IJBPAS, October, 2018, 7(10): 1832-1843

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DETERMINATION OF ROSUVASTATIN CALCIUM IN RABBIT'S SERUM BY DEVELOPING A NEW RP-HPLC/UV METHOD: VALIDATION AND OPTIMIZATION OF VARIOUS PARAMETERS

SARFRAZ RM[1*], AKRAM MA[2], HASAN ZU[3], AKRAM MR[1] AND QAISAR MN[1]

[1]College of Pharmacy, Faculty of Pharmacy, University of Sargodha40100, Punjab, Pakistan

[2]Department of Pharmacy, Gomal University, Dera Ismail Khan

[3]Global Pharmaceuticals, Industrial Area Islamabad, Pakistan

***Correspondence: Rai Muhammad Sarfraz, E Mail: sarfrazrai85@yahoo.com**

Received 27th March 2018; Revised 12th April 2018; Accepted 17thMay 2018; Available online 1st Oct. 2018

https://doi.org/10.31032/IJBPAS/2018/7.10.4489

ABSTRACT

A novel, precise, accurate and rapid isocratic reversed-phase high performance liquid chromatographic/ultraviolet (RP-HPLC/UV) method was developed, optimized and validated for determination of rosuvastatin inrabbit's serum. Effect of different experimental parameters and various particulate columns on the analysis of these analyte was evaluated. The method showed adequate separation for rosuvastatin and best resolution was achieved with Brownlee analytical C18 column (150×4.6mm, 5µm) using acetonitrile–water (50:50, v/v; pH adjusted to 3.0with orthophosphoric acid) as a mobile phase at a flowrate of 1.0 ml/min and wavelength of 243 nm. The calibration curves were linear over the concentration ranges of 1.8–260 ng/ml for rosuvastatin. The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) for rosuvastatin were 0.6 and 1.8 ng/ml respectively. All the analytes were separated in less than 6.0 min. The proposed method could be applied for routine laboratory analysis of rosuvastatin and in rabbit serum samples, pharmaceutical formulations, drug–drug interaction studies and pharmacokinetics studies.

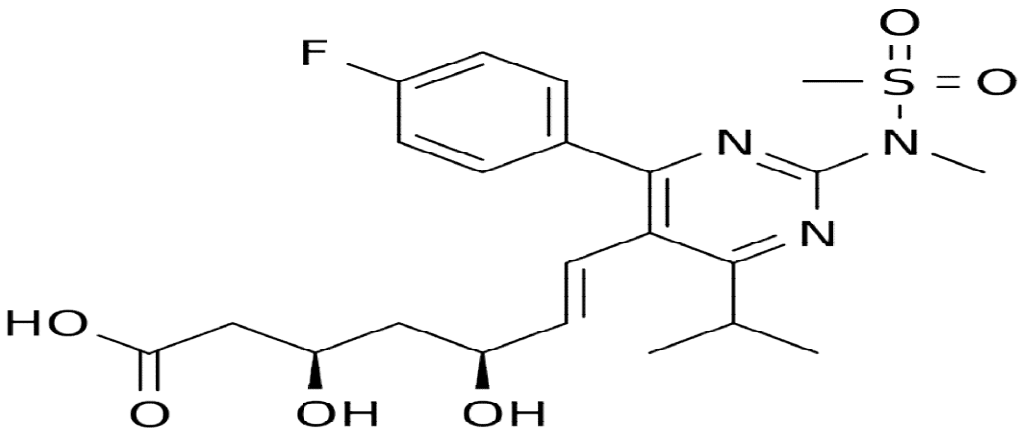
Keywords: Rosuvastatin calcium, high performance liquid chromatography, extraction, mobile phase, pharmacokinetics

