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Stability Indicating RP-HPLC Method Development and Validation: Strategy to Minimize Run Time and Retention Time Using Telmisartan in Combination and Single Dosage form and Determine its Stability

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Abstract : : A simple, specific, linear, precise and accurate reverse phase liquid chromatographic method was developed to minimize run time and retention time of Telmisartan and Hydrochlorothiazide in combination and single dosage forms. The chromatographic separation was performed using Prudent C 8 Column (60 x 3 mm, 5 µm particle size). Mobile phase used was Gradient composed of buffer and Solution A was selected and a flow rate of 1.3 ml/minute was monitored with injection volume of 5 µl. Detection was carried out at 270 nm for Hydrochlorothiazide (HCTZ) and 298 nm for Telmisartan (TMS). The method was validated as per ICH guidelines. The retention time for TMS was observed as 3.6min and for HCTZ was observed as 1.2 min. Linearity range was observed in concentration of 50 - 150 µg/ml. The percentage recovery of TMS was 99% and for HCTZ was 102.6%. The proposed method was validated and successfully applied to the estimation of Telmisartan and Hydrochlorothiazide in tablet dosage forms.

Key Word: Telmisartan, Hydrochlorothiazide, method development, Validation, Force Degradation

I. INTRODUCTION

Telmisartan is used in the treatment of Hypertension (high blood pressure), prevention of heart attack and stroke and Heart failure.

Telmisartan is an angiotensin receptor blocker (ARB). It relaxes blood vessels by blocking the action of a chemical that usually makes blood vessels tighter. This lowers the blood pressure, allowing the blood to flow more smoothly to different organs and the heart to pump more efficiently.

Analytical methods keep on updating with time as per the requirements so as to develop a simple, reliable, cost effective, reproducible and above all a method bearing a high level of accuracy and precision.

Our study aimed to develop a rapid, robust, selective, sensitive, and precise HPLC method for the determination of Telmisartan and Hydrochlorothiazide.

Hydrochlorothiazide is a thiazide diuretic (water pill) that helps prevent your body from absorbing too much salt, which can cause fluid retention. Hydrochlorothiazide is used to treat high blood pressure (hypertension). Hydrochlorothiazide is also used to treat fluid retention (edema) in people with congestive heart failure, cirrhosis of the liver, or kidney disorders, or edema caused by taking steroids or estrogen.

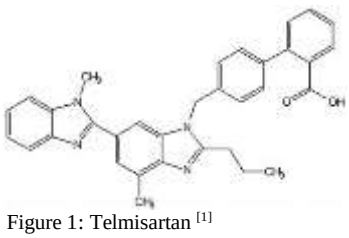

Figure 1: Telmisartan [1]

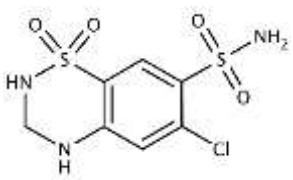

Figure 2: Hydrochlorothiazide [2]

II. MATERIAL AND METHOD DEVELOPMENT

The HPLC analysis was carried with Waters 2695 with software version Empower 2- PDA detector and Shimadzu LC-2010C HT HPLC system with UV detector and auto sampler integrated with software LCsolution Version 1.25. The column used is Prudent C 8 Column (60 x 3 mm, 5 µm particle size) and detection was carried out at 270 nm for Hydrochlorothiazide (HCTZ) and 298 nm for Telmisartan (TMS). The injection volume of sample was 5 µl and the run time was 5 minutes. A Gradient mobile phase consisted of buffer and solution A. The mobile phase was filtered through 0.45 µm nylon membrane filter and degassed before use.

Reagents and chemicals

Telmisartan standard and hydrochlorothiazide standard was taken from commercial source and tablets Telmed H and Telmikind were obtained from Local Market from Mumbai. HPLC grade Acetonitrile, Methanol was obtained from Finar Ltd. All other chemicals used were AR grade.

Preparation of Buffer solution

2gm Ammonium dihydrogen phosphate in 1000 ml of water. Adjust pH 3 with phosphoric acid.

Preparation of mobile phase

Gradient mobile Phase is used for the development.

Mobile Phase A: Buffer
Mobile Phase B: Acetonitrile: Methanol (50:50)

Diluent 1

0.1gm sodium hydroxide in 500mL of Methanol

Diluent 2

Mobile Phase A: Mobile Phase B (50: 50)

Gradient Program

Time	*Flow*	*%A*	*%B*
0	*1.3*	*90*	*10*
1	*1.3*	*90*	*10*
2	*1.3*	*40*	*60*
3	*1.3*	*40*	*60*
4	*1.3*	*90*	*10*
5	*1.3*	*90*	*10*

Preparation of standard stock solution

Weighed & transferred accurately about 40 mg of Telmisartan working standard and 12.5 mg of Hydrochlorothiazide working standard in to 100 ml volumetric flask. Added 70 ml of diluent 1 and sonicated until it dissolved completely. Diluted up to the mark with diluent 1 & mixed well.

Preparation of final standard solution

2 ml solution from standard stock solution and transferred it to 50 ml volumetric flask and dilutde it with diluent 2 up to the mark and sonicated it for 5 minutes.

Preparation of sample solution

Weighed and transferred 5 intact Tablet in 250mL of VF, added 13ml of 0.1N Sodium Hydroxide solution and shake until the tablets have completely disintegrated. Added 200ml of Methanol and sonicated for 10mins & stirred vigorously for 30min. diluted up to the mark with methanol, centrifuged a portion of the solution at 4000rpm.
Further diluted 2ml of above solution into 10ml with Diluent 2. Since both the tablets has same Label Claim, hence preparation for both the sample was same.

