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

Poster Note 71689

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Overview

Purpose: To develop a Hydrophilic Interaction Chromatography (HILIC) method coupled with Charged Aerosol Detection (CAD) for the analysis of two non-chromophoric impurities of Metoprolol.

Methods: A novel mixed mode HPLC column was used in HILIC mode to separate metoprolol and impurities A, M and N. An integrated UHPLC system with a UV and universal charged aerosol detection offering multi-mode detection for the simultaneous analysis of both non-chromophore and chromophore compounds was employed.

Results: A HILIC method for the determination of metoprolol and impurities A, M and N using multi-modal UV and charged aerosol detection is described. Multi-modal UV and charged aerosol detection in an integrated system provides a suitable means for the analysis drugs consisting of both chromophore and non-chromophore species. The detectors are orthogonal and complimentary in nature so that more compounds in the sample can be detected.

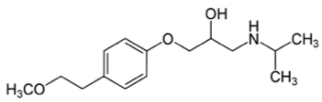
Introduction

The drug, metoprolol succinate USP, is a selective beta$_1$-adrenoreceptor blocking agent which reduces chest pain and lowers high blood pressure.[1,2] Quantification of Metoprolol 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. The European Pharmacopeia (EP) indicates that impurities M and N are analyzed by thin layer chromatography (TLC) which is not a suitable technique to provide quantitative data at lower concentrations.[5] The USP monograph modernization program has indicated that a chromatographic method is more desirable.[6]

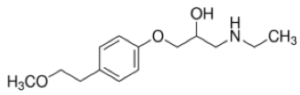
A novel mixed mode HPLC column, Thermo Scientific™ Acclaim™ Trinity P2 column was used in HILIC mode to separate metoprolol and impurities A, M and N. This column consists of high-purity porous spherical silica particles coated with charged nanopolymer particles which provide a HILIC/SAX/WAX tri-modal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

The new Thermo Scientific™ Vanquish™ UHPLC system provides an excellent integrated platform by incorporating a Binary pump, split loop Autosampler, Column compartment and both Diode Array and Charged Aerosol Detectors. This system is standardized with fluidics that are biocompatible. The arrangement of both diode array and charged aerosol detectors in the Vanquish system provides a more comprehensive analysis of the sample since they are both orthogonal and complimentary in nature. The DAD incorporates LightPipe™ technology for enhanced sensitivity and signal to noise performance. The system provides sensitive DAD with linearity up to 3 AU for those compounds with suitable chromophores. The Charged Aerosol Detector 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 provides consistent analyte response independent of chemical characteristics and gives greater sensitivity over a wider dynamic range. An analytes response does not depend on optical properties, like with UV-vis absorbance, or the ability to ionize, as with mass spectrometry (MS). 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.

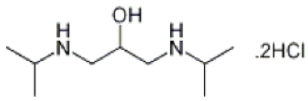
FIGURE 1. Structures of Analytes: Metoprolol and Selected Impurities.



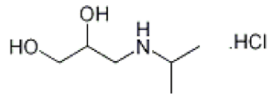
Metoprolol



Metoprolol EP Impurity A



Metoprolol EP Impurity M



Metoprolol EP Impurity N

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Methods

Liquid Chromatography using the Vanquish binary UHPLC system including:

- 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)
- Charged Aerosol Detector H, (P/N VH-D20-A)

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 ºC
Flow rate:	1.0 mL/min
Mobile Phase A:	100 mM ammonium formate pH = 3.7
Mobile Phase B:	acetonitrile
Detector settings:	CAD,10 Hz data rate, 5 s response time, 35 °C evaporation temp.,1.00 PFV DAD, 10 mm, LightPipe, 280 nm, data collection rate 10 Hz, 0.05 s response time, slit 4 nm

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.2

FIGURE 2. Cut-away Diagram of the Charged Aerosol Detector.

