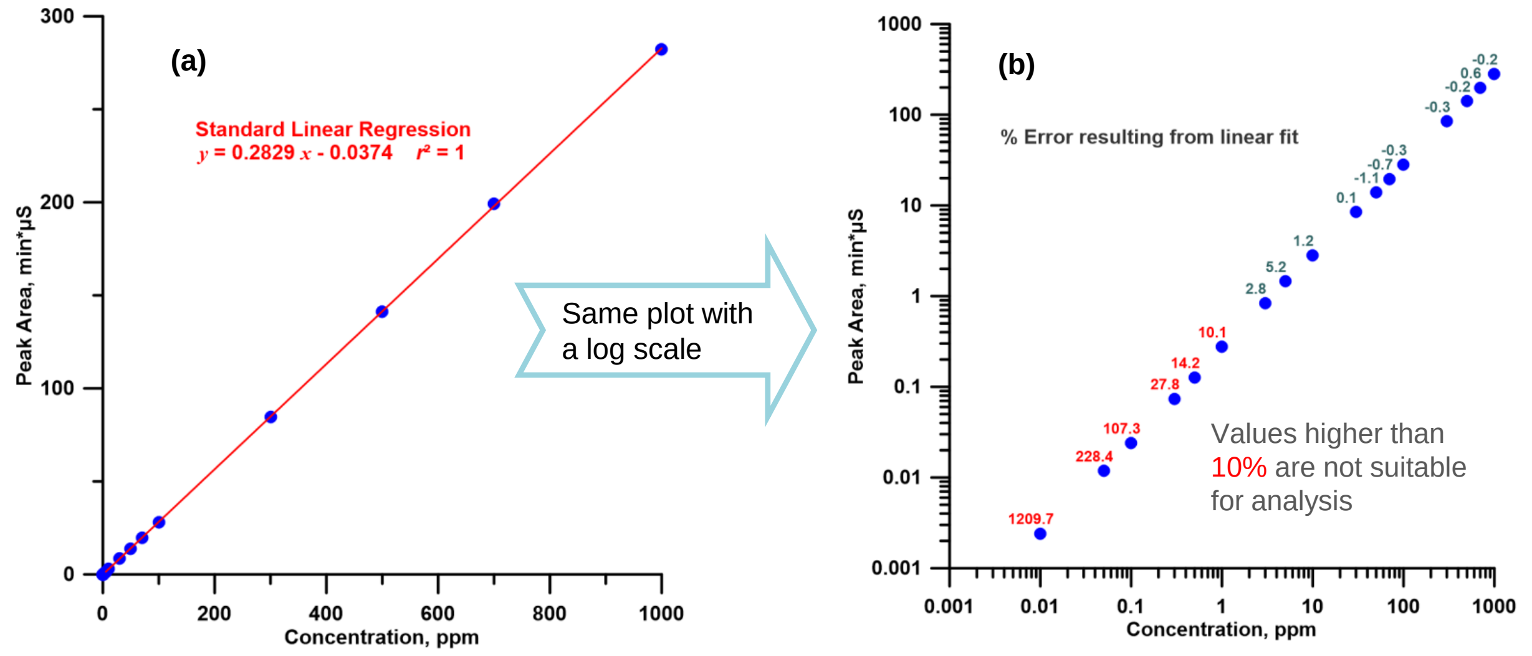


Figure 1. Area-based calibration plot for chloride; (a) shows the fit with linear axes (b) the axes are logarithmic.

As it can be seen in Figure 1, with R^2 =1 (which, in theory, should indicate a perfect overall fit), only the high data points have a low percent error.

Interpolation of Nearest Data Points for Large Data Set

For the highest accuracy, when a sample is to be quantitated, the relevant calibration curve is generated using only a few points around the observed signal of the sample peak. For linear relationships, the minimum requirement is one point on each side of the sample. As can be seen in Figure 2, a linear interpolation reduces the %Error from 107.3% to -0.8%.

