

Pharma and biopharma

# Method transfer and optimization of deoxycholic acid analysis using HPLC-CAD

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## Keywords

Deoxycholic acid analysis, cholic acid analysis, high performance liquid chromatography (HPLC), charged aerosol detector (CAD), evaporation temperature (EvapT), HPLC method transfer, power function value (PFV), power value (PV), United States Pharmacopoeia (USP)

## Application benefits

- Demonstrating the simplicity of method transfer from a Thermo Scientific™ Vanquish™ Charged Aerosol Detector H to a Thermo Scientific™ Vanquish™ Charged Aerosol Detector HP.
- Demonstrating the effortless method transfer from the previous power function value (PFV) concept to the current power value (PV) concept supported by the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), getting more results with less solvents and analysis time.
- Highlighting the signal-to-noise ratio (S/N) performance of the Vanquish Charged Aerosol Detector HP.

## Goal

To provide guidance on transferring detector settings of the United States Pharmacopoeia Monograph method for deoxycholic acid from the Vanquish Charged Aerosol Detector H to the Vanquish Charged Aerosol Detector HP. Additionally, this guidance covers optimizing these settings to enhance overall method performance.

## Introduction

The United States Pharmacopeial Convention monograph (USP 40-NF 35 or USP-NF Deoxycholic Acid) outlines a high-performance liquid chromatography with charged aerosol detection (HPLC-CAD) method<sup>1</sup> for the accurate measurement of deoxycholic acid (also known as desoxycholic acid) and its primary impurity, cholic acid (Figure 1).

Key detector settings that influence detection performance metrics such as detection limit, resolution, and linearity include:

- The evaporation temperature (EvapT) for setting the temperature of the evaporation tube and influencing sensitivity related to volatility factors.
- The filter constant for balancing noise and adjusting the detector's response time to changes in analyte concentration.

- The PV for optimizing the linear range within the detector's dynamic range for a given application.

These parameters are crucial for successful method transfer and optimization. Consequently, this work offers guidelines for method transfer and optimization from the Vanquish Charged Aerosol Detector H to the Vanquish Charged Aerosol Detector HP (Figure 2).

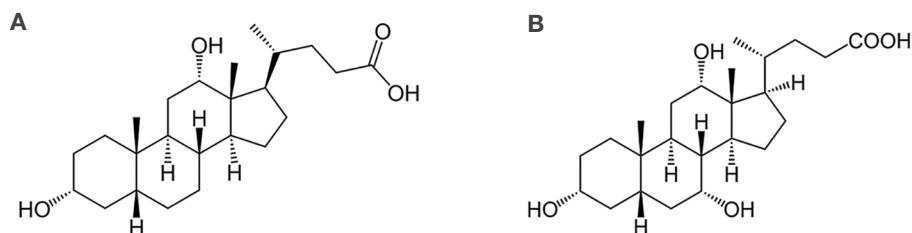


Figure 1. The chemical structures of deoxycholic acid (A) and cholic acid (B)

