thermoscientific

Charged Aerosol Detection and Method Transfer of Compendial, including USP, Methods

Mirko Pietsch, Katherine Lovejoy, Ian Acworth, Paul Gamache. Thermo Fisher Scientific, Dornierstr. 4, D-82110 Germering, Germany

INTRODUCTION

Purpose: To reproduce updated USP methods and provide guidance for method transfer guidance.

Methods: Two methods are presented in detail for drugs that lack chromophore; 1) analysis of amounts of organic impurities in metoprolol and 2) analysis of amount of deoxycholic acid and organic impurities

Results: Detectors most commonly used with HPLC systems include UV detectors and mass spectrometers. HPLC-based regulatory methods frequently rely on the UV detector because it is inexpensive and accurate. The charged aerosol detector is also increasingly being incorporated into regulatory methods because it is inexpensive, easy to use and offers universal detection and uniform response for all non-volatile analytes. This work shows two published United States Pharmacopoeia (USP) methods using the charged aerosol detector (CAD) and describes strategies for method transfer between detector models.

As part of the USP monograph modernization effort, a proposed change to the official USP Metoprolol Succinate monograph that was published in the Pharmacopeial Forum 41, Chapter 3 includes a method for the determination of organic impurities that lack UV chromophores. The older TLC method is replaced by a hydrophilic interaction chromatography (HILIC) method coupled with a CAD. An application note (1) replicates the updated USP method and related publication, both of which used older models of CAD, and provides guidance for transfer of the method to the new generation CAD.

The 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, and several minor impurities. An application note (2) replicates the original USP method, which used a Thermo Scientific™ Corona™ ultra RS Charged Aerosol Detector, and provides guidance for transfer of the method to the new generation Thermo Scientific™ Vanquish™ Flex CAD (VCAD), which is identical to the Thermo Scientific™ Corona™ Veo™ RS Charged Aerosol Detector.

BASIC CORONA VEO METHOD DEVELOPMENT

See Thermo Fisher Scientific Technical Note 157 (3)

- Run a calibration curve with 35 ˚C, power function 1.0, signal filter 5.0
- Optimize power function, look for linearity (optional)
- Run a standard with very low concentration
- Optimize evaporation temperature, based on S/N of that standard
- Optimize signal filter based on S/N of that standard
- Measure performance: linearity, precision, impurity quantification, robustness

Table 1. Filter setting conversions.

Corona CAD / CAD plus	Corona ultra	Corona ultra RS	Rate for Corona CAD / CAD plus / ultra / ultra RS	Corona Veo / Vanquish CAD
n/a	None	0	0.1 s	0.1 s
n/a	Low	1	0.2 s	0.2 s
n/a	Medium	2	0.4 s	0.5 s
n/a	High	3	1.0 s	1.0 s
None	Corona (Default)	4 (Default)	3.6 s	2.0 s
Low	n/a	5	5.9 s	3.6 s (Corona)
Medium	n/a	6	10.1 s	5.0 s (Default)
High	n/a	7	18.87 s	10.0 s

INSTRUMENTATION

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 Flex (P/N VF-P20-A)

• Split Sampler FT (P/N VF-A10-A)

• Column Compartment (P/N VH-C10-A-02)

and either

Charged Aerosol Detector Vanquish Flex 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)

SAMPLE PREPARATION

Samples, standard solutions, system suitability solutions, calibration solutions, diluents and mobile phases were prepared as described in the USP publications.

METHOD, DEOXYCHOLIC ACID

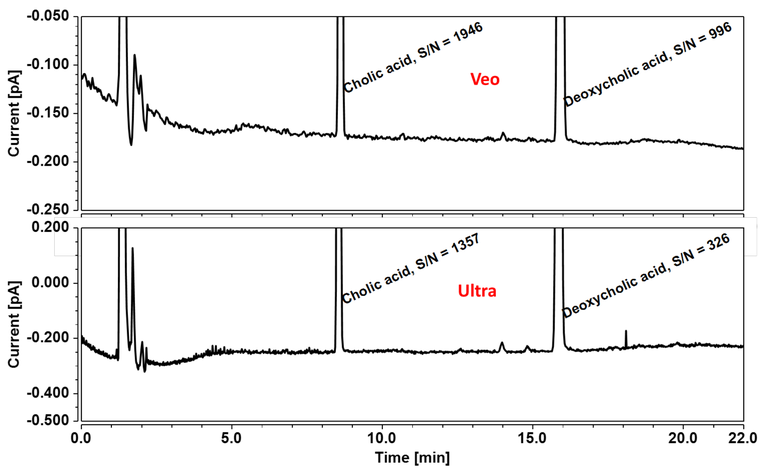
Chromatographic Conditions	
Column	Thermo Scientific™ Acclaim™ 120 C18, 4.6 x 150 mm, 3 µm
Mobile phase	A: 0.1% (v/v) formic acid in water B: 0.1% (v/v) formic acid in acetonitrile
Gradient	Time (min) %A %B 0 75 25 2 55 45 14 42 58 24 0 100 35 0 100 35 75 25 38 75 25
Column temp.	25 ˚C, forced air mode

