Analytical Method Development And Validation For The Simultaneous Estimation Of Hydrochlorothiazide, Amilodipine Besylate &Telmisartan In Tablet Dosage Form By Rp-Hplc

Dinesh P[1],Vijey Aanandhi M[2],Shanumgasundaram P[1]*

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India. Pin code- 600117

Corresponding Author :

Dr.P.Shanumugasundaram,
Department of Pharmaceutical Chemistry and Analysis,
School of Pharmaceutical Sciences,
Vels Institute of Science, Technology and Advanced Studies (VISTAS),
Pallavaram, Chennai - 600 117.
Mobile:9840126575
Email:director.sps@velsunv.ac.in

ABSTRACT

A novel simple, and accurate RP-HPLC technique was established for the simultaneous quantification of pharmaceuticals in a bilayer tablet comprising Hydrochlorothiazide, Amlodipine. and Telmisartan. Good chromatographic separation was accomplished in the isocratic mode using 0.022M monobasic sodium phosphate dihydrate phosphate with 2ml of triethylmine 1L adjusted with phosphoric acid tp a PH of 6.0 buffer and Acetonitrile (60:40) as the mobile phase, Intersil C18 (250 mm 4.6 mm id. 5 um particle size) column as the stationary phase, and a flow rate of 1.0 millilitre per minute at a detection wavelength of 257 nm. The reported periods of retention for hydrochlorothiazide, amlodipine, and telmisartan were 4.025 minutes, 7.486 minutes, and 14.084 minutes, respectively. As per ICH recommendations, this procedure has been verified. The method delivers good resolution between the medicines with low retention time. The validation parameter findings show that the suggested method is linear, exact, accurate, precise, robust, specific, and selective. As a consequence, the suggested study's findings support the notion that the established approach is an effective strategy for simultaneously estimating the dosages of Hydrochlorothiazide, Amlodipine, and Telmisartan in tablet form. The proposed study's findings support the idea that the approach devised is an accurate approach for simultaneously estimating the concentrations of Hydrochlorothiazide, Amlodipine, and Telmisartan in a bilayer tablet dosage form.

KeyWords : Amlodipine, Hydrochlorothiazide,Telmisartan, Method development, Method validation, RP-HPLC

INTRODUCTION

The fundamental goal of analytical technique development is to determine whether any analytical method utilized in the pharmaceutical industry can be used to measure APL Rapid increase in pharmaceutical industries production of drugs in and around the world cause increase in demand to seek novel and systematic analytical techniques in the pharmaceutical industries. The development of analytical methods has thus evolved into the core function of analysis.

Hydrochlorothiazide is chemically 6-chloro-1,1- dioxo-3,4-dihydro-2H-126,2,4-benzothiadiazine-7- sulfonamide. Hydrochlorothiazide is a thiazide diuretic: it is also known as water pill effectively prevents your body from absorbing too much salt and which can lead to fluid retention. High blood pressure can be treated with Hydrochlorothiazide. By lowering high blood pressure, it lowers the risk of future heart attacks and strokes. The structure of hydrochlorothiazide is given in Fig:1

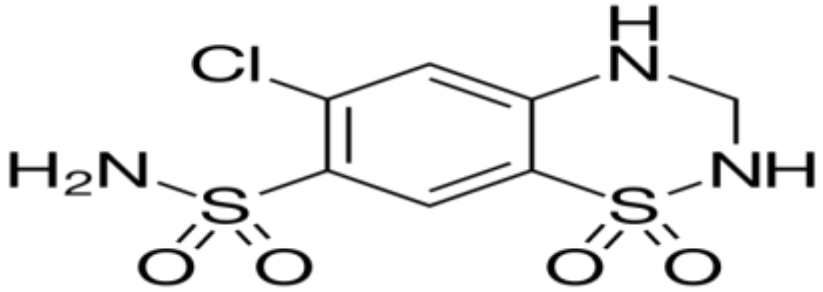


Figure 1:hydrochlorothiazide

Amlodipine is chemically Benzene sulfonic acid;3-O-ethyl 5-0-methyl 2-(2-aminoethoxymethyl)-4- (2-chlorophenyl)-6-methyl-1,4-dihydropyridine- 3,5-dicarboxylate A long-acting 1,4 dihydropyridine calcium channel blocker is Amlodipine. Amlodipine is a long-acting CCB that can be used to treat exertion-related angina and mild to moderate essential hypertension (chronic stable angina). The structure of Amlodipine is given in Figure.2

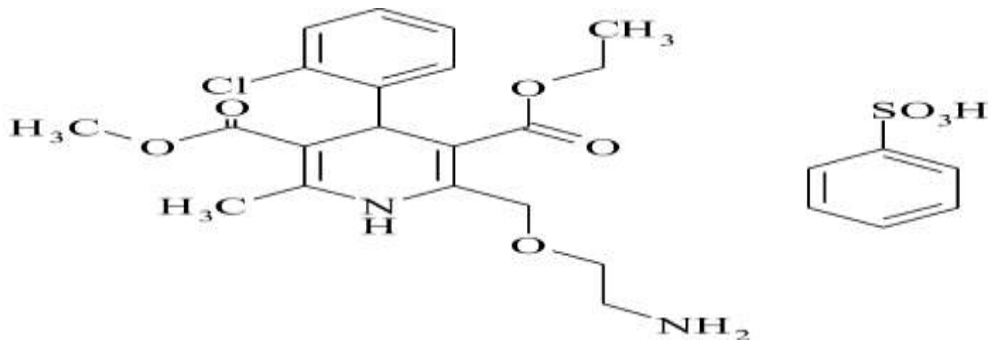


Figure 2:Amloipine Besylate

Telmisartan is chemically 2-[4-[[4-methyl-6-(1- methylbenzimidazol-2-yl)-2 propylbenzimidazol-1- yl] methyl] phenyl] benzoic acid. For the treatment of high blood

pressure, make use of Telmisartan. Bringing down high blood pressure reduces risk of heart attacks, renal issues and strokes. The category of drugs known as angiotensin receptor blockers (ARBS) includes Telmisartan. It works by relaxing blood arteries to enables easier blood flow. The structure of Amlodipine is given in the Figure 3