INTRODUCTION

Rosuvastatin calcium salt, belong to the statin class of drugs used to treat hypercholesterolemia both in patients with established cardiovascular disease as well as those who are at a high risk of developing atherosclerosis. These drugs inhibit the rate limiting key enzyme known as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase involved in cholesterol biosynthesis. Statins cause reduction in low density lipoproteins-C (LDL-C), total cholesterol (TC) and triglycerides (TG) and elevation in high-density lipoprotein-C (HDL-C). Chemically it is abis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methyl-sulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoicacid] calcium salt[1–5]. Besides lipid lowering effects, statins also have potential roles independent of cholesterol reduction as anti-oxidative [6–8], anti-tumor [9], anti-inflammatory [8, 10, 11], immunomodulator [7,12], anti-malarial [13] and bone forming agents [14]. Thus, due to theirso many beneficial effects, there is growing interest in developing analytical methods for statins monitoring. Until the approval of rosuvastatin in 2003, other statins were most efficacious used [15] but recent studies reported rosuvastatin as a potent inhibitor of HMG-CoA reductase having a higher LDL-lowering effects as compared with other statins [16],which demonstrates that rosuvastatin is one of the leading drugs in the statins class.

To date several HPLC–UV and mass spectrophotometric methods have been developed for the quantification of rosuvastatin either alone or in combination with other drugs in different matrices. LC/MS/MS methods reported for quantification of rosuvastatin in biologicalmatrices include its determination either alone [17], in combination with other drugs such as fenofibric acid [18], or its metabolite N-desmethyl rosuvastatin [19]. HPLC-UV methods have also been reported for the determination of rosuvastatin in pharmaceuticals [20], rat plasma [21] and in human plasma along with gemfibrozil [22].

Our suggested method is rapid, versatile, specific, precise and accurate for simultaneous determination of rosuvastatin and in rabbit's serum as well as for rosuvastatin based rapid release tablets. The method was validated according to standard guidelines and various experimental parameters were optimized with the aim that the reported method could be applied for routine laboratory analysis of this statin, pharmacokinetic drug–drug interaction studies and in pharmaceutical dosage forms.



MATERIALS AND METHODS

Materials

Rosuvastatin calcium of 99 % purity was giftedby Getz pharmaceuticals. Pvt. Ltd. (Karachi, Pakistan) and Acetonitrile, diethyl ether, absolute ethanol, dichloromethane, chloroform, diethyl acetate were purchased from Sigma–Aldrich (Oslov, Norway). HPLC grade ultrapure water was prepared by Milli-Q® system (Millipore, Milford, MA, USA).

Animal used to evaluate various pharmacokinetic parameters

12 male albino rabbits of average weight 2.5 ± 0.010 kg were used for this study. Rabbits were divided into 2 groups of 6 rabbits each (n=6). All the rabbits were fasted overnight with adlibitum access to water during experiment and animals were fed 4 hr after oral dose. Each group of animals had received a single dose comprised of 20 mg of rosuvastatin. Samples were withdrawn at 0, 0.5, 1, 2, 3, 4, 5, 6, 12 and 24 hr.

Instrumentation and analytical conditions

Agilent 1100 series HPLC system consisted of LC-10 AT VP pump, DGU-14 AM on-line degasser, Rheodyne manual injector fitted with a 20 µL loop, and SPD-10 AVP UV–VIS detector and a Hypersil BDS C 8 (250 X 4.6 mm) column was utilized for separation. Chromatographic system was integrated via Shimadzu model CBM- 102 Communication Bus Module to P-IV computer loaded with CLASS-GC software (Version 5.03) for data acquisition and mathematical calculations. Centrifuge Machine (Model 4000-China), Vortex Mixer (Seouline Bio Scirnce-Korea), pH Meter (WTW pH 300-Germany), Ultrasonic Bath (Fisher Scientific FS 28 H-Germany), Electric Balance, (Percia XB 120A), Membrane Filter (Sartorius, 0.45 µm filters-Germany), Distillation Plant, Micropipettes (Softpet- Finland), Filtration

Assembly (Pyrex-France), Distillation Plant (WDA/4 R & M England).

Preparation of standard solutions

The stock solutions of rosuvastatin were prepared by dissolving appropriate amount corresponding to 1.0mg/ml concentration of working standards in methanol. All stock solutions were stored at 2–8 °C. The stock solutions were further diluted with the mobile phase acetonitrile–water (50:50, v/v; pH adjusted to 3.0 with orthophosphoric acid) to give a series of standard mixtures having a final concentration in the range of 2.0–500 ng/ml.