METHOD DEVELOPMENT

A stability indicating HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide was developed and validated. Various mobile phase combination were tried to develop new method of Telmisartan and Hydrochlorothiazide on C8 column. In order to achieve acceptable peak shapes and suitable run time various buffer systems are also tried systematically. Gradient mobile phase consisted of buffer and solution A *indicated that peak shape was proper with lesser run time. Therefore* Gradient mobile phase consisted of buffer and solution A *at a flow rate of 1.3 ml/min was selected as optimized mobile phase.Prudent* C 8 Column (60 x 3 mm, 5 µm particle size) *was used as the stationary phase to reduce the run time. To analyze drugs, detection was tried at various wavelengths but* 270 nm for Hydrochlorothiazide and 298 nm for Telmisartan *was selected as the*

detection wavelength as drug showed maximum absorption. The retention time was found to be 3.6min for Telmisartan and for Hydrochlorothiazide was observed as 1.2 min. *The chromatograms obtained were shown in figure (3) and figure (4). The system suitability parameters were shown in Table (1) and Table (2).*

Table 1: System Suitability Parameters of Telmisartan

Parameter	***Telmisartan***
Retention Time	3.63
% RSD	0.77
% Assay	104.4

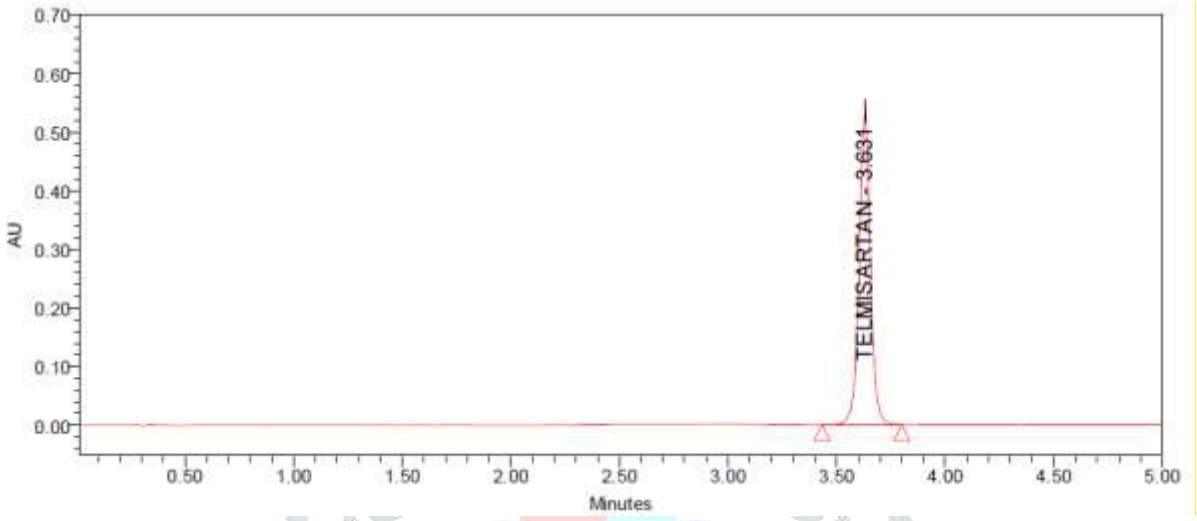

Figure 3: Representive chromatogram of Telmisartan standard.

Table 2: System Suitability Parameters of Hydroclorothiazide

Parameter	***Hydrochlorothiazide***
Retention Time	1.20
% RSD	0.89
% Assay	93.5

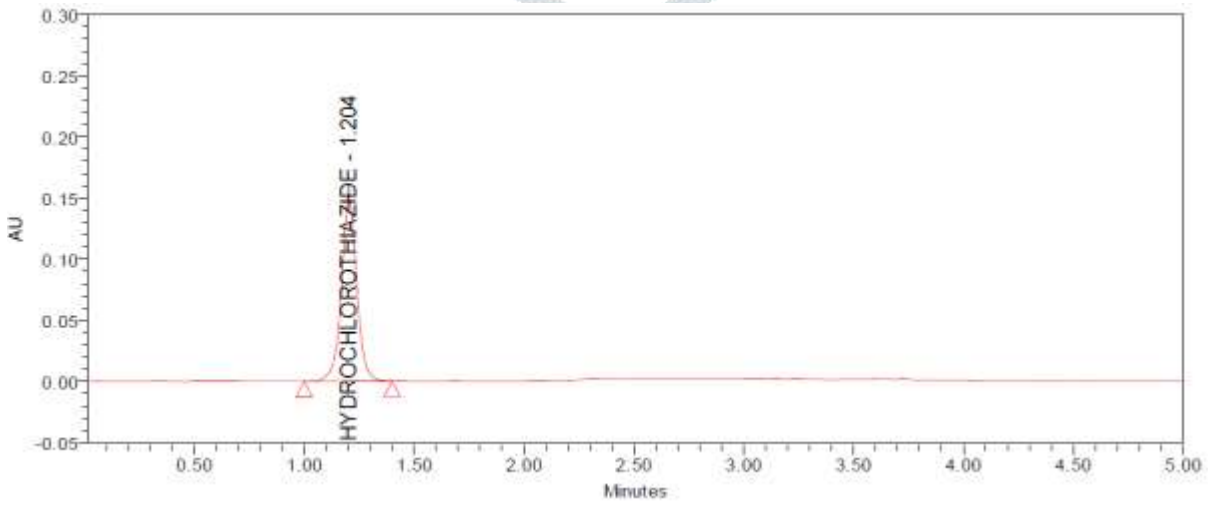

Figure 4: Representive chromatogram of Hydrochlorothiazide standard.

III. MATERIAL VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristic of a method (expressed in terms of analytical parameters) to meet the requirement for the intended application of the method.
They were tested using the optimize chromatographic conditions and instruments.

Specificity

Spectral purities of Telmisartan and hydrochlorothiazide peaks were evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurities as per the methodology. In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparations procedure to evaluate possible interfering peaks.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of Telmisartan and hydrochlorothiazide was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations such as 80, 120, 160, 200, 240 µg/ml for Telmisartan and 25, 37.5, 50, 62.5, 75 µg/ml for Hydrochlorothiazide were prepared as per table (3) and (4) and analyzed. Correlation coefficient & %Y-axis should be within the limit.

