

Human Journals

Research Article

May 2022 Vol.:21, Issue:3

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Stability Indicating HPLC Method for Simultaneous Estimation of Antihypertensive Drugs



IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals



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Submitted: 25 April 2022
Accepted: 30 April 2022
Published: 30 May 2022

Keywords: HPLC, UV Spectrophotometry, Telmisartan, Hydrochlorothiazide

ABSTRACT

A Simple precise and accurate HPLC method has been developed for simultaneous estimation of Telmisartan and Hydrochlorothiazide. The separation was achieved by using the HPLC system LC SHIMADZU UFLC-2000 Prominence LC-20AD Binary Gradient System. SPD20A detector with Rheodyne injector and Enable C18 G column 250x 4.6mm, 5µm. An injection volume of 20µL was injected and eluted with the mobile phase of Acetonitrile: Potassium dihydrogen phosphate (pH 3.0) in the ratio of 60:40 v/v at the flow rate of 1.0ml/min and UV detection at 282 nm. The linearity was found to be in the concentration range of 4–20µg/mL for Telmisartan and Hydrochlorothiazide respectively. The peaks of Telmisartan and Hydrochlorothiazide were found well separated with retention times of 5.7min and 3.1min respectively.



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Stability indicating HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide was developed and validated.

Instrument Specification

Instrument	SHIMADZU UFLC-2000 Prominence LC-20AD SPD 20A Binary Gradient System
Injector	Rheodyne
Column	Enable C-18 G column 250×4.6mm,5μm
Detector	PDA Detector
Injection volume	20μL
Flow Rate	1.0 mL/min
Detection Wavelength	282 nm

Chemicals and Reagents used:

1. Acetonitrile, HPLC grade (Sd Fine-Chem Ltd)
2. Methanol, HPLC grade (Sd Fine-Chem Ltd)
3. Potassium Dihydrogen phosphate (Thermo Fisher Scientific India Pvt. Ltd, Mumbai)
4. Millipore water
5. Telmisartan (Hetero Pharma)
6. Hydrochlorothiazide (Aurobindo Pharma)

Telma – H tablets were made by Glenmark Pharmaceuticals Ltd, Baddi, India, Purchased from the local market.

Methodology:

A method was developed for simultaneous estimation of Telmisartan and Hydrochlorothiazide on HPLC by selecting the appropriate λ max, optimum mobile phase, and flow rate which gives

peaks with good Resolution, HETP, Tailing factor, and other related System suitability parameters.

Selection of Mobile Phase:

Telmisartan and Hydrochlorothiazide are marketed as combined dosage formulations. The proposed method for estimation of Telmisartan and Hydrochlorothiazide required adequate resolution between the two drug peaks in the chromatogram. Several solvent systems were tried to obtain optimum resolution.

Mobile phase combinations used

Mobile Phase used	Ratio
Methanol	100%
Methanol : Water	50:50
Acetonitrile	100%
Acetonitrile : water	50 : 50
Ammonium acetate : Acetonitrile	50 : 50
Phosphate buffer (pH 5.0) : Acetonitrile	50 : 50
Phosphate buffer (pH 4.5): Acetonitrile	55 : 45
Phosphate buffer (pH 3.0) : Acetonitrile	40:60

Peaks of Telmisartan and Hydrochlorothiazide were well resolved with the solvent system of Acetonitrile and Potassium dihydrogen phosphate (pH 3.0) in the ratio of 60:40 (v/v).

Selection of Wavelength:

The wavelength at which Telmisartan and Hydrochlorothiazide showed maximum absorption was selected by obtaining overlay spectra of Telmisartan and Hydrochlorothiazide each of 10µg/mL concentrations in the mobile phase.

A standard stock solution of Telmisartan:

Accurately, 25 mg of Telmisartan was weighed into a clean and dry 25 mL volumetric flask, and dissolved with a sufficient volume of methanol. The volume was then made up to 25 mL with

mobile phase to get the concentration of 1000 $\mu\text{g/mL}$. 5mL of stock solution was diluted in a 50 mL volumetric flask with methanol to get a concentration of 100 $\mu\text{g/mL}$.

Working standard solution: 1mL of the stock solution was further diluted in a 10 mL volumetric flask with methanol to get a concentration of 10 $\mu\text{g/mL}$.

A standard stock solution of Hydrochlorothiazide:

Accurately, 25 mg of Hydrochlorothiazide was weighed into a clean and dry 25 mL volumetric flask, and dissolved with a sufficient volume of methanol. The volume was then made up to 25 mL with mobile phase to get the concentration of 1000 $\mu\text{g/mL}$. 5mL of stock solution was diluted in a 50 mL volumetric flask with methanol to get a concentration of 100 $\mu\text{g/mL}$.

Working standard solution: 1 ml of the stock solution was further diluted in a 10 mL volumetric flask with a mobile phase to get a concentration of 10 $\mu\text{g/mL}$.

Procedure:

Telmisartan 10 $\mu\text{g/mL}$ and Hydrochlorothiazide 10 $\mu\text{g/mL}$ solutions were scanned individually on a Shimadzu 1800 UV–Visible spectrophotometer in the wavelength range of 200 to 400 nm. The overlay spectra of these two drugs were recorded and presented in Fig.2 (Page No. 69).

Determination of Retention Time

The time at which Telmisartan and Hydrochlorothiazide showed peaks with the good resolution was determined by injecting 10 $\mu\text{g/mL}$ solution into a chromatograph at the flow rate of 1.0 mL/min, UV detection at 282 nm.

Preparation of Mobile Phase:

Potassium dihydrogen phosphate buffer (KH_2PO_4) pH 3.0 (adjusted with phosphoric acid) was prepared by dissolving 6.8 gm of Potassium dihydrogen phosphate (KH_2PO_4) in 1000 mL of water. The mobile phase was prepared in the ratio of 40:60 (Potassium dihydrogen phosphate buffer pH 3.0: Acetonitrile) filtered, degassed, and sonicated for 10 min.

(a) Determination of Retention Time for Telmisartan:

A standard stock solution of Telmisartan:

Accurately, 25 mg of Telmisartan was weighed into a clean and dry 25 mL volumetric flask, dissolved with sufficient volume of the mobile phase. The volume was then made up to 25 mL with mobile phase to get a concentration of 1000 µg/mL. 5mL of stock solution was diluted in a 50 mL volumetric flask with a mobile phase to get a concentration of 100µg/mL.

Working standard solution: 1 ml of the stock solution was further diluted in a 10 mL volumetric flask with a mobile phase to get a concentration of 10 µg/mL.

Procedure:

20 µL of working standard solution of Telmisartan (10µg/mL) was injected into the chromatograph with a flow rate of 1 mL/min. The chromatogram was recorded and the retention time was recorded and presented in Fig. 3 (Page No.70).

(b) Determination of Retention Time for Hydrochlorothiazide:

A standard stock solution of Hydrochlorothiazide:

Accurately, 25 mg of Hydrochlorothiazide was weighed into a clean and dry 25mL volumetric flask, and dissolved with a sufficient volume of the mobile phase. The volume was then made up to 25mL with mobile phase to get the concentration of 1000 µg/mL. 5mL of stock solution was diluted in a 50 mL volumetric flask with a mobile phase to get a concentration of 100µg/mL.

Working standard solution: 1 ml of the stock solution was further diluted in a 10 mL volumetric flask with a mobile phase to get a concentration of 10 µg/mL.

Procedure:

20 µL of working standard solution of Hydrochlorothiazide (10µg/mL) was injected into the chromatograph with a flow rate of 1.0 mL/min. The chromatogram was recorded and the retention time was recorded and presented in Fig. 4 (Page No.71).

(c) Determination of Retention Time for Telmisartan and Hydrochlorothiazide in combination:

The retention time for Telmisartan and hydrochlorothiazide was first determined individually and then in combination to confirm the standard retention time for Telmisartan and Hydrochlorothiazide.

