International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

RP-HPLC Method Development and Validation for The Simultaneous Estimation Telmisartan and Efonidipine in Its Bulk and Dosage Form.

Marella Alekhya[1], Ch. Gopinath*[2], Mahesh M[3]
[1,2,3] *Department of Pharmaceutical Analysis, JNTU OTRI, Anantapur, Andhra Pradesh.*

Submitted: 05-09-2023 Accepted: 15-09-2023

ABSTRACT:A Novel, selective, accurate and rapid Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) method for the analysis of Efonidipine Hydrochloride Ethanolate and Telmisartan in binary mixture has been developed and validated. The chromatographic system consisted of Inertsil ODS (4.6*250mm, 5µ) column and the separation was achieved by using ambient temperature with a mobile phase containing mobile Phase 70% buffer 30% CAN, Phosphate Buffer pH 3.0. The samples were monitored at 225 nm for detection at a flow rate of 1.2 mL/min and the retention time was about 8 mins for Efonidipine Hydrochloride Ehanolate and Telmisartan respectively. The calibration curve was linear over the concentration range 50-250 and 100-500 for Efonidipine Hydrochloride Ehanolate and Telmisartan respectively. The proposed method is accurate in the range of 99.89% - 100.23% recovery and precise (%RSD of intraday variation and % RSD of inter day variation were found to be within the acceptance criteria). Therefore, this method can be used as a more convenient and efficient option for the analysis of Efonidipine Hydrochloride Ehanolate and Telmisartan in Quality control laboratory.

KEYWORDS: Efonidipine Hydrochloride Ethanolate, Telmisartan, RSD, accuracy

I. INTRODUCTION

EFONIDIPINE:

Efonidipine is a calcium channel blocker of the dihydropyridine class, commercialized by Shionogi & Co. (Japan). Initially, it was marketed in 1995 under the trade name, Landel. The drug has been shown to block T-type in addition to L-type calcium channels. It has also been studied in atherosclerosis and acute renal failure. This drug is also known as NZ-105, and several studies have been done on its pharmacokinetics in animals.

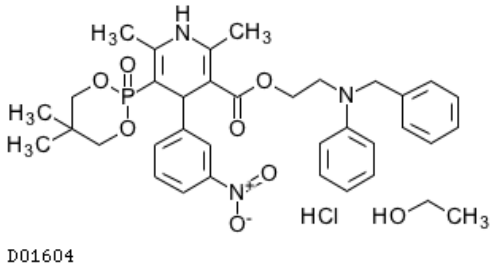

Fig.1

IUPAC Name : 2-[benzyl(phenyl)amino]ethyl 5-(5,5-dimethyl-2-oxo-1,3,2lambda5-dioxaphosphinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate hydrochloride.
Chemical formula : $C_{34}H_{39}ClN_3O_7P$
Molecular weight : 668.12
PKa : 6.0
Solubility : insoluble water, sparingly Soluble in organic Compounds
Melting point : 195°C
GENERIC NAME: Efonidipine
BRAND NAME EFnacor 40
TELMISARTAN

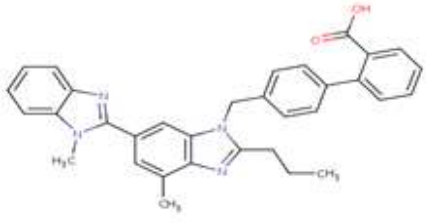

Fig.2

IUPAC Name: 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl] methyl} phenyl) benzoic acid
Chemical formula : $C_{33}H_{30}N_4O_2$
Molecular weight : 514.6169
Melting point : 261-263 °C

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

Description: Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects.

Solubility : In Soluble in water and N,N-dimethyl formamide**,**Soluble in methanol
pKa: 7.7

Mechanism of action : Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance. Telmisartan does not inhibit the angiotensin converting enzyme, other hormone receptors, or ion channels. Studies also suggest that telmisartan is a partial agonist of PPARγ, which is an established target for antidiabetic drugs. This suggests that telmisartan can improve carbohydrate and lipid metabolism, as well as control insulin resistance without causing the side effects that are associated with full PPARγ activators.

