Straightforward implementation of a compendial LC method for metolazone impurity analysis

With the Thermo Scientific Vanquish Core HPLC system operated by Empower 3

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Application benefits

- Reliable and highly reproducible chromatographic results were obtained when implementing the liquid chromatography impurity method from the European Pharmacopoeia monograph of metolazone
- Thermo Scientific™ Vanquish™ Core HPLC system can be controlled in regulated environments, and data can be processed by Waters Empower™ 3 with SII for Empower 1.2

Goal

Highlight the reliability of the Vanquish Core HPLC system in routine LC analysis performed in a Waters Empower environment

Introduction

Pharmacopeial monographs provide numerous established and approved analytical methods and guidelines to control the quality of active pharmaceutical ingredients (API). Analytical laboratories, especially those performing routine analysis for quality control purposes, often rely on these validated regulatory methods as their application eliminates the necessity of developing and validating their own methods, which usually is elaborate and time consuming. It is expected that these methods could be successfully implemented with appropriate hardware. Here, seamless

implementation of a liquid chromatographic analytical method published by the European Pharmacopoeia (EP) is demonstrated using the Vanquish Core HPLC system under the control of Empower 3 software using the Thermo Scientific™ Standard Instrument Integration (SII) for Empower 3 software plugin. The API metolazone is a diuretic drug that is used in the treatment of high blood pressure. The method published in the monograph serves to separate the API and five known impurities to allow accurate quantitation.[1]

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Methanol, Optima™ LC/MS grade (P/N A456-212)
- Fisher Scientific™ Potassium dihydrogen orthophosphate for HPLC (P/N P/4806/50)
- Ph. Eur. reference standard: Metolazone for system suitability (SST) CRS batch 1[2] (P/N Y0000702)

Equipment

- Vials (amber, 2 mL), Fisher Scientific™ (P/N 11545884)
- Snap cap with septum (Silicone/PTFE), Fisher Scientific™ (P/N 10547445)

Sample preparation

Following the steps outlined in the monograph, 3 mg of the reference standard, containing the API metolazone and the impurities A, B, C, D, and E, were dissolved in 1 mL of methanol.[1]

Instrumentation

A Thermo Scientific Vanquish Core HPLC system was used for the analysis:

- Thermo Scientific™ Vanquish™ System Base Core (P/N VC-S01-A)
- Thermo Scientific™ Vanquish™ Quaternary Pump C (P/N VC-P20-A)
- Thermo Scientific™ Vanquish™ Sampler CT (P/N VC-A12-A)
- Thermo Scientific™ Vanquish™ Column Compartment C (P/N VC-C10-A-03)
- Thermo Scientific™ Vanquish™ Variable Wavelength Detector C (P/N VC-D40-A) with standard flow cell, SST, 10 mm, 11 µL, 120 bar (P/N 6077.0250)

Chromatographic conditions

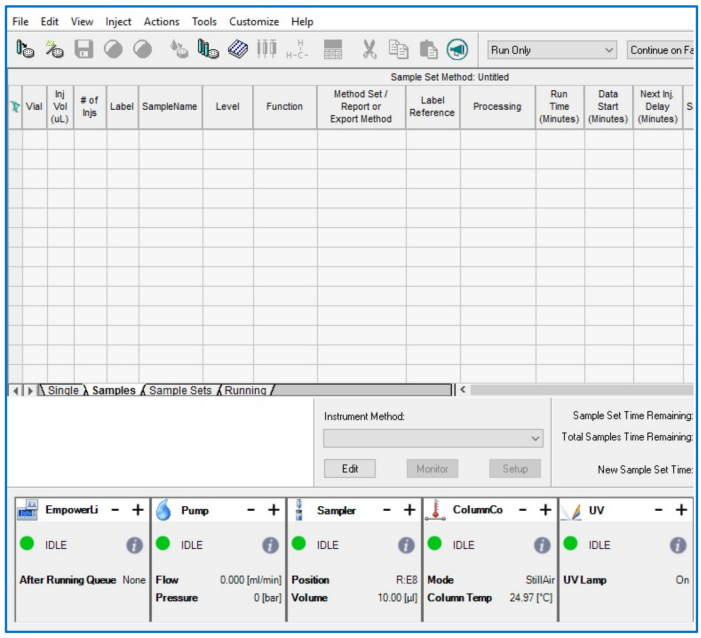
Parameter	Value
Column	Thermo Scientific™ Hypersil™ ODS C18, 4.6 × 250 mm, 5 µm, 120 Å (P/N 30105-254630)
Mobile phase	A: 5.44 g/L KH_2PO_4 in water B: Methanol
Flow rate	1.5 mL/min
Gradient	*Time (min)* / *% B* 0 / 30 5 / 30 25 / 50 35 / 50 38 / 30 48 / 30
Column temperature	30 °C with passive pre-heater (forced air)
Autosampler temperature	8 °C
Detection	Wavelength: 230 nm Data collection rate: 5 Hz Response time: 1 s
Injection volume	10 µL
Needle wash	Off

