Spectroscopy and high - performance liquid chromatography method for determination of HMG – CoA REDUCTASE INHIBITORS (statins)

Praveen kumar[1]*, meenu[2], babita [3]

[1] Prof. at SGRRU, [2] Prof. at SGRRU, [3] students

[1] Department of pharmaceutical sciences

SGRRU, Patel Nagar, Dehradun. India

ABSTRACT

Hydroxyl methyl glutaryl CoA reductase inhibitors are a class of lipid – lowering medication that is thought to reduce illness and motility those who are at high risk of cardiovascular disease. The great importance of these drugs requires development of effective analytical methods involving high sensitivity and resolution. The spectroscopy and the liquid chromatography are well established methods in the field of pharmaceutical analysis. The spectroscopic and high performance liquid chromatographic methods for determination of statins, their related impurities and co-administered drugs in the bulk drug forms and pharmaceutical formulations are reviewed.

KEY WORDS - pravastatin's, lovastatin's, atorvastatin, simvastatin, rosuvastatin

INTRODUCTION

Introduction in 1980s class of compounds are the efficacious and best tolerated hypolipidaemic drugs .They competitively inhibit conversion of 3 – Hydroxy - 3 - methyl glutaryl coenzyme A (HMG – CoA) to mevolonate (rate limiting step in CH synthesis) by the enzyme HMG – CoA reductase.

This result in compensory increase in LDL receptor expression on liver cell increase receptor mediated uptake and catabolism of IDL and DLD. Over long term feedback ,induction of HMG – CoA reductase tends to increase CH synthesis , but a steady - is finally attained with a dose – dependent lowering of LDL – CH levels. The statins work by blocking an enzyme, HMG-CoA reeducates that is the rate-limiting step in the manufacture of cholesterol. Statins reduce LDL-cholesterol, total cholesterol, and triglycerides and slightly increase high-density lipoprotein (HDL-c). Statins may also have anti-inflammatory effects.

A recent good-quality systematic review found that all statins are equally effective at lowering C-reactive protein levels, but do not affect fibrinogen or several other markers of inflammation.2 No study has evaluated whether the effect of statins on any marker is related to their effect on cardiovascular outcomes. The third report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) was released in September 2002.3 The report stresses that the intensity of treatment is directly related to the degree of cardiovascular risk. Target LDL-c levels depend on the patient's risk of heart disease, medical history, and initial LDL-c level.

(Lescol, Lescol XL) lovastatin (Mevacor, Alticor) pravastatin (Pravachol) rosuvastatin (ZD4522) (Crestor) simvastatin (Zocor)

Fluvastatin (Lescol XL) and lovastatin (Altocor) are available in extended-release as well as immediate-release forms. Lovastatin and pravastatin are natural statins found in fungi; simvastatin is a semisynthetic statin based on lovastatin, and atorvastatin, fluvastatin, and rosuvastatin are fully synthetic. Usua For most patients who are prescribed a statin, the target will be 130 mg/dL or 100 mg/dL. In ATP-III, patients who have Type II diabetes without CHD; peripheral or carotid vascular disease; and patients who have multiple risk factors and a 10-year risk of CHD > 20% are said to have "CHD equivalents," meaning that the criteria for using drug therapy and the LDL target (<100 mg/dL) is the same as for patients who have a history of CHD. Atorvastatin (Lipitor) fluvastatin l starting doses are rosuvastatin 10 mg, atorvastatin 10 mg, and 20 mg of the other statins.