Optimized (Veo) and Original (Ultra RS) CAD Settings		
	Veo / VCAD	Corona Ultra RS
Evaporation Temperature	50 ˚C	Not applicable
Power Function Value	1.2	1.0
Digital Filter	5	3
Data Collection	10 Hz	10 Hz

Deoxycholic acid Cholic acid

RESULTS, DEOXYCHOLIC ACID

Figure 1. Chromatograms showing 10 µg/mL deoxycholic acid and impurities on VCAD/Veo and Corona Ultra RS.



RESULTS, DEOXYCHOLIC ACID, CONTINUED

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

Sample	Found, Veo / VCAD	Found, Ultra RS	Acceptance Criteria
1	98.4%	98.7%	97.0-103.0%
2	50.3%	50.1%	97.0-103.0%

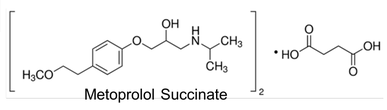
Table 3. Results of system suitability testing for deoxycholic acid.

	Ultra RS	Veo/ VCAD Flex	USP Condition
RSD of area	0.28% (mean, N = 6)	0.63% (mean, N = 6)	≤ 3.0%
S/N ratio	32 (lowest value of 3 injections)	42 (lowest value of 3 injections)	≥ 10

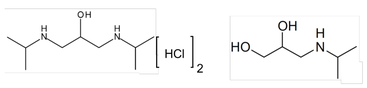
METHOD, METOPROLOL

Chromatographic Conditions	
Column	Halo Penta HILIC 4.6 x 150 mm, 5 µm, polyol column with pentahydroxypentyl derivatization. Thermo Scientific™ Accucore™ 150 Amide HILIC LC column also satisfies the USP requirements with 20 µL injection volume
Mobile phase	85% Acetonitrile, 15% 0.1 M ammonium formate in water, pH 3.2
Column temp.	25 ˚C, forced air mode
Method length, isocratic	12 minutes

	Optimized Corona Veo Settings	Corona Ultra RS Settings
Evaporation Temperature	35 ˚C	Not applicable
Power Function Value	1.3	1.0
Digital Filter	5	3
Data Collection	10 Hz	10 Hz



Metoprolol Succinate



Metoprolol USP Related Compound H

Metoprolol USP Related Compound I

RESULTS, METOPROLOL

Figure 2. Chromatogram of system suitability solution (0.1 mg/mL metoprolol succinate, 0.01 mg/mL H and 0.01 mg/mL I).

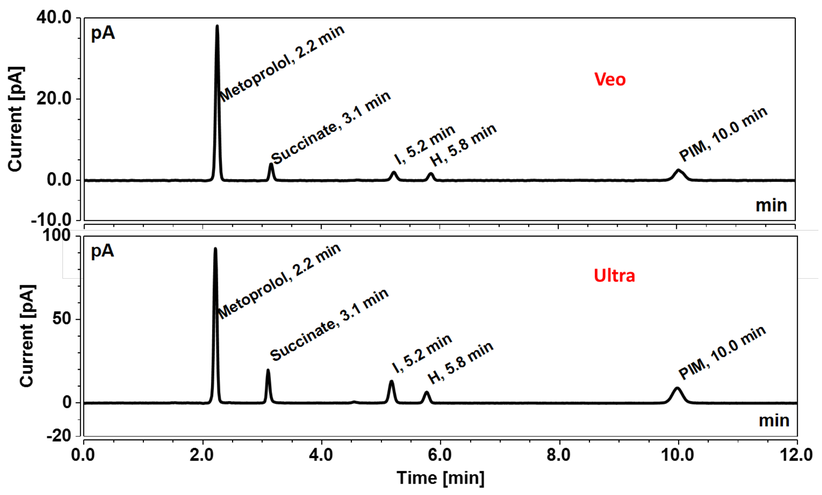


Figure 3. Linear, unweighted calibration curves and relative deviation residual plots for metoprolol related compound I. Comparison of Corona Ultra RS and Corona Veo / VCAD.

