Metoprolol and Select Impurities Analysis Using a Hydrophilic Interaction Chromatography Method with Combined UV and Charged Aerosol Detection

Bruce Bailey, Ph. D.
Thermo Fisher Scientific, Chelmsford, MA, USA

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Key Words
Vanquish UHPLC System, Metoprolol, Metoprolol EP Impurity A, Metoprolol EP Impurity M, Metoprolol EP Impurity N, Corona Charged Aerosol Detector

Goal
To develop an impurity profiling method for metoprolol and EP impurity A, which contain chromophores and two non-chromophoric impurities, using a hydrophilic interaction chromatography (HILIC) method coupled with charged aerosol detection (CAD).

Introduction
The drug metoprolol succinate USP is a selective beta$_1$-adrenoreceptor antagonist that reduces chest pain and lowers high blood pressure.[1,2] Several pharmacopoeias (United States Pharmacopoeia, European Pharmacopoeia, and British Pharmacopoeia) have indicated acceptable levels of impurities allowed by drug manufacturers. Impurity profiling of a drug substance is important since the presence of unwanted chemicals, even at small amounts, may influence the efficacy and safety of the pharmaceutical product. Quantification of metoprolol and metoprolol EP impurity A by HPLC with UV detection has been described but some impurities do not possess a detectable UV chromophore.[3,4] Both metoprolol impurities M and N are non-aromatic α-hydroxyamines, as shown in Figure 1. The European Pharmacopeia (EP) indicates that impurities M and N are analyzed by thin layer chromatography (TLC), which is not a suitable technique to produce reliable quantitative data at lower concentrations.[5] The USP monograph modernization program indicates that a liquid chromatographic method is more desirable.[6]

A novel mixed-mode HPLC column, the Thermo Scientific™ Acclaim™ Trinity™ P2 column, was used in HILIC mode to separate metoprolol and impurities A, M, and N. This column consists of high-purity, porous, spherical silica particles coated with charged nanopolymer particles that produce a HILIC/SAX/WAX tri-modal phase. Thus, the Acclaim Trinity P2 column possesses HILIC, anion-exchange, and cation-exchange mixed-mode retention mechanisms.

The Thermo Scientific™ Vanquish™ UHPLC system is an excellent integrated platform, incorporating a binary pump, split loop autosampler, column compartment, and both diode array and charged aerosol detectors, as shown in Figure 2. This system is standardized with fluidics that are biocompatible, which helps prevent any further drug degradation during analysis. The arrangement of both diode array and charged aerosol detectors in the Vanquish system are both orthogonal and complimentary in nature and offers a more comprehensive analysis of the sample. The diode array detector incorporates LightPipe™ technology for enhanced sensitivity and signal-to-noise performance. The Thermo Scientific Vanquish Charged Aerosol Detector, as shown in Figure 3, is a sensitive universal detector designed for UHPLC and provides a wide dynamic range for those compounds that lack a chromophore. Charged aerosol detection (CAD) is a mass-sensitive technique for determining levels of any non-volatile and many semi-volatile analytes after separation by liquid chromatography. This technique produces consistent analyte response independent of chemical characteristics and gives greater sensitivity over a wider dynamic range than ELSD. The presence of chromophoric groups, radiolabels, ionizable moieties, or chemical derivatization is not needed for detection. Thus non-chromophore drug impurities can be easily monitored by CAD.

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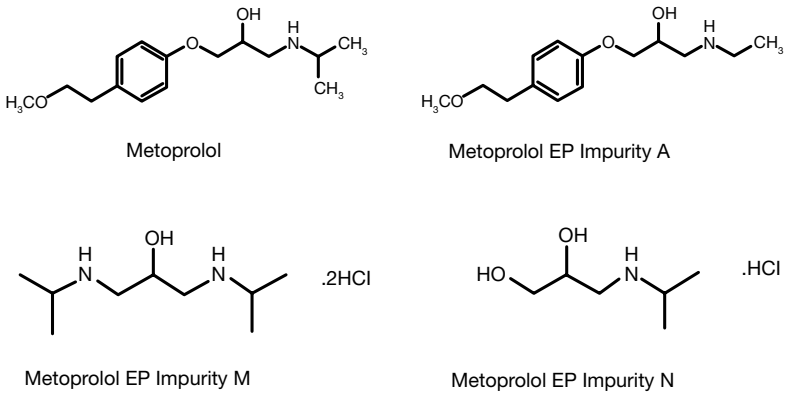

Figure 1. Structures of analytes: metoprolol and selected impurities.

