

Charged aerosol detection – use of the power function and robust calibration practices to achieve the best quantitative results

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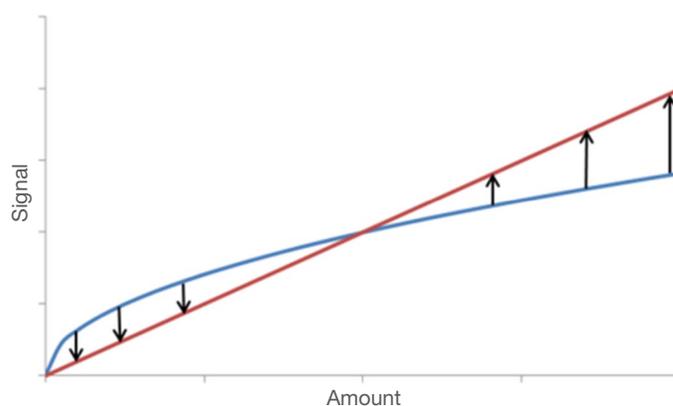
Keywords: Charged aerosol detection, charged aerosol detector, calibration, linearity, linear range

Goal

To describe how to use the power function to optimize the linear response range of charged aerosol detection and to provide guidance on evaluating and choosing calibration curve-fitting models in order to achieve the most accurate quantitative results.

Introduction

This technical note describes use of the power function (PF) with charged aerosol detection (CAD). The PF is a data acquisition parameter that affects the internal signal processing of CAD. The PF value (PFV) is a user-defined setting that allows optimization of the inherent (analog and digital) signal output. The sole purpose of the PF is to optimize the range over which the CAD response



is sufficiently linear for a given method and its range of quantitation. Since non-linear response also affects peak shape and signal-to-noise ratio, an optimal PFV also provides more accurate and simplified calculation of measures of chromatographic performance (e.g., resolution) and limits of detection. This technical note describes:

- How the PF can be used to optimize CAD performance
- How to determine an optimal PFV
- Recommended practices for achieving accurate quantitation

CAD response characteristics

CAD response is commonly described as approximately linear, or quasi-linear, over a range of 1.5 to 2 orders of magnitude ($10^{1.5}$ – 10^2), but non-linear over a wider range. The expected shape of CAD response curves can largely be explained by a combination of two relationships: 1) a cube root relationship between the mass of analyte in a dried aerosol particle and its diameter (d); 2) the amount of charge acquired per particle as a function of d , a relationship that differs between particles with $d \geq 10$ nm and those with $d < 10$ nm. Since the proportion of smaller particles ($d < 10$ nm) within an aerosol residue (i.e., distribution of dried aerosol particles) changes as a function of analyte amount, the shape of CAD response curves changes gradually over the dynamic range.

It should be noted that response curves obtained with evaporative light scattering detection (ELSD) can be similarly explained where the cube root relationship between mass and d is identical to CAD. Like particle charging with CAD, the relationship between light scattering efficiency and d also differs for different ranges of d . For ELSD, these ranges correspond to Rayleigh, Mie, and refraction/reflection light scattering mechanisms. Due to large exponential differences in efficiency between these mechanisms, ELSD response curves are more complex and the dynamic and quasi-linear ranges are more limited than CAD. The sensitivity of ELSD is also fundamentally limited compared to CAD due to an exponential drop in light scattering efficiency that occurs for particles with $d < \sim 50$ nm. In the case of CAD, an exponential drop in charge per particle also occurs but only for particles with $d < 10$ nm. A detailed description of this theory is provided in Chapter 1 of the book *Charged Aerosol Detection for Liquid Chromatography and Related Separation Techniques*.¹

Figure 1 shows a CAD response curve for a sulfonamide drug, sulfamerazine, where peak area response ($\text{pA} \times \text{min}$) is the dependent variable and analyte mass injected (m_{inj}) is the independent variable. The range of m_{inj} was 1.56 to 200 ng on column (2.0 μL injection of 0.78 to 100 $\mu\text{g}/\text{mL}$), which represents more than 2 orders of magnitude. As expected, CAD response is quasi-linear over only part of this range.

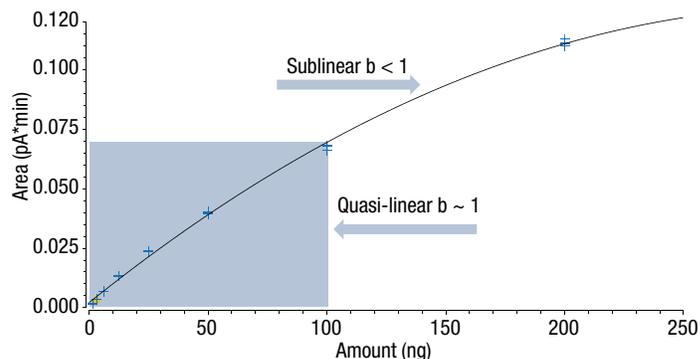


Figure 1. CAD response curve for the sulfonamide drug, sulfamerazine

CAD response can be described by a power law equation (Eqn. 1) where response is equal to m_{inj} raised to an exponent (b) and multiplied by a sensitivity coefficient (a).

$$\text{Response} = a (m_{\text{inj}})^b \quad \text{Eqn. 1}$$

The sensitivity coefficient (a) is related to the magnitude, or scale, of the y-axis in Figure 1. This technical note will mainly focus on the exponent (b), which describes the shape of the curve. When $b = 1.0$, the response is linear and the slope (a) is the response factor ($\text{peak area}/m_{\text{inj}}$), which is constant over the entire range. When $b \neq 1$, the response is non-linear and therefore the response factor changes over the range of study. When $b < 1$ the response is termed sub-linear, and when $b > 1$, supra-linear. The downward curvature toward higher m_{inj} in Figure 1 is an example of sub-linear response, while the upward curvature for metoprolol impurity M in Figure 2 indicates supra-linear response. The more b differs from 1, the greater is the degree of curvature or deviation from linear response. For example, if $b = 2$, then a 2-fold change in analyte amount would result in a 4-fold change in response, but if $b = 1.2$ only a 2.3-fold change would occur. Importantly, over the full dynamic range of CAD not only does the response factor change ($b \neq 1$), but so does the value of b or degree of curvature. This prohibits the use of a single PFV to linearize response over the full dynamic range and necessitates optimization of the PFV for a given method. The change in degree of curvature of CAD response is related to, as mentioned above, the change in the proportion of smaller ($d < 10$ nm) particles within an aerosol residue as a function of analyte amount.

