IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Development And Validation Of Analytical Method For Estimation Of Efonidipine, Telmisartan And Chlorthalidone In Synthetic Mixture

Vishal Baman[1], Heena Ninama[1], Prof. Ritika Gajre[2], Prof Mitali Dalwadi[2]

Dr. Umesh Upadhyay[3]

Student[1], Professor[2], Principal[3]

Sigma Institute of Pharmacy, Bakrol, Vadodara, Gujarat, 390019, India.

ABSTRACT: A simple, sensitive and accurate Development and validation of analytical method for estimation of Efonidipine, Telmisartan and Chlorthalidone in synthetic. A reversed-phase high performance liquid chromatography method is developed and validated for the determination of three drugs. With the help of RP- HPLC it gives us to good resolution and better separation of three drugs. The separation was conducted by using Cybersil C18 column (250mm x 4.6mm x 5µm) with mobile phase consisting Potassium dihydrogen phosphate: Methanol: Acetonitrile (30:30:40 v/v/v) (pH :3). The mobile phase was delivered at flow rate of 1.0 ml/min. The eluent was monitored at wavelength 254 nm and found a sharp and symmetrical peak of Efonidipine, Telmisartan and Chlorthalidone were found to be 6.88 min, 5.34 and 8.25 min respectively. The method was validated for linearity, accuracy, precision, system suitability. The method was found to be linear over the concentration range for the drugs efonidipine (5-30 µg/ml), telmisartan (10-60 µg/ml) and chlorthalidone (10-60 µg/ml) with coefficient R2 for EFO (0.9962) TEL (0.9947), and CTD (0.9992). Therefore, proposed method can be successfully used for routine analysis of Efonidipine, Telmisartan and Chlorthalidone in bulk as well as synthetic mixture.

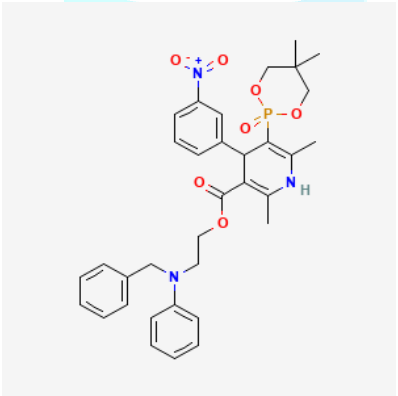
[**Keywords**: Efonidipine (EFO),Telmisartan (TEL), and Chlorthalidone(CTD),reversed–phase high-performance liquid chromatography (RP-HPLC).]

INTRODUCTION: Efonidipine is basically described as 2-(N-benzylanilino)ethyl 5-(5,5-dimethyl-2-oxo-1,3,2λ5-dioxaphosphinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate. It is anti hypertensive. Efonidipine lowers blood pressure in cerebral resistance vessels and prevents hypertension induced brain damage.

Efonidipine exhibits antihypertensive effect through vasodilatation by blocking L-type and T-type calcium channels. Efonidipine is primarily metabolized in the liver. Biliary route is the main pathway of excretion. No significant amount of unchanged drug was excreted in urine. In the urine collected for 24 h after an oral dosing, 1.1 % of the dose was excreted as deaminated Efonidipine, and 0.5% as a pyridine analogue of deaminated Efonidipine. Telmisartan is basically described in 2-[4-[[4-methyl-6-(1-methylbenzimidazole-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid.

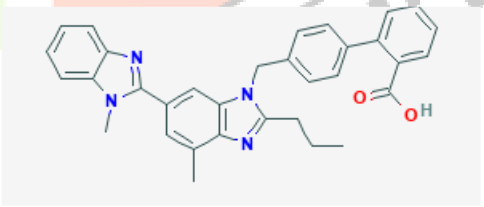
It is class of Angiotensin II(AT1 receptor) blockers. Following either intravenous or oral administration of 14C- labeled telmisartan, most of the administered dose (>97%) was eliminated unchanged in feces via biliary excreation; only minute amounts were

found in the urine (0.91% and 0.49%) of total reactivity, respectively. telmisartan is a medication used to treat high blood pressure, heart failure, and diabetic kidney disease. It is a reasonable initial treatment for high blood pressure. It is taken by mouth.Chlorthalidone is basically described in 2-chloro-5-(1-hydroxy-3-oxo-2H-isoindol-1-yl)benzene sulfonamide. Soluble in methanol, slightly soluble in alcohol, practically insoluble in water, ether and chloroform. Chlorthalidone prevents reabsorption of sodium and chloride through inhibition of the Na+/Cl- symporter in the cortical diluting segment of the ascending limb of the loop of Henle. Reduction of sodium reabsorption subsequently reduces extracellular fluid and plasma volume via an osmotic, sodium-driven diuresis. a prescription drug used to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. It is also used to reduce extra salt and water in the body caused by conditions such as heart failure, liver disease, and kidney disease.



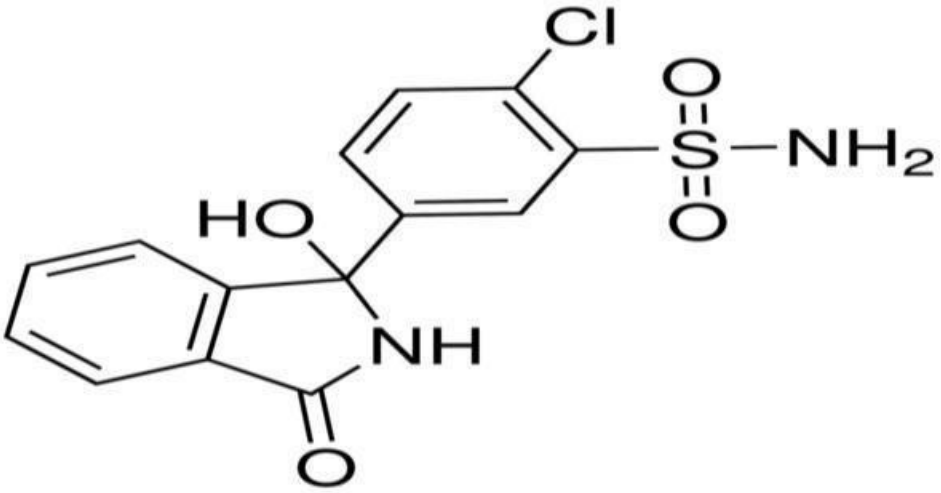
STRUCTURE:

EFONIDIPINE HYDROCHLORIDE



STRUCTURE:

TELMISARTAN ETHANOLATE



STRUCTURE OF CHLORTHALIDONE

MATERIALS AND METHODS:

Sample of Efonidipine HCl Ethanolate and Telmisartan procured from **Zuventus Healthcare Ltd**., Maharashtra. Chlorthalidone as gift sample from **CTX Lifescience** Pvt Ltd.

Experimental condition:

Apparatus: HPLC manufactured by Cyber Lab having LC-100 model no. was used in these method development .Cyber-Sil, C18 column (250mm x 4.6mm, 5 µg) was used as an stationary phase. For Identification of api by using UV Visible Spectrophotometer and FT-IR UV Visible Spectrophotometer is manufactured by Shimadzu having UV 1700 model no and FT-IR is manufactured by Agilent Technologies having Cary 630 model no.

Chemicals: HPLC Grade Water, Methanol, Acetonitrile which is manufactured by Ranchem Ltd. AR Grade Potassium Dihydrogen Phosphate, Orthophosphoric acid which is manufactured by Ranchem Ltd.

IDENTIFICATION OF API:

MELTING POINT STUDY: Melting point of Efonidipine, Telmisartan and Chlorthalidone was carried out by capillary tube method in paraffin bath. The melting point study was performed in Thieles tube that was filled with liquid paraffin. 50 mg of powdered drug was filled in capillary that was attached with the tip of thermometer with the help of thread. Then thermometer was placed in Thiele's tube and was heated. Temperature at which the drug powder melted was noted down. It was performed in triplicate.

SOLUBILITY STUDY: Solubility of Efonidipine, Telmisartan and Chlorthalidone was performed using various solvents like water, methanol, acetonitrile etc.

IR SPECTRA: Drug was placed in sample compartment of FT-IR instrument, where it was scanned in the range of 4000 - 650 cm^{-1}. Principle IR peaks were observed for drug are shown in table and from this data it was concluded that drugs were foundto be authentic.

UV ABSORPTION STUDY:

Accurately weighed 10 mg of EFO, TEL and CHLO were transferred separately in 10 ml volumetric flasks, dissolved in small volume of methanol and then volume was adjustedto the mark with methanol to obtain concentration of 1000 µg/ml. These solutions werefurther diluted to obtain concentration of 10 µg/ml. These standard solutions of EFO, TEL and CHLO in methanol were scanned in UV range, 200-400 nm in 1 cm cell using methanol as blank and maximum absorbance was measured for selection of λmax of EFO, TEL and CHLO.

METHOD DEVELOPMENT AND VALIDATION:

SELECTION OF DILUENT: Based on solubility, Efonidipine HCl Ethanolate, Telmisartan and Chlorthalidone was sparingly soluble in Acetonitrile. Hence, Acetonitrile was selected as diluent.

PREPARATION OF STOCK SOLUTION: Accurately weighed and transferred about 20 mg of Efonidipine Hydrochloride Ethanolate, 40 mg Telmisartan and 12.5 mg of chlorthalidone in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of Efonidipine Hydrochloride Ethanolate was 200 µg/ml, for Telmisartan 400 µg/ml and 125 µg/ml, for chlorthalidone. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Efonidipine Hydrochloride Ethanolate was 20 µg/ml and for Telmisartan 40 µg/mL and 12.5 µg/ml, for chlorthalidone. The optimum wavelength was selected for the estimation was 254 nm where gives maximum absorbance, which was obtained by scanning solution in the range of 200-400 nm in UV spectrophotometer.

- **Preparation Efonidipine Hydrochloride Ethanolate Standard Solution:** Accurately weigh and transfer 20 mg of Efonidipine Hydrochloride Ethanolate into a 100 mL volumetric flask. Add about 70 % diluent and sonicate to dissolve. Dilute up to mark with diluent and mix well. The solution formed will have concentration of Efonidipine Hydrochloride Ethanolate 100 µg/ml.
- Take 0.5, 1, 1.5, 2, 2.5, 3 ml above linearity solution to get series of concentration 5 – 30 ppm for EFO. Dilute the solution were filtered through 0.45 µm membrane filters.
- **Preparation Telmisartan Standard Solution**: Accurately weigh and transfer 10 mg of Telmisartan into a 100 mL volumetric flask. Add about 70 % diluent and sonicate to dissolve. Dilute upto mark with diluent and mix well. The solution formed will have concentration of Telmisartan 100 µg/ml.
- **Preparation Chlorthalidone Standard Solution:**
- Accurately weigh and transfer 12.5 mg of Chlorthalidone into a 100 ml volumetric flask. Add about 70 % diluent and sonicate to dissolve. Dilute up to mark with diluent and mix well. The solution formed will have concentration of Chlorthalidone 100 µg/ml.

  Take 0.25, 0.5, 1, 1.5, 2, 2.5 ml above linearity solution to get series of concentration 3.125 – 31.25 ppm for CTD. Dilute the solution were filtered through 0.45 µm membrane filter.

