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Development and Validation of a stability indicating RP-HPLC method for simultaneous determination of Telmisartan, Chlorthalidone and Cilnidipine in pharmaceutical combined dosage forms

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ABSTRACT

The study describes development and subsequent validation of a stability indicating reverse-phase HPLC method for simultaneous estimation of the Telmisartan, Chlorthalidone and Cilnidipine in combined solid dosage forms using RP-HPLC. Separation was accomplished on Kromasil 250 x 4.6 mm, 5µm C_{18} column using 0.1% OPA buffer and acetonitrile (57:43 v/v) as mobile phase pumped through at a flow rate of 1.2 ml/min at 30°C. Optimized wavelength was 238nm. Retention time of Telmisartan, Chlorthalidone and Cilnidipine were found to be 3.106min, 2.573min and 3.924 min respectively. %RSD of the Telmisartan, Chlorthalidone and Cilnidipine were found to be 0.87, 0.96 and 0.94 respectively. % recovery was obtained as 100.18%, 100.06% and 100.13% for Telmisartan, Chlorthalidone and Cilnidipine respectively. The proposed method also proved to be suitable as a rapid and reliable quality control method.

Keywords: Telmisartan, Chlorthalidone, Cilnidipine, RP-HPLC, Simultaneous determination, Degradation studies.

INTRODUCTION

Telmisartan is chemically nominated as (4-{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl) benzoic acid (Figure 1).Its molecular formula is C33H30N4O2 and molecular weight is 514.62. It is a diabetes angiotensin receptor blocker that shows high affinity for the angiotensin II type 1 (AT1) receptors, has a long duration of action, and has the longest half-life of any angiotensin II receptor blocker (ARB) [1]. In clinical studies, Telmisartan shows comparable antihypertensive activity to other major antihypertensive classes, such as angiotensin converting enzyme (ACE) inhibitors, beta-blockers, and calcium antagonists [2].

Chlorthalidone or Chlorthalidone is a sulphamyl benzophenone derivative, chemically known as [2-chloro-5-(1-hydroxyl-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide] (Figure 2). It is a thiazide-like diuretic, as it acts in a similar manner to the thiazide drugs but does not include the benzothiadiazine structure. It is used in the treatment of edema coupled with congestive heart failure, cirrhosis of the liver, fluid retention caused due to kidney disease and hypertension by reducing the electrolyte salts and water in the body. It also used in the treatment of diabetes insipidus and prevents the formation of calcium kidney stones in people with

increased levels of calcium in their urine (hyper calciuria) [3, 4].

Cilnidipine is chemically nominated as 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine carboxylic acid 2-methoxy ethyl (2E)-3-phenyl-propenyl ester (Figure 3). It is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals that supply blood vessels [5].

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) suggests that stress testing is an essential part of development strategy and is carried out under more severe condition than accelerated conditions. These studies provide information to establish its inherent stability characteristics of the molecule such as the degradation pathways, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures [6–7]. According to ICH guidelines stress testing should include the effect of temperature, light, oxidizing agents and susceptibility across a wide range of pH values and separation of drugs from degradation products [8]. It is also suggested that analysis of stability sample should be carried out using validated stability testing methods.

The literature survey reveals that there is no analytical method available for estimation of Telmisartan, Chlorthalidone and Cilnidipine in combined pharmaceutical dosages. The reported methods available for the individual estimation of Telmisartan [9-16], Chlorthalidone and Cilnidipine [17-22] either alone or in combination [23] with other drugs in pharmaceutical dosage forms or individually in biological fluids [24]. Stability indicating and simultaneous RP-HPLC estimation of Telmisartan and Cilnidipine, Cilnidipine and Chlorthalidone in combined tablet dosage form have been reported [25-28]. To our knowledge there has been no Stability indicating RP HPLC method development and validation reported for the simultaneous estimation of Telmisartan, Chlorthalidone and Cilnidipine combination in which ICH recommended that stress conditions be applied. Therefore, the stability indicating method was also developed by applying different stress conditions like acidic, alkali, H_2O_2, thermal, and photo degradation. We have planned to develop a new, simple, precise, economic and accurate Stability indicating RP-HPLC method development and validation for the estimation of Telmisartan, Chlorthalidone and Cilnidipine pharmaceutical dosage forms according to ICH [29] Guidelines.

EXPERIMENTAL

Materials and methods

Active pharmaceutical ingredients Telmisartan, Chlorthalidone and Cilnidipine were obtained as a gift samples from Spectrum pharma research solutions, Hyderabad. The pharmaceutical dosage form from NIKSAN Pharmaceutical India Pvt Ltd was purchased from local pharmacy. The solvents used in this work were of HPLC grade and obtained from Merck Specialties Private Limited, Mumbai.

Instrumentation and chromatographic conditions

The analysis was performed on a high performance liquid chromatography system consisting of Waters 2695 with 2996 module Photo Diode Array detector equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat. The data acquisition and analysis was performed by using Empower2 software. The chromatographic separation was performed on Kromasil 250 x 4.6 mm, 5µ C_{18} column using 0.1% OPA buffer and acetonitrile (57:43 v/v) as mobile phase at a flow rate of 1.2 ml/min gave acceptable retention time and good resolution between Telmisartan, Chlorthalidone and Cilnidipine. The column temperature was maintained at 30°C. The method was optimized at 238nm. The run time was taken as 8min.

Preparation of Standard stock solutions:

Accurately weighed and transferred 40mg of Telmisartan, 6.5mg of Chlorthalidone and 10 mg of Cilnidipine working Standards into a 50ml clean dry volumetric flask, add $3/4^{th}$ volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Preparation of Sample stock solutions Sample Preparation:

1tablet was weighed, powdered and it was (370mg) transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Preparation of buffer

Buffer: (0.1%OPA)

1ml of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

Method validation
The method was validated according to ICH guidelines. The different validation characteristics which were performed include: Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantification, Robustness and the stability indicating capability.

System suitability parameters
The system suitability parameters were determined by preparing standard solutions of Telmisartan, Chlorthalidone and Cilnidipine and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Linearity
The linearity of the method was determined by preparing three individual series of solutions in the range of Chlorthalidone (3-20µg/ml), Telmisartan (20-120µg/ml) and Cilnidipine (5-30µg/ml). The obtained peak areas were plotted against concentration.

