

## Ion chromatography

# Enhanced calibration precision: Leveraging RSE and WLS for optimal function optimization

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Analytical methods, calibration metrics, Chromeleon CDS, data quality, inverse calibration, regression models, relative standard error, sampling variability, statistical consistency, trace analysis

**Goal**

This technical note outlines the advantages of employing relative standard error (RSE), weighted least squares (WLS) approximations, and inverse calibrations in enhancing the accuracy and precision of calibration in chromatographic analysis. It discusses the shortcomings of conventional metrics and introduces a more dependable and straightforward method for evaluating and optimizing calibration processes.

**Introduction**

Rigorously calibrating instruments against known standards is crucial to ensure accurate and reproducible analytical results. The emphasis should be on the quality of the back-calculated results rather than the fit quality of the calibration itself.<sup>1</sup> Traditional methods that use unweighted regression, correlation coefficients, and relative standard deviation (RSD) might fall short, especially at lower concentrations. In contrast, relative standard error (RSE) offers a more precise way to evaluate calibration curves.<sup>2,3</sup>

The RSE (Equation 1; see the appendix for all equations) standardizes precision across various scales and calibration functions by expressing standard error as a percentage of the mean. Unlike the traditional correlation coefficient ( $r$ ) and its square ( $r^2$ ) (Equation 2), which fail in non-linear or low-concentration scenarios, RSE enhances accuracy by focusing on relative deviations.<sup>4</sup>

The coefficient of determination does not optimally measure precision, particularly for calibrations based on averaged calibration factors (ACF). RSD is relevant only for ACF calibrations (Equation 3),<sup>5</sup> and falls short for curves derived from least squares approximations.<sup>4</sup>

RSE is effective across various regression models, including weighted least squares (WLS), ensuring consistency and simplifying data interpretation. Organizations like the International Union of Pure and Applied Chemistry (IUPAC) recommend avoiding correlation coefficients, and regulatory frameworks such as the United States

Environmental Protection Agency's (US EPA) 40 CFR Part 136 endorse RSE for improved compliance and accuracy.<sup>6,7</sup> An RSE  $\leq 15\text{--}20\%$  is typically advised.<sup>8</sup> RSE allows direct comparisons between curve-fitting approaches and enhances precision across the calibration range. Considering factors like sample size and population variability, RSE provides a robust framework for calibration data evaluation.<sup>9</sup>

Although ordinary least squares (OLS) are still standard in many laboratory calibrations, they require constant variance, which is not typical of most methods.<sup>10</sup> Instead, WLS is more suitable as it addresses OLS limitations, such as the disproportionate impact on lower calibration levels. WLS reduces errors in chemical analysis by accounting for increasing response variance with concentration, ensuring reliable results even at trace levels.<sup>1,2,11,12</sup>

Forcing regression through the origin can compromise quality. While OLS and WLS may yield similar results for short-range calibrations, WLS offers more benefits for longer ranges, especially at low concentrations. Quadratic curve fits, like the  $1/\text{Amount}^2$  weighted approach, can maintain consistent relative weights, improving calibration precision.<sup>10,11,13,14</sup>

In conclusion, following RSE and WLS best practices enhances data quality and accuracy, particularly at lower concentrations.

In this technical note, we will illustrate how RSE and WLS effectively improve the quality of data derived from ion chromatography (IC), high-performance liquid chromatography (HPLC), and gas chromatography (GC) analyses.

## Software

Data evaluation was performed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) version 7.3.2. The software's RSE definition (Equation 4) includes weighting factors, expanding its use to calibration functions that must pass through the origin.<sup>5</sup>

RSE calculators are available online for those requiring an understanding of RSE or alternatives to Equation 4.<sup>15</sup>

## Results and discussion

### IC

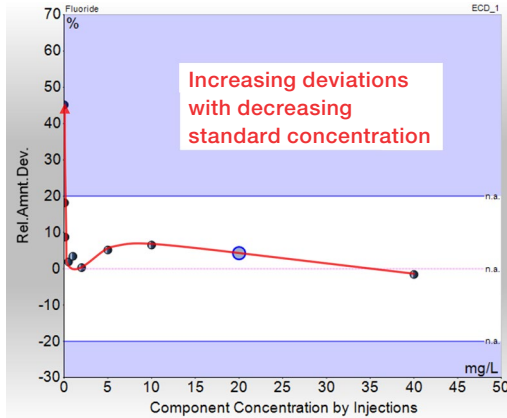
We analyzed representative anions using the Thermo Scientific™ Dionex™ IonPac™ AS23 column, utilizing a carbonate/bicarbonate eluent under isocratic conditions to evaluate RSE. The anions of interest were detected via suppressed conductivity.

The calibration range extended from 0.01 mg/L to 40 mg/L, providing a broad concentration range for analysis (Figure 1). This extensive range poses challenges for accurate calibration, as deviations at lower concentrations can adversely affect the reliability of results. The following section will explore ways to optimize fluoride calibration as a case study.



Figure 1. Chromeleon CDS Studio View illustrates anion separation on a Dionex IonPac AS23 column. Eluent used: 4.5 mM  $\text{Na}_2\text{CO}_3/0.8$  mM  $\text{NaHCO}_3$  with a flow rate of 1 mL/min and an injection volume of 50  $\mu\text{L}$ . Detection was performed with suppressed conductivity using a Thermo Scientific™ Dionex™ ADRS600 Suppressor, and data were acquired at a rate of 5 Hz. The standard concentration was 20 mg/L for each ion, with the processing method and calibration report shown in the default layout.

Initially, we employed a linear calibration curve that passed through the origin, a practice still common in many labs. Through the standard report tab in Chromeleon CDS, we adhered to conventional methods, assessing the RSD of calibration and the calibration coefficient. The analysis revealed an RSD between 5% and 12%, suggesting moderate precision. Meanwhile, the  $r^2$  values were approximately 0.999, indicating a robust linear relationship between measured and expected values. However, closer inspection of the relative amount deviation chart—a feature available in Chromeleon CDS—uncovered significant deviations at lower fluoride concentrations (Equation 5, Figure 2).



