Pharma and biopharma

Thermo Fisher SCIENTIFIC

HPLC transfer and optimization of deoxycholic acid analysis using HPLC – Charged Aerosol Detector

Kelechi Amatobi, Florian Broghammer, Katherine Lovejoy
Thermo Fisher Scientific, Germering, Germany

Abstract

Purpose: To demonstrate the method transfer and optimization process for deoxycholic acid analysis using HPLC-CAD, moving from the Thermo Scientific™ Vanquish™ Charged Aerosol Detector (H/F) to the enhanced Thermo Scientific™ Vanquish™ Charged Aerosol Detector HP. This study also highlights the improvements in sensitivity, detection limits, and overall analytical efficiency achieved through the upgraded instrumentation. Notably, the power function value (PFV) used in signal processing was adjusted from the original setting in the Vanquish CAD to a new optimized power value (PV) in the Vanquish CAD HP, contributing to improved performance and data quality.

Methods: A Thermo Scientific™ Vanquish™ Flex UHPLC system equipped with both the Vanquish Charged Aerosol Detector and HP was used to perform method transfer and optimization for deoxycholic acid analysis. Chromatographic separation was achieved using a Thermo Scientific™ Acclaim™ 120 C18 column under gradient elution with formic acid-modified aqueous and organic phases.

Results: The Vanquish Charged Aerosol Detector HP demonstrated excellent performance with an R^2 of 1.000, ensuring a strong linear relationship between analyte concentration and peak area. Optimized settings provided high sensitivity and selectivity. Both Vanquish CAD HP and the earlier generation Vanquish CAD detectors met USP requirements, validating the successful method transfer and optimization for deoxycholic acid analysis.

Introduction

The United States Pharmacopeial Convention monograph outlines a high-performance liquid chromatography with charged aerosol detection (HPLC-CAD) method [1] for the accurate measurement of deoxycholic acid (also known as desoxycholic acid) and its primary impurity, cholic acid.

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 CAD to the Vanquish CAD HP.

Materials and methods

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 and 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

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.

Test Method(s)

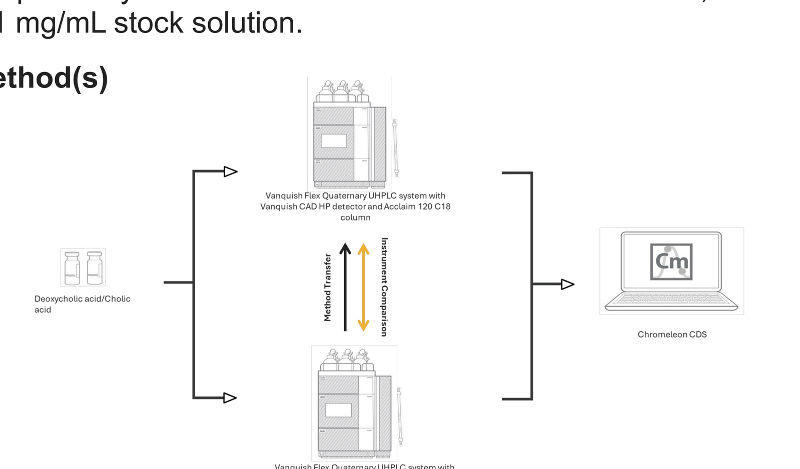


Figure 1: Schematic overview of the deoxycholic acid analysis method transfer. The process uses a Thermo Scientific Vanquish LC system, coupled with a Vanquish Charged Aerosol Detector HP or Vanquish Charged Aerosol Detector and an Acclaim C18 column.

Table 1. Chromatographic conditions.