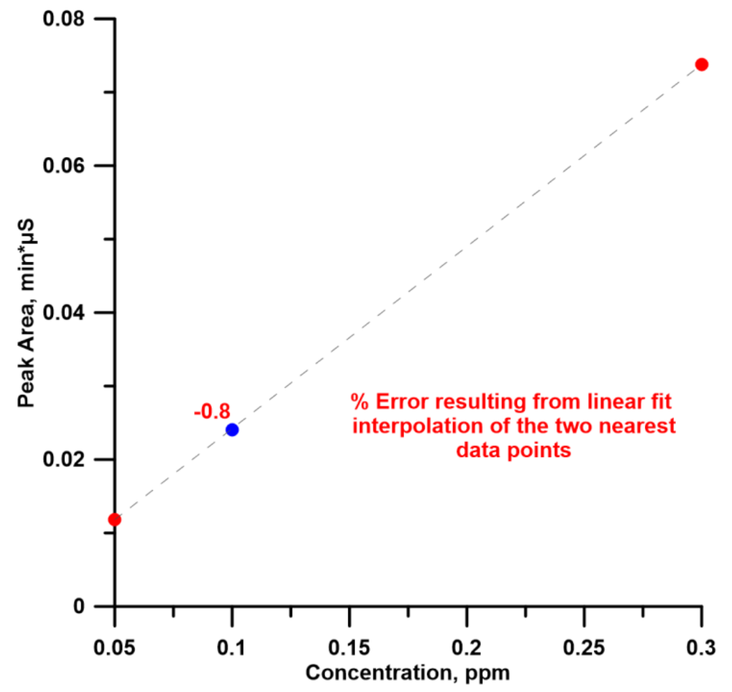


Figure 2. Linear fit interpolation of a chloride data point from Figure 1.

1/x² Weighting

The solution to high relative error is often a weighted linear regression (most commonly $1/x^2$ weighting).

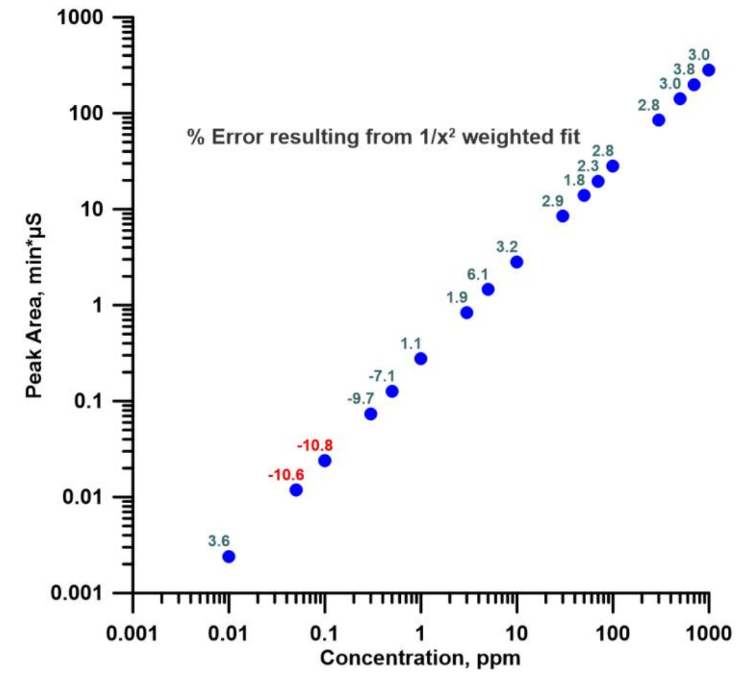


Figure 3. $1/x^2$ weighting applied to the chloride data point from Figure 1.

Appropriate Choice of Quantitation Method (1)

Since the inception of quantitative chromatography, the height and/or area of a peak have been used for quantitation. Area is a true representation of the solute quantity while the peak height is an oft-used substitute.

Peak Area Quantitation

Over a large concentration span, area linearity is better and area is preferred for better accuracy and precision.

Peak Height Quantitation

Height is better than area especially if peaks are poorly resolved; it is less affected by asymmetry and overlap. Noise filtration may affect peak heights but not area.

Appropriate Choice of Quantitation Method (2)

Width Based Quantitation at Fixed Heights

When the analyte response across the measurement range is not strictly linear, WBQ can offer superior overall performance (lower root mean square relative error over the entire range) compared to area- or height-based linear regression methods, rivaling weighted linear regression.

