Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Development and Validation RP-HPLC Method for Simultaneous Estimation of Telmisartan and Nifedipine In Synthetic Mixture

Zeel T. Doshi*, Jignesh S. Shah[1], Dilip G. Maheshwari[2]

Received on: 05-04-2016
Accepted on: 28-04-2016
Published on: 15-06-2016

Corresponding Author:

Zeel T. Doshi,
Department of Quality Assurance,
L. J. Institute of Pharmacy,
Ahmedabad, Gujarat,
India

e-mail: zeeldoshi2011@gmail.com

ABSTRACT

A simple, specific and accurate Reverse Phase High Performance Liquid Chromatography Method was developed for the simultaneous determination of Telmisartan and Nifedipine in Synthetic Mixture. The using Phenomenex Luna C_{18} (250 mm x 4.6 mm, 5 µm) column in Isocratic mode, with Mobile Phase containing ACN: Water: Methanol in the ratio of (10:20:70 v/v/v) pH 3.8 adjusted with Orthophosphoric acid at detection wavelength 234 nm with flow rate is 1 ml/min and run time is 15 min. the average retention time was found to b 2.563 min and 4.403 min for TEL and NIFE respectively. The calibration was linear in concentration range of 4-20 µg/mL for TEL and 2-10 µg/mL for NIFE. The low RSD (< 2%) Value indicates that the method is precise. The recoveries for TEL and NIFE were found to be in the range of 99-100%. The proposed method was Validated and successfully applied for the estimation of Telmisartan and Nifedipine in Synthetic Mixture.

Key-words: Telmisartan, Nifedipine, RP-HPLC.

Cite this article as:
Zeel T. Doshi, Jignesh S. Shah, Dilip G. Maheshwari Development and Validation RP-HPLC Method for Simultaneous Estimation of Telmisartan and Nifedipine In Synthetic Mixture, Asian Journal of Pharmaceutical Technology & Innovation, 04 (18); 2016, 01-10 . www.asianpharmtech.com

1 Department of Quality Assurance & Pharma Regulatory Affairs, L. J. Institute of Pharmacy, Ahmedabad.

2 Head of Department of Quality Assurance and Pharm Regulatory Affairs, L. J. Institute of Pharmacy, Ahmedabad.

INTRODUCTION: [1,2]

Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Angiotensin, formed in the blood by the action of angiotensin converting enzyme (ACE), is a powerful chemical that attaches to angiotensin receptors found in many tissues but primarily on muscle cells of blood vessels. Angiotensin's attachment to the receptors causes muscle cells to shorten and narrow the blood vessels (vasoconstrict), which leads to an increase in blood pressure (hypertension). Telmisartan blocks the angiotensin receptor. By blocking the action of angiotensin, telmisartan widens blood vessels (vasodilate) and reduces blood pressure.

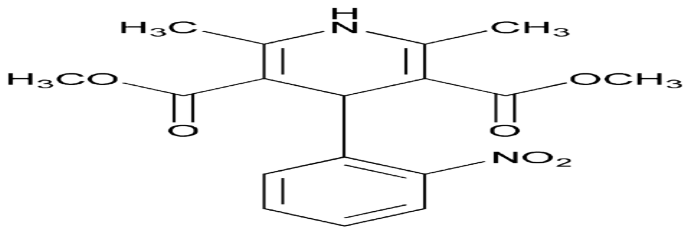

Figure 1: Structure Of Telmisartan

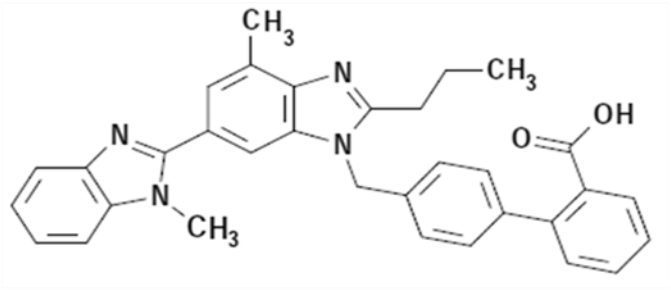

Figure 2: Structure Of Nifedipine

Nifedipine is a dihydropyridine calcium channel blocker that primarily blocks L-type calcium channels. Its main uses are as an antianginal and antihypertensive, although a large number of other indications have recently been found for this agent, such as Raynaud's phenomenon, premature labor, and painful spasms of the esophagus such as in cancer and tetanus patients. It is also commonly used for the small subset of pulmonary hypertension patients whose symptoms respond to calcium channel blockers. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

Therefore when these both the drugs are used in combination with each other, it produces synergistic effect in hypertension resulting into lowering the blood pressure without producing metabolic disease. In other hand, this combination may provide greater blood pressure reduction than the drugs used in monotherapy.

