Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2015, 6(4):13-18

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Simultaneous determination of Telmisartan, Amlodipine Besylate and Hydrochlorothiazide in tablet dosage form by using stability-indicating HPLC method

Shivaji Jadhav[1]*, Megha Rai[1], P. B. Kawde[3] and Mazahar Farooqui[1,2]

[1]Dr. Rafiq Zakaria College for Women, Aurangabad
[2]Post Graduate and Research Centre, Maulana Azad College, Aurangabad
[3]R & D Centre, Glenmark pharmaceuticals, Nasik

ABSTRACT

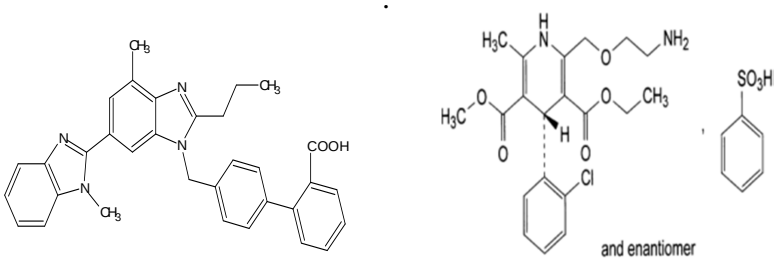
A simple, precise and rapid stability-indicating HPLC method was developed for the simultaneous quantitative determination of Telmisartan, Amlodipine and Hydrochlorothiazide from their innovative Pharmaceutical combination drug product, with the presence of degradation products. The separation was achieved on simple gradient method. The detector wavelength was 260 nm. The total runtime was 35min. and RT of Hydrochlorothiazide, Amlodipine and Telmisartan are 4.4, 8.2 and 18.7 min. respectively. The described method was validated with respect to system suitability, specificity, linearity, precision and accuracy.

Keywords: Validation, Telmisartan, Amlodipine, Hydrochlorothiazide and HPLC

INTRODUCTION

Cardiovascular diseases (CVDs) are the disorders of heart and blood vessels and primarily include coronary heart disease, hypertension, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. CVDs are the major cause of death in developed countries and also are rapidly emerging as a main cause of death in the developing world[1]. A novel formulation is developed using drugs Telmisartan, Amlodipine and Hydrochlorothiazide for CVDs[2].Telmisartan (TLM) is a nonpeptide angiotensin II receptor antagonist. It controls high blood pressure and works by blocking the effect of hormone called angiotensin-II. This angiotensin-II tends to constrict the blood vessels while promoting retention of salt and water, actions that tends to raise the blood pressure. Telmisartan prevents these effects and keeps blood pressure lower. (**Figure 1**) AMD is in a class of drugs called beta-blockers. Beta-blockers affect the heart and circulatory system. Amlodipine Besylate is used to lower blood pressure, lower heart rate, reduce chest pain, and to reduce the risk of re- current heart attacks (**Figure 1**)HYD is a thiazide diuretic. It decreases the amount of fluid in the body by increasing the amount of salt and water lost in the urine. Hydrochlorothiazide is used to lower blood pressure[3-5].

In the pharmaceutical and other fields, there is demand for the development of simultaneous analytical method to minimise the cost and time. Literature survey reveals that a variety of Spectrophotometry and chromatographic methods including UV, colorimetric determination, ratio derivative, and a stability-indicating HPLC methods have been reported for determination TLM, AMD and HYD either single or in double combination with other drugs[6-8]. But no HPLC method has been reported for simultaneous quantitative determination of TLM, AMD and HYD. Hence a rapid simple reproducible HPLC method was developed for simultaneous quantitative determination of TLM, AMD and HYD in pharmaceutical dosage forms in the presence of degradation products[9-11].



(a)Telmisartan **(b)Amlodipine** **(c)Hydrochlorothiazide**

Figure 1. Structures of Telmisartan, Amlodipine and Hydrochlorothiazide

MATERIALS AND METHODS

*Chemicals and Reagents***:** Standards and tablet (80 mg of Telmisartan, 5 mg of Amlodipine Besylate, 12.5mg of Hydrochlorothiazide).The HPLC grade acetonitrile and methanol, KH_2PO_4/Monobasic, OPA, Triethylamine and Watermilli Q.