CAD power function

The PF is a data acquisition parameter that may be used to help 'linearize' the signal output of CAD over a method's target range of quantitation. The general equation for the PF is shown below (Eqn. 2) where the default signal is that obtained using a PFV = 1.0. The denominator normalizes the output to prevent the signal from exceeding the full scale range where 500 is a constant that reflects the instruments' full scale current (nA).

$$\text{Signal Output} = (\text{Default Signal})^{\text{PFV}} / 500^{(\text{PFV}-1.0)} \quad \text{Eqn. 2}$$

The PFV can be thought of as a simple multiplication factor that is applied to the exponent b. The optimal value is the reciprocal of the power law exponent b that is observed for a given method. By applying the optimal PFV (1/b) to the power law equation (Eqn. 1), the exponent becomes 1.0, which represents linear response. This is shown in the following equation (Eqn. 3). For example, when using the instrumental default PFV of 1.0, if the observed exponent b is 0.8, then the optimal PFV would be $(1.0/0.8) = 1.25$.

$$\text{Response} = a (m_{\text{inj}})^{(b \times 1/b)} = a (m_{\text{inj}})^{1.0} \quad \text{Eqn. 3}$$

As previously mentioned, the value of b with CAD is not constant but changes gradually with analyte amount. Therefore, a single PFV cannot perfectly linearize response and is not sufficient to approximately linearize the full dynamic range.

Influence of analyte volatility on the shape of the response curve

For any analyte detected with CAD, the value of b (Eqn. 1), which describes the shape of the response curve, is at a maximum at the low end of the range of m_{inj} and gradually decreases toward higher m_{inj} . Analytes, however, can behave as non-volatiles or semi-volatiles depending on their physicochemical properties and instrumental conditions.² At higher instrumental evaporation temperature (T_e) settings, more analytes will behave as semi-volatiles. Depending on the analyte's observed volatility behavior, different strategies are necessary.

Semi-volatiles

Figure 2 shows response curves for four different analytes. The response for one of these analytes (impurity M, 1,3-Bis[(1-methylethyl)amino]-2-propanol dihydrochloride) has an upward curvature where $b > 1$. This supralinear response, which is especially noticeable at low m_{inj} , is a clear characteristic of a semi-volatile. At higher m_{inj} (not shown) the response of semi-volatiles is expected to transition through a quasi-linear ($b \sim 1$) and finally a sublinear ($b < 1$) range. Unlike non-volatiles, the response curve shape of semi-volatiles significantly depends on the analyte's physicochemical properties and therefore is rather unique to a single substance. Because of this higher inter-analyte variability and the more complex, often sigmoidal, curve shape expected for semi-volatiles, **use of a PFV other than 1.0 is generally not recommended for these analytes.** For the analytes in Figure 2, a first choice would be to use a lower evaporation temperature (T_e), which should result in impurity M behaving more like a non-volatile.

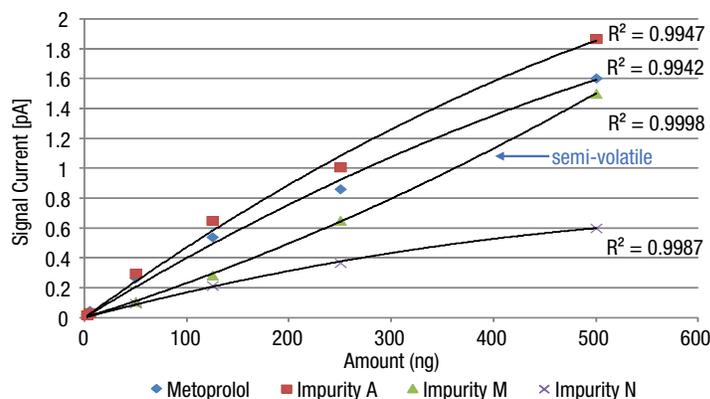


Figure 2. Response curves for non-volatile and semi-volatile analytes. Note: Differences in response magnitude for these analytes are attributed to use of a solvent gradient method without inverse gradient compensation and, for Impurity M, volatility.

Non-volatiles

Three of the curves in Figure 2 (Metoprolol, Impurity A, Impurity N) and the curve for sulfamerazine in Figure 1 are characteristic of analytes that behave as non-volatiles. For these analytes (with PFV = 1.0), the response is quasi-linear at lower analyte levels and is sub-linear at higher levels. According to theory, b for non-volatiles is expected to range from ~ 1.1 near the limit of detection to ~ 0.6 at the high end of the dynamic range.¹ For these analytes, the optimal PFV setting (1/b) for any method is therefore expected to be within the range of 1.0 to ~ 1.7 and in practice is more commonly between 1.0 and 1.6. It then follows that higher PFV settings, i.e., closer to 1.6, will be

optimal when the quantitation range is limited to higher analyte amounts and for chromatographic methods that elute analytes as more concentrated solute bands (e.g., gradient UHPLC). Since b is not constant, the optimal PFV is a “best fit” for a given method and target range of quantitation. This is therefore a compromise and can be viewed as a means to shift the ($\sim 10^2$) range over which CAD response is quasi-linear. The choice of PFV should be made using best practices for calibration, which are detailed in the following section. **Importantly, since all non-volatiles should have similarly shaped response curves, an optimal PFV should apply to all non-volatiles measured with a given method.**

Recommended calibration practices

In order to obtain accurate quantitation with any detection technique, it is important to choose an appropriate calibration model by performing a robust evaluation for quality of fit. The following are recommended practices for any quantitative method^{3,4} that are especially important to use with CAD and are discussed in more detail in Chapter 3 of Reference 1.