Column	Acclaim 120 C18 4.6 × 150 mm, 3 µm column (P/N 059133)
Solvent A	0.1% (v/v) formic acid in water
Solvent B	0.1% (v/v) formic acid in acetonitrile
Gradient	

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

Flow rate	1 mL/min
Column temperature	30 ℃, forced air mode (fan speed 5), 30 ℃ active pre-heater
Autosampler temperature	8 ℃
Injection volume	25 µL
CAD Settings	**Channel start settings** **PV (Vanquish CAD HP):** CAD_1 = 1.5, CAD_2 = 1.8, CAD_3 = 2.1, CAD_4 = 2.4 **PFV (Vanquish CAD):** CAD_1 = 1.0, 1.2, 1.4, and 1.6 were applied sequentially (only one channel can be obtained at a time).
	Data collection rate: 20 Hz **Filter settings:** 2, 3.6, 5, 10 s **Gas regulation mode:** Analytical **Evaporation temperature:** 50℃

Chromatography Data System

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

Results

System suitability test

System Suitability Tests (SST) are crucial for ensuring the reproducibility and resolution of HPLC methods. In this analysis, a mixture of 0.01 mg/mL deoxycholic acid and cholic acid was tested under specific chromatographic conditions, showing excellent separation (**Figure 2**) with deoxycholic acid eluting at 15.62 minutes and relative retention times matching USP criteria. SST parameters such as repeatability and sensitivity met USP requirements, with both CAD and CAD HP configurations proving suitable for assaying deoxycholic acid (**Table 2**).

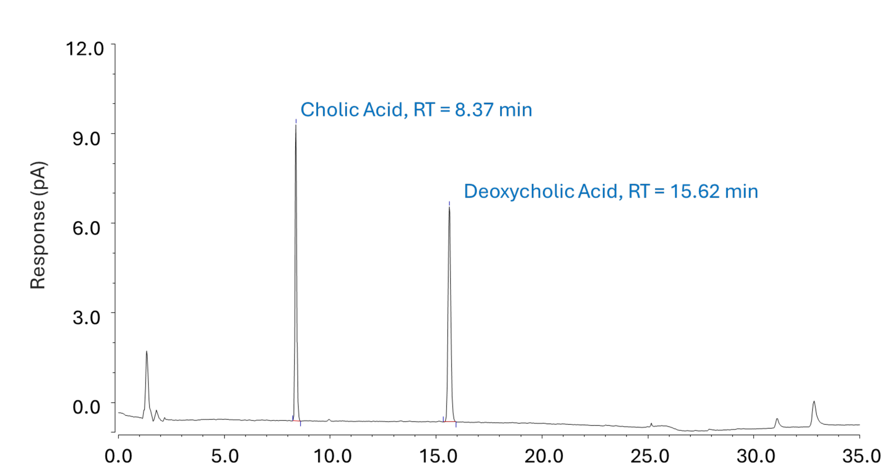


Figure 2: Chromatograms showing the separation of deoxycholic acid and cholic acid in VCAD HP.

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

	Vanquish CAD	Vanquish CAD 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

Method transfer parameters from Vanquish CAD to Vanquish CAD HP

Previous technical notes [2] [3], a previous application note [4], and chapter 3 of the book "Charged Aerosol Detection for Liquid Chromatography and Related Separation Techniques" [5] 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 CAD to CAD HP, the following parameters were optimized: PV, EvapT, and filter constant.

Power value

As noted earlier, the PV in the Vanquish CAD P series is an optimized version of the PFV used in previous CAD models. It combines PFV with a power function ratio (PFR) to enhance linearity, particularly for semi-volatile compounds. Both PV and PFV are settings tailored to optimize signal output and improve quantitation range linearity.

The Vanquish CAD P series provides three user-accessible modes in the method editor for setting PV, including options compatible with previous CAD models.

- Easy mode
- Advanced mode
- Legacy mode

Easy mode offers a single adjustable PV, while ***Advanced mode*** provides four channels with PV settings ranging from 1.5 to 2.4 — equivalent to PFVs of 1.0 to 1.6 in earlier CAD models. ***Legacy mode*** allows users to enter PFVs from previous CAD versions (**Figure 3**), which are then automatically converted to corresponding PVs for the CAD P series.