SELECTION OF MOBILE PHASE: Mobile phase selection involved selection of buffer, pH of buffer,

selection of solvent and buffer to solvent ratio. Proper selection of the HPLC method depends upon the nature of the sample, its molecular weight and solubility. For pH control buffer is required. As the acidic compound retain at low pH while base retained at high pH. The mobile phase was selected on the basis of good separation, peak purity, Tailing factor, theoretical plate etc. Various mobile phases were tried in different composition and different pH to achieve sharp peak of Efonidipine HCl Ethanolate and Telmisartan.

SELECTION OF WAVELENGTH: The optimum wavelength was selected for the estimation **254 nm** where gives maximum absorbance, which was obtained by scanning solution in the range of 200-400 nm in UV spectrophotometer.

PREPARATION OF BUFFER:

Weigh accurately and transfer about 1.36 g of potassium dihydrogen orthophosphate and 2 ml of triethylamines in 800 ml of water, adjust the pH 3 with orthophosphoric acid and add water sufficient water to produce 1000 ml.

PREPARATION OF MOBILE PHASE: Prepare a mixture of buffer, methanol and Acetonitrile in the volume ratio 30:30:40 % v/v/v. Mix well and sonicate to degas the mixture.

SELECTION OF COLUMN: Efonidipine, Telmisartan and Chlorthalidone are polar in nature. So, C18 analytical column were selected for HPLC method. The column was used Cybersil C18 column (250 mm × 4.6 mm, 5 µm) was used for the development of the method**.**

METHOD VALIDATION:

CALIBRATION CURVE: From the above prepared stock solution, pipette out appropriate volume of aliquot from standard stock solution of each of individual drug volumetric flask and transfer it to different volumetric flask of 10ml and volume adjusted upto mark with methanol, six different concentrations for TEL prepared with ranges from 10 – 60 µg/ml, EFO With range from 5-30 µg/ml and for CTD with ranges from 3.125-31.25 µg/ml were prepared from their individual respective stock solutions.

SYSTEM SUITABILITY TEST: The system-suitability tests are integral part of gas and liquid chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests are based on concept that the equipment, electronics, analytical operations, and sample to be analysed constitute an integral system that can be evaluated as such. The system suitability parameters like resolution, theoretical plates and asymmetric factor were calculated and compared with standard values.The system suitability test was carried out on freshly prepared working standard stock solution of TEL(40 µg/ml), EFO(20 µg/ml) and CTD (12.5 µg/ml), respectively.

LINEARITY: The calibration curve obtained for Telmisartan in the range of 10-60 µg/ml, Efonidipine Hydrochloride Ethanolate 5- 30 µg/ml and chlorthalidone 3.125 – 31.25 µg/ml. The correlation coefficient of Telmisartan, Efonidipine Hydrochloride Ethanolate and chlorthalidone was found to be 0.9985 and 0.9997 respectively. The calibration curve was established by plotting Peak area versus Concentration (n= 6) and straight-line regression equation was obtained. The calibration range was made in such a way that the ratio of combination was maintained throughout analysis.

PRECISION: In RP – LC method, repeatability has been carried out by injection repeatability. Repeatability was determined by analysing solution containing mixture of for Telmisartan 40 µg/mL,

Efonidipine Hydrochloride Ethanolate was 20 µg/ml and 12.5 µg/ml, for chlorthalidone. Precision was estimated in terms of intraday and interday precisions. **Inter – Day Precision** was determined by analysing sample solutions of Efonidipine, telmisartan and chlorthalidone at three levels covering low, medium, and high concentrations over the 3 different successive days. **Intra – Day Precision** of 10,30,60 µg/mL Telmisartan, 5, 15,30 µg/mL Efonidipine HCl Ethanolate and 3.125, 12.5, 25 µg/mL chlorthalidone (CTD) of respectively as a mixture of drugs were analyze at three different time intervals in a day and RSD was calculated.

ACCURACY: The accuracy of method was determined by calculating recoveries of drug by standard addition method at three different level 50 %, 100 % and 150 % of standards to pre-quantified sample solution of synthetic mixture. For TEL 10µg/ml, 20 µg/ml and 30µg/ml were spiked to pre-quantified sample solution of synthetic mixture for MON 20 µg/ml, For EFO 5 µg/ml,10 µg/ml 15 µg/ml were spiked to pre-qualified sample solution of synthetic mixture for EFO 10 µg/ml and For CTD 3.125µg/ml, 6.25 µg/ml and 9.375µg/ml were spiked in pre-quantified sample solution of synthetic mixture for BEP 6.25 µg/ml, respectively.

LOD and LOQ: The LOD was estimated from the set of 3 calibration curves used to determine method linearity.

The LOD may be calculated as, **LOD = 3.3 × (SD/Slope)**

The LOQ may be calculated as, **LOQ = 10 × (SD/Slope)**

ROBUSTNESS: According to ICH, the robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberated variations in method parameters here changes in different conditions were considered:

1.Change in Flow rate (1 mL/min ± 1)

2.Change in Mobile phase composition (30:70 % v/v ± 1ml)

3.Change in Wavelength (254 nm ± 3)

SPECIFICITY: Specificity were ensured by the use of a standard, diluent and placebo to examine the % interference of excipients. The specificity of proposed method was determined by analyzing spiking of placebo to standard and calculate the % interference.

ANALYSIS OF SYNTHETIC MIXTURE: Synthetic mixture was prepared by mixing Telmisartan (40.0 mg), Efonidipine HCl Ethanolate (20.0 mg) and chlorthalidone (12.5 mg) with starch (140.0 mg), Hydroxy propyl methyl cellulose E5 (30.0 mg), Polly vinyl pyrrolidone (20.0mg) magnesium stearate (2.5 mg) and talc (1.0mg), dissolved in 25.0 ml of distilled water and then diluted to the mark in a 100.0ml standard flask and sonicated for 5 min., filtered and filtrate was used for validating the above-mentioned methods. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Efonidipine Hydrochloride Ethanolate was 20 µg/ml and for Telmisartan 40 µg/mL and 12.5 µg/ml, for chlorthalidone.

RESULTS AND DISCUSSION:

IDENTIFICATION OF DRUG:

MELTING POINT STUDY: The observed melting point of each mentioned drugs were similar to the standard melting point reported for respective drugs as evident from Table 6.1

Table 1 Melting Point Study

Drugs	Reported Melting Point (°C)	Observed Melting Point (°C)
Efonidipine Hydrochloride Ethanolate	151 °C [25]	153-155 °C
Telmisartan	258◦C -264◦C []	260◦C -263◦C
Chlorthalidone	239 °C [40]	236-240 °C

N = 3, Mean of 3 replicates

SOLUBILITY STUDY: The solubility of substance fundamentally depends on the physical and chemical properties of the solute and solvent as well as temperature, pressure and the pH of the solution. The solubility profile is used for solvent selection in method development. The solubility of each drug in different solvent in shown in Table 4.2.

Table 2 Solubility Study

Drugs	Efonidipine Hydrochloride Ethanolate	Telmisartan	Chlorthalidone
Water	Insoluble	Insoluble	Soluble
Methanol	Soluble	Slightly Soluble	Soluble
Acetonitrile	Slightly soluble	Slightly soluble	Slightly soluble

UV Absorption Study: UV spectra of drugs in methanol depicted that the wavelength maxima of EFO, TEL and CHLO were at 254 nm, 298nm and 275 nm respectively as shown in Figure 6.1.

For High Performance Liquid Chromatography 254 nm was selected wavelength.

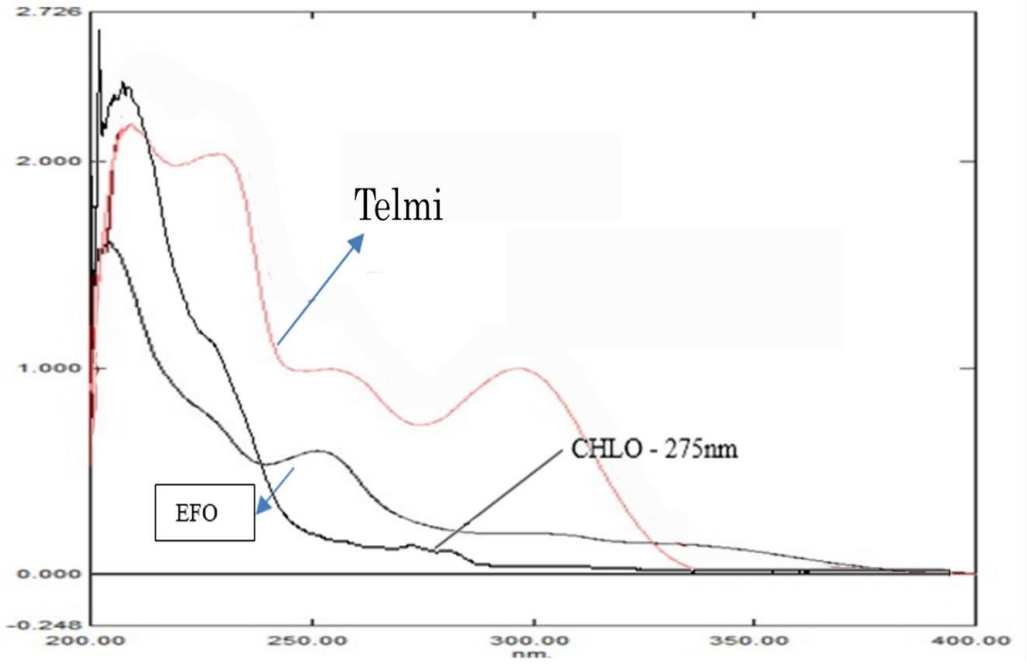


Figure 1 Overlain UV Spectrum in methanol

IR SPECTRA: An IR spectrum of reference sample shown in figure 6.2, figure 6.3 and figure 6.4 observed frequency was within the standard frequency range. So, concluded that given sample content was Efonidipine Hydrochloride Ethanolate, Telmisartan and Chlorthalidone results are shown in table 2, 3 and 4.

