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Research Article

Analytical Method Development and Validation for the Simultaneous Estimation of Telmisartan and Hydrochlorothiazide by RP-HPLC Method in Bulk and Tablet Dosage Form

[1]Taraka Ramesh G*, [2]Y Rajendra Prasad

[1]Principal scientist, Analytical R & D, Etico Life Sciences, Hyderabad, Telangana
[2]Professor, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India.

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ABSTRACT

The chromatographic conditions were successfully developed for the separation of Telmisartan and Hydrochlorothiazide by using C_{18} Column (150mm x 4.6mm)5µm, flow rate was 1ml/min, mobile phase ratio was Methanol: Phosphate buffer P^H 4.5 (70:30 v/v), detection wavelength was 268 nm. The Spectroscopic method was done in solvent using methanol and the instrument lab spectrophotometer UV5 software. The instrument used was WATERS HPLC Auto Sampler, Alliance 2695, photo diode array detector, Empower-software version 2. The retention times were found to be about 2.15 min and 3.0 min. The % purity of Telmisartan and Hydrochlorothiazide was found to be 99.86% and 100.1% respectively. The system suitability parameters for Telmisartan and Hydrochlorothiazide such as theoretical plates and tailing factor were found to be 7752, 1.3 and 6467 and 1.2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Telmisartan and Hydrochlorothiazide was found in concentration range of 2µg-25µg and 5µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery was found to be 99.86% and 99.96% respectively. %RSD for repeatability and precision was found to be < 2.LOD values were 0.012 and 0.018 and LOQ value was 0.022 respectively for Telmisartan and Hydrochlorothiazide.

Keywords: Telmisartan, Hydrochlorothiazide, HPLC

INTRODUCTION

Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension. The usually effective dose telmisartan is 40–80 mg once daily. Some patients may already benefit at a daily dose of 20 mg. In cases where the target blood pressure is not achieved, telmisartan dose can be increased to a maximum of 80 mg once daily. Telmisartan is contraindicated during pregnancy. Like other drugs affecting therenin-angiotensin system (RAS), telmisartan can cause birth defects, stillbirths, and neonatal deaths. It is not known whether the drug passes into the breast milk. Also it is contraindicated in bilateral renal artery stenosis in which it can cause renal failure.

Address for Correspondence: Taraka Ramesh G, Principal Scientist, Analytical R & D, Etico Life Sciences, Hyderabad, Telangana; E-mail: rameshgmp@gmail.com

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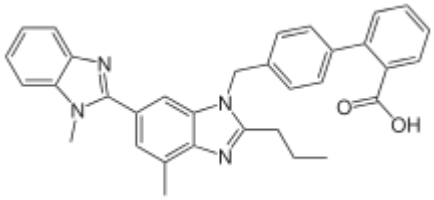
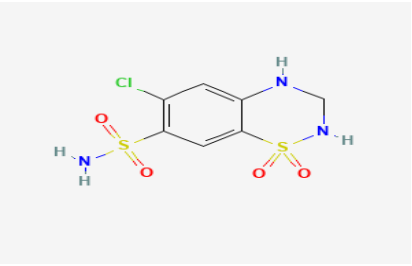
Hydrochlorothiazide is a diuretic medication often used to treat high blood pressure and swelling due to fluid build-up. Other uses include diabetes insipidus, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine. For high blood pressure it is often recommended as a first line treatment. HCTZ is taken by mouth and may be combined with other blood pressure medications as a single pill to increase the effectiveness. Potential side effects include poor kidney function, electrolyte imbalances especially low blood potassium and less commonly low blood sodium, gout, high blood sugar, and feeling faint initially upon standing up.[2] While allergiesto HCTZ are reported to occur more often in those with allergies to sulfa drugs this association is not well supported. It may be used during pregnancy but is not a first line medication in this group. It is in the thiazide medication class and acts by decreasing the kidneys' ability to retain water. This initially reduces blood volume, decreasing blood return to the heart and thus cardiac output. Long term, however, it is believed to lower peripheral resistance.



Telmisartan



Hydrochlothiazide

Analytical methods

The technique employed in qualitative and quantitative analysis is based upon the performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained.

Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods there can be no “second quality” in drugs. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production.

Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the Physico-chemical methods, the most important are optical (Refractometry, Polarimetry, Emission, Fluorescencemethods of analysis, Photometry including Photocolorimetry and Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular. The combination of Mass Spectroscopy with Gas Chromatography and Liquid Chromatography are the most powerful tools available. The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of pharmacopeia
3. The analysis should be economical.
4. The selected method should be precise and selective.

MATERIALS AND METHOD

Apparatus: The instrument used for the study was Waters HPLC Auto Sampler, Alliance 2695, photo diode array detector with Empower-software version-2.

Reagents and Materials: The solvents used were Methanol, Ortho phosphoric acid, Potassium dihydrogen ortho phosphate, Tri Ethyl Amine of HPLC Grade and HPLC Water.

Selection of chromatographic condition: Proper selection of the method depends upon the nature of the sample, its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.

Selection of detection wavelength: The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected.
Standard solutions of Telmisartan and Hydrochlorothiazide were scanned in the UV range (200-400nm) and the spectrums obtained were

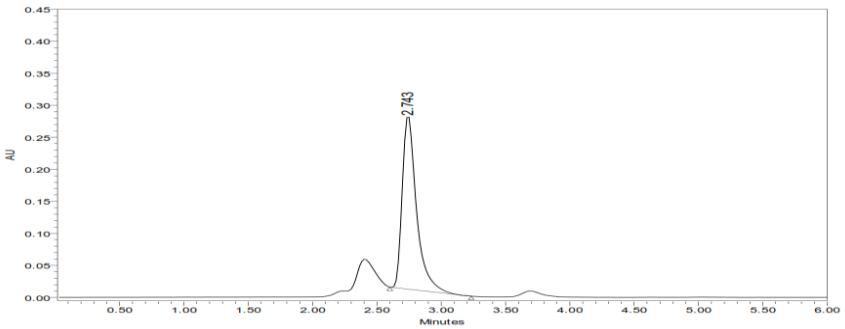
overlaid and the overlain spectrum was recorded. From the overlain spectrum, 268 nm was selected as the detection wavelength for the present study.

Selection of mobile phase: Initially the mobile phase tried was methanol and water, methanol and Methanol, buffer and water in various proportions. Finally, the mobile phase was optimized to Buffer: Methanol in proportion 30:70 v/v respectively at pH 4.5 adjusted with Orthophosphoric Acid.

Chromatographic trials for Simultaneous Estimation of Telmisartan and Hydrochlorothiazide by RP-HPLC.

Trial-1 Chromatographic conditions

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	YMC C_{18} Column(250mm x 4.6mm)5µ
Mobile Phase	Water: Methanol (30:70 v/v)
Detector	PDA
Column temperature	Ambient
Wavelength	268nm
Type of elution	Isocratic
Injection volume	10µl
Run time	10min

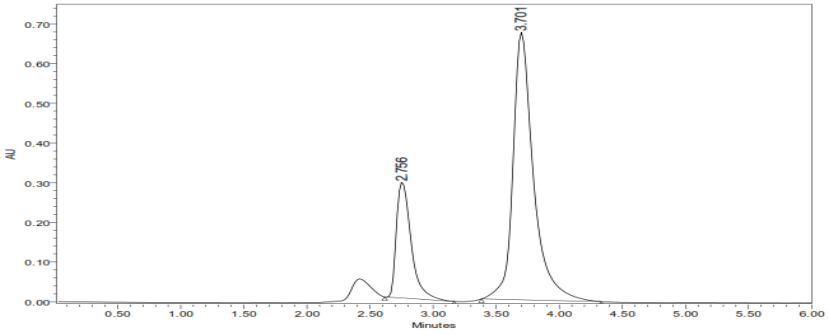


Chromatogram of Trial-1

Observation: The separation of two analytical peaks was not proper, So the mobile phase ratio has been changed for next trial.