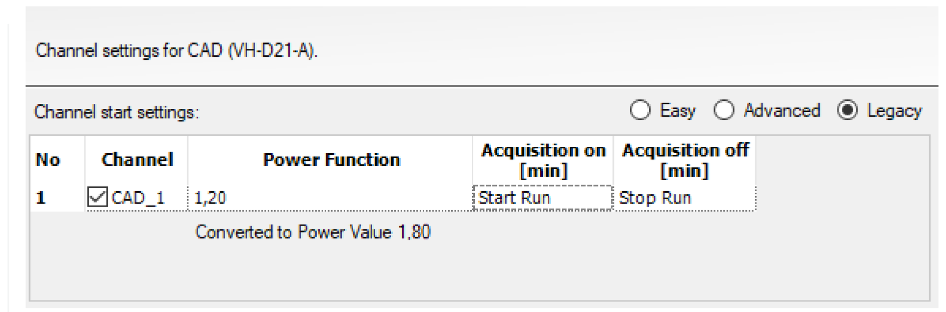


Figure 3: Legacy Mode in CAD P series for seamless method transfer

Calibration curve optimization for CAD and CAD HP at varying PFVs and PVs

To determine the optimal PFV and PV for the deoxycholic acid method, calibration curves (0.5–10 µg/mL) were analyzed at low analyte levels. Linearity was evaluated using R^2 and residual plots. The Vanquish CAD was tested at PFVs of 1.0–1.6, while the Vanquish CAD HP was evaluated at corresponding PVs of 1.5–2.4. Unlike Vanquish CAD, which required separate runs, CAD HP collected data for four PVs simultaneously, improving efficiency. Optimal linearity (R^2 = 1.000) and random residuals were observed at PFV 1.2 (Vanquish CAD) and PV 1.8 (Vanquish CAD HP) (**Figure 4 & 5**). Other settings showed reduced fit, with residuals exceeding the ±15% limit

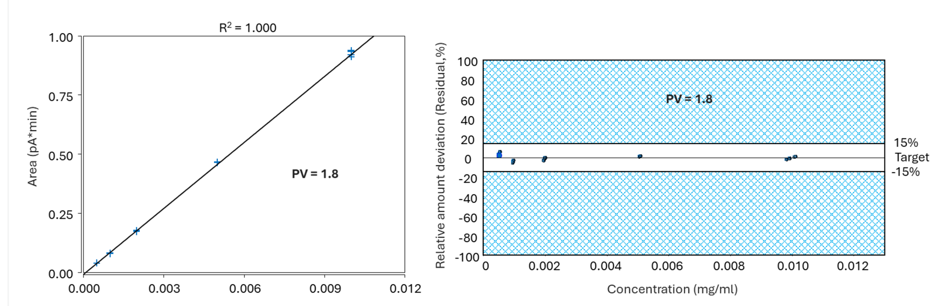


Figure 4: Calibration curve and residual plot showing the optimal PV at 1.8 for deoxycholic acid in Vanquish CAD HP.

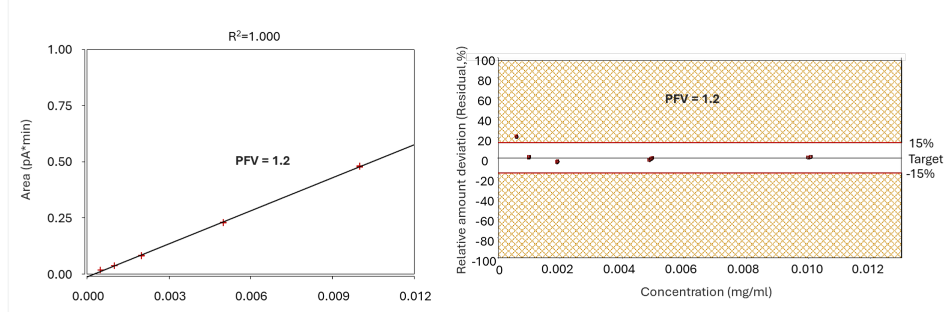


Figure 5: Calibration curve and residual plot showing the optimal PFV at 1.2 for deoxycholic acid in Vanquish CAD.