Methods

Equipment, Software, and Consumables

Vanquish UHPLC system, including:

- System Base (P/N VH-S01-A)
- Binary Pump H (P/N VH-P10-A)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Diode Array Detector HL, 320 nm (P/N VH-D10-A)
- LightPipe flow cell, standard (10 mm; P/N 6083.0100)
- Charged Aerosol Detector H (P/N VH-D20-A)
- Threaded glass HPLC vials with septa and caps, (P/N 60180-508)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2 SR2 MUa

Reagents and Standards

- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity or better
- Methanol, Optima™ LC/MS grade, (Fisher Scientific P/N A456-4)
- Acetonitrile, Optima LC/MS grade, (Fisher Scientific P/N A955-4)
- Ammonium formate, Optima LC/MS grade, (Fisher Scientific P/N A115-50)
- Formic acid, Optima LC/MS grade, (Fisher Scientific P/N A117-50)
- Metoprolol succinate, (USP 1441298)
- Metoprolol EP Impurity A, (Molcan MTP01)
- Metoprolol EP Impurity M, (TLC PharmaChem Inc. M-0815)
- Metoprolol EP Impurity N, (TLC PharmaChem Inc. M-0816)

HPLC Method Conditions

Column	Acclaim Trinity P2, 3 µm, 3.0 x 50 mm and Trinity P2, 3 µm, 3.0 x 100 mm in series
Adiabatic temperature	40 °C
Flow rate	1.0 mL/min
Mobile phase A	100 mM ammonium formate, pH = 3.7 with formic acid
Mobile phase B	Acetonitrile
Injection volume	5.00 µL
Detection CAD	Vanquish Charged Aerosol Detector
Power function	1.00
Data rate	25 Hz
Filter constant	5.0 s
Evaporator temperature	60 °C
Gas reg. mode	Analytical
Detection DAD	Vanquish Diode Array Detector
Path length	10 mm, LightPipe
Wave length	280 nm
Data rate	20 Hz
Filter constant	0.2 seconds
Slit width	4 nm

Preparation of Solutions and Reagents

Stock Standard Solutions

All standards were prepared using methanol as the diluent. Stock solution concentrations of 1.00 mg/mL of analyte (organic portion, including counterions) were prepared by accurately weighing the individual analytes into separate 30 mL polypropylene containers and then adding appropriate amounts of methanol using a volumetric pipette.

Working Standard Solutions

A 100 µg/mL working solution of the four different stock solutions was prepared by placing 1.0 mL each of stock standard into a 10 mL volumetric flask, adding water/acetonitrile (50:50, v/v) solution to mark, capping and mixing. A list showing the dilutions of stock standards for preparation of the calibration curves is presented in Table 1.

Table 1. Preparation of working standard solutions.

Concentration	1st addition	2nd addition	Instruction
100 µg/mL	None	None	See above
50 µg/mL	1 volume of 100 µM solution	Add 1 volume water/ acetonitrile (50:50, v/v)	Mix well
25 µg/mL	1 volume of 50 µM solution	Add 1 volume water/ acetonitrile (50:50, v/v)	Mix well
10 µg/mL	1 volume of 100 µM solution	Add 9 volume water/ acetonitrile (50:50, v/v)	Mix well
5 µg/mL	1 volume of 10 µM solution	Add 1 volume water/ acetonitrile (50:50, v/v)	Mix well
1 µg/mL	1 volume of 10 µM solution	Add 9 volume water/ acetonitrile (50:50, v/v)	Mix well
0.5 µg/mL	1 volume of 1 µM solution	Add 1 volume water/ acetonitrile (50:50, v/v)	Mix well