Acceptance criteria different validation parameters

Validation Parameter		Acceptance Criteria
Specificity		No degradation peak should be detected
Precision	System	NMT 2% (% RSD)
	Method	
	Intra-day	
	Inter-day	
Robustness		Within 95-105% (% Assay)
System Suitability	Tailing Factor	NMT 2.0 %
	Theoretical Plates	> 6000

Validation of an analytical method for the assay of Telmisartan and Hydrochlorothiazide:

An HPLC method has been developed for simultaneous estimation of Telmisartan and Hydrochlorothiazide using C18G column [ENABLE 250 mm (length) x 4.6mm (I.D), 5µm (Particle Size)], mobile phase Acetonitrile: Potassium dihydrogen phosphate pH 3.0 (60:40) v/v, UV detection wavelength at 282 nm, at a flow rate of 1.0 mL/min.

Validation of an analytical method is a process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application.

Typical Analytical Parameters used in Assay Validation are:

1. Accuracy
2. Precision
 - a) System precision
 - b) Method precision
 - c) Intermediate precision (Ruggedness)
3. Specificity
4. Limit of Detection (LOD)
5. Limit of Quantification (LOQ)
6. Linearity and Range.
7. Robustness

Application of the method for simultaneous determination of telmisartan and hydrochlorothiazide on marketed formulation:

Sample: Telmisartan and Hydrochlorothiazide Tablets (Telma – H)

Brand name: Telma – H (Telmisartan 40mg and Hydrochlorothiazide 12.5mg).

Manufacturer: - Glenmark pharmaceutical ltd.

Preparation of Sample Solution:

Sample stock solution:

Tablets of Telma – H containing Telmisartan and Hydrochlorothiazide and triturate the tablets, weighed powder equivalent to 25mg of Telmisartan and Hydrochlorothiazide was transferred into a clean and dry 25mL volumetric flask and dissolved in a few mL of Methanol by sonication for 3 min. The resulting solution was filtered through a Whatman filter and the volume of the filtrate was made up to 25mL with Methanol.

Working sample solution: 0.2, 0.4, 0.6, 0.8 and 1.0 mL of sample stock solution were transferred to separate 10 mL volumetric flasks and volume made up to 10 mL with Methanol.

Procedure: Absorbance of working sample solutions at 297 nm and 270 nm was recorded as A₁ and A₂. Using the simultaneous equation method the concentration of Telmisartan and Hydrochlorothiazide was calculated in serial concentrations.

RESULTS

Stability indicating HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide was developed and validated.

Instrument Specification:

Chromatographic Conditions for Sample Analysis

Instrument	SHIMADZU UFLC-2000 Prominence LC-20AD SPDM 20A Binary Gradient System
Injector	Rheodyne
Column	Enable C-18 G column 250 mm (length) x 4.6 mm (I.D) , 5 µm (Particle Size)
Detector	PDA Detector
Wavelength	282 nm
Injection volume	20 µL
Flow rate	1.0 mL/min
Mobile phase	Acetonitrile: potassium dihydrogen phosphate (60:40)

Chemicals and Reagents used:

1. Acetonitrile, HPLC grade (Sd Fine-Chem Ltd)
2. Methanol, HPLC grade (Sd Fine-Chem Ltd)
3. Millipore water

4. Telmisartan (Hetero Pharma)
5. Hydrochlorothiazide (Aurobindo Pharma)

The standard sample of Telmisartan was obtained as gift samples from (Hetero Pharma); Hydrochlorothiazide (Aurobindo Pharma) and Tablets Telma- H (Glenmark Pharmaceuticals Ltd) were purchased from the local market.

Development of HPLC Method for Simultaneous estimation of Telmisartan and Hydrochlorothiazide:

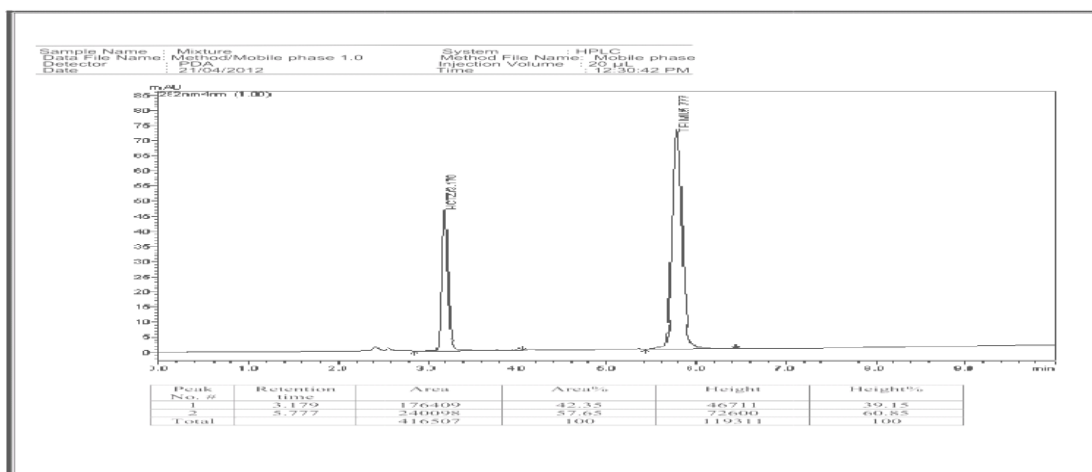
A method was developed for simultaneous estimation of Telmisartan and Hydrochlorothiazide on HPLC by selecting the appropriate λ max, optimum mobile phase, and flow rate which gives a good resolution.

Selection of Mobile Phase:

Several solvent systems were tried to get optimum resolutions of Telmisartan and Hydrochlorothiazide in the present method. The observations obtained with various mobile phases in different ratios are given below.

Different Mobile Phases used and their observations

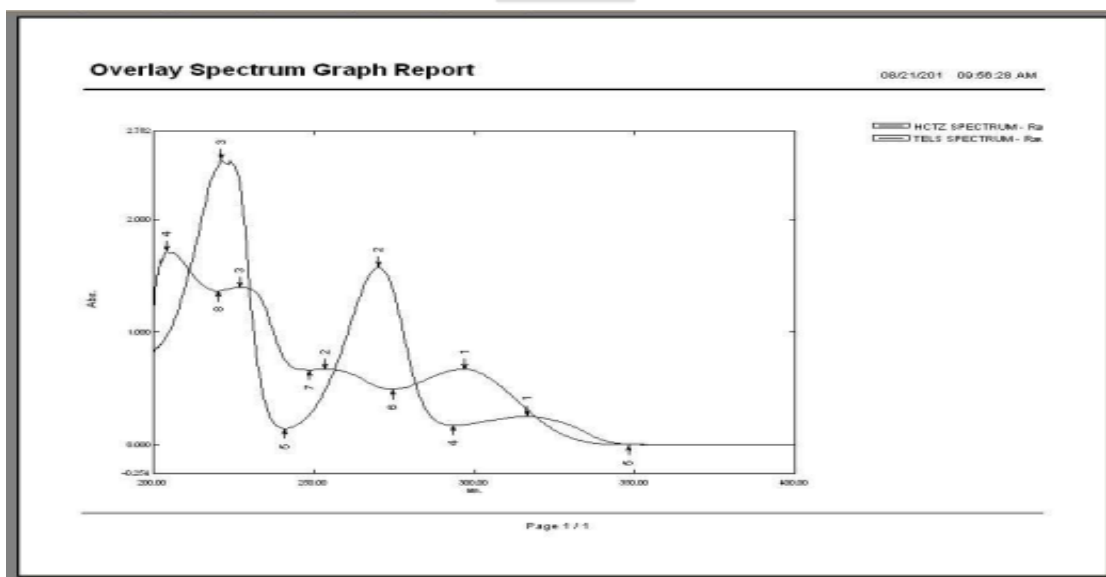
Mobile Phase used	Ratio	Observation
Methanol	100%	Merging
Methanol: Water	50:50	Poor resolution
Acetonitrile	100%	Single peak
Acetonitrile: water	50: 50	Poor resolution
Ammonium acetate : Acetonitrile	50 : 50	Poor resolution
Phosphate buffer (pH 5.0) : Acetonitrile	50 : 50	Peak splitting
Phosphate buffer (pH 4.5): Acetonitrile	55 : 45	Merging
Phosphate buffer (pH 3.0) : Acetonitrile	40:60	Satisfactory resolution



Chromatogram showing Telmisartan and Hydrochlorothiazide peaks with good resolution with the mobile phase Acetonitrile and Phosphate buffer (pH 3.0) (60:40 v/v).