Preparation of linearity solutions

Preparation of Standard stock solutions: Accurately Weighed and transferred 40mg of Telmisartan, 6.5mg of Chlorthalidone and 10 mg of Cilnidipine working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes. Flasks were made up with diluent and labelled as Standard stock solution 1, 2 and 3.
From three stock solutions pipette out 0.25ml, 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.50ml into 10ml volumetric flask to get 25%,50%, 75%, 100%, 125%, 150% of standard solutions.

Precision
a) Method precision (repeatability)
The method precision/ repeatability can be determined by injecting six working standard solutions and six sample injections. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated.
b) Intermediate precision
The intermediate precision can be determined by injecting six working standard solutions and six sample injections on different days by different operators or by different instruments. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated. The results obtained were within the acceptance criteria.

Accuracy
Accuracy was tested by the standard addition method at three different levels 50, 100 and 150%. The percentage recoveries of Telmisartan, Chlorthalidone and Cilnidipine present in the pharmaceutical dosage form were calculated.

Limit of detection and limit of quantification
Limit of detection (LOD) and limit of quantification (LOQ) of Telmisartan, Chlorthalidone and Cilnidipine were determined by calibration curve method. Solutions of Telmisartan, Chlorthalidone and Cilnidipine were prepared in linearity range and injected in triplicate. Average peak area of three analyses were plotted against concentration

Method robustness
The robustness can be determined by varying the following parameters:
Robustness of the developed method was determined by making small deliberate changes in flow rate (±0.1ml/min), column temperature (±5%), organic mobile phase ratio (±10%), along with the optimized method.

Degradation procedure:
Oxidation:
To 1 ml of stock solution of Cilnidipine, Chlorthalidone and Telmisartan, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 600^{o}c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml, 13µg/ml & 80µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:
To 1ml of stock solution Cilnidipine, Chlorthalidone and Telmisartan, 1ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 600^{o}c .The resultant solution was diluted to obtain 20µg/ml,13µg/ml & 80µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:
To 1 ml of stock solution Cilnidipine, Chlorthalidone and Telmisartan, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 20µg/ml, 13µg/ml & 80µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:
The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 20µg/ml, 13µg/ml & 80µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the 20µg/ml, 13µg/ml & 80µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml, 13µg/ml & 80µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 20µg/ml, 13µg/ml & 80µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSIONS

The present work was focused on development of a stability indicating RP-HPLC method for the simultaneous estimation of Telmisartan, Chlorthalidone and Cilnidipine in pharmaceutical dosage forms. The solubility of the active pharmaceutical ingredient was checked in different solvents like methanol, water, acetonitrile and in different ratios but finally the standard was first dissolved in methanol and made up with water: ACN (50:50). So it was chosen as a diluent. Different mobile phases like methanol and water, acetonitrile and 0.01N potassium dihydrogen ortho phosphate buffer and acetonitrile and sodium dihydrogen phosphate buffer were used in compositions at a flow rate of 1ml/min but the peak resolution, retention time and tailing factor were not satisfactory, so at last acetonitrile and ortho phosphoric acid was selected as a buffer at flow rate of 1.2ml/min. The chromatographic separation was performed on Kromosil®"(250mm x 4.6mm x 5µ) kept at 30°C with a run time of 8 minutes. Finally the method was optimized by altering the mobile phase composition / ratio and the optimized wavelength of three drugs Chlorthalidone, Telmisartan and Cilnidipine was found to be at 238nm.

System suitability parameters
The system suitability tests were conducted before performing the validation and the parameters were within the acceptance criteria like retention times were 2.573 min, 3.106 min and 3.924 min for Chlorthalidone ,Telmisartan and Cilnidipine, plate count was >2000, peak tailing was <2 and the %RSD of peak areas of six injections were ≤ 2% (Table 1). Hence the proposed method can be successfully applied to routine analysis. Chromatograms are shown in Fig 4(a), 4(b), 4(c).

Linearity range
The linearity range was in the interval of Chlorthalidone (5-30 µg/ml), Telmisartan (20-120µg/ml) and Cilnidipine (3-20µg/ml), respectively. These were represented by a linear regression equation as follows: y (Chlorthalidone) = 32080x + 8450.5 (r^2 = 0.999), y (Telmisartan) 32801x + 47284 (r^2 = 0.9993) and y (Cilnidipine) = 18607x + 8827.8 (r^2 = 0.9992). Regression line was established by least squares method and correlation coefficient (r^2) for Chlorthalidone, Telmisartan and Cilnidipine were found to be greater than 0.999. Hence the curves established were linear (Table 2, Graph 1). Chromatograms are shown in Fig 5.

Precision
Six replicates injections at the same concentration were analyzed on same day and two different days for verifying the variation in the precision and the % RSD for Chlorthalidone, Telmisartan and Cilnidipine were within acceptable limit of ≤2. Hence the method is reproducible on different days with different analyst and column. This indicates that the method is precise (Table 3).

Accuracy
The percentage recoveries for Chlorthalidone, Telmisartan and Cilnidipine were found to be 100.06%, 100.18% and 100.13% respectively (Table 4, 5, 6). The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method. Chromatograms are shown in Fig 6.

Limit of detection (LOD) and limit of quantification (LOQ)
The determined values of LOD and LOQ were calculated by using slope and Y-intercept. The LOD and LOQ values for Chlorthalidone were found to be 0.03 and 0.10µg/ml, Telmisartan were found to be 0.80 and 2.42 µg/ml, Cilnidipine were found to be 0.13 and 0.40µg/ml respectively (Table 7).

Robustness Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample and standard Chlorthalidone,

Telmisartan and Cilnidipine solution (Table 8, 9, 10). After this the results obtained were compared with that of optimized method. It was confirmed that by the deliberate changes in the parameters there was no any significant changes in standard deviation, relative standard deviation, theoretical plates, retention time and USP tailing factor.

Assay

The Content of Chlorthalidone, Telmisartan and Cilnidipine in the pharmaceutical dosage form were found by using the developed method. The percentage purity of Chlorthalidone, Telmisartan and Cilnidipine were found to be 99.60%, 99.89% and 99.90% and %RSD values for Chlorthalidone, Telmisartan and Cilnidipine were within limit of ≤2.