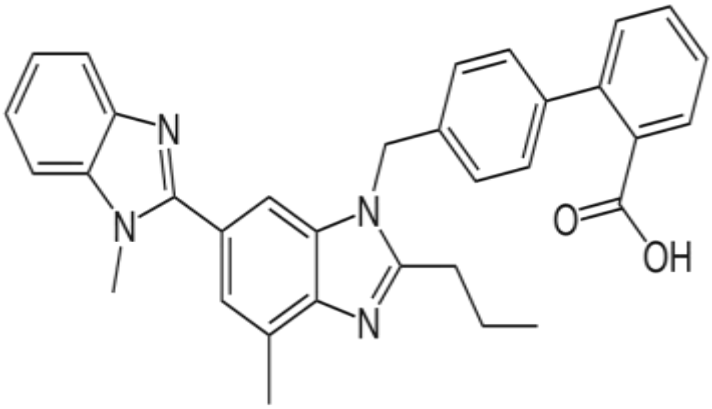


Figure 3:Telmisartan

Due to the multiple components contained in dosage forms, developing an analytical approach for the simultaneous assessment of different medications in a polypill is difficult. It is a combination of Telmisartan, Amlodipine and Hydrochlorothiazide which are antihypertensive medicines. The review of literature revealed that many approaches were reported for the estimate of individual drugs such as Amlodipine, Hydrochlorothiazide, and Telmisartan, as well as when combined with other medications. There is a need to develop a technique with less time consumption and less organic solvent consumption since the methods. that have been developed in the past were found to have longer retention times, higher consumption of organic solvents, and poor resolution. The present work aimed to develop stability indicating HPLC method for simultaneous estimation of the drugs in combined solid oral dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Acetonitrile - Analytical Research Grad, Potassium dihydrogen phosphate - Analytical Research Grad**,** Orthophosphoric acid - HPLC Grade, Water - HPLC Grade**,** Hydrochlorothiazide,amilodipine and telmisartan - Working Reference standard from Sai Mirra Innopharm in India.

Instruments and Apparatus Required: The high performance liquid chromatography system (shimadzu - prominence) using the lab solution software. The forced degradation study was carried out in high performance liquid chromatography system(waters) using empower software. The uv spectrophotometer was from shimadzu.

Instrumentation and Chromatographic conditions:

The HPLC system (Shimadzu i prominence) equipped with the UV/Visible dual absorbance detector, Intersil C18 (250 x 4.6mm; 5 µm), was used to achieve chromatographic separation.

Before use, a 0.45 µ membrane filter was used to filter the mobile phase, which was composed of monobasic sodium phosphate dihydrate buffer pH 6 and acetonitrile in a 60:40 v/v ratio. This mobile phase was then degassed and injected onto the column at a rate of 1.0 ml/min. At 257 nm, the detection was recorded. Prior to the injection of the drug solution, the injection loop's volume was 20 micro L.

Preparation of Mobile Phase:

Buffer: 0.022 M monobasic sodium phosphate dihydrate and 2ml of triethylamine was added to 1 litre of water. The pH was adjusted to 6.0 with orthophosphoric acid. The mixture of buffer and Acetonitrile was mixed in the given ratio and degassed in the ultrasonic water bath for 5 minutes. The solution was filtered under vacuums using a 0.45µ filter

Diluent: 3ml of triethylamine was added to 500 ml of purified water. To the mixture, 500ml of acetonitrile was added and mixed.

Standard Solution Preparation:

Standard Stock solution: 25 mg of Hydrochlorothiazide WRS, 14 mg of Amlodipine WRS and 160 mg of Telmisartan were weighed accurately and transferred into a 100 mL volumetric flask. Add 25 mL of diluent was into the flask. With the diluent, dissolve and dilute to volume.

Standard solution: Transfer 5 mL of sample stock solution to a 20 mL volumetric flask & dilute to volume with diluents.

Sample Solution Preparation:

Sample stock solution: 10 tablets were transferred to a 200 mL volumetric flask then 50 mL of diluent was added and the mixture was shaken mechanically for 15 minutes or until dissolved. After mixing, the volume was made up with diluent. Centrifuge or pass through a suitable filter of 0.45- um pore size.

Sample solution: 5ml of the test sample stock solution was pipetted into a 50ml volumetric flask and diluted with diluents to the mark. Procedure: The standard and the sample solutions were injected separately into the liquid chromatograph and the peak area for major peaks were recorded.

Test Procedure : 20 µl of the standard, sample, blank and placebo preparations in duplicate were injected separately into the HPLC system and the peak responses for Hydrochlorothiazide, Amlodipine and Telmisartan were measured. Hydrochlorothiazide, Amlodipine and Telmisartan were estimated using the newly Method developed RP-HPLC technique which was carried out on Intersil C18 column (4.6 x 250millimeter, 5 µm) column in isocratic mode using mobile phase The analytical composition of buffer and acetonitrile in ratio according 60:40 with a flow rate of 1 ml/min at 257nm.

Forced Degradation Study

A forced degradation study was carried out on Hydrochlorothiazide, Amlodipine and Telmisartan under various stress conditions like acid and base hydrolysis, oxidation, thermal and photolysis. Stress testing of Hydrochlorothiazide, Telmisartan and Amlodipine under different conditions using Phosphate buffer: acetonitrile (60:40) as the mobile phase solvent system suggested the degradation behaviour.

Acid degradation:

Acid degradation was performed by adding 1. 4/11 to the accurate weighted working standards, Test sample and Placebo. The mixture was kept at 60°C for 1 hr and the mixture was neutralized with 1M NaOH and the chromatogram was recorded.

Base degradation:

Base degradation was performed by adding 1M NaOH to the accurate weighted working standards, Test sample and Placebo. The mixture was kept at 60°C for 1 hr and the mixture was neutralized with 1M HCl and the chromatogram was recorded.

Oxidative degradation:

Oxidative degradation was performed by adding 3% H202 to the working standard, Test sample and Placebo and the mixture was kept at 60°C for 1 hr and the chromatogram was recorded.

Thermal degradation:

Thermal degradation was done by heating the working standard, Test sample and Placebo at 105°C for 5 hrs in an oven, and the chromatogram was recorded.

Photolytic degradation:

Photolytic degradation was done by subjecting working standard, Test sample and Placebo to 350nm UV-Visible light for 2hrs and the chromatogram was recorded.

Method Validation:

The purpose of validating a method of analysis is to show that it is appropriate for the intended use. The analytical method validation was carried out according to ICH Q2(R1) guidelines.

System suitability:

The system suitability parameters such theoretical plate, tailing factor, theoretical plates and resolution were evaluated from six replicate injections.

Specificity:

The capacity to measure the target analyte precisely and specifically in the presence of other compounds such as excipients that could be anticipated to be present in the sample matrix is

known as specificity. To determine if there is any interaction with the retention time of the API's, specificity parameters were tested from the standard, blank and placebo solutions.

Linearity:
The Linearity was determined. For Hydrochlorothiazide, Amlodipine and Telmisartan in the range of 50% to 150% of the operating concentration. A graph was plotted with concentration versus area and determine the correlation coefficient square & y-intercept.