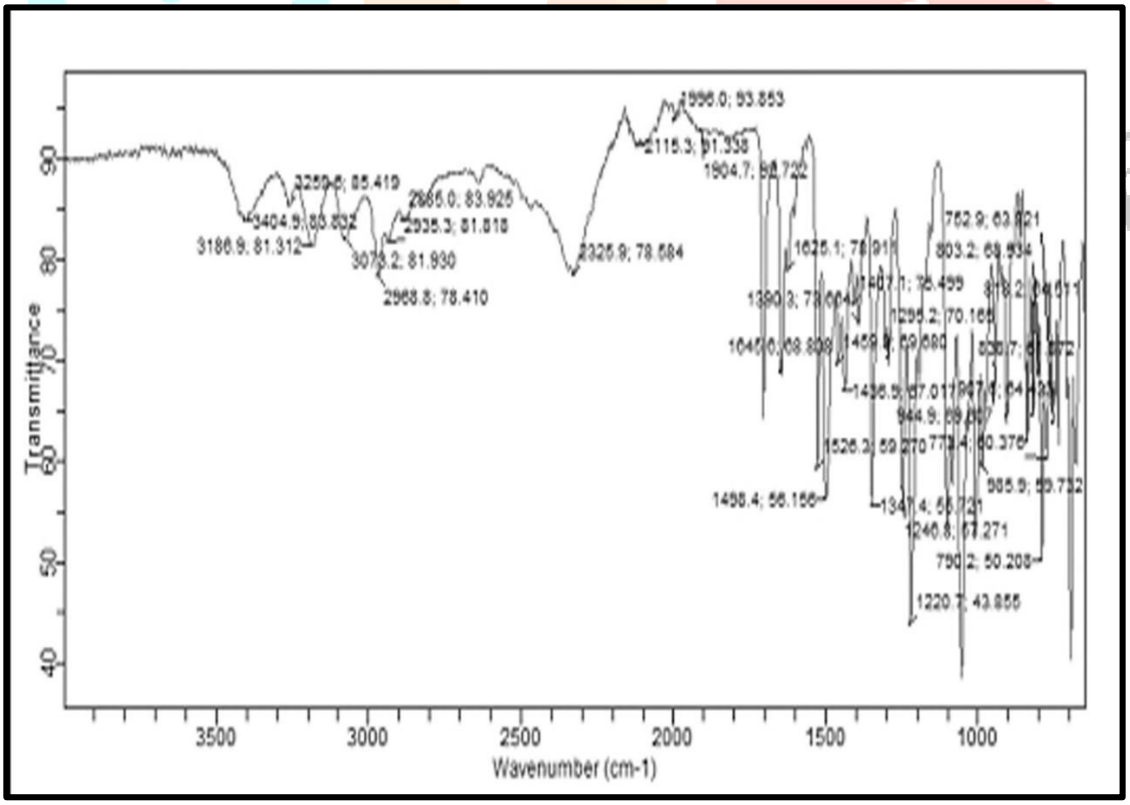


Figure 2 IR

Spectrum of Efonidipine Hydrochloride Ethanolate

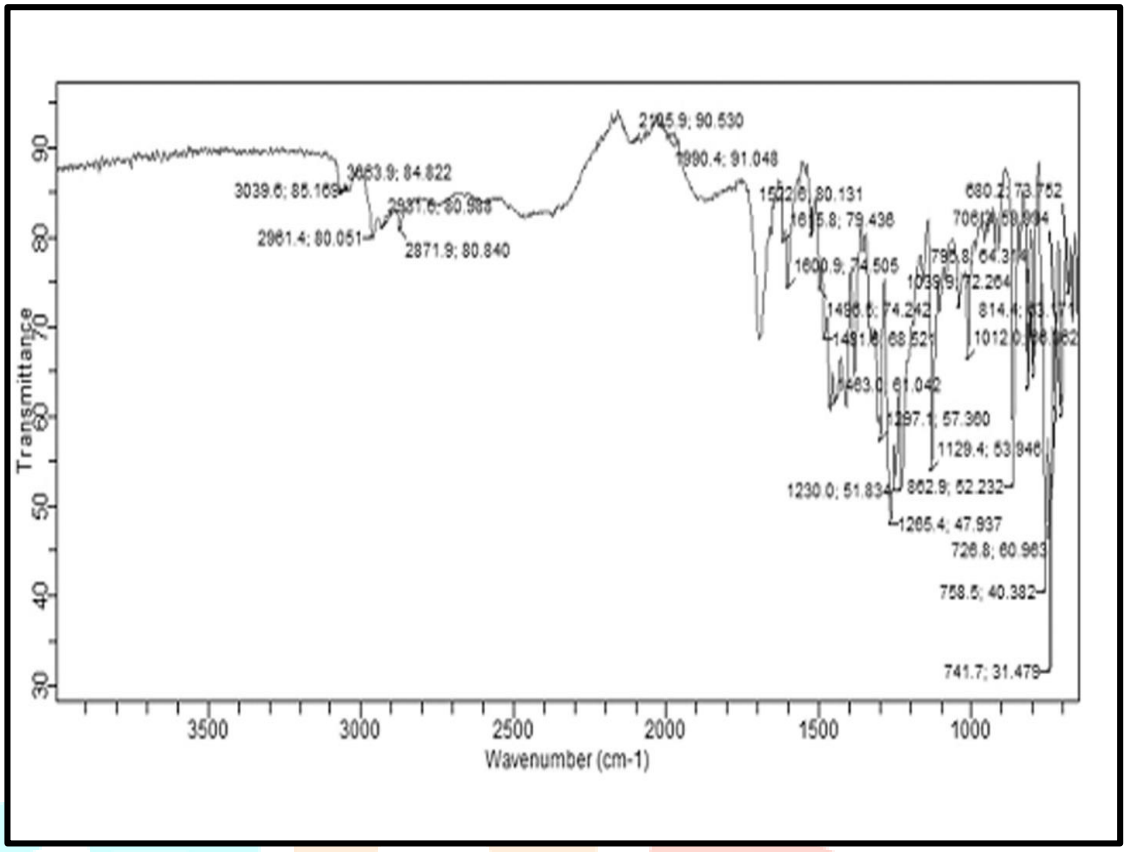


Figure 3 IR Spectrum of Telmisartan

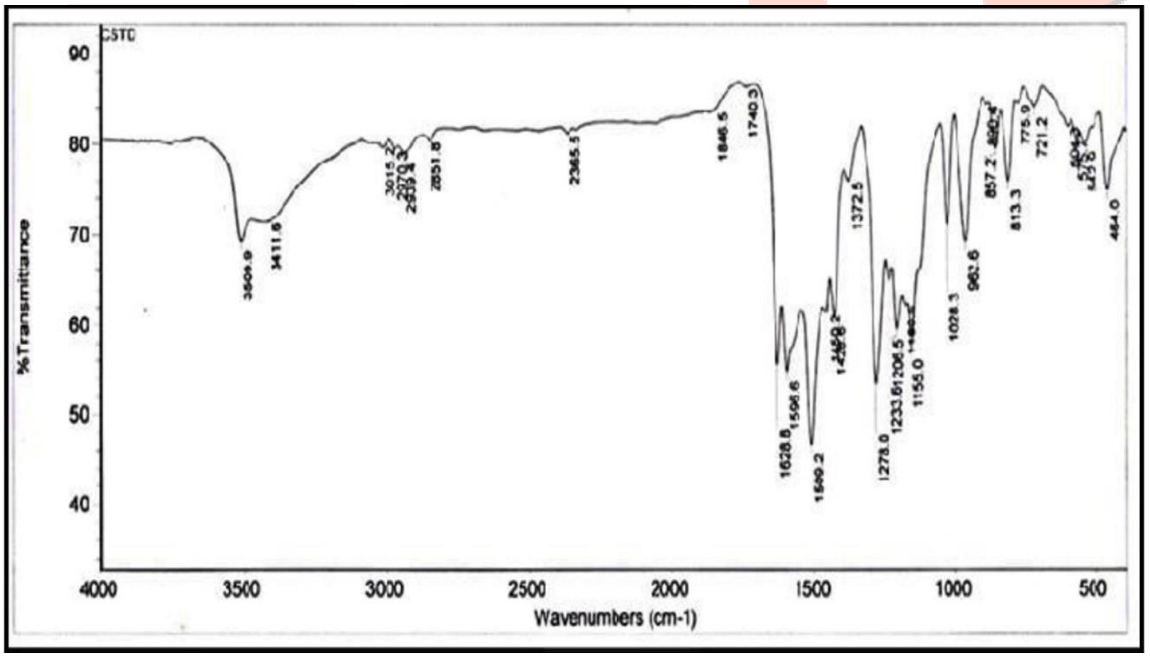


Figure 4 IR Spectrum of Chlorthalidone

OPTIMIZATION OF RP-HPLC CHROMATOGRAPHIC CONDITION:

Table 3 Method Development Trial

Trial No.	Condition	Observation
1	Column: C_{18} (250 mm x 4.6 mm),5 µm Mobile Phase: Water: Methanol (10:90 % V/V) Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 20 µl	Low retention time of Drug and peak shape was not proper only Chlorthalidone eluted Efonidipine and Telmisartan Was not found.
2	Column: C_{18} (250 mm x 4.6 mm),5 µm Mobile Phase: Water: Acetonitrile (10:90 V/V) Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 20 µl	Low retention time of Drug and peak shape was not proper only Chlorthalidone and Efonidipine eluted Telmisartan Was not Found.
3	Column: C_{18} (250 mm x 4.6 mm),5 µm Mobile Phase: Potassium Dihydrogen Phosphate: Acetonitrile (60:40 %V/V) 4 pH of buffer Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 20 µl	Higher retention time of Drug and peak shape was not proper. Telmisartan, Chlorthalidone and Efonidipine eluted
4	Column: C_{18} (250 mm x 4.6 mm),5 µm Mobile Phase: Potassium Dihydrogen Phosphate: Methanol (60:40 %V/V)-4 pH of buffer Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 20 µl	Higher retention time of Drug and peak shape was not proper. Telmisartan, Chlorthalidone and Efonidipine eluted
5	Column: C_{18} (250 mm x 4.6 mm),5 µm Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (10:40:50 %V/V) 3.5 pH of buffer Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 20 µl	Shorter retention time of Drug and peak shape was proper but resolution not good Telmisartan, Chlorthalidone and Efonidipine eluted
6	Column: C_{18} (250 mm x 4.6 mm),5 µm Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (20:30:50 %V/V) 3 pH of buffer Flow Rate: 1 ml/min	Shorter retention time of Drug and peak shape was proper but resolution not good Telmisartan, Chlorthalidone and Efonidipine eluted

	Wavelength: 254 nm Injection Volume: 20 µl	
7	Column: C_{18} (250 mm x 4.6 mm),5 µm Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:30:40 %V/V)- 3 pH of buffer Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 20 µl	Shorter retention time of Drug and peak shape was proper, resolution good Telmisartan, Chlorthalidone and Efonidipine eluted

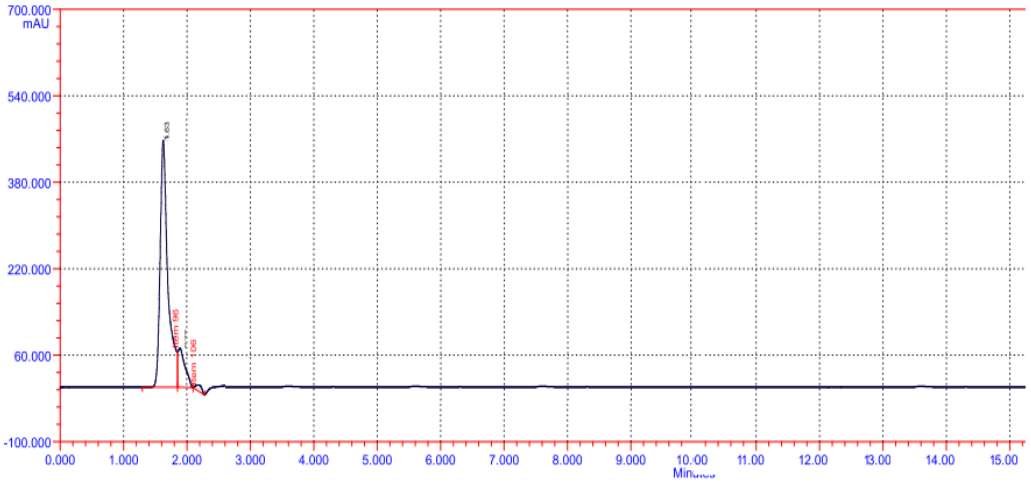


Figure 5 *Trail 1* Mobile Phase: Water: Methanol (10:90 % V/V)

