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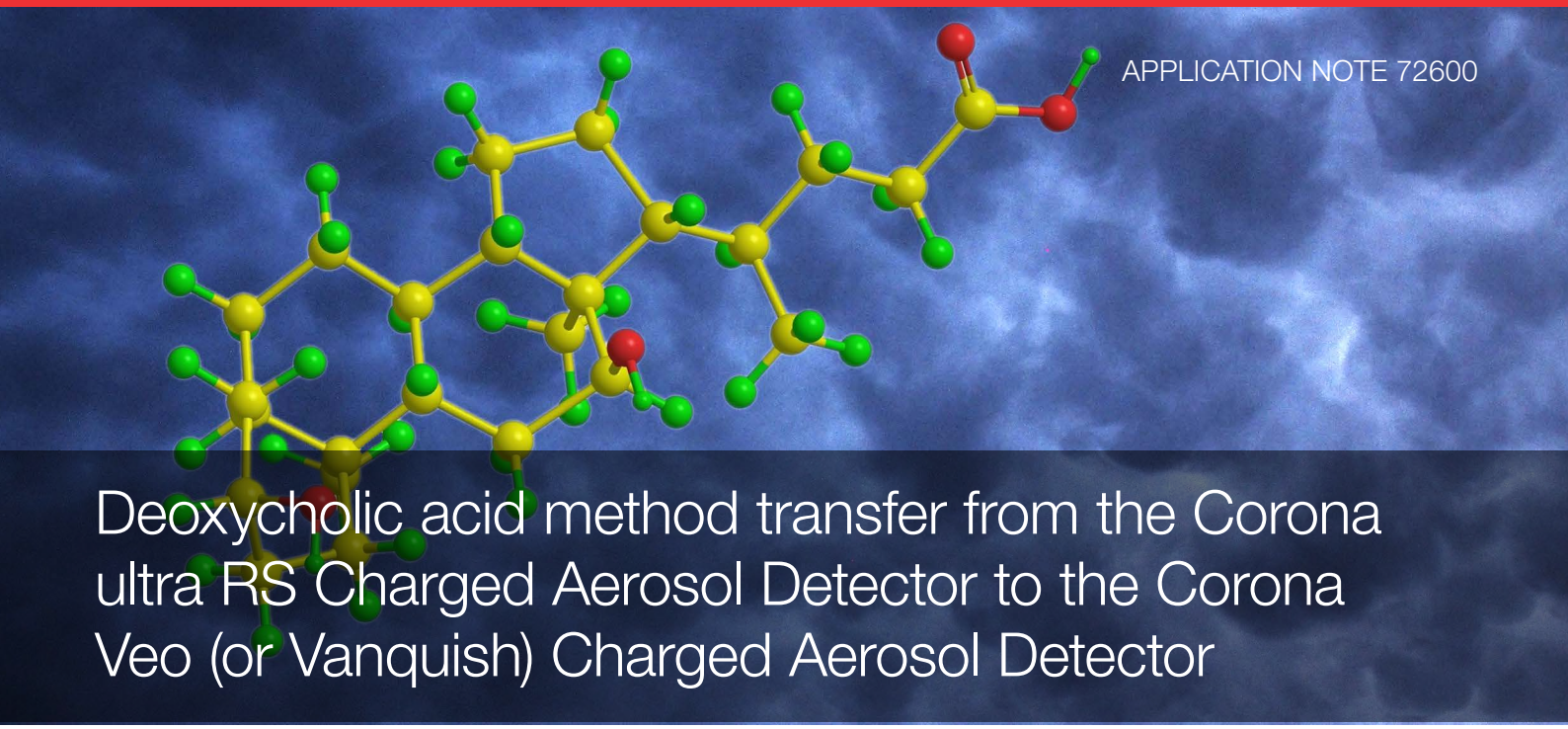

APPLICATION NOTE 72600

Deoxycholic acid method transfer from the Corona ultra RS Charged Aerosol Detector to the Corona Veo (or Vanquish) Charged Aerosol Detector