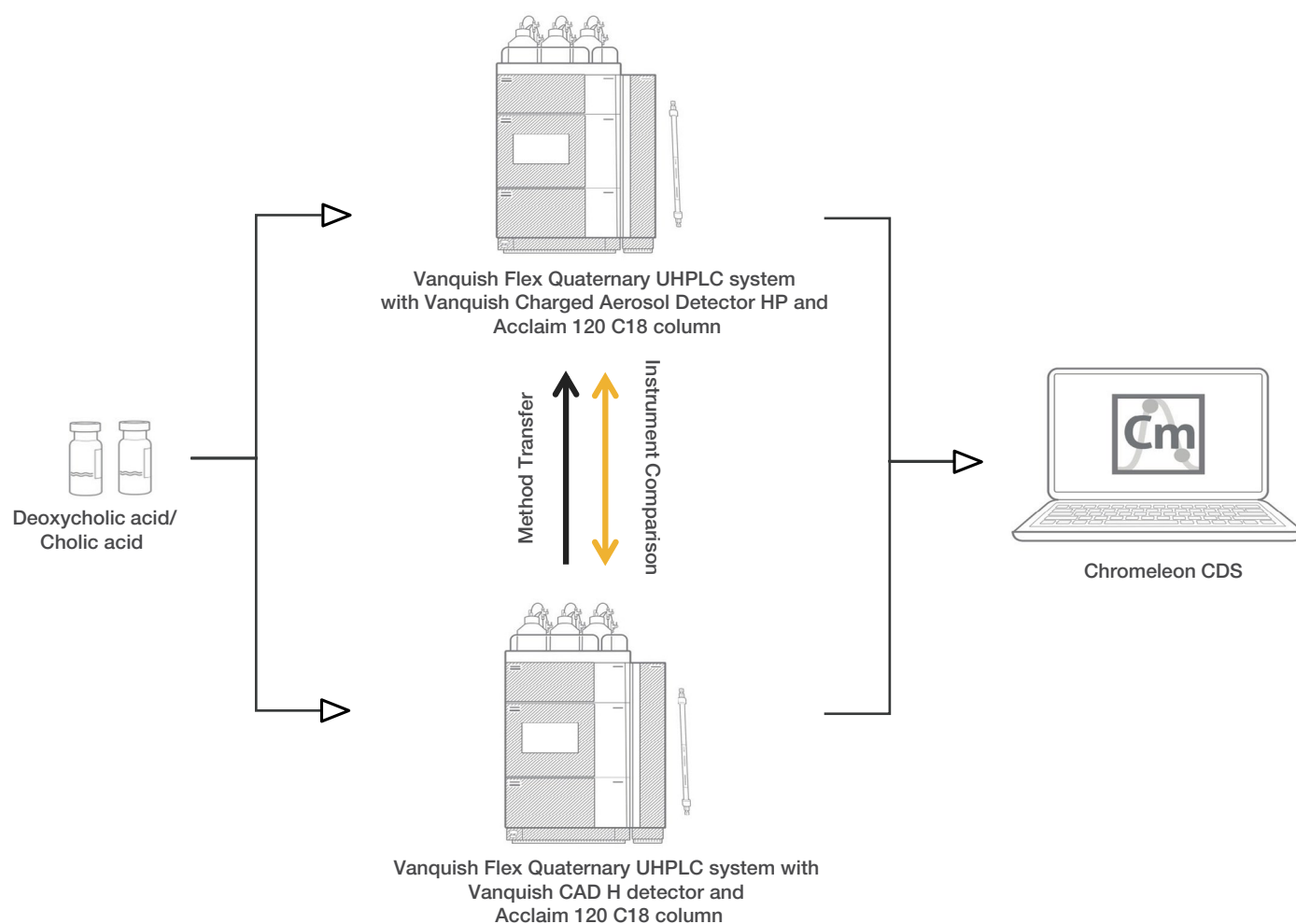


Figure 2. Schematic overview of the deoxycholic acid analysis method transfer. The process uses a Vanquish LC system, coupled with a Vanquish Charged Aerosol Detector HP or H and an Acclaim C18 column.

## Experimental

### Chemicals

- Deionized water, 18.2 MΩ.cm, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification (P/N 50136149)
- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (P/N A955)
- Fisher Scientific™ Formic acid, Optima™ LC/MS grade (P/N A117)
- Sigma-Aldrich Cholic acid, USP Reference Standard grade (P/N 1133503)
- Sigma-Aldrich Deoxycholic acid, USP Reference Standard grade (P/N 1171273)

### Sample handling

- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes, 100–1000 µL (P/N 4641100N)
- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes, 10–100 µL (P/N 4641070N)
- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes, 1–10 µL (P/N 4641030N)
- Fisherbrand™ Mini Vortexer (P/N 14-955-152)
- Thermo Scientific™ SureSTART™ 2 mL Amber Glass Short Thread Screw Top Vials, 100/pack, Level 2 (P/N 6ASV9-2P)
- Thermo Scientific™ SureSTART™ Blue Polypropylene 9 mm AVCS™ Screw Caps with Soft Blue Silicone/Clear PTFE Septa, 100/pack, Level 3 (P/N 6PSC9ST101)

### Instrumentation

- Thermo Scientific™ Vanquish™ Flex UHPLC system consisting of:
  - System Base Vanquish Horizon/Flex (P/N VF-S01-A-02)
  - Vanquish Quaternary Pump F (P/N VF-P20-A)
  - Vanquish Split Sampler FT (P/N VF-A10-A-02)
  - Vanquish Column Compartment H (P/N VH-C10-A-03)
  - Vanquish Charged Aerosol Detector HP (P/N VH-D21-A-01)
  - Vanquish Charged Aerosol Detector H (P/N VH-D20-A)

### Sample preparation

#### Stock standard solutions

Stock standard solutions were prepared as 1 mg/mL solutions of deoxycholic acid or cholic acid in an 80/20 v/v methanol/water diluent. To do this, 10 mg of standard (deoxycholic acid or

cholic acid) was added to a 10 mL volumetric flask, which was filled to the mark with the diluent. The diluent was prepared by mixing 800 mL of methanol with 200 mL of water.

### Working standard solutions

The working standard solutions were prepared at a concentration of 0.01 mg/mL by adding 1 mL of the stock standard solution to a 100 mL volumetric flask and filling to the mark with diluent, as required by the compendial method. Calibration solutions of 0.01, 0.005, 0.002, 0.001, and 0.0005 mg/mL were prepared by serial dilution in 100 mL volumetric flasks, starting from a 1 mg/mL stock solution.

