International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

Analytical Method Development and Validation for Simultaneous Estimation of Cilnidipine and Fimasartan Potassium Trihydrate in Synthetic Mixture

Ankita A. Patel[1], Yagnesh Modi[1], Mitali Dalwadi[2], Dr. Chainesh Shah[2], Dr. Umesh Upadhyay[3]
[1]Student,[2]Professer,[3]Principal
Department Of Quality Assurance, Sigma Institute Of Pharmacy,Bakrol, Ajwa, Vadodara-390019 Gujarat, India

Date Of Submission: 05-05-2021 Date Of Acceptance: 20-05-2021

ABSTRACT
To develop and validate a simple and rapid isocratic reversed phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of cilnidipine & fimasartan potassium trihydrate in synthetic mixture, the chromatographic separation was achieved by using mobile phase Potassium dihydrogen phosphate in distilled water (pH 5.0) and phosphate buffer with acetonitrile. The mobile phase was pumped at a low flow rate & elements were monitored at 254 nm. Retention times were 3.31 & 6.26 min for cilnidipine & fimasartan respectively. %RSD was observed in the range of 0.50 & 1.51 for cilnidipine and fimasartan respectively. The %recoveries found to be as for cilnidipine 98.22-101.73% & fimasartan potassium trihydrate 98.50-101.61%. All the parameters are validated as per ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms.
(KEYWORD: Reversed phase High Performance Liquid Chromatography (RP-HPLC), Cilnidipine, Fimasartan Potassium Trihydrate)

I. INTRODUCTION

The multi-components formulations are flooded in Indian pharmaceutical retail market and have gained a lot of importance because of its inherent compliance about the patient's acceptability and economicity. These combinations are available in the various dosage forms. The quantitative 2 analysis of such multicomponent formulations is very important for the confirmation about the quality and efficacy. There are different analytical methods have been reported for single drug formulations but due to complexity in the multicomponent formulation, method development is a challenge for the analytical chemist. Official books also do not provide methods of the simultaneous analysis. Most of the methods available for the analysis of active ingredients of such formulations are applicable only after prior separation, which makes it tedious, expensive and time consuming.

The analytical techniques used for estimation of drugs consist of classical and instrumental methods of analysis. In classical methods the various methods used are titrimetric, volumetric, gravimetric and different instrumental techniques employed, are spectrophotometry, gas liquid chromatography (GLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC) etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances.

Cilnidipine: The chemical name of Cilnidipine is 2-Methoxyethyl (2E)-3-phenyl-2-propane-1-yl 2, 6-dimethyl-4-(3-nitrophenyl) - 1, 4-dihydro-3, 5-pyridine dicarboxylate. Cilnidipine is a slow-acting Ca2+ channel blocker, anti-hypertensive, vasodilator, dual blocker of L-type voltage-gated Ca2+ channels in vascular smooth muscle and N-type Ca2+ channels in sympathetic nerve terminals that supply blood vessels.It was jointly developed by Fuji Viscera Pharmaceutical Company, Japan and Ajinomoto, Japan and approved in 1995. Cilnidipine acts on the L-type calcium channels of blood vessels by blocking the incoming calcium and suppressing the contraction of blood vessels, thereby reducing blood pressure. Cilnidipine also works on the N-type calcium channel located at the end of thesympathetic nerve, inhibiting the emission of norepinephrine and suppressing the increase in stress blood pressure.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

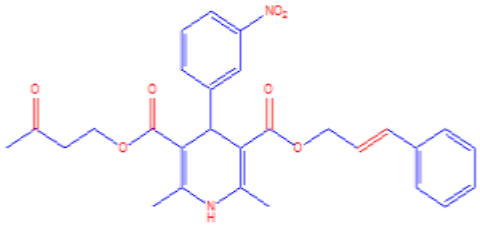

Figure 1 Chemical structure of Cilnidipine

Fimasartan Potassium Trihydrate

Fimasartan is an angiotensin II receptor antagonist (ARB) drug employed in the treatment of both hypertension and heart failure. It has been found to be safe when administered with hydrochlorothiazide (a diuretic) in clinical trials. Fimasartan was initially approved September 9th, 2010 in South Korea and is marketed under the brand name Kanarb by Boryung Pharmaceuticals.Angiotensin II activates AR1 leading to vasoconstriction and increased noradrenaline release which further increases vasoconstriction via action at α1-adrenergic receptors 1,2. It also stimulates secretion of aldosterone which acts to increase sodium and water reabsorption in the renal tubules 2. Fimasartan bind to and antagonizes AR1 preventing vasoconstriction and reducing aldosterone secretion to increase natriuresis leading to a reduction in blood volume. Together these effects produce an anti-hypertensive effect.