Optimizing evaporation temperature and signal filtering for improved sensitivity and signal quality

Evaporation temperature (EvapT) and signal filtering are critical parameters in optimizing CAD performance during method transfer. Both CAD and CAD HP support EvapT settings from ambient (+5℃) to 100℃; 50℃ is recommended for this application to ensure stable S/N ratios while minimizing analyte loss and noise. Signal filter settings were evaluated at 0.25 µg/mL using a 20 Hz data rate and 50℃ EvapT. A digital filter at 5s provided the best balance of noise reduction, peak shape, and resolution. This setting is also the default when "link data collection parameters" is enabled (**Figure 6**), streamlining setup.

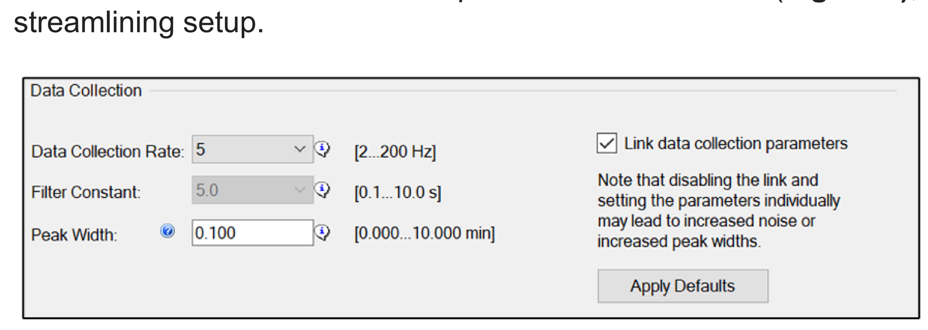


Figure 6: Data collection settings (e.g. filter constant) in Vanquish CAD P series.

Sensitivity comparison between Vanquish CAD HP and Vanquish CAD demonstrated by accelerated USP method for deoxycholic acid and cholic acid

Sensitivity is a key parameter in analytical detection, indicating the lowest concentration of an analyte that can be reliably detected (LOD) and accurately quantified (LOQ). To demonstrate the sensitivity of the Vanquish CAD HP, an accelerated USP method was used as shown in **Table 3**. The outcome is that the Vanquish CAD HP detector demonstrates greater sensitivity than the earlier generation Vanquish CAD. This becomes obvious due to the higher peak responses for both analytes (**Figure 7**) and the related numeric results for LOD and LOQ (**Table 4**).

Table 3. Chromatographic conditions of accelerated USP method by applying the method speed up tool integrated in Chromeleon

Column	Thermo Scientific™ Hypersil GOLD™ C18 2.1 × 50 mm, 1.9 µm column (P/N 052130)
Solvent A	0.1% (v/v) formic acid in water
Solvent B	0.1% (v/v) formic acid in acetonitrile
Gradient	

Time [min]	B [%]
0.000	25.0
0.141	45.0
0.985	58.0
1.689	100.0
2.463	100.0
2.463	25.0
2.674	25.0
5.000	25.0

Flow rate	1.2 mL/min
Column temperature	60℃, Still Air mode, 60℃ active pre-heater
Autosampler temperature	8℃
Injection volume	2.39 µL
CAD Settings	**Channel start settings** **PV (Vanquish CAD HP)**: CAD_1 = 1.5, CAD_2 = 1.8, CAD_3 = 2.1, CAD_4 = 2.4 **PFV (Vanquish CAD)**: CAD_1 = 1.2
	Data collection rate: 50 Hz, **Filter settings:** 1.0 s, **Gas regulation mode:** Analytical, **Evaporation temperature:** 50℃