Selection of Wavelength:

Standard solutions of Telmisartan and Hydrochlorothiazide (10 μ g/mL) were scanned in the wavelength range of 200-400 nm using phosphate buffer and Acetonitrile in the ratio of 1:1 as diluents and the UV spectrum obtained is presented below.



Overlay Spectra of Telmisartan and Hydrochlorothiazide

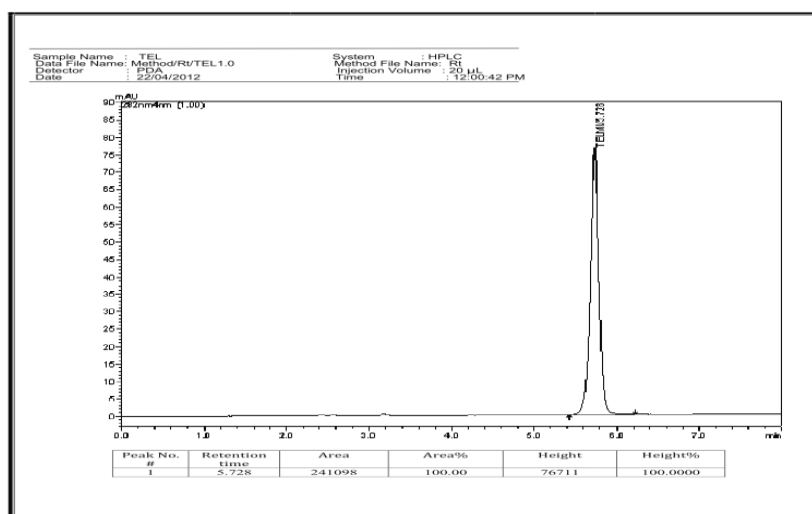
Report: The 10 µg/mL solutions of Telmisartan and Hydrochlorothiazide showed isobestic point at 282 nm which was selected as wavelength maxima for determination of Telmisartan and Hydrochlorothiazide.

Determination of Retention Time:

The optimum retention time at which Telmisartan and Hydrochlorothiazide showed good resolution was selected for the proposed method. The chromatograms obtained individually for Telmisartan and Hydrochlorothiazide and in combination are presented below.

(a) Determination of Retention Time for Telmisartan:

20 µl of standard solution Telmisartan (10 µg/mL) was injected into the chromatograph at a flow rate of 1.0 mL/min and UV detection at 282 nm. The chromatogram obtained is presented below.



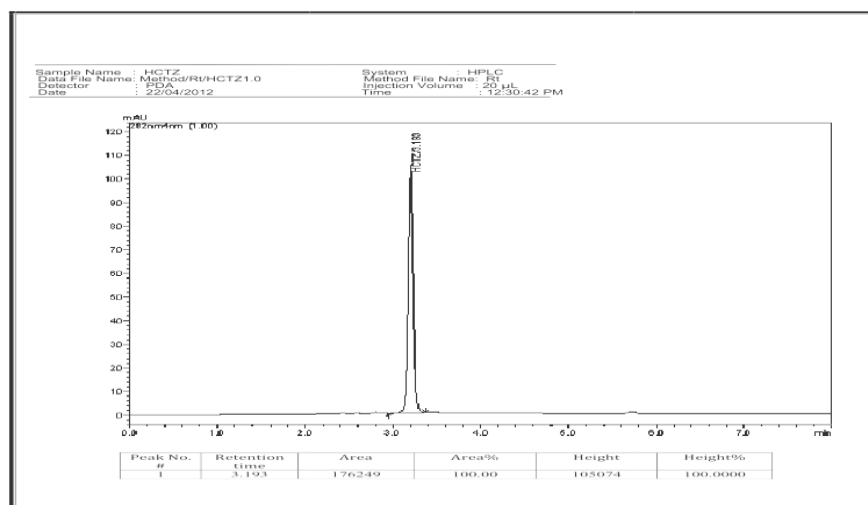
Chromatogram for a retention time of Telmisartan

Report:

The Retention time for Telmisartan was found to be 5.728 min.

(b) Determination of Retention Time for Hydrochlorothiazide

20 µl of standard solution of Hydrochlorothiazide (10 µg/mL) was injected into the chromatograph at a flow rate of 1.0 mL/min and UV detection at 282 nm. The chromatogram obtained is presented below.

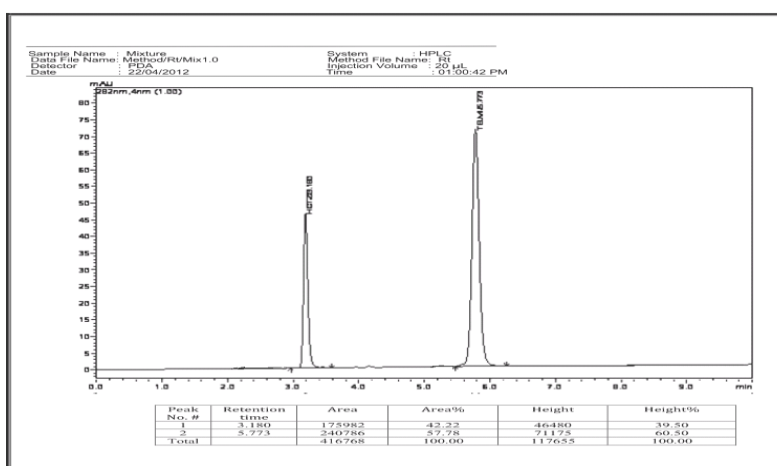


Chromatogram for retention time of Hydrochlorothiazide

Report: The Retention time for Hydrochlorothiazide was found to be 3.193 min.

(c) Determination of Retention Time for Telmisartan and Hydrochlorothiazide in combination:

20 μ L of standard mixture solution of Telmisartan and Hydrochlorothiazide (10 μ g/mL) was injected at a flow rate of 1mL/min and UV detection at 282 nm. The chromatogram obtained is presented below.

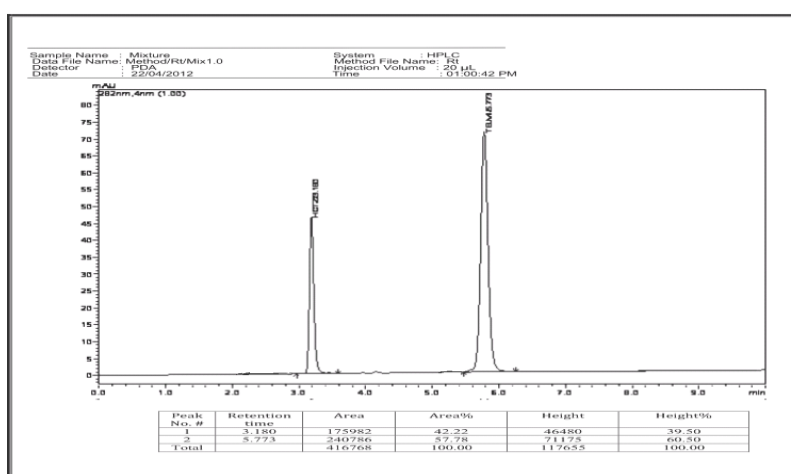


Chromatogram for a retention time of Telmisartan and Hydrochlorothiazide in combination

Report: The retention time for Telmisartan was found to be 5.773 min and the retention time for Hydrochlorothiazide was found to be 3.180 min indicating no change in retention time for Telmisartan and Hydrochlorothiazide in combination.

Determination of Telmisartan and Hydrochlorothiazide in combination.