Based on literature survey, it was found that drugs have been analysed by few methods like UV Spectrophotometry Method, RP-HPLC, HPTLC, LC-MS etc in bulk and pharmaceutical dosage form and human plasma individually and combination with other drug but there is no reported method for simultaneous estimation of Telmisartan and Nifedipine in synthetic mixture.

INSTRUMENT:

- HPLC (Shimadzu)
  Model: SPD-20A, LC-20AD
  Column: Phenomenex Luna C_{18} (250 mm x 4.6 mm, 5 µm)
  Detector: U.V Detector
  Software: Spinchrome
- Hamilton Syringe
- Analytical Weighing Balance (Wensar DAB-220)
- Sonicator (Equitron)

- Digital pH Meter (Systronic)
- High Vacuum Pump (Parag engineering)

CHEMICALS AND MATERIALS:

- Acetonitrile – Avantor Performance Material India Ltd. (HPLC grade)
- Methanol – Finar Ahmedabad. (HPLC grade)
- Water – Astron Chemical India. (HPLC grade)
- OPA (75% Ortho Phosphoric Acid) - AR Grade, Astron Chemical India.
- Telmisartan and Nifedipine were supplied by Alembic Pharmaceuticals, Vadodra and Mediwin Pharmaceutical, Ahmedabad respectively.

SELECTION OF DETECTION WAVELENGTH:

- The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 234 nm both the drug give good peak height and shape. So, 234 nm was selected for detection of Telmisartan and Nifedipine in RP-HPLC system.
  (Figure 3)

MOBILE PHASE SELECTION:

Various mobile phases, such as Methanol: Water, Acetonitrile: Water, ACN : Water : Methanol in different proportion was tried. The combination of ACN: Water: Methanol in the ratio of (10:20:70 v/v/v) pH 3.8 adjusted with Orthophosphoric acid provided optimum polarity for proper migration, separation and resolution of Telmisartan and Nifedipine. Under these conditions, the eluted peaks were well defined and resolved. Absorbance is measured at wavelength 234 nm. Flow rate is 1 ml/min and Run time is 15 min.

CHROMATOGRAPHIC CONDITION:

- **Column:** Phenomenex Luna C_{18} (240 mm × 4.6 mm, 5 µm)
- **Mobile Phase:** ACN: Water: Methanol (pH 3.8 adjusted with 10% Ortho Phosphoric Acid) (10:20:70 %v/v/v)
- **Flow Rate:** 1 ml/min
- **Detection Wavelength:** 234 nm
- **Run Time:** 15 min
- **Detector:** U.V Detector
- **Injection Volume:** 20 µL

PREPARATION OF STANDARD STOCK SOLUTION:

- **Telmisartan (100 µg/ml):**

Accurately weighed TEL(10 mg) was transferred to a 100 ml volumetric flask, and diluted to the mark with Methanol to obtain a standard stock solution (100 µg/ml).

- **Nifedipine (100 µg/ml):**

Accurately weighed NIFE (10 mg) was transferred to a 100 ml volumetric flask, and diluted to the mark with Methanol to obtain a standard stock solution (100 µg/ml).

PREPARATION AND ANALYSIS OF SYNTHETIC MIXTURE [3]:

The synthetic mixture of Telmisartan and Nifedipine was prepared in the ratio of 2:1.