Data processing and software

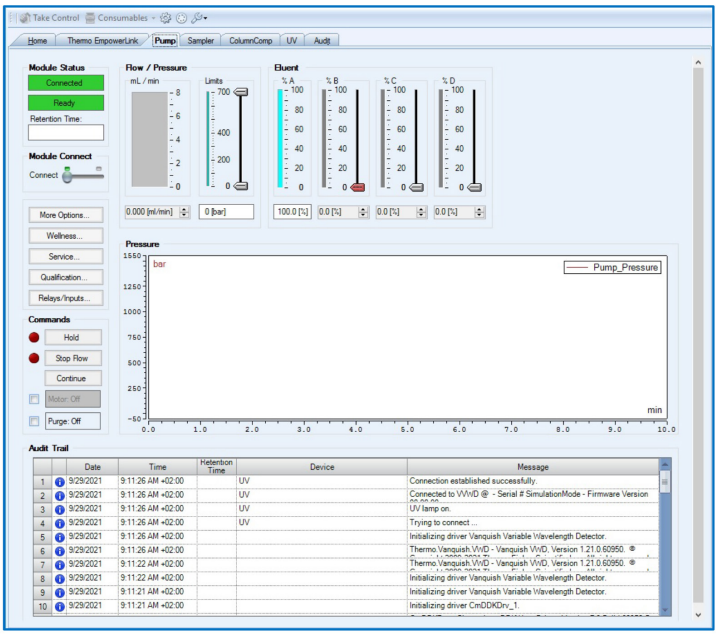
Thermo Scientific™ Standard Instrument Integration (SII) for Empower 1.2 and Waters Empower 3 FR5 Chromatography Data System (CDS) was used for data acquisition, processing, and reporting.

The SII for Empower software plugin allows the operator to configure the Thermo Scientific Vanquish Core HPLC system for control in the Empower software. As can be seen in Figure 1A, the Empower interface opens with all familiar control elements. To change settings, right-click on any of the module panels. In the instrument view of SII for Empower, the operator can then set parameters for each of the instrument modules (Figure 1B). A wizard guides through all steps to set up a new instrument method (Figure 1C). Afterwards, it can be selected on the Empower interface in the drop-down menu of the instrument methods.

