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Review Article

A Review on Method Development for the Analysis of Combine Product of Amlodipine Besilate and Rosuvastatin Calcium in Pharmaceutical Dosage form

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Abstract:
Rosuvastatin belongs to the category of statins, used in combination with calcium channel blockers, analgesics and other class of cardiovascular disease drugs in the treatment of high cholesterol and related conditions to prevent cardiovascular diseases. The clinical and pharmaceutical analysis of these drugs requires effective analytical procedures for quality control, pharmacodynamic and pharmacokinetic studies. An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental methods which were developed and used for determination of Rosuvastatin in combined dosage forms have been reviewed. This review covers various analytical methods for estimation of Rosuvastatin calcium and Fenofibrate in combined dosage forms, such as spectrophotometry, derivative spectrophotometry and various high-performance liquid chromatographic (HPLC) methods that were published from 2010 to 2014.
Key Words: Rosuvastatin, Fenofibrate, review, HPLC, Spectrophotometry.

Introduction

This review outlines various analytical methods and other related aspects of determination of Rosuvastatin calcium and Fenofibrate in combined dosage forms by chromatography and spectroscopy. Statins are introduced in the 1980s and this class of compounds is the most efficacious and tolerated hypolilipidaemic drugs. They competitively inhibit conversion of 3- Hydroxy -3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate (rate limiting step in CH synthesis) by the enzyme HMG-CoAreductase. Therapeutic doses reduce CH synthesis by 20- 50%. This results in compensatory increase in LDL receptor expression on liver cells which leads to increasedreceptor mediated uptake and catabolism of IDL and LDL.Rosuvastatin is the newer, commonly used potent statin with a plasma t1/2 of 18-24 hours. Greater LDL-CH reduction can be obtained in severe hypercholesterolemia. The daily dose for lowering LDL-CH by 30-35% is 5 mgof Rosuvastatin. Moreover, at their maximum recommended doses Rosuvastatin 40mg can reduce LDL-CH by upto 55%. All statins except Rosuvastatin are metabolized primarily by CYP3A4. Inhibitors and inducers of this

isoenzyme respectively increase and decrease statin blood levels.

The fibrates (Lipoprotein-lipase activators and isobutyric acid derivatives) primarily activate lipoprotein lipase which is a key enzyme in the degradation of VLDL resulting in lowering of circulating TGs. Activation of peroxisome proliferator activated receptor-α (PPAR-α)enhances lipoprotein lipase synthesis and fatty acid oxidation. PPAR-α may also mediate enhanced LDL receptor expression in liver. Fenofibrate is the 2 nd generation prodrug fibric acid derivative which has grater HDL-CH rising and greater LDL-CH lowering action than other fibrates with a plasma t1/2 of 20 hours. Fenofibrate appears to be the most suitable fibrate for combining with statins, because statin metabolism is minimally affected and enhancement of statin myopathy risk is lower.

A simultaneous estimation of Rosuvastatin calciumand Fenofibrate in binary mixture was developed by HPLC separation of the two drugs in reverse phase mode using Luna C18 column. Linearity was obtained in the concentration range of 1-7 µg/ml and 4-28 µg/ml for Rosuvastatin calcium and Fenofibrate respectively. Retention times of Rosuvastatin calcium and Fenofibrate were obtained as 2.60±0.03 and 7.34±0.03 min respectively. The recovery values were obtained as 99.78-101.08 % and 99.20-101.32 % for Rosuvastatin and Fenofibrate respectively. The % RSDs for Rosuvastatin Calcium and Fenofibrate were reported as 0.1984- 1.4641% and 0.0782-0.6920% respectively. The method has been successfully applied to pharmaceutical formulation and was validated according to ICH guidelines. (Anandakumar Karunakaran, 2011)