*Instrumentation***:** HPLC system of Shimadzu Japan with UV detector and Waters USA with PDA detector used consisting of a quart nary solvent manager. The output signal was monitored and processed using Empower software, water bath equipped with controller (Classic Scientific, India) was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Thermo lab, India). Thermal stability studies were performed in a dry air oven (Newtronic, India).

*Chromatographic Conditions***:** The chromatographic column used was Phenomenex Luna C8, 250 mm × 4.6 mm, 5.0 µm particles. The Mobile phase A buffer prepared by Dissolving 3.4 gm of KH_2PO_4/Monobasic in 800 ml of water, add 1.4 ml of triethylamine and adjust pH 3.5 (±0.1) with OPA and mobile B is Acetonitrile. The flow rate was 1.0 mL·min–1.The gradient program (T/%B) was set as 0/35, 12/35, 14/60, 22/60, 25/35 and 35/35. The detector wavelength was 237 nm. Diluents A is dilute 5.0 ml of Triethylamine to 2000 ml with water and 40 volumes of methanol and Diluents BMix buffer solution and Acetonitrile in ratio (65: 35 v/v).

Preparation of Standard

*Standard preparation (solution-a):*Weigh accurately 160 mg Telmisartan working standard and 25.0 mg of working standard of Hydrochlorothiazide and transfer it into 200 ml dry volumetric flask, add 170 ml of diluents A, keep the flask in ultrasonic bath for 5 minutes to dissolve the drug completely and make up the volume with diluents A.

*Amlodipine standard preparation (solution-b)***:**Weigh accurately 27.72 mg Amlodipine besilate working standard (equivalent to 20 mg of Amlodipine) and transfer it into 200 ml dry volumetric flask, add 170 ml of diluents A, keep the flask in ultrasonic bath for 5 minutes to dissolve the drug completely and make up the volume with diluents A

*Mix standard preparation***:** Pipette out 10 ml of Solution-a and 5 ml of Solution-b into 50 ml volumetric flask and make up the volume with Diluents B.

Preparation of Sample Solution: Weigh 20 tablets and calculate the average weight. Weigh and Transfer 5 tablet (equivalent to 400 mg of Telmisartan, 62.5 mg of Hydrochlorothiazide and 25mg of Amlodipine) into a 500 ml dry volumetric flask. Add 350 ml of diluent A, shake mechanically for 10 minutes sonicate it for 15 to 20 minutes with intermittent shaking and make up the volume with diluent A. Allow to settle for 15 minutes. Pipette out 5 ml of above solution in 25 ml volumetric flask and make up the volume with diluent B. Filter the above solution through 0.45um Teflon filter paper.

RESULTS AND DISCUSSION

Chromatographic Conditions: The mobile phase conditions were optimized so that the three drugs would be separated in short run time. The UV spectra of the solutions showed the absorptions 225 and 271 nm for

Hydrochlorothiazide, 238 nm for Amlodipine, 257.5nm and 298 nm for Telmisartan, for UV max. (Figure 3)Under the optimum chromatographic conditions, the retention times of Hydrochlorothiazide, Amlodipine and Telmisartan are 4.4, 8.2, and 18.7 min. respectively (Figure 2). The retention times of individual analyte are confirmed by injecting individual working solutions (Figure 4)