20 μ L of Telmisartan (10 μ g/mL) and Hydrochlorothiazide(10 μ g/mL) mixture solution was injected at a flow rate of 1.0mL/min using a mobile phase of Acetonitrile and Phosphate buffer in the ratio of 60:40 v/v and UV detection at 282nm. The chromatogram and peak area obtained are presented below.



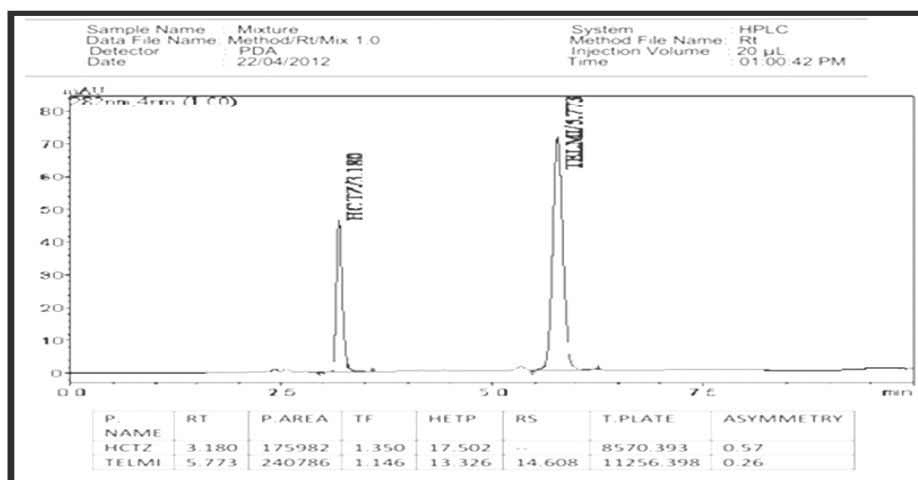
Chromatogram of Telmisartan and Hydrochlorothiazide in combination

Report:

It was found that peaks of Telmisartan and Hydrochlorothiazide were well resolved in the solvent system of acetonitrile and phosphate buffer (pH: 3.0) in the ratio of 60: 40 v/ v, with 14.608 resolution.

System suitability:

This parameter ensures that the analytical system is working properly and can give accurate and precise results. A 20 μ L solution of Telmisartan and Hydrochlorothiazide (10 μ g/mL) each was injected and the system suitability parameters like theoretical plates per column and tailing factor resolution were calculated from the following chromatogram.



Chromatogram for System Suitability Parameters for Telmisartan and Hydrochlorothiazide

System Suitability Data of Telmisartan and Hydrochlorothiazide

System Suitability Factor	Telmisartan	Hydrochlorothiazide	Acceptance Criteria
Tailing factor	1.146	1.350	2
HETP (mm)	13.32	17.50	-
Resolution	14.608		-
Theoretical plates	11256.398	8570.393	>6000
Asymmetry	0.26	0.57	1

Report:

The system suitability parameters were calculated for Telmisartan and Hydrochlorothiazide in the LC software solutions (version 1.25) and the results obtained were found to be within the acceptance criteria.

Validation of an analytical method for the assay of Telmisartan and Hydrochlorothiazide.

The HPLC method developed was validated by performing the various method validation parameters like specificity, LOD, LOQ, linearity, range, precision, robustness, accuracy, and system suitability parameters as per ICH guidelines. This is to ensure that the performance

characteristics of the HPLC method developed meets the requirements for the intended analytical applications.

The following parameters were performed for method validation:

1. Accuracy
2. Precision
 - a) System precision, b) Method precision, c) Intermediate precision (Ruggedness)
3. Specificity
4. Limit of Detection (LOD)
5. Limit of Quantification (LOQ)
6. Linearity and Range
7. Robustness

Accuracy:

This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (80%, 100%, and 120%) and the % recovery of Telmisartan and Hydrochlorothiazide was calculated. 20 μ L of three different solutions of Telmisartan and Hydrochlorothiazide were injected repeatedly into the chromatograph, the peak area and chromatogram were recorded and are presented below.

Recovery study data for Telmisartan

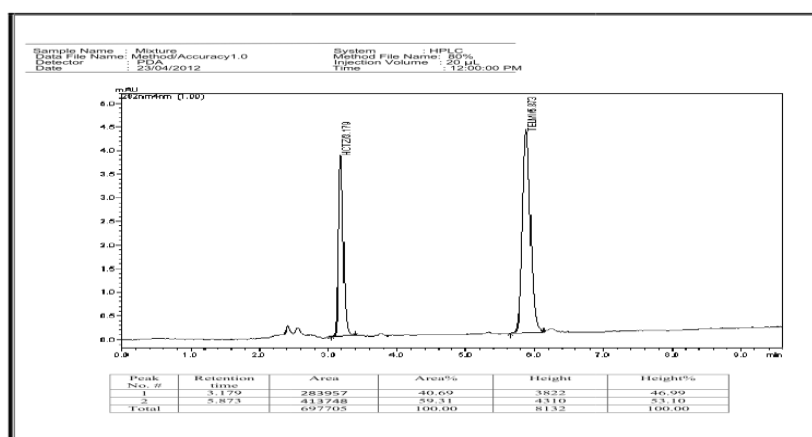
Level	Standard conc (µg/mL)	Sample conc (µg/mL)	Total Conc (µg/mL)	Peak Area*	Mean (µg/mL)	STD	% RSD	% Recovery
I	8	10	18	413748	18.22	0.10	0.54	102.75
II	10	10	20	448743	19.88	0.04	0.20	98.81
III	12	10	22	494645	22.05	0.06	0.28	100.49

*Average of three readings

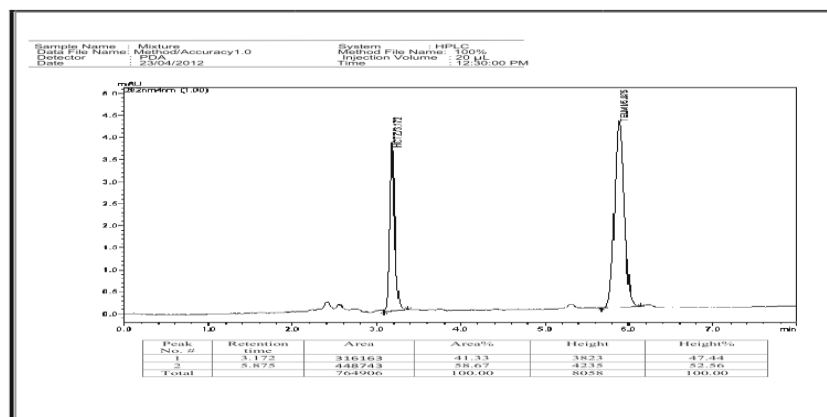
Recovery study data for Hydrochlorothiazide

Level	Standard conc (µg/mL)	Sample conc (µg/mL)	Total Conc (µg/mL)	Peak Area*	Mean (µg/mL)	STD	% RSD	% Recovery
I	8	10	18	283957	17.98	0.09	0.54	99.83
II	10	10	20	316163	20.38	0.03	0.17	103.85
III	12	10	22	341748	22.29	0.27	1.25	102.42

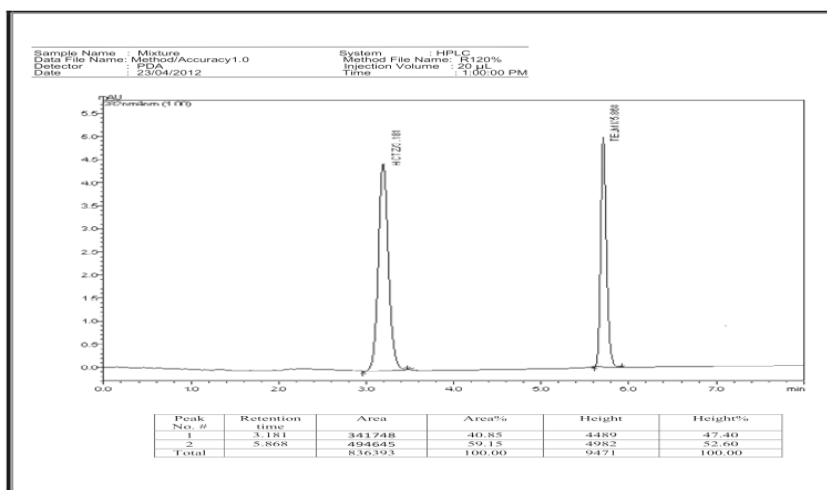
*Average of three readings



Chromatogram for Recovery Studies at 80% level



Chromatogram for Recovery Studies at 100% level



Chromatogram for Recovery studies at 120% level

Report:

The mean percentage recovery for Telmisartan and Hydrochlorothiazide at three different levels was found to be between 98.81 to 102.75 % and 99.83 to 103.85 % respectively, which are well within the limit of 95% to 105%, and hence the method was found to be accurate.