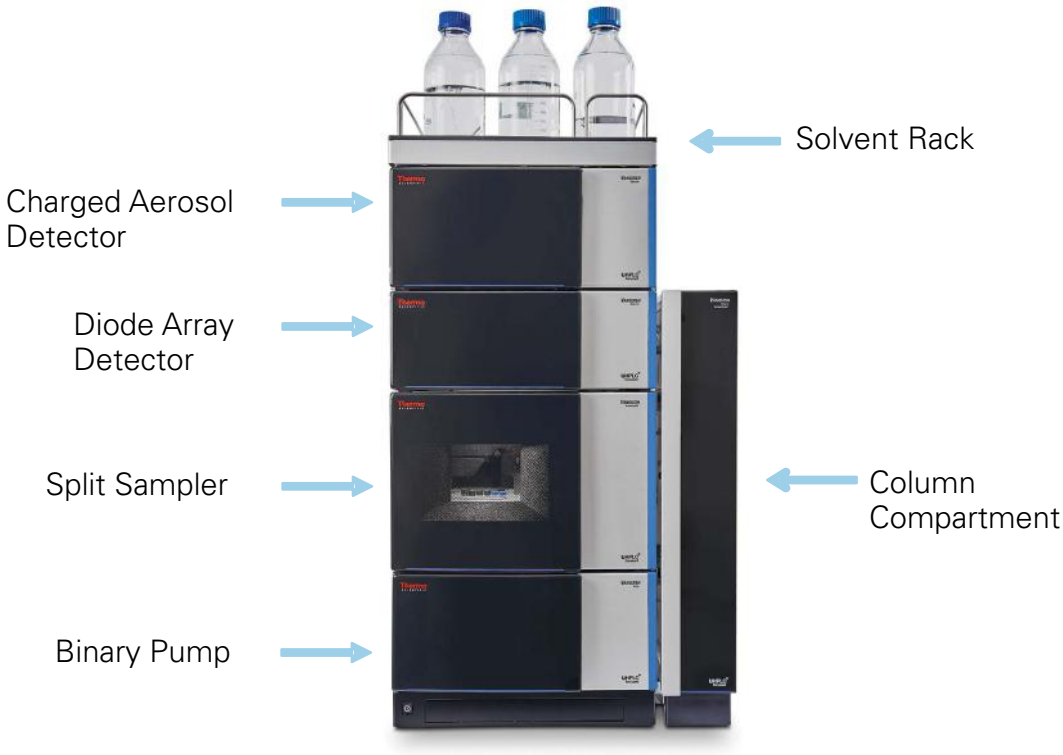

Figure 2. Integrated Vanquish UHPLC system with multi-modal analyte detection.