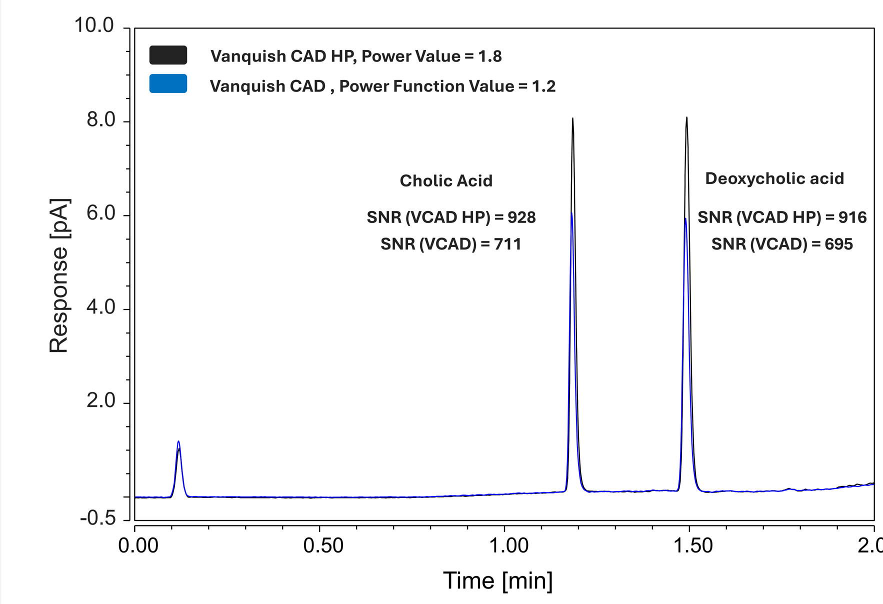


Figure 7: Chromatogram of deoxycholine and choline from Vanquish CAD HP and CAD

Table 4. Sensitivity comparison of Vanquish CAD HP and CAD detectors

Detector type	Analytes	LOD (µg/ml)	LOQ (µg/ml)
Vanquish CAD HP	Deoxycholic Acid	0.3	1.1
	Cholic Acid	0.2	1.0
Vanquish CAD	Deoxycholic Acid	0.4	1.4
	Cholic Acid	0.3	1.2

Conclusions

This study highlights the successful optimization and method transfer of the USP-NF deoxycholic acid assay using Vanquish CAD detectors. Through careful evaluation of chromatographic performance, detector settings, and calibration parameters, the Vanquish CAD HP was shown to deliver enhanced performance across multiple criteria:

Key Takeaways:

- **Excellent Sensitivity and Response:**
  Vanquish CAD HP demonstrated significantly lower LOD/LOQ values and higher peak responses compared to Vanquish CAD, making it the detector of choice for low-level analyte quantification.
- **Enhanced method optimization Flexibility:**
  Vanquish CAD HP allows simultaneous data collection for multiple PVs, ensuring faster method development and optimization.
- **Seamless Method Transfer:**
  Transitioning from Vanquish CAD to CAD HP was achieved by optimizing power value (PV), EvapT, and signal filter settings, ensuring improved performance.

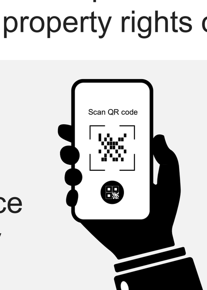
Together, these findings demonstrate that the Vanquish CAD HP not only supports efficient method transfer but also elevates performance, sensitivity, and usability—making it an ideal solution for modern HPLC applications.

References

1. USP, "USP-NF Deoxycholic Acid," The United States Pharmacopeial Convention, Rockville, MD, 2024.
2. B. Bailey, P. Gamache, I. Acworth and C. M. U. Thermo Fisher Scientific, "Guidelines for method transfer and optimization—from earlier model Corona detectors to Corona Veo and Vanquish charged aerosol detectors," Thermo Fisher Scientific, Chelmsford, MA, USA, 2017.
3. P. Gamache, T. Muellner, B. Eggart, K. Lovejoy and I. Acworth, "Charged aerosol detection – use of the power function and robust calibration practices to achieve the best quantitative results," Thermo Fisher Scientific TN 73299, 2019.

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.



Learn more at thermofisher.com/cadpseries