

Pharma

Simplifying detector modernization: Transferring a validated USP monograph method for deoxycholic acid across generations of charged aerosol detectors

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Keywords

United States Pharmacopeia (USP),
deoxycholic acid, charged aerosol
detector (CAD), assay, organic
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ingredient (API), quality control (QC)

Application benefits

- **Confident detector modernization** through transfer of a validated USP monograph method for deoxycholic acid from a Thermo Scientific™ Corona™ Veo™ RS Detector to a Thermo Scientific™ Vanquish™ Charged Aerosol Detector HP
- **Method continuity in regulated laboratories**, supported by confirmed USP system suitability requirements and comparable analytical performance across detector generations
- **Reduced method transfer effort and manual setup risk** using automated method translation functionality in the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS)

Goal

Demonstrate seamless transfer of a validated USP monograph method for deoxycholic acid from the Corona Veo RS Detector to the Vanquish Charged Aerosol Detector HP while maintaining system suitability and analytical performance.

Introduction

Although compendial methods are intended for long-term use, analytical instrumentation continues to evolve. As laboratories modernize aging systems, a reliable and compliant transfer of established methods to new generations of instruments is essential to ensure continuity of analytical workflows and regulatory confidence.

The United States Pharmacopeial (USP) monograph for deoxycholic acid, USP 40-NF 35,¹ describes a high-performance liquid chromatography (HPLC) method using charged aerosol detection for assay and organic impurity analysis. Deoxycholic acid purity assessment (assay) and impurity profiling (organic impurities) of deoxycholic acid is an important part of pharmaceutical quality control. HPLC with charged aerosol detection is well suited for this application because it enables detection of analytes that lack strong UV chromophores.

Transferring an established compendial method between charged aerosol detectors (CADs) of different generations can be challenging because some detector settings directly affect peak response, linearity, and, therefore, quantitation results. Careful alignment of parameters such as the evaporation temperature, signal filtering, and the applied power function is essential to ensure consistent method performance following method transfer.

This application note demonstrates a structured method transfer workflow from the Corona Veo RS Detector to the Vanquish CAD HP for the USP-NF deoxycholic acid HPLC-CAD method. By confirming system suitability and analytical performance after transfer, the study provides a practical approach for detector modernization while supporting method continuity in regulated pharmaceutical laboratories (Figure 1). This work builds on previously published method transfer studies between generations of CADs and further demonstrates the continuity of analytical performance across evolving CAD platforms.^{2,3}

Experimental

Chemicals

- Deionized water, 18.2 MΩ.cm, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification (Cat. No. 50136149)
- Fisher Chemical™ Formic acid (FA), Optima™ LC/MS grade (Cat. No. A117)
- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (Cat. No. A955)
- Fisher Scientific™ Methanol, Optima™ LC/MS grade (Cat. No. A456)
- Sigma-Aldrich Deoxycholic acid, USP Reference Standard grade (Cat. No. 1171273)
- Thermo Scientific™ Chemicals Deoxycholic acid. “For research and development use only” (Cat. No. 10616732)

