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

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Hydrochlorothiazide and Telmisartan by using Agilent C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: ACN, detection wavelength was 274nm.The Spectroscopic method was done in solvent using methanol and the instrument lab india 3000+ with uv win software. The instrument used for HPLC , WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 1.866 mins and 2.496 mins. The % purity of Hydrochlorothiazide and Telmisartan was found to be 99.87% and 100.27% respectively. The system suitability parameters for Hydrochlorothiazide and Telmisartan such as theoretical plates and tailing factor were found to be 4260, 1.2 and 5085 and 1.2, the resolution was found to be 7.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Hydrochlorothiazide and Telmisartan was found in concentration range of 50µg-250µg and 15µg-55µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 98.56% and 99.96%, %RSD for repeatability was 1.2, % RSD for intermediate precision was 1.9. The precision study was precision, robustness and repeatabilty.LOD value was 3.72 and 0.0242 and LOQ value was 7.40 and 0.0202 respectively.

Keywords: Telmisartan, HPLC, Hydrochlorthiazide

A R T I C L E I N F O

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1. 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.

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 drugsthis 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 vascular resistance.

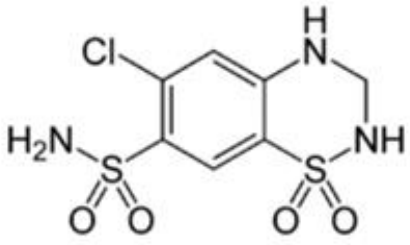

Figure 1: Structure of Telmisartan

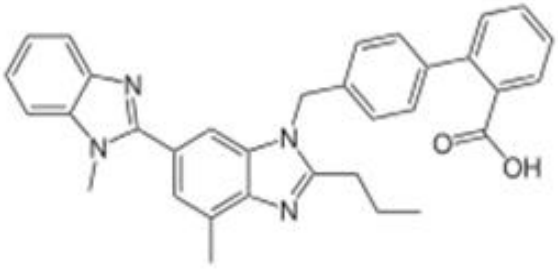

Figure 2: Structure of Hydrochlor Thiazide

Analytical methods

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results.

The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte (s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report.

The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many standard analytical methods have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities,instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own in-house methods or adapt existing ones for specific purposes.

Method development

It forms a significant part of the work of most analytical laboratories, and method validation *and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;
- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;

2. Materials and Methods

Apparatus: The instrument used for the study was the instrument used for HPLC, WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2.

Reagents and Materials

The solvent used was distilled water, 0.1 N sulphuric acid and 0.1 N sodium hydroxide AR grades.

1. Selection of mobile phase:

- Methanol : ACN (70 : 30v/v)
- Below 2: siloxane linkages are cleaved.
- Above 8: dissolution of silica.
- pH selected: 3 ±0.05
- pH controls the elution properties by controlling the ionization characteristics.
- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution.

2. Selection of wavelength:

10 mg of Hydrochlorothiazide and Telmisartan was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Hydrochlorothiazide and Telmisartan. The isobestic point was taken as detection wavelength.

3. Selection of column:

Heart of HPLC made of 316 grade stainless steel packed with stationary phase.

Silica based columns with different cross linking's in the increasing order of polarity are as follows:

←---- Non-polar-------moderately polar------Polar--------→

C_{18}< C_8< C_6< Phenyl < Amino <Cyano< Silica

In reverse phase chromatography, hydrophobic interaction between drug molecule and the alkyl chains on the column packing material. Column is selected based on solubility, polarity and chemical differences among analysts and Column selected: Agilent C18 column (4.6 x150 mm) 5 µ.

4. Selection of solvent delivery system:

a. Always preferable solvent delivery system.
b. More chance of getting reproducible result on retention time of analytes.
c. More economic than gradient technique.

5. Selection of flow rate:

Acceptable limit: - Not more than 2.5 ml/min

- Flow rate selected was 1ml/min
- Flow rate is selected based on

Reasons:

- For earlier elution of analyte and elution of all impurities within 6.0 min.
- Information from the reference method in literature.

6. Selection of diluents:

- Selection of diluent is based on the solubility of the analyte
- Diluent selected: Methanol: ACN (70 : 30v/v)

7. Selection of column temperature:

Preferable temperature is ambient or room temperature.

Reasons:

- ❖ To elute all impurities along with analyte with in 10.0 min of run time.
- ❖ Less retention time
- ❖ Good peak shape
- ❖ Higher theoretical plates.
- ❖ Good resolution.

