Int J Pharm Bio Sci 2013 July; 4(3): (P) 935 - 946

Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DEVELOPMENT AND VALIDATION OF DERIVATIVE SPECTOSCOPIC METHOD FOR SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND RAMIPRIL IN COMBINED DOSAGE FORM

DR. ATUL PATEL* AND DR. HITESH JOGIA

Shree S.K.Patel college of Pharmaceutical education and research, Hemchandracharya North Gujarat University, Gujarat, India.

ABSTRACT

A novel, simple, accurate, sensitive, reproducible, the economical spectroscopic method has been developed and validated for the determination of Atorvastatin and Ramipril in combined dosage form. First order derivative spectroscopy method is adopted to eliminate spectral interference. The method obeys Beer's Law in concentration range of 15-35 µg/ml for Atorvastatin and 7.5-17.5 µg/ml for Ramipril. The method was validated for linearity, range, accuracy, precision and specificity as per ICH guidelines. Zero crossing point for Atorvastatin and Ramipril was 223 nm and 296 nm respectively in methanol. The developed method as successfully used for the quantitative analysis of commercially available dosage form.

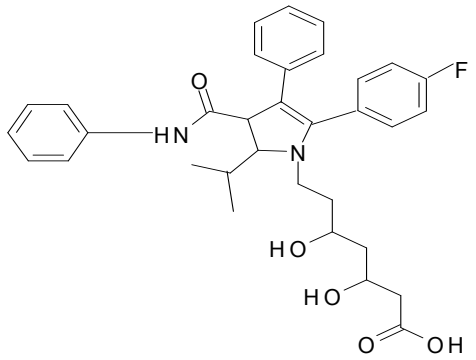
KEYWORDS: **Atorvastatin, Ramipril, Derivative Spectroscopy, Zero crossing point, combined dosage form.**

DR. ATUL PATEL
Shree S.K.Patel college of Pharmaceutical education and research, Hemchandracharya North Gujarat University, Gujarat, India.

INTRODUCTION

ATORVASTATIN

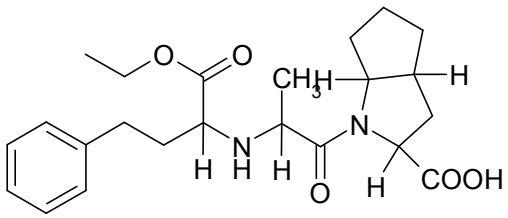
Atorvastatin is R-(R*,R*)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-IH-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. Atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. It is used to reduce LDL-cholesterol, apolipoprotein B, triglycerides and to increase HDL-cholesterol in treatment of hyperlipidemias [(1)]. Atorvastatin is a synthetic lipid-lowering agent which is about 100 times potent than the other drugs in its class and at lower costs than most of the others [(2)].



Atorvastatin

RAMIPRIL

1-[N-[1-Carboxy-3-henylpropyl] alanyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid, 1-ehtyl ester. Ramipril is an angiotensin converting enzyme inhibitor used to treat hypertension and congestive heart failure [(3)].



Ramipril

Literature survey revealed that several analytical methods such as spectrophotometry, spectrofluorimetry, High performance liquid chromatography, Raman spectroscopy, LCMS and LC-ESI-MS have been reported for the determination of either Atorvastatin [(6-30,34-45)] or Ramipril [(31-45)] alone or in combination. The aim of the present work is to develop analytical method for combined fixed dose formulation of Atorvastatin with Ramipril which is novel to the market. In this study, a simple, precise and convenient first derivative spectrophotometric method was developed and validated for its application in the simultaneous determination of Atorvastatin and Ramipril in their combined dosage forms. The method suggested was reported to have no interference of any common excipients. First derivative spectrophotometric method is fast and quick for the quality control release of the dosage form(4-5).

MATERIALS AND METHODS

Materials

Atorvastatin (ATO) and Ramipril (RAM) were supplied by Torrent Research Center (Gandhinagar, India). Tablets of combined Atorvastatin 10mg and Ramipril 5 mg were purchased from the market. Methanol (HPLC Grade, Ranchem) used as solvent.

Instruments

JASCO V-550 UV/VIS spectrophotometer having quartz cell with 1cm path length was used for the spectroscopic analysis. Mettler Toledo AX balance used for weighing. Decon F5200b sonicator used for sonication of samples. Borosilicate volumetric flasks from Emil England used for the sample preparation.