### Chromatographic conditions

Table 1. Chromatographic conditions

Parameter	Value																																					
Column	Thermo Scientific™ Acclaim™ 120 C18 3 µm, 4.6 × 150 mm (P/N 059133)																																					
Solvent A	0.1% (v/v) formic acid in water																																					
Solvent B	0.1% (v/v) formic acid in acetonitrile																																					
Gradient	<table><tr><th>Time [min]</th><th>B [%]</th></tr><tr><td>0.0</td><td>25.0</td></tr><tr><td>2.0</td><td>45.0</td></tr><tr><td>14.0</td><td>58.0</td></tr><tr><td>24.0</td><td>100.0</td></tr><tr><td>35.0</td><td>100.0</td></tr><tr><td>35.0</td><td>25.0</td></tr><tr><td>38.0</td><td>25.0</td></tr></table>	Time [min]	B [%]	0.0	25.0	2.0	45.0	14.0	58.0	24.0	100.0	35.0	100.0	35.0	25.0	38.0	25.0																					
Time [min]	B [%]																																					
0.0	25.0																																					
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14.0	58.0																																					
24.0	100.0																																					
35.0	100.0																																					
35.0	25.0																																					
38.0	25.0																																					
Flow rate	1 mL/min																																					
Column temp.	30 °C, forced air mode (fan speed 5), 30 °C active pre-heater																																					
Autosampler temp.	8 °C																																					
Injection volume	25 µL																																					
CAD settings	<table><tr><th colspan="4">Channel start settings</th></tr><tr><th>No.</th><th>Channel</th><th>PV (Vanquish CAD HP)</th><th>PFV (Vanquish CAD H)</th></tr><tr><td>1</td><td>CAD_1</td><td>1.5</td><td rowspan="4">Settings of 1.0, 1.2, 1.4, and 1.6 were applied sequentially (only one channel can be obtained at a time)</td></tr><tr><td>2</td><td>CAD_2</td><td>1.8</td></tr><tr><td>3</td><td>CAD_3</td><td>2.1</td></tr><tr><td>4</td><td>CAD_4</td><td>2.4</td></tr><tr><td colspan="4">Data collection rate: 20 Hz</td></tr><tr><td colspan="4">Filter settings: 2, 3.6, 5, 10 s</td></tr><tr><td colspan="4">Gas regulation mode (Vanquish CAD H only): Analytical</td></tr><tr><td colspan="4">Evaporation temperature: 50 °C</td></tr></table>	Channel start settings				No.	Channel	PV (Vanquish CAD HP)	PFV (Vanquish CAD H)	1	CAD_1	1.5	Settings of 1.0, 1.2, 1.4, and 1.6 were applied sequentially (only one channel can be obtained at a time)	2	CAD_2	1.8	3	CAD_3	2.1	4	CAD_4	2.4	Data collection rate: 20 Hz				Filter settings: 2, 3.6, 5, 10 s				Gas regulation mode (Vanquish CAD H only): Analytical				Evaporation temperature: 50 °C			
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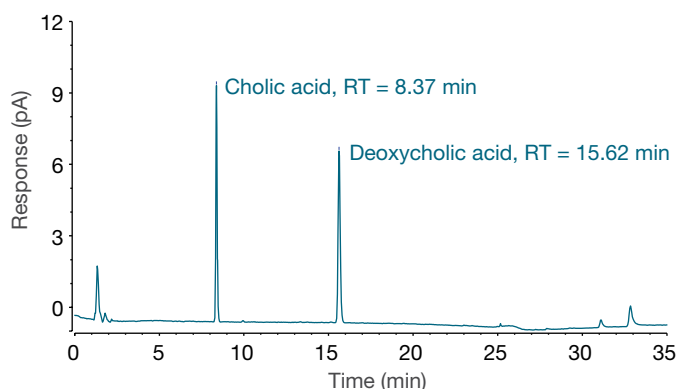
### Chromatography Data System

The Thermo Scientific™ Chromeleon™ 7.3.2 Chromatography Data System (CDS) was used for data acquisition and analysis.

## Results and discussion

### System suitability test

System suitability tests (SST) are essential for HPLC methods, ensuring that the reproducibility and resolution of the method are sufficient for accurate analysis. SST assesses the entire system, including equipment, electronics, analytical operations, and samples used for analysis, indicating the precision, specificity, and sensitivity of the method. In this specific analysis, using the chromatographic conditions in Table 1, a working standard solution mixture of 0.01 mg/mL of deoxycholic acid and its impurity, cholic acid, was tested. The resulting chromatogram showed excellent separation (Figure 3). Deoxycholic acid eluted at 15.6 minutes, which is slightly later than the USP monograph retention time of 13.0 minutes. Furthermore, the relative retention times for cholic acid and deoxycholic acid were 0.54 and 1, respectively. This is comparable to the acceptance criteria of the USP monograph, which specifies the relative retention times for cholic acid and deoxycholic acid as 0.56 and 1, respectively.



**Figure 3. Chromatogram showing the separation of deoxycholic acid and cholic acid in the Vanquish Charged Aerosol Detector HP**

System suitability parameters, such as the repeatability of the analyte peak area and sensitivity (expressed as % RSD and S/N, respectively), were determined during the system suitability test for the chromatographic method. According to the USP monograph, the requirements are not more than (NMT) 3.0% RSD for peak area at an analyte concentration of 0.01 mg/mL

and a S/N of not less than (NLT) 10 for a 0.0005 mg/mL analyte concentration. As shown in Table 2, both the instrument configurations with the Vanquish Charged Aerosol Detector H and the Vanquish Charged Aerosol Detector HP meet these criteria and are suitable for assaying deoxycholic acid using the USP-NF deoxycholic acid method.