Precision:

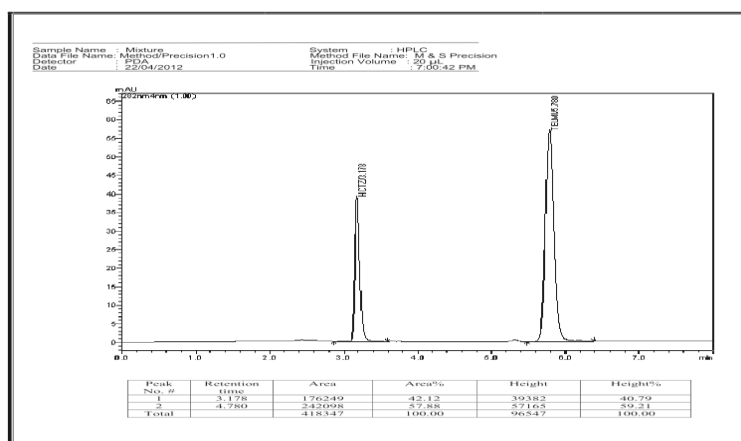
The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) it is performed to see the closeness of agreement between the series of measurements. It is determined by assaying a sufficient number of samples and their relative standard deviation is determined.

a) System precision:

b) This method validation parameter was performed to ensure the closeness of results between the true value and experimental value. Six injections of 20 µl of concentration of 10µg/mL were injected into the system. The peak area was recorded and is presented below.

System Precision Data for Telmisartan and Hydrochlorothiazide

Replicates	Telmisartan Peak area	Hydrochlorothiazide peak area
1	240876	175900
2	239987	175738
3	238976	177035
4	240098	176409
5	241006	177925
6	241098	176249
Average	240340.16	176542.66
STD	818.42	814.59
%RSD	0.340	0.461



Chromatogram of Telmisartan and Hydrochlorothiazide for System Precision

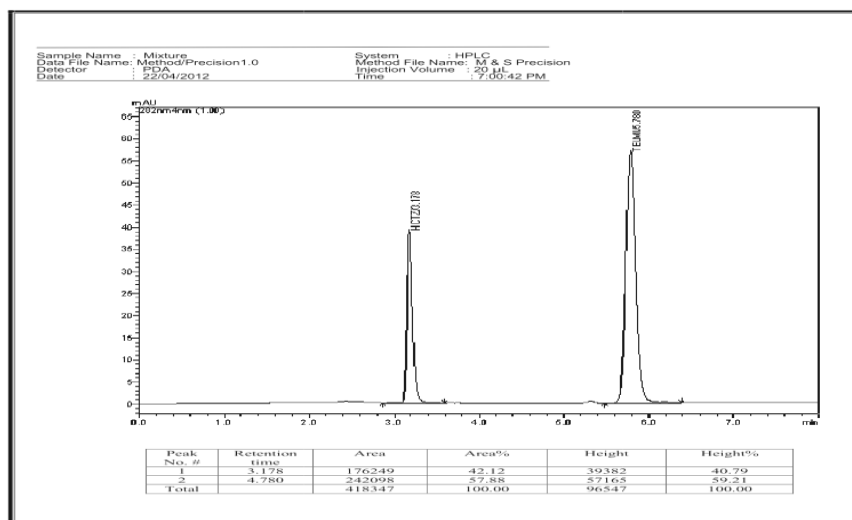
Report: The % RSD values of peak area for six replicate injections of Telmisartan and Hydrochlorothiazide were found to be **0.340** and **0.461** respectively which are well within the acceptance criteria limit of NMT 2%.

(b) Method Precision (Repeatability):

The method precision was performed to standardize methodology i.e. to check whether the developed method is precise i.e whether the method is giving consistent results. Six injections of 20 µL working standard solution of Telmisartan and Hydrochlorothiazide were injected. The peak area and Chromatograms were recorded and presented below. The % RSD for peak area and the assay was calculated.

Method Precision Data for Telmisartan and Hydrochlorothiazide

Replicates	Telmisartan		Hydrochlorothiazide	
	Peak area	Concentration (µg/mL)	Peak area	Concentration (µg/mL)
1	240876	10.017	175900	9.938
2	239987	9.974	175738	9.926
3	238976	9.926	177035	10.023
4	240098	9.980	176409	9.976
5	241006	10.023	177925	10.089
6	241098	10.027	176249	9.964
Average	240340.16	9.99	176542.66	9.98
STD	818.42	0.038	814.59	0.060
%RSD	0.340	0.388	0.461	0.607



Chromatogram of Telmisartan and Hydrochlorothiazide for Method Precision

Report:

The % RSD values of concentration for six replicate injections of Telmisartan and Hydrochlorothiazide were found to be **0.388** and **0.607** respectively which are well within the acceptance criteria limit of NMT 2%.

(c) Intermediate Precision (Ruggedness):

Intermediate precision was performed to ensure whether the precision is maintained variations like different analysts and different days. Six injections of 20µL working standard solution of Telmisartan and Hydrochlorothiazide were injected. The % RSD for peak area and the assay was calculated.

Intra-day Precision Data of Telmisartan and Hydrochlorothiazide

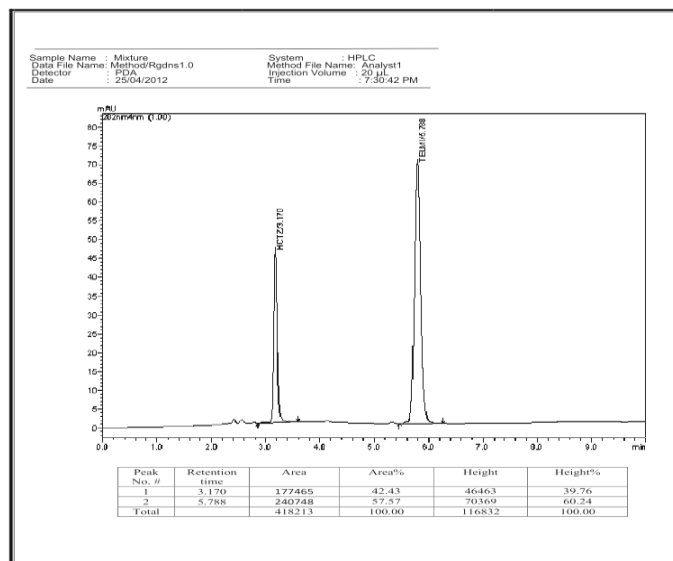
Replicates	Telmisartan		Hydrochlorothiazide	
	Peak area	Concentration (µg/mL)	Peak area	Concentration (µg/mL)
1	240748	10.011	177465	10.055
2	241693	10.055	177256	10.039
3	245259	10.225	176654	9.994
4	238533	9.905	177657	10.069
5	243674	10.149	176675	9.996
6	239754	9.963	177256	10.039
Average	241610.16	10.05	177160.5	10.03
STD	2499.51	0.118	412.24	0.030
% RSD	1.034	1.17	0.232	0.306

Report: From the intermediate precision data, the % RSD for Telmisartan and Hydrochlorothiazide was found to be **1.17** and **0.306** respectively which is well within the acceptance criteria indicating that the method is rugged.