Preparation of Standard Solutions

Stock solution of Atorvastatin: 27.25 mg of Atorvastatin calcium equivalent to about 25 mg of Atorvastatin was weighed accurately and transferred to 100 ml volumetric flask. 70 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (250 µg/ml of Atorvastatin).

Stock solution of Ramipril

25 mg of Ramipril was weighed accurately and transferred to 200ml volumetric flask. 150 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (125 µg/ml of Ramipril).

Determination of Absorption Maxima

Solutions of both drugs were prepared in methanol having concentration of 25 µg/ml for ATO and 12.5 µg/ml for RAM .The mixed solution of both drugs in same concentration was also prepared. First of all the three solutions were scanned between 200-400 nm by keeping methanol as a blank to determine the wavelength of maximum absorption for both drugs.

Derivative Spectroscopy

The Spectra in Fig.1 and Fig.2 revel that no method was possible in zero order. Derivative spectroscopic method was tried as an option for the simultaneous determination of both. The zero order spectra of both drugs (Fig.1 & Fig.2) were converted to first derivative spectra (Fig.3 & Fig.4) and overlapped to find out the zero crossover of both drugs. The ZCO for ATO 223 nm and 296 nm and for RAM

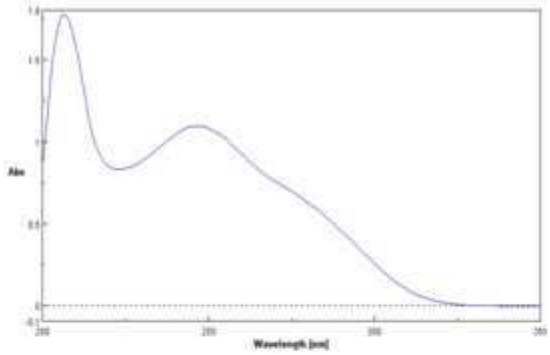


Figure 1: Zero order spectrum of ATO

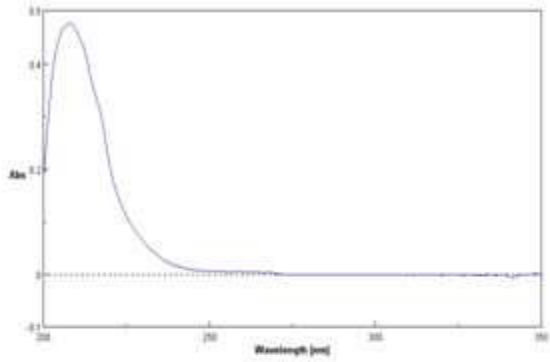


Figure 2: Zero order spectrum of RAM

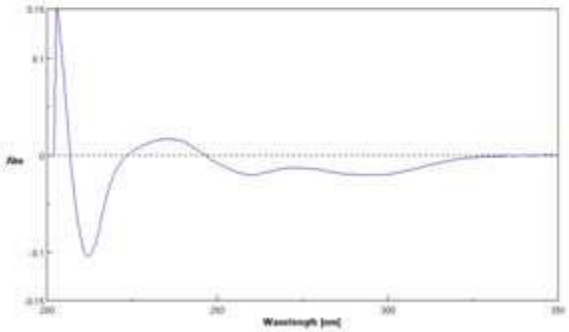


Figure 3: First order spectrum of ATO

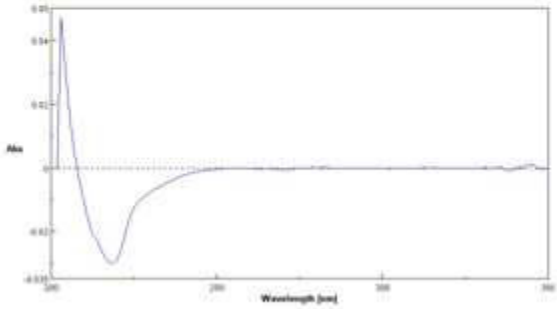


Figure 4: First order spectrum of RAM

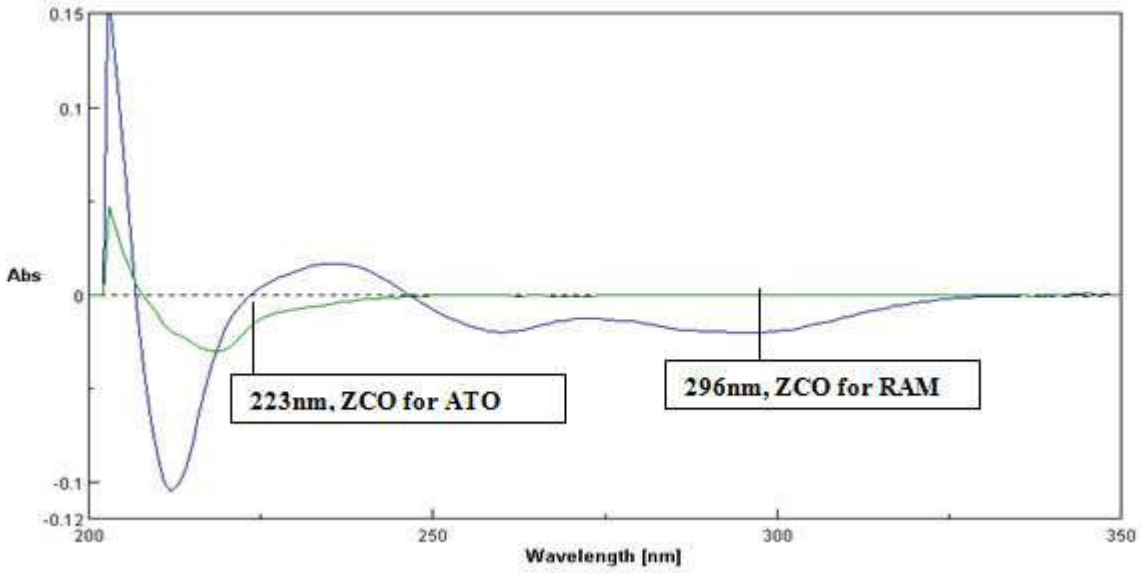


Figure 5
Overlaid first derivative spectra of ATO and RAM