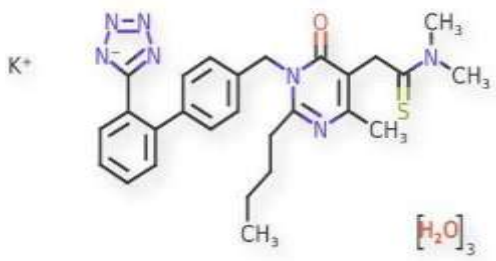

Figure2
ChemicalstructureofFimasartanPotassiumTrihydrate

SELECTION PARAMETERS OF AN ANALYTICAL METHOD

Choice of Analytical method depends on, what accuracy is required, how much sample is available what is the concentration range of the analyte, what components of the sample will cause interference, what are the physical and chemical properties of the sample matrix, how many samples are to be analyzed should be explained. Statistical evaluation, accuracy vs.precision, bias, sensitivity and application of students t test along with other statistical calculations should be done with the help of available analytical data and overviewed. The importance of calibration of instrumental methods and how analytical methods calibration can be carried out must be explained.

Usually involvement of adding one or more increments of a standard solution to sample aliquots of the same size (spiking) is done. Shimadzu HPLC 10 AT vp and UV-Visible Spectrophotometer Pharmaspec 1700 (Shimadzu) were used for HPLC and spectrophotometric estimation of marketed formulations.

Different Instrumental modes and their utility for analytical work as well as techniques in spectrophotometric methods of analysis are discussed. The developed methods are precise, rapid, simple and economical as a new analytical tool for the marketed formulations.

Once the problem is defined the following important factors are considered in choosing the analytical method. These are concentration range, required accuracy and sensitivity, selectivity time requirements and cost of analysis

Concentration range:

The ability to match the method to the optimum sample size is usually gained through experience and awareness of the different methods. Sensitivity, as it applied to an analytical method, corresponds to the minimum concentration or lowest concentration of a substance that is detectable with a specified reliability. It is often expressed numerically as a detection limit or sensitivity. Different analytical methods will provide different sensitivities and the one chosen will depend on the sensitivity that is required to solve a particular problem. Accuracy refers to the correctness of the result achieved by the analytical method.

Selectivity:

Selectivity is an indication of the preference that a particular method shows for one substance over another.

Time and cost:

Time and cost often go hand in hand usually are a reflection of the equipment, personneland space required to complete a determination.

A classic analytical problem is the simultaneous determination of two or more compounds in the same sample without previous chemical separation.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography is basically a highly improved form of column chromatography. It is operated under high pressures up to 400 atmospheres for the movement of solvent through the column unlike the gravity columns which were operated under gravity. Thus, the high pressures contribute to its fast performance. It allows using a very smaller particle size for the column packing material, in order that it gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This helps in a much better separation of the components from the mixture. The other major improvement over column chromatography concerns the detection methods, which can be used. These methods are highly automated and extremely sensitive. The essential equipment consists of an eluent, reservoir, a highpressure pump, and an injector for introducing the sample, a column containing the stationary phase, a detector and recorder. The development of highly efficient micro particulate bonded phases has increased the versatility of the technique and has greatly improved the analysis of multi-component mixtures.