GENERIC NAME : Telmisartan
Marketed Formulations:

Fig.3

II. METHODOLOGY

Table.1 INSTRUMENTS USED

S. No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module, uv detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table.2 CHEMICALS USED

S. No	Chemical	Company Name
1	Telmisartan	Glenmark
2	Efonidipine	Glenmark
3	KH_2PO_4	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

HPLC METHOD DEVELOPMENT:
Mobile Phase Optimization:
Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0), Acetonitrile in proportion 70: 30 v/v respectively.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:
Instrument used : Waters HPLC with auto sampler and uv detector.
Temperature: Ambient (25° C)
Mode of separation : Isocratic mode
Column : Inertsil ODS (4.6*250mm, 5µ)
Buffer : Phosphate buffer
pH : 3.0
Mobile phase : 70% buffer 30% ACN
Flow rate : 1.2 ml per min
Wavelength : 225 nm

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

Injection volume : 20 µl
Run time : 8 min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Phosphate buffer:

3.4g of Potassium di hydrogen ortho phosphates taken in 1000 ml of HPLC water pH was adjusted with 0.1M NAOH up to 3.0.final solution was filtered through 0.45 µm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase:

Accurately measured 700 ml (70%) of above buffer and 300 ml of Acetonitrile HPLC (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

PREPARATION OF THE TELMISARTAN& EFONIDIPINE STANDARD & SAMPLE SOLUTION:

Standard Solution Preparation:

Accurately weigh and transfer 40 mg of Telmisartan and 20 mg of Efonidipine working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer the equivalent weight of 40 mg of Telmisartan and 20 mg of Efonidipine Tablet powder into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Telmisartan and Efonidipine peaks and calculate the %Assay by using the formulae.

METHOD VALIDATION SUMMARY:

PRECISION:

Preparation of stock solution:

Accurately weigh and transfer 40 mg of Telmisartan and 20 mg of Efonidipine working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

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

INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution:

Accurately weigh and transfer 40 mg of Telmisartan and 20 mg of Efonidipine working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

ACCURACY:

Preparation of Standard stock solution:

Accurately weigh and transfer 40 mg of Telmisartan and 20 mg of Efonidipine working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 20 mg of Telmisartan and 10 mg of Efonidipine working standard into a 10 ml clean dry volumetric flask add

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 40 mg of Telmisartan and 20 mg of Efonidipine working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

III. RESULTS AND DISCUSSION

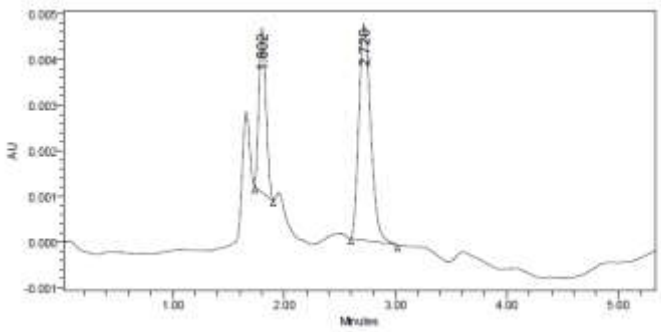

Fig.4. Trial -1

Peak Name	Retention time	Area	Height	Resolution	USP Plate count	USP Tailing
1	1.802	15674	3611		3302.90	1.16
2	2.720	34992	4724	5.67	3002.57	1.28