Forced degradation studies

The forced degradation studies were conducted and all the parameters for Chlorthalidone, Telmisartan and Cilnidipine were within the limits (Table 11). Chlorthalidone, Telmisartan and Cilnidipine have shown significant sensitivity towards the treatment of HCl, NaOH and peroxide solutions [30]. The drugs gradually undergone degradation with time and prominent degradation was observed. Chlorthalidone, Telmisartan and Cilnidipine were stable under forced thermal degradation, photolytic and neutral degradations [31-32]. From the degradation studies, Peak purity test results derived from PDA detector, confirmed that the Chlorthalidone, Telmisartan and Cilnidipine peaks were homogeneous and pure in all the analyzed stress samples. Chromatograms are shown in Fig 7.

CONCLUSIONS

A new, simple, rapid and precise stability indicating reversed-phase high performance liquid chromatographic method was developed for the simultaneous estimation of Telmisartan, Chlorthalidone and Cilnidipine in bulk and pharmaceutical dosage forms. The method is simple, accurate, linear, sensitive and reproducible as well as economical for the effective quantitative analysis of Telmisartan, Chlorthalidone and Cilnidipine in bulk and combined dosage forms. The method was validated, and all the method validation parameters were tested and shown to produce satisfactory results. The method is free from interactions of the other ingredients and excipients used in the formulations. Finally, concluded that the method is suitable for use in the routine quality control of Telmisartan, Chlorthalidone and Cilnidipine in active pharmaceutical ingredients and in pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

The authors thank Spectrum pharma research solutions, Hyderabad for providing Telmisartan, Chlorthalidone and Cilnidipine reference standards as gift samples to carry out the research work.

Table 1: System suitability parameters for Chlorthalidone, Telmisartan and Cilnidipine

S no	Chlorthalidone			Telmisartan			Cilnidipine		
Inj	RT(min)	N	Tailing	RT(min)	N	Tailing	RT(min)	N	Tailing
1	2.563	4142	1.00	3.097	4372	1.31	3.910	6912	1.10
2	2.567	4134	1.01	3.106	4332	1.35	3.924	7049	1.12
3	2.570	4648	1.02	3.111	4559	1.38	3.928	7093	1.10
4	2.573	4439	0.98	3.113	4418	1.32	3.934	7254	1.12
5	2.578	4145	0.99	3.118	4386	1.33	3.944	6980	1.09
6	2.580	3969	1.00	3.122	4445	1.34	3.945	7040	1.11

Table 2: Linearity and Statistical analysis data for Chlorthalidone, Telmisartan and Cilnidipine.

Chlorthalidone			Telmisartan			Cilnidipine		
Conc. (µg/mL)	**Peak area**	**Correlation Coefficient**	**Conc. (µg/mL)**	**Peak area**	**Correlation Coefficient**	**Conc. (µg/mL)**	**Peak area**	**Correlation Coefficient**
25	116215	0.99	25	715576	0.99	25	110776	0.99
50	227195		50	1361475		50	196682	
75	314401		75	2063485		75	285631	
100	432068		100	2713281		100	385592	
125	529314		125	3312654		125	472869	
150	629390		150	3940806		150	563947	

Table 3: Determination of repeatability and intermediate precision

Drug Name	Repeatability			Intermediate		
	Peak Area	**Std Dev**	**%RSD**	**Peak Area**	**Std Dev**	**%RSD**
Chlorthalidone	429735	5357.8	1.2	420558	7012.8	1.7
Telmesartan	2711715	31864.0	1.2	2692473	30900.4	1.1
Cilnidipine	384679	3613.4	0.9	383876	3958.7	1.0

Table 4: Recovery data for the proposed RP-HPLC method for Chlorthalidone

Concentration level (%)	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Mean %Recovery
50	6.5	6.59	101.49	99.68
		6.40	98.51	
		6.43	99.04	
100	13	13.12	100.96	100.60
		13.13	101.03	
		12.97	99.82	
150	19.5	19.12	98.09	99.90
		19.52	100.13	
		19.84	101.75	

Table 5: Recovery data for the proposed RP-HPLC method for Telmisartan

Concentration level (%)	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Mean %Recovery
50	40	40.61	101.54	99.90
		40.12	100.31	
		39.15	97.88	
100	80	81.54	101.93	100.68
		79.61	99.52	
		80.49	100.62	
150	120	118.05	98.38	99.98
		121.77	101.48	
		120.12	100.10	

Table 6: Recovery data for the proposed RP-HPLC method for Cilnidipine

Concentration level (%)	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Mean %Recovery
50	10	10.06	100.66	100.57
		9.94	99.44	
		10.16	101.61	
100	20	20.07	100.35	99.91
		19.67	98.36	
		20.20	101.02	
150	30	30.27	100.91	99.92
		30.15	100.50	
		29.50	98.36	

Table 7: Sensitivity table of Chlorthalidone, Telmisartan and Cilnidipine

Drug Name	LOD(µg/ml)	LOQ(µg/ml)
Chlorthalidone	0.03	0.10
Telmisartan	0.80	2.42
Cilnidipine	0.13	0.40

Table 8: Robustness results of the proposed RP-HPLC method for Chlorthalidone

S.no	Parameters			Peak area	RT *	% RSD
	Optimized		*Used*			
1	Flow rate (ml/min)	1.2	1.1	748941	2.58	1.5
			1.3	913918	2.53	0.8
4	Mobile phase	57:43	52:38	1532303	2.56	0.4
			62:48	522591	2.57	1.2
6	Temperature	30^0C	25^0C	726410	2.55	0.2
			35^0C	903335	2.23	0.5

***RT=Retention Time**

Table 9: Robustness results of the proposed RP-HPLC method for Telmisartan.

S.no	Parameters			Peak area	RT *	% RSD
	Optimized		*Used*			
1	Flow rate (ml/min)	1.2	1.1	4869203	2.99	0.3
			1.3	5904195	2.87	0.2
4	Mobile phase	57:43	52:38	9604089	2.88	0.8
			62:48	3299325	3.11	0.5
6	Temperature	30^0C	25^0C	4668240	2.92	0.3
			35^0C	5855092	2.85	0.2

***RT=Retention Time**

Table 10: Robustness results of the proposed RP-HPLC method for Cilnidipine.