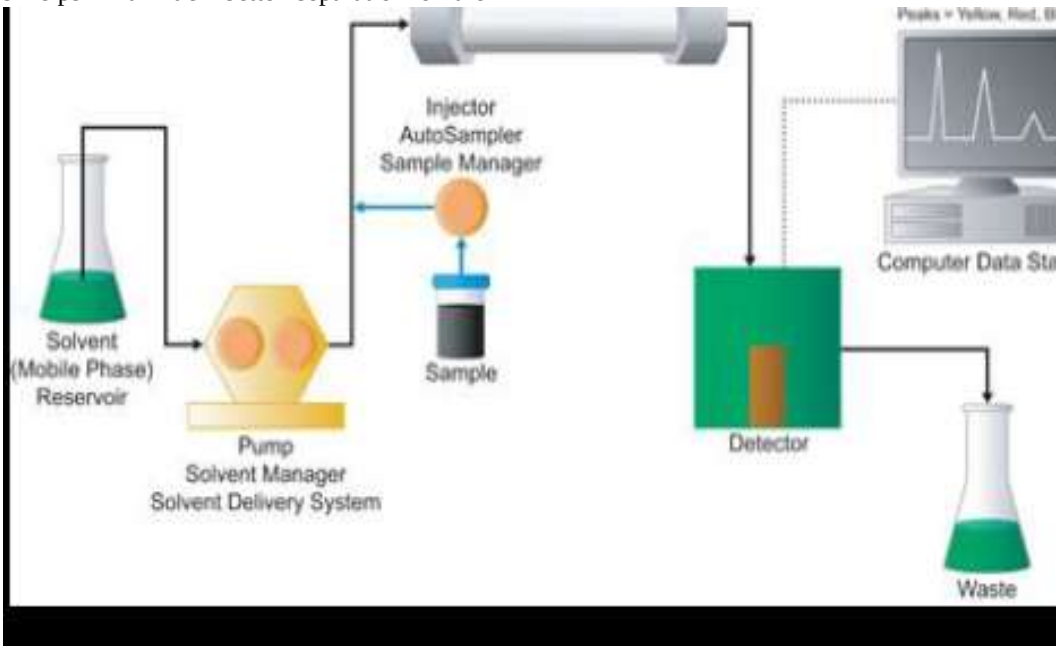

Figure 3 High Performance Liquid Chromatography

HPLC DETECTORS

The function of the detector (Refer Table-1) in HPLC is to monitor the analyte in mobile phase as it emerges from the column.

Basic Detector Requirements:

An idle LC Detector should have the following properties

- Low drift and noise level (trace analysis).
- High sensitivity.
- Fast response for high performance system.
- Wide linear dynamic range (quantitation).
- Low dead volume (minimal peak broadening and remixing of separated bands).
- Insensitivity to changes in type of solvents, flow rate and temperature.
- Operational simplicity and reliability.
- Tunable, so that detection can be optimized for different compounds.
- Preferably non-destructive.

Choosing Right Detector

- Select detector based on the chromophores presence or absence.
- Majority of pharmaceutical compounds exhibit UV spectra in the region of 200-400nm. Initial experiment can be done with lambda max of drug substance.
- Non chromophoric compounds can be analyzed with ELSD/RI/MS.
- If compound having fluorescence nature, it can be analyzed by using FLD.
- Generally, there are two types of HPLC detectors, bulk property detectors and solute property detectors.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

a. Bulk property detectors:

These detectors are based on differential measurement of a property, which is common to both the sample and mobile phase. Examples of such detectors are refractive index, conductivity and dielectric constant detectors.

b. Solute property detectors:

These detectors respond to a physical property of the solute, which is not exhibited by the pure mobile phase. These detectors measure a property, which is specific to the sample, either with or without the removal of the mobile phase prior to the detection. Solute property detectors which do not require the removal of the mobile phase before detection include spectrophotometric (UV or UV-Vis) detector, fluorescence detectors, polarogrphic, electro-chemical detectors and radio activity detectors, whilst the moving wire flame ionization detector and electron capture detector both require removal of the mobile phase before detection.

OBJECTIVES:

The present literature survey reveals that there is no HPLC method for estimation of cilnidipine and Fimasartan potassium trihydrate in combination. Main objective is that,

1. To develop a specific, accurate and sensitive chromatographic method i.e. HPLC for cilnidipine and Fimasartan potassium trihydrate in combination.
2. To Validate developed method according to ICH guideline.
3. To Apply developed HPLC method for the estimation of cilnidipine and Fimasartan potassium trihydrate in synthetic mixture.