1. Limit calibration to as small a range as possible above and below the expected sample concentration. This is a good practice even with UV detection, for example with pharmacopeial methods where the content of an active pharmaceutical ingredient is usually determined over a small range (e.g. target value $\pm 50\%$). Likewise, impurity levels are determined using a different but similarly small calibration range above and below their expected or reporting threshold levels.
2. Use the simplest curve fitting model that adequately describes the response-amount relationship over the required range of interest. A linear curve fit can be used for initial evaluation and, especially when using an optimal PFV, is often adequate for many CAD methods that require quantitation over smaller mass ranges ($\sim 10^2$). For quantitation over wider ranges, quadratic (i.e., 2nd order polynomial), log-log and point-to-point are options that are often used successfully with CAD.
3. Evaluate quality of fit by using a sufficient number of levels and replicates over the entire range with special consideration to the upper and lower limits. A good approach is to include calibration levels ($n \geq 3$ each level) in 1, 2, 5, 10, or similar, increments within each order of magnitude across the full mass range of interest, making sure to include points around the desired lower sensitivity limits. The curve should be fit to the individual data points as opposed to first averaging the response between replicates at each level.
4. Do not use aggregate measures [e.g., coefficient of determination (r^2), residual sum of squares (RSS)] as the only metrics for assessing quality of fit since deviations, especially near lower analyte amounts, may be under-represented. As widely described,^{3,4} a curve fit with an r^2 of 0.9990 may still be poor, with the largest error typically near the low end. Reliance only on r^2 is especially problematic when using a log-log curve fitting model. For these reasons, it is highly recommended to use residuals plots as a primary means to assess the quality of fit as will be discussed below. Residuals plots are useful and recommended for any curve fitting model.
5. Most HPLC response data can be described as heteroscedastic, where peak area variability is greater for higher analyte amounts.³ This larger variance may exert too much influence on a least-squares regression line. For this reason, it is often useful to use weighted regression (e.g., $1/\text{amount}$, $1/\text{amount}^2$) to counteract the influence of higher amounts on the curve fit.^{3,4} This often provides a better fit to the lower amounts.

How to determine an optimal PFV

As described earlier in the section *Influence of analyte volatility on the shape of the response curve*, there are two principle guidelines for choosing a meaningful PFV setting depending on the analyte properties:

1. The practical range of useful PFV settings for non-volatiles should be between 1.0 and 1.6.
2. Use of a PFV other than 1.0 is not recommended for analytes behaving as semi-volatiles, which are easily identified by their supralinear response.

The general approach is therefore to assess quality of fit using the recommended practices described above for PFV settings between 1.0 and 1.6. The optimal PFV can be determined experimentally or by using the “Power Law” feature within the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. To determine the PFV experimentally, it is suggested to acquire data using PFV settings of 1.0, 1.2, and 1.4. Higher (up to 1.6) or intermediate settings may then be investigated if needed. The Power Law feature simplifies the process by allowing one to simply acquire data at PFV 1.0 and then simulate the data with different Chromeleon Power Law settings. The optimal value should then be verified experimentally. The basic steps involved in use of the Power Law feature are described in Appendix A.

Optimization of the PFV using the Chromeleon Power Law feature is described here for the analysis of three sulfonamide drugs where PFV_{exp} refers to data acquired with a given experimental PFV setting and PFV_{calc} refers to data calculated using the Chromeleon Power Law feature. A representative chromatogram for data acquired using PFV_{exp} 1.0 is shown in Figure 3A. Peak widths and signal-to-noise ratios for the data with PFV_{exp} 1.0, PFV_{calc} 1.4, and PFV_{exp} 1.4 are shown in Table 1.

Table 1. Peak widths at half height and signal-to-noise ratios using a time range for noise calculation of 1.0–2.0 min for chromatograms of sulfamerazine, sulfamethizole, and sulfadimethoxine at PFVs of 1.0 and 1.4

	Sulfamerazine	Sulfamethizole	Sulfadimethoxine
Peak width			
PFV_{exp} 1.0	2.7 s	2.6 s	2.5 s
PFV_{calc} 1.4	2.3 s	2.3 s	2.2 s
PFV_{exp} 1.4	2.4 s	2.3 s	2.3 s
S/N			
PFV_{exp} 1.0	65	106	151
PFV_{calc} 1.4	162	327	579
PFV_{exp} 1.4	104	189	359

Like the response curve for sulfamerazine (Figure 1), sulfamethizole and sulfadimethoxine (not shown) also demonstrated a characteristic shape for non-volatiles. Linear calibration, with and without weighting, was examined using residuals plots for PFV_{exp} 1.0, PFV_{calc} 1.2, PFV_{calc} 1.4, and PFV_{calc} 1.6. Residuals plots were created

using Chromeleon CDS as described in Appendix B. These plots can also be generated by most chromatography software applications or by spreadsheet software using calculations described by Dolan.⁵ They show the relative error or recovery of each point (level and replicate) used for calibration against the calculated least squares regression line. This allows better visualization of the data where the best fit is obtained when the points are evenly scattered above and below a 0% error or 100% recovery line. A common practice with any detection technique is to use acceptance limits for quality of fit of within $\pm 15\%$ for all points whose levels are above the desired limit of quantitation (LOQ) and $\sim 20\%$ at the LOQ.^{5,6}