Common excipients such as Lactose (95 mg), MCC [Micro Crystalline Cellulose] (65 mg), Magnesium Stearate (5 mg), Talc (5 mg) were added in the motor pestle along with the drug Telmisartan (20 mg) and Nifedipine (10 mg).
Accurately weighed equivalently weight of Telmisartan (20 mg) which contain Telmisartan (20 mg) and Nifedipine (10 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was sonicated till the drug dissolves and was made upto mark with methanol.
This solution was filtered through Whatmann filter paper. The concentration of Telmisartan was 200 µg/ml and Nifedipine was 100 µg/ml.
From above synthetic mixture solutions take 1.2 ml and transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with mobile phase to make final concentration of Telmisartan 12 µg/ml and Nifedipine 6 µg/ml.(Table 6)

METHOD TELIDATION [4]:
The developed method was Valiidated with respect to linearity, accuracy, precision, limit of detection and limit of quantification in accordance with the ICH Q2 (R1) guideline.

- **Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

- **Linearity & Range**

The linearity of Telmisartan and Nifedipine was found to be in the range of 4-20 µg/ml and 2-10 µg/ml respectively. Plot the calibration curve of area vs concentration (µg/ml). Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

Preparation of calibration curve
Aliquots of stock solution of Telmisartan (100 µg/ml) 0.4, 0.8, 1.2, 1.6 and 2.0 ml and Nifedipine (100 µg/ml) 0.2, 0.4, 0.6, 0.8 and 1.0 ml were pipette out in same five different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of about 4, 8, 12, 16, and 20 µg/ml for Telmisartan and 2, 4, 6, 8 and 10 for Nifedipine. 20 µl of each solution were injected into HPLC system by hamilton syringe and analysed. Calibration curve was obtained by plotting respective peak area Vs Concentration in µg/ml and regression equation was obtained.

- **Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, Reproducibility (Interday precision), Repeatability.

1) Intraday Precision: Solutions containing 8, 12, 16 µg/ml of TEL and 4, 6, 8 µg/ml of NIFE were analyzed three times on the same day and %R.S.D was calculated.
2) Interday Precision: Solutions containing 8, 12, 16 µg/ml of TEL and 4, 6, 8 µg/ml of NIFE were analyzed on three different successive days and %R.S.D was calculated.
3) Repeatability: Solutions containing 12 µg/ml of TEL and 6 µg/ml of NIFE were analyzed for six times and %R.S.D. was calculated..

- **Limit of Detection (LOD)**

Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 x (σ/ S)

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

- **Limit of Quantification (LOQ)**

Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 x (σ/ S)

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

- **Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the Value which is accepted either as a conventional true Value or an accepted reference Value and the Value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the Values were measured for Telmisartan (**8 µg/ml**) and Nifedipine (**4 µg/ml**). This performance was done in triplicate.

Preparation of stock solution of mixture (Stock 1)

- The synthetic mixture of Telmisartan and Nifedipine was prepared in the ratio of 2:1.
- Common excipients such as Lactose (95 mg), MCC [Micro Crystalline Cellulose] (65 mg), Magnesium Stearate (5 mg), Talc (5 mg) were added in the motor pestle along with the drug Telmisartan (20 mg) and Nifedipine (10 mg).
- Accurately weighed equivalently weight of Telmisartan (20 mg) which contain Telmisartan (20 mg) and Nifedipine (10 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was sonicated till the drug dissolves and was made upto mark with methanol.
- This solution was filtered through Whatmann filter paper. The concentration of Telmisartan was 200 µg/ml and Nifedipine was 100 µg/ml.

.

Preparation of Standard stock solution Telmisartan (100 µg/ml): (Stock 2)

Accurately weighed TEL (10 mg) was transferred to a 100 ml volumetric flask and was diluted to half and sonicated and made upto the mark with Methanol to obtain a standard stock solution.

Preparation of Standard stock solution Nifedipine (100 µg/ml): (Stock 3)

Accurately weighed NIFE (10 mg) was transferred to a 100 ml volumetric flask and was diluted to half and sonicated and made upto the mark with Methanol to obtain a standard stock solution.

- Each flask was made up to 10 ml with Methanol.
- Each procedure was carried out for 3 times (n=3).

- **Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

It should show the reliability of an analysis with respect to deliberate variation in method parameter.

In case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in mobile phase;
- Influence of variations in mobile phase composition;

- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

➢ **System suitability tests**

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates.

