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Research Article

Development and Method Validation of Atorvastatin Calcium and Telmisartan in Tablet Dosage form by RP-HPLC Method

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ABSTRACT
A simple, economical, accurate, precise and robust reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Atorvastatin Calcium (ATC) and Telmisartan (TEL) in pharmaceutical dosage form. The HPLC separation was achieved on Chemsil C18 column (150 mm x 4.6 mm id, 5 µ particle size) with isocratic condition at ambient temperature using mobile phase as a buffer (0.02 M ammonium acetate buffer pH 4.0 by glacial acetic acid) : Acetonitrile : Tetrahydrofuran in the ratio (400:400:14 v/v/v). The analysis was performed at flow rate 1.5 ml/min. Quantification was achieved with UV detection at 246 nm. Retention time of Atorvastatin Calcium and Telmisartan were found to be 5.70 ± 0.20 minute and 6.72 ± 0.20 minute respectively. The linearity was studied in the concentration range 10-60 µg/ml and 40-240 µg/ml for Atorvastatin Calcium and Telmisartan respectively. The assay was validated for the parameters like system suitability, linearity, accuracy, precision, robustness, LOD & LOQ.

Keyword: RP-HPLC; Atorvastatin Calcium; Telmisartan; Method Validation

INTRODUCTION

Atorvastatin Calcium (ATC), chemically it is calcium salt of (βR, δR)-2-(4-flurophenyl)-α, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl-amino) carbonyl] -1H-pyroll-1-heptanoic acid trihydrate [1]. It is an antihyperlipidemic, that is it reduces level of bad cholesterol (low-density lipoprotein or LDL) and triglycerides in the blood, while increasing of good cholesterol (high-density lipoprotein or HDL)[4]. It is official in IP [1], BP[3] and USP[2]. Telmisartan (TEL), chemically it is 4-{[4-methyl-6-(1-methyl-1H-benzimidazole-2-yl)-2-propyl-1H-benzimidazole-1-yl]methyl}-2-biphenyl carboxalic acid [1]. It is an antihypertensive. It is a new angiotensin II receptor antagonist that is highly selective for type I angiotensin II receptor. Angiotensin II is the principle pressure agent of the rennin-angiotensin system with effects that include vasoconstriction, stimulation o synthesis and release of aldosterone cardiac stimulation and renal reabsorption of sodium [10]. It is official in IP [1], BP [3] and USP [2]. Telmisartan and Atorvastatin are introduced into the market in combined dosage form, which is widely used in

the treatment of hypertension. Literature review reveals that the methods have been reported for Telmisartan and Atorvastatin alone or in combined dosage forms are such as RP-HPLC, Spectrophotometric, HPTLC, Fluorimetry and ion-pair Chromatographic method [6-20].

In the present study attempt were made to develop and validated rapid, economical, precise and accurate new method for simultaneous estimation of ATC and TEL by RP-HPLC method. The developed RP-HPLC method utilised economical solvent system having advantages like better retention time, very sharp peak, symmetric peak shapes and resolution. The developed method was validated according to ICH guidelines [5].

MATERIALS AND METHOD

Materials

Working standards of ATC (potency = 95.70%) and TEL (potency = 99.94%) obtained as a gift samples from Glenmark pharmaceutical Ltd. (Mumbai, India). HPLC grade Acetonitrile, Methanol, Tetrahydrofuran, AR grades of glacial acetic acid and Ammonium acetate were procured from Merck Ltd. Mumbai India. Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a 0.45µ membrane filter paper (make-MDI). The commercial fixed dose combination product Telista plus 40 tablet (Marketed by Lupin Ltd. Mumbai) containing 10 mg ATC and 40 mg TEL was procured from the local market.

Method

The HPLC system used for analysis consisted of Shimadzu LC-2010 CHT, autosampler, UV detector with LC solution software for data acquisition and processing. The chromatographic separation was performed on Chemsil C18 column (150 mm x 4.6 mm id, 5 µ particle size) with isocratic condition at ambient temperature. The analysis was performed at flow rate 1.5 ml/min. Quantification was achieved with UV detection at 246 nm. Retention time of Atorvastatin Calcium and Telmisartan were found to be 5.70 ± 0.10 minute and 6.72 ± 0.10 minute respectively. Analytical balance of Mettler Toledo and digital pH meter of Eutech instruments pH tutor was used for analysis purpose. Chromatographic conditions are summarized in below Table.