Range:
One solution with higher concentration and one with lower concentration (as prepared under Linearity) in 6 replicates each was injected and the peak areas were recorded. Calculate the related standard deviation for the 6 areas.

Accuracy:
The accuracy of the assay method was determined by adding known amounts of Hydrochlorothiazide, Telmisartan and Amlodipine to the placebo at 50%, 100% and 150% of actual concentration. The standard preparations and test preparations were injected separately in 6 replicates. The chromatograms were recorded and responses were measured.

Precision:
Six independent assay determinations using a homogeneous sample of Telmisartan, Amlodipine and Hydrochlorothiazide tablets was performed. The content of Telmisartan, Amlodipine and Hydrochlorothiazide in the tablets was determined by each of the 6 analysis. The RSD was calculated for the 6 replicate values which was obtained.

Intermediate precision:
To evaluate the analyst, instrument and day variability of the method, the assay was performed in six replicates by another analyst using a different HPLC system on a different day. Inject the standard solution and the sample solutions in 6 replicates separately and record the peak area for major peaks. Calculate the content of Hydrochlorothiazide, Amlodipine and Telmisartan per tablet in all six preparations.

Solution stability:
The sample solutions were prepared and their stability was to be tested for the initial hour, 4 hours, 8 hours, 12 hours, 24 hours, 48 hours and 72 hours. The percentage of deviation was also be measured.

Filter integrity:
After passing through 0.45µm PVDF, 0.45 µm Nylon and 0.45 µm PTFE filters, the filtered samples were injected. The areas obtained for the filtered samples were compared against the centrifuged sample.

Robustness:

By making minor, purposeful modifications to the flow rate, wavelength, column temperature, and mobile phase composition, the method's robustness was demonstrated. The samples were injected in six replicates and % RSD was calculated.

Results And Discussion:

The purpose of this study is to develop and validate an analytical method for the simultaneous determination of Hydrochlorothiazide, Amlodipine, and Telmisartan in bulk and combined bilayer tablet dose form.

Chromatographic Method Development:

Different mobile phases were used to run the standard drugs at various pH levels, along with organic mobile phase modifiers including acetonitrile and water. Additional trials were carried out to minimize tailing by altering pH, however these changes caused the peaks to split or the resolution to diminish between the two drugs. It was also attempted to modify the organic phase, however this led to peaks merging or to no changes in the drug's tailing. The peak forms were observed to be symmetrical under the specified chromatographic conditions.

Selection of Wavelength:

The UV Spectrophotometer was used to scan the individual standard solutions of the specified drugs at a concentration of 10 mg/ml in the selected mobile phase. On the basis of the drug's increased response, detection was performed at 257 nm.

Optimized Conditions:

On the Intersil C18 column (4.6 x 250 millimeter, 51 um), acceptable chromatographic conditions with good shapes were obtained obtained after extensive optimization. Monobasic sodium phosphate dihydrate buffer: Acetonitrile (60:40) was used as the mobile phase, and the flow rate was maintained at 1 mL per minute. At a wavelength of 257 nm, the detection was observed. The injection volume was set at 20 µL. The reference solution contained 60µg/mL of Hydrochlorothiazide, 25µg/mL of Amlodipine, and 400 µg/mL of Telmisartan, respectively. The retention periods for the working reference standard were reported to be 4.076, 8.652 and 13.39 minutes for Hydrochlorothiazide, Amlodipine, and Telmisartan, respectively.WThe retention times for the test sample containing Hydrochlorothiazide,amilodipine and telmiartan were minutes, respectively. The chromatogram of test sample is given in Figure.4. Percentage purity of Hydrochlorothiazide, Amlodipine and Telmisartan in the standard samples were found to be 99.72%, 101.32% and 99.40%. Percentage purity of Hydrochlorothiazide, Amlodipine and Telmisartan in the given tablet sample were found to be 100.46%, 99.40% and 101.76%. No peak asymmetry was observed. No other impurity interference was seen. Table.1 showcase the optimized chromatographic conditions.

Method Validation:

System suitability:

System suitability tests were carried out to ascertain the adequate resolution and repeatability of the above-suggested approach. Investigations were done on the following parameters: retention time, number of theoretical plates, tailing factor, resolution and presented in Table 2. It was reported that the %RSD was less than 2% for the retention time of Hydrochlorothiazide, Amlodipine, and Telmisartan. The above mentioned parameters for Hydrochlorothiazide, Amlodipine and Telmisartan were all within acceptable ranges.

TABLE 1:SYSTEM SUITABILITY PARAMETERS OF HCTZ,AML &TEL

PARAMETERS	HYDROCHLOROTHIAZIDE	AMLODIPINE	TELMISARTAN
Relative standard deviation	0.04%	0.07%	0.05%
Tailing factor	1.273	1.742	1.851

Specificity

By comparing the test sample's retention time to that of reference drugs, the peaks of the test drugs were assessed. The retention times of the standard and test samples showed a good correlation. It was noted that the Hydrochlorothiazide, Amlodipine, and Telmisartan peaks were unaffected by the diluent or excipient peaks. Table.2 represents the observations of the specificity samples. The chromatogram of standard and test sample has without any interference

Table 2: Specificity parameters of Hydrochlorothiazide,amilodipine and telmisartan

Injection No.	**Response of the peak with Retention time**	**Influence of placebo**
1.Blank	No peaks observed	-
2.Placebo	No peaks observed	No influence
3.Standard solution	Three peaks observed. First peak at 4.024 minutes is due to Hydrochlorothiazide, Second peak at 7.480 minutes is due to Amlodipine besylate and the third peak at 14.088 minutes is due to Telmisartan.	-
4.Test solution	Three peaks observed. First peak at 4.025 minutes is due to Hydrochlorothiazide, Second peak at 7.486 minutes is due to Amlodipine besylate and the third peak at 14.084 minutes is due to Telmisartan.	No influence due to placebo

Remarks: There is no interference of the placebo. Hence the method is specific.

