Application Note 20525

Separation of Metformin and Voglibose Using a Thermo Scientific Accucore HILIC HPLC Column

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Key Words
Metformin, Voglibose, Accucore HILIC HPLC Column

Abstract
This application note demonstrates the use of the Accucore™ HILIC HPLC column for the separation of two highly polar anti-diabetic drugs, metformin and voglibose.

Introduction
Voglibose is an α-glucosidase inhibitor, used as an oral anti-diabetic drug used for the treatment of diabetes mellitius type 2[1,2]. It inhibits the activity of α-glucosidase that catalyze the decomposition of disaccharides into monosaccharides in the intestine, thereby delaying the digestion and absorption of carbohydrates, resulting in improvement of postprandial hyperglycemia.

Metformin is the oral anti-hyperglycemic drug used in the management of type 2 diabetes and is in the class of compounds known as biguanides. Metformin improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial blood glucose. Metformin also decreases hepatic glucose production, decreases intestinal absorption of glucose and improves peripheral glucose uptake and utilization.

A study of voglibose on postprandial hyperglycemia and serum lipids in type 2 diabetic patients[3], has shown that as well as reducing the postprandial hyperglycemia, it has therapeutically beneficial effects on reducing triglycerides (TGL) level during combination therapy with sulphonyl-ureas or biguanides.

As there is no robust analytical method reported for the separation of voglibose and metformin when taken in combination, the present study was aimed at providing a separation of voglibose and metformin using a HILIC mode of chromatography. Since the molecule of voglibose neither has a chromophore nor any fluorescent group in its structure, mass spectrometry was selected as a detection technique.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing.

Accucore HILIC provides enhanced retention of polar and hydrophilic analytes and improved sensitivity for MS detection.

This application note demonstrates the successful separation of voglibose and metformin using an Accucore HILIC 2.6 µm HPLC column.

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Experimental Details

Consumables	Part Number
Fisher Scientific Optima LC/MS grade acetonitrile	A955-4
Water, from TKA Water Purification System	
Fisher Scientific Optima LC/MS Ammonium formate	A115-50
Metformin hydrochloride	Akums Pharma
Voglibose	Akums Pharma
Thermo Scientific Micro+™ Vial 300 µL, Fused Insert	60180-507
Thermo Scientific 9mm Screw Top Cap W/ PTFE/Silicone septa	60180-516

Preparation of Samples

Separate stock solutions of metformin hydrochloride and voglibose were prepared in 50:50 (v/v) methanol/water at a concentration of 1 mg/mL. A working standard, containing 1 µg/mL metformin and 2 µg/mL voglibose, was prepared by serial dilution of the above stock solutions in 85:15:0.1 (v/v/v) acetonitrile/water/formic acid.

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela 1250 pump, interfaced to both a Thermo Scientific Accela Open Autosampler, and, a Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.	
Column:	Accucore HILIC 2.6 µm, 50 x 2.1 mm	17526-052130
Mobile phase:	17:83 (v/v) 100 mM ammonium formate, adjust pH to 3.2 with formic acid/acetonitrile	
Flow rate:	0.4 mL/min	
Column temperature:	Ambient	
Injection volume:	5 µL	
Syringe volume:	100 µL	
Loop Size:	20 µL	
Syringe flush:	Wash 1: 90:10 (v/v) acetonitrile/water	
	Wash 2: 50:50 (v/v) methanol/water	
Cool stack temperature:	10 °C	
Detection:	MS	
Column backpressure:	57 bar	

MS Conditions

Thermo Scientific TSQ Vantage	
Ion Source Type	**HESI-2**
Polarity	Positive
Spray Voltage (V)	3000
Vaporizer Temperature (°C)	275
Sheath Gas Pressure (Arb)	35
Ion Sweep Gas Pressure (Arb)	1.0
Auxiliary Gas Pressure (Arb)	10
Capillary Temperature (°C)	350
Declustering Voltage (V)	0
Collision Pressure (mTorr)	1.5