Sample preparation

A simple one step liquid–liquid extraction (LLE) procedure was carried out for the extraction of rosuvastatin from serum samples. A volume (50µl) of the working solution (to give a final concentration of 500 ng/ml) was added to 200µl of serum and mixed for approximately 10 s. Then absolute ethanol (600µl) was added and vortex-mixed for 2 min for deproteination. Next1.0 ml of diethyl ether (extraction solvent) was added, vortex-mixed for 5min and centrifuged at 3500 rpm at 0 °C for 5min. The organic layer was separated, collected in the same tube and evaporated to complete dryness under the gentle stream of nitrogen on a heating block maintained at 40 °C. After drying, the residue was reconstituted in 500 µl of mobile phase, vortex-mixed for 2 min and 20 µl sample was injected onto HPLC system.

Chromatographic conditions

Chromatographic separation was performed with different proportions of acetonitrile–water and methanol–water as a mobile phase with different flow rates in the range of 0.8–1.6ml/min in an isocratic mode. The injection volume was kept in the range of 10–50µl. The column oven temperature was varied in the range of 25–35 °C and the eluate was monitored using UV detection at various wavelengths in the range of 220–280 nm. Various experimental parameters were optimized for simultaneous determination of rosuvastatin.

Method validation

The suggested analytical method was validated according to international guidelines with respect to certain parameters such as specificity/selectivity, linearity, LLOQ, LLOD, precision, accuracy, sensitivity, recovery and robustness/ruggedness [23].

Linearity

The linearity of the method was established by spiking a series of standard mixtures of rosuvastatin (2.0–500 ng/ml), extracting and analyzing in triplicate. Calibration curves for standard solutions and spiked serum samples

were then acquired by plotting their response ratios (ratios of the peak area of the analytes to internal standard) against their respective concentrations. Linear regression was applied and slope (a), intercept (b), correlation coefficient (r) and standard error (Es) were determined.

Precision

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision (intra-day and inter-day reproducibility). In order to determine injection repeatability, serum samples spiked with 500 ng/ml of rosuvastatin were injected 10 times into HPLC system and repeatability of the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained. Similarly, analysis repeatability was verified by analyzing eight serum samples spiked with 500 ng/ml of rosuvastatin prepared individually, determined as amount recovered and expressed as mean and %RSD calculated from the data obtained. For the intermediate precision (intra-day and inter-day reproducibility), serum samples spiked at three different concentration levels were analyzed three times a day in triplicate injections over three consecutive days and expressed as mean ± SD and % RSD calculated from data obtained.

Specificity/selectivity

The specificity/selectivity of the analytical method was investigated by confirming the complete separation and resolution of all the desired peaks of the analytes in mobile phase and spiked rabbit's serum.

Accuracy

Accuracy was determined in terms of percent recovery. Blank rabbit's serum was spiked with the analytes at five different concentration levels (2.0, 64, 145, 250, 500 ng/ml of rosuvastatin. Another set of standard mixtures at the same concentration levels was also prepared in the mobile phase (acetonitrile–water, 50:50, v/v; pH adjusted to 3.0 with orthophosphoric acid). The serum was extracted with the procedure noted above and injected onto the HPLC system in triplicate. Percent recoveries for rosuvastatin was calculated using the following formula:

$$\% \textbf{ Recovery} = \frac{\boldsymbol{A}}{\boldsymbol{B}} \times \mathbf{100}$$

Where A is the response ratio of the analyte in serum sample, B is the response ratio of the analyte in standard mixture.

LLOD and LLOQ

Detection and quantification limits were determined through dilution method using S/N approach by injecting a 20 µl sample.

LLOD was considered as the minimum concentration with a signal to noise ratio of at least three (S/N≈3), while LLOQ was taken as a minimum concentration with a signal to noise ratio of at least ten (S/N≈10).

Stability

The stability studies of rosuvastatin spiked serum samples were carried out over a period of 48 h at 25 °C (room temperature under laboratory light), 2–8 °C (refrigerator) and−80 °C (frozen) and standard solutions for one month at 2–8 °C.