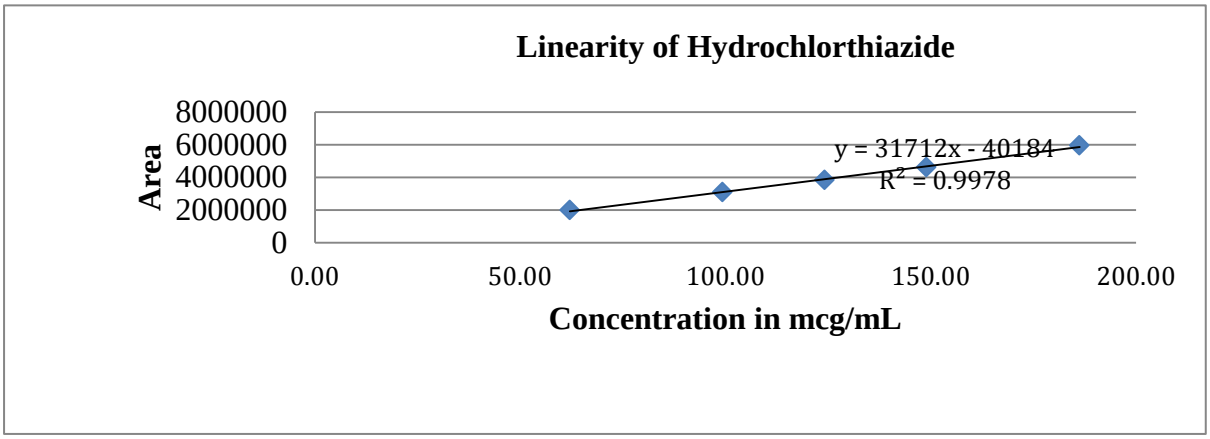
Linearity:

Calibration curves for Hydrochlorothiazide,Telmisartan and Amlodipine were found to be linear. Each drug concentration had an outstanding peak-to-area ratio correlation as shown by the R2 value consistently equal to 0.99. The peak area was represented on the y axis of a graph with the drug concentration on the x axis which is given in Figure.8. The calibration curves for Hydrochlorothiazide, Amlodipine, and Telmisartan were all found to be linear in the concentration ranges of 20 to 100 µg/mL, 10 to 40 µg/mL, and 200 to 600 ug/mL respectively with linear correlation coefficients of 0.99 for each drug. The report of analysis of the linearity is given in Table.3.

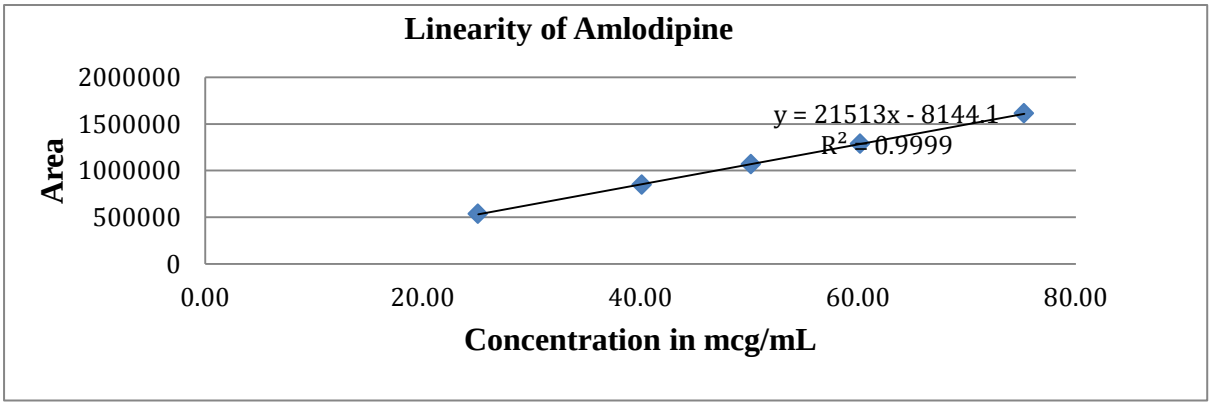
Table 3: Linearity of response of Hydrochlorothiazide, Amlodipine besylate, and Telmisartan

Sample ID	Hydrochlorothiazide		Amlodipine Besylate		Telmisartan	
	Concentration, in mcg/mL	Area	Concentration, in mcg/mL	Area	Concentration, in mcg/mL	Area
50 % of operating concentration	62.09	1993951	25.09	536406	198.03	12309605
80 % of operating concentration	99.35	3089328	40.14	849334 8	316.85	19370362
100 % of operating concentration	124.18	3835214	50.17	106979 0	396.06	24298162
120 % of operating concentration	149.02	4621714	60.21	128752 9	475.27	293060504
150 % of operating concentration	186.27	5949138	75.26	161324 7	594.09	36679109
Correlation coefficient (*r* >0.995)	**0.9978**		**0.9999**		**0.9999**	
y- intercept (NMT ±2.0 %)	**-1.05%**		**-0.76%**		**-0.17%**	

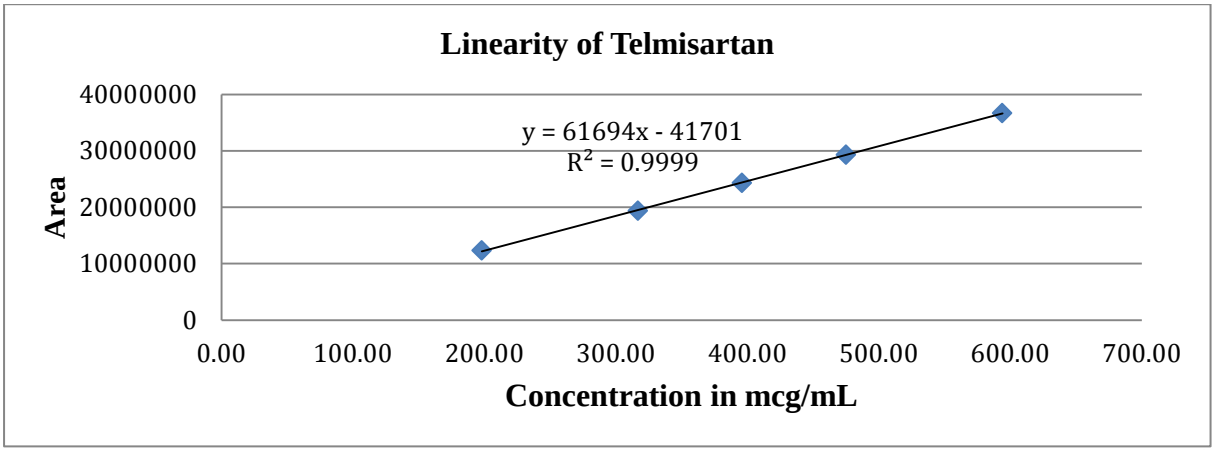
The plot between concentration and response is as follows –



(a)



(b)



(c)

Figure 4:Caliberation curve(a)Linearity graph of Hydrochlorothiazide (b)Linearity graph of Amlodipine (c)Linearity graph of Telmisartan