APPARATUS AND INSTRUMENTS:

- **UV-Visible double beam spectrophotometer with matched quartz cells instrument (Model: UV 1800)**

• Make: Shmadzu, Kyoto, Japan.
• Software: UV Probe version 2.3.4
• Wavelength range: 3.99 Abs.
• Scan speed: 40nm/min
• Photometric accuracy: ±0.003

- **High Performance Liquid Chromatography**:

Model: Cyber Lab LC- 100
Column: C18 (250 mm × 0.46 cm)
Detector: SPD 20A UV Detector Injector: Rheodyne injector
Analytical balance: Electronic analytical balance (shimadzu)
Glassware: Volumetric flasks and pipettes

- **Melting Point Apparatus:** Veego VMP-D
- **Analytical Balance:** Mettler Toledo electronic balance (ME204, Mettler Toledo Group, Mumbai, India)
- **pH meter:** Hanna instruments, Mauritius.
- **pH strip:** Merck, India.
- **Sonicator:** Digital ultrasonic cleaner, India.
- **Hot air oven:** Kumar laboratory Oven, India

Table 1Reagents and Materials

Sr. No.	Chemicals and reagents	Grades	Manufacturers
1.	Methanol	HPLC	Merck Ltd., Mumbai
2.	Acetonitrile	HPLC	Merck Ltd., Mumbai
3.	Water	HPLC	Merck Ltd., Mumbai
4.	Cilnidipine	API	Pure ChemPvt Ltd, Ankeshwar
5.	Fimasartan Potassium Trihydrate	API	Pure ChemPvt Ltd, Ankeshwar
6.	Potassium dihydrogen phosphate	AR	Merck Ltd., Mumbai
7.	Orthophosphoric Acid	AR	Merck Ltd., Mumbai
8.	Sodium dihydrogen phosphate	AR	Merck Ltd., Mumbai
9.	Disodium hydrogen phosphate	AR	Merck Ltd., Mumbai

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

IDENTIFICATION OF DRUG:

Identification of procured drugs were performed using melting point study, UV- Visible spectroscopy and IR spectra study.

Melting Point Study

Melting point study was performed by using Veego VMP-D instrument.

The observed melting point of each selected drugs were similar to the standard melting point reported for respective drugs as shown in Table 2.

Table 2 Melting Point of Drug

Drugs	Reported Melting Point (˚C)	Observed Melting Point (˚C)
Cilnidipine	107 – 112 ˚C	109 – 110 ˚C
Fimasartan potassium trihydrate	267 – 269 ˚C	267 - 268 ˚C

UV Visible Spectroscopy spectrum

UV spectra of both drugs in methanol depicted that the wavelength maxima of CIL and FIMA was at 240 nm and 262 nm respectively as shown in Figure 4 and compare with reported shown in Table 3.