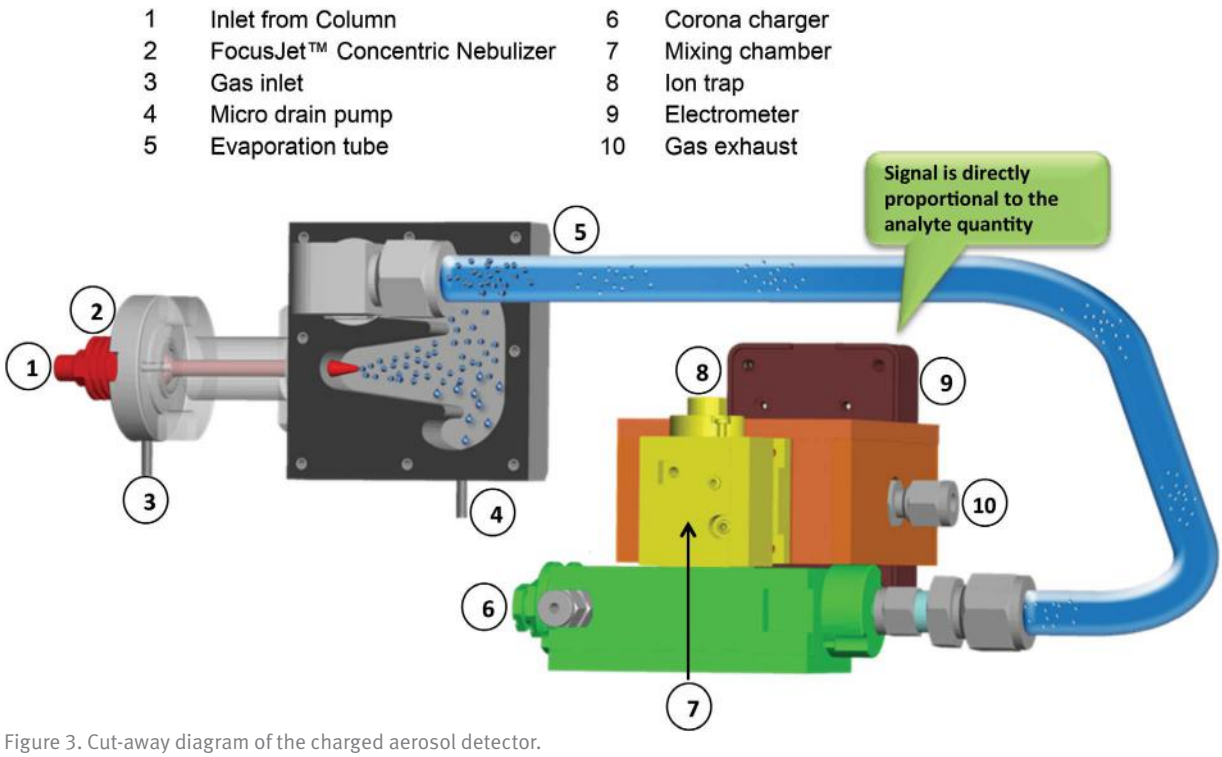

Figure 3. Cut-away diagram of the charged aerosol detector.

Results and Discussion

The separation of metoprolol and several impurities was accomplished within 10 minutes using HILIC mode on the Acclaim Trinity P2 column as illustrated in Figure 4. Note that the sample for Impurity A contained a significant amount of impurity M as well as an undefined impurity near the solvent front. The method was simplified by optimizing the pH and ionic strength of the ammonium formate buffer so that isocratic mobile phase conditions could be used. These HILIC conditions also contribute to optimal detector conditions since the higher levels of organic solvent increase the efficiency of the CAD nebulizer and produce a high signal component.

Using the HILIC technique described, metoprolol and several impurities (A, M and N) could all be detected and quantified by CAD, while only metoprolol and impurity A responded on the UV detector as shown in Figure 5.