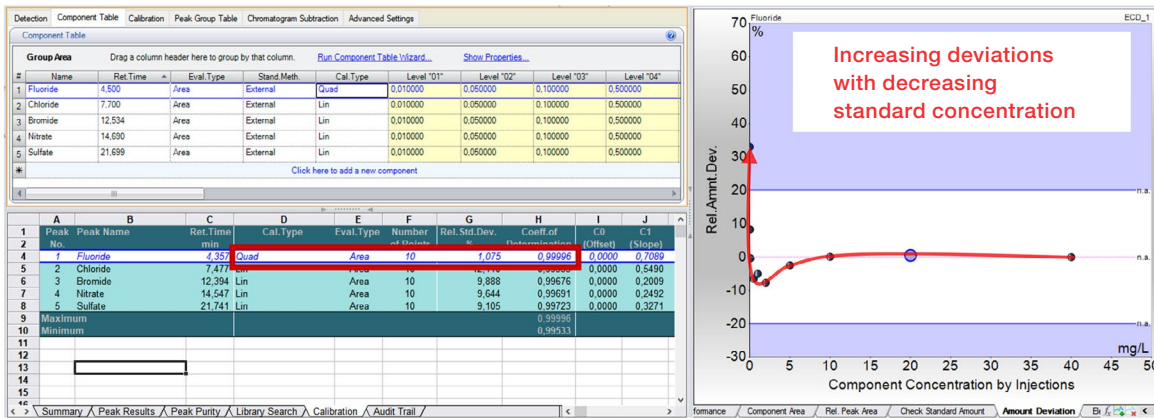
**Figure 2. The relative amount deviation plot is set up in Chromeleon CDS for fluoride calibration.** Due to the linear calibration type that forces through the origin, uncertainty significantly increases as standard concentration decreases.

Building on previous insights, we examined the benefits of using quadratic calibration curves. Transitioning from a linear to a quadratic curve for fluoride resulted in a reduced RSD of 1.1%, suggesting improved precision. Additionally, the  $r^2$  value increased to 0.9999, implying a stronger relationship between measured and expected values. However, the relative amount deviation analysis still showed deviations at lower concentrations, indicating further improvement is needed (Figure 3A).

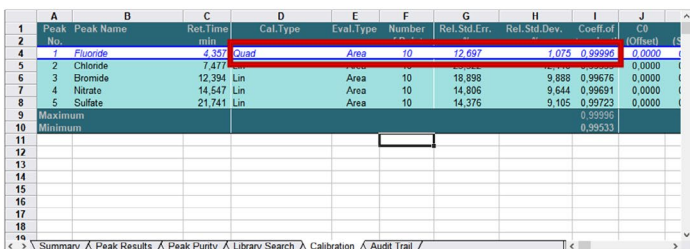
Including RSE in our evaluation provided better insights into calibration quality. By adding a column for standard RSE in the calibration tab (Figure 3B), we compared RSE values to RSDs. This comparison revealed a marked difference, with the RSE indicating approximately a 12.7% deviation for fluoride compared to the RSD of 1.1%. It is essential to note that RSD does not apply to linear or quadratic least squares approximations typically used for calibration curves.

Further examination of calibration curve properties highlighted potential error sources. Forcing data through the origin of the fluoride component can introduce errors. Switching from a forced to an "ignored origin" led to a higher RSE. This discrepancy is due to the lack of weighting in the quadratic calibration curve. Applying a weighting factor of  $1/\text{Amount}^2$  successfully addressed the uneven error distribution across the concentration range.

**(A) Original calibration and factory view**



**(B) RSE added to the calibration tab**



**Figure 3. The change to a quadratic fit and the subsequent results.** (A) Shows the conventional calibration report; (B) Introduces the RSE to the calibration tab.

By optimizing the calibration curve with a quadratic fit, adding an offset, and applying appropriate weighting, we achieved an improved RSE of 1.6% across the calibrated range. The relative amount deviation plot shows a random scatter around the target value, demonstrating enhanced calibration quality and fewer systematic errors (Figure 4).

### Interim summary of data analysis

Table 1 summarizes the fluoride calibration experiments, comparing linear and quadratic adjustment methods. The methods include linear adjustment through the origin (LIN), linear with an intercept (LIN WithOffset), and linear with an intercept combined with  $1/\text{Amount}^2$  weighting (LIN WithOffset  $1/\text{Amount}^2$ ), as well as the results for the quadratic adjustment.

The evaluation is based on the original peak area integration, facilitating straightforward comparisons. The table details the RSE for each calibration method. The LIN method has an RSE of 16.8%, which complies with the EPA's  $\leq 20\%$  criterion. Conversely, the LIN WithOffset method fails to meet this standard. The best performance stems from LIN WithOffset  $1/\text{Amount}^2$ , achieving an RSE of 1.9%.

While RSD and the coefficient of determination exhibit slight changes for the initial two calibrations, indicating apparent

linearity and precision, the RSE and relative amount deviation plots reveal a different story (Figures 2 to 4). When evaluating weighted calibration functions, RSD and  $r^2$  can be misleading, as evidenced by a rise in RSD and a minor decrease in  $r^2$  for LIN WithOffset  $1/\text{Amount}^2$ . The gray coloring in Table 1 indicates the respective values.

Similar challenges are apparent with quadratic least squares approximation, where discrepancies between RSE, RSD, and  $r^2$  are also evident. Comparing linear and quadratic WLS shows slightly better precision with the quadratic approach (RSE = 1.6% vs. 1.9%).

Relying solely on RSD and  $r^2$  for decision-making can lead to less optimal calibration models, impacting overall precision. Comparing RSE with RSD and  $r^2$  illustrates that RSE-based decisions optimize precision, whereas RSD and  $r^2$  may bias towards unweighted calibrations, increasing deviations at lower concentrations. Table 1 details the concentrations calculated using each curve fit and the corresponding relative amount deviations for the smallest and largest fluoride calibration standards.

These results emphasize the critical role of using RSE as the primary criterion for evaluating calibration models.