Table 3: Linearity Concentration Levels of Telmisartan

% level	*Volume of stock solution*	*Diluted to (ml)*	*Final concentration in ppm*
50%	0.5 ml	10	80
75%	0.75 ml	10	120
100%	1.0 ml	10	160
125%	1.25 ml	10	200
150%	1.5 ml	10	240

Table 4: Linearity Concentration Levels of Hydrochlorothiazide

% level	*Volume of stock solution*	*Diluted to (ml)*	*Final concentration in ppm*
50%	0.5 ml	10	25
75%	0.75 ml	10	37.5
100%	1.0 ml	10	50
125%	1.25 ml	10	62.5
150%	1.5 ml	10	75

Accuracy

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre analyzed sample and contents were reanalyzed by the proposed method. To check the accuracy of the developed methods and to study the interference of formulation excipients, analytical recovery experiments were carried out as per ICH guidelines Three different solutions of were prepared in triplicate at level of 50%, 100% and 150% of its predefined concentration and the percentage mean and individual recovery was calculated. Data from the linearity was considered for accuracy.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by carrying out six independent assays of Telmisartan and Hydrochlorothiazide. The mean area and % relative standard deviation (RSD) was calculated. % RSD should be ≤ 2 %.

Intermediate precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day was taken from the analysis of "Repeatability". The second set of experiments was performed by a different analyst or on different instrument. The standard deviation, relative standard deviation and mean value difference was calculated from the results obtained on each day.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was observed with the variations like pH, flow and column temperature etc.

IV. RESULT AND DISCUSSION

The objective of the method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. Telmisartan and Hydrochlorothiazide showed maximum absorbance at 298 and 270 nm respectively.

Specificity

By comparing the chromatograms of blank solution, placebo solution, reference solution & test solution it is observed that there is no interference of any peaks at the retention time of Telmisartan and Hydrochlorothiazide. The retention time of the main peaks in the chromatogram obtained with the reference solution & test solution are matching. This confirmed the specificity of the method.