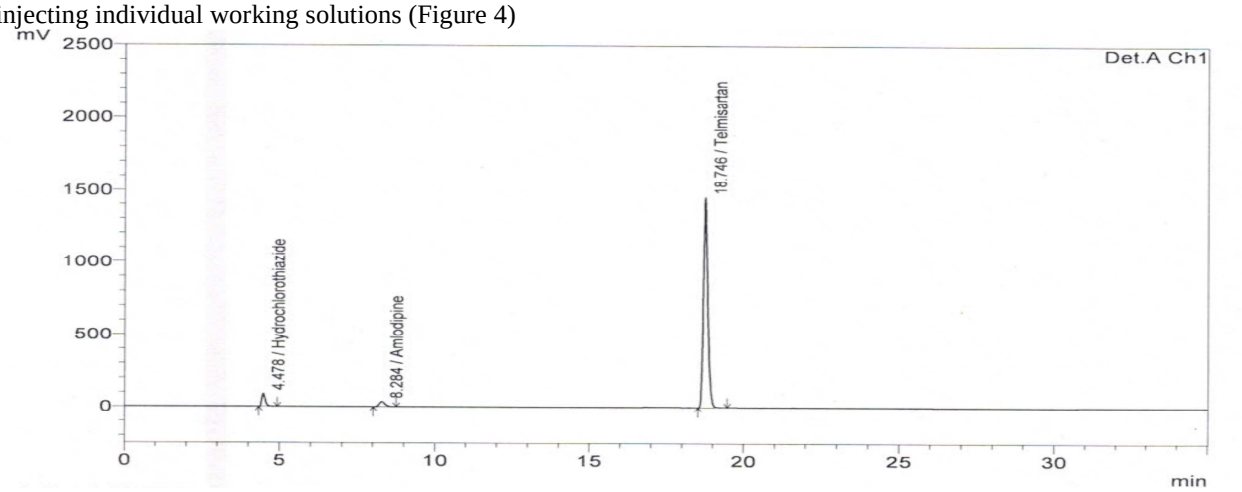
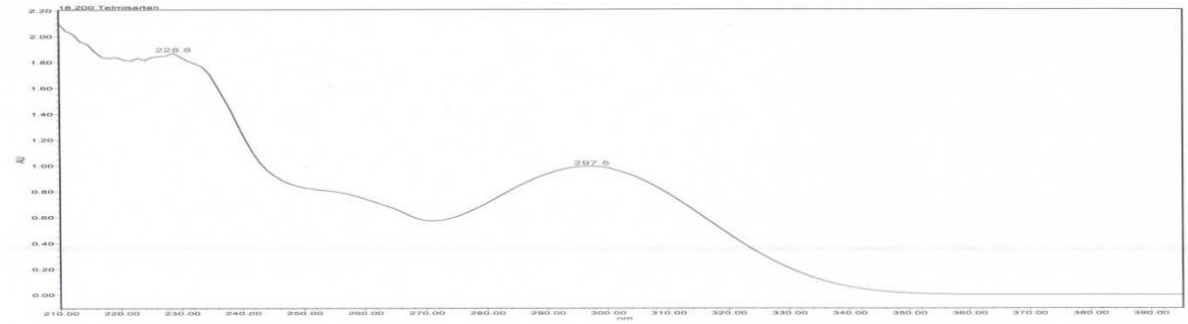
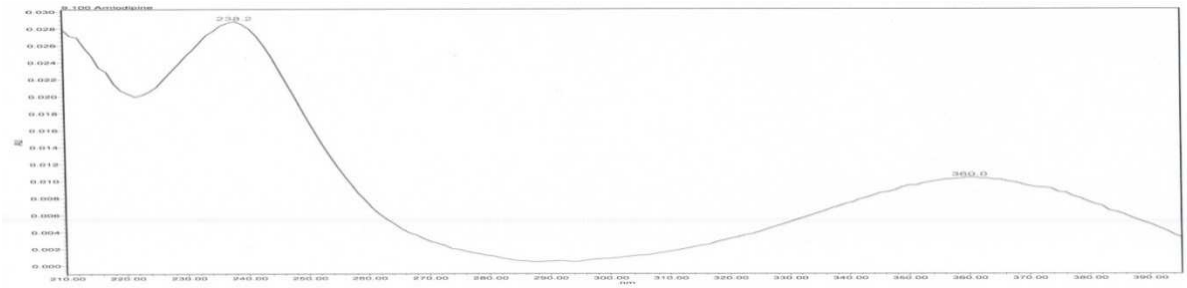


Figure-2: Chromatogram of Hhydrochlorothiazide, Amlodipine and Telmisartan in pharmaceutical sample solution and their retention time