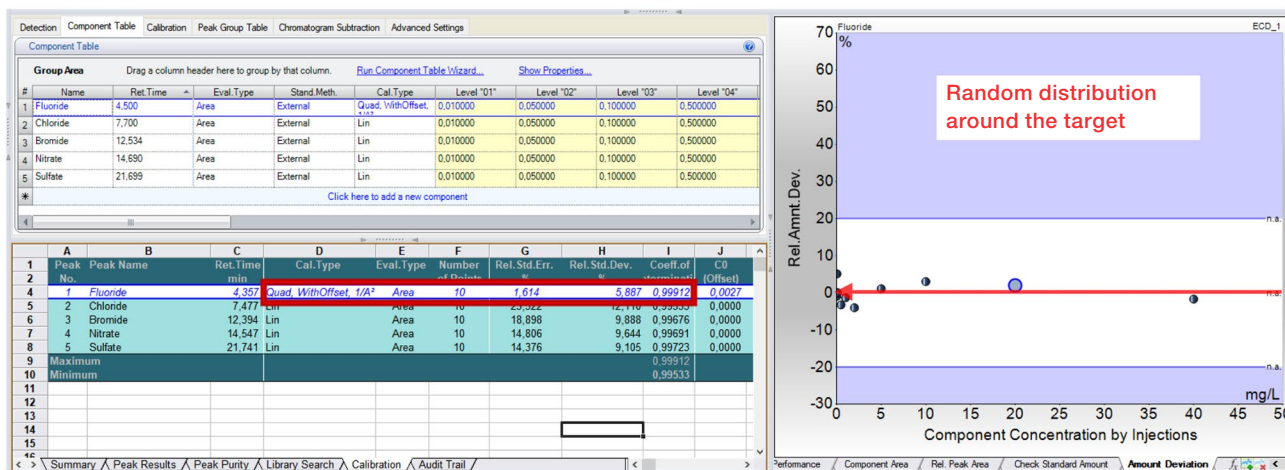


Figure 4. The impact of Weighted Least Squares (WLS) on calibration and relative amount deviation. The WLS approach used a quadratic fit with  $1/\text{Amount}^2$  weighting, resulting in a low RSE across the calibration range and a random distribution of amount deviation around the target value.



Table 1. Comparing the impact of the chosen calibration type on the parameters RSE, RSD, and the coefficient of determination

Cal. type	Eval. type	Number of points	Lowest calibration level (0.01 mg/L)		Highest calibration level (40 mg/L)		RSE	RSD	r <sup>2</sup>
			Calculated (mg/L)	Rel. amount deviation	Calculated (mg/L)	Rel. amount deviation			
Lin	Area	10	0.0145	+45.2	39.4	-1.6	16.8	5.3	0.999
Lin. WithOffset	Area	10	n.a.*	n.a.*	39.4	-1.4	609	5.3	0.999
Lin. WithOffset 1/Amount <sup>2</sup>	Area	10	0.0099	-1.2	38.05	-4.9	1.9	6.1	0.999
Quad	Area	10	0.0133	+33	39.98	-0.06	12.7	1.1	0.9999
Quad. WithOffset	Area	10	0.0628	+528	39.99	-0.04	204	1.0	0.9999
Quad. WithOffset 1/Amount <sup>2</sup>	Area	10	0.0099	-0.9	39.3	-1.7	1.6	5.9	0.999

- RSE meets EPA requirements
- RSE misses EPA requirements
- RSE: lowest deviation and no trend in the relative amount plot (see Figure 4 for Quad WithOffset 1/Amount<sup>2</sup>)
- RSD and r<sup>2</sup> incorrectly indicate better performance than RSE.
- RSD highest. r<sup>2</sup> lowest: misleading toward non-weighted calibrations

\*In this example, the curve fit Lin WithOffset yields negative concentrations for very low standards, which are reported as "n.a." The first standard concentration positively reported by the CDS is level 4 (0.5 mg/L) with a relative amount deviation of -32%.

### HPLC

RSE and WLS were also used to calibrate alkylphenones through rapid separation liquid chromatography (RSLC). Separating nine alkylphenones takes less than 12 seconds, requiring a high data acquisition rate of 100 Hz for reliable peak area integration.

We chose butyrophenone as a representative component to illustrate the method. The calibration covers a concentration range spanning roughly 1.5 orders of magnitude, smaller than the previous IC example (Figure 5).