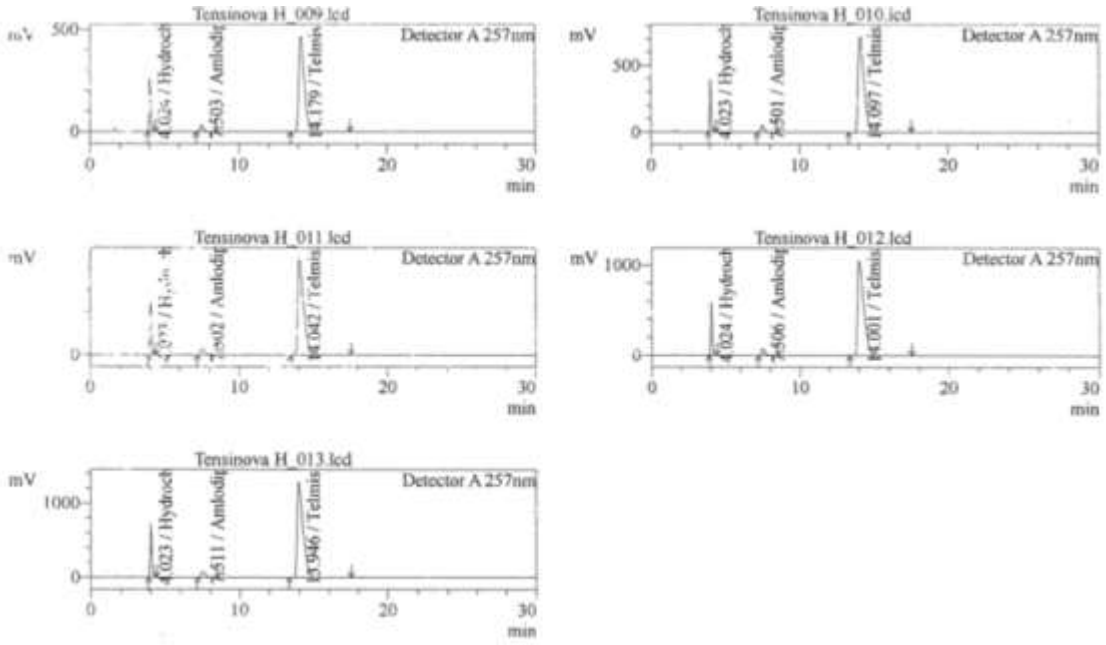


Figure 5: Linearity chromatogram of Amilodipine Besylate , Hyrochlorothiazide and Telmisartan

Range

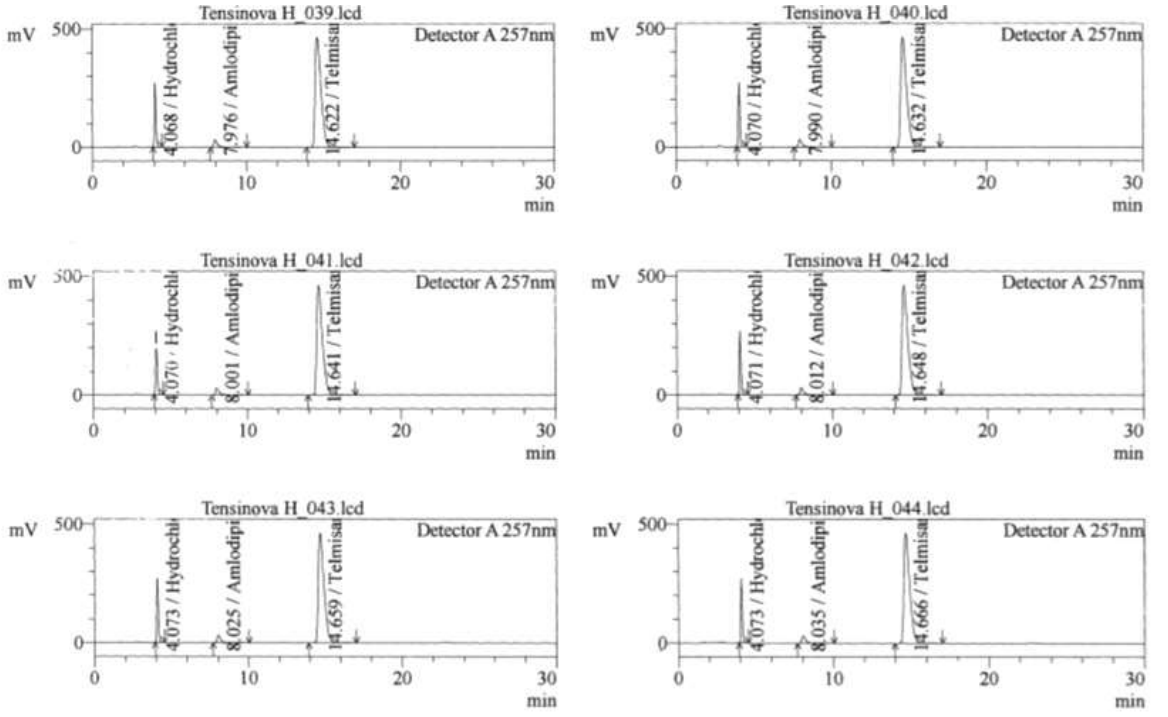
One solution with higher concentration and one with lower concentration (as prepared under Linearity) in 6 replicates each was injected and the peak areas were recorded. Calculate the related standard deviation for the 6 areas which is shown in table 4.

Acceptance Criteria for Range: RSD for 6 areas at two linearity levels - NMT 2.0 %