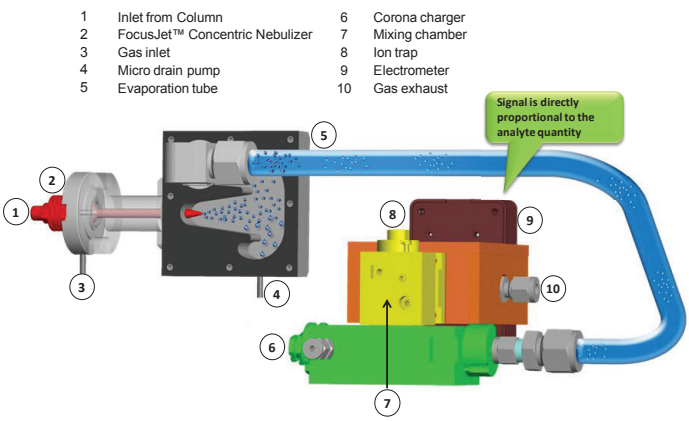
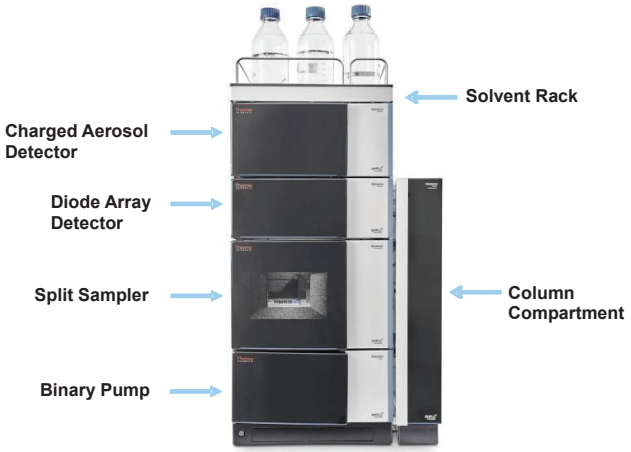


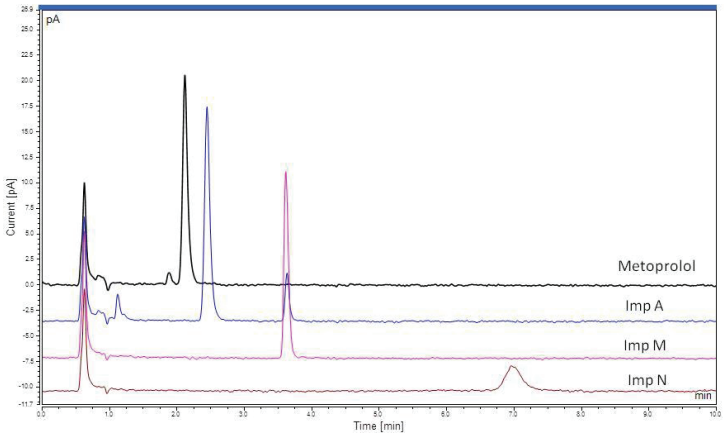
FIGURE 3. Integrated Vanquish UHPLC System with Multi-Modal Analyte Detection.



Results

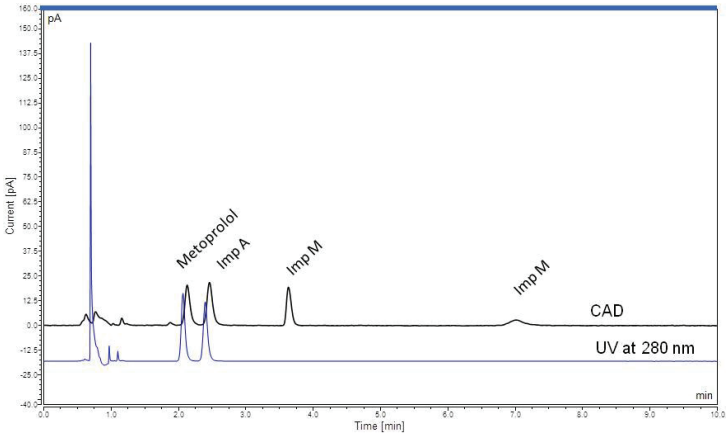
The separation of Metoprolol and several impurities was accomplished within 10 minutes using HILIC mode on the Trinity P2 column as illustrated in Figure 4. Note that the sample for Impurity A contained a significant amount of Impurity M as well as an undefined impurity near the solvent front. The method 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 provide optimal detector conditions since the higher levels of organic solvent increase the efficiency of the CAD nebulizer and provide a high signal component.

FIGURE 4. Analysis of Metoprolol and Select Impurities using Charged Aerosol Detection.