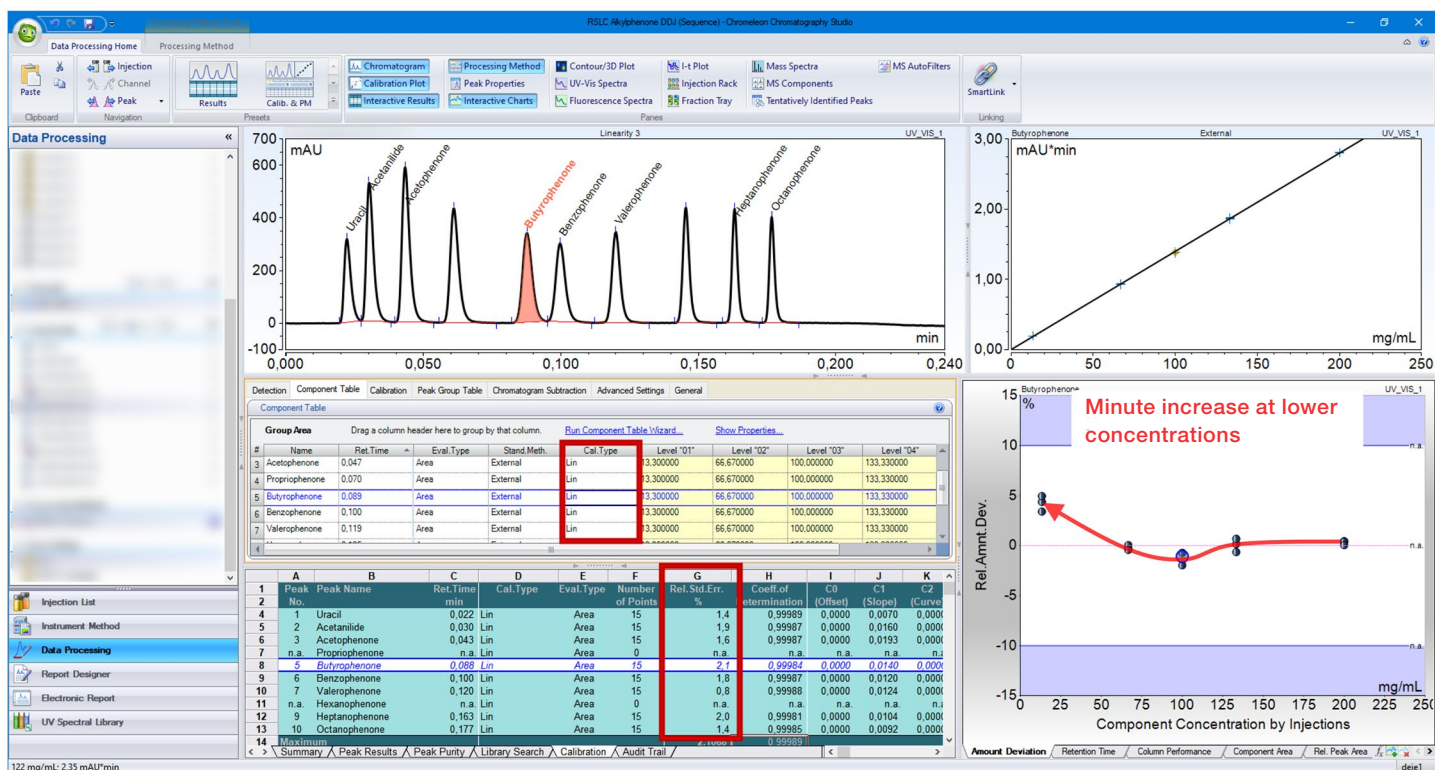


Figure 5. Chromleon CDS Studio View for alkylphenones, concentrations (see Component Table), processing method, Component Table, and Relative Amount Deviation plot with RSD replaced by RSE. Column: Thermo Scientific™ Acclaim™ 120 C8, 3 μm (2.1 × 150 mm) column; Temp.: 100 °C; Gradient (ACN/H<sub>2</sub>O): started at 40% ACN, ramped to 95% in 0.121 min, held isocratically until 0.144 min, then equilibrated to initial conditions by 0.24 min; flow: 3.7 mL/min; injection volume: 1 μL, UV detection at 245 nm, and a data acquisition rate of 100 Hz.

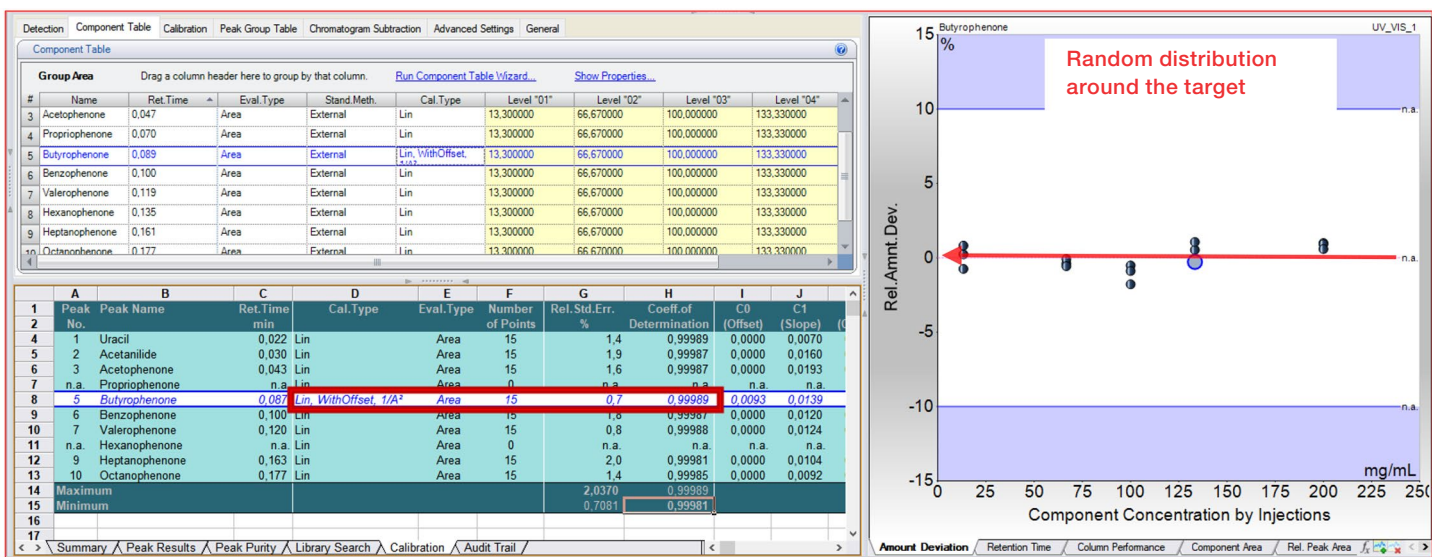
Upon reviewing the relative amount deviation plot, we noted a slight increase in deviation at lower concentrations. The RSE for butyrophenone is 1.9%, already well within the EPA's recommended limits. There are two options to improve calibration performance, especially at lower concentrations: recalibrate or use WLS with RSE for results comparison. The traditional method employed a linear calibration passing through the origin. However, when shifting to a linear approximation with an offset, the RSE increased slightly to 3.3%.

A  $1/\text{Amount}^2$  weighting factor improved precision at low concentrations, reducing the RSE to 0.98%. The relative amount deviation plot showed minimal deviation and an even distribution across the calibration range (Figure 6).

Previous recommendations suggested using quadratic WLS even for linear functions. Testing this by switching from a linear to a quadratic calibration curve showed no significant effect on the relative amount deviation plot or RSE (Figure 6(B)). As Burrows noted, "There is no penalty to using a quadratic [fit] even if the function is completely linear."<sup>16</sup>

Applying RSE and WLS substantially improved calibration precision. However, it is prudent to determine if such refinements are necessary, especially if the initial setup already meets RSE criteria. While these adjustments are beneficial, they might be most justifiable when higher precision is needed for analysis.