TABLE 1: MECHANISM OF ACTION

HMG-CoA Reductase Inhibitor Dose / LDL reduction %	**Important Facts**
Atorvastatin (Lipitor) **10 mg / 35-39%** **20 mg / 43%** **40 mg / 50%** **80 mg / 55-60%**	Metabolized in CYP3A4; high drug interaction risk High potency Multiple trials to support CV risk reduction
Cerivastatin (Lipobay, Baycol)	Removed from market 2001 due to rhabdomyolysis risk
Lovastatin (Mevacor, Altocor) **10 mg / 21%** **20 mg / 24-27%** **40 mg / 30-31%** **80 mg / 40-42%**	Low to moderate potency Metabolized in CYP3A4; high drug interaction risk Significantly raises cyclosporine levels in transplant patients
Pitavastatin (Livalo, Pitava) **1 mg / 29%** **2 mg / 36-39%** **4 mg / 41-45%**	Newest statin Moderate potency Few drug interactions
Pravastatin (Pravachol, Lipostatin) **10 mg / 22%** **20 mg / 29%** **40 mg / 34%** **80 mg / 37%**	Low potency Thought to have fewest side effects Established safety in patients with liver disease Few drug interactions
Simvastatin (Zocor) **5 mg / 26%** **10 mg / 29%** **20 mg / 38%** **40 mg / 30-41%** **80 mg / 36-47%**	Moderate potency High incidence of side effects (myalgias) Metabolized in CYP3A4; high drug interaction risk The 80 mg dose not recommended by FDA in 2011 due to myalgias/interactions

Analytical methods

Spectroscopic methods

Direct, derivative and chemo metric spectroscopic methods have been used for the analysis of different statins in their pure and pharmaceutical dosage forms.

1.Ultraviolet spectroscopy method

Simple spectrophotometric methods have been developed for determination of ATV in bulk and tablets formulations using methanol [1] or methanol: water (50:50) as solvent [2] the absorbance maximum of ATV has been found at 244 nm and 248 nm respectively. Similar methods have been elaborated for determination of SMV. The estimation of SMV has been carried out using different solvents – methanol (method I) at 236 nm, 2-propanol (method II) at 230 nm and conc.H2SO4 (method III) at 415 nm [3]. A simple spectrophotometric method for the assay of ROS in pharmaceutical formulations [4] has been developed using water as solvent. The absorbance maximum of ROS has been observed at 244 nm. Derivative spectrophotometric and absorbance ratio methods have been developed for the estimation of ATV in tablets [5] and combination of ATV and Ezetimibe in binary mixtures, bulk powder and pharmaceutical dosage forms [6 – 7].

FLV and Zofenopril have been determined simultaneously in two-component mixtures and in pharmaceutical preparations using the first, second and third derivatives of the zero-order spectra. FLV has been determined at wavelengths 339.03, 252.57 and 258.50 nm respectively [8]. A pH independent spectrophotometric method has been developed for the determination of PRV in pharmaceutical formulations. The method is based on the measurement of absorbance at isosbestic point. Isosbestic point of PRV has been determined by zero-order spectrophotometric method and difference spectrophotometric method and has been observed at 249 nm [9].

TABLE 2: Analysis of single component HMG CoA reducatase Inhibitors by UV-Spectroscopy methods

s.no	Drug	Method	Description	Ref.no
1	Estimation of Pravastatin by spectrophotometric method	Ultraviolet Spectroscopy	Detection wavelength: 737nm Linearity range: 5-25µg/ml Co-relation Coefficient: 0.999.	[10]
2	Simvastatin in bulk and tablet dosage form	Stability indicating UV spectrophotometric method	Detection wavelength : 237 nm Linearity range: 3-18 µg/ml Correlation coefficient 0.9998 Limit of Detection: 0.73 µg/ml Limit of Quantification: 2.07 µg/ml	[11]
3	Validated Simple UV Spectrophotometric Method for the Estimation of Pitavastatin in bulk and Pharmaceutical Dosage Form	UV-spectrophotometric method	Detection wavelength: 249.5nm Solvent : 0.1N HCL Linearity range: 2-12µg/ml. Co-relation Coefficient: 0.9996 %Recovery : 99.83 + 0.39 % LOD: 0.122 µg/mL LOQ: 0.371 µg/mL	[12]
4	Simple UV Spectrophotometric Determination of Rosuvastatin Calcium in Pure Form and in Pharmaceutical Formulations	UV Spectrophotometric Determination	Detection wavelength: 244 nm Solvent: methanol Linearity range: 2-18 µg/mL. Co-relation Coefficient: 0.9978 Molar absorptivity: 7.2646 x 104 L/mol.cm	[13]
5	UV Spectrophotometric estimation of Rosuvastatin Calcium and Fenofibrate in bulk Drug and Dosage Form using Simultaneous Equation Method	UV Spectrophotometric	Detection wavelength : Rosuvastatin : 244nm Fenofibrate : 286.7nm Solvent : methanol Linearity range: Rosuvastatin : 1-10µg/ml Fenofibrate: 2-20µg/ml Co-	[14]