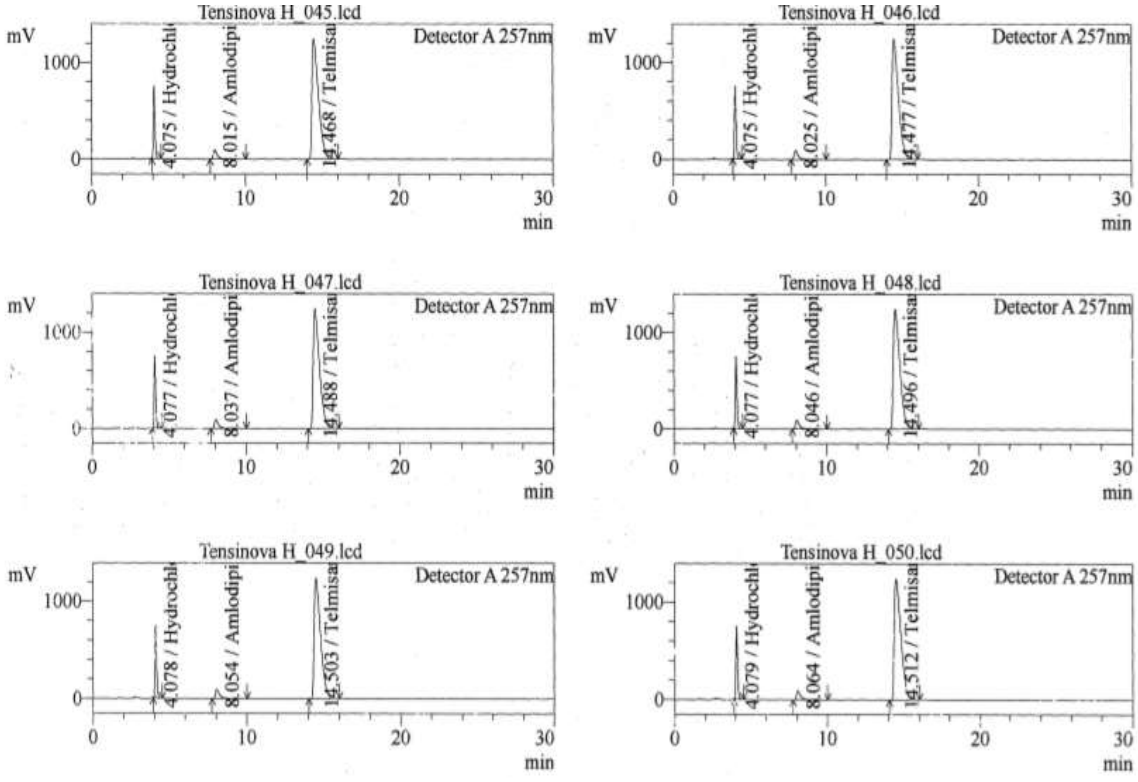
Table 4 : Range of Least and Highest Concentration Peak Area of 6 replicates

S.No	Hydrochlorothiazide		Amlodipine besylate		Telmisrtan	
	50 % Standard	150 % Standard	50 % Standard	150 % Standard	50 % Standard	150 % Standard
1	2003190	5676048	538502	1604014	12358259	36519376
2	2002814	5673016	538385	1602971	12357290	36506276
3	2002276	5673374	538095	1601042	12359143	36486979
4	2002092	5671415	537769	1599230	12360812	36466293
5	2002109	5670904	537922	1597202	12361205	36455733
6	2001580	5669160	537825	1594552	12360583	36426459
Average	**2002344**	**5672320**	**538083**	**1599835**	**12359549**	**364768853**
RSD (NMT 2.0 %)	**0.03 %**	**0.04%**	**0.06%**	**0.22 %**	**0.01%**	**0.09%**

Remarks: Correlation Coefficient square, y-intercept and RSD are within the acceptable limit.



STANDARD 50 %



STANDARD 150%

Figure 6: Range chromatogram of Amilodipine Besylate , Hyrochlorothiazide and Telmisartan

Accuracy

Recovery study validates the method's accuracy. Specific amounts of the reference drugs were added separate concentrations of 50%, 100%, and 150% The area of the solutions was calculated, along with the recovery percentage.

Table 5: Accuracy Study Values of Hydrochlorothiazide

S. No.	Sample ID		Amount added (mg)	Amount recovered (mg)	Recovery (98.0 % to 102.0 %)	Mean and RSD
1	50	A1	1.2374	1.2439	100.53	**Mean = 100.57 %** **RSD** = 0.04%
2		A2	1.2413	1.2484	100.57	
3		A3	1.2393	1.2468	100.60	
4	100	A1	2.4747	2.4599	99.40	**Mean = 100.12 %** **RSD** = 0.62 %
5		A2	2.4827	2.4940	100.46	
6		A3	2.4787	2.4911	100.50	
7	150	A1	3.7121	3.6420	98.11	**Mean = 98.19 %** **RSD** = 0.11%
8		A2	3.7240	3.6543	98.13	
9		A3	3.7180	3.6554	98.32	

Table 6: Accuracy Study Values of Amlodipine

S. No.	Sample ID		Amount added (mg)	Amount recovered (mg)	Recovery (98 % to 102 %)	Mean and RSD
1	50	A1	0.5039	0.4975	98.74	**Mean = 98.65 %** **RSD** = 0.56 %
2		A2	0.5074	0.5032	99.16	
3		A3	0.5071	0.4973	98.07	
4	100	A1	1.0078	1.0008	99.31	**Mean = 99.48 %** **RSD** = 0.20%
5		A2	1.0149	1.0118	99.70	
6		A3	1.0142	1.0084	99.43	
7	150	A1	1.5116	1.5181	100.43	**Mean = 99.47 %** **RSD** = 0.83%
8		A2	1.5223	1.5060	98.93	
9		A3	1.5213	1.5072	99.07	

Table 7: Accuracy Study Values of Telmisartan

S. No.	Sample ID		Amount added (mg)	Amount recovered (mg)	Recovery (98 % to 102 %)	Mean and RSD
1	50	A1	3.9419	3.9347	99.82	**Mean = 99.47 %** **RSD** = 0.54%
2		A2	3.9517	3.9415	99.74	
3		A3	3.9562	3.9109	98.86	
4	100	A1	7.8838	7.7547	98.36	**Mean = 100.00 %** **RSD** = 1.82%
5		A2	7.9035	7.8779	99.68	
6		A3	7.9123	8.0676	101.96	
7	150	A1	11.8257	11.7035	98.97	**Mean = 98.58 %** **RSD** = 0.34%
8		A2	11.8552	11.6663	98.41	

9		A3	11.8685	11.6765	98.38	

Remarks: The recovery and RSD for recovery at each level meets the acceptance criteria