Chromatographic peaks are modeled as two different independent generalized Gaussian distribution functions, representing respectively the leading/trailing halves of the peak.

$$h = \begin{cases} h_{max} * e^{-\frac{|t|^m}{a}}, & t < 0 \\ h_{max} * e^{-\frac{t^n}{b}}, & t \geq 0 \end{cases}$$

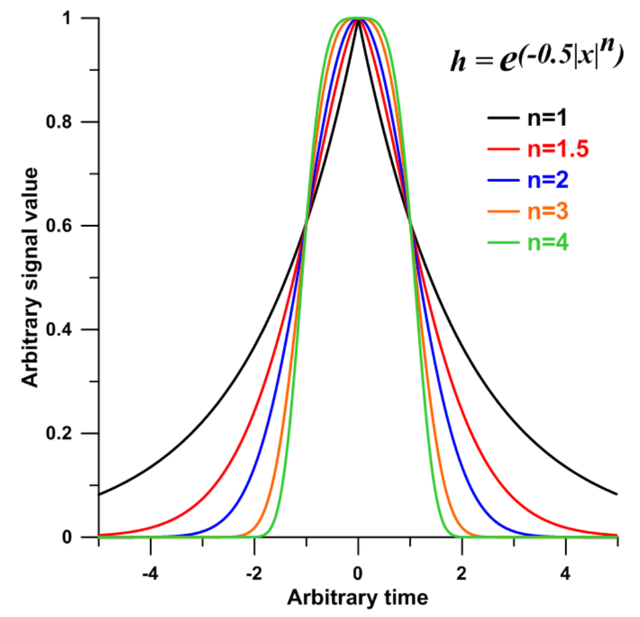
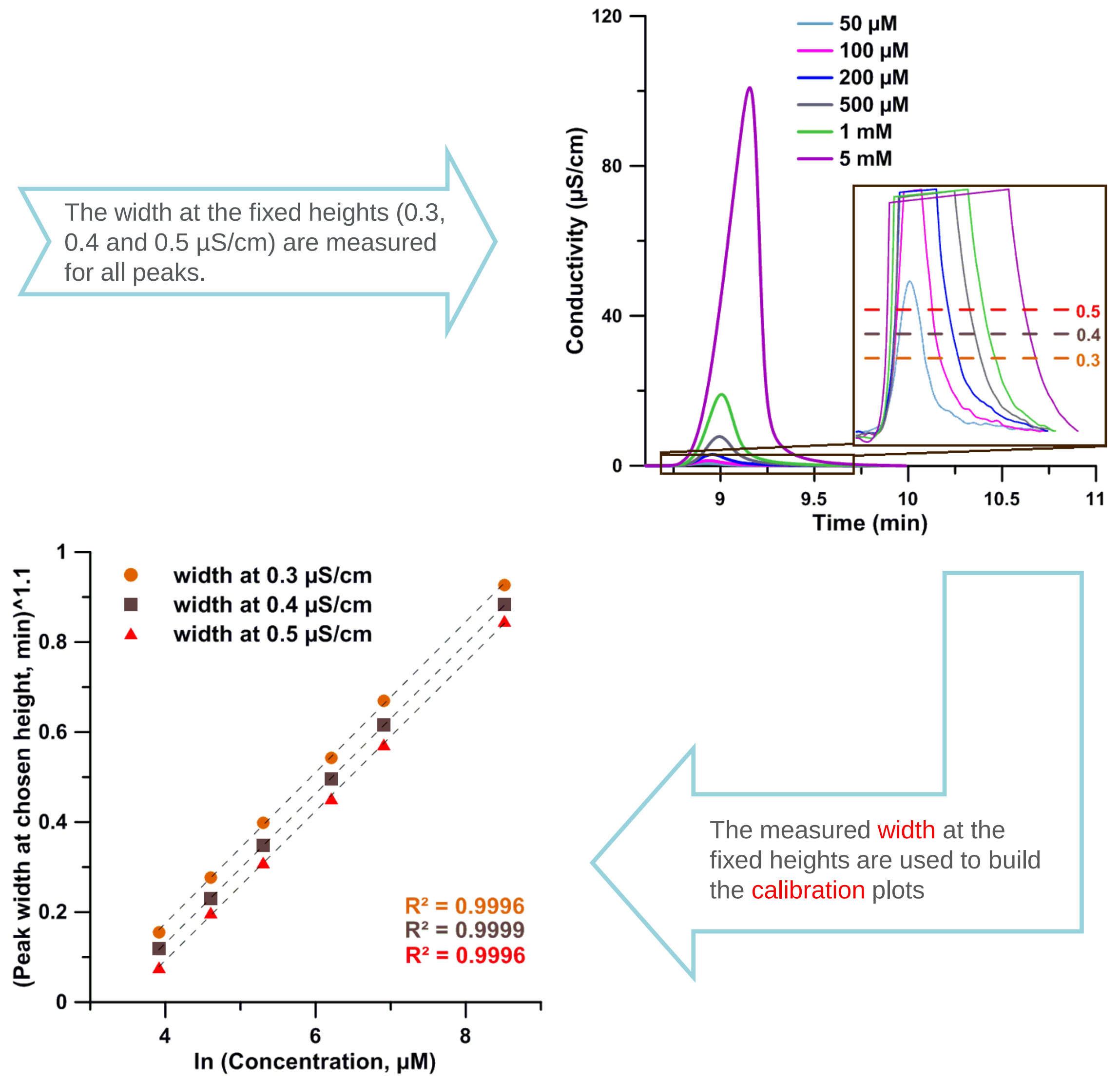