Chromatographic Conditions:

HPLC system	LC-2010 CHT , Shimadzu
Software	LC Solution
Detector	UV Detector
Wavelength	246 nm
Pump	Isocratic Pump
Stationary phase	Chemsil C18(150 mm x 4.6 mm id, 5 µ particle size)
Mobile phase	0.02 M Ammonium acetate buffer (pH 4.0 ± 0.05): ACN:THF (40:40:1.4 v/v/v)
Flow rate	1.5 ml/min
Injection volume	20 µl
Diluent	Methanol: Water (80:20 v/v)
Column temperature	25^0c

Standard solution preparation

To prepare a stock solution for assay, weight accurately equivalent to 50 mg of Atorvastatin working standard and transferred into 100 ml volumetric flask, to this 50 ml diluent was added to dissolve the substance by sonication for 5 minutes and volume was made upto the mark by diluent (solution A). weight accurately 100 mg of Telmisartan working standard and transferred to 100 ml volumetric flask, to this 40 ml diluent was added to dissolve the substance by sonication for 5 minutes and the volume was made upto the mark by diluent (solution B). 5 ml of solution A and 10 ml of solution B was taken

in 50 ml volumetric flask with the help of pipette and the volume was made upto the mark by diluent (solution C).

Preparation of tablet dosage form

To prepare a stock solution for assay, 20 tablets (Telista plus) were weight and mixed well. The average weight was determined and they were finally powdered. An aliquot of powder equivalent to 25 mg of Atorvastatin and 100 mg of Telmisartan were transferred into 100 ml volumetric flask, to this 40 ml diluent was added to dissolve the substance by sonication for 10 minutes and diluted with diluent (solution X). The resolution solution was stirred for 1 hour Typical chromatogram of ATC and TEL are and centrifuged at 5000 RPM for 10 minutes. 5 ml of solution X was taken into 25 ml volumetric flask and the volume was made upto the mark by diluent.

Method Validation

The developed method was validated according to International Conference on Harmonisation (ICH) (Q2) B guidelines for validation of analytical procedures. As per the ICH guidelines the method validation parameters checked were linearity, accuracy, precision, assay, LOD, LOQ and robustness [5].