(A) Calibration: Linear WithOffset; WLS ( $1/\text{Amount}^2$ )



(B) Calibration: Quadratic WithOffset; WLS ( $1/\text{Amount}^2$ )

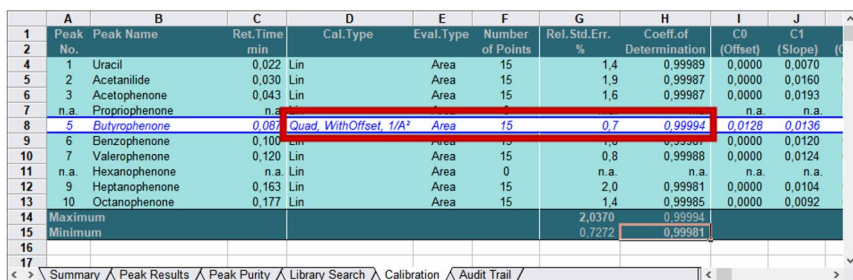


Figure 6. Impact of WLS on calibration and amount deviation for alkylphenones. (A) Represents WLS with a linear fit and  $1/\text{Amount}^2$  weighting. (B) Shows WLS with a quadratic fit and the exact weighting. Both scenarios demonstrate low RSE over the calibration range and a random distribution of the amount deviation around the target value.

## GC

We included a gas chromatographic (GC) example to demonstrate the universal applicability of RSE in evaluating chromatographic calibration experiments. Figure 7 shows the separation of ethanol, which takes about 40 seconds after a headspace injection. The calibrated concentration range spans from 100 mg/L to 5,000 mg/L, the broadest range in our examples.

We subjected the original method to RSE evaluation using OLS that constrained the calibration function to pass through the origin (Figure 7A). The RSE obtained was 7.6%, which is well within the EPA guideline of less than 20%. However, the relative amount deviation plot in Figure 7A showed increasing deviation at lower standard concentrations.

The original calibration data set was re-analyzed using WLS with a quadratic fit and  $1/\text{Amount}^2$  weighting to enhance precision. This adjustment reduced the RSE to approximately 0.7%. It produced a relative amount deviation plot in Figure 7B that displayed minor deviations and a random scatter around the target value of 0% across the calibrated range. This demonstrates the improved accuracy and reliability of the optimized calibration method.

### **Inverse calibrations – or, if nothing else fits**

In chromatography, even quadratic WLS may not always produce optimal results in certain instances. One example is the determination of ammonia by cation exchange chromatography and suppressed conductivity detection.

The dissociation of ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) creates a non-linear calibration function in suppressed conductivity due to complex equilibrium dynamics involving  $\text{NH}_4\text{OH}$ ,  $\text{NH}_4^+$ , and  $\text{OH}^-$  ions. The concentration and temperature influence the degree of dissociation of  $\text{NH}_4\text{OH}$  molecules, resulting in a non-uniform conductivity change governed by the dissociation reaction's equilibrium constant. Consequently, the conductivity response does not follow a quadratic pattern and cannot be accurately represented by a weighted quadratic calibration. More sophisticated models are necessary to capture this system's behavior.

Figure 8A illustrates a representative chromatogram and calibration curve for ammonium, detailing experimental conditions and the calibrated range. Despite employing quadratic WLS calibration and a  $1/\text{Amount}^2$  weighting factor, fitting issues became evident, especially at higher concentrations, indicated by an inflection point near the peak calibration value. Therefore, the quadratic curve could not be assessed for RSE, and the RSE for ammonium is labeled as "n.a."

An often overlooked yet significant option in Chromeleon CDS is "Inverted curve fit," which can enhance calibration results. Some experts even recommend it as a standard practice.<sup>17</sup> This option is accessible under the processing method's General or Calibration tab, contingent on system configuration.

Selecting the "inverted" option over the "normal" setting improves fitting, reducing RSE and enhancing performance overall (Figure 8B). This improved fitting boosts data precision and accuracy, yielding more reliable and consistent outcomes.