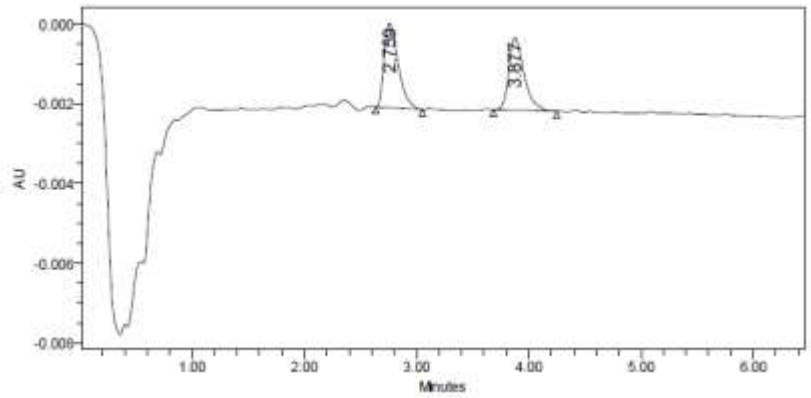

Fig.5. Trial -2

Peak Name	Retention time	Area	Height	Resolution	USP Plate count	USP Tailing
1	2.759	18499	2143		2320	1.59
2	3.877	17100	1825	4.71	4005.65	1.49

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

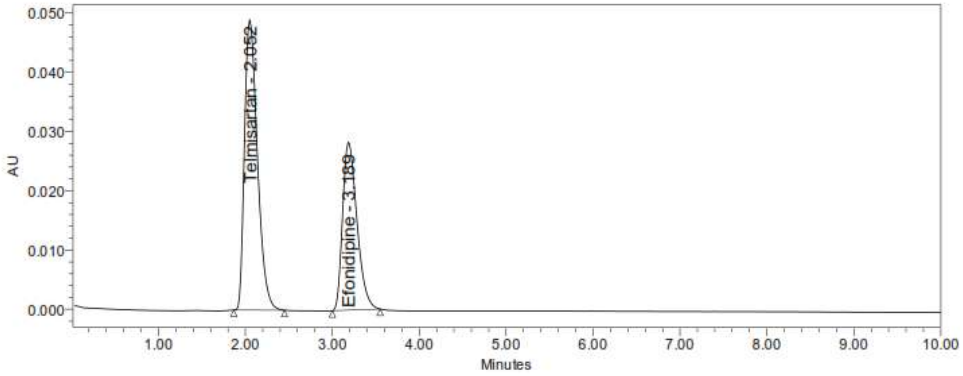

Fig.6. Optimized Chromatogram

Peak Name	Retention time	Area	Height	Resolution	USP Plate count	USP Tailing
1	2.052	191265	23272		3471	1.14
2	3.189	107234	5566	4.44	2143	1.16