8. Selection of test concentration and injection volume:

Test concentration is finalized after it is proved that API is completely extractable at the selected test concentration.

- Test concentration is fixed based upon the response of API peak at selected detector wavelength.
- And the test concentration selected is 10 ppm.
- Injection volume selected was 10µL.
- Reason: good peak area, retention time, peak symmetry.

9. Chromatographic trials for simultaneous estimation of Hydrochlorothiazide and Telmisartan by RP- HPLC.

Trial-1

Chromatographic conditions

Column : Zodiac sil RPC18 4.6×250mm 5µm
Mobile phase ratio : MeOH: H_2O (50:50%v/v)
Detection wavelength : 274nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10min
Retention time : 2.743 min

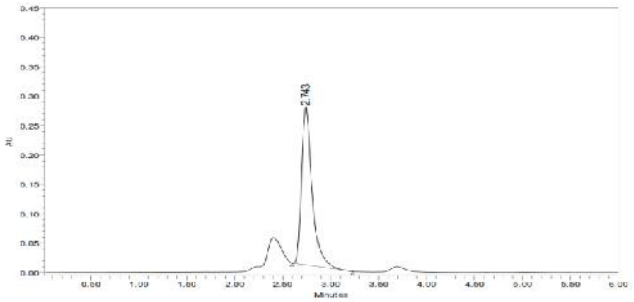

Figure 3: Chromatogram showing trial 1 injection

Observation: The trial showing only one peak in the chromatogram, so more trials were required for obtaining peaks.

Trial - 2

Chromatographic conditions

Column : Zodiacs C18 4.6x150mm 5µm
Mobile phase ratio : ACN: H_2O (50:50%v/v)
Detection wavelength : 240 nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 8.0 min
Retention time : 2.756 min & 3.701 mins

Observation: In this trial both Hydrochlorothiazide and Telmisartan were eluted but there is no proper resolution. Still more trials were required for better resolution in peaks.

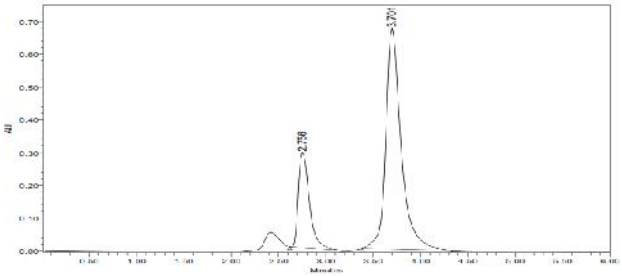

Figure 4: Chromatogram showing trial-2 injection

Trial-3

Chromatographic conditions

Column : symmetry, C18 4.6x150mm, 5µm
Mobile phase: ACN: pH 3 phosphate buffer (65:35% v/v)
Detection wavelength: 240 nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10 min
Retention time : 2.844 mins & 3.842 mins

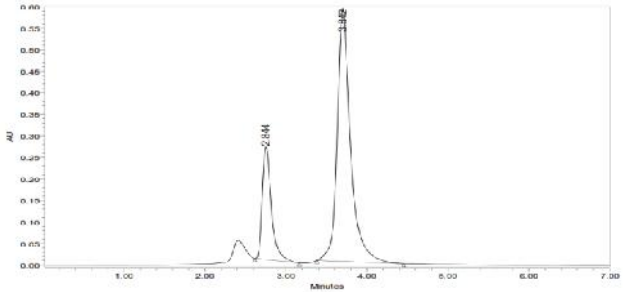

Figure 5: Chromatogram showing trial-3 injections

Observation: The separation was good; peak shape was good, still more trials were required to reduce the retention times of peaks.

Trial -4(optimized method):

Chromatographic conditions

Column : Agilent C18 column (4.6×150mm) 5µ
Mobile phase ratio: Meoh: ACN (70: 30 % V/v)
Detection wavelength : 240 nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10min
Retention time : 1.870 & 2.495 min

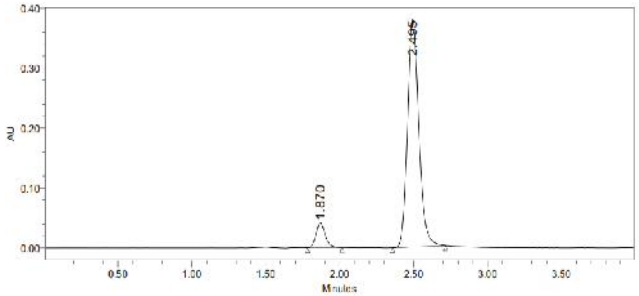

Figure 6: Chromatogram showing trial-4 injection

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.