### Method transfer parameters

Previous technical notes,<sup>2,3</sup> a previous application note,<sup>4</sup> and chapter 3 of the book *Charged Aerosol Detection for Liquid Chromatography and Related Separation Techniques*<sup>5</sup> offer valuable guidance on the key parameters to consider when transferring methods between different charged aerosol detector modules. Consequently, to transfer the USP-NF deoxycholic acid method from the Vanquish Charged Aerosol Detector H to the Vanquish Charged Aerosol Detector HP, the following parameters were optimized: PV, EvapT, and filter constant.

### Power value

The PV for the Vanquish Charged Aerosol Detector P series and the power function value (PFV) for earlier CAD models are distinct user-defined settings that optimize the analog and digital signal output of the CAD. The primary purpose is to linearize the CAD signal output over the desired quantitation range for a given method, which is useful if a quadratic or log-log fit is not an option for the calibration curve fitting model. Linearizing the CAD response is advantageous because the CAD behaves approximately linearly within specific ranges while being non-linear in others. Although linearization is not strictly required, optimizing the PV is strongly recommended for quantitation methods that depend on the relative response between peaks within a single run.

To enhance user accessibility and facilitate seamless translation, the Vanquish Charged Aerosol Detector P series offers three distinct channel settings/modes:

- Easy mode
- Advanced mode
- Legacy mode

**Table 2. Results of system suitability testing following the conditions of Table 1 (n=10)**

	Vanquish Charged Aerosol Detector H	Vanquish Charged Aerosol Detector HP	USP requirements	Detector response setting
% RSD of area	0.42	0.62	<3.0	PFV = 1.2, PV = 1.8
S/N ratio	148	210	>10	PFV = 1.2, PV = 1.8

The Easy mode features a single adjustable channel for the PV. The Advanced mode provides four channels, each capable of setting individual PVs. In the Advanced mode of the method editor, the PV settings are restricted to a range between 1.5 and 2.4. This range of Advanced mode PV settings corresponds to legacy PFVs between 1.0 and 1.6, respectively, to prevent significant deviations from a linear response, which are not recommended.<sup>3</sup> Notably, the Legacy mode permits the input of previous CAD versions' PFVs, which are automatically converted to the PVs of the Vanquish Charged Aerosol Detector P series. This mode supports PFV inputs ranging from 1.0 to 1.6. The Vanquish Charged Aerosol Detector P series applies an enhanced set of PVs by multiplying the PFVs of legacy CAD by a factor of 1.5, referred to as the power function ratio (PFR). Consequently, a PFV of 1.2 in previous CAD versions corresponds to a PV of 1.8 in the Vanquish Charged Aerosol Detector P series. Detailed specifications are presented in Table 3, while Figure 4 illustrates a screenshot of the Legacy Mode within the Vanquish Charged Aerosol Detector P series method editor.

**Table 3. Comparison of PFVs between previous CAD versions (legacy CAD) and the PVs in the Vanquish Charged Aerosol Detector P series**

User setting	Power function value (legacy CAD)	Power value (Vanquish Charged Aerosol Detector P series)
Easy mode	Not available	1.80 (default)
Advanced mode	Not available	1.50 ... 2.40
Legacy mode	1.00 ... 1.60	1.50 ... 2.40

When evaluating changes in PFVs or PVs, it is crucial to study the response effects at low analyte levels and select the best curve fit model. Concentration levels of 0.5, 1, 2, 5, and 10 µg/mL were used to derive the calibration curve. The coefficient of determination ( $R^2$ ) from calibration plots and residual plots from

residual analysis were used to assess the goodness of fit. These analyses were conducted at various PFVs and PVs. PFVs of 1.0, 1.2, 1.4, and 1.6 were used for the Vanquish Charged Aerosol Detector H, while corresponding PVs of 1.5, 1.8, 2.1, and 2.4 were used for the Vanquish Charged Aerosol Detector HP. Notably, the Vanquish Charged Aerosol Detector HP has the advantage of acquiring data for up to four PVs simultaneously, which is more efficient than the Vanquish Charged Aerosol Detector H, which requires four separate runs to acquire data at four different PFVs.

The PFV of 1.2 for the Vanquish Charged Aerosol Detector H and PV of 1.8 for the Vanquish Charged Aerosol Detector HP showed the best fit, as indicated by the  $R^2$  and residual plots. The coefficient of determination for both calibration curves for both was equal to 1, indicating a linear relationship between analyte concentration and peak area (Figures 5 and 6). Additionally, the residual plots for both showed residuals randomly scattered around the zero line ( $y=0$ ), suggesting that the chosen linear model was a good fit, and reliable predictions can be made from the calibration curve (Figures 5 and 6). Other PFVs (1.0, 1.4, 1.6) and PVs (1.5, 2.1, 2.4) were less optimal, as indicated by the coefficient of determination and residuals outside the acceptable  $\pm 15\%$  range for this analysis.