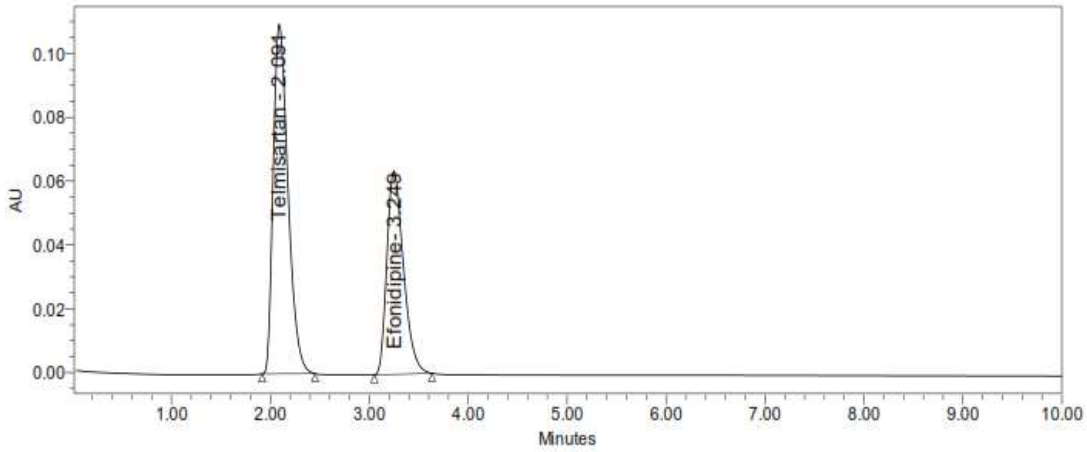

Figure 7: Chromatogram for Sample

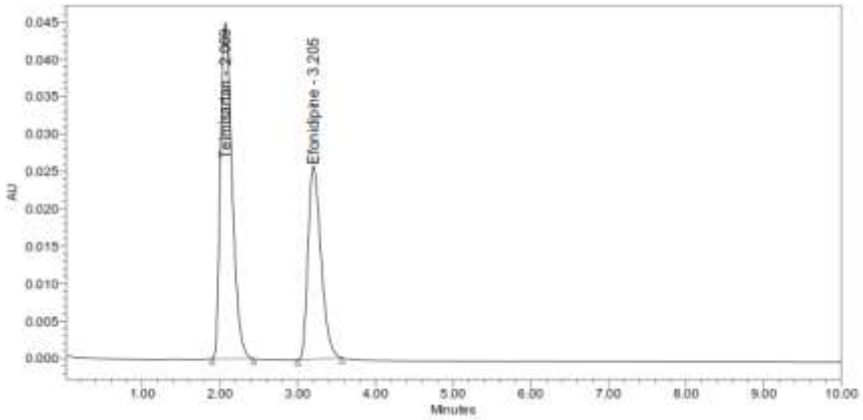

Figure.8. Chromatogram of Telmisartan and Efonidipine (100&50µg/ml)

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

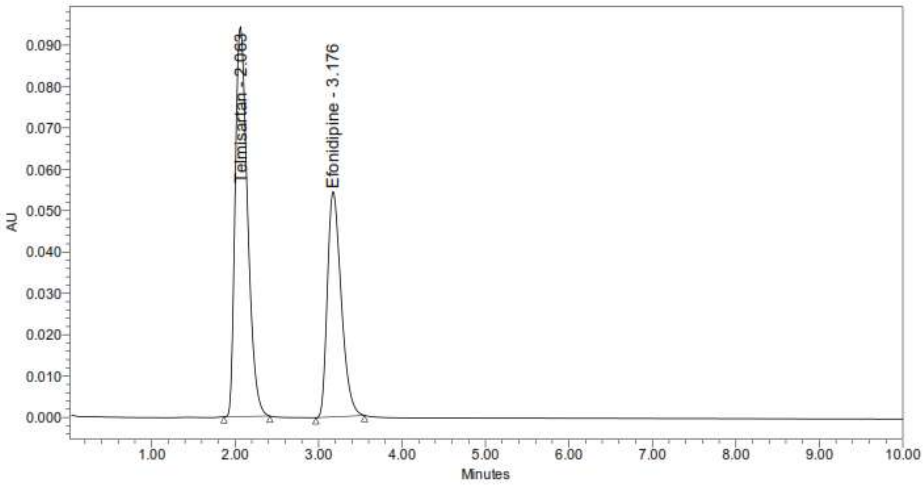

Figure 9. Chromatogram of Telmisartan and Efonidipine (200&100µg/ml)