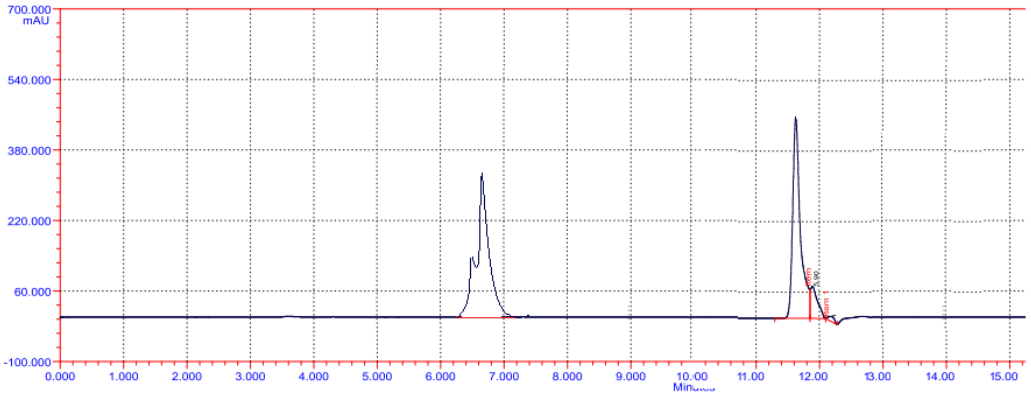


Figure 6 *Trail 2:* Water: Acetonitrile (10:90 V/V), HPLC Chromatogram

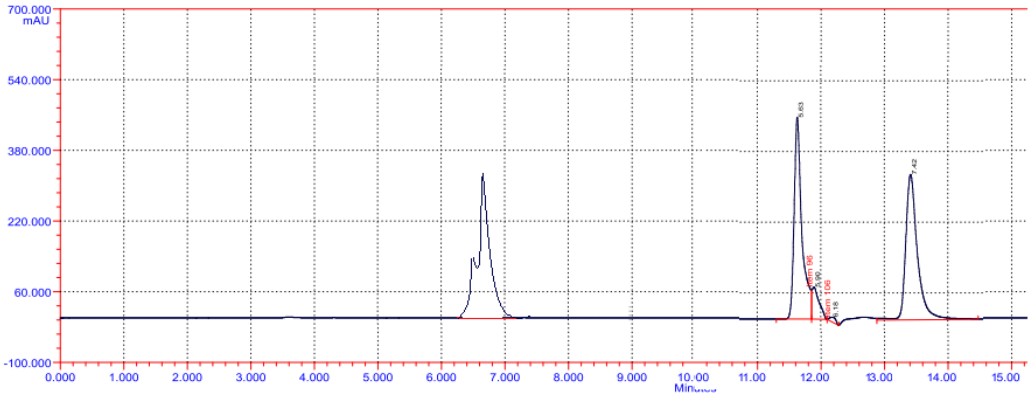


Figure 7 Trail 3: Mobile Phase: Potassium Dihydrogen Phosphate: Acetonitrile (60:40 % V/V) 4 pH of buffer, HPLC Chromatogram

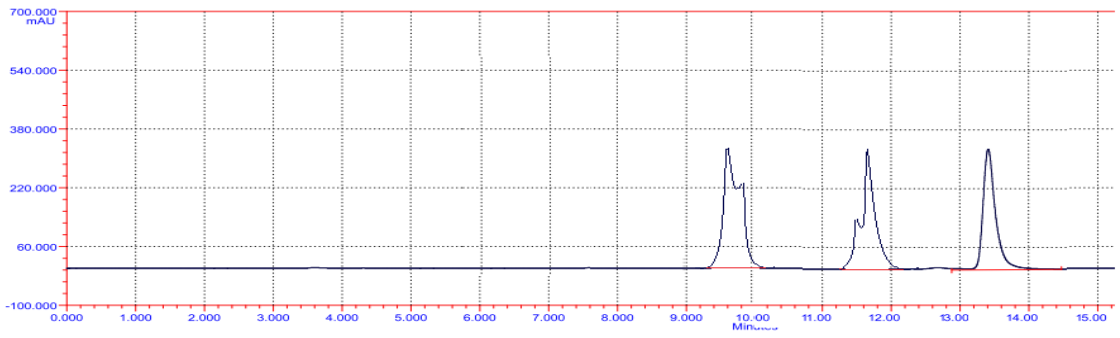


Figure 8 *Trail 4:* Mobile Phase: Potassium Dihydrogen Phosphate: Methanol (60:40 %V/V)-4 pH of buffer, HPLC Chromatogram

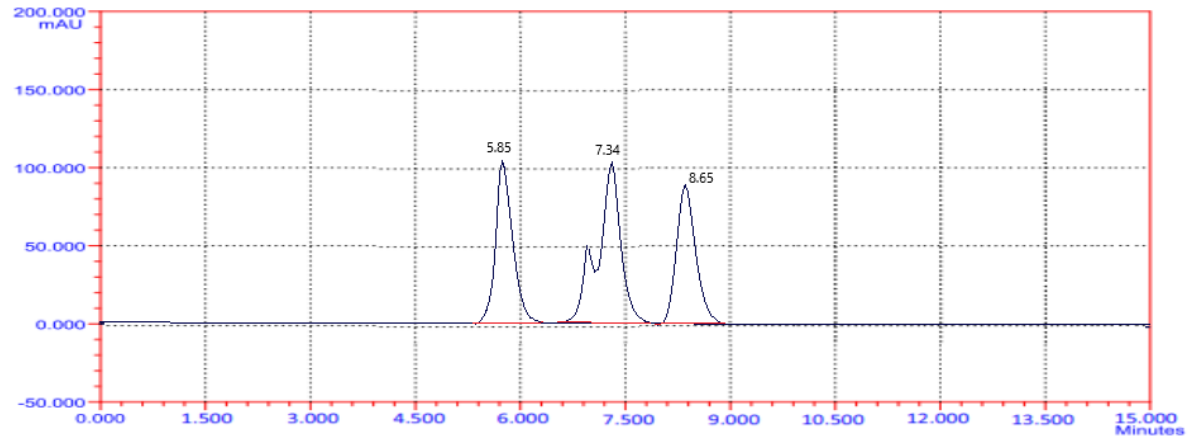


Figure 9 *Trail 5:* Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (10:40:50 %V/V) 3.5 pH of buffer, HPLC Chromatogram

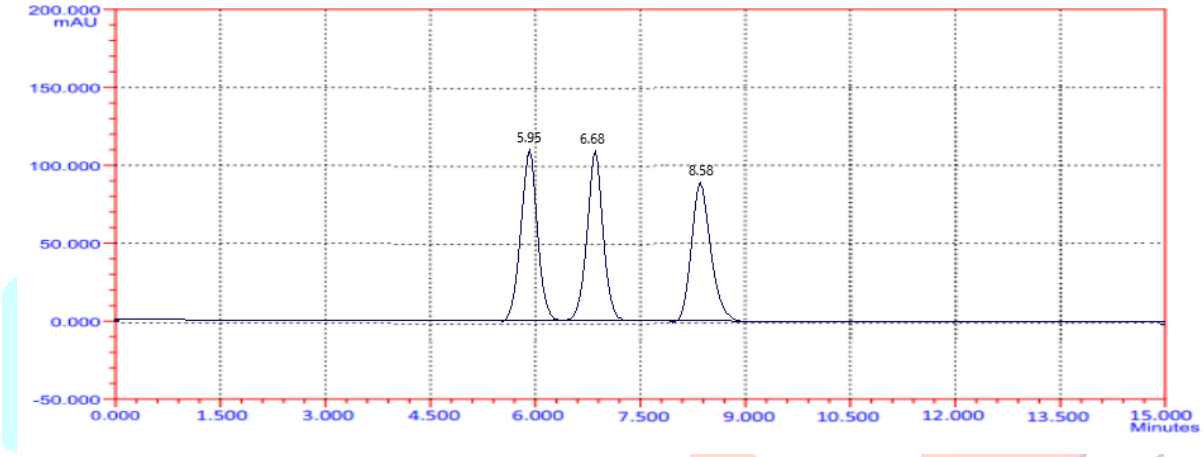


Figure 10 *Trail 6:* Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (20:30:50 %V/V) 3 pH of buffer, HPLC Chromatogram

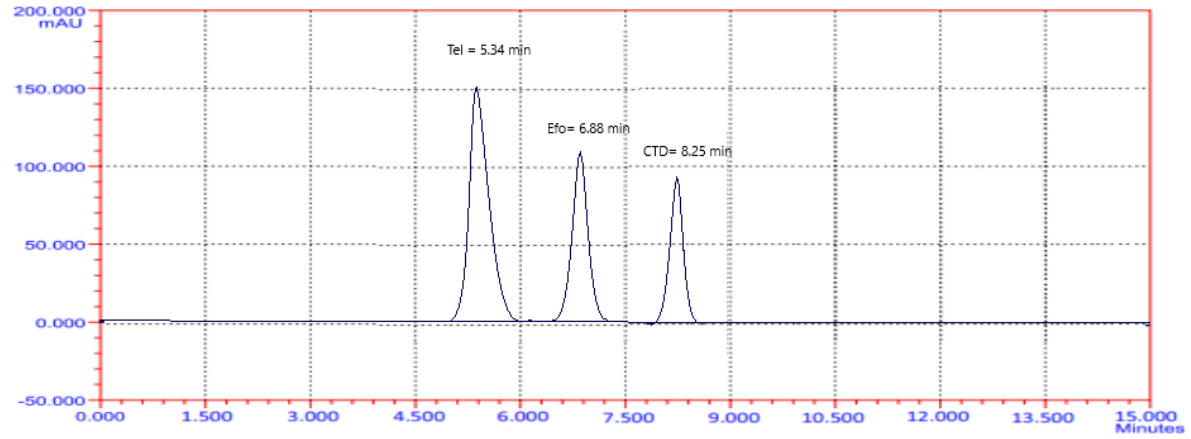


Figure 11 *Trail 7:* Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:30:40 % v/v/v)- pH-3 adjusted with o-phosphoric acid, HPLC Chromatogram