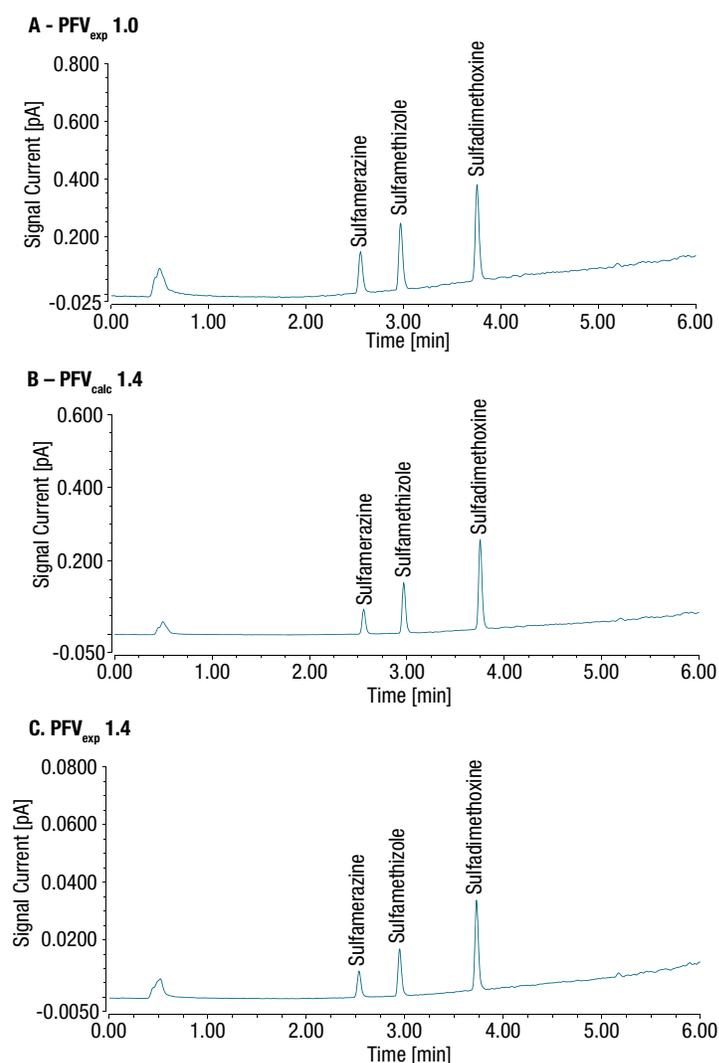
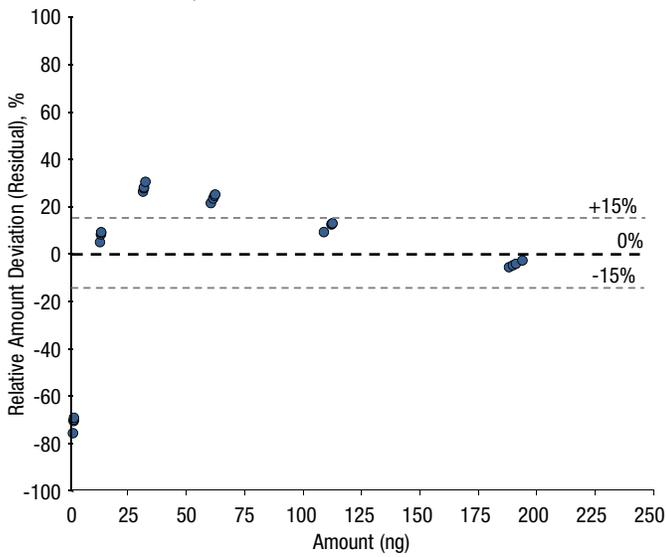


Figure 3. Optimization of the PFV using the Chromeleon CDS Power Law. Representative chromatograms from mixture of three sulfonamides (6.25 ng each). A) Data acquired using an instrumental PFV setting of 1.0 (PFV_{exp} 1.0); B) Data acquired with PFV_{exp} 1.0 and calculated using a Chromeleon Power Law exponent of 1.4 (PFV_{calc} 1.4); and C) Data acquired using an instrumental PFV setting of 1.4 (PFV_{exp} 1.4)

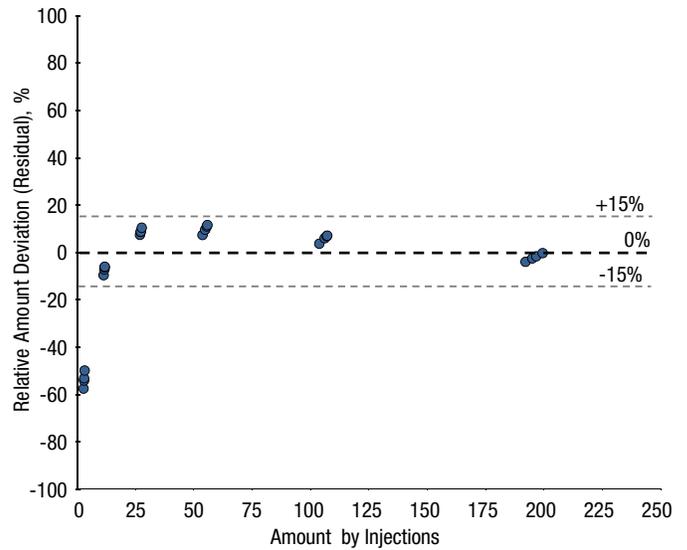
Figure 4 shows representative residuals plots for sulfamerazine using each of the PFV_{exp} and PFV_{calc} settings. Very similar results were obtained for all three analytes as expected since they are non-volatiles. The results show poor quality of fit with PFV_{exp} 1.0 where the lowest two levels (1.56 and 3.13 ng) were well outside of an acceptable range and a large error (~70%) was found for 6.25 ng (Figure 4A). Similar results were obtained with PFV_{calc} 1.2 (Figure 4B) and PFV_{calc} 1.6 (Figure 4D). In all

cases, weighting (not shown) improved the fit at the low end but not sufficiently. With PFV_{calc} 1.4 (no weighting), only the lowest level (1.56 ng ~20% error) was outside of a 15% limit, which is considered to be an acceptable quality of fit for this analysis (Figure 4C). Further improvement was obtained by using PFV_{calc} 1.4 with 1/amount weighting and, using this setting, a high quality of fit was found for all three analytes as shown in Figure 5.