## Evaporation temperature

The EvapT in a CAD is a crucial parameter that impacts detection sensitivity and selectivity. Proper optimization ensures efficient solvent removal while preserving analyte integrity. Low EvapT settings can produce a more uniform response and enable broader analyte measurement but may increase noise from semi-volatile impurities. High EvapT settings reduce noise but may result in signal loss for semi-volatile analytes, especially at low concentration levels. The ideal EvapT depends on the properties of the solvent and analytes and is often determined experimentally. Modern CAD instruments offer flexibility in adjusting the EvapT, enhancing their adaptability and ensuring accurate and reliable quantitative analysis.

No	Channel	Power Function	Acquisition on [min]	Acquisition off [min]
1	<input checked="" type="checkbox"/> CAD_1	1,20	Start Run	Stop Run

Converted to Power Value 1.80

**Figure 4. Legacy Mode in Vanquish Charged Aerosol Detector P series for seamless method transfer**

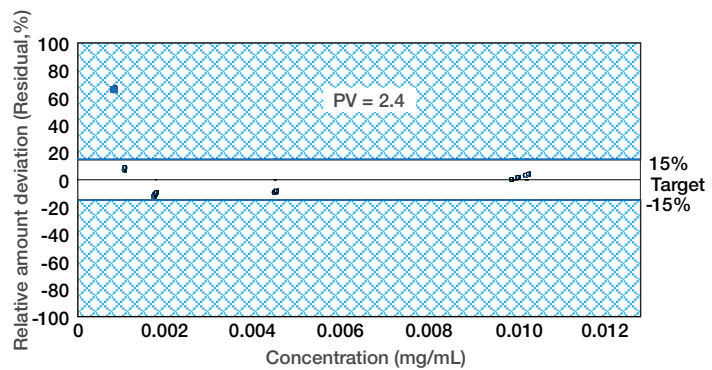
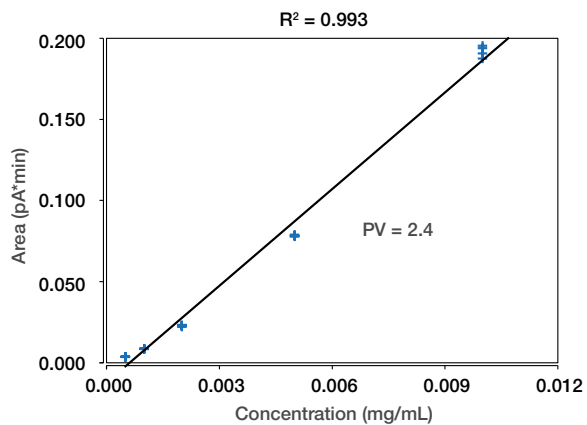
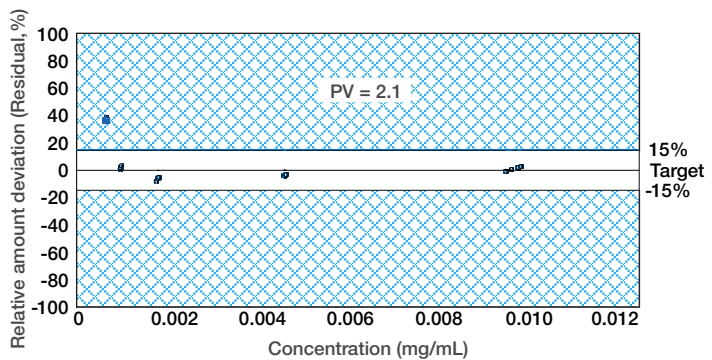
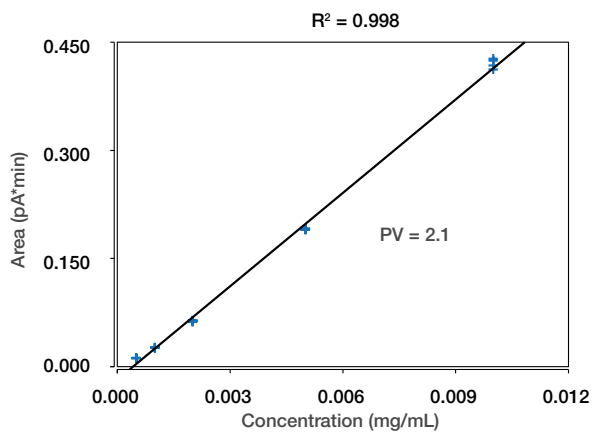
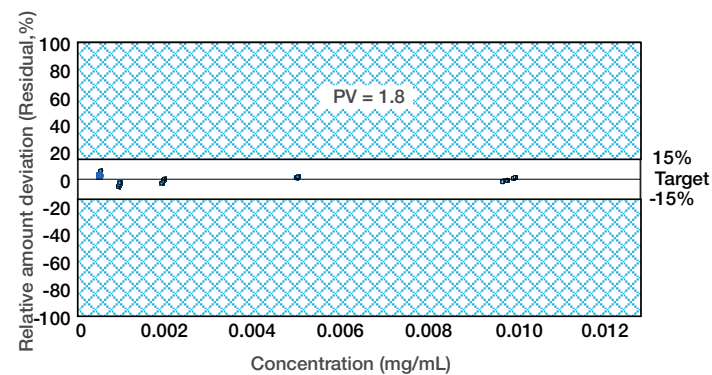
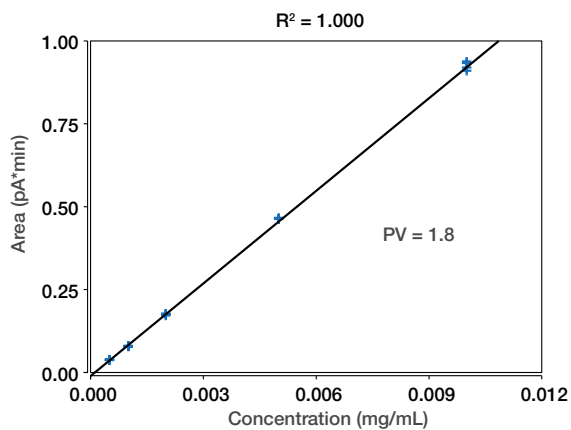
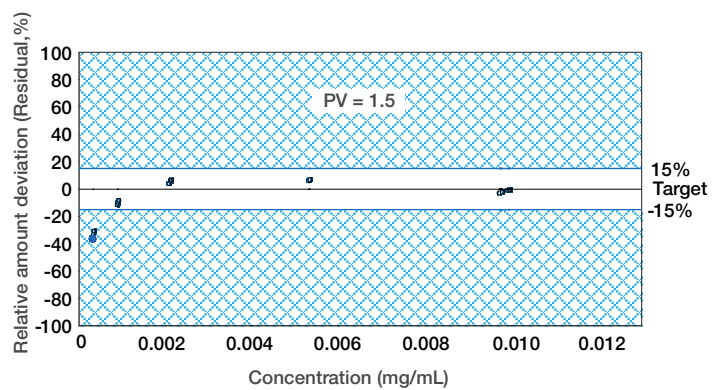
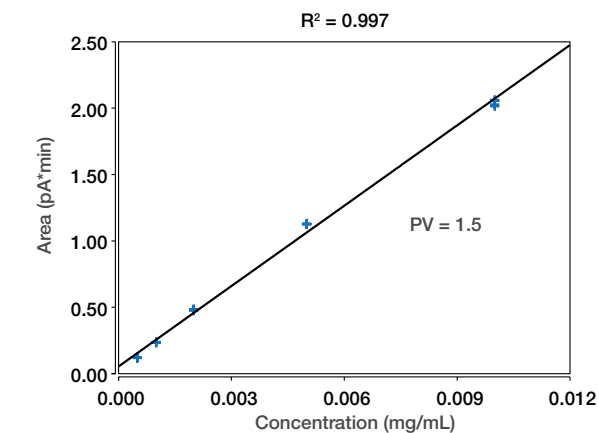


