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APPLICATION NOTE 72763

Metoprolol impurity testing by charged aerosol detection: method transfer and optimization of a USP method

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United States Pharmacopoeia, USP modernization, beta blocker, Lopressor™, Toprol™, metoprolol succinate, metoprolol tartrate, anti-doping, impurity testing

Goal

Simple step-by-step optimization for the Corona Veo/Vanquish CAD of a USP method for impurity analysis of the small molecule pharmaceutical metoprolol.

Introduction

As part of the United States Pharmacopoeia (USP) monograph modernization effort, USP 41(3) In-Process Revision includes a proposed change to the official USP Metoprolol Succinate monograph (USP 38, page 4370) for the determination of organic impurities (USP related compounds H and I) that lack UV chromophores (Figure 1). The older TLC method is replaced by one that uses a hydrophilic interaction chromatography (HILIC) method coupled with a charged aerosol detector (CAD).

This application note replicates the updated USP method and related publication,[2] both of which used older models of CAD (e.g., Thermo Scientific™ Dionex™ 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 equivalent to the Thermo Scientific™ Dionex™ Corona™ Veo™ CAD. This method also works flawlessly and without modification on a Thermo Scientific™ Vanquish™ Horizon CAD, which is equivalent to the Thermo Scientific™ Dionex™ Corona™ Veo™ RS CAD. The following Corona Veo/VCAD data acquisition parameter settings are recommended for optimal performance: power function value (PFV) = 1.30;

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evaporation temperature (Evap T) = 35 °C; Filter = 5 s. A doubling of the injection volume is also recommended for any model CAD. Using these values, the Corona Veo/VCAD easily meets all USP requirements. This work also optimizes CAD digital filter settings to ensure resolution of metoprolol from other substances, besides impurities H and I, likely to be present in real samples (USP related compounds A, B, C, D, and succinate). Metoprolol (Figure 1) is an active pharmaceutical agent present in the commercial products Lopressor™ as the tartrate salt and Toprol™ as the succinate salt.

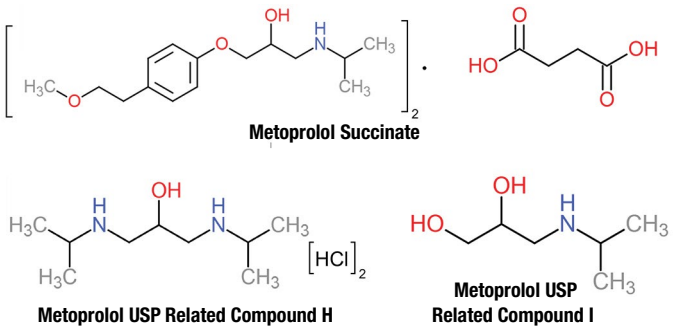

Figure 1. Metroprolol compounds

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)
- Vanquish Flex Quaternary Pump (P/N VF-P20-A)
- Vanquish Flex Split Sampler (P/N VF-A10-A)
- Vanquish Column Compartment (P/N VH-C10-A)
- Thermo Scientific™ Chromeleon™ Chromatography Data System Software 7.2.8

and either

- Vanquish Flex Charged Aerosol Detector 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)

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 Water Purification System
- Metoprolol succinate, USP Reference Standard (P/N 1441298)
- Metoprolol Related Compound H: purchased as the dichloride form of European Pharmacopoeia Metoprolol Impurity M (CAS 73313-36-7). LGC Standards (P/N MM0027.28)
- Metoprolol Related Compound I: purchased as European Pharmacopoeia Metoprolol Impurity N (CAS 6452-57-9). Enamine Store, Monmouth Junction, New Jersey, USA (P/N EN300-138953)
- Metoprolol Related Compound A (CAS 109632-08-8): Enamine Store (P/N EN300-223895)
- Metoprolol Related Compound B (CAS 56718-76-4): LGC Standards (P/N MM0027.17)
- Metoprolol Related Compound C (CAS 29122-74-5): Enamine Store (P/N EN300-649742)
- Metoprolol Related Compound D (CAS 1486464-40-7): USP Reference Standard (P/N 1441265). Note: This standard is sold containing about 25% of an impurity that coelutes with metoprolol related compound H and is UV-transparent.