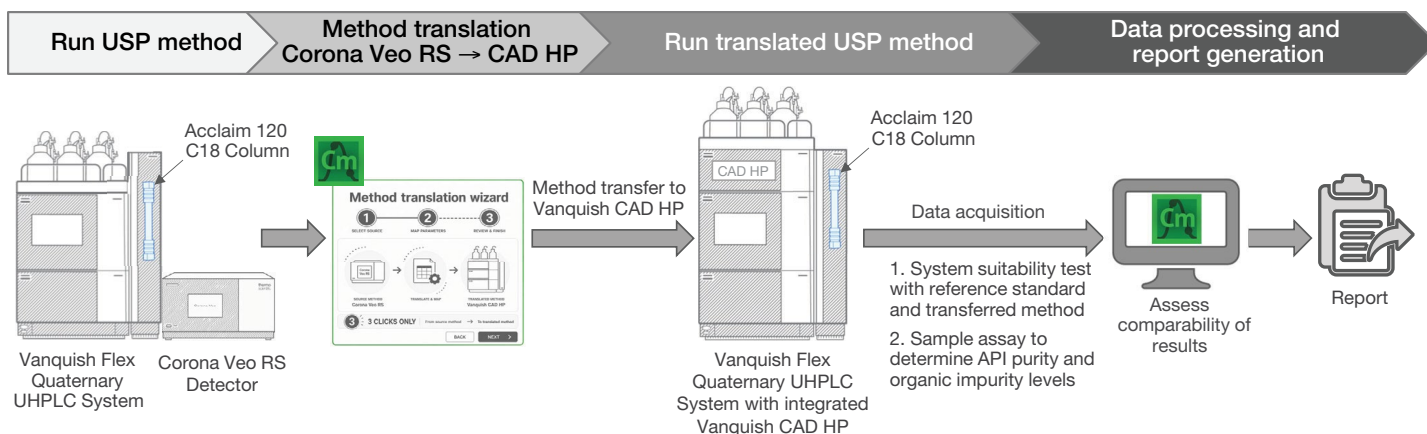


Figure 1. Summary of the applied workflow for the transfer of the USP method for deoxycholic acid for assay and organic impurities¹ from the Corona Veo RS Detector to the Vanquish CAD HP.

Sample handling

- Thermo Scientific™ Finpipette™ F2 Variable Volume Single-Channel Pipette: 100–1,000 µL (Cat. No. 4642090)
- Thermo Scientific™ Finpipette™ F2 Variable Volume Single-Channel Pipette: 10–100 µL (Cat. No. 4642070)
- Thermo Scientific™ Finpipette™ F2 Variable Volume Single-Channel Pipette: 1–10 µL (Cat. No. 4642030)
- Fisherbrand™ Mini Vortex Mixer (Cat. No. 14-955-152)
- Thermo Scientific™ SureSTART™ 2 mL Amber Glass Short Thread Screw Top Vials, 100/pack, Level 2 (Cat. No. 6ASV9-2P)
- Thermo Scientific™ SureSTART™ Blue Polypropylene 9 mm AVCS™ Screw Caps with Soft Blue Silicone/Clear PTFE Septa, 100/pack, Level 3 (Cat. No. 6PSC9ST101)

The Fisher Scientific product codes can be unique to different countries; the codes given above should be compatible across the EU and USA.

Instrumentation

Thermo Scientific™ Vanquish™ Flex UHPLC System consisting of:

- Thermo Scientific™ Vanquish™ System Base (Cat. No. VF-S01-A-03)
- Thermo Scientific™ Vanquish™ Quaternary Pump F (Cat. No. VF-P20-A)
- Thermo Scientific™ Vanquish™ Split Sampler FT (Cat. No. VF-A10-A-03)
- Thermo Scientific™ Vanquish™ Column Compartment H (Cat. No. VH-C10-A-03)
- Thermo Scientific™ Vanquish™ Charged Aerosol Detector HP (Cat. No. VH-D21-A-01)
- Thermo Scientific™ Corona™ Veo™ RS Detector (Cat. No. 5081.0020)

Standard and sample preparation

Two sample types were used in this study: a USP reference standard for system suitability testing and calibration, and a commercial sample for assay and organic impurity analysis according to the USP monograph for deoxycholic acid. For each sample type, a 1 mg/mL stock solution was prepared in 80/20 (v/v) methanol/water (diluent) by transferring 10 mg into a 10 mL volumetric flask and filling to the line.

Working solutions were then prepared at 0.01 mg/mL by transferring 1 mL of each stock solution into a separate 100 mL volumetric flask and filling to the line with diluent. In addition, a USP reference standard solution with 0.005 mg/mL was prepared by diluting 50 µL of the 0.01 mg/mL USP working solution with 950 µL of diluent.

Calibration solutions at 0.01, 0.005, 0.002, 0.001, and 0.0005 mg/mL were prepared by serial dilution from the 1 mg/mL USP stock solution using 100 mL volumetric flasks.

Chromatographic conditions

Table 1. Chromatographic conditions.

Parameter	Setting
Column	Thermo Scientific™ Acclaim™ 120 C18 4.6 × 150 mm, 3 µm (Cat. No. 059133)
Solvent A	0.1 % (v/v) FA in water
Solvent B	0.1 % (v/v) FA in acetonitrile
Gradient	Time [min] B [%]
	0.0 25
	2.0 45
	14.0 58
	24.0 100
	35.0 100
	35.0 25
	38.0 25
Flow rate	1 mL/min
Column temperature	30 °C (forced air mode) with active pre-heater at 30 °C
Autosampler temperature	6 °C
Needle wash solution	80/20 (v/v) water/methanol
Needle wash mode	Both
Injection volume	25 µL
Detector settings	PFV Corona Veo RS: 1.2 PV Vanquish CAD HP: 1.8 Data collection rate: 20 Hz Filter setting: 5 s EvapT: 50 °C

Chromatography Data System

The Chromeleon 7.3.2 CDS was used for data acquisition and analysis.