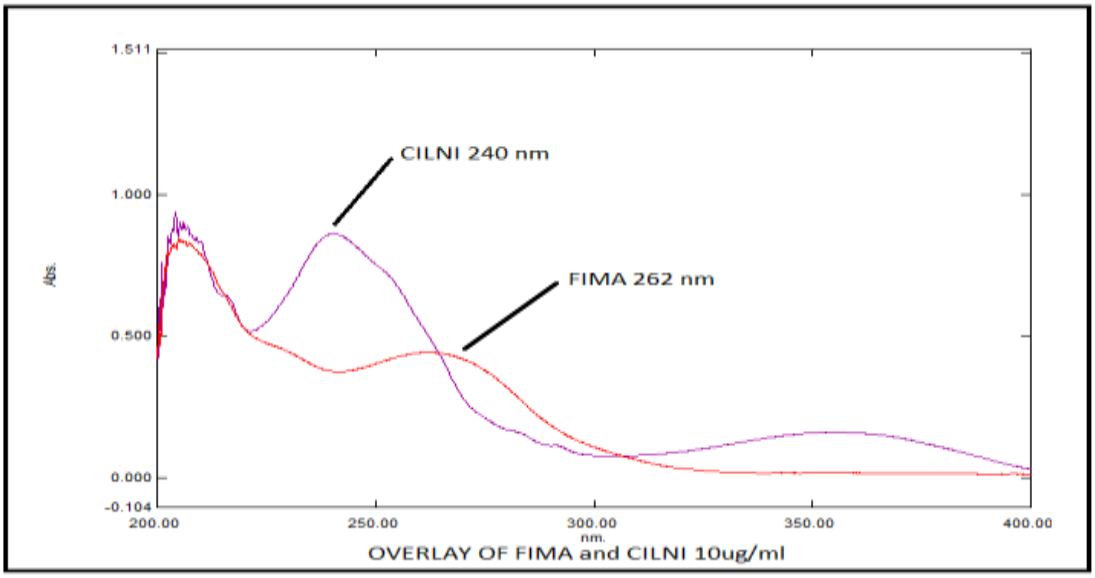

Figure IIOverlain UV spectrum of Cilnidipine (10 µg/ml) and Fimasartan potassium (10 µg/ml) in methanol

Table 3Wavelength maxima of Drug

Drugs	Reported wavelength maxima	Observed wavelength maxima
Cilnidipine	242 nm	240 nm
Fimasartan potassium trihydrate	262 nm	262

Infrared Spectroscopy

The Infra-red spectroscopic studies of solid samples were done by Bruker FTIR. The drug sample was the placed in sample holder and transferred to the sample compartment. Both samples were scanned in the region of 4000-400 cm^{-1} using Bruker FTIR. IR spectra of FIMAand CIL sample were scanned and it was compared

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

with the standard IR spectra of FIMA and CIL drugs as shown below in Figure 5,6 & 7 respectively.