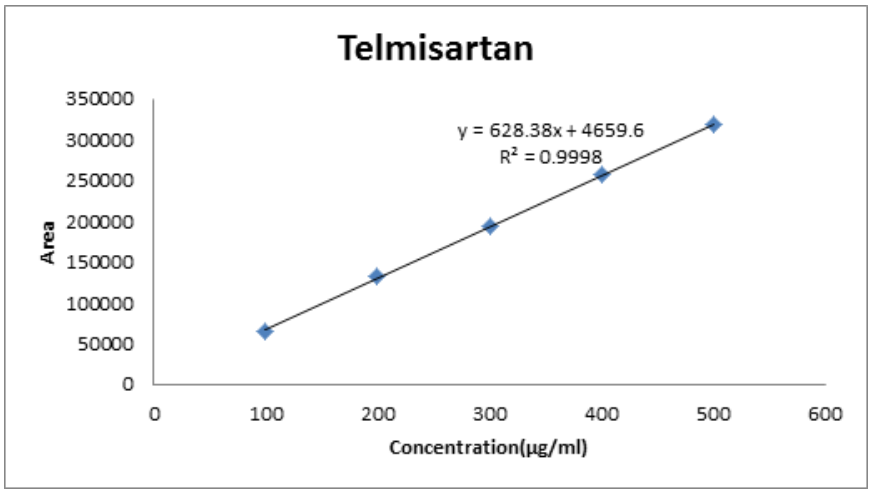

Fig.10. Linearity results of Telmisartan

Table.3. Linearity results of Efonidipine

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	50	32441
2	II	100	67728
3	III	150	100630
4	IV	200	134448
5	V	250	172463
Correlation Coefficient			0.999

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

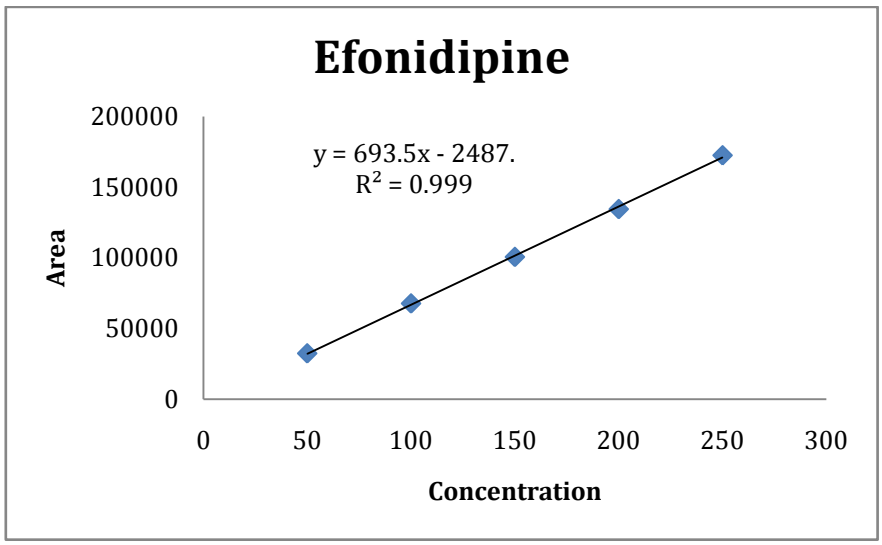

Fig.11 Linearity results of Efonidipine

Table.4

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	50	32441
2	II	100	67728
3	III	150	100630
4	IV	200	134448
5	V	250	172463
Correlation Coefficient			0.999

Table 5 : The results are summarized for Telmisartan and Efonidipine

Injection	Area for Telmisartan	Area for Efonidipine
Injection-1	192345	104533
Injection-2	192432	104232
Injection-3	192971	104531
Injection-4	192899	104399
Injection-5	192898	104018
Injection-6	192333	104689
Average	192646.3	104400.3
Standard Deviation	305.8	241.9
%RSD	0.2	0.2