A



B



C

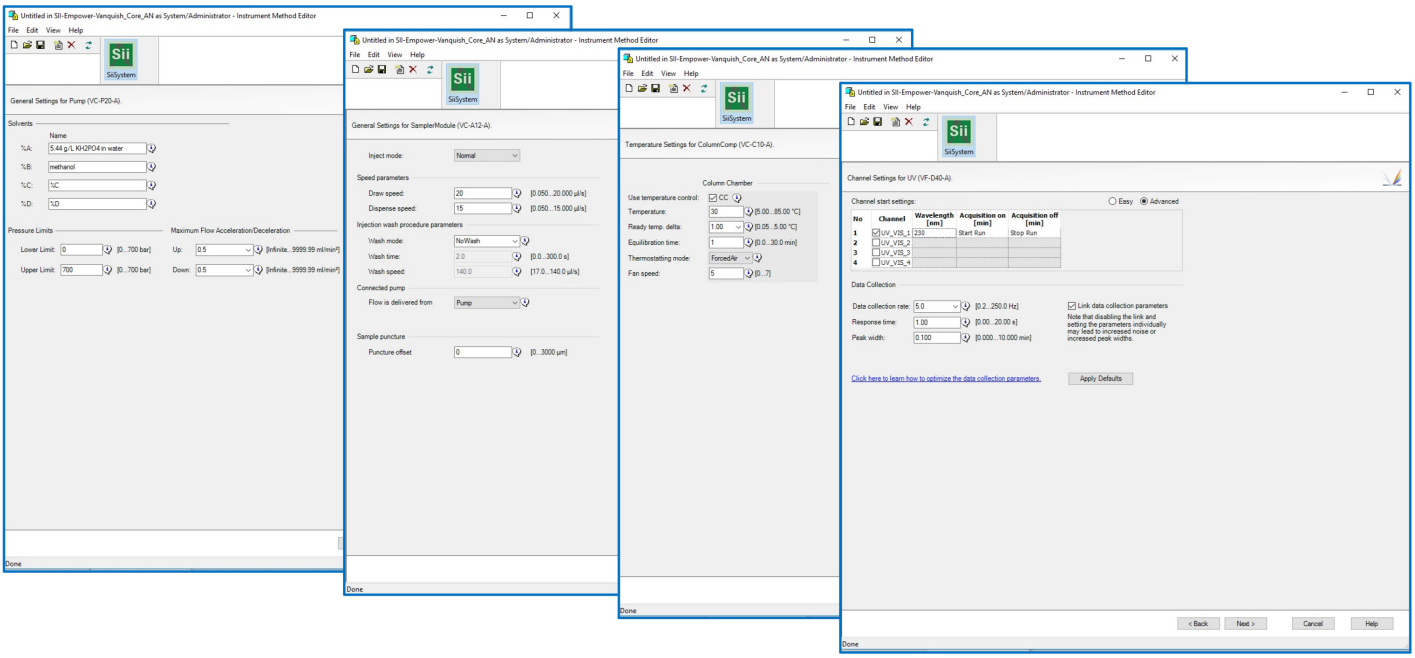


Figure 1. Screenshots of (A) Empower interface, (B) SII Instrument View, and (C) Instrument method wizard

Results and discussion

The method for impurity analysis of metolazone was set up as outlined in the Ph. Eur. Monograph,[1] and six consecutive sample injections were executed. Figure 2 displays an example chromatogram obtained.

The system suitability criteria for method acceptance as outlined in the Ph. Eur. monograph require a minimum resolution for 1.6 for impurity peaks E and C and a minimum resolution of 1.5 for impurity peaks A and B.[1] These criteria were easily met with significantly higher resolution values than the minimum acceptance limit would require (2.8 and 1.9).

The excellent repeatability of retention times and peak areas of the impurities, expressed as relative standard deviations (RSDs) over the six consecutive injections, is shown in Figure 3. Retention time RSDs range from 0.01% to 0.02%, while peak area RSDs were found between 0.1% and 0.4%.