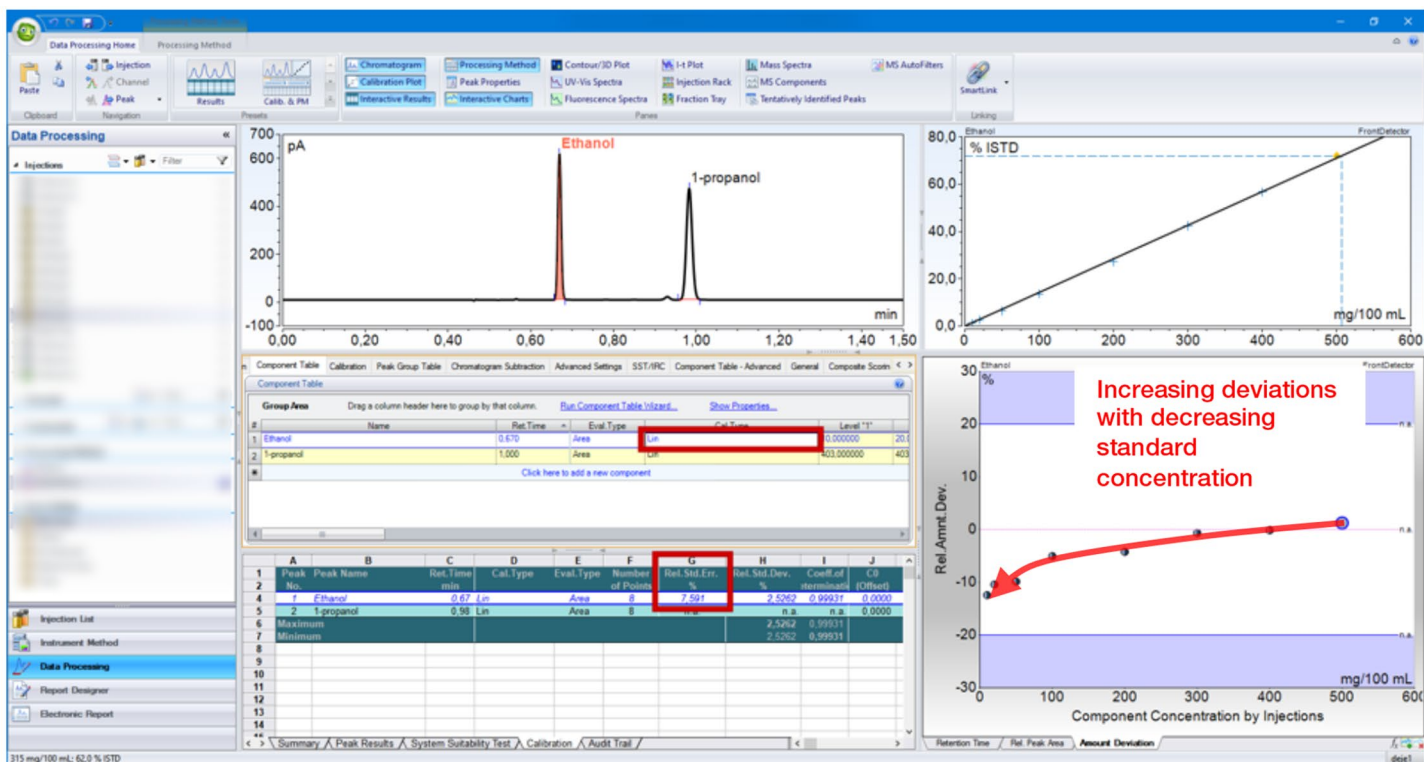
The inverted fitting method offers better calibration curve adjustments, especially when other methods fail or reduce precision. Traditional calibration regresses measured data on known concentrations, while inverse calibration regresses known concentrations on measured data. Inverse calibration can outperform traditional methods in prediction accuracy, replicates, and interpolations, even with limited data points. Traditional calibration, however, tends to produce fewer systematic biases.