Figure 4. Plots of independent generalized Gaussian distribution functions.



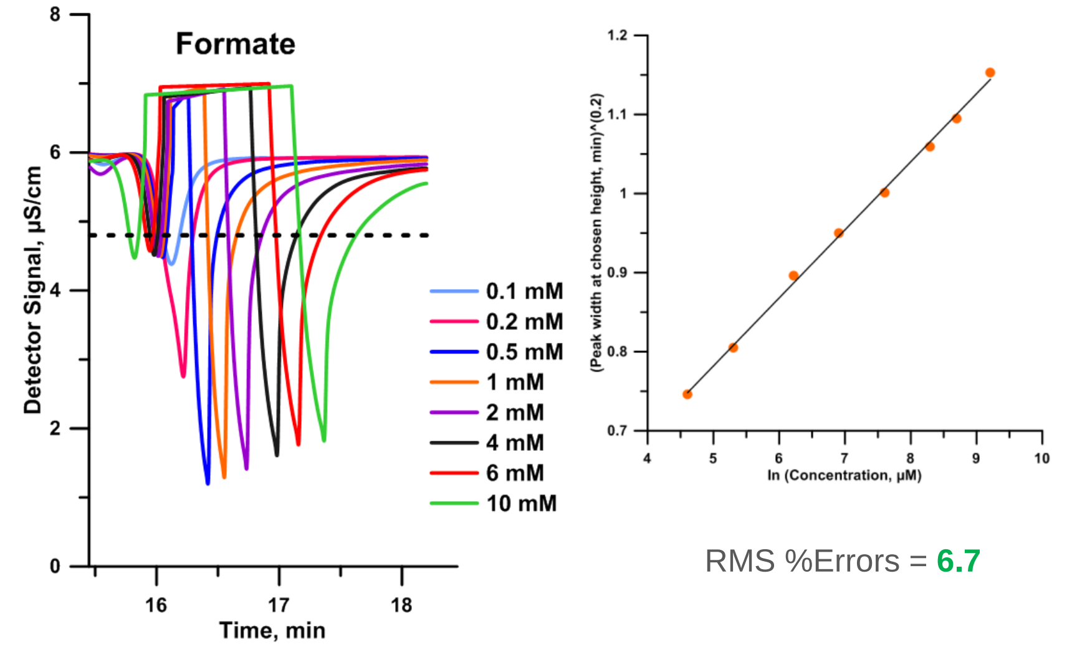
The width at the fixed heights (0.3, 0.4 and 0.5 µS/cm) are measured for all peaks.