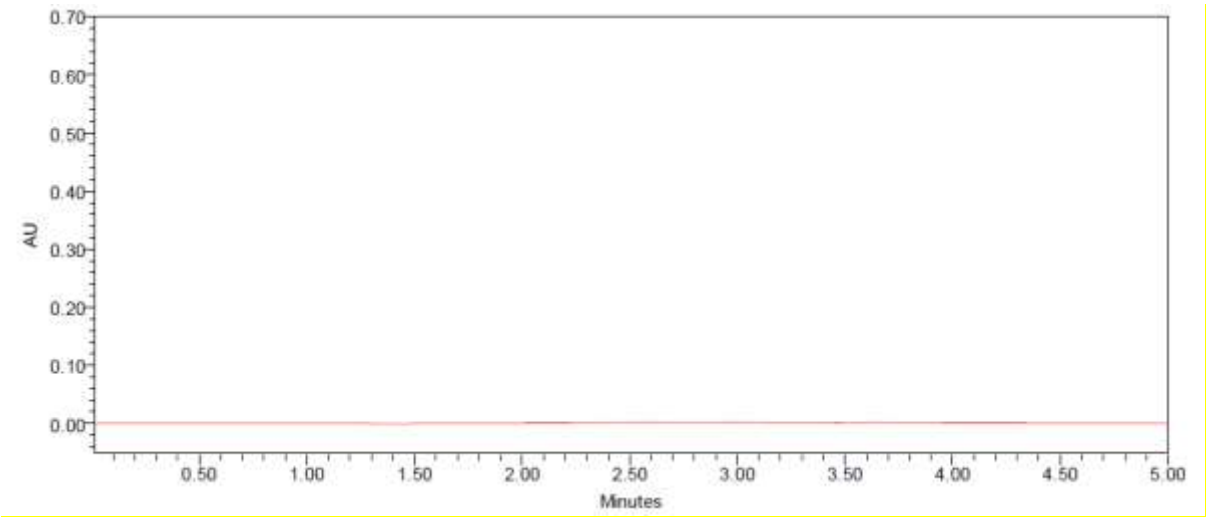

Figure 5: Chromatogram of blank solution

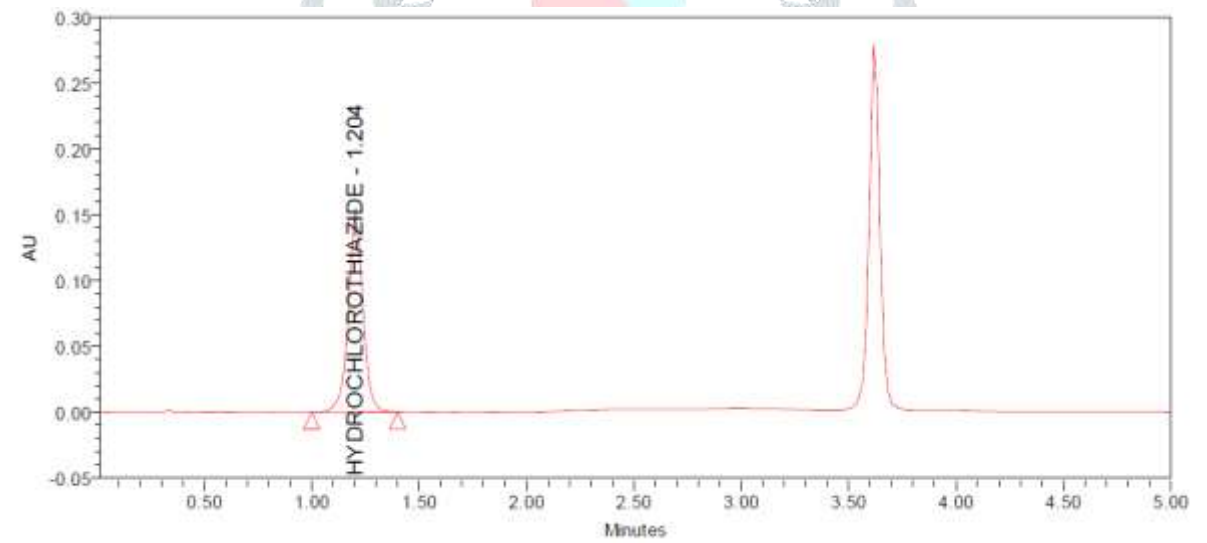

Figure 6: Chromatogram of standard solution of Hydrochlorothiazide

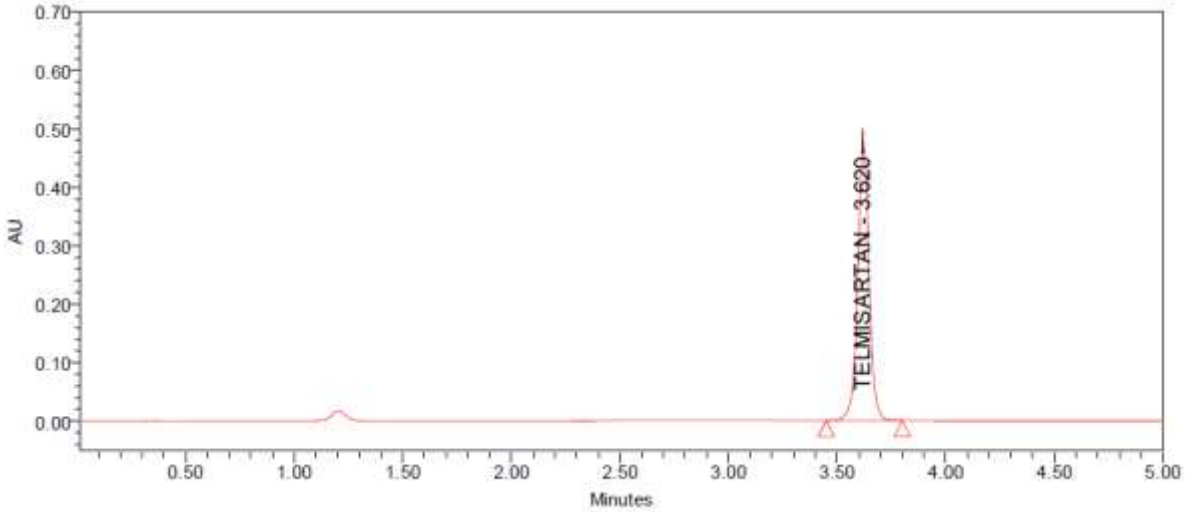

Figure 7: Chromatogram of standard solution of Telmisartan

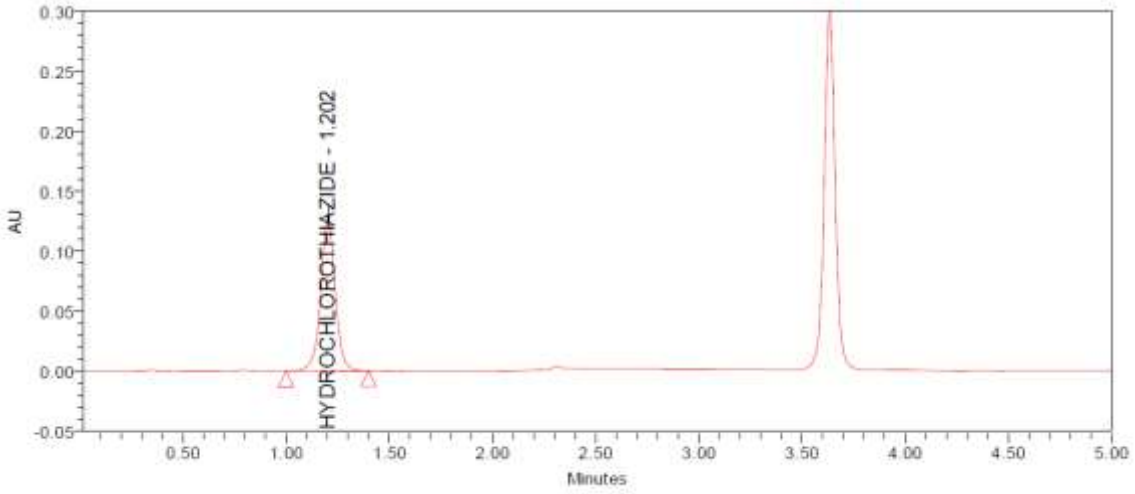

Figure 8: Chromatogram of sample solution (Hydrochlorothiazide) Telmed H

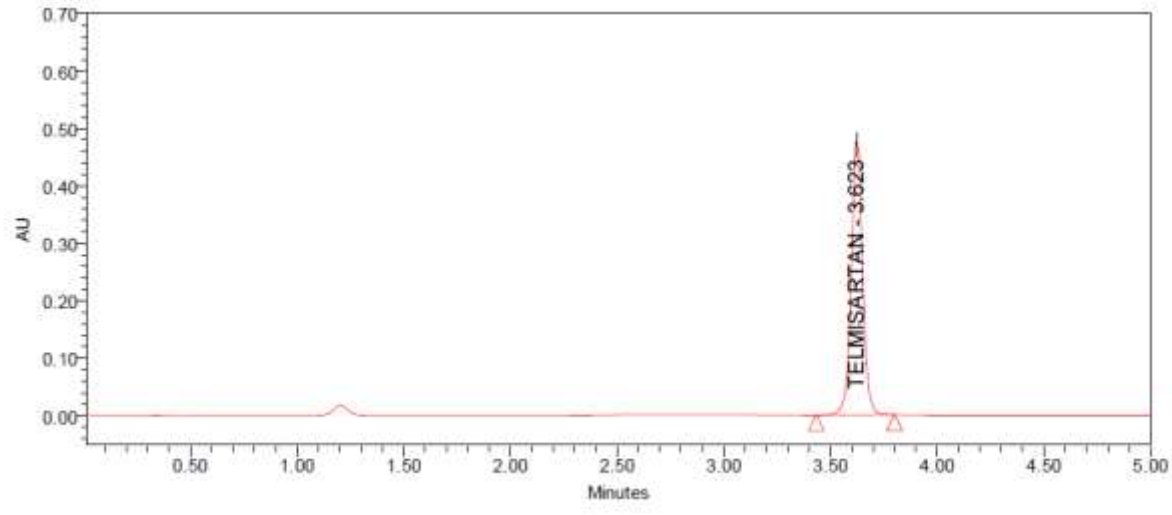

Figure 9: Chromatogram of sample solution (Telmisartan) Telmed H

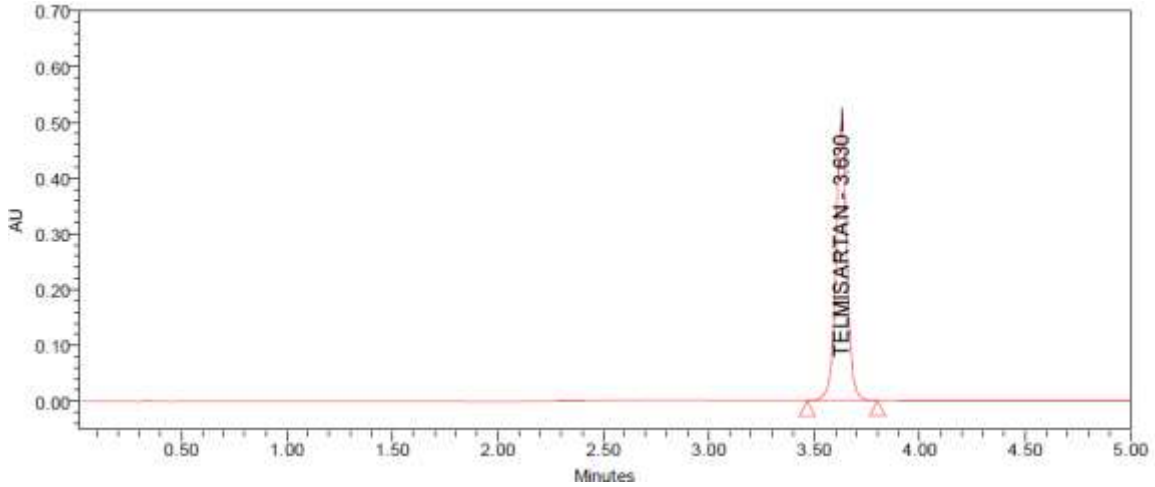

Figure 10: Chromatogram of sample solution of telmikind

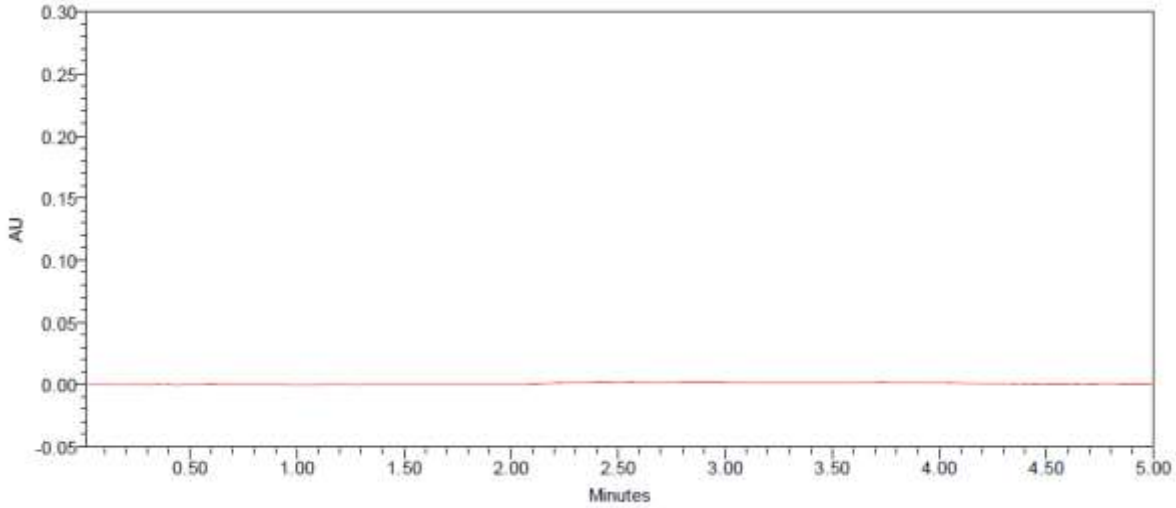