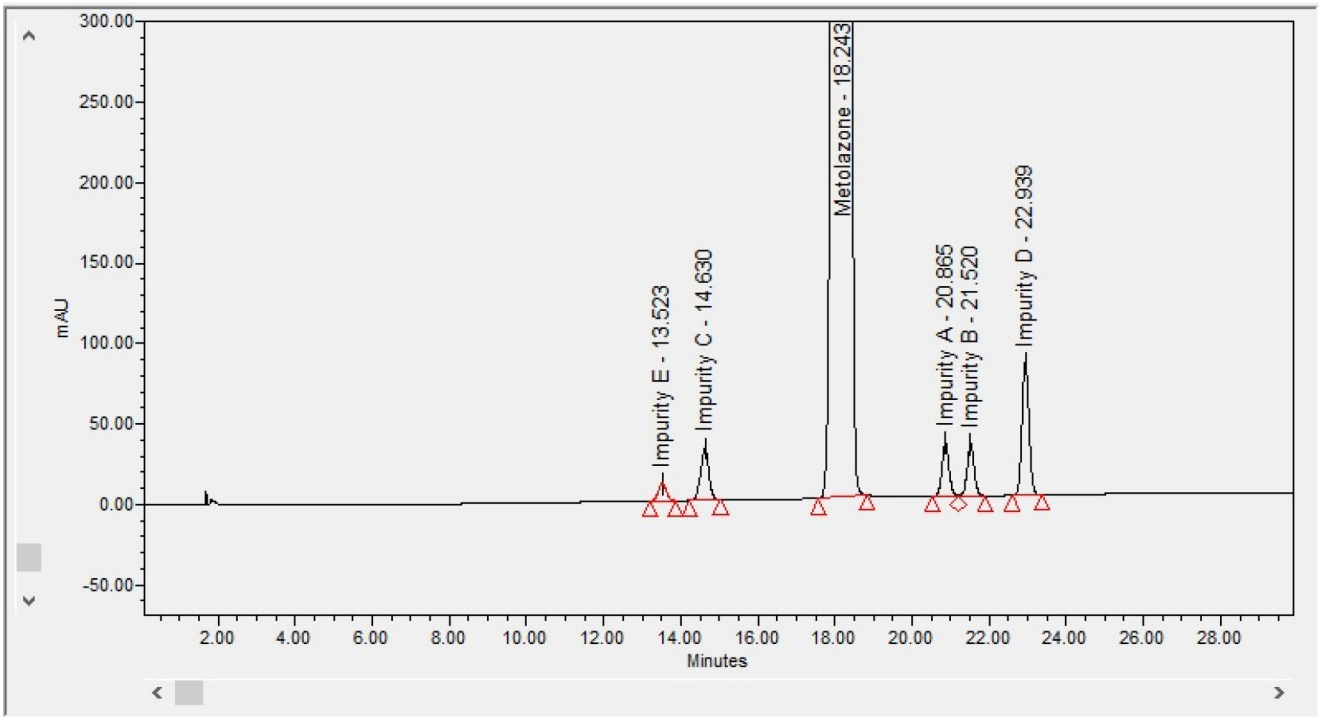


Figure 2. Chromatogram of API metolazone and its five impurities

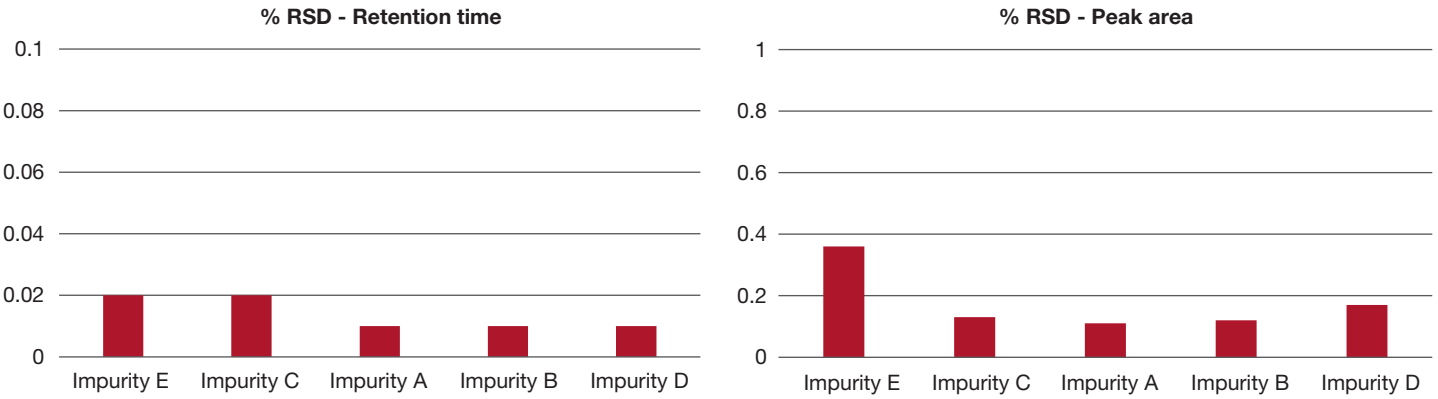


Figure 3. Relative standard deviations (RSDs) of retention times and peak areas of impurities over six injections obtained by the Vanquish Core HPLC system

Conclusion

The Ph. Eur. method for metolazone impurity analysis was implemented with the Vanquish Core HPLC system operated by Empower 3 software with SII for Empower. System suitability criteria were met and reliable and repeatable results were obtained, assuring that the system fulfills requirements of any quality control laboratory.

References

1. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.) Online, 9th edition 2018 (9.0), monograph 1757: Metolazone.
2. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.); 7, Allée Kastner CS 30026, F-67081 Strasbourg (France).

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