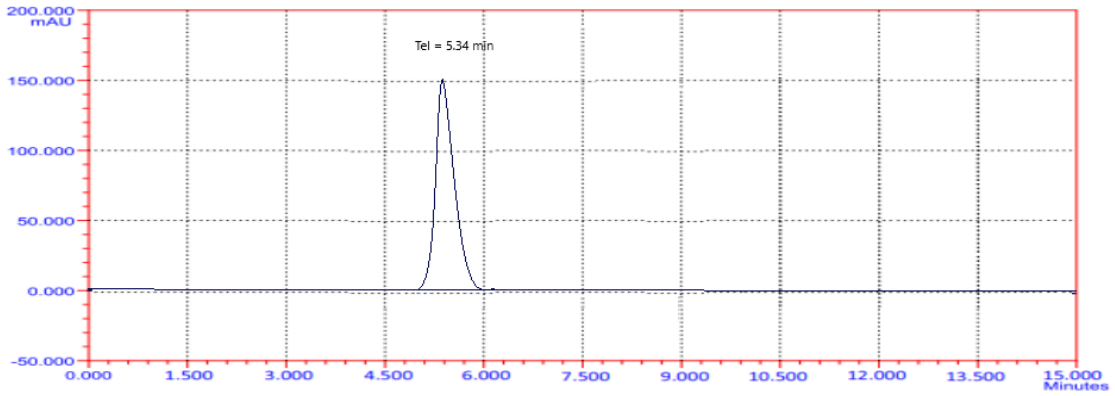


Figure 12 Telmisartan HPLC Chromatogram on optimized Mobile Phase

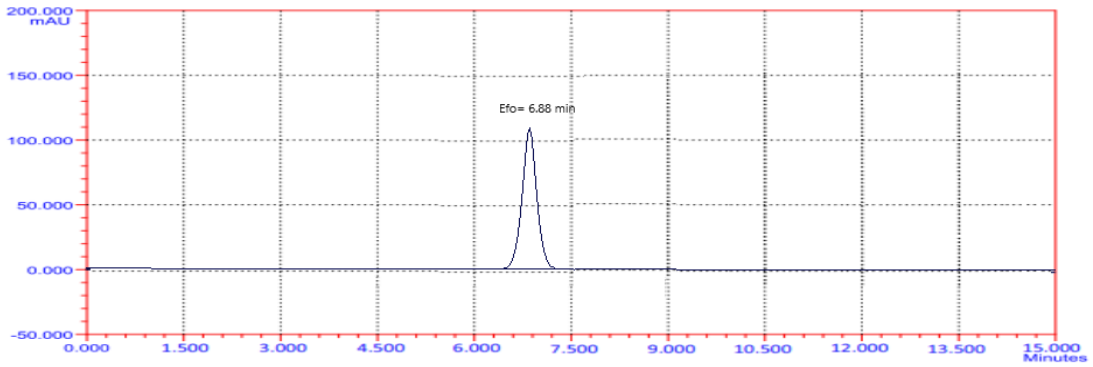


Figure 13 Efonidipine HCL Ethanolate HPLC Chromatogram on optimized Mobile Phase

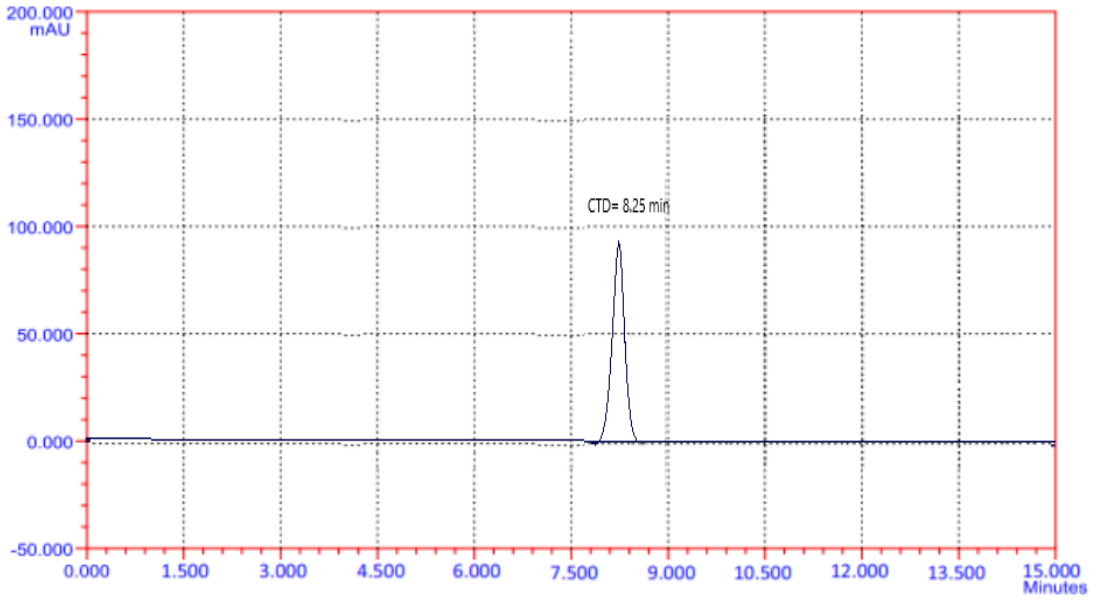


Figure 14 Chlorthalidone HPLC Chromatogram on optimized Mobile Phase

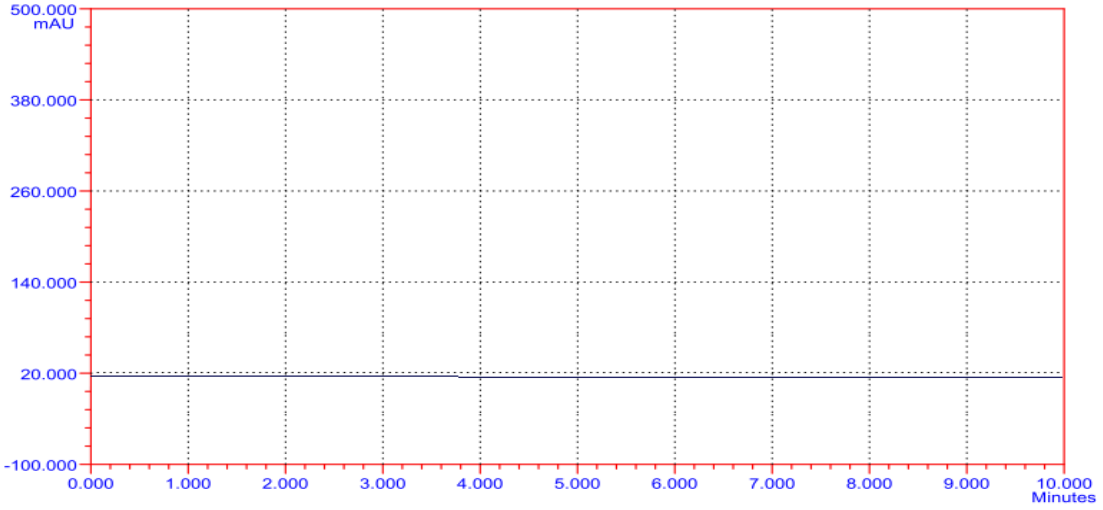


Figure 15 Blank HPLC Chromatogram on optimized Mobile Phase

Method Development Trial

Table 4: Optimization of RP-HPLC chromatographic condition

Sr. No.	Chromatographic parameter	Optimize Condition		
1	Flow Rate	1 ml/min		
2	Detection Wavelength	254nm		
3	Mobile Phase composition	Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:30:40 % v/v/v)- pH-3 adjusted with o-phosphoric acid		
4	Column	C_{18} (250 mm×4.6 mm×5 µm)		
5	Injection Volume	20 µl		
6	pH of buffer	3± 0.02		
7	Retention time (min)	TEL	EFO	CTD
		5.34	6.88	8.25

METHOD VALIDATION

1.1.1. Linearity

The calibration curve obtained for Telmisartan in the range of 10-60 µg/ml, Efonidipine Hydrochloride Ethanolate 5- 30 µg/ml and chlorthalidone 3.125 – 31.25 µg/ml. The correlation coefficient of Telmisartan, Efonidipine Hydrochloride Ethanolate and chlorthalidone was found to be 0.9985 and 0.9997 respectively. The calibration curve for TEL, EFO and CTD given in Fig. no. 6.16, 6.17 and fig. no 6.18. The overlay HPLC chromatogram of drugs was given in fig no. 6.19.

Table 5 Linearity Data of Telmisartan

	Peak Area ± SD	**RSD**
10	64463.65 ± 1181.94	1.83
20	94683.38 ± 611.53	0.65
30	120876.28 ± 420.80	0.35
40	152481.00 ± 896.58	0.59
50	178387.77 ± 1100.43	0.62
60	196548.57 ± 1373.38	0.70

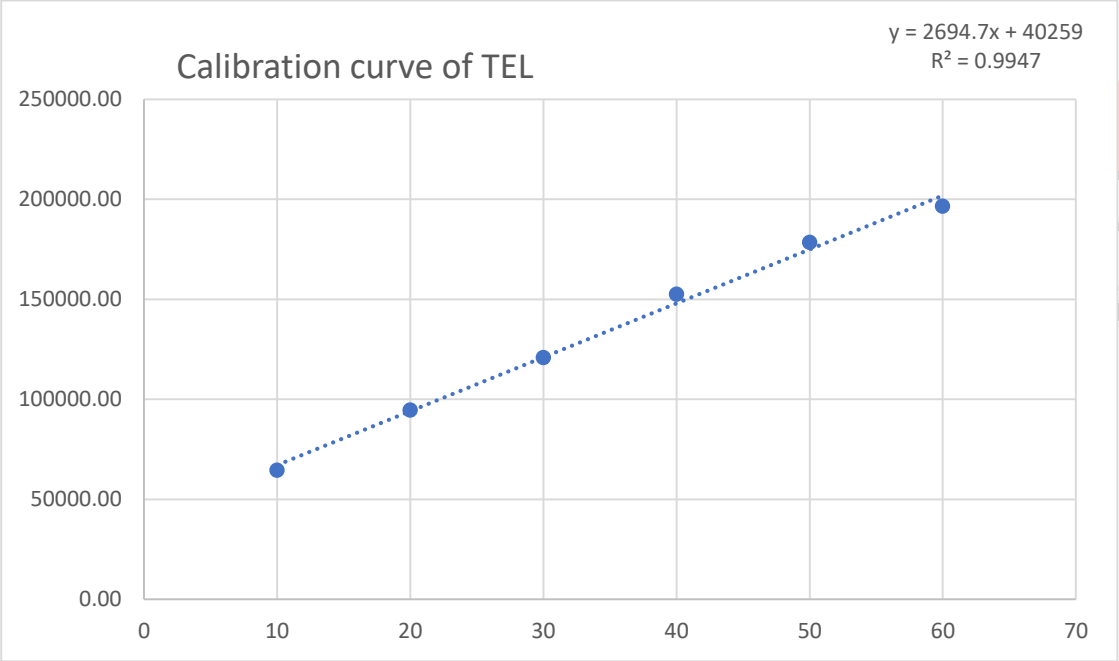


Figure 16 : Calibration Curve of Telmisartan (TEL)

Table 2 Linearity Data of Efonidipine Hydrochloride Ethanolate

Conc.	**Peak Area** ± SD	**RSD**
5	27527.22 ± 456.37	1.66
10	45538.08 ± 775.51	1.70
15	62018 ± 570.95	0.92
20	82659 ± 557.96	0.68
25	105143 ± 1078.48	1.03
30	117940 ± 609.24	0.52