Trial-2 Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Agilent C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Buffer: Methanol P^H 3.0 (40:60 v/v)
Buffer	Potassium dihydrogen orthophosphate ph2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	260nm
Injection volume	20µl
Run time	10min

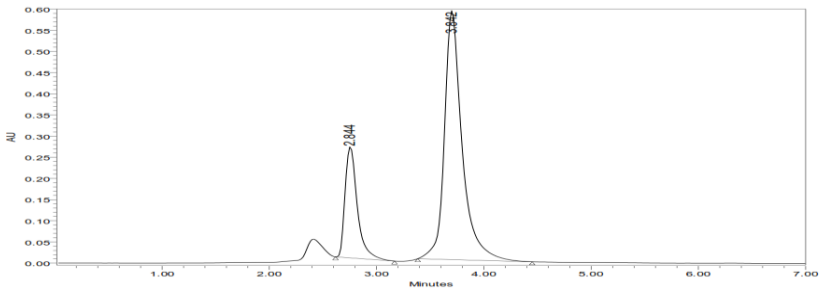


Chromatogram of Trial-2

Observation: The separation of two analytical peaks was not proper, So the mobile phase ratio has been changed for next trial.

Trial-3 Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Agilent C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Buffer P^H 4.0: ACN (60:40 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with OPA
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	260 nm
Injection volume	20µl
Run time	10min



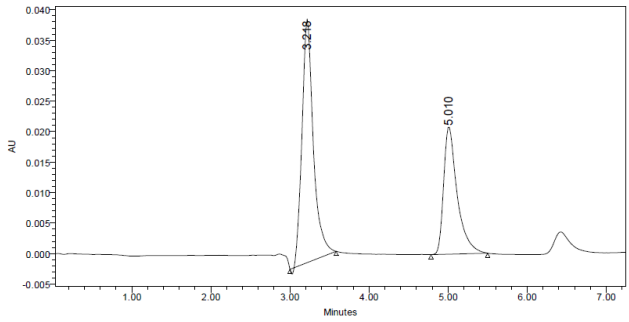
Chromatogram of Trial-3

Observation: The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

Trial-4 Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Inertsil C_{18} Column (150mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer: Methanol P^H 4.0 (40:60 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjust with orthophosphoric acid

Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	268 nm
Injection volume	20µl
Run time	10min

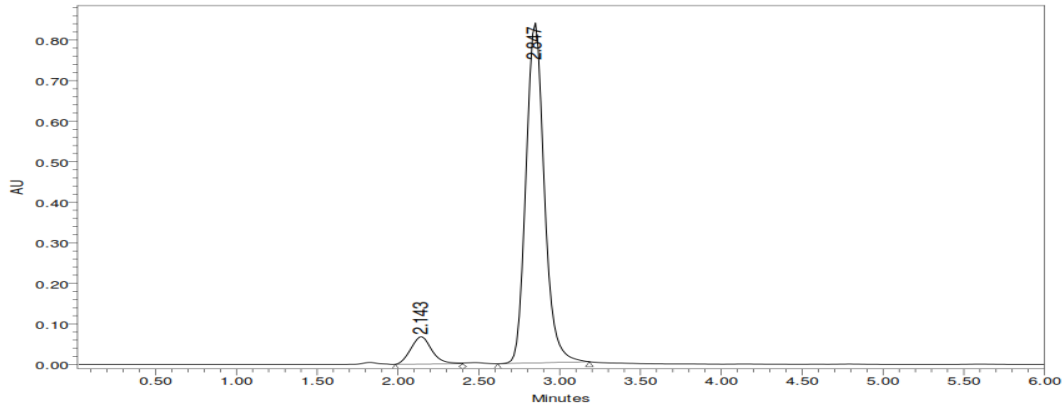


Chromatogram of Trial-4

Observation: The separation of two analytical peaks is occurred but fronting occurs in Telmisartan peak.

Trial-5 Chromatographic condition (OPTIMIZED METHOD)

Parameters	**Description**
Flow rate	1.2 ml min^{-1}
Column	Inertsil C_{18} Column (150mm x 4.6mm)5µm.
Mobile Phase	Phosphate buffer: Methanol P^H 4.5 (30:70 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 4.5 adjust with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	268 nm
Injection volume	10 µl
Run time	10 min



Chromatogram of Trial-5 (Optimized)

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Procedure

Preparation of Buffer: About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 4.5 was adjusted with Orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase: Mobile phase consist of buffer: Methanol of P^H 4.5 (30:70) was taken sonicated and degassed for 10min and filtered through 0.45 µm nylon membrane filter