S.no	Parameters			Peak area	RT*	% RSD
	Optimized		*Used*			
1	Flow rate (ml/min)	1.2	1.1	656517	3.94	2.5
			1.3	842365	3.74	1.2
4	Mobile phase	57:43	52:38	1416617	3.79	1.5
			62:48	467907	3.91	1.4
6	Temperature	30^0C	25^0C	661393	3.82	1.0
			35^0C	838444	3.71	0.2

***RT=Retention Time**

Table 11: Forced Degradation results of proposed RP-HPLC method

Degradation Condition	Chlorthalidone			Telmisartan			Cilnidipine		
	%Drug Degrad	Purity of		% Drug Degrad	Purity of		% Drug Degraded	Purity of	
		Angle	Threshold		Angle	Threshold		Angle	Threshold
Control Sample	-	-	-	-	-	-	-	-	-
Acid	2.51			3.26	0.157	0.294	3.96	1.511	1.984
Alkali	3.67			2.96	0.351	0.917	2.18	1.618	1.924
Oxidation	2.68	0.981	1.850	3.38	0.336	0.224	1.54	1.407	1.659
Thermal	0.45	0.738	0.910	0.35	0.197	0.332	0.98	0.792	1.019
UV	0.44	0.197	0.488	0.01	0.194	0.309	0.74	0.787	0.868
Water	0.72	0.953	1.461	0.37	0.187	0.306	0.90	0.624	0.836

Figure 1, 2, 3: Structures of Telmisartan, Chlorthalidone and Cilnidipine

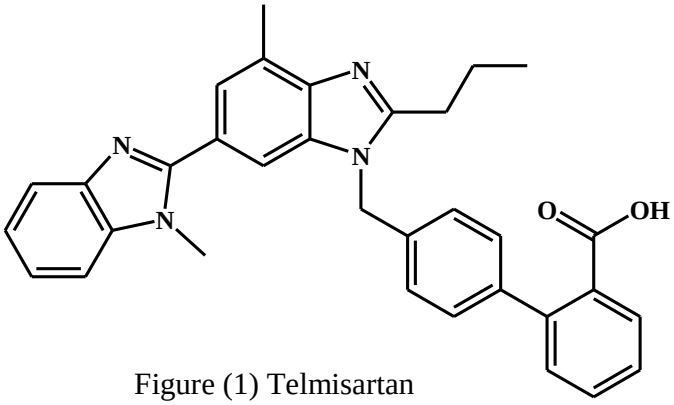


Figure (1) Telmisartan

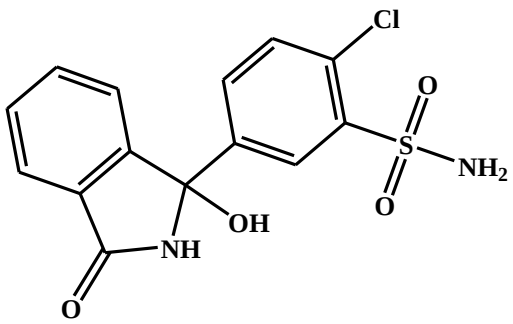


Figure (2) Chlorthalidone

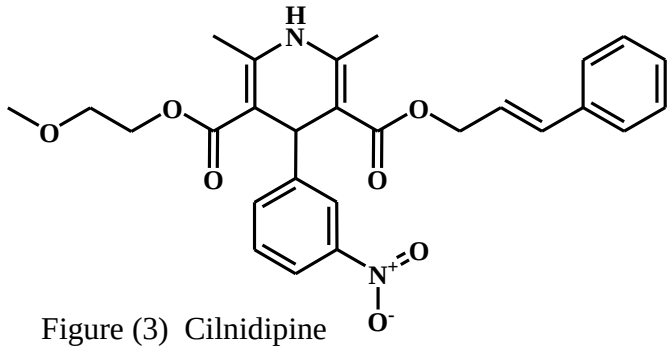


Figure (3) Cilnidipine

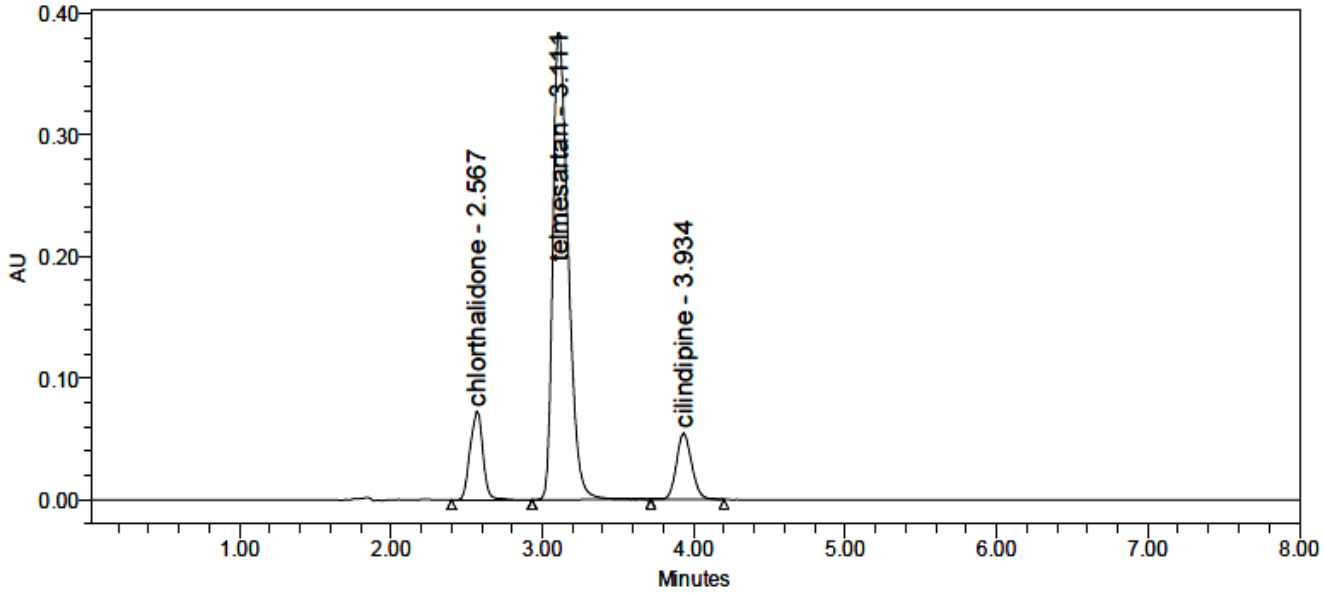


Figure 4 (a): RP-HPLC Chromatogram of Chlorthalidone, Telmisartan and Cilnidipine