Authors

[1]Katherine Lovejoy, [2]Paul Gamache, [1]Tibor Muellner, and [2]Ian Acworth

[1]Thermo Fisher Scientific, Germering, Germany

[2]Thermo Fisher Scientific, Chelmsford, MA

Keywords

United States Pharmacopoeia, desoxycholic acid, method transfer

Goal

To provide guidance for transferring the United States Pharmacopeia (USP) Monograph method for deoxycholic acid, also known as desoxycholic acid, from the Thermo Scientific™ Corona™ ultra RS™ Charged Aerosol Detector (CAD) to the Thermo Scientific™ Corona™ Veo™ Charged Aerosol Detector or Thermo Scientific™ Vanquish™ Charged Aerosol Detector (VCAD).

Introduction

The United States Pharmacopoeia (USP) monograph USP 40-NF 35 describes the use of an HPLC-CAD method for the measurement of both deoxycholic acid, its primary impurity, cholic acid (Figure 1), and several minor impurities. This application note replicates the original USP method, which used a Corona ultra RS CAD, and provides guidance for transfer of the method to the new generation Vanquish Flex CAD (VCAD), which is identical to the Corona Veo CAD.

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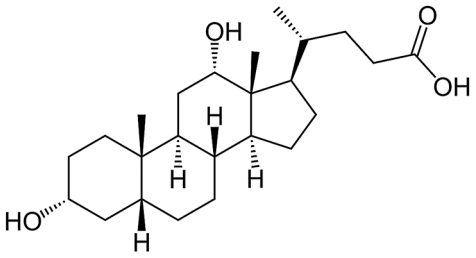
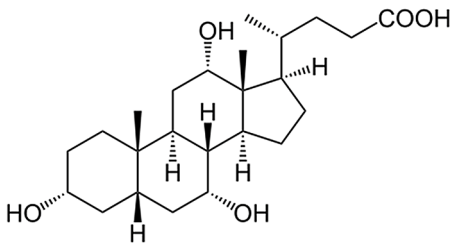


Deoxycholic acid



Cholic acid

Figure 1. The chemical structures of deoxycholic acid and cholic acid.

Experimental

Equipment

Chromatographic separation was performed on a Thermo Scientific Vanquish Flex Quaternary UHPLC system including:

- System Base Vanquish Flex (P/N VF-S01-A)
- Quaternary Pump F (P/N VF-P20-A)
- Split Sampler FT (P/N VF-A10-A)
- Column Compartment (P/N VH-C10-A)
- Thermo Scientific™ Chromeleon™ Chromatography Data System Software 7.2 SR 5

and either

- Vanquish Charged Aerosol Detector F with concentric flow nebulizer (P/N VF-D20-A, identical to Corona Veo Charged Aerosol Detector, P/N 5081.0010)

or

- Corona ultra RS Charged Aerosol Detector (P/N 70-9406), no longer sold

Reagents and standards

- Acetonitrile, Fisher Scientific™ LC-MS grade (P/N A/0638/17)
- Formic acid, Acros Organics™, 99% for analysis grade (P/N 270480010)
- Water, Ultra-pure (18.2 MΩ·cm at 25 °C) from a Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure (P/N 50136171) Water Purification System
- Cholic acid, Sigma-Aldrich®, USP Reference Standard grade (P/N 1133503)
- Deoxycholic acid, Sigma-Aldrich, USP Reference Standard grade (P/N 1171273)

Conditions

Column:	Thermo Scientific™ Acclaim™ 120 C18*, 4.6 × 150 mm, 3 µm (P/N 059133)		
Mobile Phase A:	0.1% (v/v) Formic acid in water		
Mobile Phase B:	0.1% (v/v) Formic acid in acetonitrile		
Gradient Profile:	*Time (min)*	*% A*	*% B*
	0.0	75.0	25.0
	2.0	55.0	45.0
	14.0	42.0	58.0
	24.0	0.0	100.0
	35.0	0.0	100.0
	35.0	75.0	25.0
	38.0	75.0	25.0
Flow Rate:	1.0 mL/min		
Column Temp.:	30 °C, forced air mode, 30 °C active pre-heater		
Inj. Volume:	25 µL		
Corona ultra RS CAD:	PFV = 1.00; Filter = 3 s; Neb. Temp. = On, 25 °C		
Corona Veo CAD/VCAD:	PFV = 1.20; Filter = 5 s; Evap T = 50 °C		

The USP column requirement is for a 4.6 × 150 mm column with 3 µm particle size of type L1, which is fulfilled by the Acclaim 120 C18 4.6 × 150 mm column with 3 µm particle size.