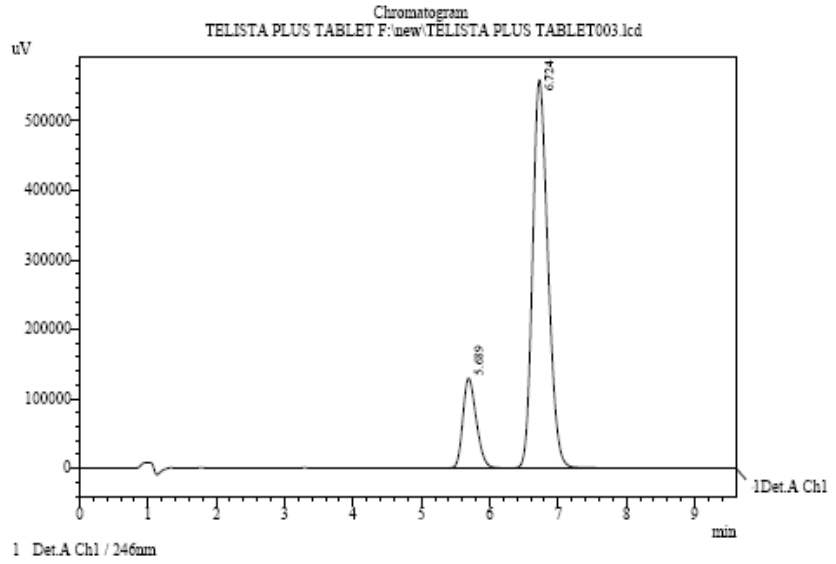

Fig. 1: Chromatogram of Standard solution

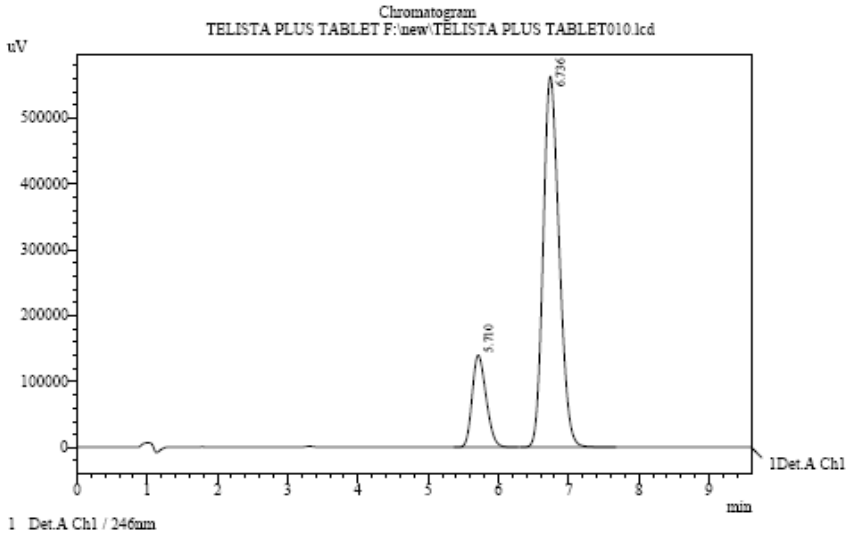

Fig. 2: Chromatogram of Sample solution

RESULTS AND DISCUSSION

System suitability

System suitability test of the chromatographic system was performed before each validation run. Six replicate injections of standard preparation was injected and tailing factor, theoretical plates, resolution and % RSD of peak area were determine for the same. Acceptance criteria for system suitability parameters that are tailing factor should not be more than 2, theoretical plates should be not less than 2000, resolution should be not less than 2 and % RSD of peak area should not be more than 2.0%. System suitability test parameters for ATC and TEL for the developed method are reported in table 1.

Table 1: System suitability parameters of ATC and TEL

Parameters	ATC ± % RSD (n=6)	TEL ± % RSD (n=6)
Retention time (min)	5.704 ± 0.470	6.724 ± 0.135
Tailing factor	1.279 ± 0.503	1.226 ± 0.357
Theoretical plates	3821.667 ± 0.636	4067.167 ± 0.922
Resolution		2.596 ± 1.918

Linearity (Calibration Curve)

For constructing calibration curve, series of six dilutions in the concentration range 10-60 (10, 20, 30, 40, 50, and 60) µg/ml for ATC and 40-240 (40, 80, 120, 160, 200, and 240) µg/ml for TEL was taken. Calibration curve were constructed by plotting peak area vs. concentration of ATC and TEL and regression equation calculated from straight line equation. Linearity curves for ATC and TEL shown in figure no. 3 and 4 respectively.

The method showed good linear response in the concentration range 10-60 µg/ml for ATC (r2 = 0.9998) & 40-240 µg/ml for TEL (r2 = 0.9999)