Results and discussion

The USP monograph method for assay and organic impurity profiling of deoxycholic acid was transferred from a Corona Veo RS Detector to a Vanquish CAD HP while maintaining the same HPLC configuration. System suitability testing was performed to confirm compliance with USP requirements. In addition, critical analytical performance characteristics were evaluated to verify that the method delivers comparable performance across two generations of Thermo Scientific™ Charged Aerosol Detectors.

Method translation from Corona Veo RS Detector to Vanquish CAD HP

The method translation wizard in Chromeleon CDS enables automated translation of a method from the Corona Veo RS Detector to the Vanquish CAD HP in only three clicks, as shown in Figure 2. Differences between the detector generations, including parameter nomenclature, units, and technical settings, are automatically recognized and converted to the corresponding format during the translation process. Upon completion, the method is immediately ready to run on the Vanquish CAD HP without additional manual adjustments of the translated detector parameters. This automated translation process

simplifies detector modernization by reducing the risk of manual transcription errors and supporting consistent method setup during transfer.

Analytical performance verification

Following method translation to the Vanquish CAD HP configuration, a system suitability test (SST) was performed in accordance with the USP monograph. The USP working standard solution at 0.01 mg/mL was injected six times to determine the relative standard deviation (RSD) of the deoxycholic acid peak area. In addition, the USP reference standard solution at 0.005 mg/mL was injected six times to determine the signal-to-noise (S/N) ratio. The results in Table 2 demonstrate that the transferred method met both USP SST requirements.

Table 2. Results of system suitability testing after method transfer to Vanquish CAD HP. The respective standard solution was injected six times. The USP requirements of RSD peak area and S/N ratio passed.

USP SST requirement	Vanquish CAD HP result	Pass /Fail
RSD peak area ≤ 3%	0.89	PASS
S/N ratio ≥ 10	40	PASS