Zero cross over (ZCO) point was defined as a particular wavelength at which one component has a response (positive or negative) while the response of other drug is zero. At ZCO we can measure the response of one component while the response of other remains zero, but for simultaneous determination of both drugs there must be two ZCOs at which one can be quantified while the response of the other would remain zero. In this case when two first derivative responses were overlapped following ZCO points were selected as shown in fig.5. The ZCO of both drugs were evaluated at various concentration levels of each drug. Wavelengths selected for quantitation were 296 nm for ATO (ZCO for RAM) and 223 nm for RAM (ZCO for ATO). The selection of ZCO was evaluated by preparing various solutions containing different concentrations of each component. The derivative response of each drug at ZCO point was derived and plotted against respective concentration. The regression equation and correlation coefficients were derived from this data. They suggest no interference of one drug at ZCO point of other drug at all concentration levels (Table 1). Series E was selected as a calibration tool for the UV spectrophotometer. The standard preparation was selected at concentration level of 25 μg/ml for ATO and 12.5 μg/ml for RAM.

Validation

Method was validated according to ICH Guidelines. According to ICH Guidelines for the assay method was validated with respect to linearity, range, precision, accuracy, and specificity.

Linearity

Linearity of concentration versus first derivative response was plotted in various series (A-E).
The stock solutions were diluted in the following concentration range, and data were evaluated by regression analysis. Data suggested no interference of one drug at ZCO point of other drug at all concentration levels. Data related to regression analysis are tabulated in table 1.

Table 1
Data related to various calibration curves for ATO and RAM.

Series	Concentration (μg/ml)		Regression Equation	Correlation coefficient
	ATO	RAM		
A	15-35	0	y =0.0008x + 0.002 (ATO)	0.9999
B	0	7.5-17.5	y=0.0013x + 0.0048 (RAM)	0.9994
C	15-35	12.5	y =0.0008x + 0.0001 (ATO)	0.9999
D	25	7.5-17.5	y=0.0014x + 0.0039 (RAM)	0.9999
E	15-35	7.5-17.5	y =0.0008x - 0.0005 (ATO)	0.9992
			y=0.0017x - 0.0001 (RAM)	0.9993