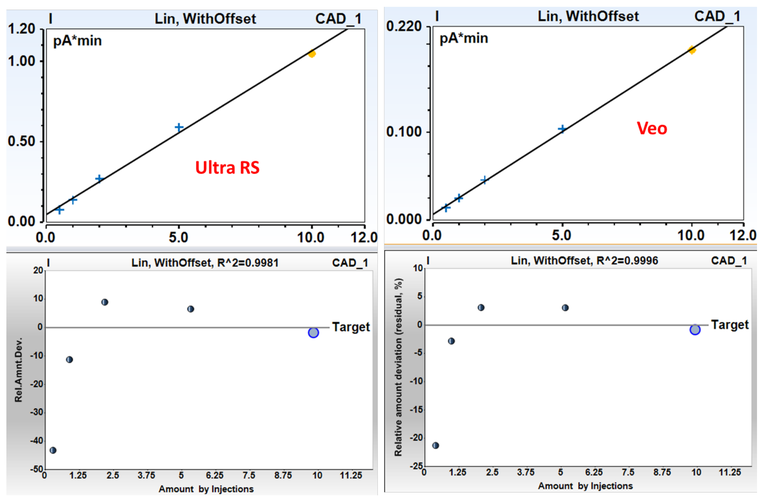


Figure 4. Comparison of very similar chromatograms of the system suitability test solution for Corona Veo / VCAD and Corona Ultra RS.

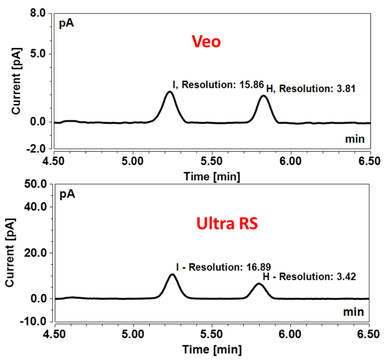


Table 4. Results of system suitability testing for metoprolol method.

	%RSD, peak area, 2 µg/mL H and I	Resolution between H and I
Corona ultra RS	2.56% for H 1.92% for I (mean, N = 6)	3.42
Corona Veo / VCAD	2.82% for H 2.71% for I (mean, N = 6)	3.81
Doubled Injection Volume, Corona Veo / VCAD	1.90% for H 2.03% for I (mean, N = 6)	5.41
USP Condition	≤ 3.0%	≥ 2.0

RESULTS, METOPROLOL, CONTINUED

Response curves for compound I using the 10 µL injection volume are shown in Figure 3. A linear fit is applied. The correlation coefficient, R2, is 0.9981 for Corona Ultra RS and 0.9996 for Corona Veo / VCAD. Note: An R2 near 1, by itself, does not necessarily prove linearity as this metric is based on the assumption that the data show equal absolute error throughout the concentration range. Since most HPLC analyses show somewhat higher absolute error at higher amounts, it is generally recommended to closely examine goodness of fit especially at the extremes of the required quantitation range.

Peak resolution between H and I was better with Corona Veo / VCAD than with the Corona ultra RS. Peak area reproducibility was the same. Both parameters improve with a doubled injection volume, a change that is explicitly allowed by USP. No adverse effects on retention time, resolution, peak shape or quantitative accuracy were found when doubling the injection volume for either model of detector. The method is robust with respect to injection volume.

Table 5 shows good reproducibility for quantification of metoprolol related compound I for the 20 µL injection volume. Acceptable reproducibility is also obtained with the 10 µL injection volume.

Figure 5. Corona Veo / VCAD chromatograms showing the benefit of doubling the injection volume (from 10 µL to 20 µL) for the metoprolol method.

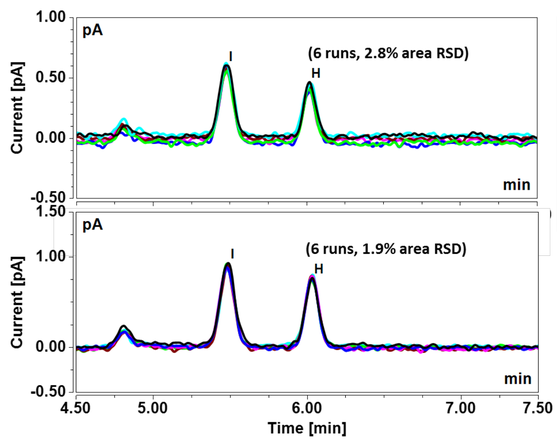
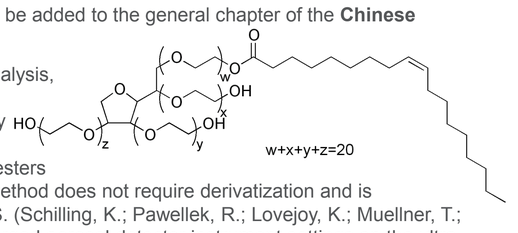


Table 5. Quantification of metoprolol impurities with 20 µL injection volume.