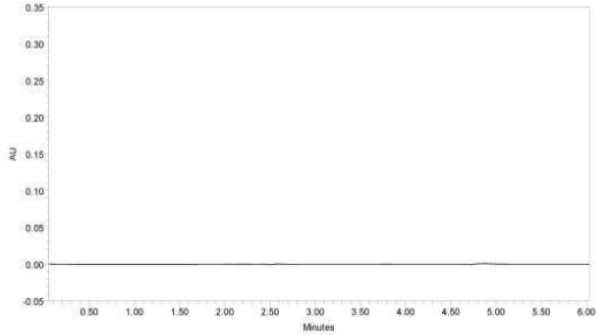
Standard Preparation: Weigh accurately 10 mg of Hydrochlorothiazide and 2 mg of Telmisartan working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution)

Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents

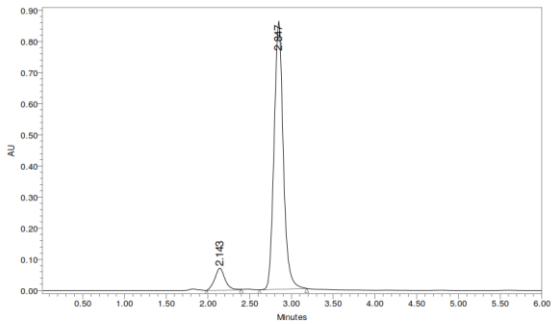
RESULTS AND DISCUSSION

Method Validation Parameters

Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.



Chromatogram of Blank



Chromatogram of Sample

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Serial dilutions of Telmisartan and Hydrochlorothiazide (2-25µg/ml and 5-100 µg/ml) were injected into the column and detected at a wavelength set at 268 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

Range: Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 2-25µg/ml and 5-100 µg/ml for Telmisartan and Hydrochlorothiazide respectively

Accuracy: Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

b) Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Telmisartan and Hydrochlorothiazide at each level should be not less than 95.0% and not more than 105.0%.

Assay procedure

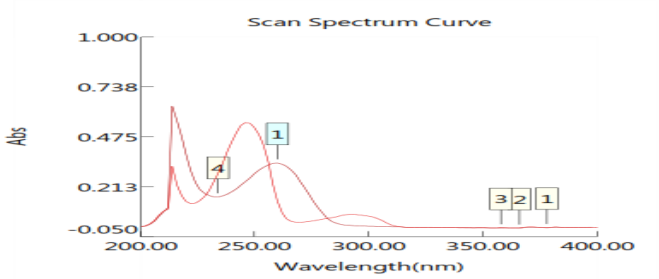
20µL of the standard and sample solutions of Telmisartan and Hydrochlorothiazide were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the Tablets were calculated using the peak areas.

Precision: Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions.

The % RSD of peak areas of six samples was calculated. The method precision was performed on Telmisartan and Hydrochlorothiazide formulation.

Acceptance criteria: The % RSD for the area of six sample injections results should not be more than 2.

Selection of solvent: Solutions of Telmisartan and Hydrochlorothiazide were prepared in different solvents like methanol, ethanol, acetonitrile and UV spectrum of each were recorded by scanning between 200-400 nm.



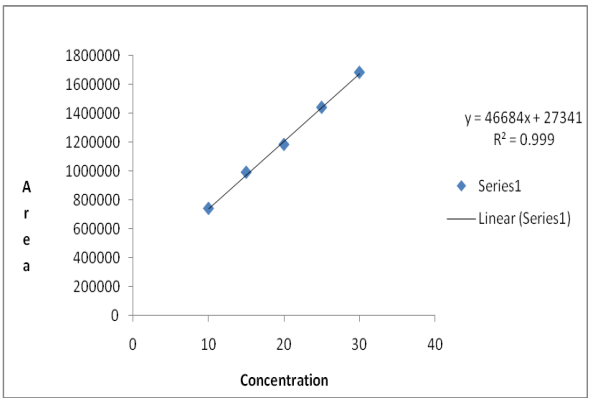
Overlain Spectra of Telmisartan and Hydrochlorothiazide in Methanol