A simple, fast and precise reverse phase liquid chromatographic method was developed for the simultaneous determination of Rosuvastatin calcium and Fenofibrate in bulk and in formulations. The chromatography was carried out at 400 C. Separation of these drugs was performed on BDS C-18 column (250×4.6 mm, 5 µ) as stationary phase using a mobile phase comprising of acetonitrile and water in the ratio of 70:30 v/v at a flow rate of 1.5 ml/min. The detection wavelength of Rosuvastatin calcium and Fenofibrate was 287 nm for both the drugs. The retention times for Rosuvastatin calcium and Fenofibrate were found to be 2.0 and 8.5 min respectively. The linearity range for boththe drugs was reported as 1 - 5 µg/ml. The proposed method was found to be accurate, precise and rapid for simultaneous determination of Rosuvastatin calcium and Fenofibrate in the commercial formulations. (Borole T.C,2011)

A rapid, specific, sensitive and simple high performance liquid chromatographic method was developed for simultaneous estimation of Rosuvastatin calcium and Fenofibrate in tablet formulations. The separation was achieved by Phenomenex C18 column (250×4.6 mm, 5 µm) with a mobile phase consisting of methanol and 0.02 M ammonium dihydrogen phosphate buffer mixture (75:25 v/v) at a flow rate of 1.0 ml/min. The detection was carried out at 272 nm. Retention time of Rosuvastatin calcium and Fenofibrate were obtained as 4.18 min and 5.18 min respectively. The linear dynamic range was 12-32 µg/ml and 174-464 µg/ml for Rosuvastatin calcium and Fenofibrate respectively. The method was validated for accuracy, precision, ruggedness and robustness. The accuracy studies showed 99.00 % recovery for Rosuvastatin and 100.00 % recovery for Fenofibrate. The % RSDs for Rosuvastatin and Fenofibrate were reported as 0.7 % and 0.62 %respectively. LOD and LOQ were reported as 0.5 and 1.0 µg/ml respectively for Rosuvastatin and for Fenofibrate as5 and 12 µg/ml respectively. The proposed method was successfully applied for the simultaneous determination ofboth drugs in commercial tablets. The results of the analysis have been validated statistically and by recovery studies. (Devika GS, 2011)

An isocratic RP-HPLC method was developed using Jasco HPLC system with HiQ sil C18 HS (250×4.6mm, 5 µ) column .The mobile phase consisted of a mixture of acetonitrile and water in the ratio of 70:30 v/vat a flow rate of 1.5 ml/min. The eluates were monitored at a

wavelength of 287 nm. The retention times for Rosuvastatin calcium and Fenofibrate were reported as 2.28 min and 13.06 min respectively. The method exhibited good linearity (r2 = 0.99) in the range of 1-10 µg/ml. The proposed method was validated as per ICH guidelines. The stress testing of Rosuvastatin calcium and Fenofibrate was carried out under acidic, alkaline, oxidative, neutral, photolytic and thermal conditions. Rosuvastatin calcium and Fenofibrate were well resolved from respective degradation products. The results indicated that this method was simple, rapid, precise and accurate and also a stability indicating one for determination of Rosuvastatin calcium and Fenofibrate in bulk and in pharmaceutical dosage forms. (Ladke Abhijeet, 2012)

A simple, precise, accurate and rapid HPLC method was developed and validated for simultaneous determination of Rosuvastatin calcium and Fenofibrate in combined tablet dosage form. This method showed adequate separation for Rosuvastatin calcium and Fenofibrate. Best resolution was achieved with Hypersil C18 column (250×4.6 mm, 5 µm) using a mixture of Acetonitrile and water (90:10 v/v) as a mobile phase at a flow rate of 1.0 ml/min. The detection of drugs was carried out at 240 nm.The retention times for Rosuvastatin calcium and Fenofibrate were 2.30 min and 4.92 min respectively. The recovery studies showed 99 % and 100 % recovery for Rosuvastatin Calcium and for Fenofibrate respectively. The % RSDs for Rosuvastatin Calcium and Fenofibrate were reported as 0.454 % and 0.764 % respectively. LOD was reported as 0.227 µg/ml for Rosuvastatin calcium and 3.17 µg/ml for Fenofibrate. LOQ was reported as 0.688 µg/ml for Rosuvastatin calcium and 9.6252 µg/ml for Fenofibrate. The results of the analysis were validated statistically and by recovery studies. The proposed method can successfully be used to determine the drug contents in marketed formulations. (Mohd. Moinuddin, 2012)