Table 1: TSQ Vantage ionisation parameters

MS Acquisition Parameters

Identification was performed by selected reaction monitoring (SRM) using the precursor-to-product combinations shown below:

Compound	[M+1] m/z	Product m/z	Collision energy	S-Lens
Metformin	130.089	71.090	21	56
Voglibose	268.157	92.070	21	87

Table 2: TSQ Vantage acquisition parameters

Scan type:	SRM
Peak width:	Q1 - 0.7 (FWHM)
	Q3 - 0.7 (FWHM)
Scan width:	0.02 m/z
Scan time:	0.1 s
MS acquisition time:	3.5 minutes

Data Processing

All data were processed using Thermo Scientific LCQuan (v. 2.6) software. Algorithm for integration - ICIS

Results

The analysis was performed on an Accucore HILIC 2.6 µm, 50 x 2.1 mm HPLC column. As shown in Figure 1, metformin and voglibose were analyzed in less than 3.5 minutes. Table 3 shows the results from six replicate injections.

	Metformin	Voglibose
Retention time (minutes)	1.05 (K'=2.9)	1.96 (K'=6.3)
%RSD on retention time	0.42	0.52

Table 3: Retention time results for metformin and voglibose

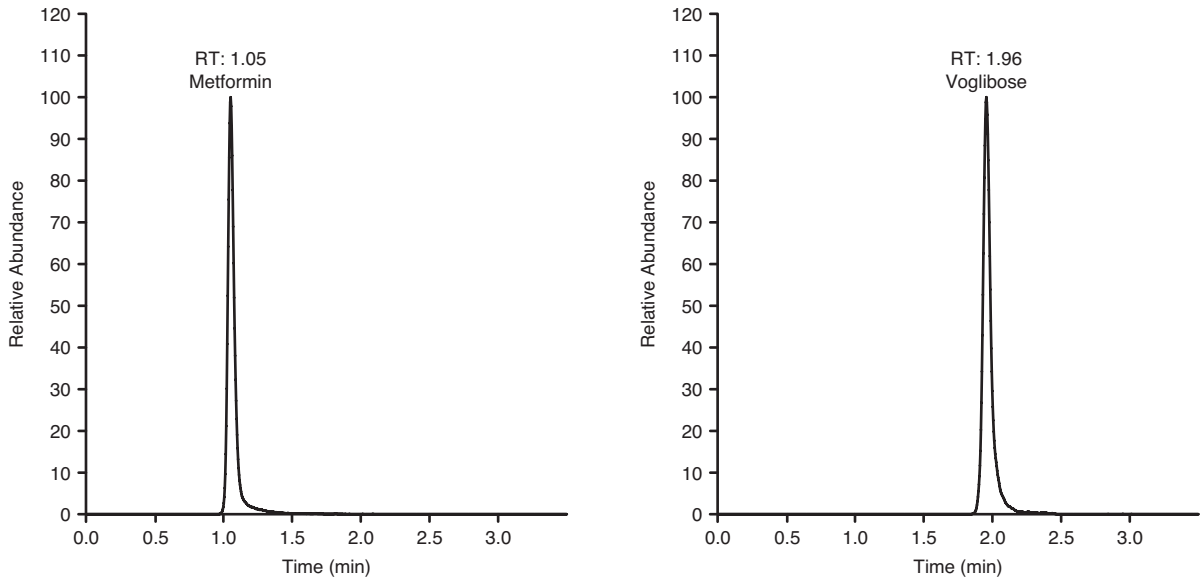

Figure 1: SRM chromatograms of metformin and voglibose on Accucore HILIC 2.6 µm, 50 x 2.1 mm column

Conclusion

Six replicate injections of metformin and voglibose showed that Accucore HILIC produced stable and reproducible results. This demonstrates that Accucore HILIC is an excellent choice of column for the separation of voglibose and metformin.

References

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