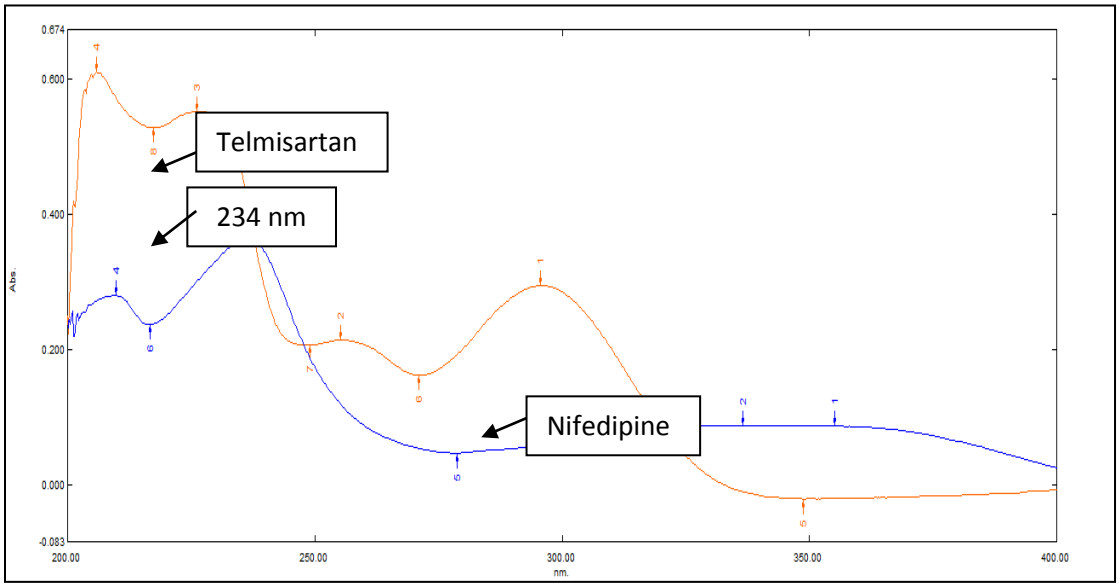

Figure 3: Selection of Wavelength

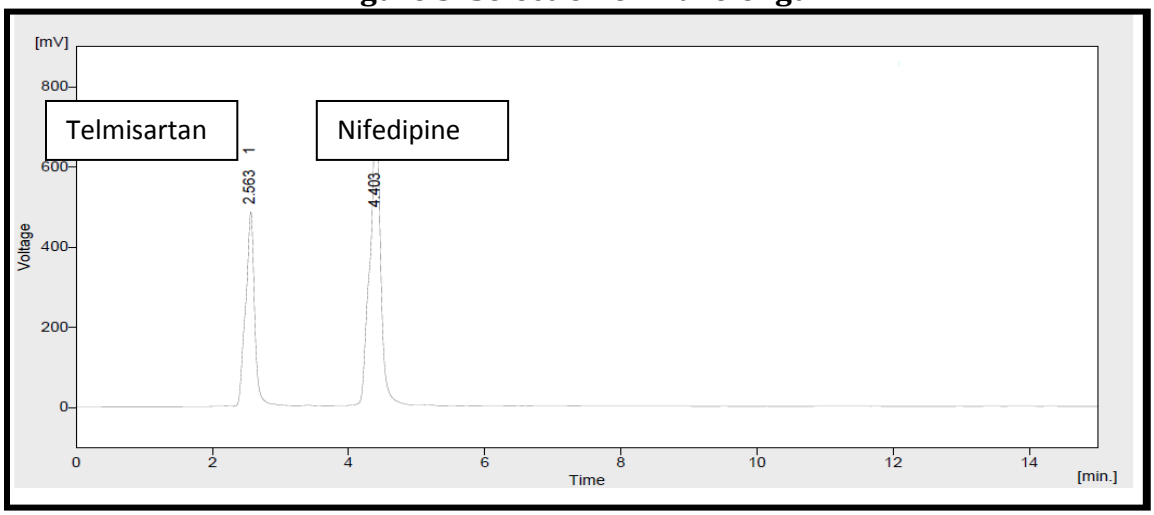

Figure 4: Chromatogram of TEL (20 µg/ml) and NIFE (10 µg/ml) in ACN: Water: Methanol (pH 3.8 adjusts with ortho phosphoric acid) (10:20:70 %v/v/v)