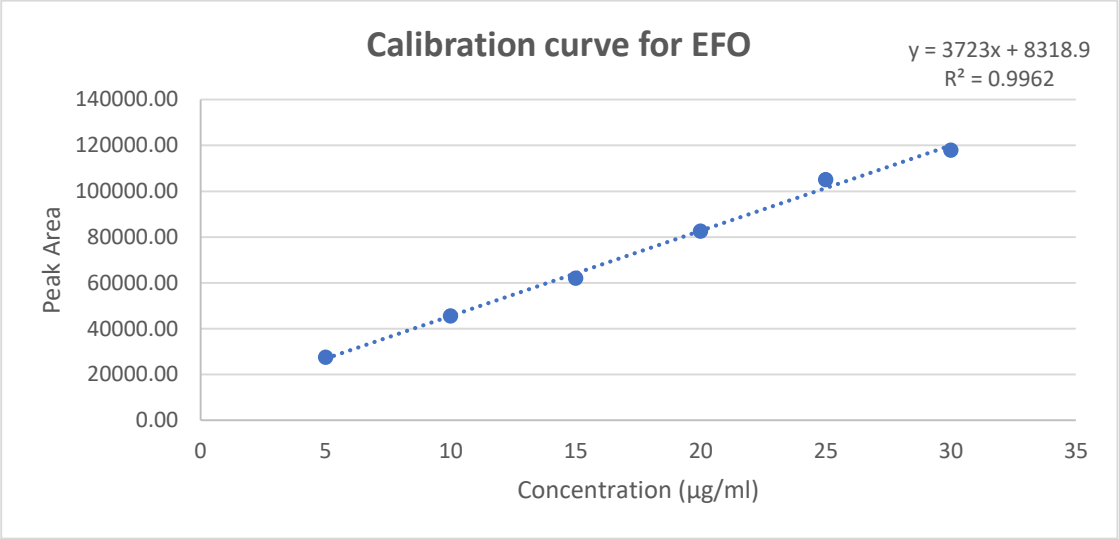


Figure 17: Calibration Curve of Efonidipine Hydrochloride Ethanolate

Table 7 Linearity Data of Chlorthalidone

Conc.	**Peak Area** ± SD	**RSD**
3.125	11163.65 ± 163.12	1.46
6.25	19233.38 ± 332.89	1.73
12.5	37209.95 ± 561.20	1.51
18.75	57520 ± 624.98	1.09
25	77921.1 ± 852.34	1.09
31.25	95932.73 ± 878.44	0.92

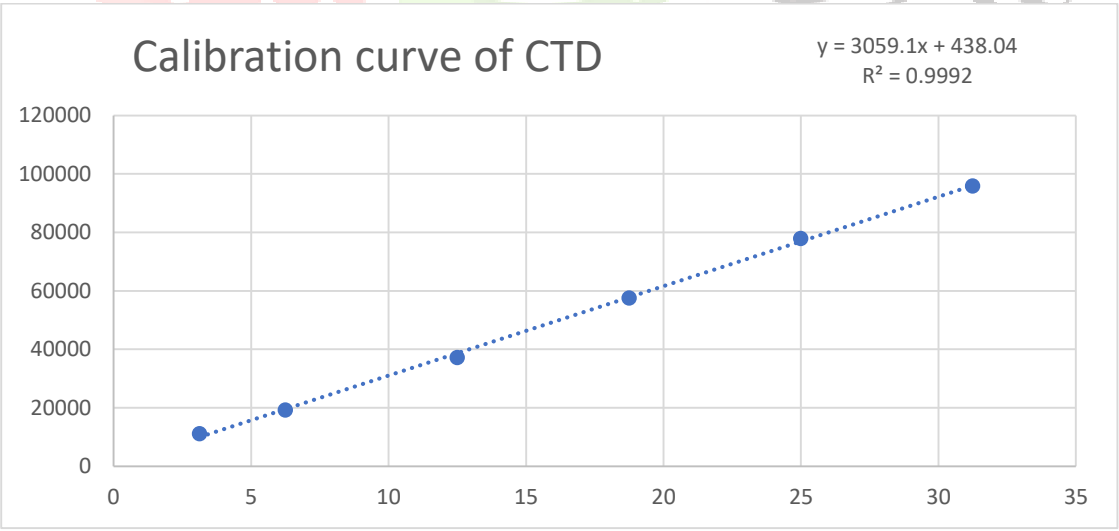


Figure 18: Calibration Curve of chlorthalidone (CTD)

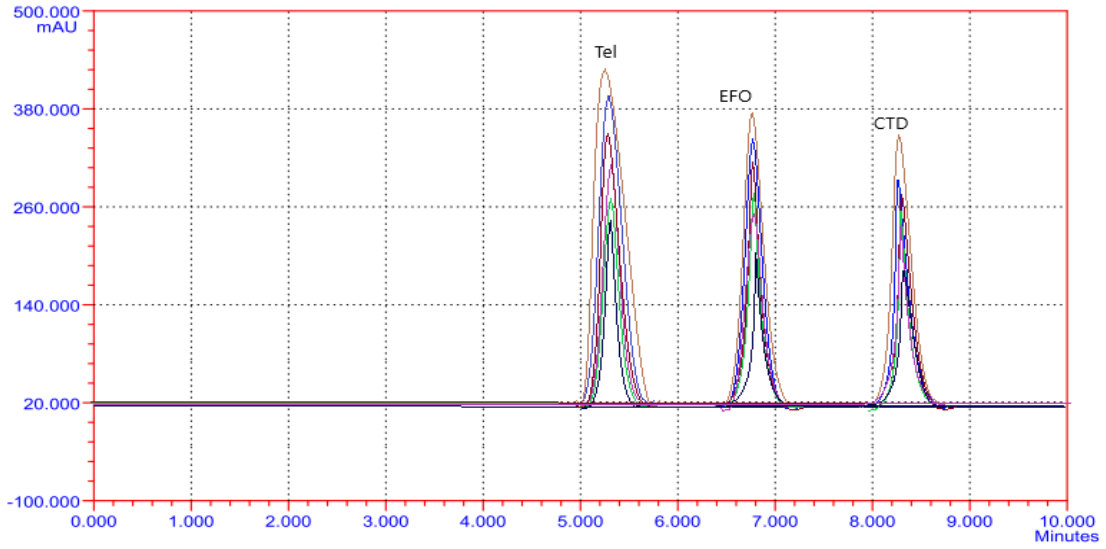


Figure 19: The overlay HPLC Chromatogram of Telmisartan in the range of 10-60 µg/ml, Efonidipine Hydrochloride Ethanolate 5- 30 µg/ml and chlorthalidone 3.125 – 31.25 µg/ml on optimized Mobile Phase

1.1.2. Specificity

➢ Specificity were ensured by the use of a standard, diluent and placebo to examine the % interference of excipients. It was proved by comparing chromatogram of blank, standard solution and sample preparation solution, there was no any interference of excipients with peak of TEL, EFO and CTD.

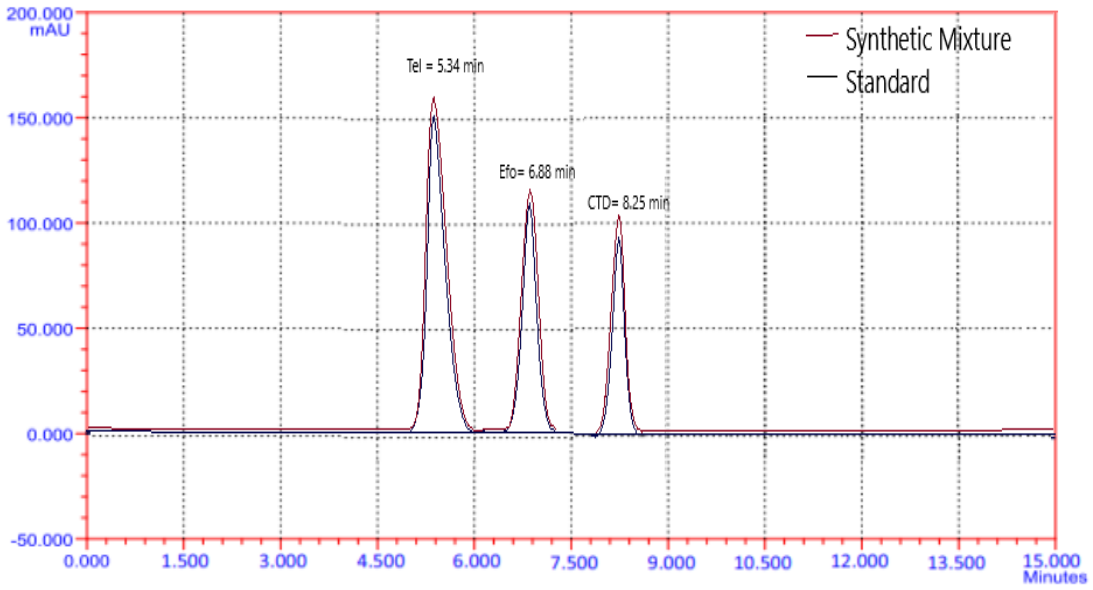


Figure 20: The overlay HPLC Chromatogram of standard and Synthetic mixture

1.1.3. Accuracy

➢ Accuracy of method was carried out at three level (50 %,100 % and 150 %).

% Recovery for TEL was found to be in range of 100.10-102.93 %, for EFO it was found to be range of 97.90 - 103.37 % and CTD it was found to be range of 98.03 -101.39 % are shown in Table 6.12.

Table 8: Accuracy data

Level (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	% Recovery
Telmisartan (TEL)						
0	20	0	20	94349.57	20.25	101.23
50	20	10	30	120822.83	30.03	100.10
100	20	20	40	150970.13	41.17	102.93
150	20	30	50	177519.67	50.99	101.97
Efonidipine Hydrochloride Ethanolate (EFO)						
0	10	0	10	45226.64	9.91	99.13
50	10	5	15	62991.82	14.69	97.90
100	10	10	20	82881.32	20.03	100.14
150	10	15	25	104533.23	25.84	103.37
Chlorthalidone (CTD)						
0	6.25	0	6.25	19349.57	6.18	98.91
50	6.25	3.125	9.375	29050.44	9.35	99.77
100	6.25	6.25	12.5	37922.83	12.25	98.03
150	6.25	9.375	15.625	48903.14	15.84	101.39

1.1.4. Precision

Repeatability was determined by analysing solution containing mixture of for Telmisartan 40 µg/mL, Efonidipine Hydrochloride Ethanolate was 20 µg/ml and 12.5 µg/ml, for chlorthalidone. Peak area of same concentration was measured six times and % RSD was calculated shown in Table 6.13.

Table 9: Repeatability Data of Telmisartan (TEL), Efonidipine Hydrochloride Ethanolate (EFO) and Chlorthalidone (CTD)

Telmisartan (TEL)			Efonidipine Hydrochloride Ethanolate (EFO)			Chlorthalidone (CTD)		
Sr. No	Conc. (µg/ml)	Area	Sr. No	Conc. (µg/ml)	Area	Sr. No	Conc. (µg/ml)	Area
1	20	94298.7	1	10	45371.53	1	6.25	19598.7
2	20	94174.2	2	10	44916.85	2	6.25	19474.2
3	20	94575.8	3	10	45391.53	3	6.25	18975.8
4	20	94449.3	4	10	45721.58	4	6.25	19449.3
5	20	94738.6	5	10	44851.33	5	6.25	18738.6
6	20	95863.7	6	10	46975.65	6	6.25	19163.7
Average	44178.60		Average	45538.08		Average	19233.38333	
SD	147.58		SD	775.51		SD	332.89	
% RSD	0.334		RSD	1.70		RSD	1.73	

➢ Intra – Day Precision of 10,30,60 µg/mL Telmisartan, 5, 15,30 µg/mL Efonidipine HCl Ethanolate and 3.125, 12.5, 25 µg/mL chlorthalidone (CTD) of respectively as a mixture of drugs were analyze at three different time intervals in a day and RSD was calculated shown in Table.