a)Telmisartan



(b)Amlodipine



(c)Hydrochlorothiazide

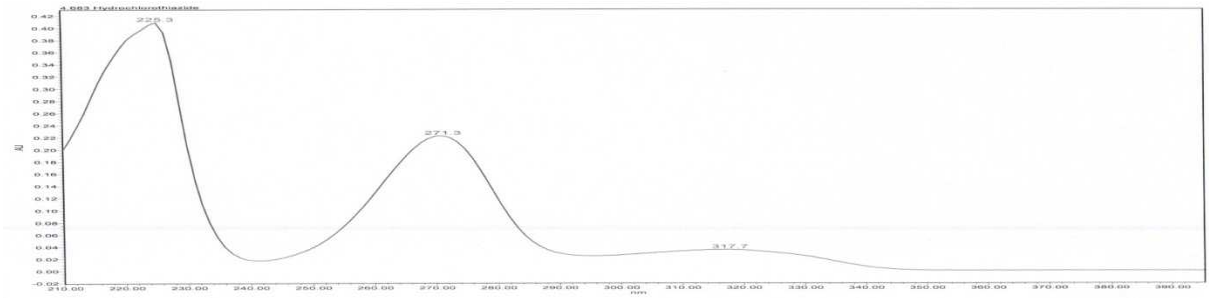
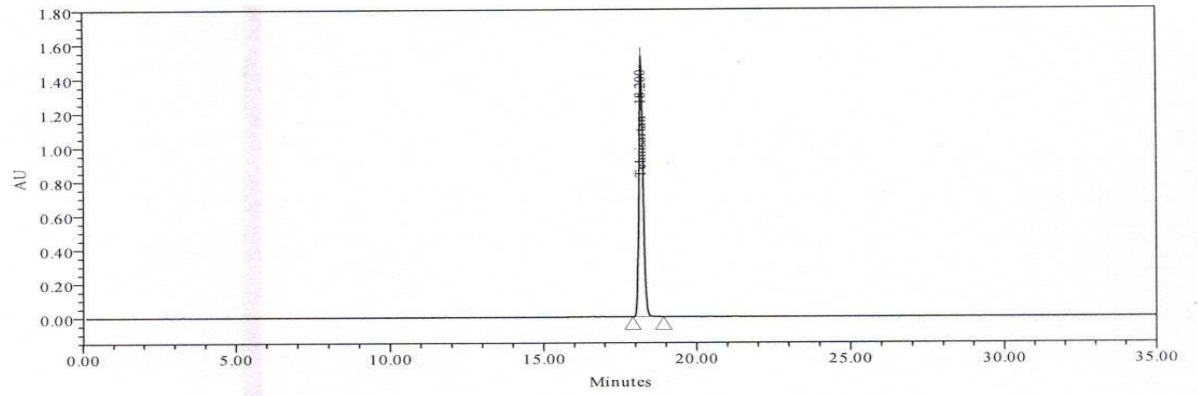
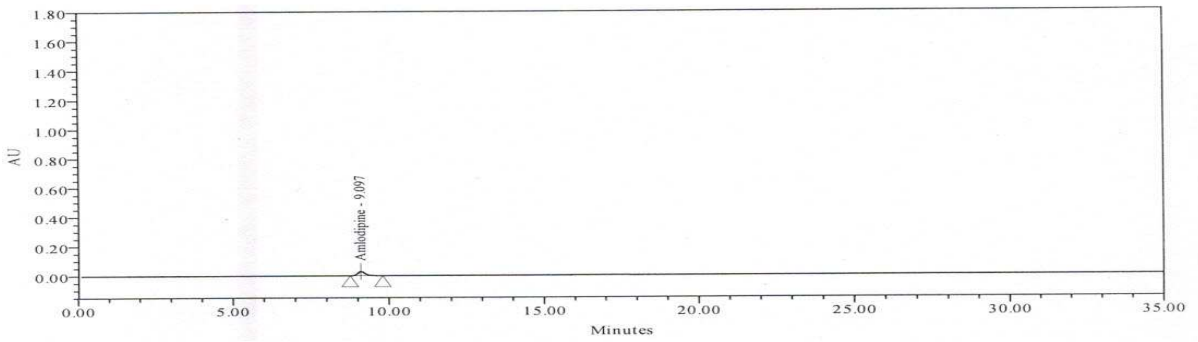