Figure 5. Calibration curves and residual plots at PV 1.5, 1.8, 2.1, and 2.4 for deoxycholic acid in the Vanquish Charged Aerosol Detector HP



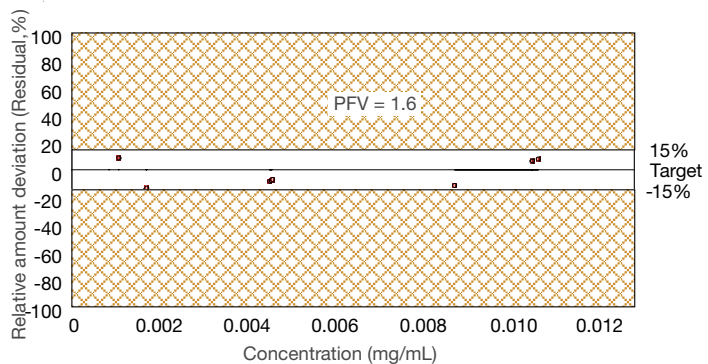
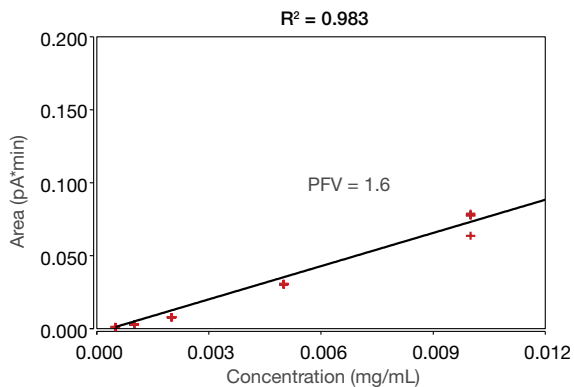
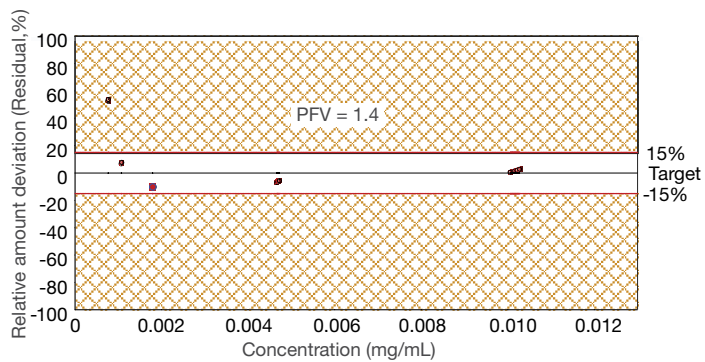
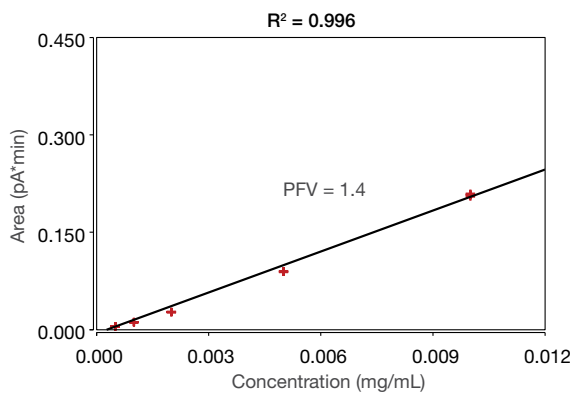
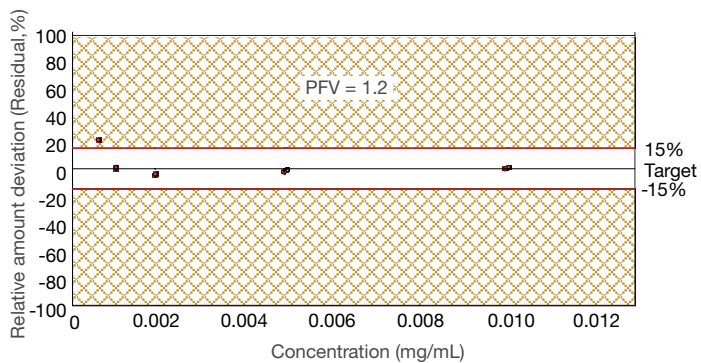
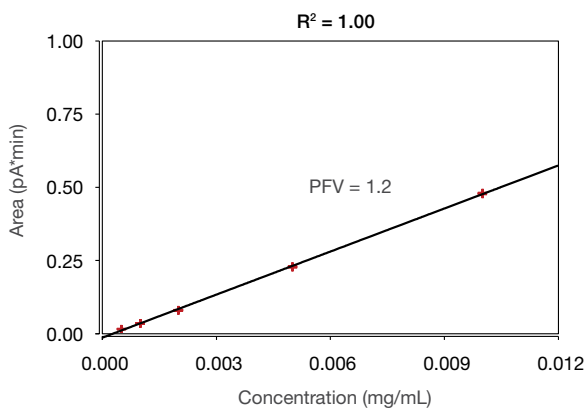
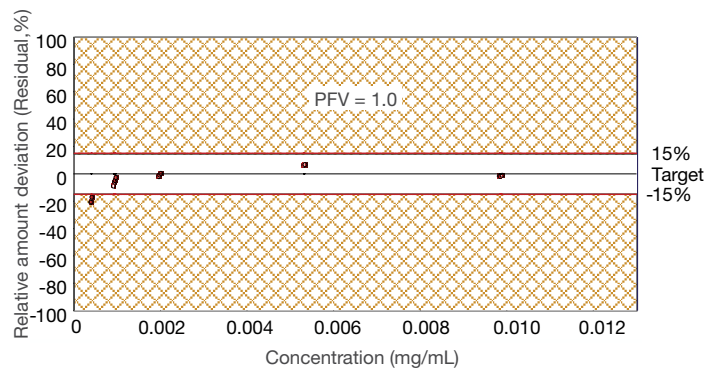
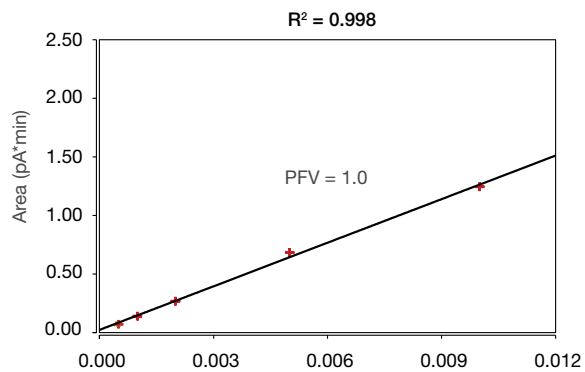


Figure 6. Calibration curves and residual plots at PFV 1.0, 1.2, 1.4, and 1.6 for deoxycholic acid in the Vanquish Charged Aerosol Detector H

Both the Vanquish Charged Aerosol Detector H and the Vanquish Charged Aerosol Detector HP have a settable EvapT from ambient (+ 5 °C) to 100 °C. For this method transfer application, five temperature points were selected: 35 °C, 42 °C, 50 °C, 60 °C, and 70 °C. At a concentration of 0.25 µg/mL, all EvapT settings achieved a S/N ratio of 50 or above for both detectors, exceeding the USP requirement of NLT 10 for a concentration of 0.5 µg/mL (LOD). For the Vanquish Charged Aerosol Detector HP, an EvapT of 60 °C provided the highest S/N, while for the Vanquish Charged Aerosol Detector H, an EvapT of 70 °C yielded the highest S/N. However, to avoid analyte degradation at higher temperatures and increased noise at lower temperatures, an EvapT of 50 °C is recommended for this application, as it shows consistent S/N between the Vanquish Charged Aerosol Detector HP and the Vanquish Charged Aerosol Detector H.

