APPLICATION BRIEF

Straightforward implementation of a compendial LC method for metolazone impurity analysis

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

Sylvia Grosse, Maria Grübner Thermo Fisher Scientific, Germering, Germany

Keywords: HPLC, Vanquish Core HPLC system, metolazone, European Pharmacopoeia, routine analysis, pharmaceutical, SII for Empower, Empower 3 FR5

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 1² (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 B: Methanol	H_2PO_4 in water						
Flow rate	1.5 mL/min							
Gradient	Time (min) 0 5 25 35 38 48	% B 30 30 50 50 30 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.

В

ile Edit	View	v Inject	t Action	ns Too	ls Custo	mize	Help								i 🔊 Tak	e Control 🚪	Consumables + 🎲 😳	8.		
b %	. 8		•	·5 0	6 🖉	ЮЙ н	H -C-	X	Þ	B 🕣	Run Only		~	Continue on	Bome	Themo E	npowerLink Pump	Sampler Column	Comp UV Audit	
									Sample	Set Methor	t Untitled				Mod	ule Status	Row / Pressure		Buent	10 10
Vial V (u	nj /ol lnj iL)	of Labe	el Sample	Name	Level	Functi	ion	Method Se Report o Export Meth	t / r nod Re	Label	Processing	Run Time (Minutes	Data Start (Minutes	Next Inj. Delay (Minutes)	S. Rete	Ready ntion Time:	- 8 - 6	- 700 (C) - 400	100 - 100 - 80 - 80 - 60 - 60	100 100 100 100 100 100 100 100
															Mod	ect	-2	200	40 20 0 0	
	-			-											M	ore Options Wellness	0.000 [ml/min] 🚖	0 [bar]	100.0 [%] 0.0 [%] 🜩	0.0 [1]
	-	_		_												Service	1550 bar			Pump_Pressure
	-			_											R	slays/inputs	1250			
	-			-											•	Hold Stop Bow	750			
▶ <mark> \ Si</mark>	ngle)	Sample	s (Sam	ple Sets	s 🕻 Runnir	ng /				<						Continue	250			
								Instrument M	ethod:			To	Sample Set tal Samples	Time Remainin Time Remainin		Purge: Off	-50	1.0 2.0	3.0 4.0	min 5.0 6.0 7.0 8.0 9.0 10.0
												_			Audi	t Trail		10 start or 1		
								Edit		Monitor	Setup		New S	Sample Set Tin	e:	Date	Time	Time	Device	Message
							_								1	9/29/202	9:11:26 AM +02:00	UV		Connection established successfully.
- W						11	ŭ .				<i>c</i>	1	4		2	0 9/29/202	9:11:26 AM +02:00	UV		Connected to VVVU (gr - Senai # SimulationMode - Firmware Version
En	npower	u -	+ 0	Pump		+	1 5	Sampler	- +	🤳 Co	umnCo -	+	Ø UV	- +	3	0 9/29/202	9-11-26 AM +02:00	UV		Traine to assess
															4	0 9/29/202	9.11.26 AM +02.00	00		Initiation driver Vienevich Vienekle Weveleenth Detector
IDL	E	(0	IDLE		0	• 1	DLE	0	IDLI	E	0	IDLE	0	5	 3/23/202 3/23/202 3/23/202 3/23/202 	9:11:26 AM +02:00 9:11:26 AM +02:00 9:11:22 AM +02:00			Thermo Vanquish. W/D - Vanquish W/D, Version 1210.60950. Thermo Vanquish. W/D - Vanquish W/D, Version 1210.60950.
After Du	maine (June N	Ela		0.000 Im	l/min1	Donitie	-	D.CO	Mada	0		/1	0	1	9/29/202	9:11:22 AM +02:00			Initializing driver Vanguish Variable Wavelength Detector.
Hater Ru	and the	AUCUC N		~	0.000 [m	wining	r osibo		n.c8	Mode	5		Lamp	0		9/29/202	9:11:21 AM +02:00			Initializing driver Vanguish Variable Wavelength Detector.
			Pre	ssure	([bar]	Volum	ne	10.00 [µl]	Column	emp 24.97	[°C]			3	9/29/202	9:11:21 AM +02:00			Initializing driver CmDDKDry 1.

С



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).

Find out more at thermofisher.vanquishcore

For Research Use Only. Not for use in diagnostic procedures. ©2022 Thermo Fisher Scientific Inc. All rights reserved. Waters is a registered trademark of Waters Corp. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. AB000340-na-en 0322S