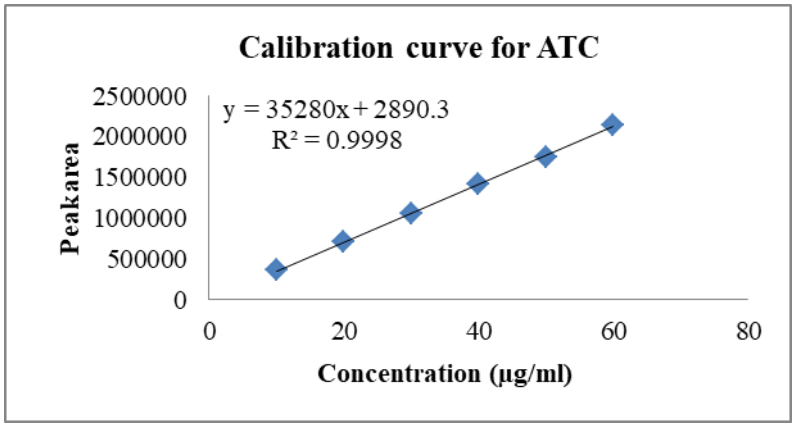

Fig. 3: Linearity curve of ATC

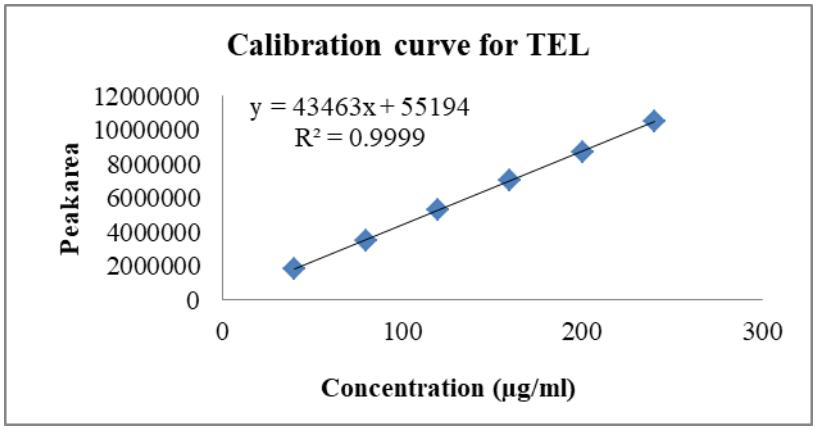

Fig.4: Linearity curve of TEL

Table. 2: Regression parameters of calibration curve

Parameters	ATC	TEL
Linear range (µg/ml)	10-60	40-240
Slope	35280	43463
Intercept	2890.3	55194
Correlation Coefficient (r^2)	0.9998	0.9999

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of ATC and TEL by the standard addition method. The accuracy of the analytical method was assessed by determination of recovery for three concentrations (corresponding to 80,100 and 120% of test solution concentration). For each concentration, three sets were prepared. The mean recovery and % RSD of recoveries of ATC and TEL were reported.

The results of recovery of ATC and TEL with the %RSD are given in below table.

Table 3: Accuracy study data of ATC

Accuracy level	Set No.	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	Mean recovery (%)	SD	RSD (%)
80%	1	80	79.92	99.9	100.4	0.611	0.608
	2	80	80.88	101.1			
	3	80	80.24	100.3			
100%	1	100	98.30	98.3	99.3	1.112	1.120
	2	100	100.50	100.5			
	3	100	99.10	99.1			
120%	1	120	118.32	98.6	99.1	0.585	0.591
	2	120	119.64	99.7			
	3	120	118.68	98.9			

Table 4: Accuracy study data of TEL

Accuracy level	Set No.	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	Mean recovery (%)	SD	RSD (%)
80%	1	320	324.48	101.4	100.6	0.802	0.797
	2	320	322.24	100.7			
	3	320	319.36	99.8			
100%	1	400	405.20	101.3	99.8	1.286	1.288
	2	400	395.60	98.9			
	3	400	397.20	99.3			
120%	1	480	484.32	100.9	100.9	0.700	0.694
	2	480	487.68	101.6			
	3	480	480.96	100.2			

Precision

The precision of analytical method express the degree of agreement among individual test when the procedure is applied repeatedly to multiple sampling of homogenous samples. Precision are considered at three levels that is system

precision, method precision (repeatability) and intermediate precision (reproducibility).

System precision

The system precision of the instrument was checked by repeatedly injecting (n =6) standard solutions of the ATC and TEL under the same chromatographic condition and calculate the % RSD of peak area which should not be more than 2%.

Method precision (Repeatability)

The method precision of the analytical method was determined by analysed six sets of sample preparation against the same standard. Assay of all six sample preparation was determined and mean of assay, standard deviation and %RSD for the same was calculated.

Intermediate Precision (Reproducibility)

Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different instrument under same experimental conditions. Assay of all replicate sample preparation was determined and mean assay, standard deviation and %RSD for the same was calculated.

The method was found to be precise and %RSD was found to be less than 2% was shown in below tables.

Table 5: Data of system precision study

Sr. No.	Area of ATC	Area of TEL
1	1755615	8687517
2	1761837	8707308
3	1759043	8695944
4	1753459	8698669
5	1760735	8714866
6	1757547	8683935
Mean	1758039.3	8698039.8
Standard deviation	3155.803	11691.736
%RSD	0.180	0.134

Table 6: Data of method precision study

Sr. No.	Wt of sample in mg	Avg. Area of ATC	Avg. Area of TEL	% Assay of ATC	% Assay of TEL
1	667.62	1837082	8839969	100.1	101.5
2	673.51	1845032	8834951	101.0	101.9
3	672.60	1819619	8726720	99.7	100.8
4	672.33	1793259	8638661	98.3	99.8
5	676.71	1864703	8830359	101.6	101.4
6	675.20	1827673	8617555	99.8	99.2
Mean				100.1	100.8
Standard Deviation				1.128	1.069
%RSD				1.128	1.061