Figure-3: PDA spectra of Telmisartan, Amlodipine and Hydrochlorothiazide

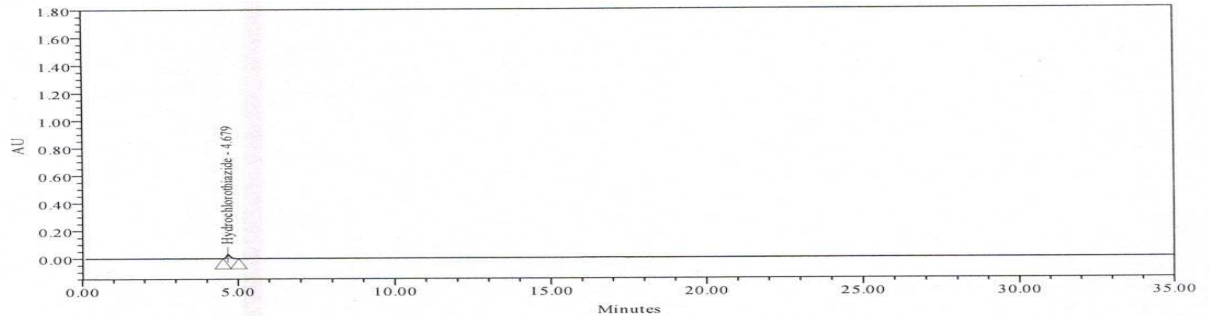
(a)Telmisartan



(b)Amlodipine



(c)Hydrochlorothiazide



Figure–4: Chromatograms of individual standard and their retention times

Validation

*System suitability***:** In order to find the adequate peak separation (resolution) and repeatability of the proposed method, suitability parameters including retention factor, selectivity and asymmetry factor were investigated & the results were summarised in Table 1.

Table -1: System suitability test parameters

System suitability test parameters	HYD	AML	TLM
Retention time (min)(mean ± S.D. n=5)	4.481 ± 0.003	8.290 ± 0.022	18.751 ± 0.006
Repeatability of Retention time RSD % (n=5)	0.076	0.270	0.332
Repeatability of Peak area, RSD % (n=5)	0.067	0.084	0.030
Resolution	---	13.737	34.303
Tailing factor (asymmetry factor)	1.387	1.319	1.351
USP plate count	7066	9443	77722

*Specificity***:** Retention time of Telmisartan, Hydrochlorothiazide, and Amlodipine peaks in sample preparation are comparable with standard preparation. Therefore, the HPLC method for the determination of assay for Telmisartan, Hydrochlorothiazide, and Amlodipine in Telmisartan Amlodipine and Hydrochlorothiazide Tablets is specific.

*Forced degradation studies***:** Stress testing of a drug substance can help to identify the likely degradation products, which can help to establish the degradation pathways and the intrinsic stability of the molecule. Acid induced degradation, Base induced degradation, and Hydrogen peroxide induced degradation, Photo-degradation, Humidity Degradation (25 ° C/92% for 24 hrs.)was carried out with respective condition and peak purity results mentioned in Table-2.

Table-2: Peak purity results of TLM, AML & HYD in forced degradation study

Name	Purity Angle			Purity Threshold			Purity Criteria		
	TLM	AML	HYD	TLM	AML	HYD	TLM	AML	HYD
Acid degradation	0.270	0.641	0.114	1.013	1.685	1.081	Pass	Pass	Pass
Base degradation	0.301	0.640	0.119	1.013	1.695	1.069	Pass	Pass	Pass
Peroxide degradation	0.212	0.832	0.191	1.012	1.909	1.084	Pass	Pass	Pass
Thermal degradation	0.238	0.571	0.109	1.014	1.600	1.074	Pass	Pass	Pass
Photolytic degradation	0.280	0.557	0.106	1.012	1.589	1.072	Pass	Pass	Pass
Humidity degradation	0.179	0.562	0.097	1.013	1.609	1.069	Pass	Pass	Pass

Linearity Linearity solutions were prepared from stock solution at six concentration levels from 50 to150% of analyte concentrations. The slope, Y-intercept and correlation coefficient were calculated and summarized in Table 3.

Table-3: Linearity for Telmisartan, Hydrochlorothiazide, and Amlodipine

Analyte	Concentration range	Correlation Coefficient	Slope	Intercept
HYD	12.63-37.89 µg/mL	0.99970	25465	50617
ALM	4.97-14.91 µg/mL	0.99990	51095	8475
TLM	79.99-239.98 µg/mL	0.99900	79305	1378863

Precision The precision of the assay method was evaluated by carrying out six independent assays of TLM, AML, and HYD test samples against qualified standard.