			relation Coefficient Rosuvastatin : 0.998	

2. Spectrofluorimetric methods

Spectrofluorimetric procedure for determination of ATV in pharmaceutical formulations has been developed. In this method, the native fluorescence characteristics of ATV have been studied in both acidic and basic media. High sensitivity has been obtained with 5% acetic acid and the fluorescence intensity has been measured at λex 276 nm and λem 389 nm [15]. On the other hand, spectrofluorimetric methods have been elaborated for the determination of ROS, Ezetimibe and PTV in pharmaceutical preparations.

The first method is based on measuring the fluorescence of the drugs at their optimum excitation and emission wavelengths. Fluorescence intensity has been measured at λex 315 nm, 260 nm, and 245 nm, and at λem 362 nm, 309 nm, and 373 nm for ROS, Ezetimibe, and PTV respectively. The second method has been developed for simultaneous determination of ROS and Ezetimibe The fluorescence has been measured at gem 309 nm for Ezetimibe and 432 nm .

Statins are a class of drugs mostly used for treating hyperlipidemia, and rosuvastatin is the newest drug in the market belonging to this class. In this present work, a method was developed based on the molecular fluorescence technique, with the objective to quantify rosuvastatin in urine samples. For this purpose, the study of several parameters was made to the maximum analytical signal (under reaction with sulfuric acid during 40 min). One previous step to avoid matrix interference was carried out (liquid-liquid extraction). The limit of detection (LOD) and the limit of quantification (LOQ) were 0.38 and 1.28 mg L (-1), respectively. Linear relationship between rosuvastatin concentration and fluorescence intensity was found until 5.0 mg L (-1). This proposed method was tested in several samples spiked with rosuvastatin and recovery was found in the range of 90 ± 10%.

This drugs, namely, rosuvastatin calcium (RSV), ezetimibe (EZE), and pitavastatin calcium (PIT). This method is based on the native fluorescence of the drugs at their optimum excitation and emission wavelengths. Fluorescence intensity were measured at λ_{em} 362 nm, 309 nm, and 373 nm excitation at λex 315 nm, 260 nm, and 245 nm for RSV, EZE, and PIT, respectively. The calibration graphs were linear over concentration ranges 0.50–10.0, 0.25–4.0, and 0.10–3.00 μg mL−1 for RSV, EZE, and PIT, respectively. The simultaneous determination of RSV and EZE was developed. Fluorescence measured at λem 309 nm for EZE and 432 nm for RSV excitation at λex 260 nm for both.

Kinetic spectrophotometric methods

The determination of kinetic spectroscopy method ATV [15] and PRV [16] in pure and pharmaceutical dosage forms has been described. The oxidative coupling reaction of ATV with 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) in the presence of Ce (IV) in an acidic medium to form colored product with λmax at 566 nm. The reaction was followed by measuring the increase in absorbance at 566 nm as a function of time. On the other hand, the method for PRV determination is based on the formation of colored product between PRV and 4-chloro-7-nitrobenzo2-oxa-1, 3-diazole in acetone medium. The reaction was followed spectrophoto metrically by measuring the increase in absorbance at 462 nm as a function of time. The initial rate and fixed time methods have been used in both procedures. From the mentioned above it can be seen that, most of the reported spectroscopic methods are based on ultraviolet spectrophotometric methods.