Conditions

Column:	HALO™ Penta HILIC 4.6 x 150 mm, 5 µm, polyol column with 1,2,3,4,5-pentahydroxypentyl derivatization. Advanced Materials Technology, Wilmington, Delaware, USA (P/N 95814-705).
Mobile Phase:	85% acetonitrile, 15% 0.1 M ammonium formate in water, pH 3.2
Flow Rate:	1.0 mL/min
Column Temp.:	25 °C, forced air mode
Inj. Volume:	10 µL
Corona ultra RS:	PFV = 1.00; Filter = 3 s; Neb. Temp. = on, 25 °C
Corona Veo/VCAD:	PFV = 1.30; Filter = 5 s; Evap T = 35 °C. These parameters were individually optimized (see below)

Preparation of solutions and reagents

Mobile phase preparation

A 200 mL solution of 100 mM ammonium formate was prepared, adjusted to pH 3.20 with formic acid, and subsequently filtered through a 0.45 µm cellulose acetate filter. A 150 mL portion of the filtered ammonium formate buffer was added to 850 mL acetonitrile.

Diluent

The diluent was prepared by adding 850 mL acetonitrile to 150 mL water.

Stock solutions

- A 1.0 mg/mL stock solution of metoprolol succinate was prepared by adding 6.116 mg of the 99.8% pure substance (2:1 ratio of metoprolol to succinate) to a 5 mL volumetric flask and filling to the line with diluent.
- A 1.0 mg/mL stock solution of Metoprolol Related Compound H (impurity H) was prepared by adding 7.16 mg of the 99% pure dichloride salt to a 5 mL volumetric flask and filling to the line with diluent.
- A 1.0 mg/mL stock solution of Metoprolol Related Compound I (impurity I) was prepared by adding 5.26 mg of the 95% pure substance to a 5 mL volumetric flask and filling to the line with diluent.
- A 1.0 mg/mL stock solution of Metoprolol Related Compound A (impurity A) was prepared by adding 5.26 mg of the 95% pure substance to a 5 mL volumetric flask and filling to the line with diluent.
- A 1.0 mg/mL stock solution of Metoprolol Related Compound B (impurity B) was prepared by adding 5.06 mg of the 98.8% pure substance to a 5 mL volumetric flask and filling to the line with diluent.
- A 1.0 mg/mL stock solution of Metoprolol Related Compound C (impurity C) was prepared by adding 5.26 mg of the 95% pure substance to a 5 mL volumetric flask and filling to the line with diluent.
- A 1.0 mg/mL stock solution of Metoprolol Related Compound D (impurity D) was prepared by adding 5.79 mg of the 93% pure HCl salt to a 5 mL volumetric flask and filling to the line with diluent.

Standard solution, system suitability solution, calibration solution, LOD/LOQ solution

- The standard solution was prepared as 2 µg/mL each of impurities H and I by adding 20 µL of each of the stock standard solutions of H and I to a 10 mL volumetric flask and filling to the line with diluent.
- The system suitability solution was prepared as 100 µg/mL of metoprolol succinate and 10 µg/mL each of H and I, by adding 100 µL of the stock standard solutions of H and I and 500 µL of a 2 mg/mL solution of metoprolol succinate to a 10 mL volumetric flask and filling to the line with diluent.
- Calibration solutions of 100, 50, 20, 10, 5, 2, 1, and 0.5 µg/mL were prepared by serial dilution in the diluent in 10 mL volumetric flasks starting from 10 mL of a 1 mg/mL stock solution.
- Solutions for determining LOQ and LOD were prepared at 0.2 and 0.1 µg/mL by further serial dilution in 10 mL volumetric flasks.

Sample solutions

The sample solutions of 2 mg/mL metoprolol succinate used to produce the data for “E8-H” and “E8-I” were prepared by weighing 20.00 mg of the metoprolol succinate USP reference standard, adding to a 10 mL volumetric flask, and filling to the line with diluent. The samples C7, C8, C9, and E9 were prepared in the same manner and spiked with varying amounts of compounds H and I.

Results and discussion

Figure 2 shows the separation of metoprolol, succinate, and impurities H and I in the system suitability solution. Both related compounds were well separated and easily quantified.

