An Improved Fast RP-HPLC Method for Rosuvastatin and its Degradation Products Using a Thermo Scientific Accucore PFP HPLC Column

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Rosuvastatin, core enhanced technology, Accucore PFP HPLC column, anti isomer separation, HPLC

Abstract
This application note demonstrates the advantages of using an Accucore™ PFP HPLC Column for the determination of rosuvastatin and its degradation products by HPLC-UV.

Introduction

Rosuvastatin is classified as a statin, a type of agent that inhibits cholesterol production in the liver. Rosuvastatin calcium is a synthetic lipid-lowering agent approved as a treatment for hypercholesterolemia.

Rosuvastatin is an inhibitor of HMG-CoA reductase, an enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis.

Rosuvastatin reduces cholesterol by increasing the number of low-density lipoprotein (LDL) receptors on the cell-surface to enhance uptake and catabolism of LDL. It also inhibits hepatic synthesis of hepatic very-low-density lipoprotein (VLDL), which reduces the total number of VLDL and LDL particles. The treatment reduces tri-glycerides (TG) and produces increases in high-density lipoprotein cholesterol (HDL-C.)

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. Accucore PFP uses an optimized perfluorinated benzene ring for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

This application note demonstrates the successful resolution between rosuvastatin and its anti isomeric impurity using an Accucore 2.6 µm column as compared to UHPLC[1], without the high backpressures associated with the technique.

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Experimental Details

Consumables	Part Number
Water, from TKA Water Purification System	
Fisher Scientific HPLC grade potassium dihydrogen orthophosphate	13415
Fisher Scientific HPLC grade orthophosphoric acid	ZA260-500
Fisher Scientific HPLC grade acetonitrile	26892-1000
Fisher Scientific HPLC grade methanol	ZA452SK-4
Thermo Scientific Screw Top 2 mL Vial Kit w/PTFE/Silicone Closure	60180-600
Rosuvastatin procured from Customer	

Solutions
Working standard contained 1000 µg/mL of in mobile phase.

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Dionex UHPLC Plus Focused (Ultimate 3000) system	
Column:	Accucore PFP 2.6 µm, 100 x 3.0 mm	17426-103030
Buffer preparation:	weigh 2.74 g of KH_2PO_4 in 1000 ml of water and adjust pH-2.5 with o-phosphoric acid	
Mobile phase:	70:29:1 (v/v) buffer:acetonitrile:methanol	
Diluent:	50:50 (v/v) acetonitrile:water	
Flow rate:	0.7 mL/minute	
Column temperature:	30 °C	
Injection details:	2.5 µL partial loop	
Injection wash solvent:	50:50 (v/v) water:acetonitrile	
UV detector wavelength:	243 nm	
Backpressure:	approximately 230 bar	

Results

The analysis was performed on an Accucore PFP 2.6 µm, 100 x 3.0 mm column - see Figure 1, which shows the result for acid degradation. Table 1 shows the results from six replicate injections at the 1 ppm level.

Parameter	Rosuvastatin
Retention time (minutes)	7.14
%RSD on retention time	0.09
Area	827.51
%RSD on area	1.08

Table 1: Retention time and area results for rosuvastatin

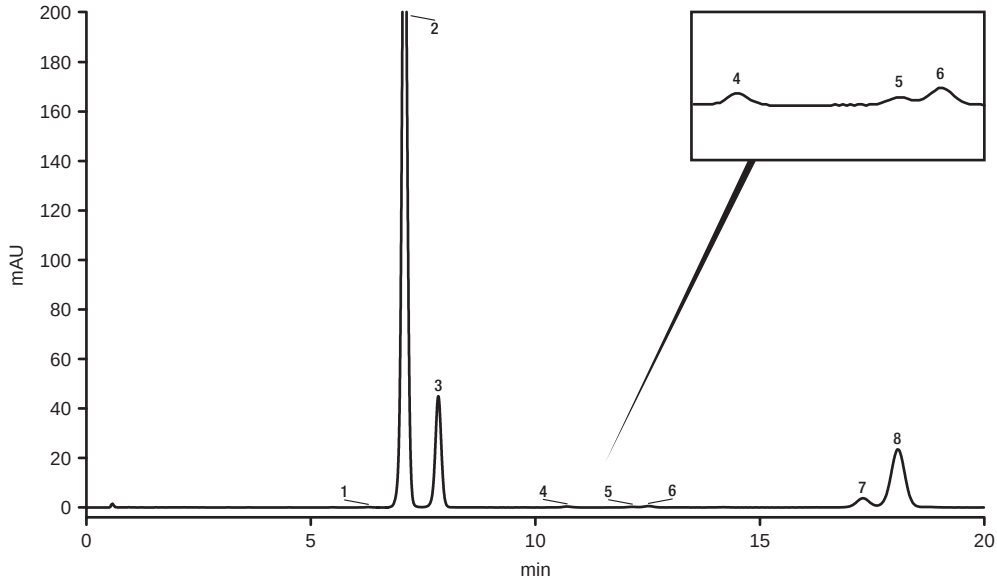

Figure 1: Chromatogram representing resolution of acid degradation for rosuvastatin

1. unknown impurity-1 2. rosuvastatin 3. anti isomer 4. unknown Impurity-2 5. unknown impurity-3 6. unknown impurity-4 7. unknown impurity-5 8. lactone impurity

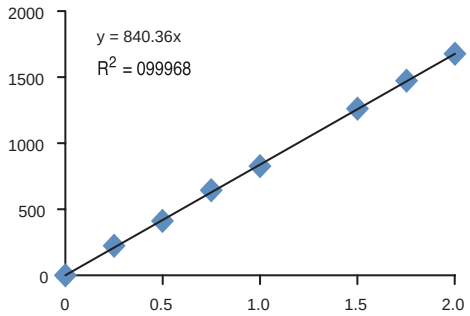

Figure 2: Linearity also established from LOQ (0.25 ppm) to 2 ppm. The value of R2 was found 0.99968

In documented degradation studies of rosuvastatin the acid degradation study showed more degradation than base or oxidation[1]. Acid degradation was carried out using 1N HCl for which results are shown below in Table 2.

Peak name	t_R(Min)	% Rel. Area Response	Resolution
Rosuvastatin	6.98	75.06	N/A
Anti Isomer of Rosuvastatin	7.73	7.72	3.3
Lactone	18.11	15.64	27.7

Table 2: Representation for the extent of degradation and resolution of degradation products with acid induced degraded sample

The method in this application note has a shorter run time than the HPLC method in the literature[2]. It also has comparable resolution of anti-isomer with reported UHPLC methods[1]. Accucore HPLC columns provide benefits in terms of lower backpressures and reduced run times, when compared to the literature methods.

Replicate injections of rosuvastatin showed that Accucore PFP produced stable and reproducible results.

Conclusion

This application note shows that Accucore PFP is an excellent choice of column for resolution of anti isomeric impurities, demonstrating UHPLC like performance at HPLC backpressures[1].

References

1. Development and Validation of a Stability-Iindicating UPLC Method for Rosuvastatin and its Related Impurities in Pharmaceutical Dosage Forms, *Quim. Nova,* **2011** ,Vol. 34, No. 2, 250-255.

2. Determination of Rosuvastatin in the Presence of its Degradation Products by a Stability-Iindicating LC Method, Mehta, T. N.; Patel, A. K.; Kulkarni, G.; Subbaiah, G.; *J. AOAC* Int. **2005**, 88, 1142.

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