A rapid, sensitive and precise analytical method for the simultaneous estimation of Rosuvastatin calcium and Fenofibrate in bulk and in pharmaceutical dosage forms was developed by RPHPLC. The method was based on HPLC separation of two drugs in reverse phase mode using Luna C18 column. Linearity was obtained in the concentration range of 1-7 µg/ml and 4-28 µg/ml for Rosuvastatin calcium and Fenofibrate respectively. The method after validation according to ICH guidelines was successfully applied to quality control of combined pharmaceutical formulations containing Rosuvastatin calcium and Fenofibrate. (Sharma, 2012)

A rapid, specific, sensitive and simple high performance liquid chromatographic method was developed for simultaneous estimation of Rosuvastatin calcium and Fenofibrate in tablet formulation. The separation was achieved by X-TERRA column RP-C18 (150×4.6 mm, 3.5 µ) with a mobile phase consisting of sodium dihydrogen phosphate buffer and Acetonitrile mixture (35:75 v/v) at a flow rate of 0.8 ml/min. The detection of the drugs was carried out at 256 nm. Retention times of Rosuvastatin calcium and Fenofibrate were found to be 2.006 and 3.856 min, respectively. The linear dynamic range was 10-50 µg/ml and 160-800 µg/mlfor Rosuvastatin calcium and Fenofibrate respectively. The method was validated for accuracy, precision, specificity, ruggedness, robustness, LOD and LOQ. The accuracy studies showed 100.5 % recovery for Rosuvastatin and 99.9 % recovery for Fenofibrate. The %RSDs for Rosuvastatin and Fenofibrate were reported as 0.24 % and 0.21 % respectively. LOD and LOQ were reported as 2.98; 9.98 µg/ml and 2.96; 10 µg/ml for Rosuvastatin and Fenofibrate respectively. The proposed method was successfully applied for the simultaneous determination of both drugs in commercial tablets. The results of the analysis have been validated statistically and by recovery studies. (Swetha Ankireddy, 2012)

An accurate, sensitive and precise RP-HPLC method has been developed and validated for the simultaneous estimation of Rosuvastatin calcium and Fenofibrate in bulk and pharmaceutical dosage forms. The separation

was achieved by Zorbax Eclipse plus C18column (100×4.6 mm, 3.5 µm) in isocratic mode, with mobile phase comprising of Acetonitrile and water in the proportions of 90:10 v/v at a flow rate of 0.6 ml/min with detection wavelength of 243 nm. The retention time of Rosuvastatin calcium and Fenofibrate were 1.93 and 4.65min respectively. The linearity range was 2-16 µg/ml for Rosuvastatin calcium and 14-112 µg/ml for Fenofibrate. The regression data showed good linear relationship witha correlation coefficient of 0.9974 for Rosuvastatin calcium and 0.9954 for Fenofibrate. The method was validated in accordance with the requirements of ICH guidelines. The accuracy studies showed 99.99 %recovery for Rosuvastatin and 99.78 % recovery for Fenofibrate. The % RSDs for Rosuvastatin and Fenofibrate were reported as 1.29 – 1.57 % (intra-day) and 0.90 – 1.89 % (inter-day) respectively. LOD and LOQ were reported as 0.12 and 0.77 µg/ml for Rosuvastatin andas 0.67 and 2.05 µg/ml for Fenofibrate respectively. Moreover, the proposed analytical method was applied to analyze he formulations that are commercially available.(Bhavna A Patel, 2013)