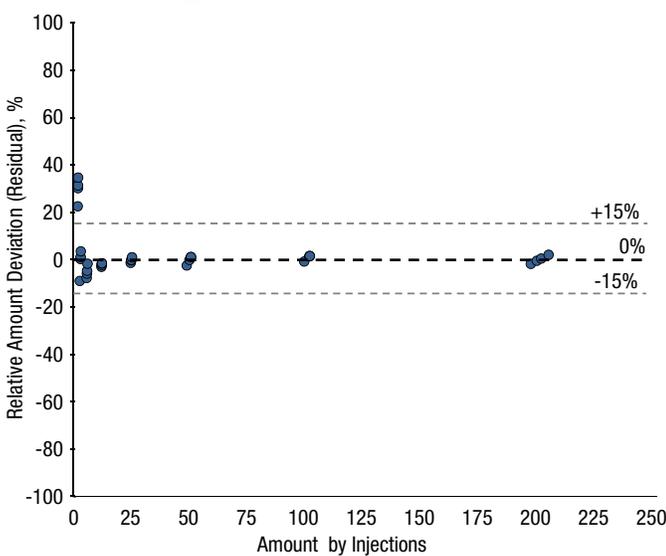
A Sulfamerazine, PFV_{exp} 1.0, no weighting



B Sulfamerazine, PFV_{calc} 1.2, no weighting



C Sulfamerazine, PFV_{calc} 1.4, no weighting



D Sulfamerazine, PFV_{calc} 1.6, no weighting

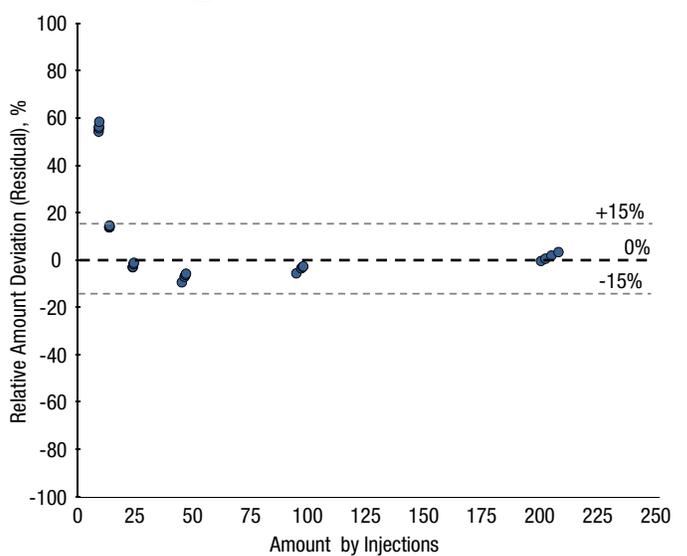
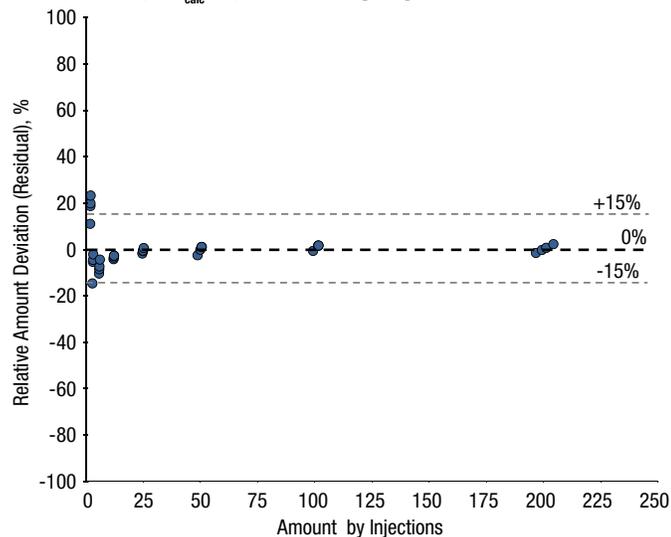
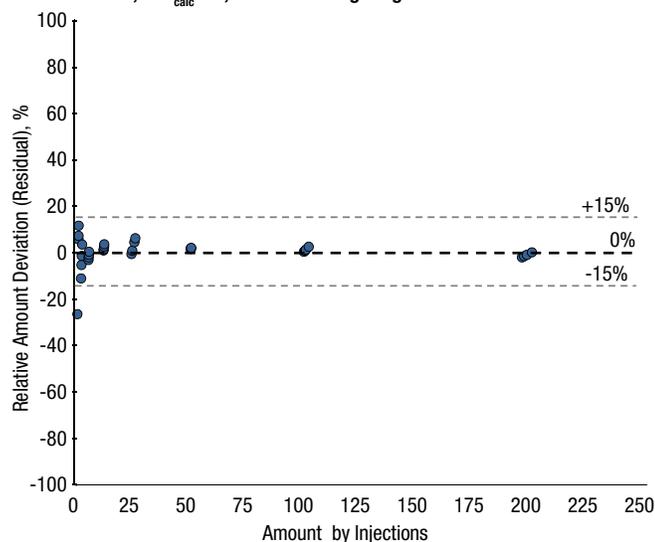


Figure 4. Residuals plots obtained for linear least squares regression without weighting of calibration data obtained for sulfamerazine at different PFV settings. A) PFV_{exp} 1.0; B) PFV_{calc} 1.2; C) PFV_{calc} 1.4; and D) PFV_{calc} 1.6. An acceptable fit was found only when using a PFV_{calc} of 1.4. See text for discussion of criteria used to evaluate quality of fit.