Preparation of solutions and reagents

Mobile phase preparation

- Mobile phase A: 1 L of 0.1% aqueous formic acid was prepared by adding 1 mL of formic acid to 1 L ultrapure water in a 1 L graduated cylinder.
- Mobile phase B: 1 L of 0.1% formic acid in acetonitrile was prepared by adding 1 mL of formic acid to 1 L acetonitrile in a 1 L graduated cylinder.

Stock standard solutions

Samples were prepared as 1 mg/mL stock solutions in the diluent, 80/20 methanol/water, by adding 10 mg of the sample or reference standard to a 10 mL volumetric flask and filling to the line with diluent. The diluent was prepared by adding 800 mL methanol to 200 mL water.

Working standard solutions

The working standard solutions were prepared as 0.01 mg/mL by adding 1 mL of the stock standard solution to a 100 mL volumetric flask and filling to the line with diluent. The 0.01 mg/mL concentration is 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.

Results and discussion

System suitability

Figure 2 shows the separation of deoxycholic acid and cholic acid standards using the Acclaim 120 C18 column. Both peaks were well separated and easily quantified. Deoxycholic acid elutes at 15.8 min, which is slightly later than the retention time stated in the USP monograph of "about 13.0 min"; the relative retention time of cholic acid of 0.54 min closely matches the USP-given value of 0.56 min. Neither of these values are required for system suitability, however. The system suitability test requires a %RSD for the signal area of not more than 3.0% for a 0.01 mg/mL solution and a signal-to-noise ratio (SNR) of not less than 10 for a 0.0005 mg/mL solution. As shown in Table 1, both the Corona ultra RS CAD and the Corona Veo CAD/VCAD are suitable for assaying deoxycholic acid and its organic impurities using USP 40-NF 35.

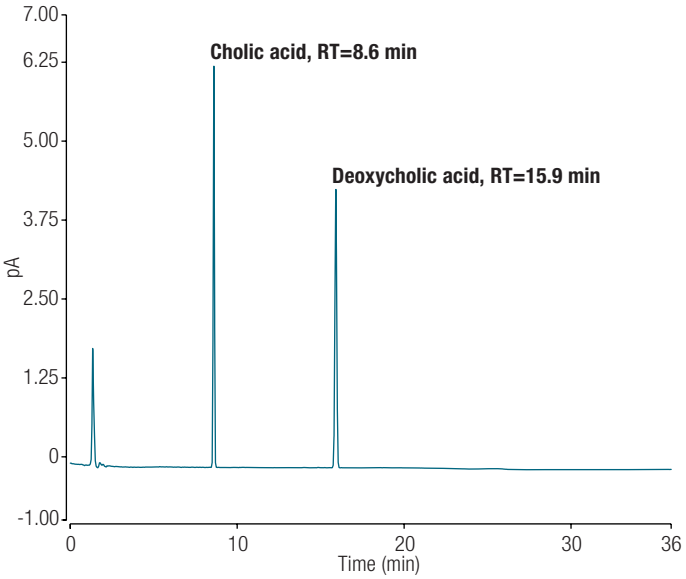


Figure 2. Chromatogram of 0.01 mg/mL cholic acid and 0.01 mg/mL deoxycholic acid.

Method transfer (from Corona ultra RS CAD to Corona Veo CAD/VCAD)

Technical Note 157[1] and Chapter 3 of Charged Aerosol Detection for Liquid Chromatography and Related Separation Techniques[2] were used to provide guidance for method transfer from the Corona ultra RS CAD to the Corona Veo CAD/VCAD. Data acquisition parameters were optimized in the following sequence.