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

Technical Note 157[3] and Chapter 3 of *Charged Aerosol Detection for Liquid Chromatography and Related Separation Techniques*[4] were used to provide guidance for method transfer from the Corona Ultra RS CAD to the Corona Veo/VCAD. Data acquisition parameters were optimized in the following sequence:

a) PFV

The first data acquisition parameter that should be optimized is the 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.[3] 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 using residual plots. Several different PFVs were evaluated including 1.10, 1.20, 1.30, and 1.40. The PFV of 1.3 produced the best calibration curve based on a robust evaluation of goodness of fit.

b) Evap T

There is little to no relationship between the Nebulizer T setting on the Corona ultra RS detector and the Evap T setting on the Corona Veo/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/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. Four concentrations of related compound I around its limit of detection were evaluated: 0.5, 1, 2, and 5 µg/mL.

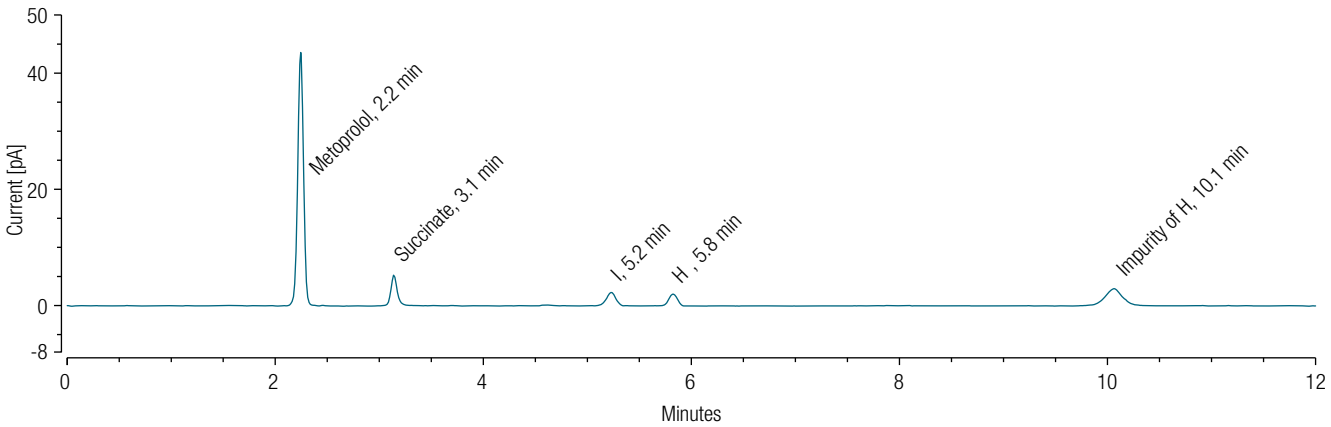

Figure 2. HPLC-Corona Veo CAD chromatogram of a 10 µL injection of the system suitability solution (0.1 mg/mL metoprolol succinate, 0.01 mg/mL H, and 0.01 mg/mL I)

The signal decreased with increasing temperature, as shown in Table 1. Similarly, the background noise decreased with increasing temperature. As described above, the mobile phase buffer was prepared by adjusting a 100 mM ammonium formate (pKa 3.77) solution to pH 3.2 with formic acid. This results in an aqueous buffer concentration of >200 mM and a final mobile phase buffer concentration of >30 mM. This rather high additive concentration (≤15 mM is typically recommended for CAD and MS) may result in a relatively high background signal and baseline noise for this application.

c) Signal filter

Four different digital filter settings were evaluated (2, 3.6, 5, and 10 s) for a 1 µg/mL concentration of related compound I. The SNR increased with higher filter settings and the noise decreased (see Table 1). The 5 s filter was chosen because it offered a balance between good SNR and acceptable separation between metoprolol and other impurities that are not quantified in this monograph, but which may be present in real metoprolol samples (metoprolol related compounds A, B, C, and D). Figure 3 shows that metoprolol is resolved from its major impurities with a filter value of 5. The 10 s filter resulted in broadened peaks that were poorly resolved.