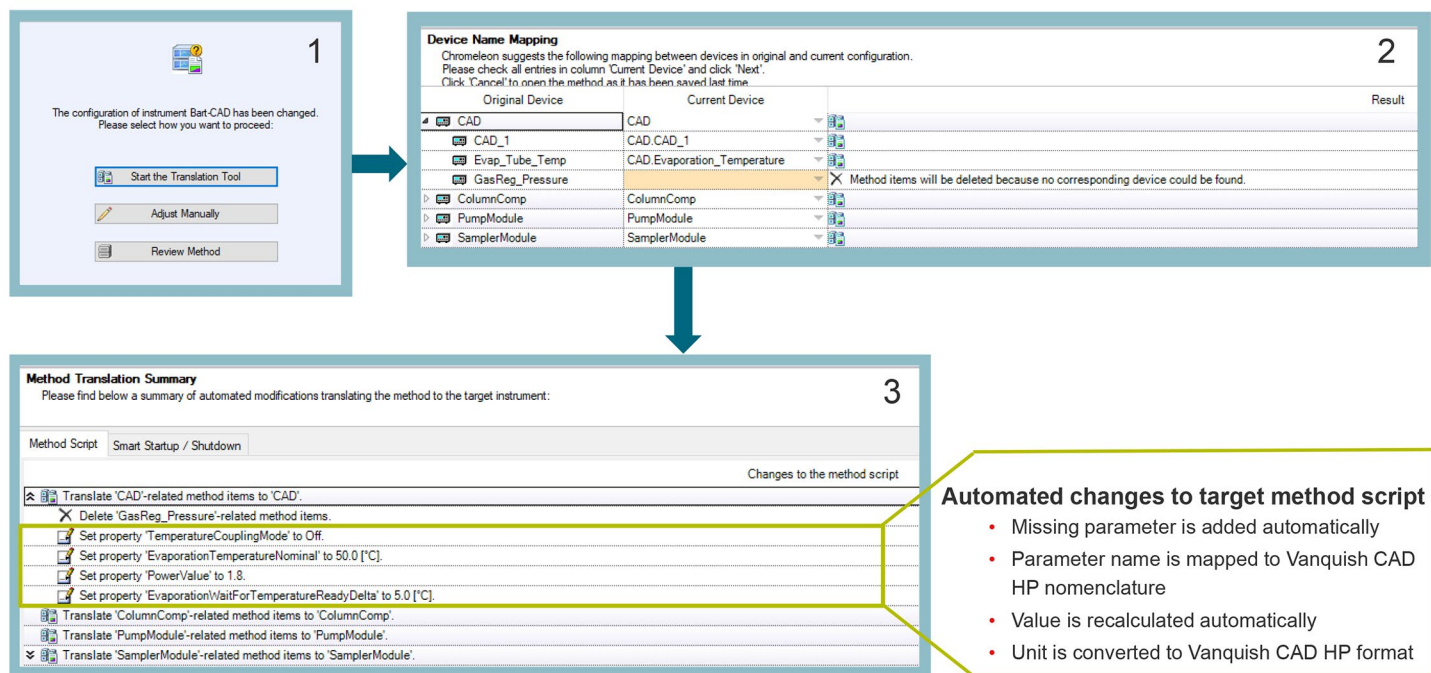


Figure 2. Three-click automated method translation from Corona Veo RS Detector (source device) to Vanquish CAD HP (target device) using the method translation wizard in Chromeleon CDS. The CDS detects the required detector translations (1), suggests source-to-target device mapping (2), and provides a translation summary (3), including added parameters, updated nomenclature, recalculated values, and unit conversions.

The Corona Veo RS Detector method uses the power function value (PFV), but the equivalent setting in the Vanquish CAD HP is termed power value (PV). Both parameters are designed to linearize the detector response across the desired quantitation range. The automatic method translation correctly converts this parameter, enabling equivalent analytical performance after transfer. As illustrated in Figure 3, both detectors showed excellent linearity, with R^2 values of 0.99981 and 0.99985, respectively. These results confirm that detector response linearization was successfully maintained after transfer from the Corona Veo RS Detector to the Vanquish CAD HP.

Assay and organic impurities determination for deoxycholic acid sample

The assay value and organic impurity profile of deoxycholic acid were assessed in a commercially available sample. Following the USP monograph, a 0.01 mg/mL sample solution was used for the assay, whereas a 1 mg/mL sample solution was used for determination of impurity levels.

Figure 4 presents the chromatogram of the 1 mg/mL sample solution obtained using the Vanquish CAD HP method, and Table 3 lists the relative retention times (RRTs) of the known and unknown impurities detected in the sample. Comparable chromatographic behavior was observed for both detectors, with the same RRTs obtained for all compounds. The results demonstrate successful method transfer while supporting compliance with USP requirements.

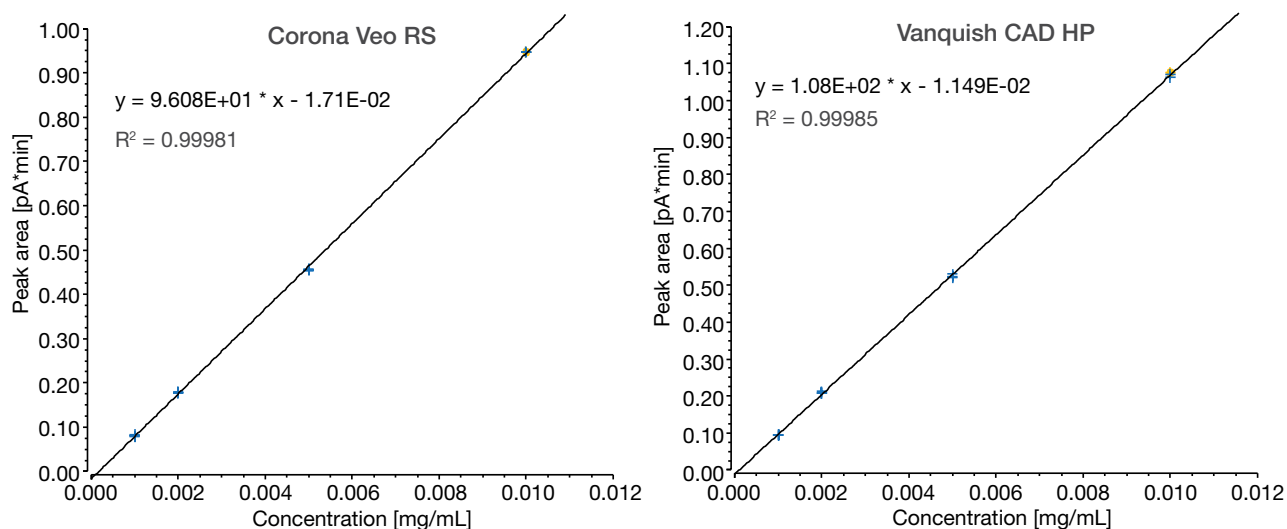


Figure 3. Calibration curves for deoxycholic acid obtained with the Corona Veo RS Detector (left) and the Vanquish CAD HP (right). Equivalent detector response linearization was achieved using a PFV of 1.2 for the Corona Veo RS Detector and a PV of 1.8 for the Vanquish CAD HP.