The measured width at the fixed heights are used to build the calibration plots

Figure 5. Width-based quantitation calibration plot of chloride standards at different fixed heights.

Quantitation of Truncated Peaks with WBQ

The signal may enter a nonlinear response region or even be truncated from detector/data system limitations. WBQ is the only option.



RMS %Errors = **6.7**

Figure 6. Weak acid detection involving post column reaction by permeative amine introduction. The signal of interest is the inverted peak. Width is measured at a fixed height below the baseline.

Evaluation of Quantitation Methods

For different data sets we evaluate the quantitation accuracy by comparing the Root mean square of the % error (RMS % Error) of Area, Height and Width quantitation.

Peaks affected by detector saturation

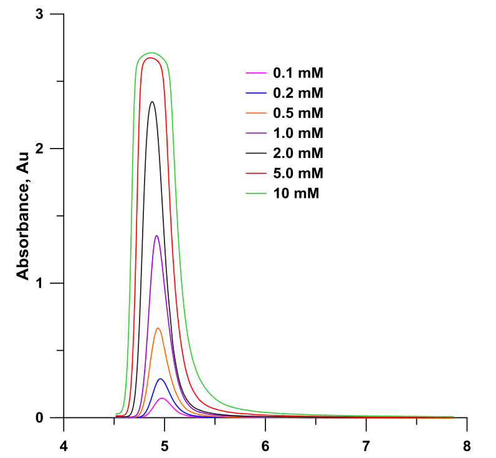


Figure 7. Nitrate peak detected at 200 nm on a Dionex AD 20 detector.

	% RMS Errors, unweighted			%RMS Errors, 1/x² weighted		
	Area	Height	Width	Area	Height	Width
Nitrate	373	1130	**11.4**	23.2	40.2	**11.5**

Table 1. RMS % Error of nitrate.

Normal Peaks

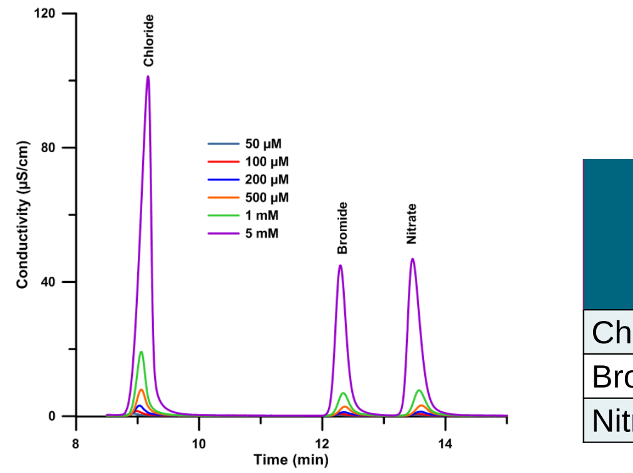


Figure 8. Ion chromatographic data for gradient elution of a 3-anion standard mixture.

	% RMS Errors, unweighted			%RMS Errors, 1/x² weighted		
	Area	Height	Width	Area	Height	Width
Chloride	53.5	31.3	**0.9**	9.5	8.1	**0.9**
Bromide	61.2	80.4	**5.4**	10.8	15.6	**5.3**
Nitrate	67.1	67.5	**5.9**	13.2	13.4	**5.9**

Table 2. RMS % Error of chloride, bromide, and nitrate

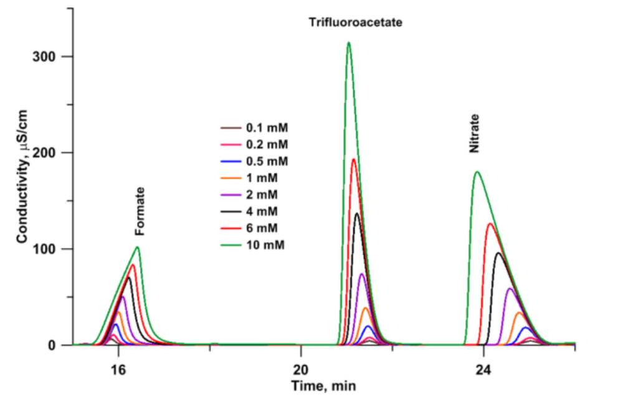


Figure 9. Ion chromatographic data for gradient elution of a 3-anion standard mixture.