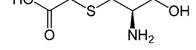
Sample ID	Veo (%)	Ultra RS (%)
F1, I	0.15	0.17
F2, I	0.18	0.18
F3, I	0.17	0.16
F1, H	0.19	0.21
F2, H	0.24	0.21
F3, H	0.22	0.20

OTHER COMPENDIAL-RELEVANT PUBLICATIONS

- Charged aerosol detection will be added to the general chapter of the **Chinese Pharmacopoeia**.
- **Polysorbate**: For fatty acid analysis, the European Pharmacopoeia describes gas chromotography after derivatization with methanol to fatty acid methyl esters (FAMEs). The UHPLC-CAD method does not require derivatization and is inexpensive relative to GC-MS. (Schilling, K.; Pawellek, R.; Lovejoy, K.; Muellner, T.; Holzgrabe, U. Influence of charged aerosol detector instrument settings on the ultra-high-performance liquid chromatography analysis of fatty acids in polysorbate 80. *J. Chrom. A* 2018, 1576, 58-66.)



- **Carbocisteine**: The European Pharmacopoeia is gradually replacing TLC tests that semi-quantitatively assess the impurity relative to a reference spot. (Schilling, K.; Pawellek, R.; Wahl, O.; Holzgrabe, U. (2018). HPLC-CAD impurity profiling of carbocisteine using SCX-RP mixed-mode chromatography, Thermo Fisher Scientific AN72706.)



- **Ibandronate**: Wahl, O.; Holzgrabe, U. Impurity profiling of ibandronate sodium by HPLC-CAD. *J. Pharm Biomed. Analysis* 2015, 114, 254-264.
- **Streptomycin sulfate**: Holzgrabe, U.; Nap, C.-J.; Kunz, Nathalie, Almeling, S. Identification and control of impurities in streptomycin sulfate by high-performance liquid chromatography coupled with mass detection and corona charged-aerosol detection. *J. Pharm. Biomed. Analysis* 2011, 56 (2), 271-279.
- **Alanine – Asparagine**: Wahl, O.; Holzgrabe, U. Amino acid analysis for pharmacopoeial purposes. *Talanta* 2016, 154, 150-163.

CONCLUSIONS

- Either the Vanquish CAD (Corona Veo CAD) or the Corona ultra RS readily perform the USP compendial procedures for content and impurity levels of deoxycholic acid and impurity levels of metoprolol.
- After method transfer, resolution, peak area reproducibility, and noise are nearly identical for the two detectors.
- For the metoprolol method, resolution and peak area reproducibility improve with a doubled injection volume (20 µL instead of 10 µL), a change that is explicitly allowed by USP. We recommend this change for both detectors.
- Use actual standards near the LOD and LOQ to determine these. CAD response is non-linear. Do not extrapolate from higher concentrations.
- Nebulizer temperature (on CADs before 2013) is not equivalent to evaporation temperature
- Evaporation temperature is a critical parameter. Use the lowest evaporation temperature that provides acceptable sensitivity.
- Nebulizer temperature is less critical, matters most with volatile solvents (THF) due to dramatic cooling in the nebulizer upon evaporation.

REFERENCES

1. Lovejoy, K.; Gamache, P.; Muellner, T.; Acworth, I. (2018) Metoprolol impurity testing by charged aerosol detection: method transfer and optimization of a USP method. Thermo Fisher Scientific AN72763.
2. Lovejoy, K.; Gamache, P.; Muellner, T.; Acworth, I. (2018) Deoxycholic acid method transfer from the Corona ultra RS Charged Aerosol Detector to the Corona Veo (or Vanquish) Charged Aerosol Detector. Thermo Fisher Scientific AN72600.
3. Bailey, B.; Gamache, P.H.; and Acworth, I.N. (2014). Guidelines for Method Transfer and Optimization – from Earlier Model Corona Detectors to Corona Veo Detectors, Thermo Fisher Scientific TN157.

ACKNOWLEDGEMENTS (if necessary)

List optional acknowledgements here, such as “We would like to thank Professor Smith from the University of Texas for supplying the purified samples.”

TRADEMARKS/LICENSING

ThermoFisher SCIENTIFIC