Table-4: Intraday and inter day precision results for TLM, AML & HYD

Day	Activename	Pre-1 %Assay	Pre-2 %Assay	Pre-3%Assay	Pre-4 %Assay	Pre-5 %Assay	Pre-6 %Assay	Mean	% RSD
Intra-Day	TLM	99.7	99.8	99.2	99.1	99.4	99.3	99.4	0.281
	AML	99.4	99.5	99.0	98.9	99.1	99.0	99.2	0.245
	HYD	100.9	101.1	100.4	100.2	100.3	100.1	100.5	0.403
Inter-Day	TLM	99.6	99.1	99.7	99.4	100.5	99.9	99.7	0.478
	AML	99.8	99.4	100.0	99.7	101.4	100.1	100.1	0.697
	HYD	99.7	99.1	99.6	99.2	101.2	99.6	99.7	0.760

Accuracy The accuracy of an analytical method expresses the nearness between the reference value and found value. The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 80%, 100% and 120% of target test concentration (321.56 to 478.35 µg/mL for TLM, 19.99 to 30.18 µg/mL for AML and 50.94 to 75.77 µg/mL for HYD) in tablets. The results obtained are shown in Table 5.

Table-5: Accuracy

Analyte	Recovery level	Amount Added (mg)	Amount Recovered (mg)	% RSD
Hydrochlorothiazide	80 %	50.94	51.227	0.65
	100%	62.91	63.11	0.35
	120 %	75.77	75.29	0.57
Amlodipine	80 %	19.99	20.014	0.26
	100%	25.05	25.056	0.40
	120 %	30.18	30.281	0.45
Telmisartan	80 %	321.56	323.77	0.76
	100%	400.23	398.27	0.40
	120 %	478.35	473.09	0.42

Solution Stability & Mobile Phase Stability The solution stability of TLM, AML, and HYD was carried out by leaving the test solution in tightly capped volumetric flask at room temperature for 30 hrs. The same sample solution was assayed for a 9, 18 and 30hours against freshly prepared standard solution. The mobile phase stability was also carried out by assaying the freshly prepared standard solution up to 48 hours The % RSD of the assay of TLM,

AML, and HYD during solution stability and mobile phase experiments were within 1% and it indicates that both standard and test preparation and mobile phase were stable for 30 hrs on bench top at room temperature.

CONCLUSION

The established HPLC method proves to be simple, linear, precise, accurate and specific. The method was validated and shows satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs TLM, AML, and HYD in presence of degradation products in stability by the industry.

Acknowledgement
The authors wish to thank the management of Glenmark Pharmaceutical Ltd. for supporting this work and Analytical Research & Development of Glenmark Pharmaceutical Ltd. is appreciated.

REFERENCES

[1] Megha Rai, P. B. Kawde; *Res J Pharm Bio Chem. Sci*, **2013**, 4(1), 560
[2] J. G. Panchal, R. V. Patel, B. R. Mistry, S. K. Menon; *Eurasian J Anal Chem*, **2012**, 7,28
[3] K. K. Kumar, C. K. Rao, G. Madhusudan: *Am. J Anal Chem* **2012**, 3,50
[4] S. Nalwade, V. R. Reddy, D. D. Rao. I. K. Rao; *Scientia Pharma*. **2011**, 79, 69
[5] S. D. Kayal, F. A. Khan, A. V. Chandewar; *Int J Pharm Res Dev*, **2011**, 3(5), 144
[6] Gajanan Kasawar and Mazhar Farooqui; *Analytical Sciences* **2009,** 25,1495
[7] Sayyed Husain, Rashid R. Munjewar, Mazahar Farooqui, *J Pharma Sci Tech*, **2012**, 1(2), 1
[8] Ganjanan B. Kasawar, Mazahar Farooqui *Orbital: The elce J of Chem*, **2014**, 6(31), 130
[9] S. B. Wankhede, S. G. Wadodkar; *Ind J Pharm Sci*, **2007**, 69(2), 298
[10] J. Swaminathan , T. Vetrichelvan; *Int. J. Pharm. Res. & Anal*, 1(1), **2011**, 7-14.
[11] R. N. Rao, S. Sen, P. Nagaraju, V. S. Reddy, P. R. Krishnamurthy & U. Bhaskar; *Asian J Chem*, **2006**, 18(2), 775
[12] L. R. Bhat, R. K. Gordge & M. C. Damle; *J Liquid Chrom & Related Tech*, **2007**, 30(20), 3059