Robustness

The robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like column oven temperature (±1 °C), flow rate (±5%) and pH of the mobile phase (±0.2 units).

RESULTS AND DISCUSSION

Several organic solvents were tried for the preparation of stock solutions of all analytes. Methanol was selected due to greater solubility of analytes in it. The corresponding working solutions of rosuvastatin were prepared by diluting their stock solutions with acetonitrile–water (50:50, v/v; pH adjusted to 3.0 with orthophosphoric acid). Acetonitrile, methanol and methanol–ethanol in different ratios were tried for protein precipitation but complete protein precipitation was achieved with absolute ethanol at least three times the volume of serum. Dichloromethane, ethyl acetate, chloroform, n-hexane and diethyl ether were evaluated either alone or in different ratios for the extraction of all the analytes from the serum. Recovery of the rosuvastatin was better when extracted with diethylether. So, best results in terms of recoveries were obtained with diethyl ether (extraction solvent). The residue was reconstituted in 500 µl of mobile phase and 20 µl sample was injected onto HPLC system.

Feasibility of different solvent systems such as acetonitrile–water and methanol–water mixtures in different compositions, pumped at different flow rates (in the range of 0.8–1.6ml/min) having variable pH range (2.0–4.0) and at different column oven temperatures (in the range of 25–35 °C) were evaluated. Best results were obtained using acetonitrile –water in the ratio of 50:50, v/v (pH adjusted to 3.0 with orthophosphoric acid) at a flow rate of 1.0 ml/min. While optimizing the composition of the mobile phase, the pH was fixed to 3.0 and while assessing the effect of pH of themobile phase, the mobile phase composition was acetonitrile –water (50:50, v/v). The retention of all analytes varied considerably by changing the pH of the mobile phase in the

range of 2.0–6.0. Since rosuvastatin (pka = 4.6) isacidic compound so its retention on the columnis likely to be pH dependent. When pH of the mobile phase was decreased from 4.0 to 3.0 the retention times of the analytes decreased unexpectedly and with further decrease in the pH to 2.0 the retention times increased once again. This behavior may be due to a change in the solubility of the analytes in the mobile phase or may be due to change in binding of the analytes to the stationary phase. Therefore, pH 3.0 was chosen as optimum pH because of the reasonable retention times, resolution and separation of rosuvastatin. Various detection wavelengths in the UV range of 220–280 nm were tried for monitoring of all analytes. Keeping in view the theoretical values of molar absorptivity co-efficient of rosuvastatin, the wavelength 243 nm was selected as the optimum wavelength for determination of rosuvastatin. Typical chromatograms of HPLC in mobile phase are shown in fig 1.

Five different types of columns were used to find better separation of drug. While selecting best column for analysis all other parameters were kept constant. Hypersil BDS C 8 (250 X 4.6 mm) column was finally selected for HPLC analysis of rosuvastatin in this method. Typical chromatograms of rosuvastatin calcium determined in rabbit's spiked plasma and samples taken after administration of 20 mg of rosuvastatin calcium are shown in fig 2 and 3 respectively. Retention time was found 5.1 minutes by using Hypersil BDS C 8 (250 X 4.6 mm). The response found was linear upto 2-500 ng/ml. correlation co-efficient was 0.998. The linearity equations and standard errors for the calibration curves of standard mixtures and spiked serum samples of rosuvastatin were measured. Average percent recoveries for rosuvastatin was greater than 98.0% while %RSD value for rosuvastatin was less than 1% indicating accuracy of the reported method. Precision data and intermediate precision (intra-day and inter-dayreproducibility) are summarized in Table 1. The %RSD values for both intra-day and inter-days were less than 2.0%, which indicates that the proposed method was precise.