Figure 11: Chromatogram of placebo solution of Telmed H

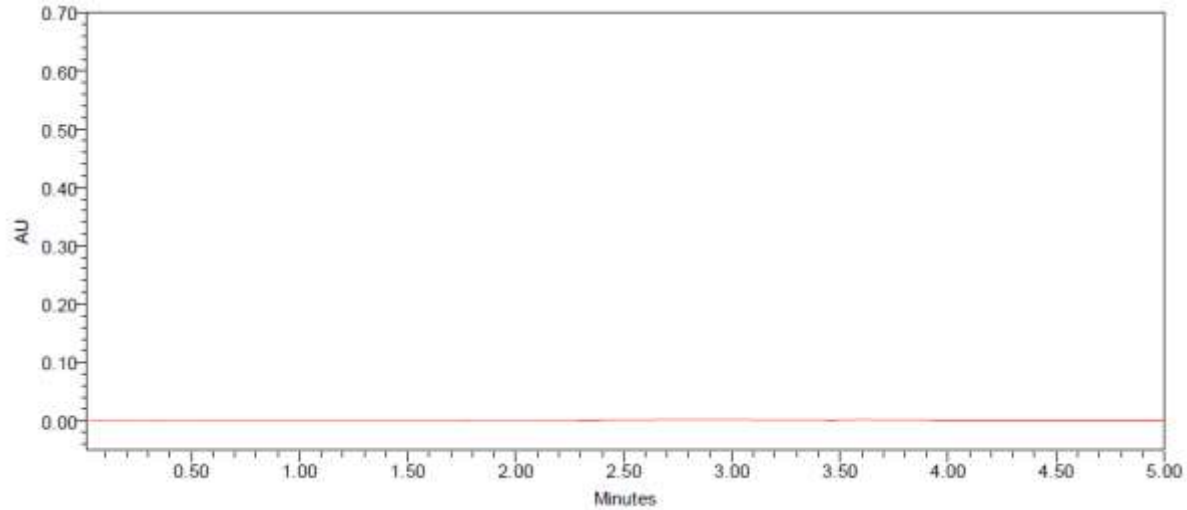

Figure 11: Chromatogram of placebo solution of Telmikind

Linearity

Five concentrations such as 80, 120, 160, 200, 240 µg/ml for Telmisartan *and 25, 37.5, 50, 62.5, 75 µg/ml for* Hydrochlorothiazide *were prepared and the linearity graph was plotted using concentration verses peak area as shown in Figure (11) and Figure (12). Graph of Residuals against concentration was also plotted as per shown in Figure (13) and Figure (14). A linear relationship was obtained between peak areas and quantity analyzed in the range of 50% to 150%*