10. Procedure

Preparation of mobile phase

Mix a mixture of above ACN 30 ml (30%) and 70 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation:

Mobile phase was used as the diluent.

Preparation of the individual Hydrochlorothiazide standard preparation

10 mg of Hydrochlorothiazide working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Telmisartan standard preparation

10 mg of Telmisartan working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the hydrochlorothiazide and Telmisartan standard and sample solution Sample solution preparation:

10 mg of Hydrochlorothiazide and 2 mg Telmisartan tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

3. Results and Discussion

Analytical Method Validation

Validation parameters

1. 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.

2. Linearity

Preparation of stock solution

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.

Preparation of Level – I (50ppm of Hydrochlorothiazide and 15 ppm of Telmisartan): 1.0 ml of stock solution was

taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level–II(100ppm of hydrochlorothiazide and 25ppm of Telmisartan)

1.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level-III(150ppm of hydrochlorothiazide and 35ppm of Telmisartan)

2.0 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level-IV(200ppm of hydrochlorothiazide and 45ppm of Telmisartan)

2.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level–V(250ppm of hydrochlorothiazide and 55ppm of Telmisartan)

3.0 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Procedure

Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

Acceptance criteria

Correlation coefficient should be not less than 0.999.

3. 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 50µg/ml-250µg/ml and 15µg/ml-55µg/ml of hydrochlorothiazide &Telmisartan respectively.

4. Accuracy

Preparation of standard stock solution

10mg of Hydrochlorothiazide and 2 mg of Telmisartan working standard were accurately weighed and 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 out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

5.2 Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of stock solution:

10 mg of Hydrochlorothiazide and 2mg of Telmisartan working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria: The % RSD for the area of five 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.

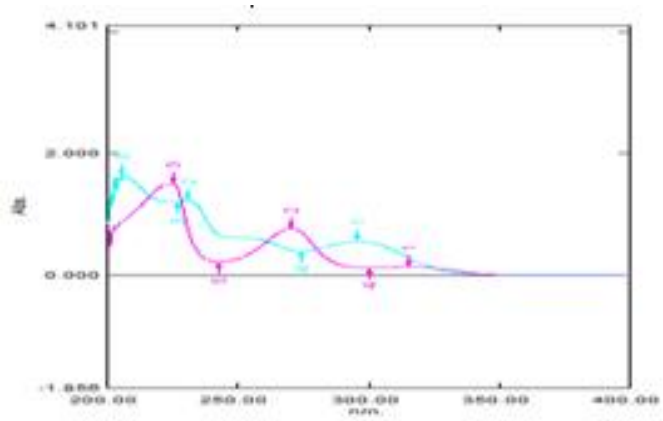

Figure 7: Overlain Normal spectra of Telmisartan and Hydrochlorothiazide in Methanol

Validation of the Method

Linearity

Telmisartan & Hydrochlorothiazide:

The linearity study was performed for concentration range of 50µg/ml -250µg/ml Telmisartan and 15µg/ml - 55µg/ml Hydrochlorothiazide and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999)respectively.

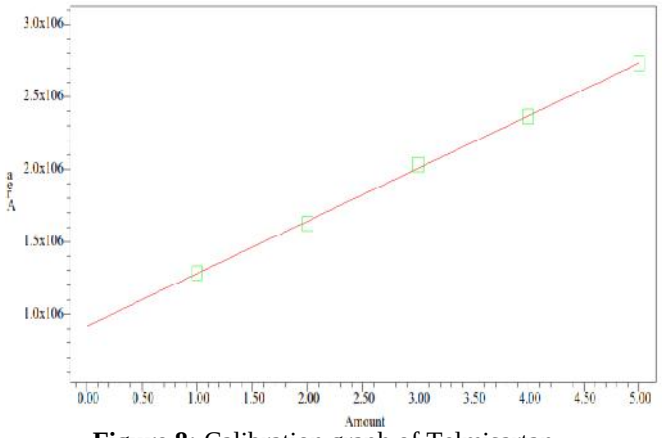

Figure 8: Calibration graph of Telmisartan

Table 1: Calibration data of Telmisartan

S.No	Linearity level	Concentration	Area
1	I	50 ppm	92490
2	II	100 ppm	73080
3	III	150 ppm	82966
4	IV	200 ppm	110813
5	V	250 ppm	102563
Correlation Coefficient			0.999

Table 2: Calibration data of Hydrochlorothiazide

S.No	Linearity Level	Concentration	Area
1	I	15 ppm	2263677
2	II	25 ppm	1624173
3	III	35 ppm	1284282
4	IV	45 ppm	2729292
5	V	55 ppm	2033027
Correlation Coefficient			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 (10mg), a known quantity of standard Telmisartan and Hydrochlorothiazide were added at 80%' 100%and 120% level and the contents were re-analyzed by the proposed method.