Power Function Value

The first data acquisition parameter that should be optimized is the Power Function Value (PFV). The PFV is used to help linearize the signal output of the CAD over the desired range of quantitation so that SNR is a more accurate measure of sensitivity limits and peak shape is a more accurate measure of chromatographic performance.[4] When evaluating changes in PFV, it is very important to study its effects on response for low levels of analyte and to choose the best curve fit model. Several different PFVs were evaluated including 1.0, 1.10, 1.15, 1.20, and 1.30. The PFV of 1.2 produced the best calibration curve based on a robust evaluation of goodness of fit.

Table 1. Results of system suitability testing.

	Corona ultra RS CAD	Corona Veo CAD/VCAD	USP Requirement
%RSD of area	0.28% (mean, N = 6)	0.63% (mean, N = 6)	< 3.0%
S/N ratio	32 (lowest value of three injections)	42 (lowest value of three injections)	> 10

Evaporation Temperature

There is little or no relationship between the nebulizer temperature (Nebulizer T) setting on the Corona ultra RS detector and the evaporation temperature (Evap T) setting on the Corona Veo CAD/VCAD detector. The Nebulizer T setting is used to prevent freezing of the nebulizer due to evaporative cooling that occurs with highly volatile solvents. It has limited use as a method control variable. The Evap T setting on the Corona Veo CAD/VCAD is an important method parameter enabling greater analytical flexibility. However, the correct choice of Evap T is essential. A low Evap T has the advantage of producing more uniform response between analytes, and the accompanying reduction in selectivity enables the measurement of a broader range of analytes. However, it can be associated with increased noise due to greater contribution from semivolatile impurities. A higher Evap T, on the other hand, is associated with decreased noise, but as more analytes behave as semivolatiles, there may be a loss of signal, especially when measuring low levels. As part of the method transfer, three different Evap Ts were evaluated – 35, 50 and 70 °C. Although an Evap T of 70 °C produced the highest SNR for both deoxycholic acid (SNR = 14 for 0.25 µg/mL) and cholic acid (SNR = 20), due to the concern that it could have an adverse effect on sensitivity for other impurities, an Evap T of 50 °C was chosen as a compromise. At 50 °C the SNR for deoxycholic acid and cholic acid at 0.25 µg/mL, a lower level than the USP-required LOD of 0.5 µg/mL, were 8 and 15, respectively. The background noise was 0.012 pA for all three Evap Ts evaluated.

Signal filter

Several different digital filter settings were evaluated (2, 3.6, 5, and 10 s). The 5 s filter was chosen because it showed a slightly better SNR of 14 for deoxycholic acid at a concentration of 0.25 µg/mL. The SNR was about 10 for the other filter settings.

Method Performance

Using PFV = 1.20, Evap T = 50 °C, and a filter of 5 s, the Corona Veo CAD/VCAD met USP criteria for precision (for 10 µg/mL deoxycholic acid, N = 6, %RSD = 0.63%); and LOD (SNR = 42 for 0.5 µg/mL deoxycholic acid).

Linearity

For all experiments, a linear plot weighted by 1/area^2 was used.[2] Because larger concentrations show larger deviations and therefore have a greater influence on the linear regression line, weighting is necessary to ensure that every concentration is equally well represented by the calibration curve. This model was chosen by following the FDA's guidelines for validation of bioanalytical methods, which require "applying the simplest model that adequately describes the concentration-response relationship using appropriate weighting and statistical tests [such as the residual plot shown in Figure 3] for goodness of fit."[3]