A simple, specific and accurate reverse phase liquid chromatographic method was developed for the estimation of Rosuvastatin calcium and Fenofibrate in combined dosage forms. The separation of the two drugs was performed in reverse phase mode using C18 column (Agilent ODS UG 5 column 250×4.5 mm dimensions) with mobile phase containing acetonitrile, methanol and water mixture (40:40:20 v/v/v) in isocratic mode at a detection wavelength of 252 nm. The retention times of Rosuvastatin calcium and Fenofibrate were 2.3 and 5.0 min respectively. Both the drugs showed good linearity inthe concentration range of 1-5 µg/ml and 8-40 µg/ml for Rosuvastatin calcium and Fenofibrate with a correlation coefficient of 0.99968 and 0.99969 respectively. The proposed method has been validated according to ICH guidelines and was applied to pharmaceutical formulation, where the method showed good precision with percentageRSD for Rosuvastatin Calcium and Fenofibrate reported as 0.815 and 0.751 % respectively. The percentage recovery reported for Rosuvastatin Calcium andFenofibrate were 99.9 – 100.7 % and 98.9 - 99.9 %respectively. The LOD was reported as 0.19 µg/ml for Rosuvastatin calcium and 0.28 µg/ml for Fenofibrate.LOQ was reported as 0.58 µg/ml for Rosuvastatin calciumand 0.87 µg/ml for Fenofibrate. The recoveries of Rosuvastatin calcium and Fenofibrate were obtained as 100.06 and 99.59 % respectively indicating that the proposed method is accurate and precise for the simultaneous estimation of Rosuvastatin calcium and Fenofibrate in bulk and pharmaceutical dosage forms. (Jajam Thriveni, 2013)

A new simple, precise, accurate and reproducible RP-HPLC method for simultaneous estimation of Rosuvastatin and Fenofibrate in bulk and pharmaceuticalformulations was developed and validated. Separation of Rosuvastatin and Fenofibrate was successfully achieved on a waters Hypersil C18 column (4.6×250 mm, 6.5 µm) with an isocratic mode utilizing orthophosphoricacid buffer (pH 3) and methanol in the ratio of 65:35 v/v at a flow rate of 1.2 ml/min. The drugs in the eluate were monitored at 238 nm. The retention times for Rosuvastatin and Fenofibrate were obtained as 1.950 and 3.858 min respectively. The method was validated and the response was found to be linear in the drug concentration range of 50-150 µg/ml for both Rosuvastatin and Fenofibrate. Thecorrelation coefficients were obtained as 0.999 for both the drugs. The % RSDs for Rosuvastatin and Fenofibrate were reported as 0.72 % and 0.94 % respectively. The LOD and LOQ for Rosuvastatin were 0.0053 and 0.017 µg/ml while for Fenofibrate were 0.00019 and 0.00063 µg/ml respectively. This method showed good percentage recovery of 99.00 % indicating that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard withthe sample. So, the method specifically determines the analyte in the sample without interference from excipientsof tablet

dosage forms. The method was extensively validated according to ICH guidelines for linearity, range,accuracy, precision, specificity and robustness. (M. Sumalatha, 2013)

A new precise, accurate and rapid validated method forthe determination ofRosuvastatin calcium and Fenofibrate has been developed by using reverse phase high performance liquid chromatography in pharmaceutical dosage forms.

Spectrophotometric Methods

A simple, precise and accurate UV spectrophotometric method for simultaneous estimation of Rosuvastatin calcium and Fenofibrate in binary mixturewas developed. It involves absorbance measurement at 243 nm (λmax of Rosuvastatin calcium) and 287 nm in methanol. The linearity wasobtained in the range of 1-6 µg/ml and 4-28 µg/ml for Rosuvastatin calcium and Fenofibrate respectively. The recovery values were obtained within the limits of 98.41 – 99.17 % and 100.02 – 100.18 % for Rosuvastatin and Fenofibrate respectively. The % RSDs for Rosuvastatin Calcium and Fenofibrate were reported as less than 2 % for both the drugs. The method has been successfully applied to pharmaceutical formulation and was validated according to ICH guidelines. (Anandakumar Karunakaran, 2011).

A new, simple and sensitive Spectrophotometric method has been developed for the determination of Rosuvastatin calcium and Fenofibrate in bulk and in pharmaceutical formulations. The drugs obeyed beer's law in the concentration range of 1-10 µg/ml and 2-20 µg/ml for Rosuvastatin and Fenofibrate respectively. Theaccuracy studies showed 100.33 % recovery for Rosuvastatin and 99.10 % recovery for Fenofibrate. The % RSDs for Rosuvastatin and Fenofibrate were reported as 0.000575 % and 0.000582 % respectively. The methodwas found to be simple, accurate, precise, economical androbust. (R.R. Sevda, 2011)