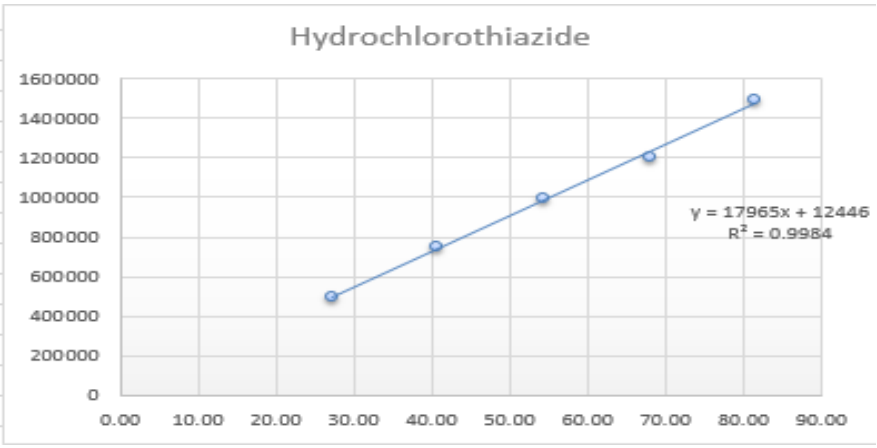

Figure 12: Linearity plot for Hydrochlorothiazide

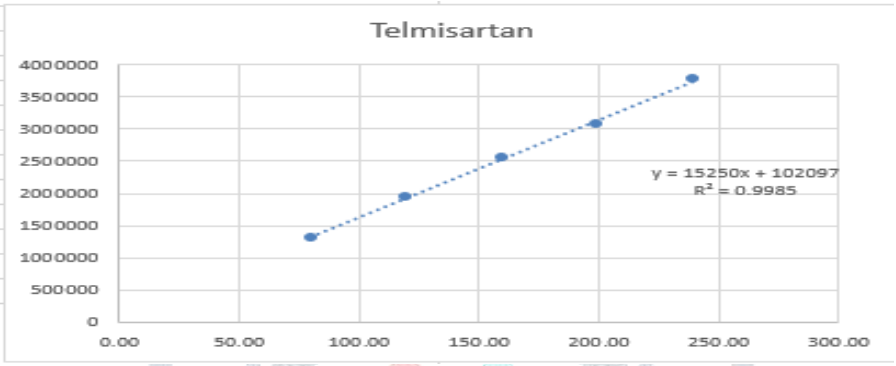

Figure 13: Linearity plot for Telmisartan

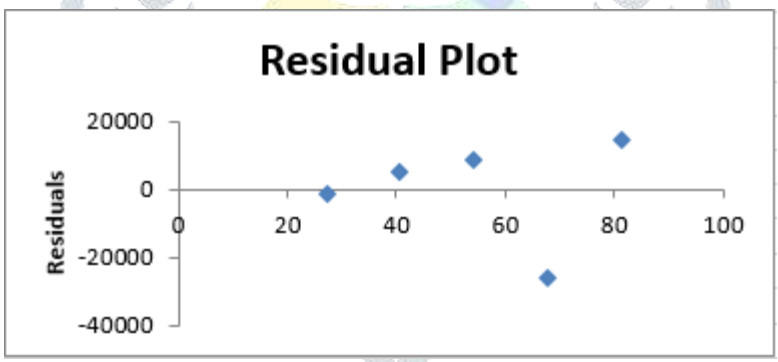

Figure 14: Plot of Residuals against concentration for Hydrochlorothiazide

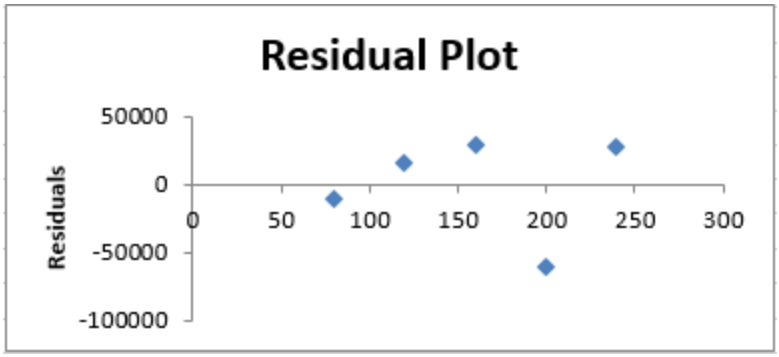

Figure 15: Plot of Residuals against concentration for Telmisartan

Table 5: Observation table for linearity of Hydrochlorothiazide

Parameter for Linearity	***Values***	***Acceptance Criteria***
Correlation coefficient R	0.999	≥ 0.999
%Y – axis intercept	1.03	≤ ± 5 %
Slope of regression line	17965.47	To be reported
Residual sum of squares	989824592.5	To be reported

Table 6: Observation table for linearity of Telmisartan

Parameter for Linearity	***Values***	***Acceptance Criteria***
Correlation coefficient R	0.999	≥ 0.999
%Y – axis intercept	3.31	≤ ± 5 %
Slope of regression line	15249.92	To be reported
Residual sum of squares	5627731593	To be reported

The method was considered to be linear in the range on 80 – 240 µg/ml for Telmisartan and 25 to 75 µg/ml for Hydrochlorothiazide as Correlation coefficient & %Y-axis intercept should be within the limit.