Inter – Day Precision of 10,30,60 µg/mL Telmisartan, 5, 15, 30 µg/mL Efonidipine HCl Ethanolate and 3.125, 12.5, 25 µg/mL chlorthalidone (CTD) of respectively as a mixture of drugs were analyze at three different days and RSD was calculated shown in Table.

For Repeatability, Intraday and Interday precision RSD was found to be less than 2 revels that the proposed method is acceptable shown in Table 6.14.

Table 10 : Intraday and Interday precision of method

Telmisartan (TEL),				
Concentration	**Intraday precision**		**Interday precision**	
	Peak Area (Mean ± SD)^n	%RSD	Peak Area (Mean ± SD)^n	%RSD
10	65255.40	0.85	63438.57	1.44
30	120822.83	0.23	121597.07	1.01
60	197223.63	0.77	197641.84	1.41
Efonidipine Hydrochloride Ethanolate (EFO)				
5	27717.63 ± 235.26	0.85	27493.47 ± 457.87	1.67
15	61658.48 ± 184.75	0.30	62377.48 ± 1017.94	1.63
30	118612.00 ± 274.42	0.23	117468.22 ± 503.79	0.43

Chlorthalidone (CTD)				
3.125	11088.73 ± 76.68	0.69	11181.90 ± 200.16	1.79
12.5	36989.50 ± 211.10	0.57	37330.40 ± 665.17	1.78
25	77253.00 ± 612.59	0.79	78589.20 ± 319.15	0.41

1.1.5. LOD and LOQ

LOD & LOQ of Telmisartan, Efonidipine HCl Ethanolate and chlorthalidone (CTD) of were determined by equation according to ICH guideline calculation of these was given in Table 6.15.

Table 10: LOD and LOQ of Telmisartan, Efonidipine HCl Ethanolate and chlorthalidone (CTD)

Drug	Telmisartan (TEL)	Efonidipine Hydrochloride	Telmisartan (TEL)
Limit of detection (LOD)	0.95 µg/ml	0.47 µg/ml	0.34 µg/ml
Limit of quantification (LOQ)	3.18 µg/ml	1.57 µg/ml	1.15 µg/ml

1.1.6. Robustness

➢ Deliberate change in parameter like flow rate, wavelength, mobile phase composition ratio and showed RSD of peak area less than 2 %, indicating that the method was robust, result is shown in table 6.25.

Table 11: Robustness

EFFECT OF CHANGE IN VOLUME OF PHOSPHATE BUFFER									
	25 ml			30 ml			35 ml		
	Peak Area	**SD**	**%RSD**	**Peak Area**	**SD**	**%RSD**	**Peak Area**	**SD**	**%RSD**
TEL (20 µg/ml)	95445.53	1005.25	1.05	95017.2	747.23	0.79	94537.90	1186.52	1.26
EFP (10 µg/ml)	45621.34	665.54	1.46	45516.19	589	1.30	45928.01	876.70	1.91
CTD (6.25 µg/mL)	19645.53	198.93	1.01	19117.2	357.62	1.87	19171.23	266.03	1.39
EFFECT OF CHANGE IN FLOWRATE									
	0.9 ml/min			**1 ml/min**			**1.1 ml/min**		
	Peak	**SD**	**%RSD**	**Peak**	**SD**	**%RSD**	**Peak**	**SD**	**%RSD**
TEL (20 µg/ml)	94778.87	**941.55**	**0.99**	95017.2	747.23	0.79	94804.57	**770.65**	**0.93**
EFP (10 µg/ml)	45488.01	637.43	1.4	45516.19	589	1.30	46094.68	806.89	1.75

CTD (6.25 µg/mL)	19412.20	224.03	1.15	19117.2	357.62	1.87	19204.57	251.70	1.31
EFFECT OF CHANGE IN DETECTION									
251 nm				**254 nm**			**257 nm**		
	Peak Area	**SD**	**%RSD**	**Peak Area**	**SD**	**%RSD**	**Peak Area**	**SD**	**%RSD**
TEL (20 µg/ml)	94871.2333	**882.65**	**0.93**	95017.2	747.23	0.79	94454.03	**282.23**	**0.3**
EFP (10 µg/ml)	45646.2389	458.59	1	45516.19	589	1.30	45321.48	439.33	0.97
CTD (6.25 µg/mL)	19507.4	80.04	0.41	19117.2	357.62	1.87	19362.4	172.53	0.89

1.2. Assay of synthetic mixture

➢ The synthetic mixture containing 40 mg Of Telmisartan, 20 mg of Efonidipine Hydrochloride Ethanolate and 12.5 mg of Chlorthalidone was analysed using the developed method, chromatogram of drug mixture indicating no interference of the excipients and Result are shown in table 6.26 and they were found satisfaction.

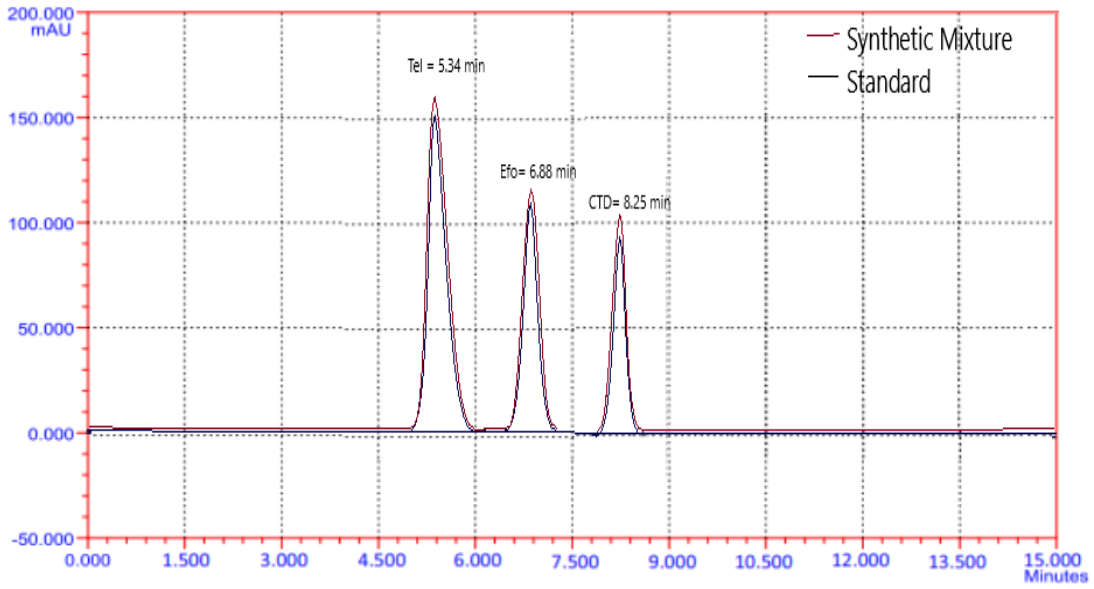


Figure 21 Chromatogram for Analysis of Synthetic mixture

Table 12: Analysis of Synthetic mixture

Drugs	Conc.	% Assay
Telmisartan	40 µg/ml	103.18 ± 0.098
Efonidipine Hydrochloride Ethanolate	20 µg/ml	100.14 ± 0.756
Chlorthalidone	12.5 µg/ml	98.46 ± 1.52

2. Conclusion

- Analytical RP-HPLC method were developed for the simultaneous estimation of Efonidipine Hydrochloride Ethanolate, Telmisartan and Chlorthalidone in Synthetic mixture. The method was developed to estimate and separate Efonidipine Hydrochloride Ethanolate Telmisartan and Chlorthalidone using RP-HPLC and developed method was validated as per ICH Q2 (R1) guideline.
- A Specific, precise, Accurate, Robust and cost-effective Reversed Phase High Performance Liquid Chromatographic method was developed for simultaneous determination of Efonidipine Hydrochloride Ethanolate Telmisartan and Chlorthalidone in their synthetic mixture.
- For RP-HPLC three drugs was separated by Cyber Lib C18 Column (250 mm × 4.6 mm,5 µm), utilizing a Mobile Phase Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:30:40 % v/v/v)- pH-3 adjusted with o-phosphoric 254 nm.
- The co-relation coefficient of 0.9947 for Telmi, 0.9962 for Efo, and 0.9992 for CTD HPLC method respectively. The % RSD Value was found for the validation parameter that indicate the preciseness of the proposed method and is applicable for routine analysis for quantitative determination of TEL, EFO and CTD. The LOD was found for Telmi 0.95 µg/ml, Efo 0.47 µg/ml and CTD 0.35 µg/ml for developed method respectively. The LOQ was found for Telmi 3.18 µg/ml, Efo 1.57 µg/ml and CTD 1.15 µg/ml for developed method respectively. The assay result shows that the methodology was selective for evalution of Efo and Telmi without hindring from the excipient. Whereas the mean percentage recovery was found to be 100.10-102.93 % for Telmi, 97.90-103.37 % for EFO and and 98.03 – 101.39 % for CTD of RP-HPLC respectively. The result of analysis was validated according to ICH Q2 R1 Guidelines. This simple and precise method can be used of both drug in quality control laboratories.

REFERENCE:

1. Chatwal GR, “Instrumental methods of chemical analysis”, *Himalaya publishing house*, **2011**, 5, 2.615-2.626.

2. https://www.omicsonline.org/open-access/analytical-method-development-and- validation-a-concise-review-2155-9872-5-233

3. Analytical method development and validation https://lubrizolcdmo.com/technical-briefs/analytical-method-development-and-validation

4.Analytical method development https://www.americanpharmaceuticalreview.com/Featured-Articles/190687-Analytical-Method-Development-Key-to-Process-Development/

5. Different types of column https://www.pharmaguideline.com/2016/01/different-types-of-columns-used-in-hplc.html

6. Skoog DA, “Principle of instrumentation analysis”, *Thomson Asia Pvt Ltd*, **2005**, 5, 300-304.

7. Jeffery GH, "Vogel's Textbook of Quantitative Chemical Analysis", *Adison Wesley Longman LTD*, **1966**,5, 216-220.

8. Troy DB, and Remington. "The science and practice of pharmacy" *B.I Publication Pvt Ltd*, **2005**,21, 647-648.

9. Galen WE, "Analytical instrumentation handbook" *Marcel Dekker Inc*, **2004**,21, 1123, 1183.

10. Giri, D, "High Performance Liquid Chromatography Principle, Types, Instrumentation and Applications", **2020**,547-562.

11. ICH Q2 (R1); "validation of analytical procedure: Text and Methodology". International Conference on Harmonization, Geneva; **2005.**

12. KD tripathi MD, "Essential of Medical Pharmacology" 6th edition, *Jaypee brothers medical publishers*, New Delhi, **2008**.