	% RMS Errors, unweighted			%RMS Errors, 1/x² weighted		
	Area	Height	Width	Area	Height	Width
Formate	110.1	388.0	**4.5**	13.7	36.5	**4.5**
Nitrate	7.2	188.2	**3.7**	**3.7**	18.9	**3.7**
Trifluoro acetate	11.4	54.5	**2.4**	3.1	6.4	**2.4**

Table 3. RMS % Error of formate, nitrate, and trifluoroacetate.

Quantitation of Impurities

Chromatographic peak shape is generally analyte concentration independent: without column overloading, a well-behaved analyte peak is scalable. The presence of a unresolved impurity in a peak subtly alters its shape.

A description of peak shape allows any departure from the pure analyte benchmark to be statistically identified and quantified.

We quantify the amount of nitrate present in a non-resolved mixture of 2000 µM bromide and 20 µM nitrate (1% impurity).
Impurity quantitation is based on shape change from the generalized Gaussian distribution function model (see figure 4). The calculated amount of Nitrate after deconvolution is 18.2 µM.

Effective impurity quantitation can be achieved down to 1%.

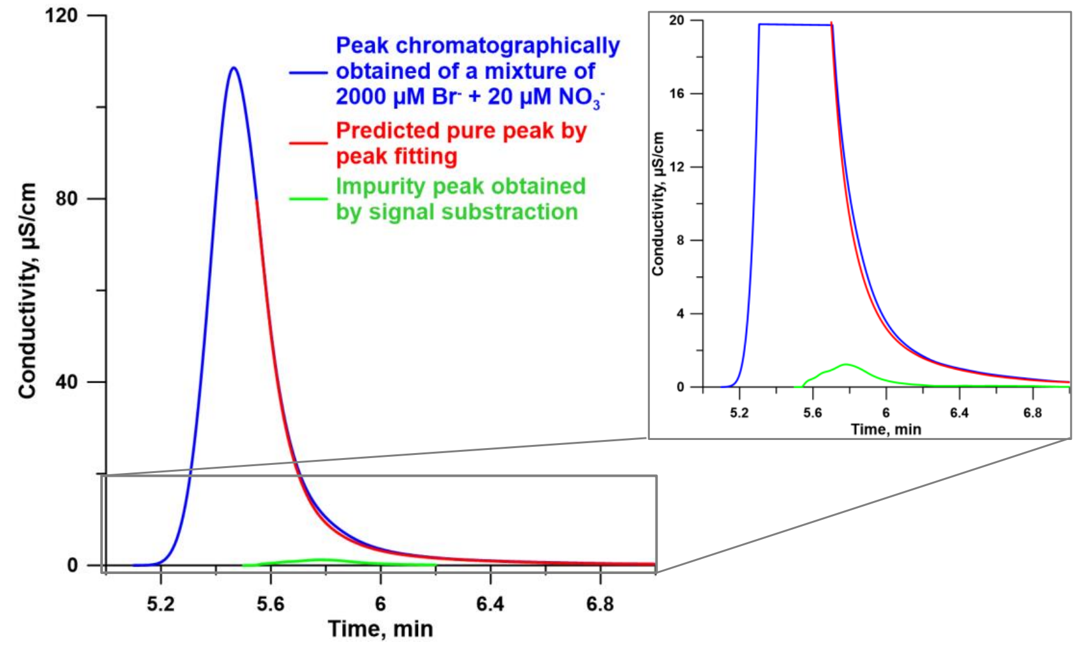


Figure 10. Quantitation of a 1% nitrate impurity in a bromide sample.

CONCLUSIONS

The methods described here are not just applicable to ion chromatography. They are valuable to Chromatography in general. $1/x^2$ weighting and data interpolation can be exploited for the reduction of relative error. WBQ often provides significantly lower relative errors. We suggest WBQ not as a panacea but as an adjunct, it is valuable in some settings and allows the detection and quantitation of impurities.

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TRADEMARKS/LICENSING

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