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

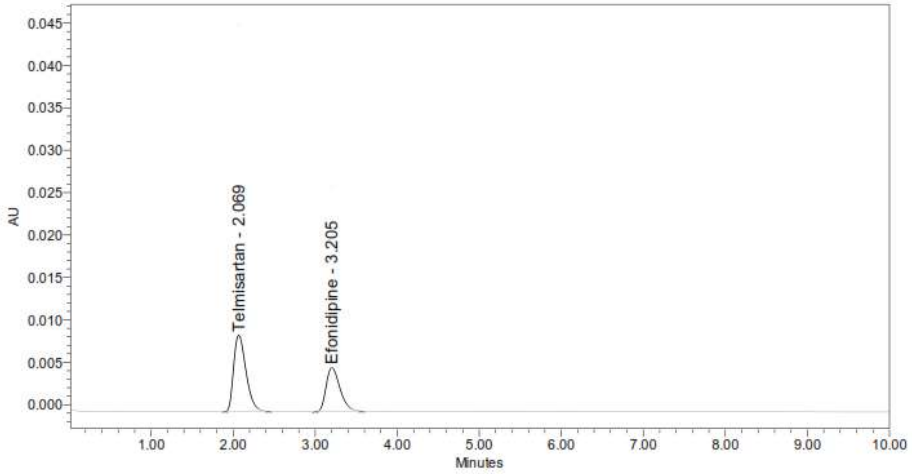

Figure 12: LOD

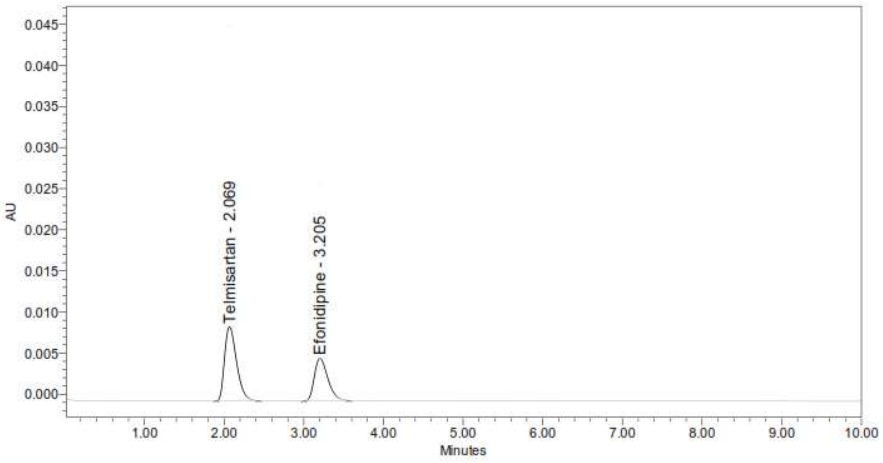

Figure13: LOQ

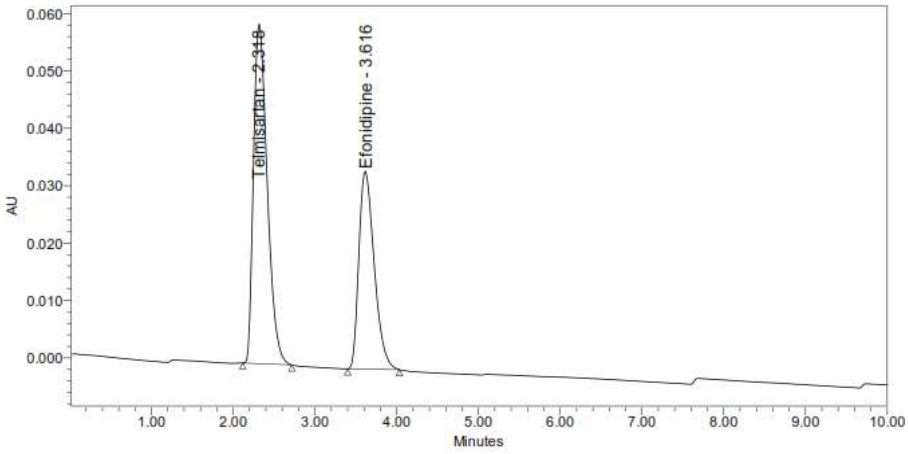

Fig.14 Less Organic

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

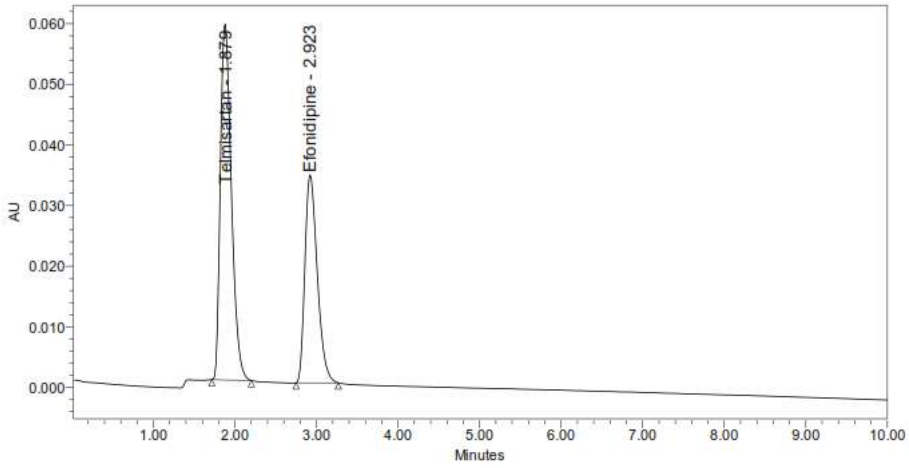

Fig.15. More organic

Table.6 System suitability results for Telmisartan

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3726.18	1.21
2	*Actual	3417.62	1.14
3	10% more	3343.64	1.34

Table.7. System suitability results for Efonidipine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	3175.92	1.31	4.96
2	*Actual	2381.56	1.11	4.42
3	10% more	34445.92	1.23	4.96

***** Results for actual Mobile phase composition (50:50 Buffer: ACN) have been considered.

IV. CONCLUSION

A new method was established for simultaneous estimation of Telmisartan and Telmisartan by RP-HPLC methods. The chromatographic conditions were successfully developed for the separation of Telmisartan and Telmisartan by using Inertsil ODS C18 column (4.6×250mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN : KH2PO4 ph 3, detection wavelength was 225nm. The instrument used forHPLC , WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.098 mins and 3.287 mins. The % purity of Telmisartan and Telmisartan was found to be 99.87% and 100.27% respectively. The system suitability parameters for Telmisartan 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 Telmisartan 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 repeatability. LOD value was 3.72 and 0.0242 and LOQ value was 7.40 and 0.0202 respectively. Hence the suggested RP-HPLC can be used for routine analysis of

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 79-89 www.ijprajournal.com ISSN: 2249-7781

Telmisartan and Telmisartan in API and Pharmaceutical dosage form.