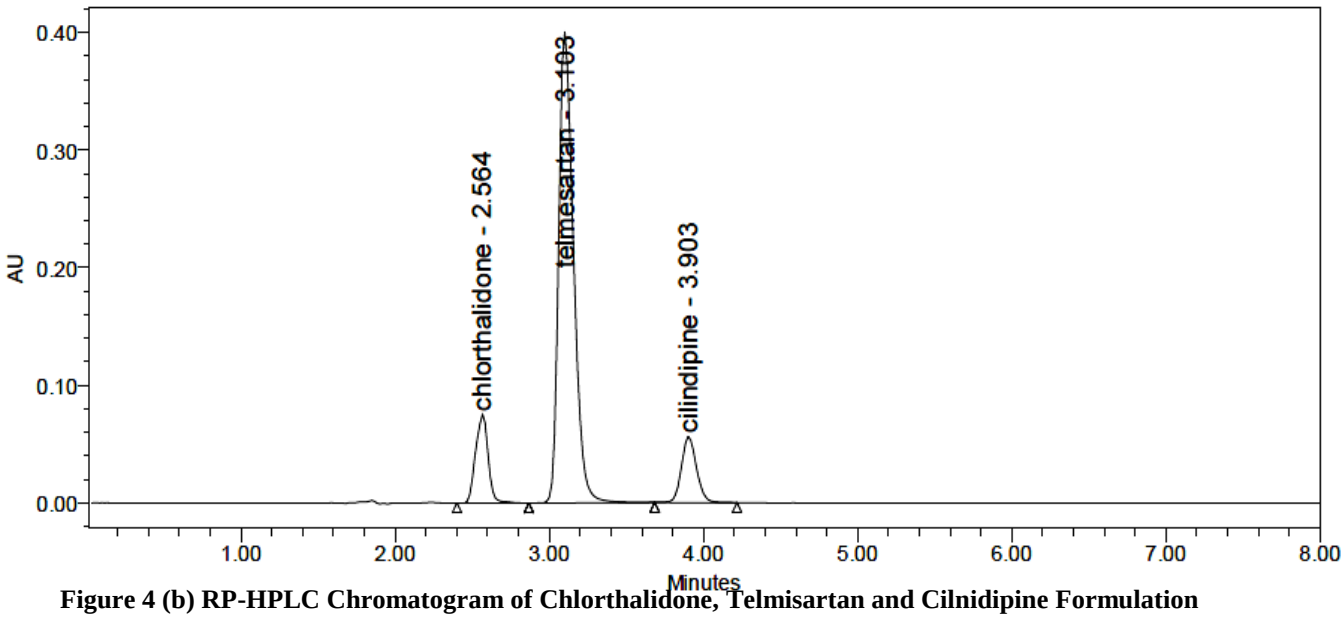


Figure 4 (b) RP-HPLC Chromatogram of Chlorthalidone, Telmisartan and Cilnidipine Formulation

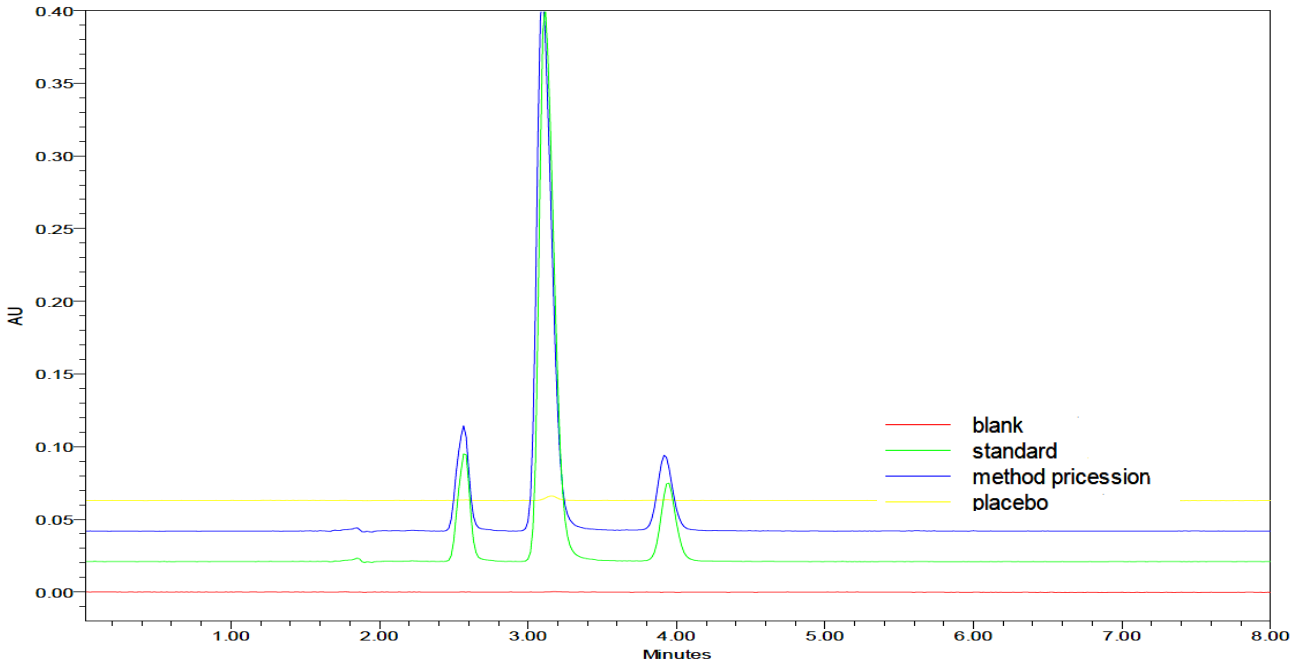


Figure 4 (c): Specificity overlay chromatogram of blank, standard, placebo and marketed sample of Chlorthalidone, Telmisartan and Cilnidipine