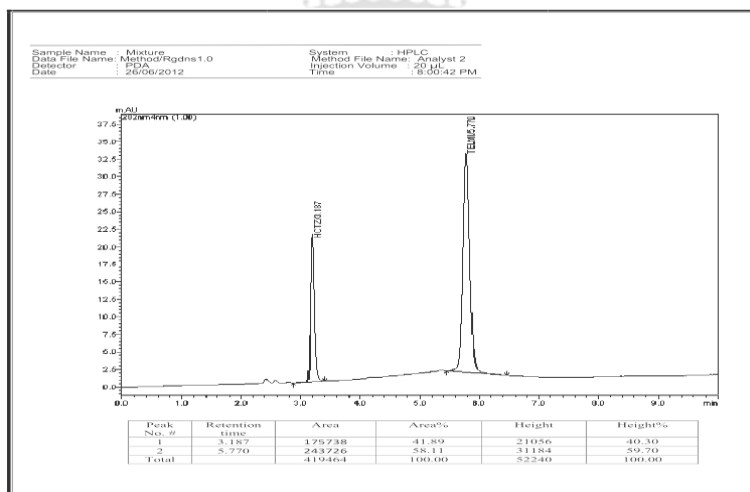
Inter-day Precision for Telmisartan and Hydrochlorothiazide

Replicates	Date interval	Telmisartan		Hydrochlorothiazide	
		Peak Area	Concentration (µg/mL)	Peak Area	Concentration (µg/mL)
1	25/04/2012	243676	10.149	176875	10.011
2	25/04/2012	240658	10.006	176652	9.994
3	26/04/2012	246542	10.285	177294	10.042
4	26/04/2012	243726	10.152	175738	9.926
5	27/04/2012	239987	9.974	177156	10.032
6	27/04/2012	241009	10.023	177842	10.083
Average		242599.66	10.09	176926.16	10.01
Standard Deviation		2493.80	0.118	709.42	0.052
% RSD		1.027	1.171	0.400	0.527

Report: From the intermediate precision data, the % RSD for Telmisartan and Hydrochlorothiazide were found to be 1.171 and 0.527 respectively which is well within the acceptance criteria indicating that the method is rugged.



Chromatogram for Intraday precision

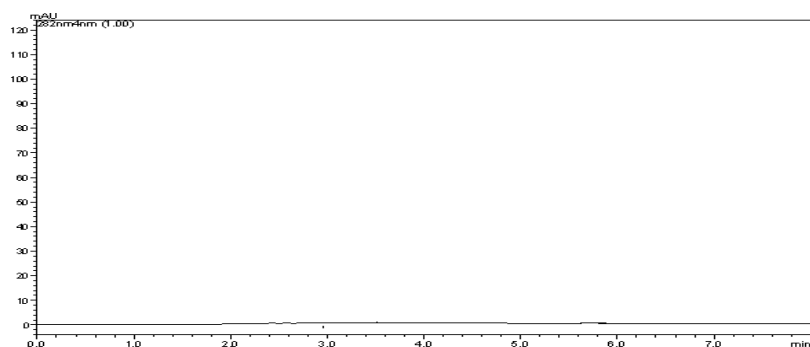


Chromatogram for Inter day precision

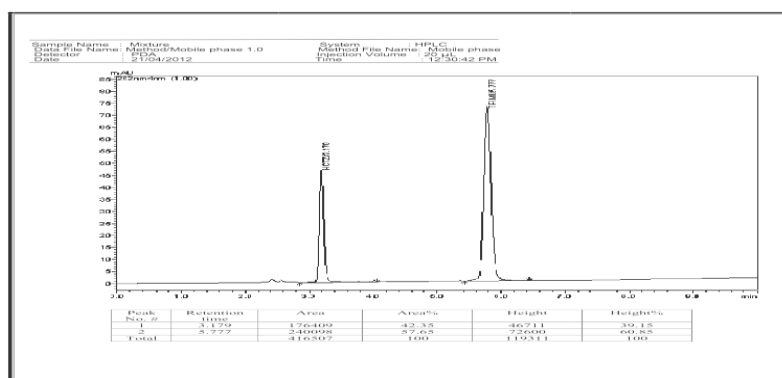
Specificity:

Specificity was performed to assess and ensure that the impurities and degraded products do not interfere with the peaks of analytes.

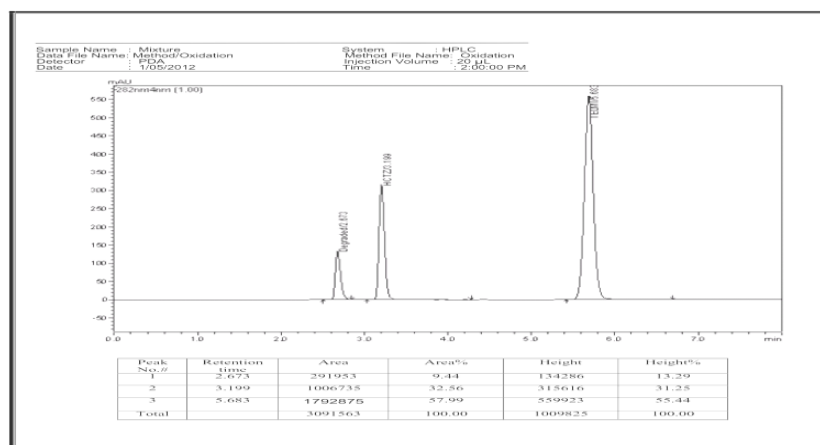
Determination: Volume of 20 μ L of sample solution of Telmisartan and Hydrochlorothiazide were injected in Blank solution, Standard sample, acidic, alkali, oxidation, heat conditions, and Purity peaks were injected into the chromatograph and the chromatograms were recorded and presented below.



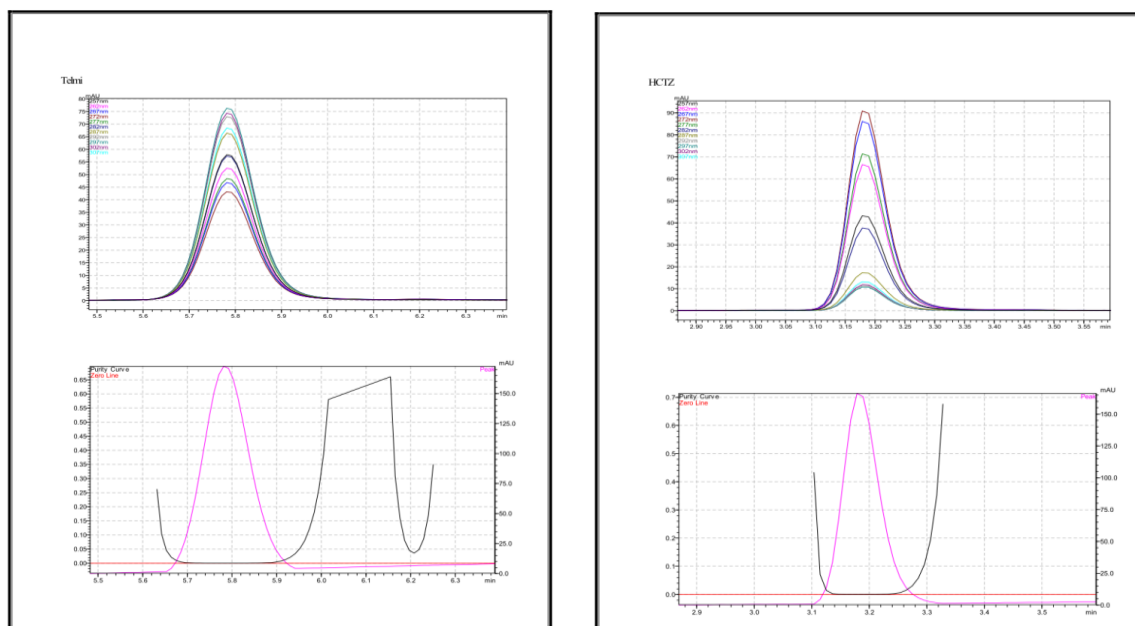
Chromatogram for Specificity (Blank)



Chromatogram for Specificity (Standard sample)



Chromatogram for Specificity (Oxidation)



Chromatograms for Specificity (TEL and HCTZ Peak profile and Peak purity)

TELMISARTAN:

Impurity: Not detected

Peak purity: 0.999998

Single point threshold: 0.999844

Minimum Peak purity index: 153

HYDROCHLOROTHIAZIDE:

Impurity: Not detected

Peak purity: 0.999999

Single point thresholds: 0.999815

Minimum Peak purity index: 245

Observation: The analytes did not have any interference with the degraded components.