A Sulfamerazine, PFV_{calc} 1.4, 1/Amount weighting



B Sulfamethizole, PFV_{calc} 1.4, 1/Amount weighting



C Sulfadimethoxine, PFV_{calc} 1.4, 1/Amount weighting

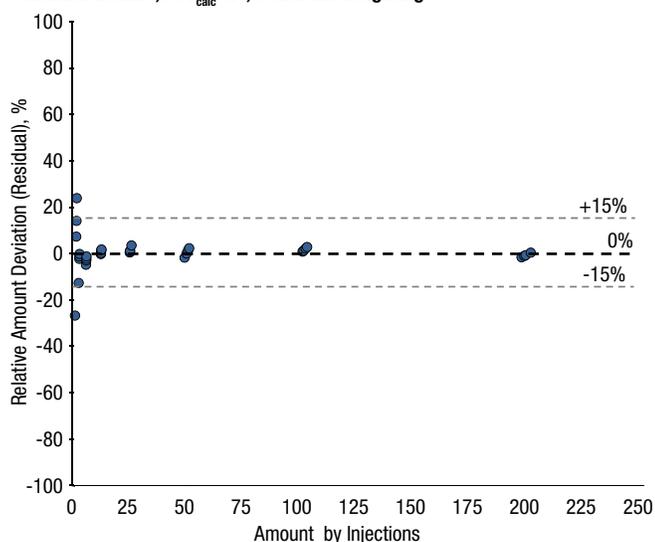


Figure 5. Residuals plots obtained for linear least squares regression with 1/amount weighting of calibration data obtained using a PFV_{calc} of 1.4. A) sulfamerazine; B) sulfamethizole; and C) sulfadimethoxine. An acceptable fit was found for all three analytes. See text for discussion of criteria used to evaluate quality of fit.

The optimal PFV_{calc} of 1.4 obtained by using the Chromeleon Power Law feature was then verified by acquiring data with a PFV_{exp} of 1.4. Acceptable results were found for all three analytes (for example, see the residuals plot for sulfamerazine in Figure 6). This demonstrates the usefulness of Chromeleon CDS to successfully and quickly determine an optimal PFV. As mentioned, an optimal PFV can be determined experimentally by acquiring data at different PFV_{exp} settings (e.g., 1.0, 1.2, 1.4, 1.6), albeit requiring more instrument time.

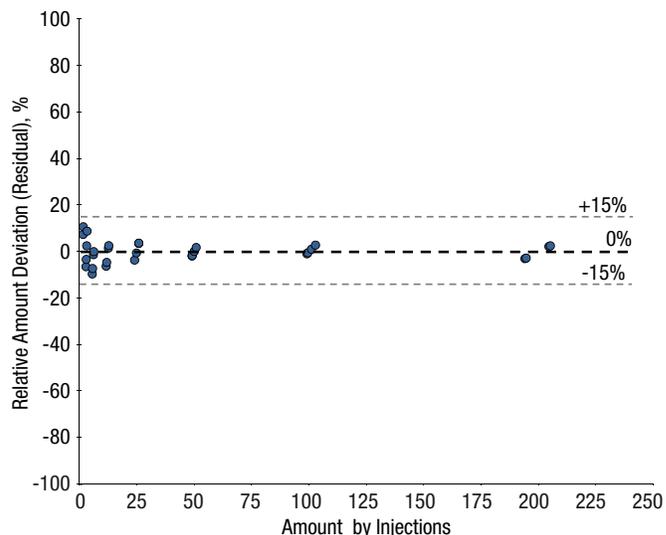


Figure 6. Residuals plot obtained for linear least squares regression with 1/amount weighting of calibration data obtained using a PFV_{exp} of 1.4 for sulfamerazine. An acceptable fit was found. See text for discussion of criteria used to evaluate quality of fit.

Figure 3 shows analysis of the same sample using PFV_{exp} 1.0, PFV_{calc} 1.4, and PFV_{exp} 1.4. The difference in signal (note y-axis scale) between PFV_{calc} 1.4 (Figure 3B) and PFV_{exp} 1.4 (Figure 3C) is related to the normalization factor in Eqn. 2 where $500^{(1.4-1.0)} = 12.01$. As expected and demonstrated by the verification results, normalization affects the magnitude of response (coefficient a in Eqn. 1) but not the shape of the curve (exponent b in Eqn. 1).

When to use a PFV other than 1.0

The main use of a PFV other than 1.0, as shown here for the sulfonamides, is to optimize the inherent (analog and digital) signal output so that a linear calibration curve can be used for routine analysis. This has additional benefits related to the effects of non-linear response on peak shape and S/N value. This is evident when comparing the chromatograms in Figure 3. The characteristic sublinear

response of CAD is known to cause slight apparent peak broadening, and with this type of response curve, true limits of detection are better than estimated based on S/N values from higher level standards.¹ As shown in Figure 3 and Table 1, the peaks in Figures 3B and 3C are sharper, baseline drift is lower, and since response has been linearized, S/N values are more accurate measures of limits of detection than Figure 3A. Use of an optimal PFV to better linearize the signal output may therefore help to avoid common pitfalls where misleading data can be obtained for metrics such as chromatographic resolution, column efficiency or limits of detection. These metrics are calculated by using the linear-scaled analyte concentration profiles and thus yield distorted results when a nonlinearly translated signal profile is used. The benefits of optimizing PFV may therefore also be considered for all methods, even those that use nonlinear calibration.

For methods that rely on relative response between peaks for quantitation, optimization of the PFV is highly recommended. Figure 7 is an example where relative peak areas are used to determine the mass distribution of polymers. With sublinear response, the smaller peaks at the edge of the distribution would likely be overestimated. Optimizing the PFV would therefore provide more accurate results.