The fluvastatin sodium (FVS) in pure form and pharmaceutical formulations. The method is based on the formation of colored product between FVS and 4-chloro-7-nitrobenzofurazan (NBD-Cl) in acetone medium at 55 ± 2ºC.This reaction is followed spectrophotometrically by measuring the increase in absorbance at 462 nm as a function of time and The rate data and fixed time methods were adopted for constructing the calibration curves. The linearity ranges was found to be 15.0–50.0 and 10.0–90.0 μg mL^{-1} for rate data and fixed time methods, respectively. The limit of detection for rate data and fixed time methods is 0.017 and 0.134 μg mL^{-1}, respectively. This proposed methods have been successfully applied to the determination of fluvastatin sodium in pharmaceutical dosage forms with no interference from the excipients.The Statistical comparison of the results shows that there is no significant difference between the proposed and official methods.

The quantitative analysis of pravastatin sodium (PVS) in pure and pharmaceutical formulations has been described in kinetic spectroscopy methods. this method were based on the formation of colored product between PVS and 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) in acetone medium at 55 ± 2 °C. The spectrophotometrically by measuring the increase absorbance at 462 nm as a function of time. The methods were adopted for constructing the calibration curves. The linearity ranges were found to be 15.0–50.0 and 10.0–70.0 μg mL−1 for initial rate and fixed time methods, respectively. The limits of detection for initial rate and fixed time methods are 0.029 and 0.086 μg mL−1, respectively. Both methods have been applied successfully for the estimation of PVS in commercial dosage forms with no interference from the excipients. The results are compared with the HPLC pharmacopoeia method.

High-performance liquid chromatography

There is an impressive increase in the use of high-performance liquid chromatography for determination of statins. HPLC has been used frequently in all fields of statins research. In the available literature there is a recent review, describing chromatographic and electrophoretic analytical methods for determination of statins, published in 2012 [17]. That's why in this paper we are going to focus on articles, published after 2012 or older ones, which we found that, have not been included in the mentioned review. There are many reported HPLC methods for separation and quantitative determination of statins in pure and in pharmaceutical dosage forms (alone or in combination with other drugs). These methods based on different stationary phases (C8, C18), different mobile systems and using UV or diode array (DAD) detectors.

Table3. HPLC methods for determination of statins

Drug/ Formulation	Application	Detector / Column	Chromatographic conditions	Ref.
ATV	Drug substance	HPLC-DAD SunFire C18 ODS (250x4mm, 5μm,)	acetonitrile: phosphoric acid 0.1% (65:35) flow rate=1.5 ml/min; λmax=238 nm	[18]
ATV	Tablets	HPLC-UV LiChrospherR 100 RP18 (250x4mm, 5μm)	0.1% acetic acid: acetonitrile (45:55), pH-3.8 flow rate=0.8 ml/min; λmax=246 nm	[19]
ATV and impurities	Drug substance	HPLC-UV C18 (250x4.6mm, 3.5μm)	A: phosphate buffer pH-5.4 B: acetonitrile: tetrahydrofuran: (90:10) gradient elution mode flow rate=1.5 ml/min; λmax=220 nm	[20]
ATV and FLV, PRV	Drug substance	HPLC-DAD ODS-AQ YMC (50x4.6mm,	ethanol: formic acid (pH-2.5, 25 mM) (50:50) flow	[21]