When choosing a method, consider the nature of the data. Classical calibration is better for extreme data points due to reduced bias. Conversely, inverse calibration is suited for central data points due to higher precision.<sup>18,19</sup>

In summary, the decision between classical and inverse calibration depends on prioritizing minimizing random errors or avoiding systematic bias within the dataset.



(A) Calibration: Linear forced Origin; OLS



(B) Calibration: Quadratic WithOffset; WLS (1/Amount<sup>2</sup>)

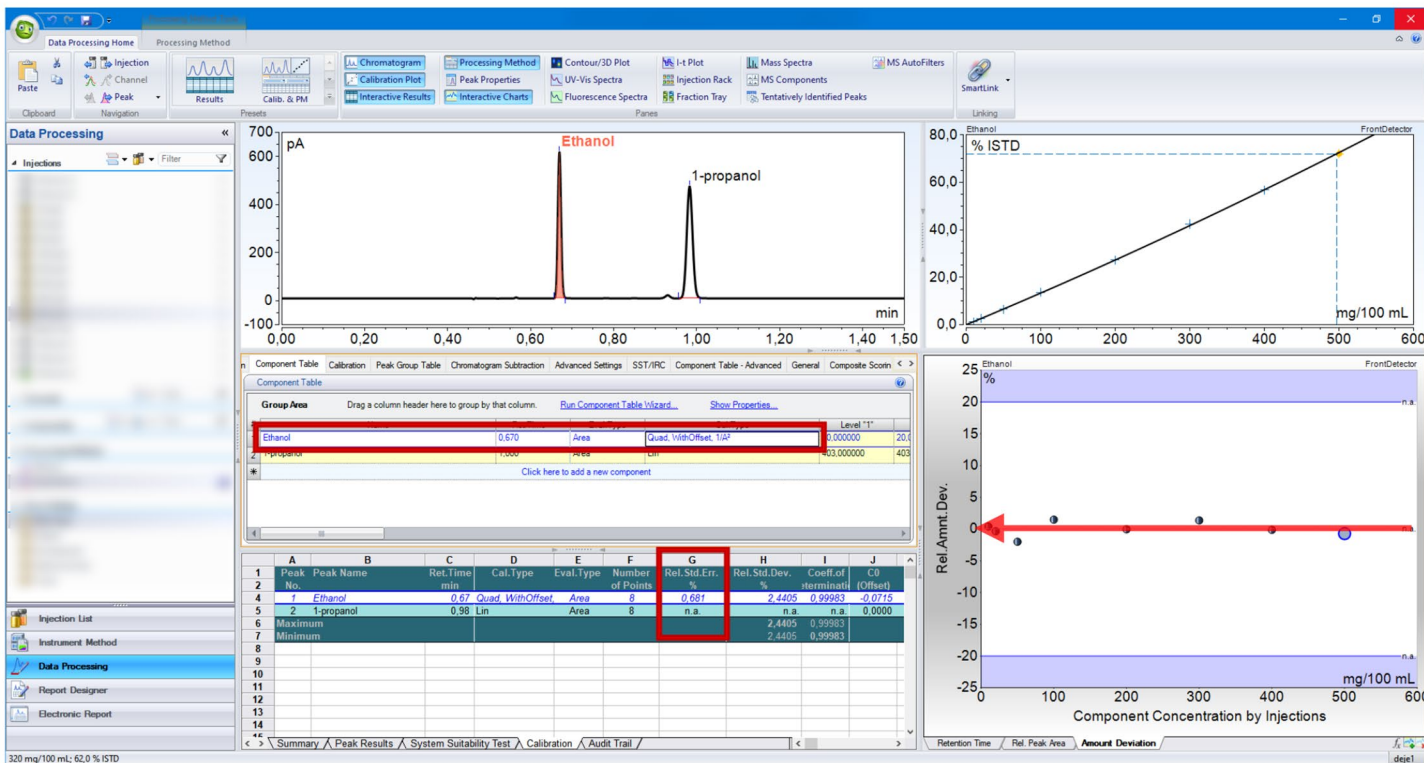
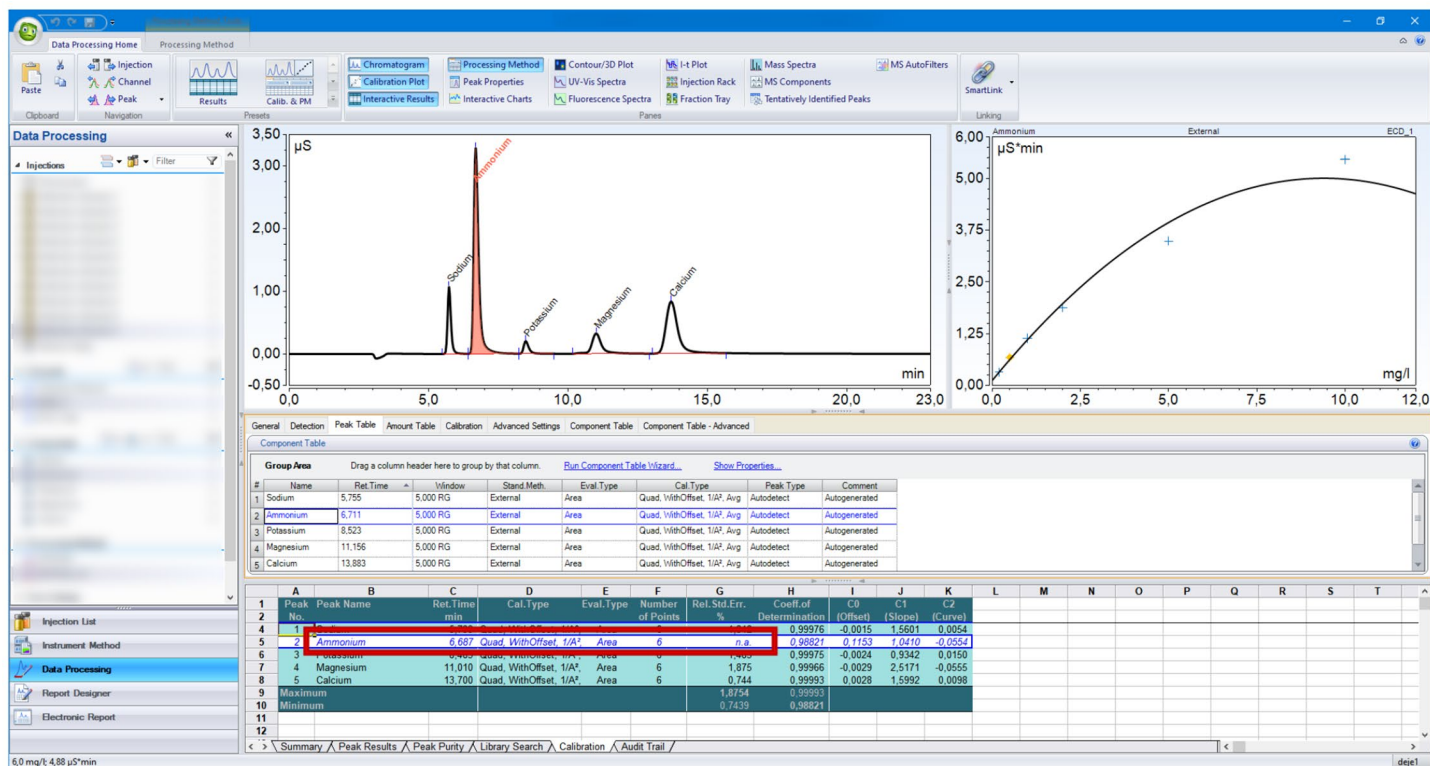