The LLOD for rosuvastatin standard solutions was found to be 0.6 ng/ml, while LLOQ were found to be 2.0 ng/ml, as shown in Table 1. Results from the stability studies of both spiked serum samples and standard solutions indicated that spiked serum samples were stable for 72 h when stored at room temperature (25 °C), refrigerator (2–8 °C) and frozen (−80 °C), while the standard

solutions demonstrated stability for two month at 2–8 °C. Minor deliberate changes in different experimental parameters such as column oven temperature (±1 °C), flow rate (±5%) and pH of the mobile phase (±0.2 units) did not significantly affect the recoveries, peak area and retention time indicating that the proposed method is robust. To calculate different pharmacokinetic parameters to establish implementation of this method in rabbit's plasma we have administer 20 mg of rosuvastatin to 6 rabbit's. We have calculated $C_{max,}$ $t_{max,}$ $t_{1/2}$and $AUC_{(0\ -\infty)}$as shown in Table 2. These results had revealed that this developed method is good for determination of different pharmacokinetic parameters in plasma.

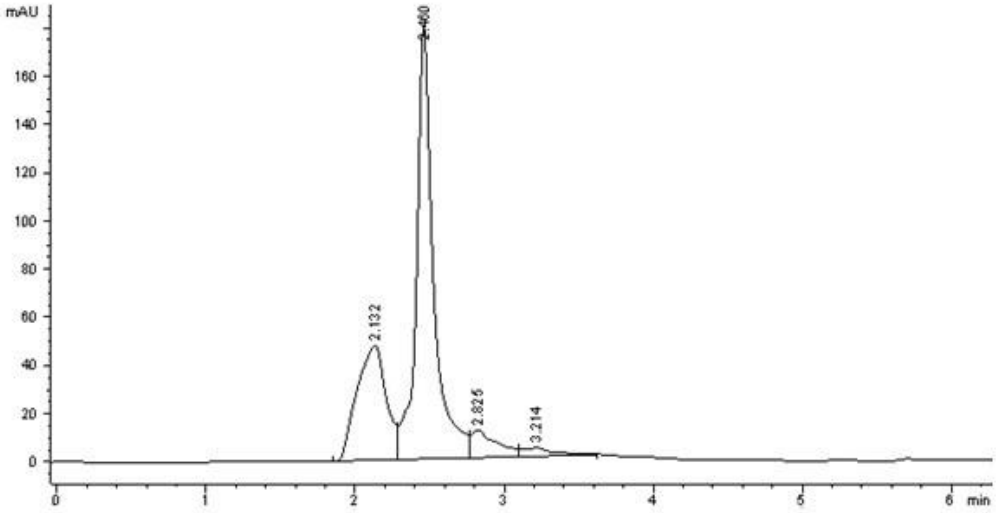


Figure 1: HPLC chromatogram of blanked Plasma

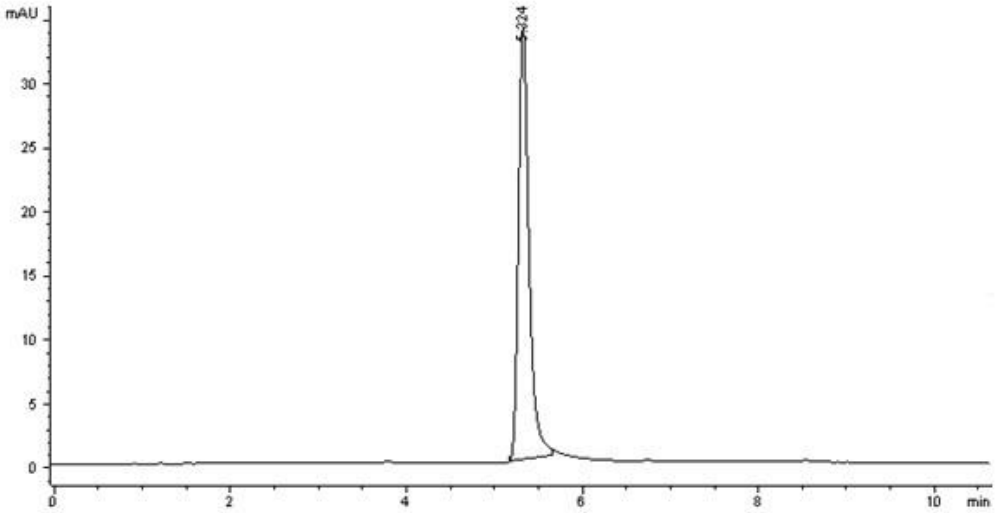


Figure 2: HPLC chromatogram of rosuvastatin in mobile phase

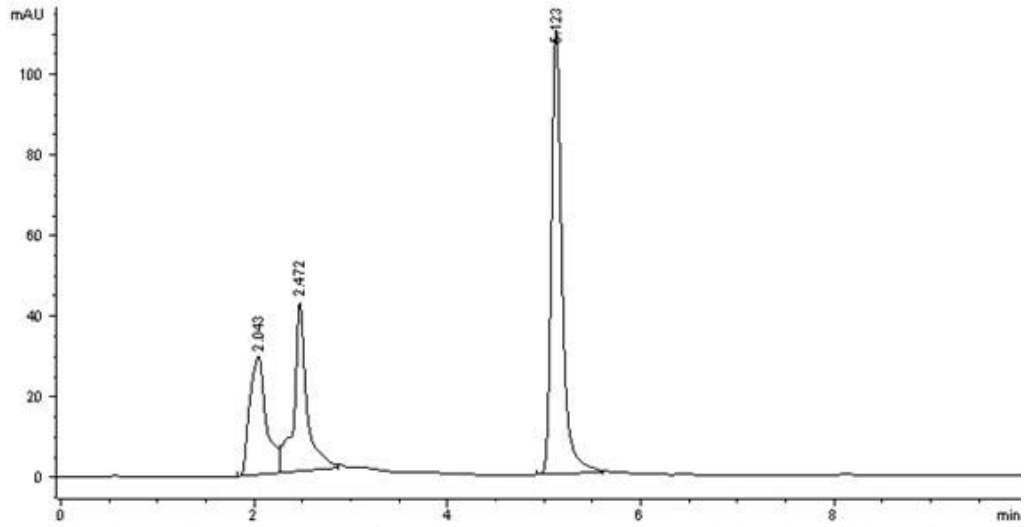


Figure 3: HPLC chromatogram of rabbit's samples taken after administration of 20 mg of drug

Table 1: Intra-day and inter-day precision data (n = 3)

Known Spiked concentration	concentration found			
	Intra-day (mean±SD)	%RSD	Inter-day (mean±SD)	%RSD