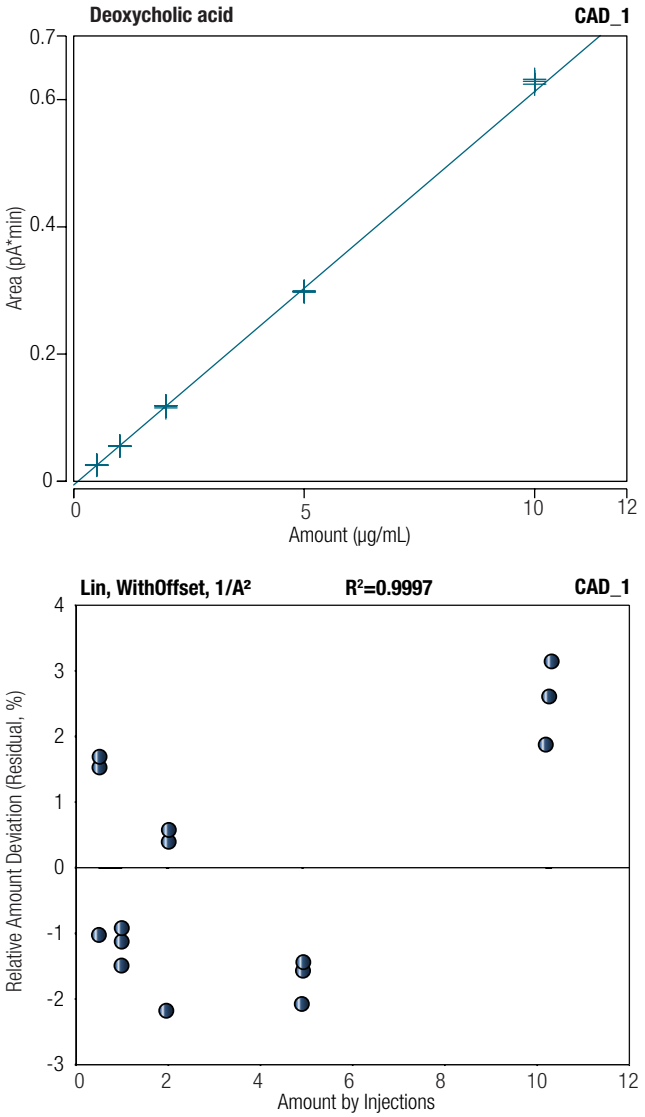


Figure 3. Calibration curve and residual plot for deoxycholic acid using a power function value of 1.20.

Robustness

No adverse effects were found (e.g., on retention time, peak shape, or quantitative accuracy) when doubling the injection volume of a 0.001 mg/mL sample of deoxycholic acid.

Quantification of deoxycholic acid

The percentage of deoxycholic acid in the portion of deoxycholic acid taken was calculated according to the following equation from the USP monograph:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P$$

r_U = peak area of deoxycholic acid from a 10 µg/mL sample solution

r_S = peak area of deoxycholic acid from a 10 µg/mL standard solution

C_S = concentration of the USP deoxycholic acid RS in the standard solution

C_U = concentration of deoxycholic acid in the sample solution

P= labeled purity of USP deoxycholic acid RS in %

As shown in Table 2, sample 1 met the acceptance criteria on the Corona Veo CAD/VCAD and on the Corona ultra RS CAD. Sample 2, a mixture of bile salts, did not meet the acceptance criteria on the Corona Veo CAD/VCAD or on the Corona ultra RS CAD. Sample 2 was found to contain approximately 50% deoxycholic acid by both the Corona Veo CAD/VCAD and Corona ultra RS CAD. These results are as expected because this was labeled as a mixture of equal parts cholic acid and deoxycholic acid. Subsequent quantification of impurities of this sample verified that the sample was 50% cholic acid.

Quantification of impurities

The percentage of each impurity in a commercial sample of deoxycholic acid, advertised as 98% pure, was calculated according to the following equation from the USP monograph and is shown in Table 3:

$$\text{Result} = [r_U / (r_S \times 100 + r_T)] \times 100$$

r_U = peak area of individual impurity from a 1 mg/mL sample stock solution of deoxycholic acid

r_S = peak area of deoxycholic acid from a 10 µg/mL sample solution

r_T = sum of peak areas of all impurities from the 1 mg/mL sample stock solution of deoxycholic acid

Table 2. Percentage of deoxycholic acid in 10 µg/mL samples.