Accuracy

The percentage recovery of Telmisartan and Hydrochlorothiazide was tabulated in table (7). The method was considered to be accurate as the % individual recovery was within the acceptance criteria of 97-103 % and the % mean recovery was within the acceptance criteria of 98 – 102 %.

Table 7: Recovery at Different Concentration Levels

Accuracy level	***% Recovery of HYdrochlorothiazide***	***% Recovery of Telmisartan***
50%	102.4	100.7
	102.6	100.5
	103.1	100.8
100%	102.9	99.4
	102.5	99.0
	102.5	98.9
150%	102.9	97.2
	102.2	97.4
	102.1	97.2
Mean recovery	102.6	99.0
Minimum recovery	102.1	97.2
Maximum recovery	103.1	100.8

Precision

The exactness of the method as defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations was well within the acceptance limit of ≤ 2 %. Refer table (8).

Table 8: Method Precision of Telmed H Tablet

Sample No.	***% Assay of Hydrochlorothiazide***	***% Assay of Telmisartan***
Sample 01	94.7	102.5
Sample 02	94.9	102.5
Sample 03	95.6	103.0
Sample 04	94.8	102.4
Sample 05	95.4	103.8
Sample 06	95.0	102.7
Mean	**95.1**	**102.9**
STD Dev	**0.31**	**0.75**
% RSD	**0.32**	**0.73**

Table 9: Method Precision of Telmikind Tablet

Sample No.	***% Assay of Telmisartan***
Sample 01	101.7
Sample 02	102.3
Sample 03	101.2
Sample 04	101.4
Sample 05	102.4
Sample 06	101.5
Mean	**101.8**
STD Dev	**0.51**
% RSD	**0.50**

Intermediate Precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. Refer table (9) for % Assay of Hydrochlorothiazide and Telmisartan and table (10) and (11) for comparison of two independent repeatabilities

Table 10: Intermediate Precision of Telmed H Tablet

Sample No.	***% Assay of Hydrochlorothiazide***	***% Assay of Telmisartan***
Sample 01	94.2	104.3
Sample 02	95.2	104.4
Sample 03	94.3	104.0
Sample 04	92.4	104.5
Sample 05	94.5	103.8
Sample 06	94.6	102.8
Mean	**93.8**	**103.7**
STD Dev	**1.22**	**0.84**
% RSD	**1.30**	**0.81**

Table 11: Intermediate Precision of Telmikind Tablet

Sample No.	***% Assay of Telmisartan***
Sample 01	100.9
Sample 02	100.2
Sample 03	101.0
Sample 04	102.0
Sample 05	101.7
Sample 06	101.7
Mean	**101.3**
STD Dev	**0.19**
% RSD	**0.19**

Table 12: Comparison of two independent repeatability of Hydrochlorothiazide for Telmed H Tablet

Parameter	***1st day Repeatability***	***2nd day Repeatability***
Number of determinations	6	6
Mean (%) assay	95.1	93.8
RSD (%)	0.32	1.30
Mean value difference (%) **Acceptance Criteria:** < 2.0 % absolute	**1.3**	

Table 13: Comparison of two independent repeatability of Telmisartan forTelmed H Tablet

Parameter	***1st day Repeatability***	***2nd day Repeatability***
Number of determinations	6	6
Mean (%) assay	102.9	103.7
RSD (%)	0.73	0.81
Mean value difference (%) **Acceptance Criteria:** < 2.0 % absolute	**0.8**	

Table 14: Comparison of two independent repeatability of Telmisartan for Telmikind Tablet

Parameter	***1st day Repeatability***	***2nd day Repeatability***
Number of determinations	6	6
Mean (%) assay	101.8	101.3
RSD (%)	0.51	0.19
Mean value difference (%) **Acceptance Criteria:** < 2.0 % absolute	**0.5**	

Robustness

Method was found to be robust as system suitability criteria was achieved for all the robustness parameters tested. Deliberate change in parameter does not have any significant effect on the method performance, which demonstrated that the developed HPLC method was robust. The results were shown in Table (12) and table(13).

Table 15: Robustness Result for Hydrochlorothiazide for Telmed H tablet

Parameter	**System suitability**		**% Assay**
	% RSD	**STD deviation**	
Flow rate			
1.2 ml/min	**0.16**	**1232.99**	**94.1**
1.4 ml/min	**0.34**	**2316.18**	**95.0**
Buffer pH			
pH 2.8	**0.18**	**1802.25**	**94.2**
pH 3.2	**0.17**	**1675.69**	**94.2**
Column oven temperature			
35	**0.39**	**2808.05**	**94.9**
45	**0.17**	**1266.49**	**95.0**

Table 16: Robustness Result for Telmisartan for Telmed H Tablet

Parameter	System suitability		% Assay
	% RSD	STD deviation	
Flow rate			
1.2 ml/min	0.78	15001.75	103.6
1.4 ml/min	0.57	9326.62	103.5
Buffer pH			
pH 2.8	0.12	3090.50	102.3
pH 3.2	0.42	10874.29	102.3
Column oven temperature			
35	0.22	3876.08	103.9
45	0.34	6000.26	104.7

Table 17: Robustness Result for Telmisartan for Telmikind Tablet

Parameter	System suitability		% Assay
	% RSD	STD deviation	
Flow rate			
1.2 ml/min	0.78	14931.94	101.6
1.4 ml/min	0.57	9326.62	102.5
Buffer pH			
pH 2.8	0.13	3381.75	100.1
pH 3.2	0.42	10874.29	99.1
Column oven temperature			
35	0.22	3876.08	100.3
45	0.34	6000.26	100.4

Stability of Analytical solution

The sample and standard preparation were tested against freshly prepared standard preparation for 24 hrs. Results found within the acceptance limit of ± 2%. Refer table (18) and (19)

Table 18: Solution Stability Results for Telmed H tablet

	Solution stability report of Hydrochlorothiazide		***Solution stability report Telmisartan***	
Stability condition	***% Assay***	***% Absolute Difference***	***% Assay***	***% Absolute Difference***
Initial	94.10	--	102.3	--
6 hrs	94.70	-0.6	102.3	0.0
12hrs	94.10	0.0	101.8	0.50
24 hrs	93.10	1.0	101.8	0.50

Table 19: Solution Stability Results for Telmikind tablet

Solution stability report Telmisartan for Telmikind Tablet		
Stability condition	***% Assay***	***% Absolute Difference***
Initial	99.6	--
6 hrs	99.40	0.2
12hrs	99.10	0.50
24 hrs	199.20	0.40

Degradation Studies:
The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Telmisartan and Hydrochlorothiazide using the method developed.

