Analytical Chemistry Research 12 (2017) 17–27

Contents lists available at ScienceDirect

Analytical Chemistry Research

journal homepage: www.elsevier.com/locate/ancr

Structural identification and estimation of Rosuvastatin calcium related impurities in Rosuvastatin calcium tablet dosage form

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ARTICLE INFO

Article history:
Received 28 August 2016
Received in revised form
11 November 2016
Accepted 14 November 2016
Available online 17 November 2016

Keywords:
Stability studies
Degradant impurity
Isolation and characterization

ABSTRACT

A precise, accurate, specific, linear, rugged and robust analytical method was developed and validated for estimation of process and degradant impurities of Rosuvastatin calcium (RSC) in Rosuvastatin calcium tablets. 150 mm length column, 4.6 mm diameter and 3.5μ particle size with C_{18} stationary phase and pH3.0 phosphate buffer as mobile phase. Column was maintained at 30 °C.All impurities are monitored at 248 nm.Impurities are separated in gradient elution mode. All degradant impurities of RSC (Anti-isomer, 5-ketoacid, lactone and meglumine adduct), process impurity (Imp-A) are well separated. Unknown impurity (Meglumine adduct) formed during stability studies was isolated using preparative HPLC and structure was characterized by NMR and Mass spectrometry (LC-MS and HRMS) studies. Method is capable of separating and estimating all the degradant and process impurities.

1. Introduction

Rosuvastatin calcium (RSC) is a synthetic lipid-lowering agent for oral administration. It inhibits HMG-CoA reductase, the rate-limiting enzyme that converts HMG-CoA to mevalonate, a precursor of cholesterol. The chemical name for rosuvastatin calcium is bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2 [methyl (methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. The empirical formula for rosuvastatin calcium is $(C_{22}H_{27}FN_3O_6S)_2Ca$ and the molecular weight is 1001.14. Rosuvastatin calcium drug substance is official in Ph. Eur monograph. Listed impurities in monograph are Anti-isomer (Imp-B), 5-keto acid (Imp-C), lactone (Imp-D) and Imp-A. Rosuvastatin calcium is available in 5, 10, 20 and 40 mg tablet dosage form. Stability studies play a crucial role in inspecting the quality of the drug product during its shelf life. Excipients used in formulating the drug product may react and form adduct with drug substance. Hence it is necessary to develop such a method which can detect and separate all the possible degradant and process impurities. Few methods are available for estimation of RSC in bulk and pharmaceutical dosage forms by HPLC [1–4], by HP-TLC [5], estimation of RSC in presence of degradation impurities [6,7]. Anti-isomer, 5-keto acid and lactone are the degradant impurities. Anti-isomer and lactone will be formed during acid degradation and 5-keto acid during oxidation. Impurity-A (Acetone adduct) is a process impurity formed during synthesis of Rosuvastatin calcium drug substance. Meglumine adduct is a degradant impurity and it will be formed during stability studies of Drug product at 40°C/75% RH condition. Meglumine is a base used as excipient to stabilize the formulation. Adduct impurity was synthesized by mixing RSC and Meglumine and keeping at 105 °C.Enriched sample was injected in preparative HPLC and collected the impurity. By using HRMS, 1H NMR and 13C NMR techniques, structure for impurity was identified. Structures, chemical names of RSC and impurities are tabulated in Table 1.

2. Instrumentation and reagents

Waters HPLC system (make: Waters) with Photo diode array detector (Model: 2996) was used. Mono basic phosphate was used in mobile phase preparation. Acetonitrile and methanol were used of gradient grade supplied by Merck. AR grade monobasic phosphate supplied by Merck was used in buffer preparation. Sonicator (model: powersonic420) was used in sample preparation. Centrifuge (make: Thermo scientific) was used to centrifuge the sample preparation. Rosuvastatin calcium and all impurities supplied by Biocon. Adduct impurity was synthesized by preparative HPLC.

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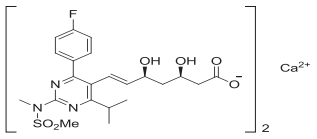
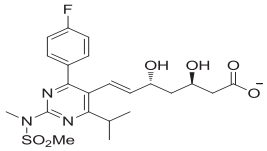
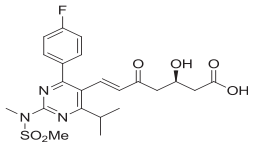
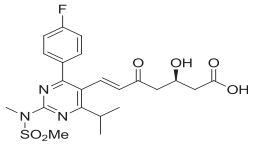
http://dx.doi.org/10.1016/j.ancr.2016.11.002

Table 1
Chemical names and structures for Rosuvastatin calcium and its related impurities.