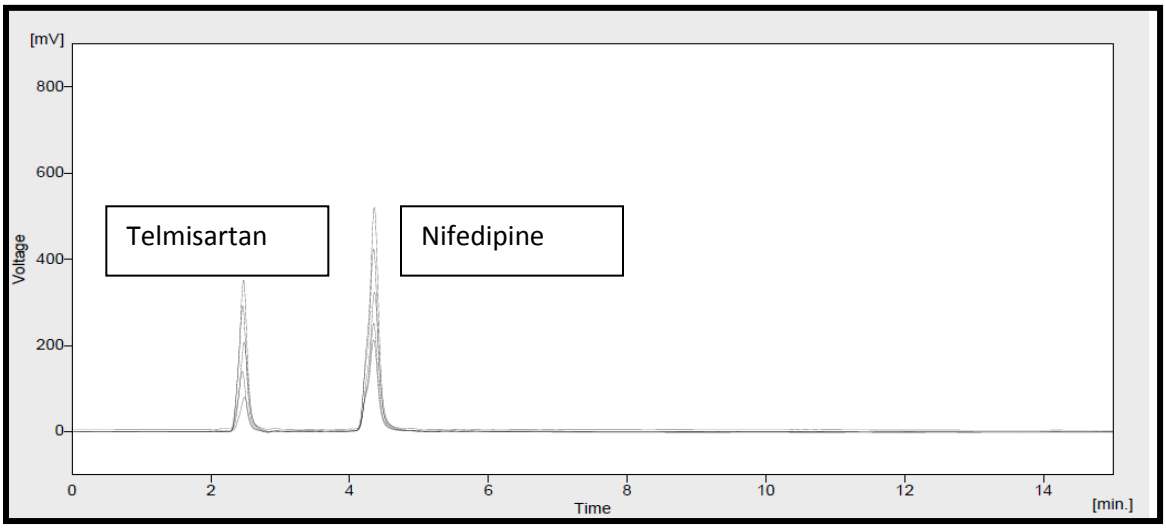

Figure 5: Overlay Chromatogram of Telmisartan and Nifedipine in ACN: Water: Methanol (pH 3.8 adjusts with ortho phosphoric acid) (10:20:70 %v/v/v)

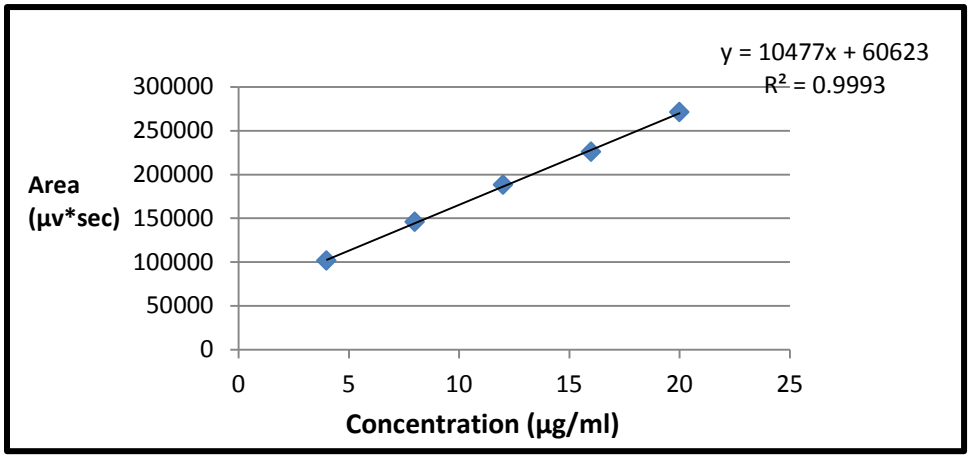

Figure 6: Calibration curve of Telmisartan (4-20 µg/ml) (7-35 µg/ml)