Two simple UV-spectrophotometric methodswere developed for simultaneous determination of Rosuvastatin calcium and Fenofibrate in pharmaceutical formulations. Methanol AR grade was used as solvent. Method-I (Q-Absorbance ratio method) involves formation of Q-Absorbance equation at two wavelengths i.e., 255.99 nm (isoabsorptive point) and 286 nm (λmax of Fenofibrate). The accuracy studies showed 100.56 % recovery for Rosuvastatin and 100.45 % recovery forFenofibrate. The inter and intra-day precision obtained forRosuvastatin Calcium and Fenofibrate were reported as 0.26-1.27 % (inter-day) ; 0.07-0.64 % (intra-day) and 0.27-1.20 % (inter-day) ; 0.06-0.70 % (intra-day) respectively. Method-II (Multicomponent Mode of Analysis) involves the measurement of absorbance at twowavelengths i.e., 243 nm (λmax of Rosuvastatin calcium)and 286 nm (λmax of Fenofibrate). The accuracy studies showed 100.87 % recovery for Rosuvastatin and 101.57% recovery for Fenofibrate. The inter and intra-day precision obtained for Rosuvastatin Calcium and Fenofibrate were reported as 0.11-1.68 % (inter-day) ; 0.09-0.64 % (intra-day) and 0.18-1.98 % (inter-day) ; 0.13-0.87 % (intra-day) respectively. In both methods, Rosuvastatin calcium and Fenofibrate followed same linearity at the concentration range of 03-18 µg/ml at theirrespective λmax values. Both these methods were found to be accurate, precise and rugged as indicated by low valuesof %RSDs. Both these methods were also found to be rapid, economical and can successfully be applied for theroutine analysis of bulk and combined tablet dosage forms. (Prashant S. Mandwal, 2012)

A simultaneous determination of Rosuvastatincalcium and Fenofibrate in fixed dose combined formulation was developed by first order derivative Spectrophotometry. The absorbance values at 233.5 nm and 254 nm of the first derivative spectrum were used forthe estimation of Rosuvastatin calcium and Fenofibrate respectively without mutual interference. This method obeyed beer's law in the concentration range of 5-30µg/ml for Rosuvastatin calcium and 5-35 µg/ml for Fenofibrate respectively. The accuracy studies showed recoveries of 100.58±0.896 % for

Rosuvastatin Calcium and 99.68±0.652 % for Fenofibrate respectively. The interand intraday precision obtained for Rosuvastatin Calciumand Fenofibrate were reported as 0.51 % ; 0.565 % and 0.297 % ; 0.289 % respectively. LOD was reported as 0.35µg/ml for Rosuvastatin calcium and 0.52 µg/ml for Fenofibrate. LOQ was reported as 1.05 µg/ml for Rosuvastatin calcium and 1.58 µg/ml for Fenofibrate. Theresults of analysis have been validated statistically and therecovery studies confirmed the accuracy of the proposed method. (Rekha Rajeevkumar, 2012)

A simple, novel, sensitive and precise validated spectrophotometric method was developed for simultaneous determination of Rosuvastatin calcium and Fenofibrate in synthetic mixture and in its dosage form. Methanol was selected as a common solvent for estimation of Rosuvastatin calcium and Fenofibrate with λmax at 243 nm and 224 nm respectively. The linearity was obtained in the concentration range of 4-12 µg/ml for Rosuvastatin and 16-48 µg/ml for Fenofibrate. The Zero crossing points of Rosuvastatin and Fenofibrate were 224.11 nm and 243.29 nm respectively. The correlation coefficients obtained for Rosuvastatin and Fenofibrate were 0.9963 and 0.9996 respectively. The % RSD for intra-day precision was 0.76-1.05 % for Rosuvastatin and0.32-1.16 % for Fenofibrate. The inter-day precision was0.76-1.82 % for Rosuvastatin and 0.42-1.47 % for Fenofibrate. The detection limit and quantification limit were found to be 1.96 ; 5.96 µg/ml for Rosuvastatin and 0.76 ; 2.32 µg/ml for Fenofibrate respectively. All the validation parameters were checked as per the ICH guidelines. The recoveries were obtained as 100.9-103.2% for Rosuvastatin and 100.9-101.3 % for Fenofibrate. No interference from the tablet excipients showed theapplicability of the method to the routine analysis of the pharmaceutical dosage forms. (Bhavna Patel, 2013)

A simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method forthe simultaneous determination of Rosuvastatin calcium and Fenofibrate in combined pharmaceutical dosage formwas developed. The derivative spectrophotometric method was based on the determination of both the drugsat their respective Zero Crossing Point (ZCP). The first order derivative spectrum was obtained in methanol and the detections were made at 243 nm (ZCP of Rosuvastatin calcium) for Fenofibrate and 239 nm (ZCP of Fenofibrate)for Rosuvastatin calcium. The linearity was obtained in the concentration range of 2-10 µg/ml for Rosuvastatin calcium and 3-15 µg/ml for Fenofibrate. The results of analysis have been validated statistically. The method wasfound to be simple, sensitive, accurate and precise as per ICH guidelines. The accuracy studies showed 100.98 % recovery for Rosuvastatin and 101.23 % recovery for Fenofibrate. The inter and intra-day precision obtained forRosuvastatin Calcium and Fenofibrate were reported as 0.29-1.17 % (inter-day) ; 0.21-0.50 % (intra-day) and 0.70-1.46 % (inter-day) ; 0.28-0.99 % (intra-day) respectively. LOD and LOQ were reported as 0.14 ; 0.42 µg/ml and 0.33 ; 1.02 µg/ml for Rosuvastatin and Fenofibrate respectively. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial pharmaceutical dosage forms. (Sumit Vyas, 2013)

Authors	Linearity range (µg/ml)		Accuracy (%)		Precision (%RSD)	
	ROS	FEN	ROS	FEN	ROS	FEN
Anandakumar Karuna karan, 2011	1-7	4-28	98.41-99.17	100.02-100.18	0.1376-0.8428	0.1480-0.6376
R.R. Sevda, 2011	1-10	2-20	100.33	99.10	0.00057	0.000582
Prashant S. Mandwal, 2012	03-18	03-18	100.87	101.57	0.11-1.68	0.18-1.98
Rekha Rajeev kumar, 2012	5-30	5-35	100.58	99.68±0.652	0.51	0.297
Bhavna Patel, 2013	4-12	16-48	100.9-103.2	100.3-101.3	0.76-1.82	0.42-1.47
Sumit Vyas, 2013	2-10	3-15	100.98	101.23	0.29-1.17	0.70-1.47
Vijaykumar Parmar, 2013	4-12	16-48	98.32-100.09	95.19-105.3	0.79-1.52	0.43-1.53

Conclusion

This review is focused at outlining the various HPLC methods, Spectrophotometric methods and other related aspects of Rosuvastatin and Fenofibrate incombined dosage forms. By analyzing the validation dataof the reported methods, it can be concluded that the RP- HPLC methods are more sensitive and rapid than spectrophotometric methods. The RP-HPLC methoddeveloped by S. Thukabai et al., for estimation of Rosuvastatin and Fenofibrate is most accurate and precise with low retention times reported for the drugs. The column used was Agilent XDB C18 column and themobile phase optimized was a mixture of 0.01 M potassium dihydrogen phosphate and methanol (55:45 v/v). In view of the various spectrophotometric methods developed for Rosuvastatin and Fenofibrate in combined dosage forms it was observed that the solvents used were either methanol or distilled water. The Spectrophotometric estimation developed by R. R. Sevdaet al., (2011) can be considered as most sensitive and precise method showing linearity in the range of 1-10 µg/ml and 2-20 µg/ml for Rosuvastatin and Fenofibrate respectively. The % RSDs were reported as 0.000575 % and 0.000582 % respectively for Rosuvastatin and Fenofibrate with methanol as solvent. The spectroscopic methods developed by Bhavna A Patel in 2013 and Prashant S. Mandwal in 2012 also showed high levels of accuracy and are also quite sensitive with low LOD values. Hence in this review it can be found that several research groups have keenly worked on the simultaneousestimation methods of Rosuvastatin and Fenofibrate by HPLC and Spectrophotometry. These methods canextensively be used for relative analysis of the drugs in bulk and in dosage forms. With the knowledge gained from this extensive information, newer analytical methodscan be developed with advancing technology.

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