Temperature stress studies:
Temperature stress studies. Sample and API were exposed to dry heat (80º C) in an oven for 24 hrs. The API and solution were then removed from the oven and analyzed as previously described.

Stress degradation by hydrolysis under acidic condition:
To 3 ml sample solution 0.5 ml of 0.1 N HCl was added in 20 ml of volumetric flask. Then, the volumetric flask was kept in hot water bath at 80^0C for 30 minutes. After 30 min, cooled at room temperature and neutralized it with 0.1N NaOH by adding 0.5ml in each and diluted with diluent up to the mark.

Stress degradation by hydrolysis under alkaline condition:
To 3 ml sample solution 0.5 ml of 0.1 N NaOH was added in 20 ml of volumetric flask. Then, the volumetric flask was kept in hot water bath at 80^0C for 30 minutes. After 30 min, cooled at room temperature and neutralized it with 0.1N HCl by adding 0.5ml in each and diluted with diluent up to the mark.

Oxidative degradation:
To 3 ml sample solution 0.5 ml of H_2O_2 was added in 20 ml of volumetric flask. Then, the volumetric flask was kept in hot water bath at 80^0C for 30 minutes. After 30 min, cooled at room and diluted with diluent up to the mark.

Table 20: Force degradation for Hydrochlorothiazide for Telmed H tablet

Condition	Time	%Degradation
Acidic Degradation	30 min	No degradation
Alkaline Degradation	30 min	2.1 %
Oxidative Degradation	30 min	1 %
Thermal Degradation	30 min	0.9 %

Table 21: Force degradation for Telmisartan for Telmed H tablet

Condition	Time	%Degradation
Acidic Degradation	30 min	2 %
Alkaline Degradation	30 min	2.9 %
Oxidative Degradation	30 min	4.2%
Thermal Degradation	30 min	2.7%

Table 22: Force degradation for Telmisartan for Telmikind tablet

Condition	Time	%Degradation
Acidic Degradation	30 min	3.6 %
Alkaline Degradation	30 min	3.3 %
Oxidative Degradation	30 min	1.5 %
Thermal Degradation	30 min	1.9 %

V. CONCLUSION

The solution stability as described previously were observed in short-term and long-term basis. All the time results of the stability studies were within the acceptable limit. According to table 20,21 drug was found to degrade sufficiently in alkaline, acidic and oxidative conditions.
In this present work a new simple, selective, precise, accurate and robust, linear, precise, HPLC method was developed and validated for the estimation of Telmisartan and Hydrochlorothiazide in pharmaceutical dosage form in accordance with the ICH guidelines.
The present work is having short run time and retention time hence reduced time and solvent. This method is cost effective and easy to determine.
The current work is worthwhile as developed HPLC method is selective, simple and rapid which can be very beneficial for the routine analysis of Telmisartan in combination as well as single dosage form.

VI. REFERENCES

[1] *ICH. Q2B Validation of Analytical Procedures: Methodology. International Conference on Harmonisation. Geneva: IFPMA; 1996.*
[2] *Indian Pharmacopeia (2018) Government of India, Ministry of Health and Family Welfare, Indian Pharmacopeia Commission, Ghaziabad.*
[3] *British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London; 2011(II): 1408, 2085.*
[4] *United State Pharmacopeia (2020) USP 42/ NF 37. US Pharmacopoeial Convention. Inc., New York*
[5] *Amrinder, S., Jha, K.K., Anuj, M., Amit, K., Journal of Scientific & Innovative Research, 2013, 2(1), 160-175.*
[6] *Bhatia NM, Shinde HV, Bhatia MS, Choudhari PB, Ingale KB, Development and validation of spectrophotometric and ion pair chromatographic techniques for estimation of Telmisartan and Hydrochlorothiazide, Ars.Pharmaceutica, 51(3), 2010, 145-154.*
[7] *Blessy M, Patel RD, Prajapati PN, Agrawal YK (2014) Development of forced degradation and stability indicating studies of drugs—a review. J Pharm Anal 4(3):159–165.*
[8] *Joshi S, Karbhari PA, Bhoir SI, Bindu KS, Das C. RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. J Pharm Biomed Anal. 2010;52:362–71*
[9] *Bhagwate S, Gaikwad NJ (2013) Stability indicating HPLC method for the determination of hydrochlorothiazide in pharmaceutical dosage form. J Appl Pharm Sci 3:88–92*
[10] *Mhaske RA, Garole DJ, Mhaske AA, Sahasrabudhe S (2012) RP-HPLC method for simultaneous determination of amlodipine besylate, valsartan, telmisartan, hydrochlorothiazide and chlorthalidone: application to commercially available drug products. Int J Pharm Sci 3:141–149*
[11] *Sujana K, Gowri Sankar D, Bala Souri O, Swathi Rani G, Stability indicating RPHPLC method for the determination of Telmisartan in pure and pharmaceutical formulation, International Journal of Pharmacy and Pharmaceutical Sciences, 3(2), 2011, 164-167.*
[12] *Patel G, Patel S, Prajapiti D, Rajendra Mehta S, RP-HPLC Method for Simultaneous Estimationof Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Forms, Journal of Pharmaceutical Sciences, 3(1), 2010, 49-53.*
[13] *ICH guideline Q1A (R2) (2003) Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva.*
[14] *ICH guideline Q2(R1) (2005) Validation of analytical procedures: text and methodology. In: International conference on Harmonization, Geneva*
[15] *Wankhede SB, et al. RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. Ind J Pharmaceut Sci. 2007;69:298.*
[16] *Vijaya Kumar M, Muley P.R; Stability indicating RP-HPLC method for determination of telmisartan in solid dosage form; The Indian pharmacist; 2005; 4(36):69-72*