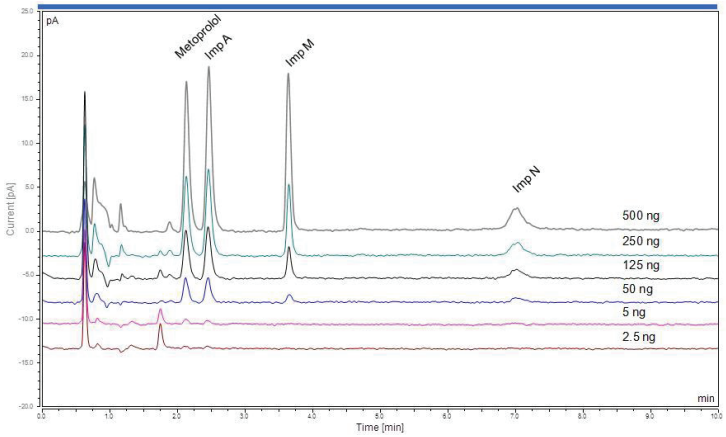
Using the HILIC technique described, metoprolol and several impurities (A, M and N) could all be detected and quantified by CAD while only metoprolol and impurity A responded on the UV detector as shown in Figure 5.

FIGURE 5. Detection of Metoprolol and Impurities by CAD and UV at 280 nm.



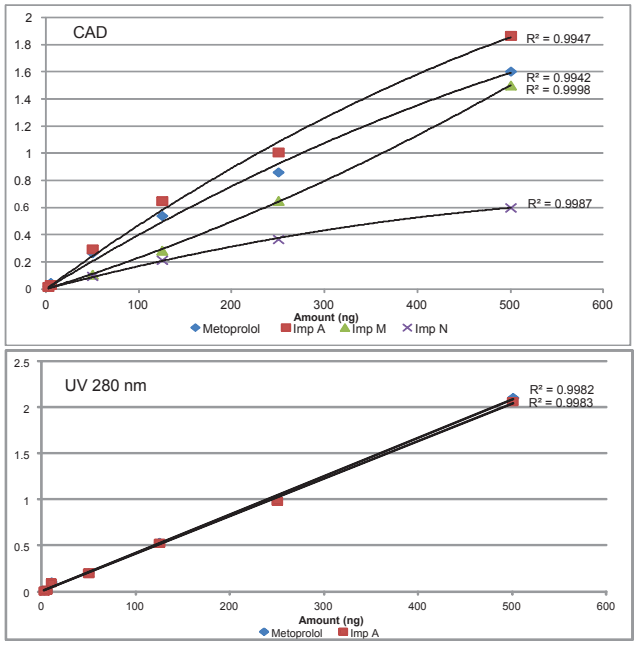
The charged aerosol detector provided good sensitivity for the analysis of all compounds as illustrated in Figure 6. Both metoprolol and impurity A could be detected at amounts as low as 2.5 ng on column while the LOD for impurity M and N was 10 and 25 ng, respectively. Since the charged aerosol detector can provide 4 orders of dynamic range , impurities of metoprolol can be measured to the 0.1 percent level relative to the API (data not shown).

FIGURE 6. Overlaid Chromatograms for Metoprolol and Impurities by CAD ranging from 2.5 – 500 ng on column.



The polynomial curve fit was used for charged aerosol detector calibration curves due to its non-linear 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 1 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.



Discussion

An isocratic HILIIC chromatographic method using both UV and Charged Aerosol Detection was developed for the drug Metoprolol and Impurities A, M and N. 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 provides a wide dynamic range capable of detecting impurities to the 0.1% level of the API. Typically, the charged aerosol detector is used to provide data for those compounds that lack a chromophore. Together, the Vanquish diode array and charged aerosol detectors are both orthogonal and complimentary in nature providing a more comprehensive analysis of both drug and impurities present in the sample.

TABLE 1. Goodness of fit and %RSD metrics for analysis of Metoprolol and Impurities by CAD and UV 280 nm.

Peak Name	Ret.Time min	Curve Type	Number of Points	Coeff.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

Conclusions

- A HILIC method for suitable quantitation of non-chromophore species EP impurities M and N is described using charged aerosol detection.
- Using an integrated system with multi-modal UV and charged aerosol detection provides a suitable means to quantify drugs like metoprolol and impurities The detectors are orthogonal and complimentary in nature and provide 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.

References

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