Method performance

Using a PFV = 1.30, Evap T = 35 °C, and a filter of 5 s, the Corona Veo/VCAD met USP system suitability criteria for precision (for 2 µg/mL H and 2 µg/mL I, N = 6, %RSD = 2.03% for I and 1.90% for H using a doubled injection volume); and resolution (for a solution of 100 µg/mL metoprolol succinate, 10 µg/mL H and 10 µg/mL I, resolution of 5.41 between H and I using a doubled injection volume). These requirements comprise the system suitability test defined in the monograph. Doubling the injection volume from 10 µL to 20 µL, a modification allowed under USP guidelines, improves the precision and is a recommended modification (see *Robustness,* below). See Table 2 for more details.

Table 1. Noise and signal-to-noise ratio (SNR) for 5 µg/mL related compound I (Evap T) or for 1 µg/mL related compound I (filter at 35 °C)

	Evap T (°C), 5 µg/mL I			Filter (at 35 °C), 1 µg/mL I			
	35	50	70	2	3.6	5	10
SNR	12.2	4.0	1.7	5.1	5.7	7.6	9.0
Noise	0.214	0.170	0.155	0.145	0.072	0.066	0.058

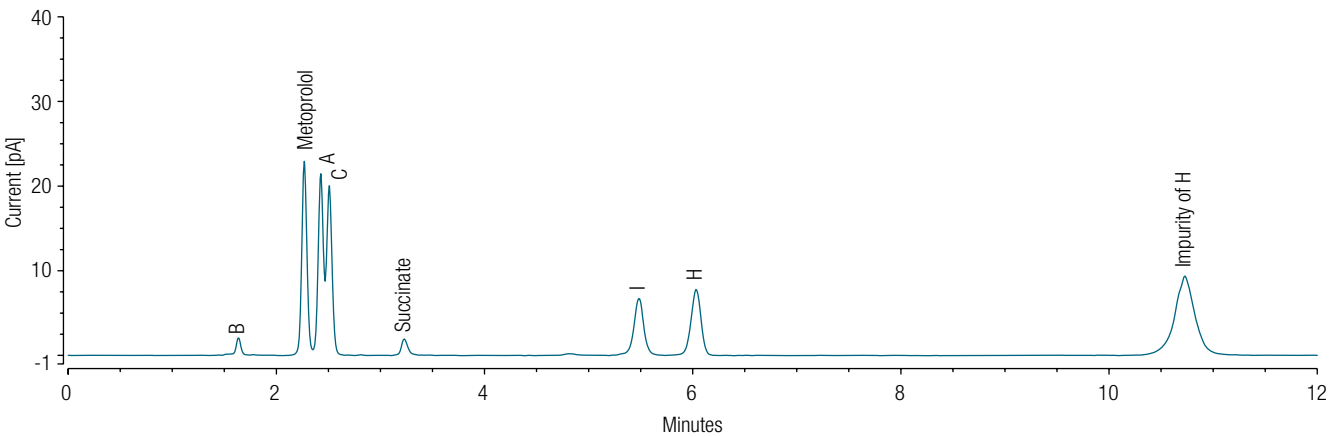

Figure 3. HPLC-Corona Veo CAD chromatogram of a 20 µL injection of 20 µg/mL of each of metoprolol succinate and related compounds A, B, C, H, and I, showing that metoprolol is resolved from its major impurities with a filter value of 5. The peak at around 11 minutes is an impurity of compound H. Related compound D was not injected because it has an impurity that interferes with quantification of related compound H.

Table 2. Results of system suitability testing

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

Response curves

Response curves for compounds H and I using the 10 µL injection volume are shown in Figure 4. A linear fit is applied in both cases. The correlation coefficient, R^2, for compound H is 0.9997 and for I is 0.9995. Note: An R^2 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.