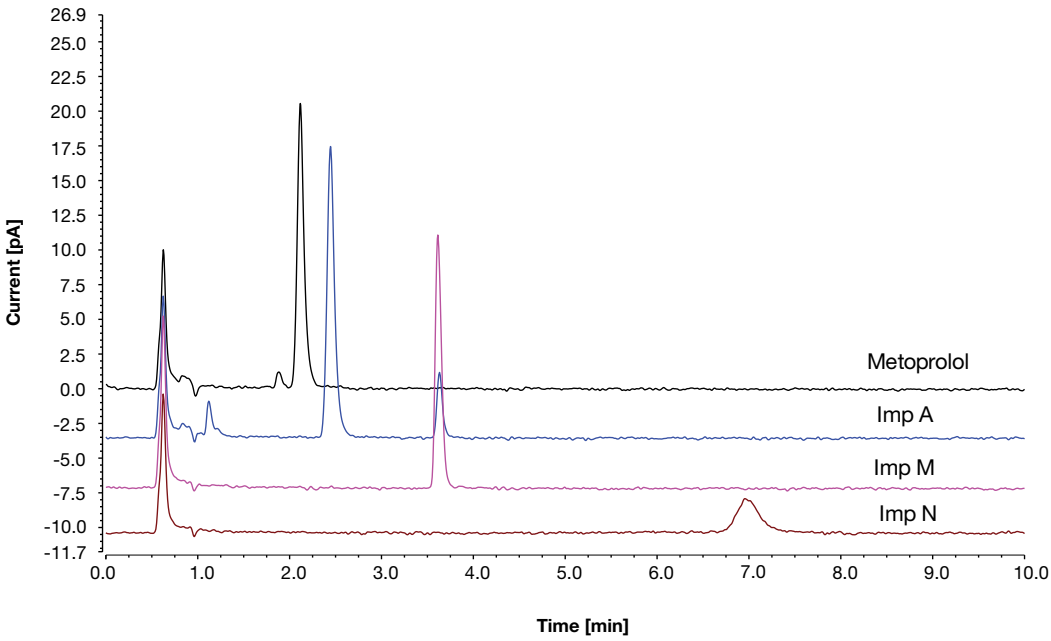

Figure 4. Analysis of metoprolol and select impurities using charged aerosol detection.

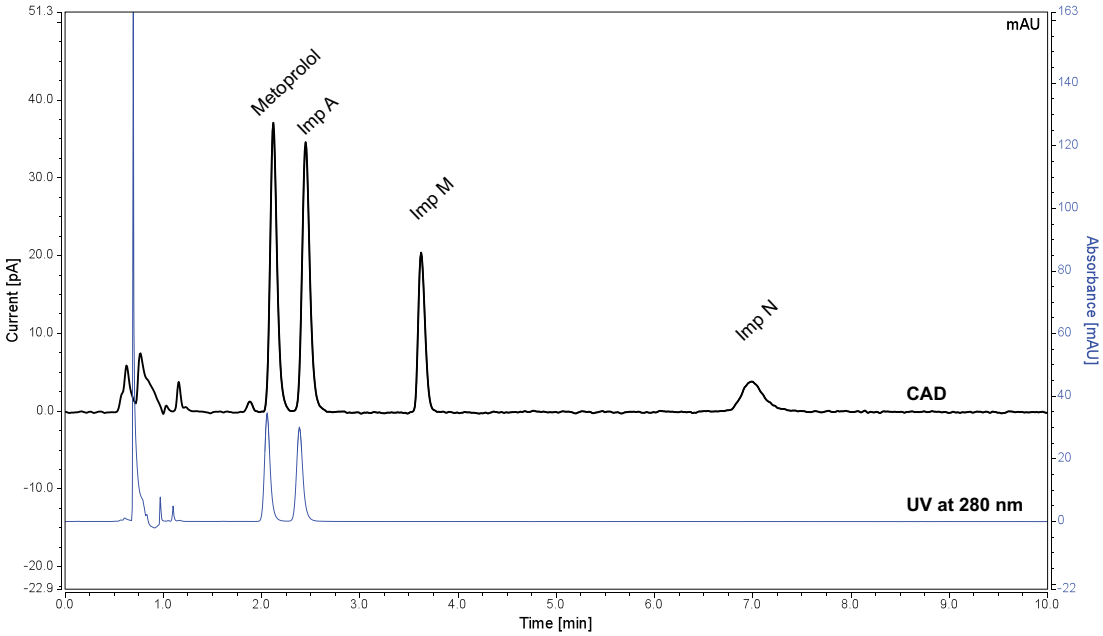

Figure 5. Detection of metoprolol and EP impurities by CAD and UV at 280 nm.

The charged aerosol detector offers good sensitivity for the analysis of all compounds as illustrated in Figure 6. Both metoprolol and impurity A could be detected at amounts as low as 2.5 ng on column, while the LOD for impurity M and N were 10 and 25 ng, respectively. Since the charged aerosol detector can present four orders of dynamic range, impurities of metoprolol can be measured to the 0.1% level relative to the API (data not shown).