REFERENCES

[1] Douglas A.Skoog, F. James Holler & Stanley R. Crouch. Instrumental analysis, India edition, 2007, pg: 13-14.
[2] Gurdeep R. Chatwal & Sham K. Anand. Instrumental Methods Of Chemical Analysis (Analytical Chemistry) , pg: 2.566-2.567.
[3] Ahuja S & Dong MW. Handbook of Pharmaceutical Analysis by HPLC. 1st edition, Academic Press Publisher.UK 2005.
[4] Satinder Ahuja & Neil Jespersen. Modern Instrumental Analysis 47 (Comprehensive Analytical Chemistry) volume-47, pg-7-8.
[5] Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors, New Delhi, 6th edition, 1986, 1-15.
[6] Douglas A. Skoog, F. James Holler, Timothy A. Nieman. Principles of instrumental analysis, Saunders Golden Sun burst Series, Philadelphia, 2nd edition, 1980, 725-760.
[7] David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists, Harcourt Publishers Limited, 2nd Edition, 1999, 221-232, 267-311.
[8] Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Edition, 1997, 1-56, 234-289,685-712.
[9] Beckett A.H, J.B. Stenlake. Practical Pharmaceutical Chemistry, 4th edition. C.B.S publications. Pg No.53-62.
[10] Remingtonn's The Science and Practise of Pharmacy,20th Edition, 2000.
[11] Connors KA. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc, New Delhi, 3rd Edition, 1994, 373-421.
[12] Rashmin.B.Patel, Mrunali R. Patel, An Introduction to Analytical method development for pharmaceutical formulations, Pharmainfo.net 2008; 17:19
[13] **Sharma B.k** Instrumental methods of chemical analysis. 19 ed: Goel Publishing House, 2003.
[14] Galen Wood Ewing, Instrumental methods of chemical analysis, 340-345.
[15] United States of Pharmacopeia, USP30-NF25, The official compendia of standards, official May 1, 2007.
[16] ICH topic Q2B, validation of analytical procedure & methodology, The European agency for evaluation of medicinal products, human medicines evaluation unit 1996.
[17] ICH: Q2A, Text on validation of analytical procedure (October 1994).
[18] T. Gopala Swamya , K. Nagarajub and A. Lakshmana Rao,RP-HPLC Method for the Simultaneous Estimation of Telmisartan and Hydrochlorothiazide in Pharmaceutical Dosage Form. International Journal of Drug Development & Research | October-December 2011, Vol. 3, Issue 4.
[19] K Konam, S R Kanala. Method Development and Validation for the Simultaneous Estimation of Azelnidipine And Telmisartan in Pharmaceutical Formulation by High Performance Liquid Chromatography. Bull. Env. Pharmacol. Life Sci., Vol10[2] January 2021: 19-27.
[20] Manish Kumar, Umesh Chandra, Arun Garg, Pankaj Gupta. (2021). Impurity profiling of Azelnidipine and Telmisartan in Fixed Dose Combination using Gradient RP-HPLC Method. Annals of the Romanian Society for Cell Biology, 15050–15067
[21] SB Wankhede, MR Tajne, KR Gupta, SG Wadodkar, RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. Indian journal of pharmaceutical sciences,Year : 2007 | Volume : 69 | Issue : 2 | Page : 298-300.
[22] A.B.N. Nageswara Rao et al, Development and Validation of RP-HPLC method for Simultaneous Determination of Hydrochlorthiazide and Eprosartan in Bulk and Pharmaceutical Dosage Form.Scholars Research Library Der Pharmacia Lettre, 2011: 3 (5) 318-325.
[23] Sharanya Gumulapuram Et Al, Stability Indicating Rp-Hplc Method Development And Validation For The Simultaneous Estimation Of Eprosartan Mesylate And Hydrochlorothiazide In Bulk And Tablet Dosage Form.International Journal of Pharmacy and Pharmaceutical Sciences ,Vol 6 suppl 2, 2014.

[24] Harsha U. Patelet al.; Simultaneous analysis of eprosartan and hydrochlorothiazide in tablets by high-performance liquid chromatography.Pharm Methods. 2011 Apr-Jun; 2(2): 143–147.
[25] Narendra Devanaboyina Et Al, Simultaneous Determination Of Olmesartan And Hydrochlorothiazide In Combined Pharmaceutical Dosage Form By Rp-Hplc Method. International Journal of Pharma and Bio Sciences, Vol 3/Issue 2/April – June 2012.
[26] Suryadevara Vidyadhara et al, Method Development and Validation for Simultaneous Estimation of Olmesartan Medoxomil and Hydrochlorothiazide by RP-HPLC.Oriental Journal of Chemistry, 2014, Vol. 30, No. (1): Pg. 195-201.
[27] Reddyvalam Lankapalli Ch Sasidhar et al, Method Development and Validation for Simultaneous Estimation of Olmesartan Medoxomil and Hydrochlorothiazide by Rp-Hplc.
[28] oriental journal of chemistry , volume 30.
[29] Janhavi R Rao at el, Simultaneous Quantitation of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide in Pharmaceutical dosage form by using HPLC.International Journal of PharmTech Research ,Vol.3, No.3, July-Sept 2011.