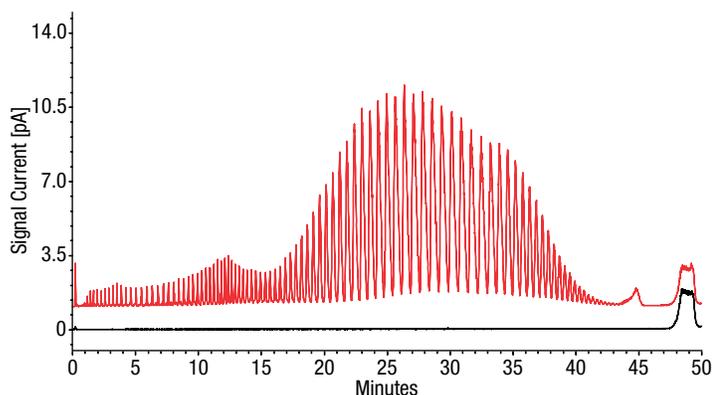


Figure 7. Chromatogram of polyethylene glycol 3000 (red) and water blank (black) using reversed-phase HPLC and CAD. Since relative peak areas are used to evaluate the mass distribution of subspecies, it is recommended to optimize the PFV in order to obtain the most accurate results.

Actions to avoid when using a PFV other than 1.0

- Any use of a PFV <1.0 will likely result in greater deviation from linear response (sublinear) and is not recommended.
- Use of a PFV >1.7 will likely result in supralinear response and sigmoidal curve shape and is therefore not recommended.
- Choice of a PFV should not be based on improving SNR or peak shape. PFV should only be chosen to linearize response rather than to artificially sharpen peaks or to exaggerate the SNR values for high level standards.

Conclusions

- The PF is a data acquisition parameter that is very useful to optimize the range over which CAD response is approximately linear for a given method.
- An optimal PFV enables use of linear calibration curves for many CAD methods and is highly recommended when using relative peak response for quantitation.
- Use of a PFV to optimize the linear response range can improve the accuracy of common metrics such as chromatographic resolution, column efficiency, and limits of detection.
- Best practices for calibration, which includes a robust evaluation of calibration curve quality of fit, can significantly improve the accuracy of quantitative results and is a key step in determining an optimal PFV.
- The Chromeleon CDS Power Law feature greatly facilitates the determination of an optimal PFV.

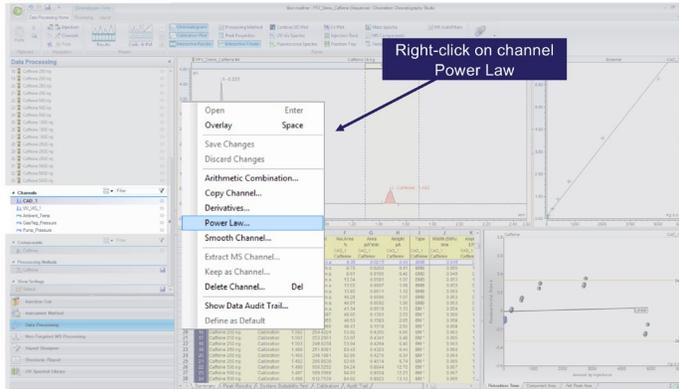
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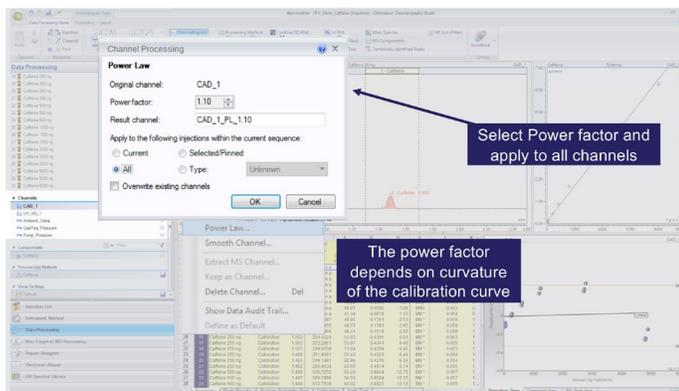
Appendix A: Use of the Power Law feature in Chromeleon CDS

Chromeleon CDS includes a Power Law processing action that applies a power factor to an existing channel such that the data points are raised by an exponent (typically 1.0 to 1.6) with the results being output to a new channel. The basic steps are as follows:

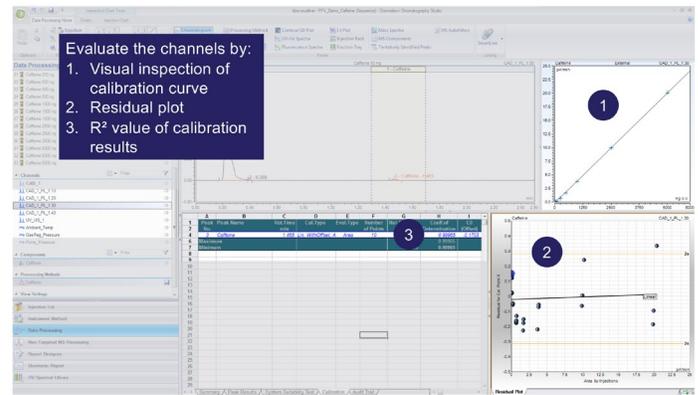
1. Within the data processing navigation pane right-click on an existing channel and select "Power Law".



2. Select the power factor value and apply to all channels.



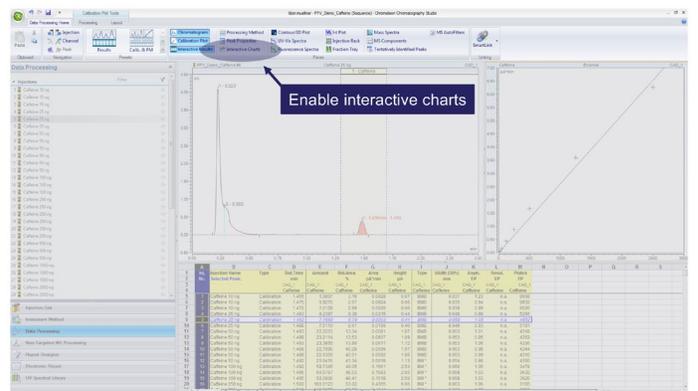
3. The new channel can then be evaluated for calibration quality of fit using residuals plots and other metrics.