13. https://pubchem.ncbi.nlm.nih.gov/compound/Efonidipine **2021.**

14. https://go.drugbank.com/salts/DBSALT001268 **2021.**

15. https://pubchem.ncbi.nlm.nih.gov/compound/65999 **2021.**

16. https://go.drugbank.com/drugs/DB00966 **2021**.

17. https://pubchem.ncbi.nlm.nih.gov/compound/2732 **2021**.

18. https://go.drugbank.com/drugs/DB00310 **2021**.

19. Rajput AS et al, "RP-HPLC method development and validation for the quantification of Efonidipine hydrochloride in HME processed solid dispersions." *Future. J. Pharm. Sci*, **2020**,6(70), 1-9.

20. Kumar A, Shoni SK, et al. "Development and Validation of Liquid Chromatography (RP-HPLC) Methodology for Estimation of Efonidipine HCl Ethanolate (EFD)." *Pharm Anal Acta*, **2017**,8(5), D.

21. Pandya CP and Rajput SJ. "Forced degradation study of efonidipine HCl ethanolate, characterization of degradation products by LC-Q-TOF-MS and NMR. J. Appl. Pharm." *Sci*, **2020**, 10(04), 75-99.

22. Patel, G. H., S. D. Adeshra, and D. B. Meshram. "RP-HPLC Method Development and Validation for Simultaneous Estimation of Efonidipine Hydrochloride Ethanolate and Telmisartan in Their Synthetic Mixture." *International Journal of Pharmaceutics and Drug Analysis*, **2021**,9, 190-5.

23. Shreya D. Adeshra, Grishma H. Patel, "Development and Validation of Three Novel UV Spectrophotometric Methods for Simultaneous Estimation of Efonidipine Hydrochloride Ethanolate and Telmisartan in Their Synthetic Mixture and Its Comparison Using ANOVA." *J. Med. Chem. Sci*, **2021**, 4(2) 145-153.

24. Indian pharmacopoeia, "Government of India, ministry of Health and Family Welfare." The Indian pharmacopoeia Commission, **2018**, 2(8) 3319-3320.

25. Japanese pharmacopoeia, "The ministry of Health, Labour and Welfare The Japanese pharmacopoeia Commission." **2016**, 13, 1656-1657.

26.) Rathod S.D, Patil P.M, "UV- Spectrophotometric Method for Estimation of Telmisartan in bulk and tablet dosage form." *Int J Pharm Sci Res*, **2012,** 3(10), 3936-3939.

27) Chivate N.D, Patil S.M, "Development of UV spectrophotometric method for estimation of telmisartan as a pure API." **2012**,5(6),3331-3333.

28) Surekha M. L, Kumaraswamy G, "Development and Validation of RP-HPLC method for the Estimation of Telmisartan in bulk and tablet dosage form." *lnt.J. Drug Dev & Res*, **2012**, 4(4), 200-205.

29) Sumaiya SS and Bharadwaj A. "A Validated RP-HPLC Method for Tablets Containing Amlodipine Besylate and Telmisartan HCl as Active Pharmaceutical Ingredient." *Modern. Chem*, App, **2020**, 8(3), 1-3.

30) Bangaruthalli et al. "Simultaneous estimation of telmisartan and atorvastatin calcium in API and tablet dosage form." *J. Drug Del. There*, **2019**, 9(1), 175-179.

31) Sivakamasundari G and Kannappan N. "Method Development and Validation for Simultaneous Estimation of Chlorthalidone, Telmisartan and Amlodipine By RP- UPLC In Pharmaceutical Dosage Form." *Int. J. Res. Pharm. Sci*, **2018**, 9(3), 686-690.

32) Thakare L, Ahmad S and Shastry VM. "Development and Validation of UV-Visible Spectrophotometric Method For Estimation of Cilnidipine And Telmisartan In Bulk And Dosage Form." *Indo Am. J. Pharm. Res*, **2017**, 7(04), 8552-8559.

33) Vatchavai RB et al. "Method development and validation for simultaneous estimation of telmisartan and amlodipine by RP-HPLC." *World J. Pharm. Sci*, **2017**, 5(4), 45-53.

34) Shaik M et al. "RP-HPLC method development and validation for simultaneous estimation of Amlodipine besylate and Telmisartan in tablet dosage form." *Indian J. Res. Pharm. Biotech*, **2017**, 5(1), 74-76.

35) Sivasubramanian et al. "Simultaneous Estimation of Irbesartan, Telmisartan, Hydrochlorothiazide and Ramipril in Combined Dosage forms by Validated HPTLC Method*." J. Anal. & Pharm. Res*, **2017**, 4(4), 1-7.

36) Damor D et al, "Method Development and Validation of Simultaneous Estimaton of Cilostazol and Telmisartan." *J. Pharm. Anal*, **2015**, 4(3), 41-48.

37) Jabir AO et al. "Method Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in Tablet Dosage Form by RP-HPLC Method." *Res. J. Pharm. Bio and Chem. Sci*, **2012**, 3(3), 509-517.

38) Syamala GJ et al. "RP-HPLC method development and validation for the simultaneous estimation of amlodipine besylate and telmisartan in API and pharmaceutical dosage form." *Int. J. pharm. Invest. Res*, **2019**, 6(4), 209-221.

39) Ravi V.B, Kumar J, Ponneri V, "Simultaneous determination of Telmisartan and amlodipine in human plasma by LC/MS/MS and its application in a human pharmacokinetics study." *Journal of Pharmaceutical Analysis*, **2012**,2(5), 319–326.

40) Chabukswar A.R, Jagdala S.C, et al, "HPLC Method development for telmisartan and amlodipine." *Research J. Pharm. and Tech*, **2010**, 3(4), 1227-1230.

41) Prabhu C, Subramanian G, Arumugam K, Kini S, "Determination of Telmisartan by HPTLC-A Stability indicating assay." *Journal of Planar Chromatography*,**2007**, 6, 477–481.

42) The Indian pharmacopoeia **2018**, 2,1616-1618.

43) British pharmacopoeia - **2009**, 1406-1407.

44) Chaitali Kharat,1 Vaishali A. Shirsat, -"A Validated RP-HPLC Stability Method for the Estimation of Chlorthalidone and Its Process-Related Impurities in an API and Tablet Formulation." *Hindawi International Journal of Analytical Chemistry*, **2020**,11,1-11.

45) Shubhangi Sidram Bamgonde, Kaveri Chandrant Dulange, "UV- Spectrophotometric Method Development and Validation for Determination of Chlorthalidone In Bulk and Pharmaceutical Dosage Form." *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, **2019**, 7 (1),8-15.

46) Sandeep Sonawane, Sneha Jadhav, Priya Rahade, -"Development and Validation of Stability-Indicating Method for Estimation of Chlorthalidone in Bulk and Tablets with the Use of Experimental Design in Forced Degradation Experiments." *Hindawi Publishing Corporation Scientific*, **2016**, 9,1-9.

47) Sirisha Tadiboyina, Gurupadayya Bannimath, "Enantiomeric Separation and Quantitative Estimation of Chlorthalidone Enantiomers by Chiral Ultrafast Liquid Chromatography." *IJRPC*, **2015**, 5 (2),361-367.

48) Narmeen S. Abdullah, Medea A. Hassan, -"Spectrophotometric determination of chlorthalidone in pharmaceutical formulations using different order derivative methods." *Arabian Journal of Chemistry*, **2014**, 1(8).237-243.

49) Anna Pratima G. Nikalje, Ramesh Gadikar, "A Simple HPTLC Method for Simultaneous Estimation of Atenolol and Chlorthalidone in Pharmaceuticals." *To Chemistry Journal*, **2020**, 6, 35-47.

50) P. H. Sakpal, A. R. Chabukswar, "Stability Indicating Rp – Hplc Method Development And Validation For Simultaneous Estimation Of Amlodipine And Chlorthalidone In Bulk And Tablet Dosage Form." *IJPSR*, **2019**, 2161-2168.

51) Sohni, S. K., R. Kumar, M. Akhtar, C. Ranjan, and G. Chawla. "Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Azilsartan Medoximil And Chlorthalidone In Bulk Form

And Formulation Using Quality By Design." *International Journal of Pharmacy and Pharmaceutical Sciences*, **2016**, 8(2), 266-72.

52) Sonali Sangle, Padmanabh Deshpande, et al, "Development And Validation Of Stability Indicating HPTLC Method For Simultaneous Determination Of Olmesartan Medoxomil And Chlorthalidone In Combined Tablet Dosage Forms." *ejpmr*, **2017**,4(7), 574-581.

53) Bhamini R. Chaudhary, Jayant B. Dave. "Development And Validation Of Stability Indicating Gradient Rp-Hplc Method For Simultaneous Estimation Of Telmisartan And Chlorthalidone In Bulk Api And Fixed Dose Combination." *World Journal of Pharmaceutical Research*, **2017**, 6(10), 1015-1029.

54) Sohni, S. K., R. Kumar, M. Akhtar, C. Ranjan, and G. Chawla. "Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Azilsartan Medoximil And Chlorthalidone In Bulk Form And Formulation Using Quality By Design." *International Journal of Pharmacy and Pharmaceutical Sciences*, **2016**, 8(2), 266-72.

55) Dr. Aneesh T. P., R, R., M, A. P., Sasidharan, A., and Choyal, M., "RP-HPLC method for simultaneous determination of losartan and chlorthalidone in pharmaceutical dosage form." *International Research Journal of Pharmacy*, **2015**.6(7),453-457.

56) Sirisha T, Gurupadayya B, Siddiraju S. "Optimized and Validated RP-UPLC Method for the Determination of Losartan Potassium and Chlorthalidone in Pharmaceutical Formulations." *Adv Pharm Bull*. **2015**,5(1),133-6.

57) Ebeid WM, Elkady EF, El-Zaher AA, El-Bagary RI, Patonay G. "Spectrophotometric and spectrofluorimetric studies on azilsartan medoxomil and chlorthalidone to be utilized in their determination in pharmaceuticals." *Anal Chem Insights*. **2014**, 9, 33-40.

58) S. Naazneen, A. Sridevi, "Stability-Indicating Rp-Hplc Method For The Simultaneous Estimation Of Azilsartan Medoxomil And Chlorthalidone In Solid Dosage Forms." *Int J Pharm Pharm Sci*, **2015**, 6 (6), 236-243.

59) 1. Miyamoto, Misao Nissan Chemical Industries Ltd., Process for producing efonidipine hydrochloride preparations, European Patent, EP97907336A. **2003**.

60) Li Shengfei, Pharmaceutical composition using efonidipine as active ingredient, its preparation method and use, Chinese Patent, CN 200610075606, **2006**.

61) Maheshkumar Gadakar, Ghanshyam WAGH, Yogesh Wakchaure, Ponpandian Thanasekaran, Dnyandeo PUNDE, Improved process for the preparation of chlorthalidone, WIPO patent, WO2018158777A1, **2018.**

62) Lawrence Solomon, Method of treating hypertension with a very low dose of chlorthalidone, US Patent, 20070004792, **2005**.

63) Nurit Perlman, Eyal Gilboa, Process for preparing telmisartan, US Patent, US20090124814A1, **2009**.