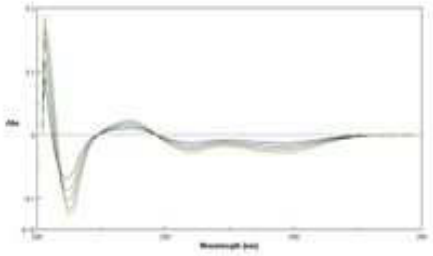


Figure 6: Series - A (ATO)

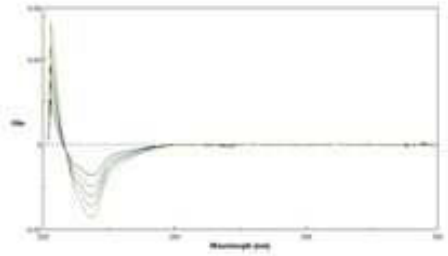


Figure 7: Series - B (RAM)

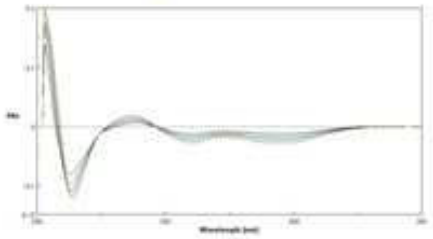


Figure 8: Series-C (Linearity of ATO, where RAM is constant)

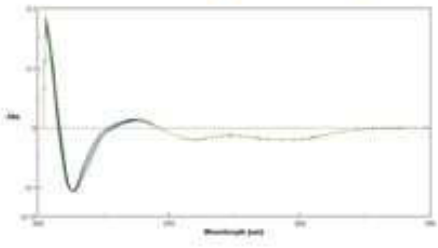


Figure 9: Series-D (Linearity of RAM, where ATO is constant)

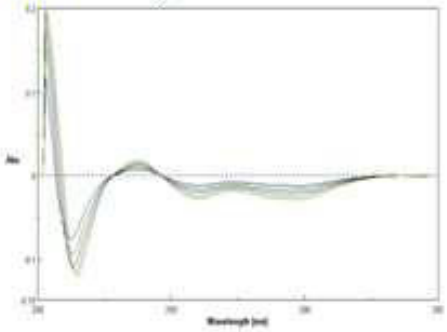


Figure 10: Series-E (Linearity of ATO and RAM)

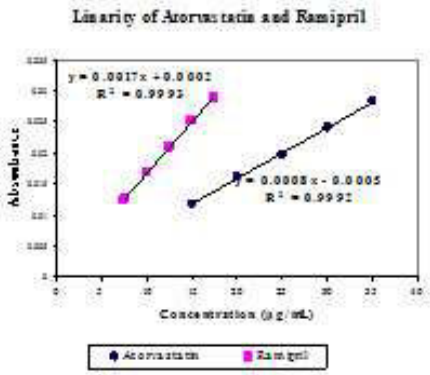


Figure 11: Linearity graph of ATO and RAM

The regression analysis of various series suggested that the FDS response was linear for both components in various concentration ranges. Fig.11 indicates the linearity graph of series E suggesting, R^2 of 0.9992 for ATO and 0.9993 for RAM.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of same homogeneous sample under the prescribed conditions. System precision is a measure of the method variability that can be expected for a given analyst performing the analysis.

System Precision

System precision was evaluated by analyzing the series E for three times. All the data were compared and % relative standard deviations were calculated.

Table 2
System precision data for ATO and RAM (n=3)

NEB (µg/ml)	Mean Absorbance for ATO	%RSD	SAM (µg/ml)	Mean Absorbance for RAM	%RSD
15	0.01199	0.50	7.5	0.01275	1.93
20	0.01609	0.28	10	0.01665	1.68
25	0.01983	0.13	12.5	0.02108	0.22
30	0.02410	0.37	15	0.02497	1.06
35	0.02849	0.25	17.5	0.02926	1.18

Method Precision

It was evaluated by analyzing the sample of same batch for six times. Data suggests high degree of method precision.

Table 3
Method precision data for ATO and RAM

No.	ATO	RAM
1	100.3	100.5
2	100.8	100.9
3	100.1	100.5
4	99.7	99.6
5	101.1	101.0
6	100.1	100.2
Mean	**100.4**	**100.5**
%RSD	**0.51**	**0.49**

Intermediate Precision

It was evaluated by analyzing the sample of by different analyst, on different day and using different instruments.