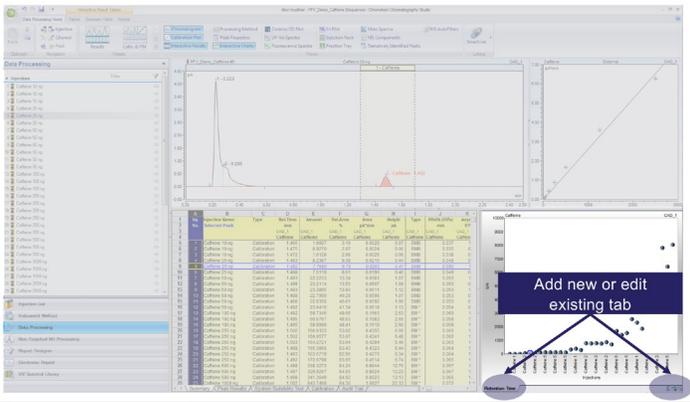
Appendix B: Creating relative residuals plots in Chromeleon CDS

Chromeleon CDS enables the use of Interactive Charts that provide a graphical representation of specific data acquired from a set of injections or a query in the Data Processing category. The following shows how to use this feature within Chromeleon CDS to create relative residuals plots.

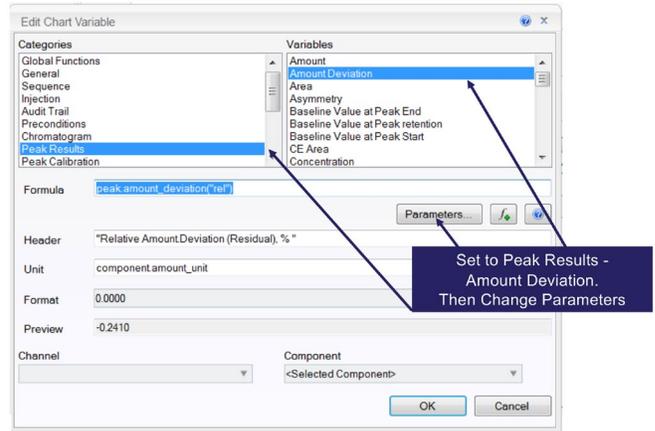
1. Within the data processing view of the Chromatography Studio, enable interactive charts.



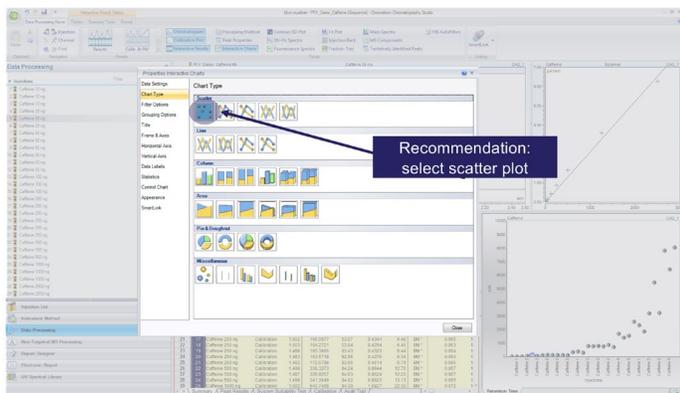
2. Within the interactive chart pane either add a new tab or edit an existing tab.



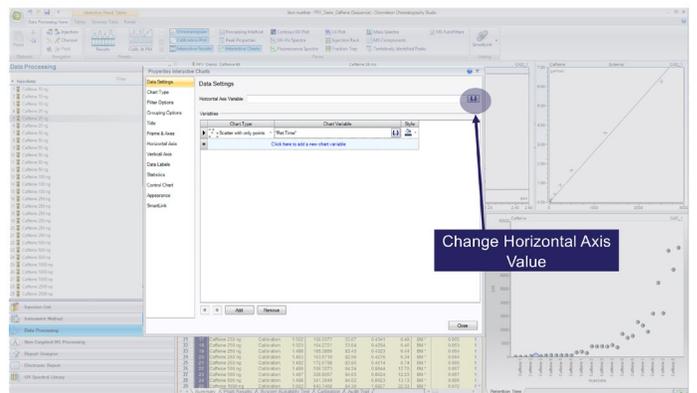
5. Select the Peak Results category and choose "Amount Deviation" as the variable. Click the parameters button and choose "Relative in % of the expected amount."



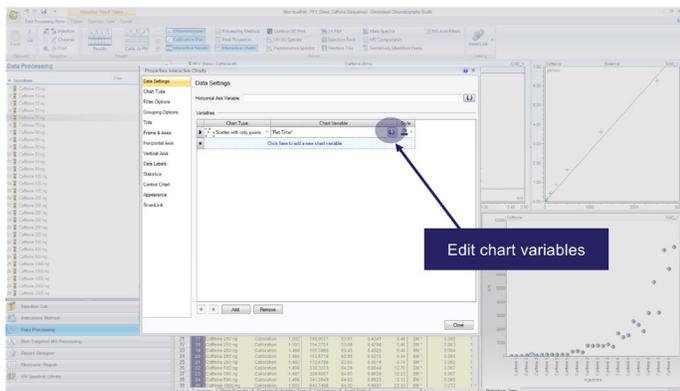
3. Right-click on the chart and select "Chart Variables" from the drop-down list, then "Chart Type" and choose "Scatter Plot".



6. Likewise, from the Chart Variables list, select "Data Settings" and choose the icon to change horizontal axis variable.



4. Likewise, from the Chart Variables list, select "Data Settings" and choose the icon to edit chart variables.



7. Select the "Peak Results" category and choose "Amount" as the variable.