S.no	Name	Chemical name	Structure
01	Rosuvastatin calcium	(E)-(3R,5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl (methylsulphonyl)amine}pyrimidin-5-yl}-3,5-dihydroxyheptane-6-enoic acid.	
02	Imp-A	(3R,5S,6E)-7-[4-(4-Fluorophenyl)-2-[[(2-hydroxy-2-methylpropyl)sulfonyl](methyl)amino]-6-(1-methylethyl)-pyrimidinyl-5-yl]-3,5-dihydroxyhept-6-enoic acid.	
03	Anti-isomer	(3R,5R,6E)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-(methanesulfonyl- methyl-amino)-pyrimidin-5-yl-3,5-dihydroxy-hept-6-enoic acid calcium	
04	5-Keto acid	Calcium ((3R,6*E*)-7 (4-(4-fluorophenyl)-6-isopropyl-2-(N mehylmethylsulfonamido)pyrimidin-5-yl)-3-hydroxy-5-oxohept-6-enoate)	
05	Lactone	*N*-{4-(4-Fluoro-phenyl)-5-[2-(4-hydroxy-6-oxo-tetrahydro-pyran-2-yl)-vinyl]-6-isopropyl-pyrimidin-2-yl}-*N*-methyl-methanesulfonamide	
06	Adduct	(3R,5S,*E*)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(N- methylmethylsulfonamido)pyrimidin-5-yl)-3,5-dihydroxy-N-methyl-N-((2S,3R,4R,5R)-2,3,4,5,6-pentahydroxyhexyl)hept-6-enamide	

3. Materials and solutions

3.1. Standard preparation (0.2% of test concentration)

Weighed accurately about100mgof Rosuvastatin calcium Working Standard into a 100 mL volumetric flask, added 75 mL of diluent, sonicated to dissolve the drug, and diluted to volume with diluent.10 mL of above solution transferred in to a 250 mL volumetric flask and diluted to volume with diluent. Pipetted 5 mL Rosuvastatin calcium standard stock solution in to 100 mL volumetric flask and diluted to volume with diluent.

3.2. Sample preparation

weighed and transferred 20tablets into a mortar and made a fine powder. Transferred sample equivalent to 100 mg of rosuvastatin calcium into 100 mL volumetric flask. Added 75 mL of diluent and sonicated for 30 min, at less than 25 °C.Diluted to volume with diluent and mixed. Centrifuged a portion of above solution with lid at 5000 rpm for 10 min.

3.3. Spiked sample preparation

Weighed and transferred 20tablets into a mortar and made a fine powder. Transferred sample equivalent to 100 mg of rosuvastatin calcium into 100 mL volumetric flask. Added required quantity of impurity stock solutions to sample solution to get 0.2% of test concentration for all impurities and 0.5% for 5-keto acid impurity. Added 75 mL of diluent and sonicated for 30 min, at less than 25 °C.Diluted to volume with diluent and mixed. Centrifuged a portion of above solution with lid at 5000 rpm for 10 min.

4. Methods

X Bridge 150 × 4.6 mm, 3.5μ HPLC column with C18 stationary phase was used to separate the entire impurities.20 mM monobasic phosphate buffer was and pH adjusted to 3.0 with dilute ortho phosphoric acid was used in mobile phase preparation. Mobile phase-A consists of buffer and Methanol in the ratio of 80:20. Mobile phase-B consists of buffer, acetonitrile and methanol in the ratio of 15:25:60. Column temperature was maintained at 30 °C.Sample temperature was maintained at 5 °C.All the impurities are monitored at 248 nm.Gradient elution mode was used to separate the impurities.20 μL sample was injected into HPLC system.

5. Results

Developed method is capable of separating all the degradant and process impurities of RSC. Method is validated as per ICH guidelines and found to be precise, specific, accurate, linear, robust and rugged. Limit of detection and Limit of quantification were established for all the impurities and RSC. %RSD for Imp-A, adduct, Anti isomer, 5-keto acid and lactone were found to be 0.6, 0.6, 0.6, 0.5 and 0.7 respectively. No placebo peaks are found at the retention times of Imp-A, adduct, Anti isomer, 5-keto acid and lactone impurities. Recovery values for all the impurities at each spike level were found to be between 85% and 115%.Method is found to be linear from LOQ level to 200% of test concentration for all the impurities. Correlation coefficient values are found to be more than 0.997. Reported the slope and intercept values LOQ and LOD values are established for impurities and RSC. LOQ values are found to be less than reporting threshold. Changes were done in mobile phase composition, pH variation, and column temperature and flow rate