Table 7: Data of intermediate precision study

Sr. No.	Wt of sample in mg	Avg. Area of ATC	Avg. Area of TEL	% Assay of ATC	% Assay of TEL
1	676.62	1807025	8806969	100.6	101.1
2	674.13	1779871	8730189	99.5	100.5
3	671.31	1813561	8792151	101.8	101.7
4	670.30	1774612	8706987	99.7	100.8
5	676.22	1769436	8827442	98.6	101.3
6	674.06	1811644	8663991	101.2	99.8
Mean				100.2	100.9
Standard Deviation				1.194	0.662
%RSD				1.192	0.657

Assay of ATC and TEL shown in table 8.

Table 8: Results of assay

Drugs	Label claim (mg/tab)	Amount of drug estimated (mg/tab)	% Amount found
ATC	10.0	10.07	100.7
TEL	40.0	40.72	101.8

Robustness

The robustness of the method was established by introducing small changes in various parameters like, pH of mobile phase, flow rate, wavelength, column temperature and mobile phase composition. The result obtained from assay of test solution was not affected by varying the conditions and in accordance with true value.

The robustness of the method was evaluate by calculating % assay of test solution which is not more than ± 2.0% from mean value of method precision and system suitability parameters meets the requirements.

Robustness was evaluated by varying different parameters. The results of these variations are given in table 9.

Table 9: Robustness study of ATC and TEL

Parameters	Variation	ATC		TEL	
		Retention time(min)	Assay (%)	Retention time(min)	Assay (%)
Flow rate(ml/min)	1.3	6.56	100.65	7.75	101.25
	1.5	5.68	100.01	6.71	98.19
	1.7	5.06	98.23	5.95	101.55
pH	3.8	5.68	99.36	6.71	100.62
	4.0	5.68	98.43	6.71	101.22
	4.2	5.70	99.90	6.73	100.02
Column temperature (^{0}C)	24	5.68	100.35	6.71	98.36
	25	5.68	99.56	6.71	100.34
	26	5.67	98.65	6.71	101.53
Wavelength(nm)	245	5.70	101.85	6.72	100.25
	246	5.68	98.25	6.71	98.88
	247	5.70	99.37	6.72	101.73

Limit of Detection & Limit of Quantification

Limit of Detection (LOD) is the lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of Quantification (LOQ) is the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. According to ICH recommendation, the approach based on the standard deviation (SD) of the response and slope (M) was used for determining the detection and quantification limits. LOD can be calculated according to formula LOD = 3.3 (SD/M) and LOQ = 10(SD/M). The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 & LOQ as the amount for which the signal to noise ratio was 10:1.

LOD and LOQ results of ATC and TEL are given in table 10.

Table 10: LOD and LOQ study of ATC & TEL

Drugs	LOD (µg/ml)	LOQ (µg/ml)
ATC	0.228	0.692
TEL	1.187	3.596

CONCLUSION

The developed method was suitable for the simultaneous estimation of Atorvastatin Calcium and Telmisartan in tablet dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH of mobile phase, column temperature, wavelength etc. A good peak symmetry, resolution between ATC and TEL was obtained with mobile phase Buffer (pH 4.0 ± 0.05): ACN: THF (40:40:1.4 v/v/v) at a flow rate 1.5 ml/min. The wavelength of detection selected was 246 nm. The retention time of Atorvastatin Calcium and Telmisartan was about 5.70 ± 0.20 minute and 6.72 ± 0.20 minute respectively. A validated RP-HPLC method has been developed for the determination of Atorvastatin calcium and Telmisartan in tablet dosage form. The developed method is simple, rapid, linear, accurate, precise and specific. Results from the validation experiments showed that the method is reliable and accurate therefore it can be successfully applied for the routine quality control analysis of Atorvastatin calcium and Telmisartan in pharmaceutical dosage form.