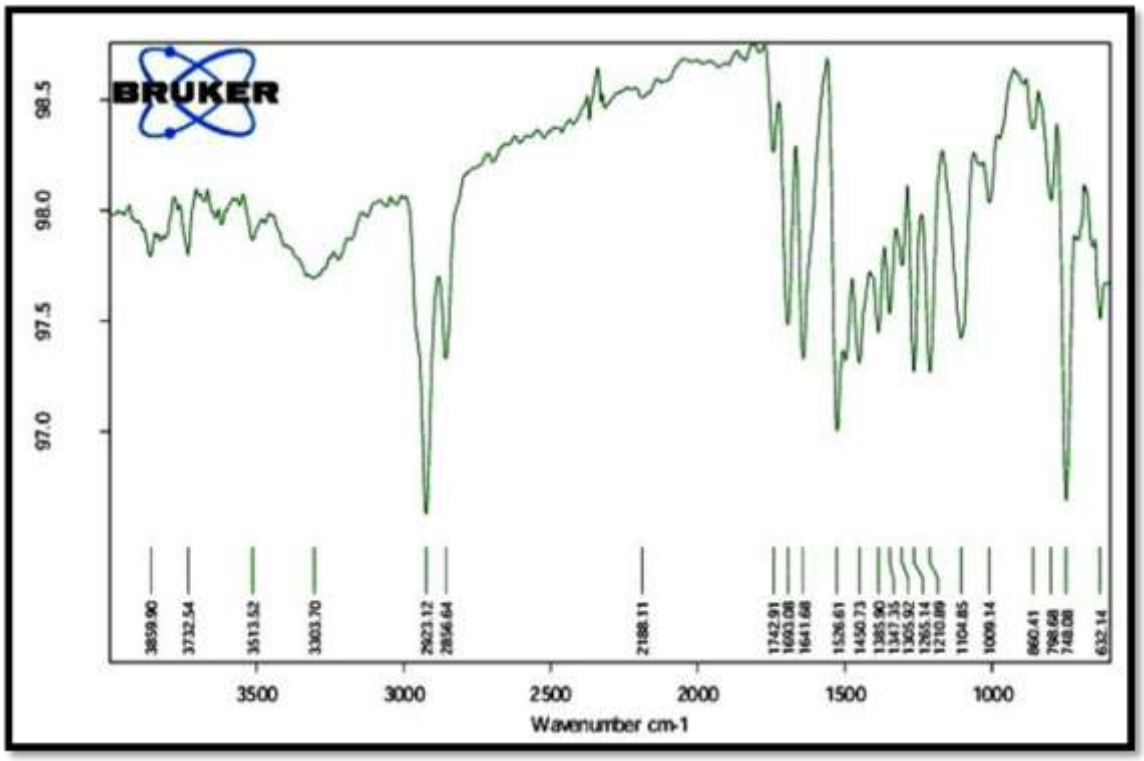

Figure 5 FTIR spectrum of Fimasartan sample

Table IIIInterpretation of IR spectra of Fimasartan

Sr. No.	Functional group	Reported wavenumber (cm-1)	Observed wavenumber (cm-1)
1.	N-H stretching secondary amine	3500-3200	3519.54
2.	O-H stretching	3330-3250	3336.52
3.	C-H stretching	3100-3000	3063.70
4.	C-H stretching	3000-2840	2955.12
5.	C-H stretching	3000-2840	2927.64
6.	C=O Stretching aromatic stretching	1650	1641.48
7.	C=C stretching	1600-1400	1537.61
8.	C-N Stretching	1250-1020	1150.14