Precision

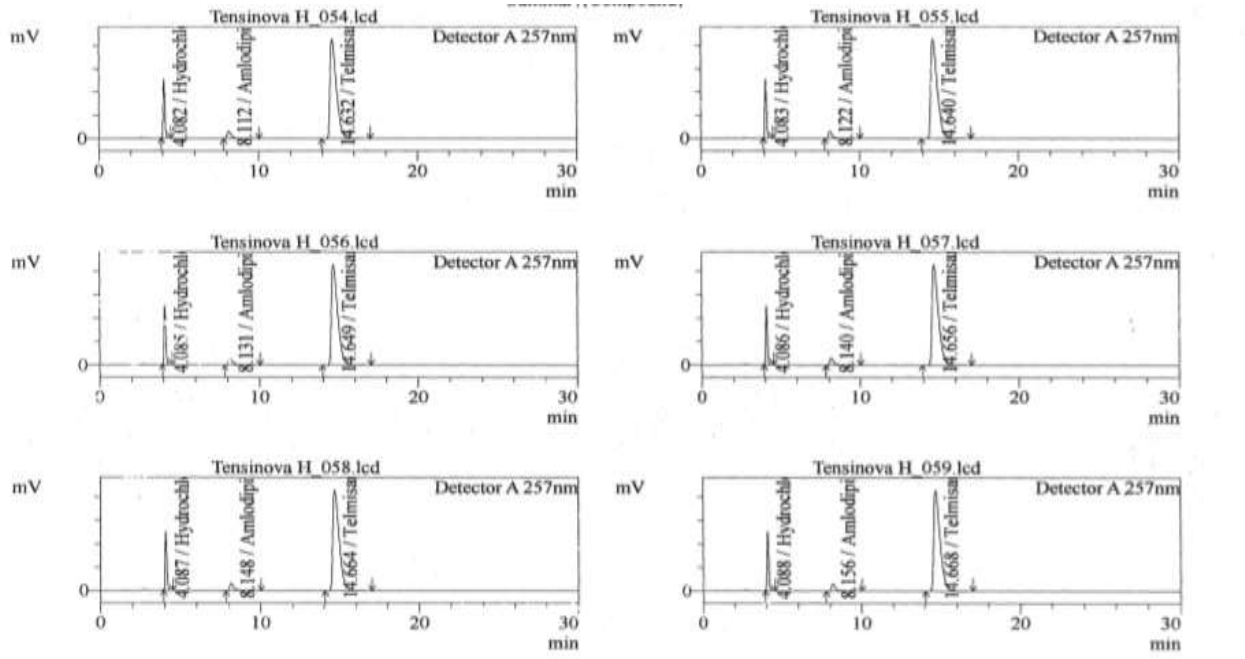
In the method validation, the method was found precise with the results obtained as % RSD 0.58% for Hydrochlorothiazide, 0.24% for Amlodipine and 0.37% for Telmisartan. The report of analysis of precision is given in Table.8.

Table.8.Report Of Analysis Of Precision

TEST NO.	Hydrochlorothiazide	Amlodipine	Telmisartan
	Content (mg/tablet)		
1	12.35	5.05	39.00
2	12.26	4.96	38.50
3	12.61	5.14	39.75
4	12.41	4.98	38.93
5	12.44	4.95	38.78
6	12.59	5.06	39.51
AVG	**12.44**	**5.02**	**39.08**
RSD	**1.09%**	**1.48%**	**0.47%**

Remarks: RSD between the assay values and overall RSD between the two sets are within acceptable limits.

STANDARD:



TEST:

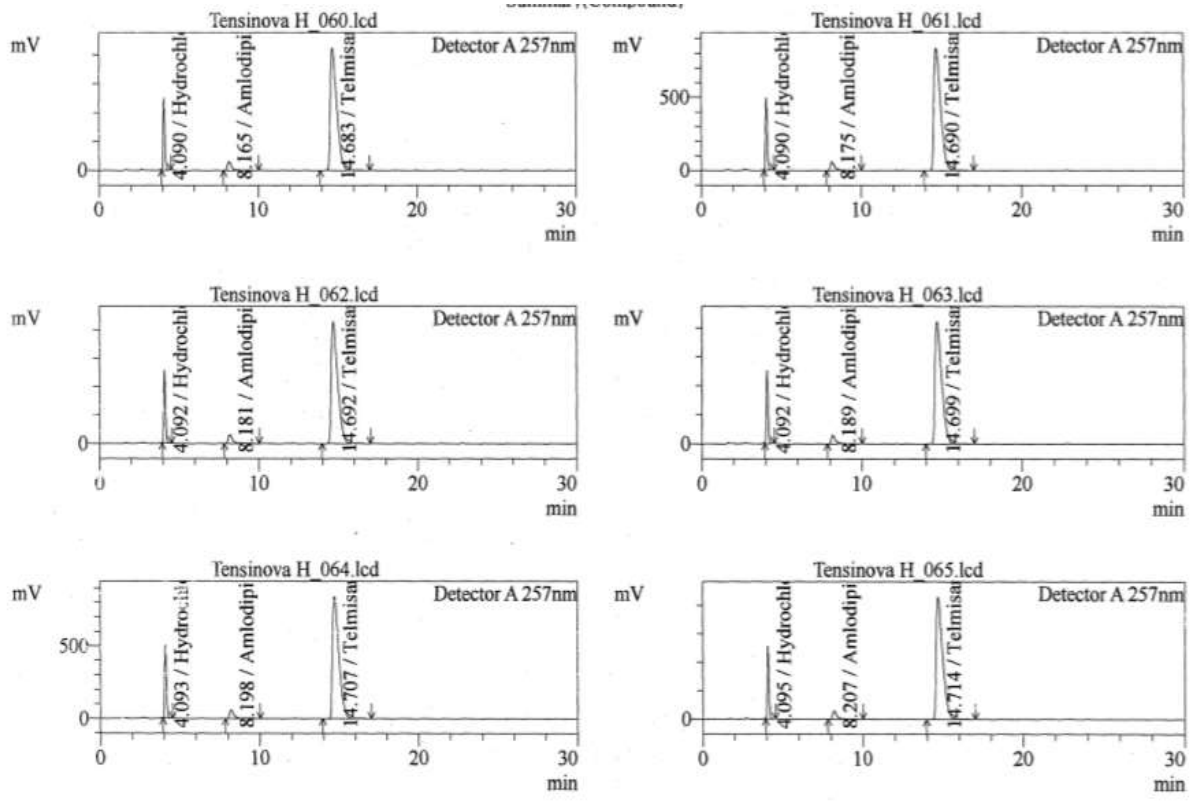


Figure 7: Range chromatogram of Amilodipine Besylate , Hyrochlorothiazide and Telmisartan

Solution stability

The sample solutions were prepared and their stability was to be tested for the initial hour, 4 hours, 8 hours, 12 hours, 24 hours, 48 hours and 72 hours. The standad solution and test olution are stable for 72 hrs.The percentage of deviation was also be measured which is shown in table 11.

Acceptance Criteria for Solution Stability: The % Deviation from the initial area at each time is NMT 2.0 %.

Table 9: Solution stability of Hydrochlorothiazide ,aimilodipine and telmisartan standard and test at different time period.

S.N0	TIME	%Deviation from the initial area					
		Hydrochlorothiazide		Amilodipine		Telmisartan	
		STD	Test	STD	Test	STD	Test
1	1 hrs	0.71%	1.77%	0.53%	0.51%	0.19%	0.12%
2	50 hrs	0.25%	0.64%	1.55%	1.82%	0.13%	0.19%
3	56 hrs	1.78%	1.22%	2.98%	1.73%	1.34%	0.56%

Remarks: The % Deviation from the initial area is within acceptable limits for both the peaks in standard and test solutions even after 12 hours. Hence the solutions are stable up to 12 hours.