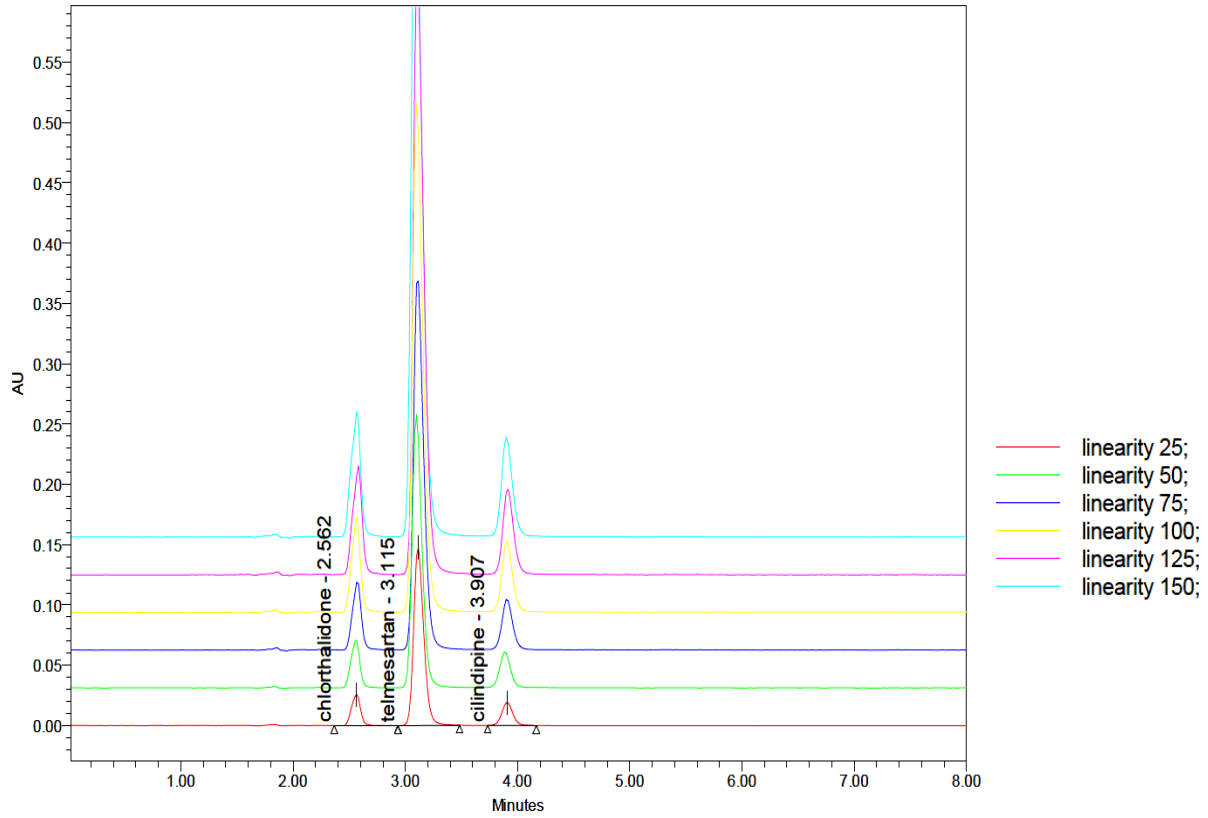


Figure 5: Linearity overlay chromatograms of Chlorthalidone, Telmisartan and Cilnidipine