### Signal filter

In a charged aerosol detector, the signal filter enhances detection by reducing baseline noise and improving the S/N ratio, ensuring accurate and reliable analyte detection. It smooths high-frequency noise while preserving the analyte signal. The filter constant determines the level of noise reduction, with smaller filter constants (e.g., 0.2 seconds) providing a faster response but more noise, and larger filter constants (e.g., 10.0 seconds) resulting in a slower response but less noise. Proper adjustment of the filter constant and data collection rate is critical to balance noise reduction with peak height, symmetry, and resolution. These settings can be tailored to specific applications, enabling improved sensitivity and precision especially in complex chromatograms. Optimizing the signal filter ensures the best analytical performance, delivering consistent and reliable quantitative results.

In this method transfer, a data collection rate of 20 Hz was used, with an EvapT maintained at 50 °C. Various digital filter settings (2, 3.6, 5, and 10 seconds) were evaluated. All selected filter settings exhibited a S/N ratio of more than 50 for both the Vanquish Charged Aerosol Detector HP and the Vanquish Charged Aerosol Detector H, exceeding the USP requirement of NLT 10 for a concentration of 0.5 µg/mL (LOD). Since filter settings affect peak width, this parameter was evaluated. Smaller filter settings, such as 2, 3.6, and 5 seconds, resulted in narrower peak widths. However, there was significant noise associated with the peaks obtained at filter settings of 2 seconds and 3.6 seconds (data not shown). At the highest filter setting of 10, the peak exhibited less noise than the filter setting of 5, but the peak width was slightly larger likely due to the slower response time caused by the high filter setting. Therefore, a filter setting of 5 is recommended for this application to get equivalent performance.

To simplify user operations, the filter constant is set to a default value of 5.0 when the "link data collection parameters" option is selected (Figure 7). This setting ensures that the data collection rate automatically adjusts the peak width while maintaining the filter constant at 5.0. However, users can manually adjust the filter constant by deselecting the "link data collection parameters" option, enabling them to control the data collection rate, filter constant, and peak width independently. It is crucial to exercise caution when manually adjusting these three parameters, as improper settings may result in increased noise or altered peak width during method transfer.

**Figure 7.** Data collection settings (e.g., filter constant) in the Vanquish Charged Aerosol Detector P series



## Conclusion

As CAD becomes more prominent in analytical methods, it is essential to provide clear guidelines for method transfer between detectors. The USP-NF deoxycholic acid method, initially developed with the Thermo Scientific™ Dionex™ Corona™ Ultra RS™ detector and successfully transferred to the Vanquish Charged Aerosol Detector F, was further successfully transferred from the Vanquish Charged Aerosol Detector H to the Vanquish Charged Aerosol Detector HP. The method transfer involved optimizing key parameters, including PV, EvapT, and signal filter constant. Chromeleon CDS streamlined the entire method transfer and optimization process, from controlling the instrument and evaluating data to graphically displaying residual plots.

The final optimized parameters for the Vanquish Charged Aerosol Detector HP were PV = 1.8, EvapT = 50 °C, and a filter constant of 5 seconds. These settings ensured that the system met the USP criteria for S/N ratio and peak area precision, with the Vanquish Charged Aerosol Detector HP demonstrating excellent performance and method transferability.

The Vanquish Charged Aerosol Detector HP demonstrated an excellent linear correlation between analyte concentration and peak area, as specified by the USP method for this application, evidenced by a coefficient of determination ( $R^2$ ) of 1.0 and well-distributed residuals within a 15% error limit. The optimized EvapT and filter settings ensured high sensitivity and selectivity while minimizing noise.

## Additional advantages of the Vanquish Charged Aerosol Detector HP include:

- Legacy mode for the Vanquish Charged Aerosol Detector P series, allowing users to input the PFV as a method parameter, matching the method parameters used in earlier versions of CAD. This legacy mode enables easy switching of methods from legacy CAD to Vanquish Charged Aerosol Detector HP.
- The ability of the Vanquish Charged Aerosol Detector HP to acquire data at different PVs in a single run saving time and solvents, which is more efficient than the Vanquish Charged Aerosol Detector H, which requires four separate runs to acquire data at four different PFVs.

In conclusion, the optimized parameters and additional features of the Vanquish Charged Aerosol Detector HP make it a highly efficient and reliable detector, ensuring seamless method transfer and excellent analytical performance.

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