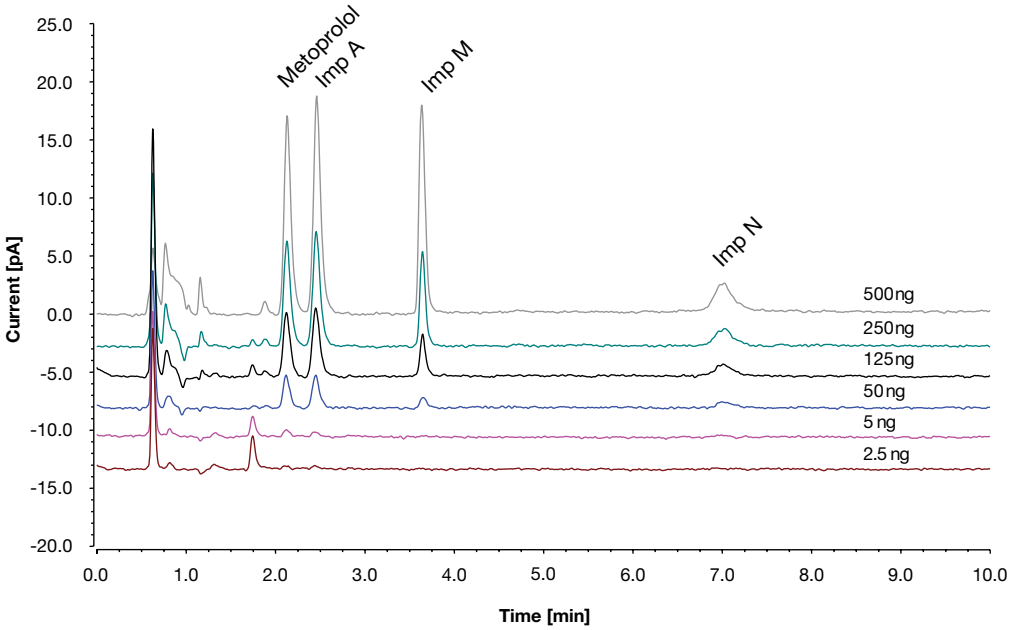

Figure 6. Overlaid chromatograms for metoprolol and impurities by CAD ranging from 2.5 to 500 ng on column.

The polynomial curve fit was used for charged aerosol detector calibration curves due to its nonlinear nature as shown in Figure 7, and a linear curve fit was used for the UV detector. Calculations related to goodness of fit are shown in Table 2 for both CAD and UV data. The coefficient of determination was greater than 0.994 for all peaks.