		3µm)	rate=1 ml/min; λmax=238 nm	
ATV and PRV, SMV	Drug substance Dosage form	HPLC-UV Poroshell 120 SB C18 (150x4.6mm, 2.7µm)	0.1% o-phosphoric acid: methanol gradient elution mode flow rate=1 ml/min; λmax=238 nm	[22]
ATV and ROS, SMV, Captopril	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5µm)	acetonitrile: water (60:40) pH-2.9 flow rate=1,5 ml/min; λmax=230 nm	[23]
ATV and ROS, SMV, Diltiazem	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5µm)	acetonitrile: water (85:15), pH-2.6 flow rate=1 ml/min; λmax=230 nm[46]	[24]
FLV				
FLV and ATV, PRV	Drug substance	HPLC-DAD ODS-AQ YMC (50x4.6mm, 3µm)	ethanol: formic acid (pH-2.5, 25 mM) (50:50) flow rate=1 ml/min; λmax=238 nm	[21]
LOV				
LOV and SMV	Tablets	HPLC- DAD LiChrosorb C18 (250x4.6mm, 5µm)	acetonitrile: water (30:70) flow rate=1.5 ml/min; λmax=240 nm	[25]
LOV and PRV, SMV	Drug substance Dosage form	HPLC-DAD LiChrospher C8 (250x4mm, 5µm)	acetonitrile: 0.1% phosphoric acid (65:35) flow rate=1.5 ml/min; λmax=238 nm	[26]
PTV				
PTV	Tablets	HPLC-UV Agilent Eclipse XDB C18 (150x4.6mm, 5µm	phosphate buffer (pH-3.4): acetonitrile (65:35) flow rate=0.9 ml/min; λmax=244 nm	[27]
PTV and related substances	Tablet	HPLC-DAD Phenomenex Kinetex C18 (75x4.6mm, 2.6µm)	A: acetate buffer (pH-3.8): acetonitrile (90:10) B: acetonitrile: water (90:10) gradient elution mode flow rate=1 ml/min; λmax=250 nm	[28]
PTV and related substances	Drug substance Dosage form	UHPLC-DAD Poroshell 120 SB-C18 (100x4.6mm, 2.7µm)	A: sodium formate: acetonitrile: formic acid (75:25:0.2); B: acetonitrile: sodium formate: formic acid (95:5:0.05) gradient elution mode flow rate=2 ml/min; λmax=250 nm	[29]
PTV and Ezetimibe	Dosage form	HPLC-DAD Phenomenex Luna C18 (250x4.6mm, 5µm)	0.1% o-phosphoric acid: acetonitrile: triethylamine (19.8:80:0.2); pH-3±0.05 flow rate=1.4 ml/min; λmax=235 nm	[30]
PRV				
PRV	Tablets	HPLC-UV Phenomenex Luna C18 (150x4.6mm, 5µm)	acetonitrile: potassium dihydrogen phosphate (30:70), pH-3 flow rate=1.5 ml/min; λmax=240 nm	[31]

PRV	Tablets	HPLC-DAD LiChrospher C18 (125x4mm, 5µm)	methanol: water: trimethylamine: glacial acetic acid (455:545:2:1.2) flow rate=2 ml/min; λmax=238 nm	[32]
ROS				
ROS	Drug substance Dosage form	HPLC-DAD Nucleodur C8 (250x4.6mm, 5µm)	0.1M formic acid: methanol (25:75) flow rate=1,0 ml/min; λmax=280 nm	[33]
ROS and ATV, SMV Captopril	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5µm)	acetonitrile: water (60:40), pH-2.9 flow rate=1,5 ml/min; λmax=230 nm	[34]
ROS and ATV, SMV, Diltiazem	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5µm)	acetonitrile: water (85:15), pH-2.6 flow rate=1 ml/min; λmax=230 nm	[23]
ROS and ATV, SMV Enalapril	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5µm)	acetonitrile: water (60:40), pH-2.8 flow rate=1.8 ml/min; λmax=230 nm	[35]
SMV				
SMV and LOV	Tablets	HPLC-DAD LiChrosorb C18 (250x4.6mm, 5µm)	acetonitrile: water (30:70) flow rate=1.5 ml/min; λmax=240 nm	[25]
SMV and ATV, PRV	Drug substance Dosage form	HPLC-UV Poroshell 120 SB C18 (150x4.6mm, 2.7µm)	0.1% o-phosphoric acid-methanol gradient elution mode flow rate=1 ml/min; λmax=238 nm	[22]
SMV and LOV, PRV	Drug substance Dosage form	HPLC-DAD LiChrospher C8 (250x4mm, 5µm)	acetonitrile: 0.1% phosphoric acid (65:35) flow rate=1.5 ml/min; λmax=238 nm	[26]
SMV and ATV, ROS Captopril	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5µm)	acetonitrile: water (60:40) pH-2.9 flow rate=1,5 ml/min; λmax=230 nm	[23]
SMV and ATV, ROS, Diltiazem	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5µm)	acetonitrile: water (85:15), pH-2.6 flow rate=1 ml/min; λmax=230 nm	[24]
SMV and ATV, ROS Enalapril	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5µm)	acetonitrile: water (60:40), pH-2.8 flow rate=1.8 ml/min; λmax=230 nm	[35]

Conclusion

This article describes different spectroscopic and HPLC methods for analysis of statins (Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin and Simvastatin) in pure forms, in different pharmaceutical dosage forms and in multicomponent mixtures. Ultraviolet, visible, spectrofluorimetric and kinetic spectrophotometric methods are presented. These methods are fast and suitable for the analysis of simple matrices without overlapping spectra. For more complex matrices, they require prolonged sample pretreatment and data processing.