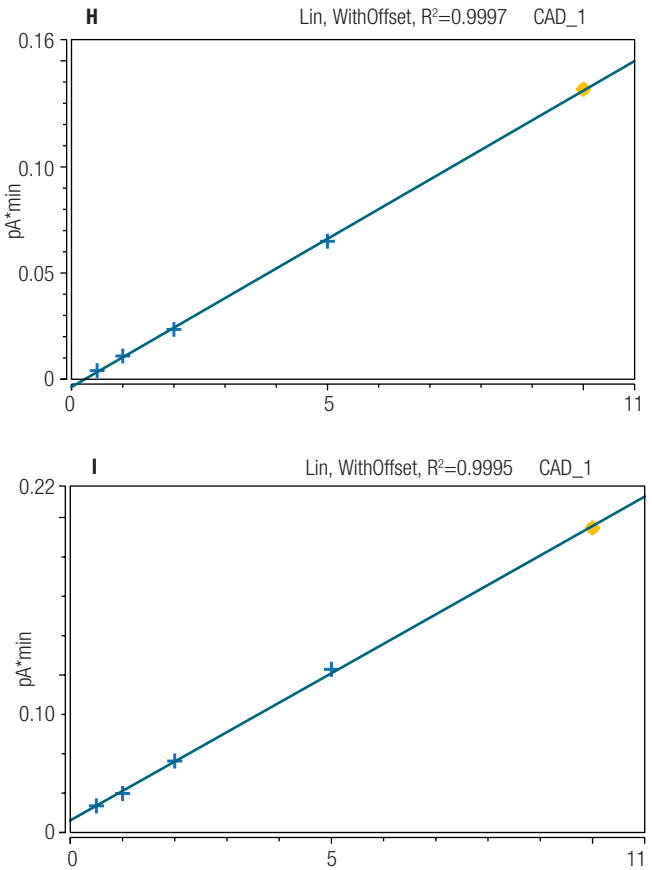

Figure 4. Five-point calibration curve (0.5, 1, 2, 5, and 10 µg/mL) for compounds H and I using a linear fit and 10 µL injection volume

Robustness

No adverse effects were found (e.g., on retention time, resolution, peak shape, or quantitative accuracy) when doubling the injection volume for the Corona Veo/VCAD or the Corona ultra RS CAD. Based on these results, we recommend that the user double the injection volume given in the monograph from 10 µL to 20 µL. Such a change is explicitly allowed by USP. The method is robust with respect to injection volume and not adversely affected by the change. The %RSD for the area of repeated injections improves (see Table 2) and the limit of quantification is 0.2 µg/mL or better.

Quantification of impurities

The standard solution was used to calculate the amount of H and I in the metoprolol succinate sample solution using the 10 µL injection volume (Tables 3 and 4).

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

Where r_U is the peak area of H or I in the sample solution,
r_S is the peak area of H or I in the standard solution
C_S is the concentration of H or I in the standard solution (mg/mL)
C_U is the concentration of metoprolol succinate in the sample solution

The results show acceptable reproducibility for the same samples measured by the Corona Veo/VCAD and the Corona Ultra RS CAD. Use of the 20 µL injection volume improved reproducibility for both instruments (Table 4).

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Table 3. Results for quantification of impurities with 10 µL injection volume

Sample ID	Corona Veo CAD (% H or I)	Corona ultra RS CAD (% H or I)
C7-I	0.13	0.20
C8-I	0.14	0.22
C9-I	0.12	0.19
E9-I	0.04	0.05
E8-I	0.00	0.00
C7-H	0.19	0.25
C8-H	0.21	0.27
C9-H	0.20	0.26
E9-H	0.06	0.06
E8-H	0.01	0.03

Table 4. Results for quantification of impurities with 20 µL injection volume

Sample ID	Corona Veo CAD (% H or I)	Corona ultra RS CAD (% H or I)
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

Conclusion

As charged aerosol detection achieves increasing prominence in USP compendial methods, it becomes increasingly important to ensure all models of charged aerosol detectors are suitable for use, as well as provide guidelines for method transfer between detectors.

The USP 41(3) In-Process Revision to USP 38 (page 4370) for determination of impurities in Metoprolol Succinate originally developed with a Corona ultra RS detector, was easily transferred from the Corona ultra RS detector to the Corona Veo/VCAD charged aerosol detector. A standard method transfer procedure was followed, resulting in final Veo/VCAD parameters of PFV = 1.30, Evap T = 35 °C, and a filter of 5 s.

Performance of the Corona Veo/VCAD readily met the standard set by the Corona ultra RS detector. Peak resolution between H and I was better with the Corona Veo/VCAD detector than with the Corona ultra RS detector, and peak area reproducibility was about the same. Both detectors easily satisfied the resolution and peak area reproducibility tests for system suitability specified in the USP compendial method. Both resolution and peak area reproducibility improve with a doubled injection volume, a change that is explicitly allowed by USP. Based on the data presented here, we recommend use of the doubled injection volume for both instruments. Either instrument can be used to perform the USP compendial assay for impurities in metoprolol succinate.

References

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