VALIDATION OF THE METHOD

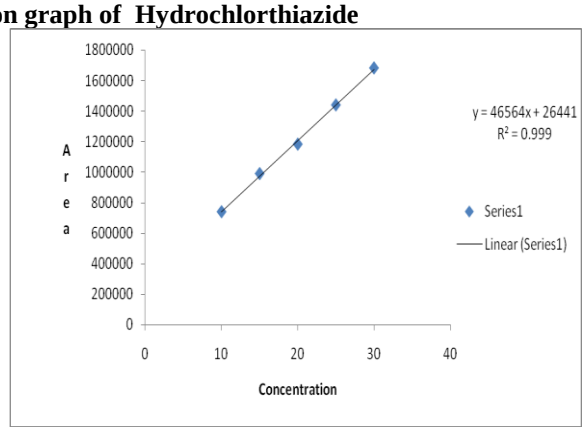
LINEARITY

Telmisartan and Hydrochlorothiazide: Serial dilutions of Telmisartan and Hydrochlorothiazide (2-25µg/ml and 5-100 µg/ml) were injected into the column and detected at a wavelength set at 268 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.999 and 0.999 respectively.

Calibration graph of Telmisartan



Calibration graph of Hydrochlorthiazide



Calibration data of Telmisartan and Hydrochlorothiazide

Telmisrtan		**Hydrochlorthiazide**	
Conc(mcg/ml)	**Area**	**Conc(mcg/ml)**	**Area**
2	1089645	5	700046
10	1202161	40	890368
15	1289999	60	993023
20	1347846	80	1109886
25	1411214	100	1232302
0.999			

Recovery studies

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder a known quantity of standard Telmisartan and Hydrochlorothiazide were added at 50%, 100% and 150% level and the contents were re-analyzed by the proposed method.

Showing accuracy results for Telmisartan

Sample Id	Conc found (µg/ml)	Concn Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	0.5	0.501	100.2		**%RSD= 0.506**
50%	0.5	0.486	98.2	99.86	
50%	0.5	0.489	98.8		
100%	1	1.0	100		**%RSD=0.64**
100%	1	0.989	98.4	99.8	
100%	1	0.99	98.4		
150%	1.5	1.494	97.8		**%RSD=1.42**
150%	1.5	1.492	98.2	99.4	
150%	1.5	1.482	100.1		

Showing accuracy results for Hydrochlorthiazide

Sample Id	Conc Obtained(µg/ml)	%Recovery of drug	Mean accuracy	%RSD
50%	4.94	98.2	**100.1**	**1.4**
50%	4.92	99.4		
50%	5.01	100.5		
100%	9.94	99.6	**99.6**	**0.3**
100%	9.92	99.2		
100%	9.96	99.4		
150%	14.79	98.2	**99.2**	**0.520**
150%	14.96	99.4		
150%	14.86	98.9		

Robustness:

System Suitability Results at different flow rates of Hydrochlothiazide

S.No	Flow rate (ml/min)	System suitability results	
		USP plate count	USP tailing
1.	0.8	6452	1.2
2.	1	5676	1.2
3.	1.2	4679	1.2

System Suitability Results at different flow rates of Telmisartan

S.No	Flow rate (ml/min)	System suitability results	
		USP plate count	USP tailing
1.	0.8	5643	1.2
2.	1	6432	1.2
3.	1.2	6652	1.2

LOD and LOQ:

Telmisartan			Hydrochlorothiazide		
Conc.(x) (µg/ml)	**Peak Areas (y)**	**Statistical Analysis**	**Conc.(x) (µg/ml)**	**Peak Areas (y)**	**Statistical Analysis**
5	1696	S = 38092 c = 608048 LOD: 0.012µg/ml LOQ: 0.022µg/ml	20	1941	S = 38092 c =359381 LOD:0.018µg/ml LOQ: 0.022 µg/ml
5	3126		20	4568	

SUMMARY AND CONCLUSION

A new method was established for simultaneous estimation of Telmisartan and Hydrochlorothiazide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Telmisartan and Hydrochlorothiazide by using Agilent C18 column (4.6×150 mm) 5µ, flow rate was 1.2ml/min, mobile phase ratio was (70:30 v/v) methanol: Buffer, detection wavelength was 268 nm. Precision and recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims.

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