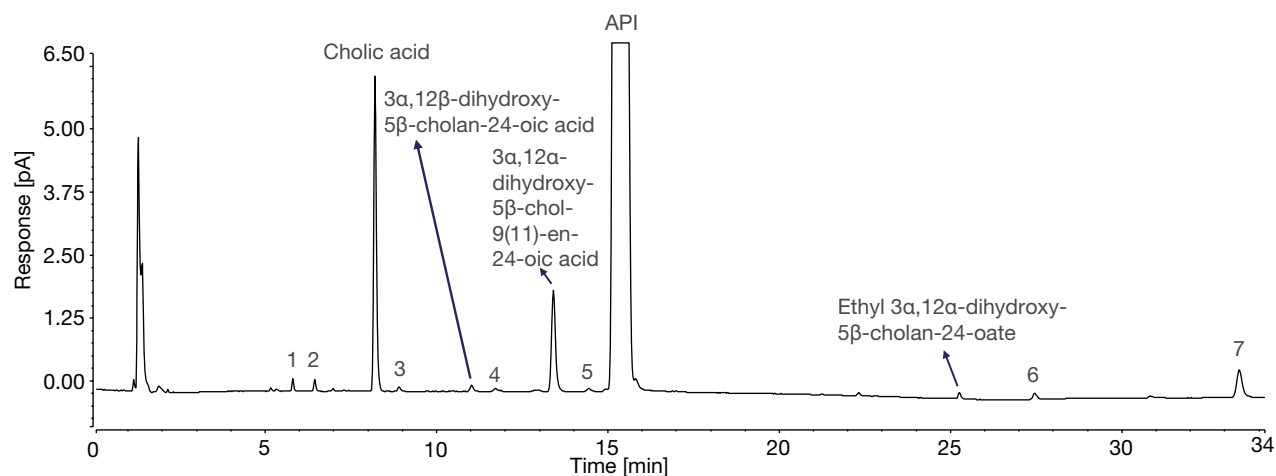


Figure 4. Chromatogram of the 1 mg/mL deoxycholic acid sample solution obtained for the Vanquish CAD HP method. Known impurities are labelled with compound name, while unknown impurities are marked by numbers. Only impurities above a S/N ratio of 10 were labelled and considered for further investigation.

Table 3. Summary of RRTs for known and unknown impurities for deoxycholic acid. The same RRTs were obtained for all compounds with both detectors.

Compound name	RRT Corona Veo RS	RRT Vanquish CAD HP	USP requirement
Cholic acid	0.53	0.53	0.56
3 α ,12 β -dihydroxy-5 β -cholan-24-oic acid	0.71	0.71	0.69
3 α ,12 α -dihydroxy-5 β -chol-9(11)-en-24-oic acid	0.87	0.87	0.87
Ethyl 3 α ,12 α -dihydroxy-5 β -cholan-24-oate	1.64	1.64	1.61
Unknown impurity 1	0.38	0.38	-
Unknown impurity 2	0.42	0.42	-
Unknown impurity 3	0.58	0.58	-
Unknown impurity 4	0.76	0.76	-
Unknown impurity 5	0.94	0.94	-
Unknown impurity 6	1.78	1.78	-
Unknown impurity 7	2.17	2.17	-

Table 4 and Table 5 summarize the assay and organic impurity level results of the investigated commercial sample obtained with the Corona Veo RS Detector and the Vanquish CAD HP, respectively. The determined deoxycholic acid content was significantly below the USP specification of 97%–103% for a pharmaceutical-grade active pharmaceutical ingredient (API). Given that the product was labelled “For research and development use only,” this finding was expected. Results obtained with both detectors were in close agreement and confirmed that the sample failed to meet the purity acceptance criteria defined by the USP monograph.