Table 4
Intermediate precision data for ATO and RAM

Analyst Change		
	%Assay	
	ATO	**RAM**
Analyst-I	99.6	100.5
Analyst-II	100.3	100.1
Mean	**100.0**	**100.3**
%RSD	**0.50**	**0.28**
Day Change		
	%Assay	
	ATO	**RAM**
Day-I	99.8	100.0
Day-II	98.9	100.6
Mean	**99.4**	**100.3**
%RSD	**0.64**	**0.42**
System Change		
	%Assay	
	ATO	**RAM**
System-I	99.6	100.9
System-II	98.4	100.2
Mean	**99.0**	**100.6**
%RSD	**0.86**	**0.49**

Specificity

Specificity of the method was evaluated by checking the interference of placebo. Placebo weight in the particular tablet was derived, by subtracting the weight of drugs from the average weight of the tablet and treated as per the procedure followed for the test sample. No absorbance observed at the wavelength for ATO and RAM.

Accuracy

To ensure the reliability and accuracy of method, the recovery studies were carried out by adding known quantity of drug with sample preparation. Accuracy of the method was determined at 3 different concentration levels of each component by standard addition method.

Table 5
Accuracy data for ATO and RAM

	Parameter	**Accuracy**		
	Drug	**ATO**		
No.	**Level of Recovery**	**Amount of Drug Added (µg/ml)**	**Amount of Drug Found (µg/ml)**	**%Recovery**
1.	Sample Preparation (80%)	20.05	19.95	99.5
2.	Sample Preparation (100%)	25.01	24.58	98.3
3.	Sample Preparation (120%)	30.09	29.88	99.3
	Drug	**RAM**		
No.	**Level of Recovery**	**Amount of Drug Added (µg/ml)**	**Amount of Drug Found (µg/ml)**	**%Recovery**
1.	Sample Preparation (80%)	10.03	9.90	98.7
2.	Sample Preparation (100%)	12.59	12.53	99.6
3.	Sample Preparation (120%)	14.74	14.84	100.7

Assay

Preparation of Standard Solutions

Stock solution of Atorvastatin: 27.25 mg of Atorvastatin calcium equivalent to about 25 ATO was weighed accurately and transferred to 100ml volumetric flask. 70 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (250 µg/ml of ATO).

Stock solution of Ramipril: 25 mg of Ramipril was weighed accurately and transferred to 200ml volumetric flask. 150 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (125 µg/ml of RAM).

Working standard solution: 5 ml of each stock solution were transfered in 50 ml volumetric flask and volume made to 50 ml with methanol (25 µg/ml of ATO and 12.5 µg/ml of RAM).

Preparation of Sample Solution

Twenty tablets of the combined dosage form were weighed and average weight was calculated. Five tablets (equivalent to 50.0 mg of ATO and 25 mg of RAM respectively) were weighed and transferred in to 200 ml volumetric flask, 150 ml of methanol was added and sonicated for 60 minutes. After cooling, the volume was made up to the mark with methanol and 10 ml was filtered through 0.45µ Millipore filter. 5ml of filtrate was diluted to 50ml with methanol to get the final concentration of 25 µg/ml of ATO and 12.5 µg/ml of RAM. This solution was used to get the first derivative test spectra.

Calculation for Assay

$$\% \text{ Assay (Atorvastatin)} = \frac{\text{(Test Absorbance X Standard Concentration X Assay of Standard)}}{\text{(Standard Absorbance X Sample Concentration)}}$$

$$\% \text{ Assay (Ramipril)} = \frac{\text{(Test Absorbance X Standard Concentration X Assay of Standard)}}{\text{(Standard Absorbance X Sample Concentration)}}$$

Convert standard concentration equivalent to Atorvastatin using equivalency.

Equivalency: 1155.36mg of Atorvastatin Calcium ≈ 1115.27mg of Atorvastatin
Where absorbance of Atorvastatin at 296 nm, and Ramipril at 223 nm, in test and standard preparation.