Figure 7. Calibration function for ethanol via GC with flame ionization detector (FID). (A) Shows LIN unweighted (OLS) with increasing deviation at lower concentrations (RSE = 7.6%). (B) Depicts WLS with a quadratic fit and 1/Amount<sup>2</sup> weighting, resulting in a low RSE (0.7%) and random distribution of amount deviation around the target value. Column: Thermo Scientific™ TraceGOLD™ TG ALC1 GC column; Injection: headspace (standard mode) with a 1 mL loop; Carrier gas: N<sub>2</sub>; Oven: 50 °C; FID: 300 °C; Airflow: 350 mL/min; Makeup gas flow: 45 mL/min; Hydrogen flow: 29 mL/min; Data acquisition rate: 25 Hz.



(A) Normal ("Standard") Curve fitting



(B) Inverted Curve fitting

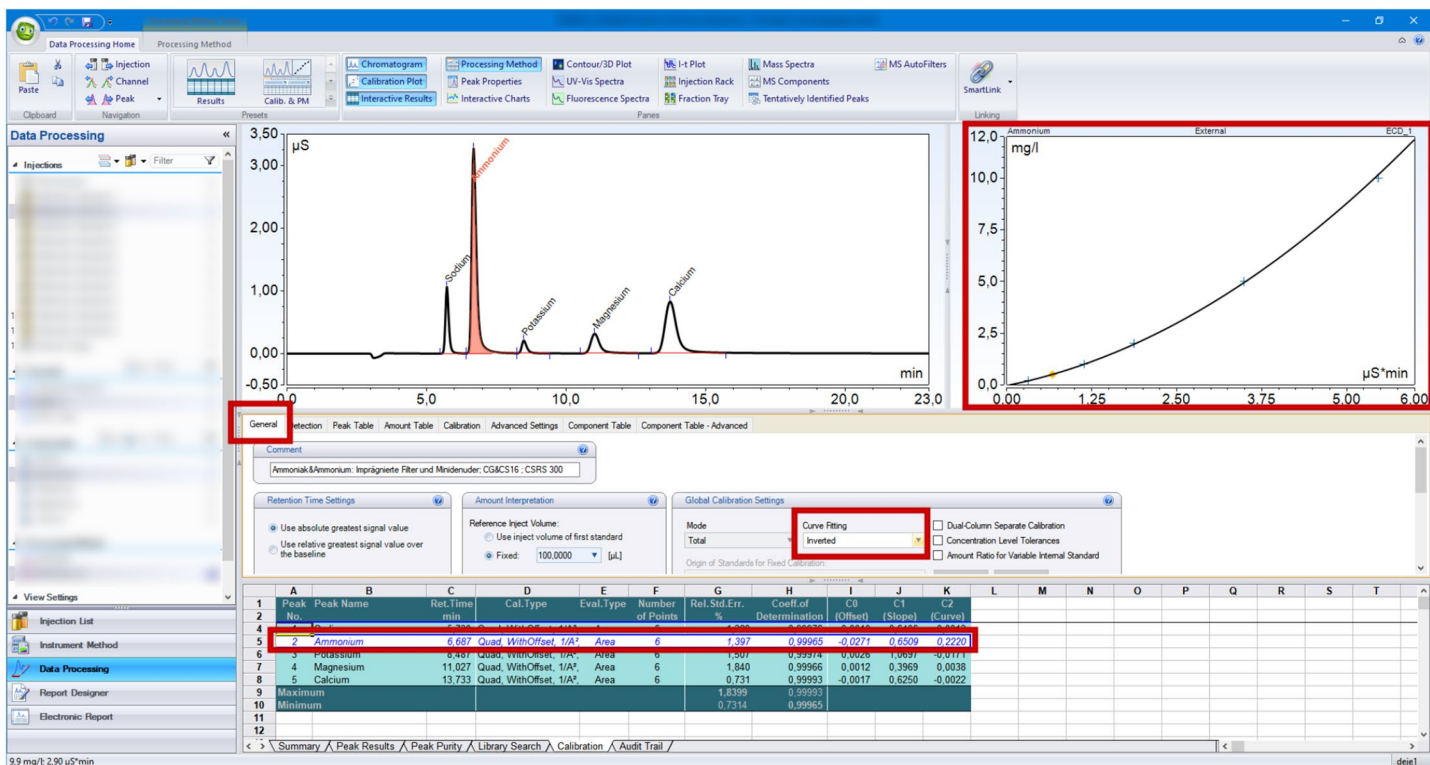


Figure 8. Calibration function for ammonium using a Thermo Scientific™ Dionex™ IonPac™ CS12A column. Eluent: 18 mM methanesulfonic acid at 1 mL/min. Detection was performed with suppressed conductivity using a Thermo Scientific™ Dionex™ CDRS600 suppressor, and data were acquired at a rate of 5 Hz. (A) shows normal curve fitting, while (B) displays inverted curve fitting.

## Conclusions

This technical note explores the advantages of using RSE, WLS approximations, and inverted calibrations to enhance calibration quality in chromatography. By optimizing calibration curves through quadratic fitting, offset adjustments, and suitable weighting factors (such as  $1/\text{Amount}^2$ ), the calibration process can be significantly improved, resulting in lower RSEs and better overall calibration quality. Incorporating RSE as an evaluation tool offers a deeper understanding of calibration reliability.

Inverted calibrations, particularly in specific chromatographic scenarios, have the potential to improve curve fitting and calibration precision. Adjusting calibration curves using the inverted fitting method can enhance the overall performance of chromatography systems, leading to more reliable and consistent results. This approach effectively aligns data points, boosting confidence in measurements.

These techniques, integrated into Chromeleon CDS, equip researchers and analysts with valuable tools to easily improve the accuracy and precision of their analytical results.

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## Appendix: Equations

$$RSE = 100\% \cdot \sqrt{\frac{\sum_{i=1}^n \left(\frac{x'_i - x_i}{x_i}\right)^2}{n - p}}$$

**Equation 1. RSE definition, as per the U.S. EPA.**<sup>8</sup> Here,  $n$  is the number of calibration points,  $p$  is the number of coefficients in the equation (average = 1, linear = 2, quadratic = 3),  $x_i$  is the specified amount, and  $x'_i$  is the calculated amount for each calibration point.

$$RSE = 100\% \cdot \sqrt{\frac{n}{n - m} \cdot \frac{\sum_{i=1}^n w_i \cdot \left(\frac{x'_i - x_i}{x_i}\right)^2}{\sum_{i=1}^n w_i}}$$

**Equation 4. The RSE definition in Chromeleon CDS.**<sup>5</sup> Here,  $n$  represents the number of calibration standard injections, and  $m$  is the number of coefficients needed (LIN:  $m = 1$ ; LOFF:  $m = 2$ ; QUAD:  $m = 2$ ; QUOFF:  $m = 3$ ; EXP:  $m = 2$ ). The variable  $w_i$  is the weight factor,  $x_i$  is the specified amount, and  $x'_i$  is the calculated amount for the  $i^{\text{th}}$  calibration injection.

$$r^2 = 1 - \frac{\sum_{i=1}^n w_i (y_i - F(x_i))^2}{\sum_{i=1}^n w_i (y_i - \bar{y})^2}$$

**Equation 2. The coefficient of determination ( $r^2$ ) as defined in Chromeleon CDS.**<sup>5</sup> In this equation,  $\bar{y}$  is the weighted average of all  $y$  values,  $n$  is the number of calibration injections, and  $w_i$ ,  $x_i$  and  $y_i$  represent the weights and values for calibration inputs.

$$\text{Relative Amount Deviation} = 100\% \times \frac{x'_i - x_i}{x_i}$$

**Equation 5. Relative Amount Deviation represents the deviation compared to the expected amount.** It is used to evaluate the quality of a calibration. The smaller this deviation, the closer the calibration point is to the calibration curve, providing a straightforward assessment of calibration precision across the range. Here  $x_i$  is the specified amount, and  $x'_i$  is the calculated amount for the  $i^{\text{th}}$  calibration injection.<sup>5</sup>

$$ACF = \frac{1}{n} \sum_{i=1}^n c_i$$

Averaged calibration factors

$$RSD = 100\% \frac{SD}{ACF}$$

Relative Standard Deviation of the calibration

**Equation 3. RSD of calibration in Chromeleon CDS.**<sup>5</sup> In these equations,  $n$  is the number of calibration levels,  $c_i$  is the calibration factor,  $SD$  is the standard deviation, and  $ACF$  is the average calibration factor

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