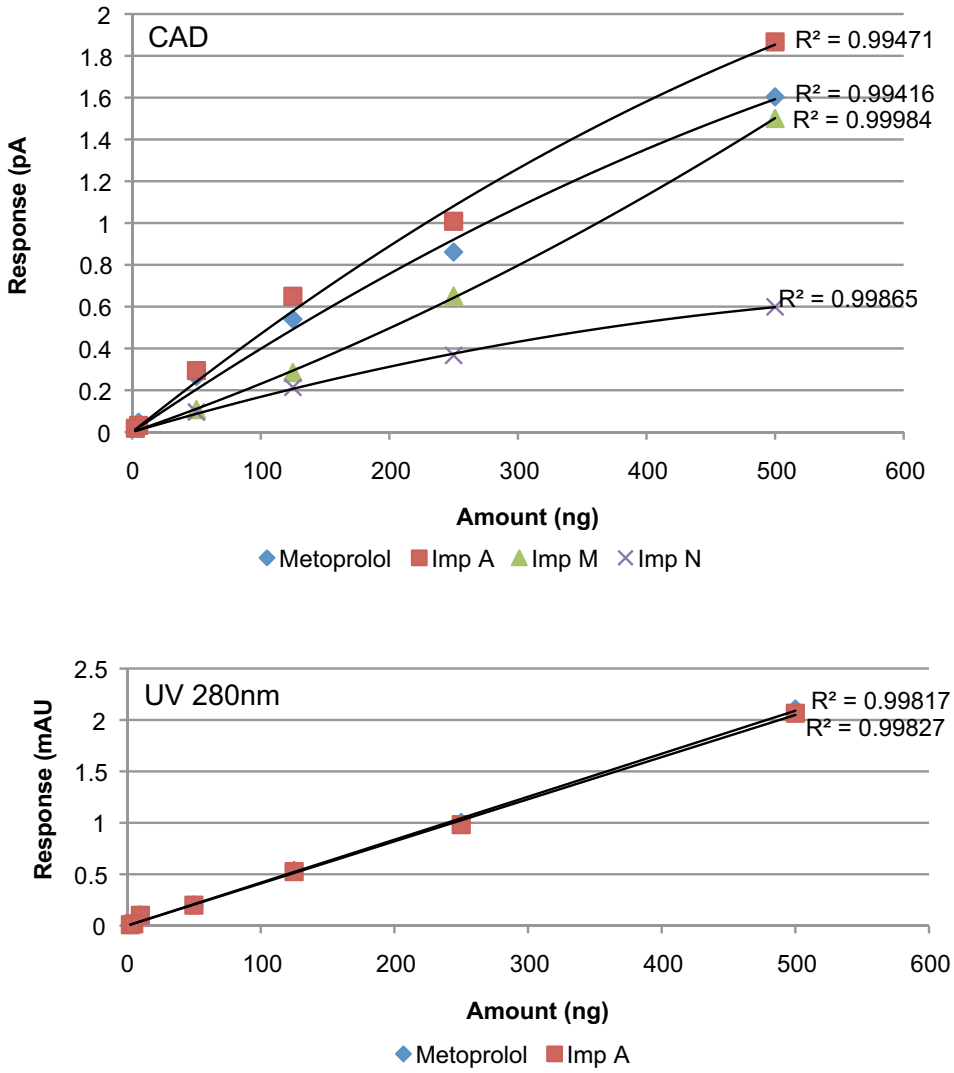

Figure 7. Calibration data for metoprolol and impurities using CAD and UV 280 nm.

Table 2. Goodness of fit metrics for analysis of metoprolol and impurities by CAD and UV 280 nm.

Peak Name	RetentionTime (min)	Curve Type	Number of Points	Coefficient of Determination
Metoprolol, CAD	2.123	Polynomial	6	0.994
Metoprolol, UV 280	2.065	Linear	7	0.998
Imp A, CAD	2.451	Polynomial	6	0.995
Imp A, UV 280	2.396	Linear	7	0.998
Imp M, CAD	3.647	Polynomial	4	1.000
Imp N, CAD	7.011	Polynomial	4	0.999

An isocratic HILIC chromatographic method using both UV and charged aerosol detection was developed for the drug metoprolol and EP impurities A, M and N. The HPLC method described simplifies impurity profiling of metoprolol succinate, replacing current TLC methods in EP. The DAD incorporates LightPipe technology for enhanced sensitivity and signal-to-noise performance. This DAD achieves the best signal-to-noise performance through the combination of lowest baseline noise, a very long light-path, and minimum peak dispersion. The charged aerosol detector is a sensitive universal detector designed for UHPLC and has a wide dynamic range capable of detecting impurities to the 0.1% level of the API. Typically, the charged aerosol detector is used to furnish data for those compounds that lack a chromophore. Together, the Vanquish diode array and charged aerosol detectors are both orthogonal and complimentary in nature, offering a more comprehensive analysis of both drug and impurities present in the sample.

Conclusion

A simple, rapid, and accurate method without derivatization was developed for the analysis of metoprolol succinate and metoprolol EP impurity A, M, and N using isocratic HILIC chromatography with UV and charged aerosol detection. The HPLC detectors are orthogonal and complimentary in nature and render a more comprehensive analysis of the sample since they can detect both chromophore and non-chromophore species using the configuration described. The integrated Vanquish system provides excellent data quality.

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