APPLICATION BRIEF 73488

Straightforward implementation of a compendial LC method for metolazone impurity analysis with the Vanquish Core HPLC system

Author: Maria Grübner,
Thermo Fisher Scientific, Germering, Germany

Keywords: HPLC, Vanquish Core HPLC system, metolazone, European Pharmacopoeia, routine analysis, pharma

Application benefits

Reliable and highly reproducible chromatographic results were obtained when implementing the liquid chromatography (LC)-based impurity method from the Ph. Eur. monograph of metolazone.

Goal

Highlight the reliability of the Thermo Scientific™ Vanquish™ Core HPLC system in routine LC analysis.

Introduction

Pharmacopoeial 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 analyses 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. In the current application brief, seamless implementation of a liquid chromatographic analytical method published by the European Pharmacopoeia (Ph. Eur.) is demonstrated using the Vanquish Core HPLC system.

The API metolazone is a diuretic drug, which 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 details

Reagents and materials	Part number
Deionized water, 18.2 MΩ·cm resistivity or higher	n.a.
Fisher Chemical™ Methanol Optima™ LC/MS grade	A456-212
Fisher Chemical™ Potassium dihydrogen orthophosphate for HPLC	P/4806/50
Ph. Eur. reference standard: Metolazone for system suitability (SST) CRS batch 1[2]	Y00007022

ThermoFisher SCIENTIFIC

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

The following instrument was used:

	Vanquish Core Quaternary
	System Base Vanquish Core (P/N VC-S01-A-02)
Pump	Quaternary Pump C (P/N VC-P20-A-01)
Sampler	Split Sampler CT (P/N VC-A12-A-02)
Column compartment	Column Compartment C (P/N VC-C10-A-03)
Detector	Diode Array Detector CG (P/N VC-D11-A-01)
Flow cell	Standard (10 mm, 13 µL, P/N 6083.0510)

Data processing and software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.3 was used for data acquisition and analysis.

Results and discussion

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

The system suitability criteria for method acceptance as outlined in the Ph. Eur. monograph require a minimum resolution of 1.6 for impurity peaks E and C and a minimum resolution of 1.5 for impurity peaks A and B. These criteria were easily met and with significantly higher resolution values than minimum acceptance limit would require (2.6 and 1.9). As presented, the Ph. Eur. method was successfully implemented with the Vanquish Core HPLC instrument.

HPLC conditions	
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	0 min – 30% B 5 min – 30% B 25 min – 50% B 35 min – 50% B 38 min – 30% B 48 min – 30% B
Column temperature	30 °C (forced air)
Autosampler temperature	8 °C
Detection	Wavelength: 230 nm, Bandwidth: 4 nm Data collection rate: 5 Hz Response time: 1 s
Injection volume	10 µL
Needle wash	Off

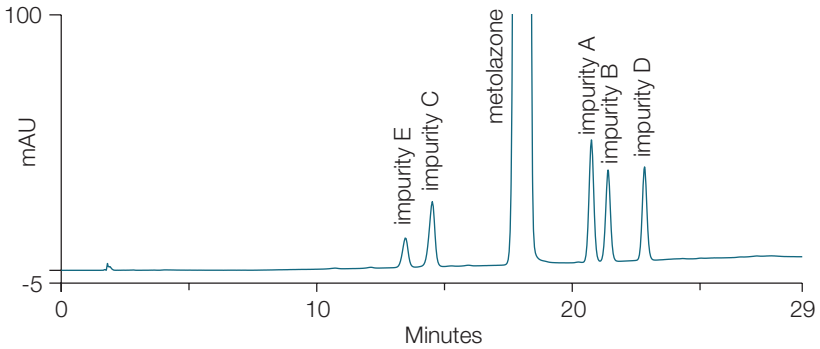

Figure 1. Example chromatogram of API metolazone and its five impurities

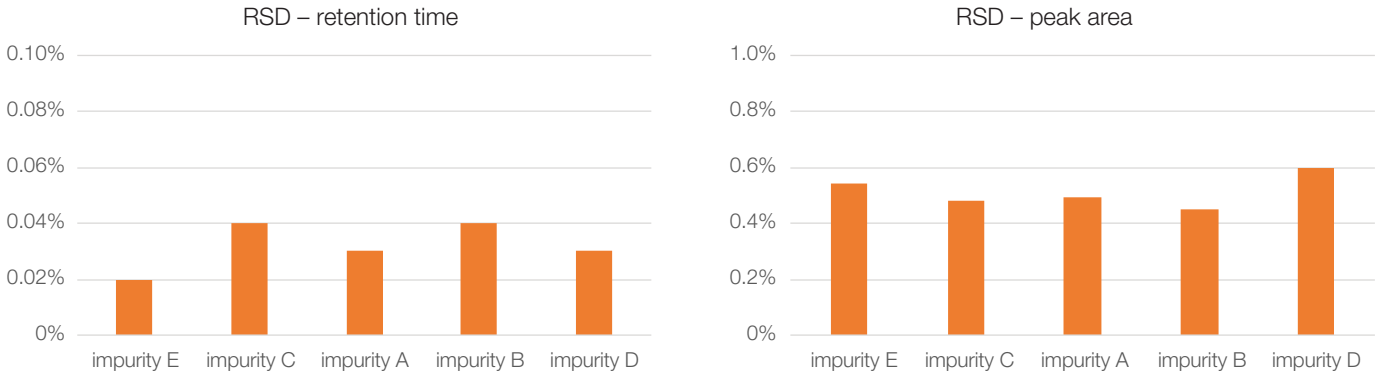

Figure 2. Relative standard deviations (RSD) of retention times and peak areas of impurities over six injections obtained by the Vanquish Core HPLC system

The excellent repeatability of retention times and peak areas of the impurities, expressed as relative standard deviations (RSD) over the six injections, is shown in Figure 2. Retention time RSDs range from 0.02% to 0.04%, while peak area RSDs were between 0.4% and 0.6%.

Conclusion

The Ph. Eur. method for metolazone impurity analysis was easily implemented with the Vanquish Core HPLC system. System suitability criteria were met, and reliable and repeatable results were obtained assuring the system to fulfill requirements when placed in a 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).

Find out more at **thermofisher.com/vanquishcore**

ThermoFisher SCIENTIFIC