RESULTS AND DISCUSSION

In this study a simple, precise, accurate and convenient first derivative spectroscopic method was developed and validated for its application in the simultaneous determination of ATO and RAM in their combined dosage form. In first order derivative spectroscopy, wavelengths selected for quantitation were 296 nm for ATO (ZCO for RAM) and 223 nm for RAM (ZCO for ATO).Both drugs follow linearity with the concentration range (ATO: 15-35 µg/ml , RAM: 7.5-17.5 µg/ml) with R^2 value of 0.9992 for ATO and 0.9993 for RAM (Table 1). The percentage RSD was found the range of 0.13-0.50 for ATO and 0.22-1.93 for RAM in system precision (Table 2). The percentage RSD was found 0.51 for ATO and 0.49 for RAM in method precision (Table 3). The percentage RSD was found the range of 0.50-0.86 for ATO and 0.28-0.49 for RAM in intermediate precision (Table 4). The mean % recovery found to be 99.0% for ATO and 99.7% for RAM (Table 5).Method is specific as placebo gave no interference with the determination of both drugs. The mean % assay was found to be 100.0% for ATO and 100.3% for RAM. The overall results of various validation parameters were summarized in table 6.

Table 6
RESULT

Parameter	Atorvastatin	Ramipril
Wavelength	296 nm	223 nm
Zero crossing point	223 nm	296 nm
Range (µg/ml)	15-35	7.5-17.5
Linearity (R^2)	0.9992	0.9993
Precision (% RSD)		
1)System precision	0.13-0.50	0.22-1.93
2)Method precision	0.51	0.49
3)Intermediate precision	0.50-0.86	0.28-0.49
Accuracy (% Recovery)	99.0	99.7

CONCLUSION

The developed method is novel, simple, accurate, sensitive, reproducible, economical which would be used for the determination of Atorvastatin and Ramipril in combined dosage form.

ACKNOWLEDGEMENT

The authors are highly thankful to Torrent Research Centre, Gandhinagar, India and Shree S.K.Patel college of Pharmaceutical education and research for providing samples and facilities to complete the work.

REFERENCES

1. H.S. Malhotra, K.L, Goa, Drugs, 61, 1835-1881(2001).
2. S.C. Sweetman, Martindale the Complete Drug Reference, 34th Edition, Pharmaceutical press, London, pp 862,866, (2005).
3. T.G.Warner, M.C.Perry, Drugs,58,3,469-475(1999).
4. Dr. Atul Patel, Development and Validation of derivative spectroscopic method for the simultaneous determination of Nebivolol hydrochloride and S- amlodipine besylate in combined dosage form, International Journal of Pharma and bio Sci 4(3),379-391, July (2013).
5. S J Rajput, A K Patel, Assay of Lamotrigine and Nicorandil by difference spectroscopy, Indian journal of pharmaceutical sciences, 66(3),342-344 (2004)..
6. Said A Hassan, Hany W.Darwish, Maissa Y Salem, Development and Validation of H-Point Standard addition method applied for the analysis of binary mixture of Amlodipine and Atorvastatin, International Journal of Pharma and bio Sci 4(2),230-243, Apr (2013).
7. Sasmita Ku Acharjya, M.Mathrusri Annapurna, Sailja Koya , Liquid Chromatographic method for simultaneous estimation of Atorvastatin Calcium and amlodipine besylate in Pharmaceutical dosage forms, 4(1),161-170, Oct-Dec (2010).
8. Jadhav, S.D., Bhatia, M.S., Thamake, S.L., Pishawikar, S.A., Spectrophotometric methods for estimation of atorvastatin calcium form tablet dosage forms, International Journal of PharmTech Research. 2(3): 1948-1953(2010).
9. Guihen E, Sisk GD, Scully NM, Glennon JD, Rapid analysis of atorvastatin calcium using capillary electrophoresis and microchip electrophoresis, Electrophoresis, 27(12):2338-47, Jun 2006.
10. Stanisz B, Kania L, Validation of HPLC method for determination of atorvastatin in tablets and for monitoring stability in solid phase, Acta Pol Pharm,63(6):471-6, Nov-Dec (2006).
11. Valesky RJ, Liu L, Musson DG, Zhao JJ, Automated enzyme inhibition assay method for the determination of atorvastatin-derived HMG-CoA reductase inhibitors in human plasma using radioactivity detection, J Pharmacol Toxicol Methods,57(1):61-9, Jan-Feb (2008) .
12. Mazurek S, Szostak R, Quantification of atorvastatin calcium in tablets by FT-Raman spectroscopy, J Pharm Biomed Anal, 15;49(1):168-72, Jan (2009).
13. Shirkhedkar AA, Surana SJ, Development and validation of a reversed-phase high-performance thin-layer chromatography-densitometric method for determination of atorvastatin calcium in bulk drug and tablets,J AOAC Int. 93(3):798-803,May-Jun (2010).
14. Chaudhari BG, Patel NM, Shah PB, Patel LJ, Patel VP, Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of Atorvastatin and Ezetimibe from their combination drug products. J AOAC Int, 90(6):1539-46, Nov-Dec (2007).
15. Pasha MK, Muzeeb S, Basha SJ, Shashikumar D, Mullangi R, Srinivas NR, Analysis of five HMG-CoA reductase inhibitors-atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin:

pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and in vitro metabolism studies, Biomed Chromatogr, 20(3):282-93, Mar (2006).
16. Khedr A, Stability-indicating high-performance liquid chromatographic assay of atorvastatin with fluorescence detection, J AOAC Int, 90(6):1547-53, Nov-Dec (2007).
17. Chaudhari BG, Patel NM, Shah PB, Chem Pharm Bull (Tokyo), Stability indicating RP-HPLC method for simultaneous determination of atorvastatin and amlodipine from their combination drug products,55(2):241-2466, Feb (2007).
18. Gupta LK, Spectroscopic characterization and quantitative determination of atorvastatin calcium impurities by novel HPLC method, Spectrochim Acta A Mol Biomol Spectrosc, 97:495-501, Nov (2012).
19. Nagaraj, Vipul K, Rajshree M,Simultaneous quantitative resolution of atorvastatin calcium and fenofibrate in pharmaceutical preparation by using derivative ratio spectrophotometry and chemometric calibrations,Anal Sci. 23(4):445-51,April (2007).
20. Sivakumar T, Manavalan R, Muralidharan C, Valliappan K, An improved HPLC method with the aid of a chemometric protocol: simultaneous analysis of amlodipine and atorvastatin in pharmaceutical formulations, J Sep Sci,30(18):3143-53. Dec (2007).
21. Nakarani NV, Bhatt KK, Patel RD, Bhatt HS, Estimation of atorvastatin calcium and fenofibrate in tablets by derivative spectrophotometry and liquid chromatography, AOAC Int. ,90(3):700-5, May-Jun (2007).
22. Wankhede S.B, Dixit N.R, Chitlange S.S,Stability indicating HPTLC method for quantitative determination of atorvastatin calcium and metoprolol succinate in capsules, Der Pharmacia Lettre.3(1): 1-7(2011).
23. Kadav AA, Vora DN,Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets, J Pharm Biomed Anal, 10;48(1):120-6, Sep (2008).
24. Patil U.P., Gandhi, S.V., Sengar, M.R., Rajmane, V.S., Gandhi, S.V., A validated densitometric method for analysis of Telmisartan and Atorvastatin calcium in fixed dose combination, Journal of the Chilean Chemical Society, 55(1): 94-96 (2010).
25. H. J. Panchal, B. N. Suhagia, Simultaneous analysis of atorvastatin calcium and losartan potassium in tablet dosage forms by RP-HPLC and HPTLC, Acta Chromatographica., 22(2): 173(2010).
26. Patole, S.M, Khodke, A.S, Potale, L.V, Damle M.C,A validated densitometric method for analysis of atorvastatin calcium and metoprolol tartarate as bulk drugs and in combined capsule dosage forms, Journal of Young Pharmacists, 3(1): 55-59(2011).
27. Sanjay G. Walode, Avinash V. Kasture, Sudhir G. Wadodkar, Stability-indicating HPTLC method for the determination of atorvastatin and ezetimibe: Application to pharmaceutical dosage forms, Journal of Planar Chromatography - Modern TLC, 25(1): 81(2012).
28. Nikalje A, Choudhari V, Validated TLC method for simultaneous quantitation of atorvastatin, ezetimibe, and fenofibrate in bulk drug and formulations, Acta Chromatographica, 23(2): 267-280 (2011).
29. Chhaya R. Macwana, Archita J. Patel, Vishal M. Parmar, Samir G. Patel, Simultaneous HPTLC analysis of Atorvastatin calcium, Ezetimibe and Fenofibrate in tablet,Journal of Liquid Chromatography & Related Technologies. , 35(4): 524 (2012).
30. Seshachalam, U., Kothapally, C.B., HPLC analysis for simultaneous determination of atorvastatin and ezetimibe in pharmaceutical formulations, Journal of Liquid Chromatography and Related Technologies,31(5): 714-721(2008).