Report:

As there is no interference with the degraded components at a retention time of 5.7 (Telmisartan) and 3.1 min (Hydrochlorothiazide), hence the proposed method was specific for the detection of Telmisartan and Hydrochlorothiazide.

Limit of Detection (LOD):

For Telmisartan:

$$\text{Limit of detection} = 6.324/21073 \times 3.3$$

$$= 0.00099 \times 10^3$$

$$= 0.99 \text{ ng/mL}$$

For Hydrochlorothiazide:

$$\text{Limit of detection} = 6.324/13427 \times 3.3$$

$$= 0.00155 \times 10^3$$

$$= 1.55 \text{ ng/mL}$$

The lowest concentration of Telmisartan that can be detected was determined from the standard curve was **0.99 ng/mL**.

The lowest concentration of Hydrochlorothiazide that can be detected was determined from the standard curve was **1.55 ng/mL**.

Limit of Quantification (LOQ):

$$\text{Limit of quantification} = \frac{\sigma}{S} \times 10$$

For Telmisartan:

$$\text{Limit of quantification} = 6.324/21073 \times 10$$

$$= 0.0030 \times 10^3$$

$$= 3 \text{ ng/mL}$$

For Hydrochlorothiazide:

$$\text{Limit of quantification} = 6.324/13427 \times 10$$

$$= 0.0047 \times 10^3$$

$$= 4.7 \text{ ng/mL}$$

The lowest concentration at which peak can be quantified is called LOQ, which was found to be **3ng/mL** for Telmisartan. And for Hydrochlorothiazide was found to be **4.7ng/mL**.

Linearity and Range:

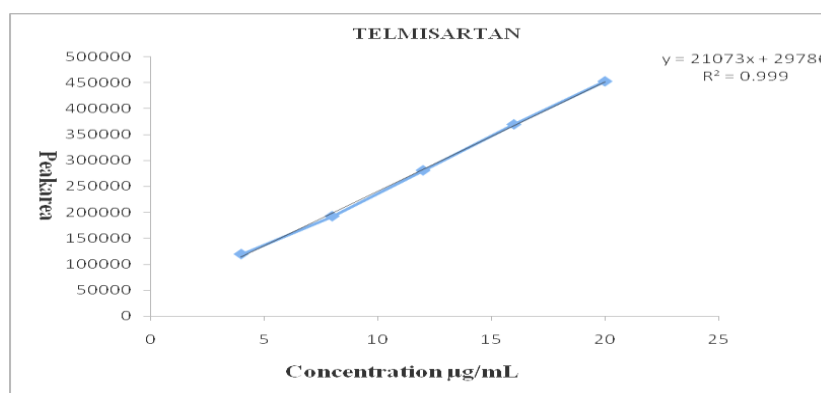
The linearity was performed to ensure that the test results are directly proportional to the concentration of the analyte sample.

20 μ L of each working standard solution of Telmisartan and Hydrochlorothiazide was injected into the chromatograph. The results obtained are tabulated below.

Linearity and Range data for Telmisartan

Volume of stock solution(ml)	Volume adjusted to(ml)	Concentration (μ g/mL)	Telmisartan Peak Area
0.4	10	4	119182
0.8	10	8	192210
1.2	10	12	280551
1.6	10	16	369214
2.0	10	20	452134

*Avg. of Three Readings

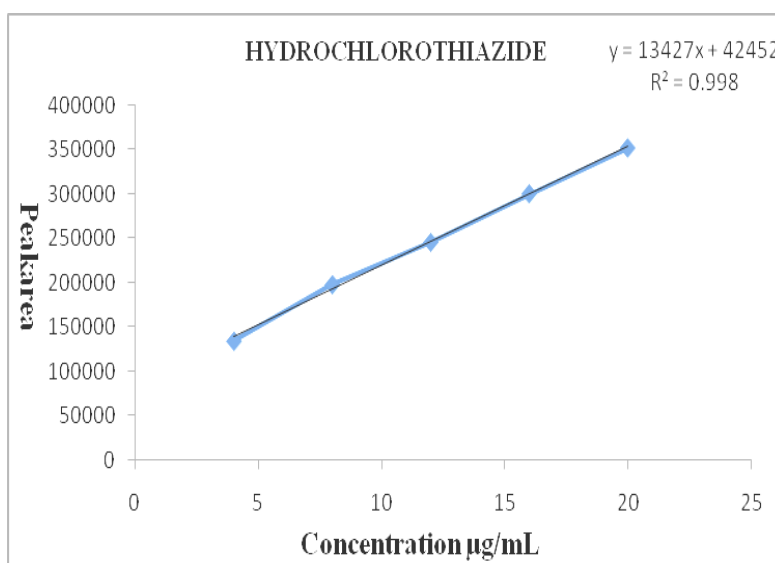


Linearity and Range Graph of Telmisartan

Linearity and Range data for Hydrochlorothiazide

Volume of stock solution (ml)	Volume adjusted to(ml)	Concentration (µg/mL)	Hydrochlorothiazide Peak Area
0.4	10	4	134410
0.8	10	8	198004
1.2	10	12	245612
1.6	10	16	300288
2.0	10	20	351800

***Avg. of Three Readings**



Linearity and Range Graph of Hydrochlorothiazide

Linearity Report for Telmisartan and Hydrochlorothiazide

Parameters	Telmisartan	Hydrochlorothiazide	Acceptance Criteria
Linearity Range	4 - 20 µg/mL	4 - 20 µg/mL	-
Regression equation	$y = 21073x + 29786$	$y = 13427x + 42452$	-
Correlation coefficient	0.999	0.998	0.999
Percentage curve fitting	99.9%	99.8%	99.0%
Intercept	29786	42452	-
Slope	21073	13427	-

Report

The linearity in response for Telmisartan and Hydrochlorothiazide was observed in the concentration range of 4–20µg/mL respectively.

Robustness:

Robustness was performed to check the ability of the system to give unaffected results for small deliberate changes in system parameters and method parameters.

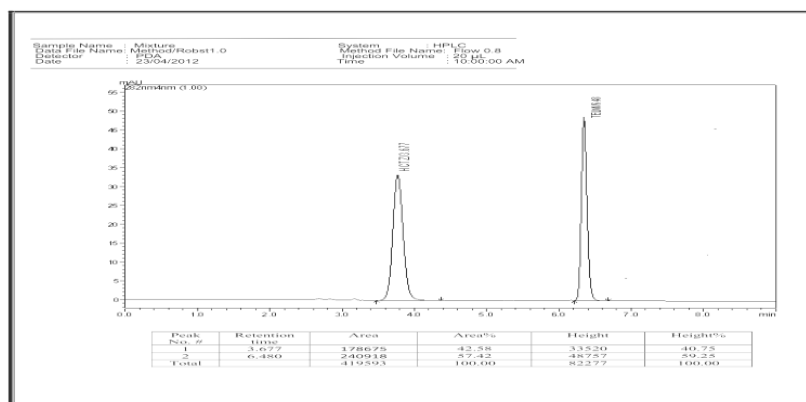
(a) Change in Flow Rate:

20 µL working standard solutions were prepared in the mobile phase and were injected into a chromatograph at flow rates of 0.8 and 1.2mL/min; the % assay was calculated and presented below.