References

1. Naveed S. Simple ultra voilet spectrophotometric assay of atorvastatin API formulation and their comparative study. Global J Med Res B 2014; 14(2): 35-38.
2. Ghanty S, Sadhukhan N, Mondal A. UV-spectrophotometric method for quantification of atorvastatin in tablets. J Pharm Sci Tech 2012; 2(1): 34-40.
3. Raju KN, Babu IS, Jyothi R, Prathyusha S. methods for estimation of simvastatin in bulk drug and its dosage by spectrophotometry form. J Pharm Chem Biol Sci 2012; 2(1): 124-129.
4. Naveed S, Qamar F. Simple UV spectrophotometric assay of rosuvastatin formulations. Global J Pharm Res 2014; 3(3): 1985-1990.
5. Sharma S, Sharma MC, Method development and validation of atorvastatin calcium using FeCl3 by UV-Visible spectrophotometric methods. Am-Euras J Toxicol Sci 2011; 3(2): 105-110.
6. Baghdady YZ, Al-Ghobashy MA, Abdel-Aleem AAE, Weshahy SA. Spectrophotometric and methods for the simultaneous determination of ezetimibe and atorvastatin calcium.by TLC densitometry J Adv. Res 2013; 4(1): 51-59.
7. Abdelwahab NS, El-Zeiny BA, Tohamy SI. spectrophotometric methods for simultaneous determination of some antihypetensive drugs. J Pharm Anal 2012; 2(4): 279-284.
8. Belal TS, Daabees HG, Abdel-Khalek MM, Mahrous MS, Khamis MM. spectrophotometric method for determination of mixtures (atorvastatin calcium and ezetimibe; candesartan cilexetil and hydrochlorothiazide) in tablets. J Pharm Anal 2013; 3(2): 118-126.
9. Stolarczyk M, Maślanka A, Apola A, Rybak W, Krzek J. for simultaneous determination of zofenopril and fluvastatin in mixtures and pharmaceutical dosage forms.by derivative spectroscopy method Spectrochim Acta a 2015; 148: 66-71.
10. Y Srinivasa Rao; G Haritha; P Nayana tara; K P R Chowdary; J V L N Seshagiri Rao.Int.J.Chem. Sci.: 1(4), 2003, 368-370
11. Vinit Chavhan; Minal Ghante. J App Pharm, April, 2014, Vol. 6; Issue 2: 235 -246
12. Yunoos Mohammad; M Sowjanya; B Sushma; K Kumar; Pavan Asian Journal of Research in Chemistry;Apr2014, Vol. 7 Issue 4, p393 13Alka Gupta; P Mishra; K Shah. E-Journal of Chemistry, 2009, 6(1), 89-92
13. R R Sevda; an S Ravetkar; P J Shirote. International Journal of ChemTech Research, April-June 2011, Vol. 3, No.2, pp. 629-635
14. .Sharaf El-Din MMK, Salama FMM, Nassar MWI, Attia KAM, Kaddah MMY. spectrofluorimetric method for the determination of atorvastatin in pharmaceutical preparations. J Pharm Anal 2012; 2(3): 200-205.
15. .Nigović B, Mornar A, Sertic M. A Current Trends and Advance review in Analytical Methods for Determination of Statins: Chromatography and Capillary Electrophoresis. Chromatography - The Most Versatile Method of Chemical Analysis. Calderon L Ed. InTech 2012: 385-428