31. M.M.Baing,V.V.Vidhya,R.T.Sane,S.N.Menon,K.Dalvi, Simultaneous RP-LC determination of Losartan Potassium, Ramipril and Hydrochlorothiazide in Pharmaceutical preparation, Chromatographia, 64 (5-6),293-296, September (2006).
32. R.Bhushan, D.Gupta, S.Singh, Biomedical chromatography, Liquid chromatographic separation and UV determination of certain antihypertensive agents, 20(2), 217-224, February (2006).
33. Al-Majed AA, Belal F, Abadi A, al-Obaid AM, The voltammetric study and determination of ramipril in dosage forms and biological fluids, Farmaco,55(3):233-8, Mar (2000).
34. Sankar AS, Vetrichelvan T, Venkappaya D, Simultaneous estimation of ramipril, acetylsalicylic acid and atorvastatin calcium by chemometrics assisted UV-spectrophotometric method in capsules, .Acta Pharm,61(3):283-96 , Sep (2011).
35. Joseph L, George M, Rao B VR, Simultaneous estimation of atorvastatin and ramipril by RP-HPLC and spectroscopy, Pak J Pharm Sci, 21(3):282-4, Jul (2008).
36. Panchal HJ, Suhagia BN, Simultaneous determination of Atorvastatin calcium and Ramipril in capsule dosage forms by high-performance liquid chromatography and high-performance thin layer chromatography. J AOAC Int. 93(5):1450-7, Sep-Oct (2010).
37. Potdar, V.H., Mule, V.S., Pishawikar, S.A., Jadhav, S.D., Thamake, S.L., RP-HPLC method for simultaneous estimation of atorvastatin calcium and ramipril from plasma,Ars Pharmaceutica., 52(2): 14-19 (2011).
38. Patole, S.M., Potale, L.V., Khodke, A.S., Damle, M.C., A validated HPLC method for analysis of Atorvastatin Calcium, Ramipril and Aspirin as the bulk drug and in combined capsule dosage forms,International Journal of Pharmaceutical Sciences Review and Research. , 4(3): 40-45 (2010).
39. Rasheed, S.H, Ramakotaiah M., Vani P.S, Arief M, Gajavalli S.R, A stability-indicating LC method for the simultaneous determination of metoprolol, atorvastatin and ramipril in combined pharmaceutical dosage form,Research Journal of Pharmaceutical, Biological and Chemical Sciences,1(4): 816-829(2010).
40. Hiral J. Panchal, Bhanubhai N. Suhagia, Natvarlal J. Patel, Simultaneous HPTLC analysis of atorvastatin calcium, ramipril, and aspirin in a capsule dosage form, JPC - Journal of Planar Chromatography - Modern TLC, 22(4): 265-271(2009).
41. Panchal, H.J., Suhagia, B.N., Patel, N.J., Rathod, I.S., Patel, B.H., Simultaneous estimation of atorvastatin calcium, ramipril and aspirin in capsule dosage form by RP-LC, Chromatographia, 69(1-2): 91-95(2009).
42. Panchal, H.J., Suhagia, B.N., Simultaneous determination of atorvastatin calcium and ramipril in capsule dosage forms by high-performance liquid chromatography and high-performance thin layer chromatography,Journal of AOAC International., 93(5): 1450-1457(2010).
43. Seshadri RK, Desai MM, Raghavaraju TV, Krishnan D, Rao DV, Chakravarthy IE, Simultaneous quantitative determination of metoprolol, atorvastatin and ramipril in capsules by a validated stability-indicating RP-UPLC method, Sci Pharm, 78(4):821-34, (2010).
44. Thamake SL, Jadhav SD, Pishawikar SA, Development and Validation of method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril from Capsule dosage form by derivative spectroscopy, Asian Journal Research chem.,2(1),52-53 Jan-Mar (2009).
45. A.Sharma, B.Shah,B.Patel, Simultaneous Estimation of Atorvastatin Calcium,Ramipril and Aspirin in Capsule dosage form using HPTLC, Der pharmachemica, 2(4),10-16,(2010).