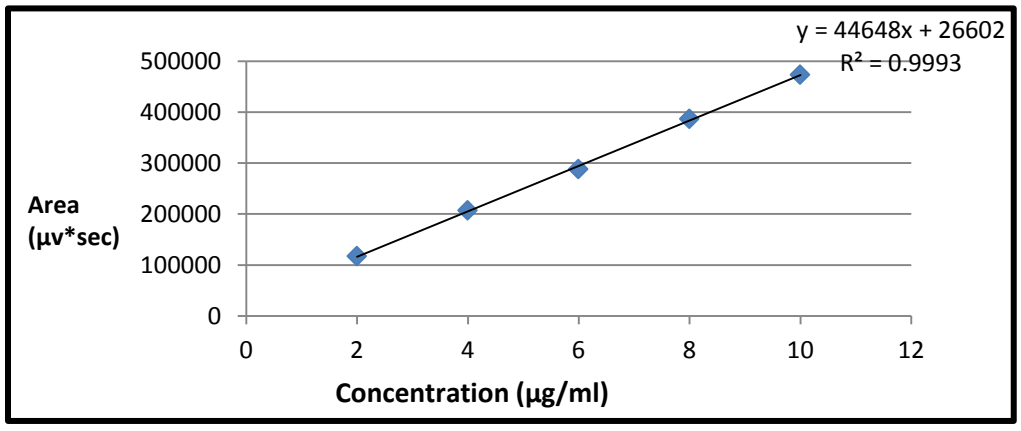

Figure 7: Calibration curve of Nifedipine(2-10 µg/ml) (1-5 µg/ml)

Table 1: Linearity Data for TEL (4-20 µg/ml) and NIFE (1.5-7.5 µg/ml)

TELMISARTAN			NIFEDIPINE		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD	Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD
4	101853.8±572.87	0.5624	2	116196.7±520.25	0.4477
8	146030.5±615.08	0.4212	4	207906.7±628.22	0.3021
12	188767.3±674.05	0.3570	6	289247.7±694.02	0.2399
16	225747.3±778.79	0.3449	8	388071.3±741.93	0.1911
20	271372±838.09	0.3088	10	473829.7±765.28	0.1615

Table 2: Precision Study for TELMISARTAN

TELMISARTAN		
INTRADAY PRECISION		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=3)**	**% R.S.D**
8	146331.7±576.48	0.3939
12	187217±680.12	0.3632
16	224989.7±700.11	0.3111
Interday precision of Telmisartan		
Conc. (µg/ml)	**Mean peak area (µv*sec) ± S.D (n=3)**	**% RSD**
8	155929.3±635.37	0.4074
12	188351±702.04	0.3727
16	235965±723.99	0.3068
Repeatability of Telmisartan		
Conc. (µg/ml)	**Mean peak area (µv*sec)±SD (n=6)**	**% RSD**
12	187784±876.33	0.4666

Table 3: Precision Study for NIFEDIPINE

NIFEDIPINE		
INTRADAY PRECISION		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=3)**	**% R.S.D**
4	207206.7±665.83	0.3213
6	288264.3±709.45	0.2461
8	387661.3±737.11	0.1901
Interday precision of Nifedipine		
Conc. (µg/ml)	**Mean peak area (µv*sec) ±SD (n=3)**	**% RSD**
4	218306.7±680.68	0.3118
6	288697.7±763.76	0.2645
8	399048±831.38	0.2083
Repeatability of Nifedipine		
Conc. (µg/ml)	**Mean peak area (µv*sec) ±SD (n=6)**	**% RSD**
6	288481±700.71	0.2428

Table 4: Recovery Study Data

Name of Drug	% Level of Recovery	Test Amt. (µg/ml)	Amt. of Drug Spiked (µg/ml)	Total Std Amt. (µg/ml)	Total Amount Recovered (µg/ml)	% Recovery ± R.S.D. (n=3)
Telmisartan	50	8	4	12	12.01	99.62±716.42
	100	8	8	16	16.04	100.30±753.52
	150	8	12	20	20.07	100.35±876.05
Nifedipine	50	4	2	6	5.99	99.86±692.82
	100	4	4	8	8.01	100.14±737.11
	150	4	6	10	10.01	00.19±781.02

Table 5: LOD and LOQ Data

Drug Name	TELMISARTAN	NIFEDIPINE
Detection limit	0.2306	0.0264
Quantitation limit	0.6990	0.0801

Table 6: Application of HPLC Method to Synthetic Mixture

Drug Name	Amount in Synthetic Mixture (µg/ml)	Amount Found (µg/ml) ± S.D. (n=3)	% Assay ± R.S.D. (n=3)
TELMISARTAN	8	8.01±576.48	100.08±0.3948
NIFEDIPINE	4	3.99±950.4384953	99.83±0.4569