16. Ferello L, Stamer SG, Repetto MF, Zubata PD, Segall AI. A reversed-phase HPLC method for the determination of atorvastatin calcium in tablets. Austin Chromatography. 2014; 1(1): 4.
17. Gupta KL. Spectro characterization and quantitative determination of atorvastatin calcium impurities by novel HPLC method. Spectrochim Acta Part A 2012; 97: 495–501.
18. Oliveira MA, Yoshida MI, BelineloVJ, Valotto RS. kinetics of atorvastatin under stress conditions and chemical analysis by HPLC. Molecules 2013; 18(2): 1447-1456.
19. LD, Ferello L, Stamer SG, Repetto MF, Zubata PD, Segall AI. A reversed-phase HPLC method for the determination of atorvastatin calcium in tablets. Austin Chromatography. 2014; 1(1): 4.
20. Gupta KL. Spectroscopic characterization and quantitative determination of atorvastatin calcium impurities by novel HPLC method. Spectrochim Acta Part A 2012; 97: 495–501.
21. Assassi AL, Roy CE, Perovitch P, Auzerie J, Hamon T, Gaudin K, Green analytical method 2015; 1380: 104–111.
22. Praveen KSN, Bhadre GDG, Vathsala DC, Mantelingu K, Rangappa KS. Simultaneous estimation of statins like pravastain, atorvastatin and simvastatin in bulk and pharmaceutical dosage form by means of high-performance liquid chromatography. J Chem Pharm Res 2013; 5(5): 359-364.
23. Sultana N, Arayne MS, Naveed S. Simultaneous determination of captopril and statins in API, pharmaceutical formulations and in human serum by RP-HPLC. J Chin Chem Soc 2010; (57): 378-383.
24. Sultana N, Arayne MS, Shafi N, Siddiqui FA, Hussain A. RP-HPLC method for the simultaneous analysis of diltiazem and statin: Application in pharmaceuticals and human serum. Anal Methods 2010; 2(10): 1571–1576.
25. Manova M, Pencheva I, Peikov P, Petrova G, Tsvetkova B. Validation of HPLC method for determination of HMG Co-A reductase inhibitors. Pharmacia 2011; LVIII (1-4): 50-54.
26. Silva TD, Oliveira MA, de Oliveira RB, Vianna-Soares CD. validation of a simple and fast HPLC method for determination of lovastatin, pravastatin and simvastatin. J Chromatogr Sci 2012; 50(9): 831–838
27. Sujatha K, Seshagiri Rao JVL., A new validated stability-indicating RP-HPLC method for the estimation of pitavastatin in tablet dosage forms. Int J Pharm Anal Res 2014; 3(1): 67-74.
28. Goud ES, Reddy DRVK, Reddy MNC. Development and validation of a reverse-phase liquid chromatographic method for determination of related substances of pitavastatin for 2 and 4 mg tablets. Int J Pharm Pharm Sci 2014; 6(1): 95-100.
29. Hariram B, Kumar RS, Shree AJ, Rao DV, Srinivas LKK. High performance method on superficially porous stationary phase for the determination of related substances in pitavastatin calcium by HPLC. Chromatographia 2015; 78(15): 1017–1029.
30. Panchal H, Suhagia BN. Validation of pitavastatin calcium and ezetimibe in binary mixture by liquid chromatography. Int J PharmTech Res 2011; 3(4): 2155-2161.
31. Raj ND, Kumaravel S, Murugan R, Narayanan SS, Vijayalakshmi R. HPLC method for the determination of pravastatin in tablet dosage forms. Int J Res Pharm Sci 2010; 1(2): 187-189.

32. Maslarska V. Development, optimization and validation of HPLC method for determina Pharm Biol Chem 2014; 3(2): 230-234.
33. Ashour S, Omar S. high-performance liquid chromatographic method for the estimation of rosuvastatin calcium in bulk and pharmaceutical formulations. Int J Biomed Sci 2011; 7(4): 283-288.
34. Kumar P, Chakraborthy GS. Validated stability indicating RP-HPLC method development for determination of rosuvastatin. Int J Adv Pharm Res 2014; 5(5): 274-282.
35. Sultana N, Arayne MS, Naveed S. Simultaneous determination of enalapril and statin's in pharmaceutical formulations by RP-HPLC. J Chil Chem Soc 2011; 56(3): 734-737.