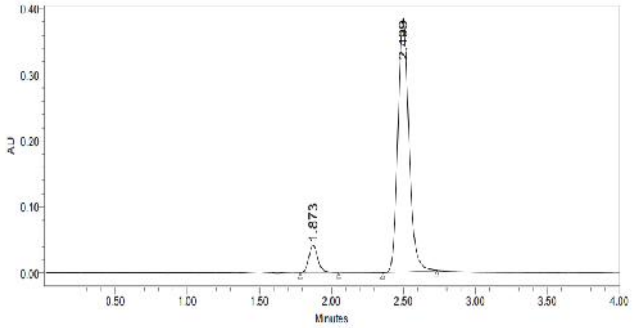

Figure 9: Chromatogram Showing Sample Injection

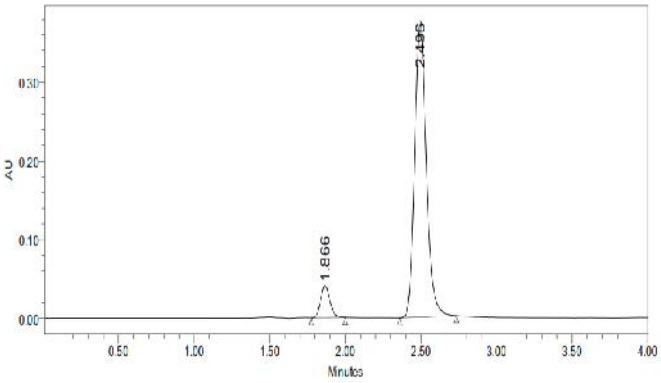

Figure 10: Chromatogram Showing Standard Injection

4. Conclusion

A new method was established for simultaneous estimation of Hydrochlorothiazide and Telmisartan by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Hydrochlorothiazide and Telmisartan by using Agilent C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: ACN, detection wavelength was 274nmThe Linearity was performed in in the range of 50 µg/ml to 250 µg/ml for Telmisartan for hydrochlorothiazide in the range of 15 µg/ml to 55 µg/ml .The co efficient of correlation was found to be less than 0.99 in both wavelengths. Precision and recovery studies also found to be with the range.

Table 3: Showing accuracy results for Hydrochlorothaizide

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	8361453	5	4.90	99.89%	98.56%
100%	15234026	10	9.96	99.28%	
150%	2126270	15	15.0	99.50%	

Table 4: Showing accuracy results for Telmisartan

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	583723	0.5	0.99	99.53%	99.96%
100%	907927	1.0	1.05	99.38%	
150%	152428	1.5	1.495	99.52%	

Table 5: Showing system suitability results for Hydrochlorothaizide

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2481	1.3
2	1	2326	**1.2**
3	1.2	2056	1.2

Table 6: Showing system suitability results for Telmisartan

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5235	1.3
2	1	4961	1.3
3	1.2	4671	1.2

Table 7: Showing system suitability results for Hydrochlorothiazide

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2247	1.4
2	***Actual**	**2294**	**1.2**
3	5 % more	2339	1.1

Table 8: Showing system suitability results for Telmisartan

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5027	1.02
2	***Actual**	**4870**	**1.03**
3	5 % more	4927	1.05

Table 9: Showing Precision details Hydrochlorothiazide

S. No	Drug Name	Rt	Peak area	S. No	Drug Name	Rt	Peak area
1	Hydrochlorothiazide	1.869	180541	1	Telmisartan	2.497	2034160
2	Hydrochlorothiazide	1.872	178697	2	Telmisartan	2.499	2032633
3	Hydrochlorothiazide	1.872	180413	3	Telmisartan	2.500	2044788
4	Hydrochlorothiazide	1.873	181984	4	Telmisartan	2.500	2039999
5	Hydrochlorothiazide	1.874	179493	5	Telmisartan	2.500	2046222
Mean			180225.6	**Mean**			2039560
Std			1234.897	**Std**			6104.307
%Rsd			0.685195	**%Rsd**			0.299295

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