Table 7: Robustness Data of TELMISARTAN and NIFEDIPINE

Condition	Variation	TELMISARTAN	NIFEDIPINE
		%Assay ± R.S.D (n=3)	%Assay ± R.S.D (n=3)
Flow Rate (1 ml ± 0.1 ml/ min)	0.9 ml/min	99.58±604.51	99.5±702.37
	1.0 ml/min	100.08±699.42	99.16±750.55
	1.1 ml/min	99.89±706.47	99.66±781.02
Detection Wavelength (257 nm ± 1 nm)	233	100.08±680.12	100.16±709.45
	234	99.89±699.42	99.83±750.55
	235	99.91±702.04	100.16±763.76
Change in Mobile Phase Composition (%v/v/v)	8 : 18 : 68	99.75±652.07	99.16±721.11
	10 : 20 : 70	99.89±699.42	99.16±750.55
	12 : 22 : 72	99.5±720.14	99.5±757.18

RESULTS:

For RP- HPLC method various mobile phase compositions was tried to get adequate separation of eluted compound. Separation of TEL and NIFE were performed by use of isocratic mobile phase prepared from ACN: Water: Methanol in the ratio of (10:20:70 v/v/v) pH 3.8 adjusted with Orthophosphoric acid at detection wavelength 234 nm with flow rate is 1 ml/min and run time is 15 min. the average retention time was found to b 2.563 min and 4.403 min for TEL and NIFE respectively. The calibration was linear in concentration range of 4-20 μg/mL for TEL and 2-10 μg/mL for NIFE. The low RSD (< 2%) TELue

indicates that the method is precise. The recoveries for TEL and NIFE were found to be in the range of 99-100%.

Table 8: Summary of Validation Parameters

Sr. No.	Parameters	TELMISARTAN	NIFEDIPINE
1	Linearity Range (µg/ml)	4-20	2-10
2	Regression equation (y = mx +c)	y = 10477x + 60623	y = 44648x + 26602
3	Correlation Coefficient (r^2)	0.9993	0.9993
4	Repeatability (% RSD, n=6)	0.4666	0.2428
5	Intraday Precision (%RSD, n=3)	0.3111-0.3939	0.1901-0.3213
6	Interday Precision(% RSD, n=3)	0.3068-0.4074	0.2083-0.3118
7	Accuracy (% Recovery, n=3)	99.62-100.35	99.86-100.19
8	LOD (µg/ml)	0.2306	0.0264
9	LOQ (µg/ml)	0.6990	0.0801

DISCUSSIONS:

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in Synthetic Mixture with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Telmisartan and Nifedipine in Synthetic Mixture.

CONCLUSION:

A simple, rapid, sensitive, accurate and precise RP-HPLC Method has been developed and Valiidated for routine analysis of TELMISARTAN and NIFEDIPINE in Synthetic Mixture. The RP-HPLC method is suitable for simultaneous estimation of TELMISARTAN and NIFEDIPINE in Synthetic Mixture without interference of each other. The developed method was successfully applied in Synthetic Mixture. The proposed Method can be utilized for the routine analysis of TELMISARTAN and NIFEDIPINE in Synthetic Mixture.

ACKNOWLEDGEMENT:

We are heartly thankful to Dr. K. Pundarikakshudu, Director of L.J Institute of Pharmacy, Ahmedabad for providing all the facilities and the Valuable Guidance during the Research work.

REFERENCES:

1. Rx-list, "The internet drug index Telmisartan", June 2012, http://www.rxlist.com/micardis.drug.html
2. Drug bank, "Nifedipine(DB01115) Drug profile", September 2013, http://www.drugbank.ca/drugs/db01115
3. Mancia, Giuseppea, Parati, Gianfranco, Bilo, Grzegorz, Choi, Jasmine, Kilama, MichaelOchan, Ruilope and Luis Me, "Blood pressure control by the nifedipine GITS–telmisartan." *Jou. Hyper.* **2011**, *29*, 600-609.
4. ICH, Q2 (R1) Validation of Analytical Procedures: Text and Methodology International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2005.