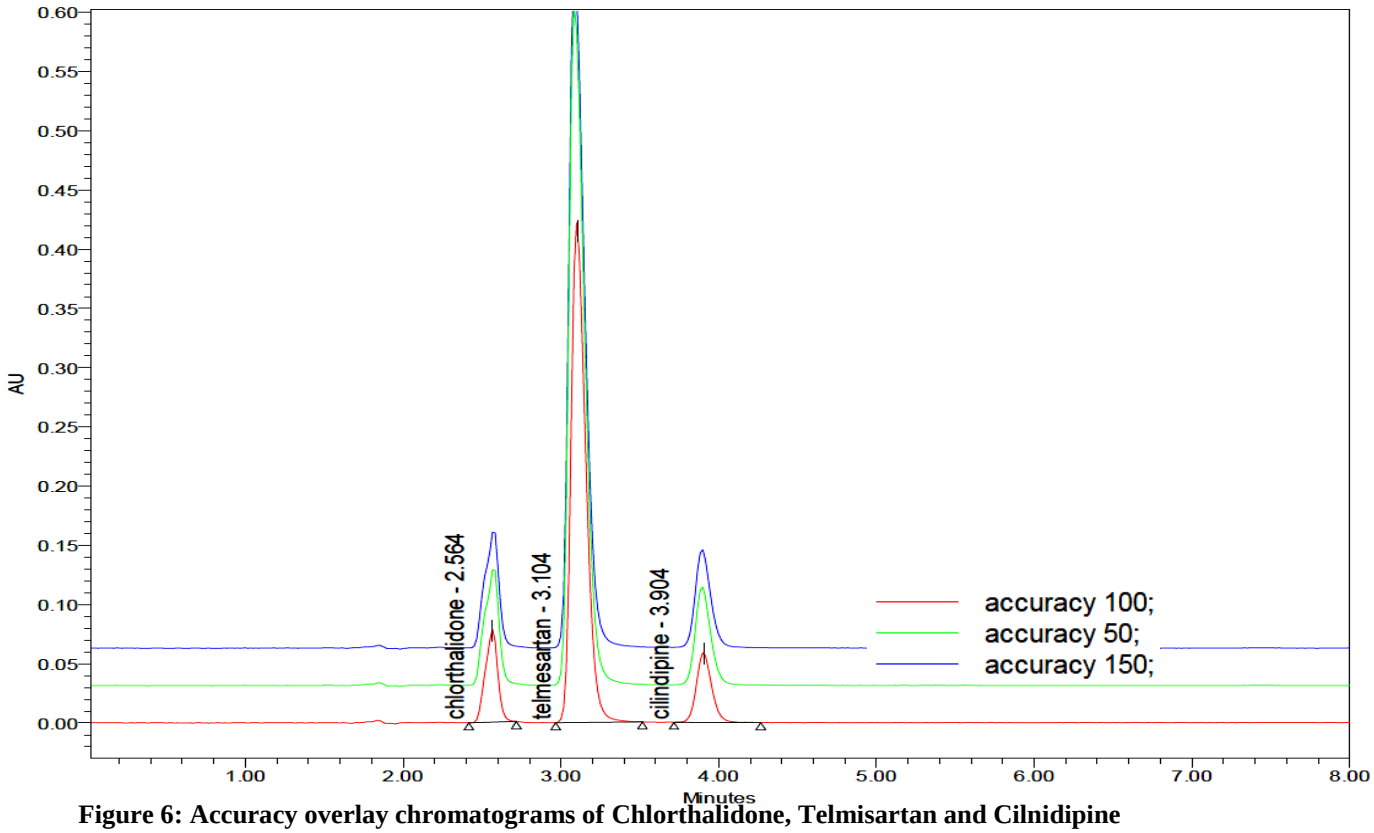


Figure 6: Accuracy overlay chromatograms of Chlorthalidone, Telmisartan and Cilnidipine

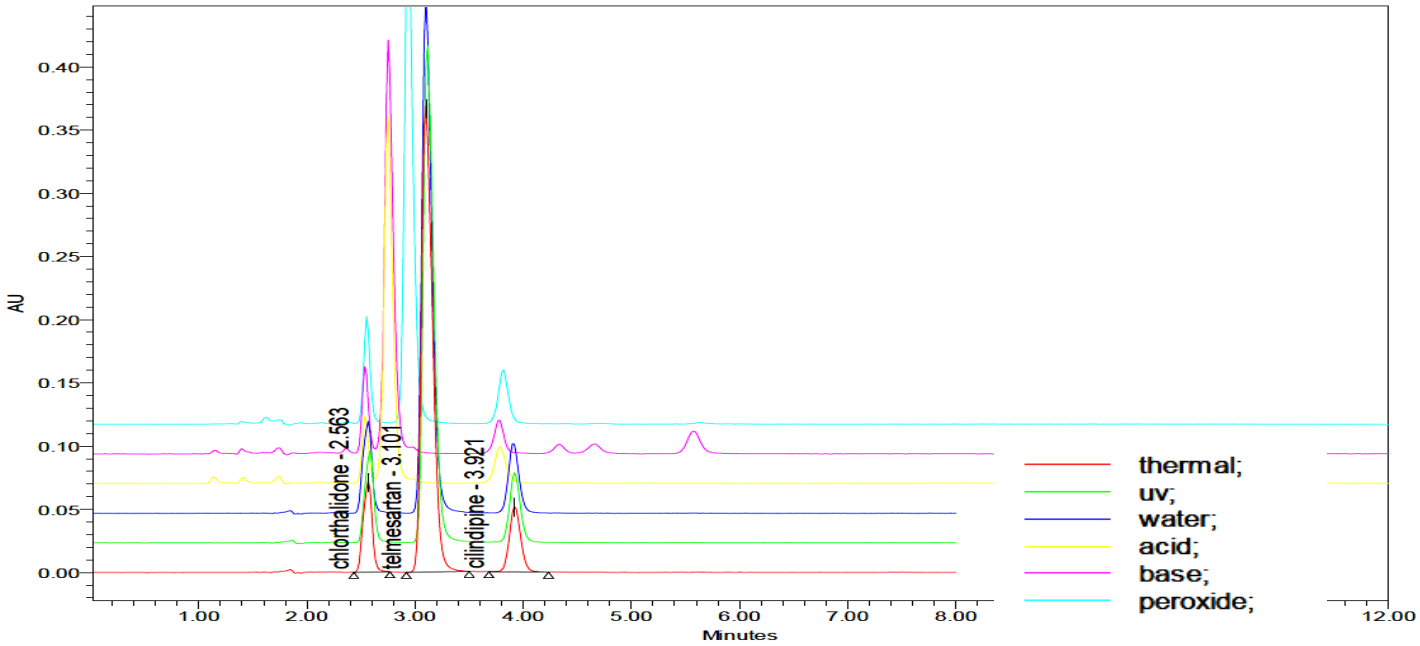
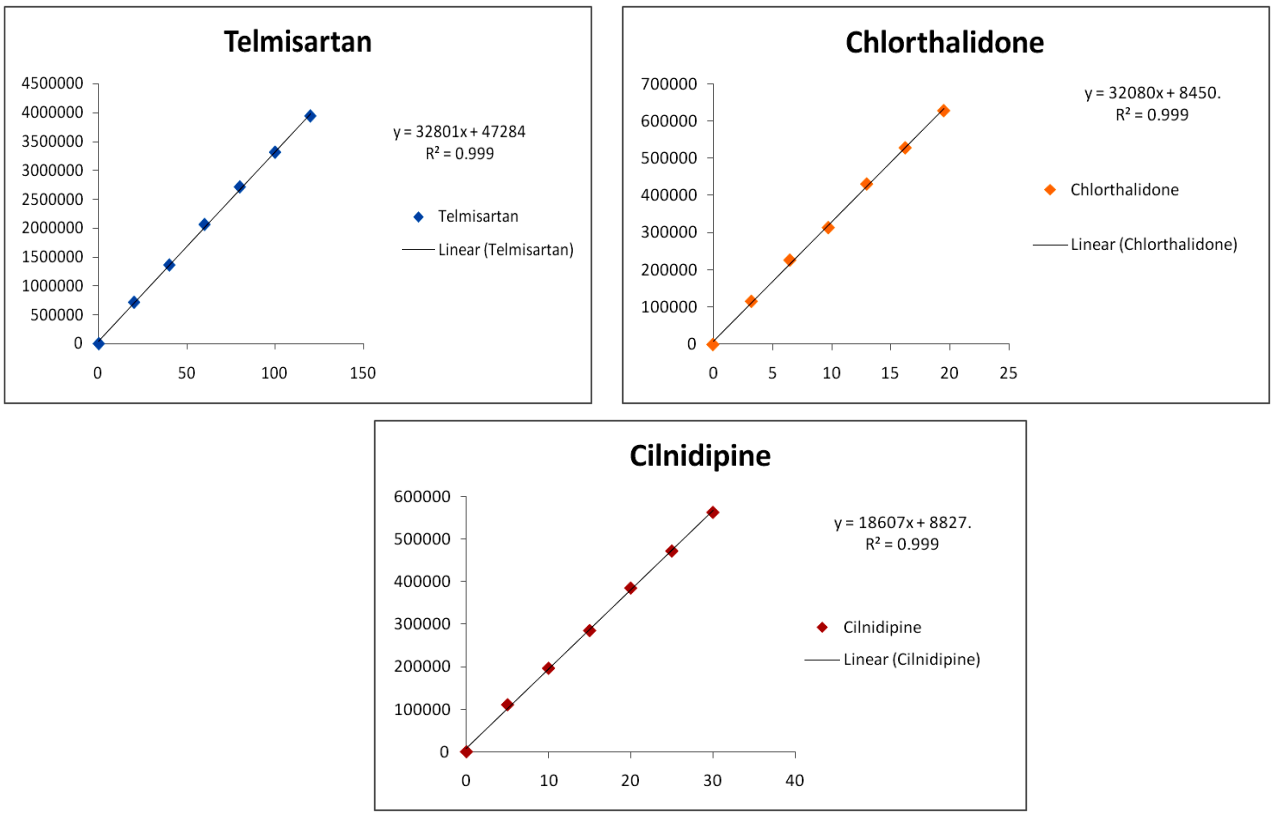