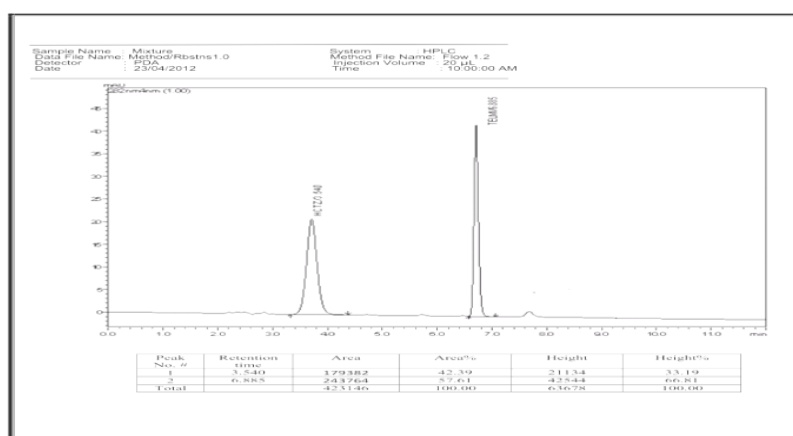
Robustness Data for Change in Flow Rate

Drug	Change inflow Rate (ml)	Peak Area*	Amount recovered	% Assay
Telmisartan	0.8 mL	240918	10.01	100.191
	1.2 mL	243764	10.15	101.541
Hydrochlorothiazide	0.8 mL	178675	10.14	101.455
	1.2 mL	179382	10.19	101.981

*Average of three readings



Chromatogram for Change in Flow rate (0.8mL/min)



Chromatogram for Change in Flow rate (1.2mL/min)

Report:

The % assay was found to be 100.19%, 101.54 % for Telmisartan, and 101.45%, 101.98% for Hydrochlorothiazide when the flow rate was changed to 0.8 mL and 1.2mL respectively, indicating that the method was found to be robust with a slight change in flow rate.

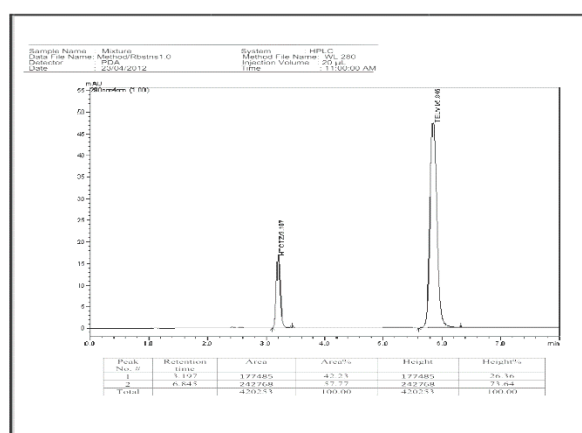
b) Change in Detection wavelength:

For the method developed, a detection wavelength of 282nm was used. For the Robustness study, the detection wavelength was deliberately changed to 280 nm and 284 nm.

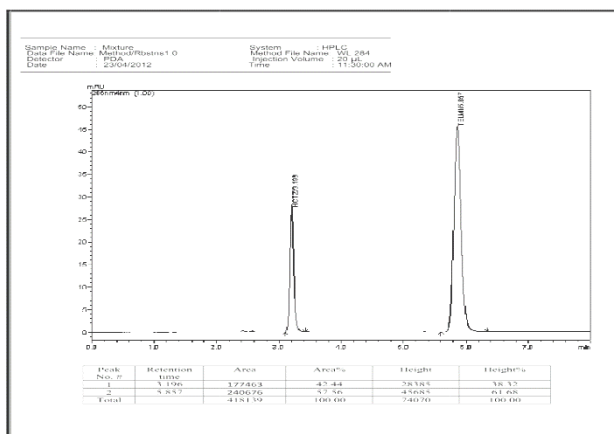
Robustness Data for Change in Detection wavelength

Drug	Change in Detection wavelength	Peak Area*	Amount recovered	% Assay
Telmisartan	280 nm	242768	10.10	101.069
	284 nm	240676	10.00	100.076
Hydrochlorothiazide	280 nm	177485	10.05	100.568
	284 nm	177463	10.05	100.552

*Average of three readings



Chromatogram for Change in Detection wavelength (280 nm)



Chromatogram for Change in Detection wavelength (284 nm)

Report:

The % assay was found to be 101.06%, 100.07% for Telmisartan, and 100.56 % and 100.55% for Hydrochlorothiazide when the detection wavelength was changed to 280 nm and 284 nm respectively, indicating that the method was found to be robust with a slight change in detection wavelength.

Application of developed HPLC method for the simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form:

The HPLC method developed and validated for the estimation of Telmisartan and Hydrochlorothiazide was applied to determine Telmisartan and Hydrochlorothiazide were in marketed formulations (Tablets).

Brand name: Telma - H (Telmisartan 40mg, Hydrochlorothiazide 12.5mg).

Manufacturer: Glenmark Pharmaceuticals.

Chromatographic conditions for sample Analysis

Instrument	SHIMADZU UFLC-2000 Prominence LC-20AD SPDM 20A Binary Gradient System
Injector	Rheodyne
Column	Enable C-18 G column 250×4.6mm,5µm
Wavelength	282 nm
Detector	PDA Detector
Flow rate	1.0 mL/min
Injection volume	20µl
Mobile Phase	Acetonitrile: Phosphate Buffer, pH 3.0 (60:40 v/v)

Procedure:

20 µL of each working stock sample solution of concentrations of 4, 8, and 12 µg/mL were injected into a chromatograph at a flow rate of 1.0 mL/min and UV detection at 282 nm. The peak area and chromatogram obtained were recorded. The concentration of the drug in the sample solution was determined and the % assay for the amount of Telmisartan and Hydrochlorothiazide present in the marketed dosage form was calculated. The results obtained are presented in the table below.

Assay report of Telmisartan and Hydrochlorothiazide

The volume of stock solution	Telmisartan			Hydrochlorothiazide		
	Peak Area*	Concentration	% Assay	Peak Area	Concentration	% Assay
0.4	114876	4.037	100.75	93840	3.827	95.50
0.8	194876	7.834	97.87	149738	7.990	99.87
1.2	280976	11.919	99.16	201335	11.833	98.33

*Average of three readings

Report:

The % assay of Telmisartan and Hydrochlorothiazide in the marketed formulation was found to be in the range of 95.50 - 99.87% respectively. The results show that there is no interference from excipients and no impurities were observed in samples for the proposed method.

Hence the developed HPLC method was found to be accurate, precise, and specific and can be routinely used for simultaneous estimation of Telmisartan and Hydrochlorothiazide in marketed formulations.

REFERENCES:

- 1 Singh GN, Gupta RP. Stability of pharmaceuticals. Dept. of Pharmaceutics, Institute of Technology, B.H.U., Varanasi U.P. Eastern Pharmacist Aug 1987; 85-9.
- 2 Teresa I, Lucas, Bishara RH, Robert HS. A Stability Program for the distribution of drug products. Pharmaceutical Technology Resource Guide 2004; 86-9.
- 3 David JM, International Stability Testing. Inter pharm Press, Inc. Buffalo Grove, Illinois. 2005. P. 235 – 237.
- 4 Satinder A, Stephen S. Degradation and impurity analysis for pharmaceutical drug candidates. Handbook of Modern Pharmaceutical Analysis; 98-9.
- 5 ICH, Stability Testing, Q1A (R2), Stability Testing of New Drug Substances and Products. Feb 2003; 1-20.
- 6 Guidelines: Validation of Analytical Procedures: Methodology, ICH Harmonized Tripartite Guidelines.
- 7 Melveger A J, Huynh-BaK. Critical Regulatory Requirements for a Stability Program. Handbook of Stability Testing in Pharmaceutical Development. 9-19.
- 8 Jessica C, Timothy G, Philip L, Joseph SR, Operational Aspects of Stability. Stability Studies, 463.
- 9 Validation of compendial Assays-Guidelines 'Pharmacopeial Convention, Rockville, MD, 1985.
- 10 Chih H, Ganlin C. Stability indicating high performance liquid chromatographic assay methods for drugs in pharmaceutical dosage forms: part-1. J food drug anal 1996; 4 (4):271-92.