Robustness

The method was found to be Robust after making small deliberate changes in the chromatographic conditions. The flow rate was changed 0.9 ml/min and 1.1 ml/min. The wavelength was changed to 255nm and 259mm and the mobile phase ratio was changed to 58: 42 and 62:38. The column. temperature changed to 28°C and 32°C and the pH- changed to 5.8 and 6.2. It is observed that there was no marked changes in the method parameters which demonstrated that the method developed is robust.

Acceptance Criteria for Robustness: Should pass the system suitability under each variable parameter.

Table 10: variation and its resulted values like % RSD, Theoretical plate and Resolution between Hydrochlorothiazide,amilodibine and telmisartan

		Hydrochlorothiazide		**Amilodipine**		**Telmisartan**	
Parameters	**Variation**	**RSD NMT 2.0 %**	**Tailing Factor**	**RSD NMT 2.0 %**	**Tailing Factor**	**RSD NMT 2.0 %**	**Tailing Factor**
Change in wavelength	255 nm	0.13 %	1.260	0.16 %	1.552	0.15%	1.677
	259 nm	0.26 %	1.256	0.19 %	1.553	0.25%	1.679
Flow rate	0.9 mL/min	0.06 %	1.263	0.19 %	1.576	0.10%	1.730
	1.1 mL/min	0.10 %	1.253	0.11 %	1.556	0.09%	1.670
pH of buffer	pH 5.8	0.17 %	1.248	0.09 %	1.582	0.06%	1.501
	pH 6.2	0.17 %	1.253	0.17 %	1.628	0.15%	1.964
Mobile phase composition	Buffer: Acetonitrile (58:42)	0.05 %	1.265	0.33 %	1.572	0.03%	1.680
	Buffer: Acetonitrile (62:38)	0.19 %	1.258	0.38%	1.597	0.16%	1.815

Remarks: Passes the system suitability under each variable parameter. Parameter: RSD(NMT 2.0%) and Tailing factor (NMT 2.0)

Filter Integrity

Filter integrity of Hydrochlorothiazide, Amlodipine besylate and Telmisartan standard solution and test solution were checked for centrifuge, PVDF 0.45 um, Nylon 0.45pm and 0.45pm PTFE. All the filters are suitable for filtering the sample solutions.

Acceptance Criteria for Filter Integrity: The % Deviation in area of the filtered solution from the area obtained in centrifuged solution is NMT 2.0 %.

Table 11: Area and % Deviation in Area of Hydrochlorothiazide,amilodipine and telmisartan Standard and Test Obtained Using Different Filter

S.NO	Filter used	Deviation in area from centrifuged sample		
		Hydrochlorothiazide	Amilodipine	Telmisartan
1	PVDF,0.45μm (Polyvinylidiene fluoride)	1.79%	1.43%	1.83%
2	Nylon 0.45 μm	1.87%	0.87%	1.80%
3	PTFE,0.45 μm (Polytetrafluoroethylene)	1.67%	0.66%	1.53%

Remarks: The areas obtained for the solutions filtered through PVDF, Nylon and Teflon filters are well within specified limits. PVDF, Nylon and Teflon filters are suitable for filtration.

CONCLUSION:

Most of the methods used in the past for estimating Hydrochlorothiazide,Amiilodipine and Telmisartan were tedious and time-consuming. Thus, a reversed phase HPLC method was developed and validated. The results of the system suitability and applicability indicated that the proposed method is suitable and applicable for routine laboratory analysis. With less retention time, the approach offers good resolution between the drugs. The proposed technique is linear, exact, accurate, precise, robust, specific, and selective, according to the results of the validation parameters which confirms that the results obtained meet the pre-established acceptance criteria. Therefore, the study results confirm that the developed method is a suitable technique for simultaneous estimation of Hydrochlorothiazide,Amiilodipine and Telmisartan in Tablet dosage form.

ACKNOWLEDGEMENT

The authors thank the management and staffs, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS) and Sai Mirra Innopharm Pvt Ltd, Chennai - 98 for providing us the instrumentation facilities to carry out this work.

CONFLICTS OF INTEREST

There was no conflicts of interest.

FUNDING

None

REFERENCES

1. Kayal SD, Khan FA, Tated AG, Bakal RL, Chandewar AV. Method development and validation for the simultaneous dosage form by RP-HPLC. Pharmaceutical Research and Development. 2011;3(5):144-153.

2. Mukhopadhyay S, Kadam K, Sawant L, Nachane D, Pandita N. Simultaneous determination of related substances of Telmisartan and Hydrochlorothiazide in tablet dosage form by using reversed phase high performance liquid chromatographic method. Journal of Pharmacy and Bioallied Sciences. 2011;3(3):375.

3. Sinha SK, Shrivastava PK, Shrivastava SK. Development and validation of a HPLC method for the simultaneous estimation of Amlodipine and Telmisartan pharmaceutical dosage form. Asian Pacific Journal of Tropical Biomedicine. 2013;2(1):312-5. Upendra B, Hemant D, Kumar DA. RP-HPLC Method

4. Upendra B, Hemant D, Kumar DA. RP-HPLC Method Development and Validation For Estimation of Telmisartan in Bulk and Tablet Dosage Form. International Journal of Drug Regulatory Affairs. 2014;1(2):61-4..

5. Kranthi M, Srinivas A. Analytical Method Development and Validation and Force Degradation Studies for Simultaneous Estimation of Amlodipine and Telmisartan in Tablet Dosage Form by using RP-HPLC International Journal of Pharmaceutical and Phytopharmacological Research. 2014; 4:2-7.

6. Chabukswar AR, Jagdale SC, Kumbhar SV, Desai DJ, Kuchekar,BS, Lokhande PD. HPLC Method Development for Telmisartan and Amlodipine. Research Journal of Pharmacy and Technology. 2013;3(4):1227-30.

7. L. Kanaka Lakshmi, et al. Analytical Method Development and Validation for the Simultaneous Estimation of Telmisartan and Hydrochlorothiazide by RP-HPLC Method in Bulk and Tablet Dosage Form. Journal of Pharmaceutical andBiomedical Analysis. 2016; 4(1):1-8.

8. Guideline ICH. Stability testing of new drug substances and products. Q1A (R2), currentstep. International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use. 2003; 4:1- 24.

9. Guideline ICH. Validation of analytical procedures: text and methodology. Q2 (R1). International Conference Harmonization of technical requirements for registration of pharmaceuticals for human use. 2005;1(20):05.