2	1.84	0.56	1.80	0.86
64	61.68	0.24	60.98	1.10
250	243.56	0.56	244.12	0.84
500	493.36	0.34	492.86	0.54

Table 2: Pharmacokinetic parameters of rosuvastatin after a single oral dose of 20 mg

Parameters	Commercial tablet
C_{max} (ng/mL)	8.45
t_{max} (h)	7.50
$t_{1/2}$(h)	19.10
$AUC_{(0-\infty)}$ (ng/h/mL)	125.46

CONCLUSION

A novel, simple, rapid and cost effective RP-HPLC/UV method was effectively developed for determination of rosuvastatin in rabbit's serum. The proposed method was optimized and validated for the various experimental parameters. Influence of pH of the mobile phase, column oven temperature and different particulate columns on the analysis of rosuvastatin was evaluated. This method can also be applied for determination of various pharmacokinetic parameters by administering different pharmaceutical dosage forms in human and other animals.

ACKNOWLEDGEMENT

Authors are very obliged to Higher Education Commission (HEC) of Pakistan for providing financial support to conduct this Research Project.

REFERENCES

[1] Olsson AG, McTaggart F, Raza A. Rosuvastatin: a highly effective new HMG-CoA reductase inhibitor.

Cardiovascular Therapeutics, 20 (4), 2002, 303-28.

[2] Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science, 292 (5519), 2001, 1160-4.

[3] Wierzbicki AS, Poston R, Ferro A. The lipid and non-lipid effects of statins. Pharmacology & therapeutics, 99(1), 2003, 95-112.

[4] Davidson MH. Rosuvastatin: a highly efficacious statin for the treatment of dyslipidaemia. Expert opinion on investigational drugs, 11(1), 2002, 125-41.