Regarding impurity determination, both detectors detected the same four known impurities and seven unknown impurities in

the sample. The level of 3 α ,12 α -dihydroxy-5 β -chol-9(11)-en-24-oic acid exceeded the USP acceptance criteria of ≤ 0.15 for a pharmaceutical-grade API, further confirming that the sample does not meet the quality requirements for pharmaceutical use.

The Vanquish CAD HP produced slightly higher signal responses than the Corona Veo RS Detector, which was reflected in the calculated levels of total unknown impurities (0.15% and 0.22%, respectively) and total all impurities (1.14% and 1.24%, respectively). Despite these small differences, the total impurity levels determined by both detectors remained well below the USP acceptance limit of 2.0%, demonstrating comparable impurity profiling performance after method transfer.

Table 4. Calculated deoxycholic acid content and organic impurity levels obtained with the Corona Veo RS Detector method for a commercially available sample labelled as “For research and development use only”. The sample failed the USP assay specification and exceeded certain impurity levels (red numbers), confirming that it is not suitable for use as a pharmaceutical-grade API.

Compound name	Amount [%] Corona Veo RS	USP acceptance criteria [%]
Assay		
Deoxycholic acid	81	97–103
Organic impurities		
Cholic acid	0.65	-
3 α ,12 β -Dihydroxy-5 β -cholan-24-oic acid	0.02	≤ 0.15
3 α ,12 α -Dihydroxy-5 β -chol-9(11)-en-24-oic acid	0.31	≤ 0.15
Ethyl 3 α ,12 α -dihydroxy-5 β -cholan-24-oate	0.01	≤ 0.15
Total unknown impurities	0.15	-
Total all impurities	1.14	≤ 2.0

Table 5. Calculated deoxycholic acid content and organic impurity levels obtained with the Vanquish CAD HP method for a commercially available sample labelled as “For research and development use only”. The sample failed the USP assay specification and exceeded certain impurity levels (red numbers), confirming that it is not suitable for use as a pharmaceutical-grade API.

Compound name	Amount [%] Vanquish CAD HP	USP requirement [%]
Assay		
Deoxycholic acid	79	97–103
Organic impurities		
Cholic acid	0.67	-
3 α ,12 β -Dihydroxy-5 β -cholan-24-oic acid	0.02	≤ 0.15
3 α ,12 α -Dihydroxy-5 β -chol-9(11)-en-24-oic acid	0.32	≤ 0.15
Ethyl 3 α ,12 α -dihydroxy-5 β -cholan-24-oate	0.01	≤ 0.15
Total unknown impurities	0.22	-
Total all impurities	1.24	≤ 2.0

Conclusion

In this study, we have demonstrated the successful transfer of the USP monograph method for assay and organic impurity profiling of deoxycholic acid from a Corona Veo RS Detector to a Vanquish CAD HP. The transfer was supported by the Chromeleon CDS method translation wizard, a functionality that automates and simplifies method translation steps.

The following key results were achieved:

- **USP system suitability requirements were met**, achieving an RSD of peak area of 0.89% and an S/N ratio of 40 on the Vanquish CAD HP.
- **Comparable linear performance was achieved on both detector generations**, with nearly identical calibration behavior and coefficient of determination (R^2) obtained after automatic conversion of the Corona Veo RS power function value setting to the corresponding Vanquish CAD HP power value setting.
- **Comparable chromatographic performance was demonstrated**, with identical relative retention times observed for all four known impurities and seven unknown impurities.
- **Assay and impurity profiling results were in close agreement between the two detectors** and led to the same conclusion: the investigated sample did not meet the USP purity specifications for a pharmaceutical-grade API.

Overall, the results support a confident modernization from the Corona Veo RS Detector to the Vanquish CAD HP, demonstrating method continuity and consistent analytical performance for routine analysis of deoxycholic acid.

References

1. United States Pharmacopeia (USP) Deoxycholic Acid Monograph, USP 40–NF 35 – Assay and Organic Impurities, Official from August 1, **2017**.
2. Thermo Fisher Scientific Technical Note 003816: [Method transfer and optimization of deoxycholic acid analysis using HPLC-CAD](#), **2025**.
3. Thermo Fisher Scientific Application Note 72600: [Deoxycholic acid method transfer from the Corona ultra RS Charged Aerosol Detector to the Corona Veo \(or Vanquish\) Charged Aerosol Detector](#), **2018**.

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