Sample	Percentage Found, Corona Veo CAD/VCAD	Percentage Found, Corona ultra RS CAD	Acceptance Criteria
1	98.4%	98.7%	97.0–103.0%
2	50.3%	50.1%	97.0–103.0%

A sample with a stated purity level of 98% was analyzed for individual and total impurities using both a Corona Veo/VCAD (Table 3) and a Corona ultra RS CAD (Table 4). Both detectors produced nearly identical results for known impurities cholic acid, 3α,12β-dihydroxy-5β-cholan-24-oic acid, and 3α,12α-dihydroxy-5β-chol-9(11)-en-24-oic acid while a higher level (0.25%) of ethyl 3α,12α-dihydroxy-5β-cholan-24-oate was found with the Corona Veo/VCAD than with the Corona ultra RS CAD (0.14%). A possible unknown impurity was found only when using the Corona ultra RS CAD (relative retention time of 2.01) and contributed to a higher total impurity determination of 1.82% compared to 1.37% with the Corona Veo/VCAD. Further study is required to determine whether these differences are related to detection or other factors. Peaks of less than 0.10% total area summed to 0.40% of total area on the Corona ultra RS CAD and to 0.39% of total area on the Corona Veo CAD/VCAD. With both detectors, the total impurity level met the acceptance criteria of not more than 2% and confirms this sample's stated purity of 98%.

Table 3. Impurities found by the Corona Veo CAD/VCAD in a commercial sample of deoxycholic acid, advertised and verified to be 98% pure.

Impurity Name	Relative Retention Time (Actual)	Relative Retention Time (Compendial)	Acceptance Criteria NMT (%)	% Found	Pass / Fail
Cholic acid	0.52	0.56	1.0	0.10	Pass
3α,12β-Dihydroxy-5β-cholan-24-oic acid	0.72	0.69	0.15	0.13	Pass
3α,12α-Dihydroxy-5β-chol-9(11)-en-24-oic acid	0.88	0.87	0.15	0.26	Fail
Ethyl 3α,12α-dihydroxy-5β-cholan-24-oate	1.62	1.61	0.15	0.25	Fail
Impurity at 33.6 min	2.15	-	0.10	0.25	Fail
Total impurities	-	-	2.0	1.37	Pass

Table 4. Impurities found by the Corona ultra RS CAD in a commercial sample of deoxycholic acid, advertised and verified to be 98% pure.

Impurity Name	Relative Retention Time (Actual)	Relative Retention Time (Compendial)	Acceptance Criteria NMT (%)	% Found	Pass / Fail
Cholic acid	0.52	0.56	1.0	0.10	Pass
3α,12β-Dihydroxy-5β-cholan-24-oic acid	0.72	0.69	0.15	0.13	Pass
3α,12α-Dihydroxy-5β-chol-9(11)-en-24-oic acid	0.88	0.87	0.15	0.26	Fail
Ethyl 3α,12α-dihydroxy-5β-cholan-24-oate	1.62	1.61	0.15	0.14	Pass
Impurity at 31.4 min	2.01	-	0.10	0.41	Fail
Impurity at 33.6 min	2.15	-	0.10	0.39	Fail
Total impurities	-	-	2.0	1.82	Pass

Conclusion

As charged aerosol detection achieves increasing prominence in compendial methods, it gets increasingly important to provide guidelines for method transfer between detectors.

The USP Monograph (USP 40-NF 35) method for deoxycholic acid, originally developed with a Corona ultra RS detector, was easily transferred from the Corona ultra RS CAD to the Corona Veo CAD/VCAD charged aerosol detector. A standard method transfer procedure was followed, resulting in final Corona Veo CAD/VCAD parameters of PFV = 1.20, Evap T = 50 °C, and a filter of 5 s.

The performance of the Vanquish CAD (Corona Veo CAD) readily met the standard set by the Corona ultra RS CAD. The signal-to-noise ratio for the low-level standards was generally better on the Vanquish CAD (Corona Veo CAD) than on the Corona ultra RS CAD and peak area reproducibility was about the same. Both detectors easily satisfied the SNR and peak area reproducibility tests for system suitability specified in the USP compendial method.

Either instrument can be used to perform the USP compendial procedures for both content and impurity levels of deoxycholic acid.

References

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