[5] McTaggart F, Jones P. Effects of statins on high-density lipoproteins: a potential contribution to cardiovascular benefit. Cardiovascular drugs and therapy, 22(4), 2008, 321-338.

[6] Schupp N, Schmid U, Heidland A, Stopper H. Rosuvastatin protects against oxidative stress and DNA damage in vitro via upregulation of glutathione synthesis. Atherosclerosis, 199(2), 2008, 278-87.

[7] Resch U, Tatzber F, Budinsky A, Sinzinger H. Reduction of oxidative stress and modulation of autoantibodies against modified low-density lipoprotein after rosuvastatin therapy. British journal of clinical pharmacology, 61(3), 2006, 262-74.

[8] Sugiyama M, Ohashi M, Takase H, Sato K, Ueda R, Dohi Y. Effects of atorvastatin on inflammation and oxidative stress. Heart and vessels, 20(4), 2005, 133-6.

[9] Weitz-Schmidt G. Statins as anti-inflammatory agents. Trends in pharmacological, 23(10), 2002, 482-487.

[10] Stalker TJ, Lefer AM, Scalia R. A new HMG-CoA reductase inhibitor, rosuvastatin, exerts anti-inflammatory effects on the microvascular endothelium: the role of mevalonic acid. British journal of pharmacology, 133(3), 2001, 406-412.

[11] Mach F. Statins as immunomodulators. Transplant immunology, 9(2), 2002, 197-200.

[12] Wong RP, Davis TM. Statins as potential antimalarial drugs: low relative potency and lack of synergy with conventional antimalarial drugs. Antimicrobial agents and chemotherapy, 53(5), 2009, 2212-4.

[13] Garrett IR, Gutierrez G, Mundy GR. Statins and bone formation. Current Pharmaceutical Design, 7(8), 2001, 715-736.

[14] Jones P, Kafonek S, Laurora I, Hunninghake D. for the CURVES investigators. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). American Journal of Cardiology, 81(5), 1998, 582-7.

[15] Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, Cain VA, Blasetto JW, Group SS. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR Trial). The American journal of cardiology, 92(2), 2003, 152-60.

[16] Oudhoff KA, Sangster T, Thomas E, Wilson ID. Application of microbore HPLC in combination with tandem MS for the quantification of rosuvastatin in human plasma. Journal of Chromatography B, 832(2), 2006, 191-6.

[17] Trivedi RK, Kallem RR, Mullangi R, Srinivas NR. Simultaneous determination of rosuvastatin and fenofibric acid in human plasma by LC–MS/MS with electrospray ionization: assay development, validation and application to a clinical study. Journal of pharmaceutical and biomedical analysis, 39(3), 2005, 661-9.

[18] Hull CK, Martin PD, Warwick MJ, Thomas E. Quantification of the N-desmethyl metabolite of rosuvastatin in human plasma by automated SPE followed by HPLC with tandem MS detection. Journal of pharmaceutical and biomedical analysis, 35(3), 2004, 609-14.

[19] Gomes FP, Garcia PL, Porto Alves JM, Singh AK, Kedor-Hackmann ER, Miritello Santoro MI. Development and validation of stability-indicating HPLC methods for quantitative determination of pravastatin, fluvastatin, atorvastatin, and rosuvastatin in pharmaceuticals. Analytical letters, 42(12), 2009, 1784-804.

[20] Kumar TR, Shitut NR, Kumar PK, Vinu MC, Kumar VV, Mullangi R, Srinivas NR. Determination of

rosuvastatin in rat plasma by HPLC: validation and its application to pharmacokinetic studies. Biomedical Chromatography, 20(9), 2009, 881-887.

[21] Vittal S, Shitut NR, Kumar TR, Vinu MC, Mullangi R, Srinivas NR. Simultaneous quantitation of rosuvastatin and gemfibrozil in human plasma by high-performance liquid chromatography and its application to a pharmacokinetic study. Biomedical Chromatography, 20(11), 2006, 1252-9.

[22] Épshtein NA. Validation of HPLC techniques for pharmaceutical analysis. Pharmaceutical Chemistry Journal, 38(4), 2004, 212-28.