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REFERENCES

1. Indian Pharmacopoeia. Ministry of Health and Family Welfare Government of India. Ghaziabad: Indian Pharmacopoeia Commission; 2010; vol. II & vol. III, p 849 & 2186.
2. The United State Pharmacopoeia. America: The United States Pharmacopoeia Commission; 2012, USP 35 NF 30, p 2263 & 4776.
3. The British Pharmacopoeia. London: The British Pharmacopoeia Commission; 2012, vol. I & II, p 2191 & 2154.
4. Available from www. Drugs. Com. Cerner multum et al. Inc. Version. 2000-2015;
5. ICH Harmonized Tripartite Guideline, Validation of analytical procedure text and methodology Q2 [R1]. In International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, 2005.
6. Patil UP, Gandhi S V, et al. Int J Chem Tech Res 2009; 1(4): 970-973.
7. Napa D, Socalingam A. Int J Res Rev Pharm Appl Sci 2012; 2(5): 959-964.
8. Mukuntha N K, Sumathi V. Rao, et al. Int J Scient Res 2014; 3(11): 357-359.
9. Chaudhari KU, Gaikwad PD, et al. Int J Pharm Technol 2010; 2(2): 255-264.

10. Vijayamirtharaj R, Ramesh J, et al. Pharmacie Globale Int J Comprehen Pharm 2010; 1(04): 1-4.
11. Saurabh K Sinha, Prabhat K Shrivashtava, et al., Asian Pacific J Trop Biomed 2012; S312-S315.
12. Anusha M, Meena Bharathi D, et al. Int J Pharm Sci Rev Res 2012; 16(2): 111-115.
13. Raj P. Ashok et al. Indian J Res Pharm Biotechnol 2013; 1(6): 786-792.
14. Panchal PJ, Suhagia BN. J Asso Official Analyt Chem 2010; 93(5): 1450-1457.
15. Patole S, Khodke A, et al., J Youngpharm 2011; 3(1): 55-59.
16. Pilli NR, Inamadugh JK, et al. Simultaneous determination of Atorvastatin, Amlodipine, Ramipril and Benzepril in human plasma by LC-MS/MS and its applications to a human pharmacokinetic study. Biomed Chromat 2011; 25(4): 439-449.
17. Shah Y, Iqbal Z, et al. Simultaneous determination of Rosuvastatin and Atorvastatin in human serum using RP-HPLC/UV detection: method development, validation and optimization of various experimental parameters. J Chromat B Anal Technol Biomed Life Sci 2011; 879 (9-10): 557-563.
18. Mhaske RA, Garole DJ, et al. RP-HPLC method for simultaneous determination of Amlodipine Besylate, Valsartan, Telmisartan, Hydrochlorothiazide and Chlorthalidone: application to commercially available drug products. Int J Pharm Sci Res 2012; 3(1): 141-149.
19. Lakshmi Sivasubramanian, Lakshmi KS. H-point standard addition method for simultaneous spectrophotometric determination of Irbesartan, hydrochlorothiazide and Telmisartan in tablet. Int J Res Pharm Chem 2014; 4(2): 373-380.
20. Asha B. Thomas, Sheetal N. et al. Simultaneous spectrophotometric estimation of Amlodipine Besylate and Telmisartan in tablet dosage form. Int J Pharma Tech 2010; 2(2): 1334-1341.
21. Vidhya K, Bhusari SR, Dhaneshwar. Validatd HPTLC method for simultaneous estimation of Atenolol, Hydrochlorothiazide and Amlodipine Besylate in bulk drug and formulation. Int J Anal Bioanal Chem 2011; 1(3): 70-76.
22. Vekariya NR, Patel MB, et al. Development and validation of TLC-densitometry method for simultaneous determination of Telmisartan and Amlodipine Besylate in bulk and tablets. J Young Pharm 2009; 1(3): 259-263.
23. Prajakta S, Nawale AA, Shrikhedkar, et al. Normal and reversed-phase HPTLC methods for simultaneous estimation of Telmisartan and Metoprolol Succinate in pharmaceutical formulation. Int Schol Res Net 2012; Article ID 815353: 1-6.
24. Patil UP, Gandhi SV, et al. A validated densitometric method for analysis of Telmisartan and Atorvastatin calcium in fixed dose combination. J Chilean Chem Soc 2010; 55 (N 1): 94-96.
25. Aniruddha R, Chabukswar SC, Jagdale, et al. Simultaneous HPTLC estimation of Telmisartan and Amlodipine Besylate in tablet dosage form. Schol Res Lib 2012; 2(3): 94-100.

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