Figure 7: Forced degradation overlay Chlorthalidone, Telmisartan and Cilnidipine



Graph 1: Linearity Graphs of Chlorthalidone, Telmisartan and Cilnidipine

REFERENCES

1. Wolfgang Wienen, Michael Entzeroth, Jacobus C. A. van Meel, Joachim Stangier. et.al. Cardiovascular Drug Reviews, 2000; 18(2):127–154
2. Marc p. Maillard, Christine Perregaux, Catherine Centeno, Joachim Stangier. et.al. J Pharmacol Exp Ther, 2002; 302(3):1089-95.
3. Massimo Cirillo, Fabiana Marcarelli, Alessandra A. Mele. et.al Hypertension, 2014; 63(4):692-7
4. Fredric L. Coe, Joan H. Parks, David A. Bushinsky et.al. Kidney Int, 1988; 33:1140—1146
5. Carlos Escobar, Rocio Echarri, Vivencio Barrios. International Journal of Nephrology and Renovascular Disease 2012; 5 69–80
6. ICH, Stability Testing of New Drug Substances and Products. International Conference on Harmonization, IFPMA, Geneva (1993).
7. ICH Harmonised Tripartite Guideline “Stability Testing Of New Drug Substances And Products Q1a(R2)" 2003.
8. Monika Bakshi, Saranjit Singh. J. Pharm. Biomed. Anal, 2002; 28:1011–1040
9. M. RamaMohana Reddy, A. Praveen Kumar, V. Krishna Reddy. et.al. Int J Phram Pharm Sci, 2012; 4(1):497–504.
10. C. D. Niranjan, S. M. Patil, J. K. Sabaji, and A. N. Chivate. Journal of Pharmacy Research, 2012; 5(6):3331–3333.
11. K. K. Pradhan, U. S. Mishra, A. Sahoo. et.al. Int. J. Res. Pharm. Sci.,, 2011; 2(4):526–530, 2011.
12. G. Mahathi, Y. Ravindra Reddy, and P. Uttam Prasad. International Journal of Pharmacy and Technology, 2011; 3(3):3370–3389, 2011.
13. M. Raja, N. Spoorthy, D. Banji, et.al. Journal of Pharmacy Research, 2012; 5(8): 4585–4587.
14. P. Vani and S. K. Kalyana. Pharmacia Letter, 2013; 5(3):81–89.
15. K. E. Parmar, R. S. Mehta, N. D. Patel. Int J Pharm Pharm Sci, 2013; 5(2):420–425.
16. Parikh, P; Sahoo, U; Zanvar, A and Seth, AK. Pharm. Sci. Monitor, 2013; 4:111-123.
17. H. S. Karmalkar, V. V. Vaidya, N. A. Gomes. et.al. Analytical Chemistry, 2008; 7(8):573–576.

18. Kyeong-Ryoon Lee , Yoon-Jee Chae , Jong-Hwa Lee. et al. Journal of Liquid Chromatography and Related Technologies, 2012; 35(2):308–320.
19. Mhaske.RA, Garole.DJ, Mhaske. AA. et.al. Int. J. Pharm. Pharmceut. Sci and Res, 2012; 3:141-149.
20. Kasimala, MB and Kasimala, BB. J. Atom. Molecule, 2012; 2:117-126.
21. Prasad PH, Patel PM, Vijaysree D. et.al. Der. Pharm. Chem, 2013; 5:139-143.
22. Singh, B; Patel, DK and Ghosh, SK. Int. J. Pharm. Pharmceut. Sci, 2009;1: 24-29.
23. Elshanawane AA, Mostaf, SM and Elgawis MS. J. AOAC Int, 2009; 92:404-409.
24. Mohamed S. Elgawish, Samia M. Mostafa and Abdalla A. Elshanawane. saudi Pharm J. 2011 ;19(1): 43–49.
25. Sahoo, S; Mishra, SK and Panda, PK. Int. J. Pharmaceut. Res. Sch, 2012; 4:1-5.
26. Reema H. Rupareliya and Hitendra S. Joshi. ISRN Chromatography Volume 2013; 2:6
27. Neelima Kudumula , Y. Rajendra Prasad. Pharmacophore 2014; 5(4):442-450
28. ICH Harmonised Triparitite Guidelines, "Validation of Analytical Procedures: Text and Methodology Q2 (R1)", 2005
29. J.R. Valdes Santurio, J.A. Martinez Perez, B. Lopez Pelaez, C. Martinez Manchado. STP Pharma. Sci. 1995; 5:391–395.
30. ICH, Stability Testing: Photostability Testing New Drug Substances and Products. International Conference on Harmonization, IFPMA, Geneva 1996.
31. H.H. Tonnerson (Ed.), the Photostability of Drugs and Drug Formulations, Taylor and Francis, London, 1996, pp. 9.