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

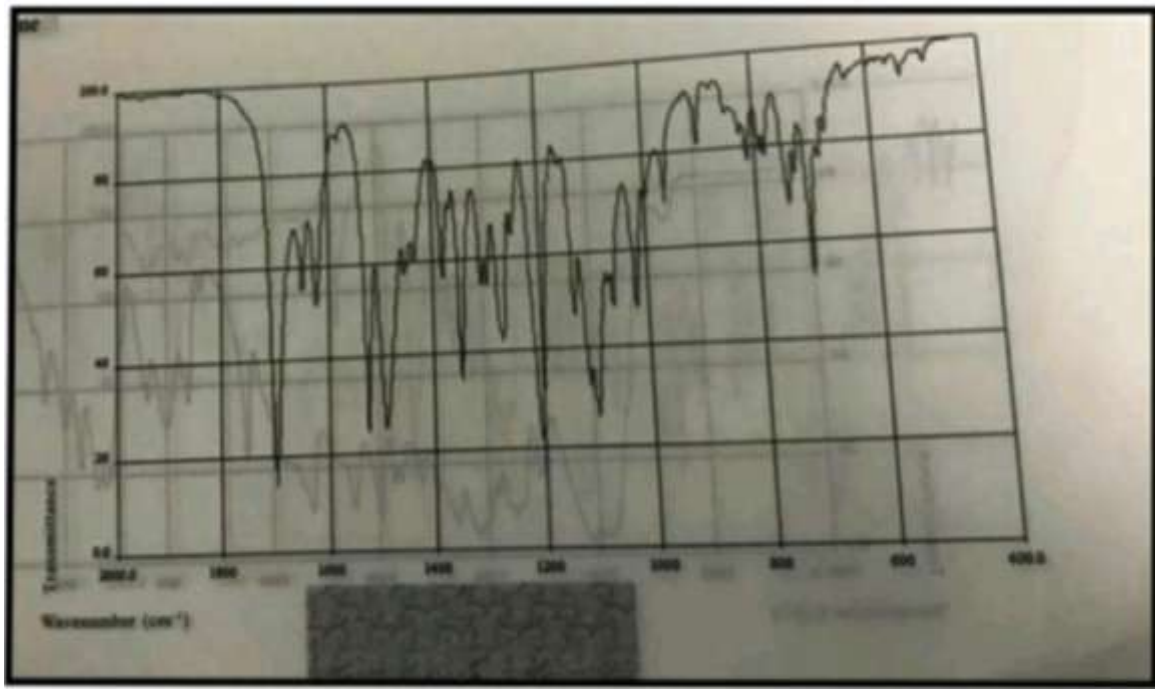

Figure 6IR spectrum of Cilnidipine standard

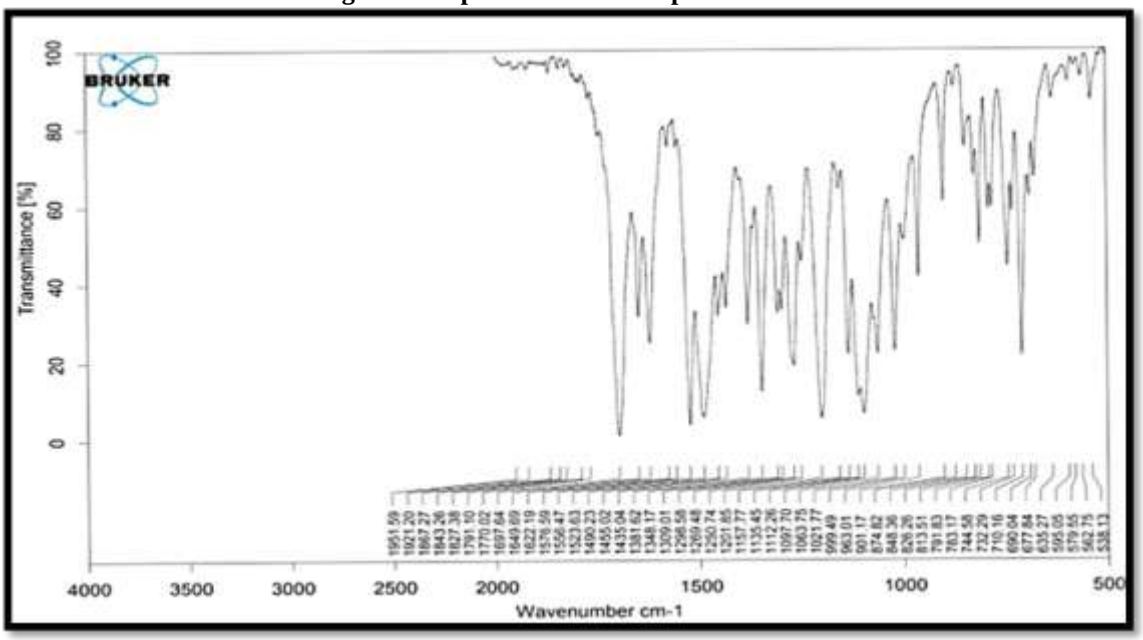

Figure 7FTIR spectra of Cilnidipine sample

Table 3Interpretation of IR spectra of Cilnidipine

Sr. No.	Functional group	Standard wavenumber (cm-1)	Observed wavenumber (cm-1)
1.	C – H Aromatic	900-690	692
2.	C – O	1320-1210	1291
3.	C=O Stretching	1870-1540	1553
4.	C=C Ring Stretching	1600,1475	1603,1450
5.	O – H Stretching	1420-1350	1348

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

6.	N=O Stretching	1290-1190	1251
7.	N-O Stretching	1357-1318	1353

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD

Selection of solvent

The solubility of Cilnidipine (CIL) and Fimasartanpotassium trihydrate (FIMA) were checked by the different solvents like methanol, Acetonitrile, chloroform, dichloromethane, water, toluene where all the drugs were freely soluble in Acetonitrile as well as methanol. Therefore, methanol has been selected as a common solvent for analysis.

Procedure for determination of Wavelength for Measurement

Accurately weighed 10mg of CIL and FIMA were transferred separately into 100ml volumetric flasks, dissolved in small volume of methanol and then volume was adjusted to the mark with methanol to obtain concentration in 100 µg/ml. These solutions were further diluted to obtain concentration of 10 µg/ml. These standard solutions of CIL and FIMA in methanol were scanned in UV range, 200-400 nm in 1 cm cell using methanol as blank and maximum absorbance was shown at 254nm $\lambda_{max}$of CIL and FIMA which was selected based on overlay spectrum.

Preparation of Mobile Phase

Dissolve 3.4 g of potassium dihydrogen phosphate in 500 ml of water and adjust the pH to 5.0 with 0.1 M potassium hydroxide. The transfer of 300ml of Phosphate Buffer (pH 5.0) and 200ml of Acetonitrile in to 500 ml reservoir of HPLC system and sonicate for 10 min for degassing.

Preparation of stock solution

Stock Solution were prepared by weighing 10mg of each Cilnidipine (CIL) and Fimasartanpotassium trihydrate (FIMA) transferred to 3 different 10ml volumetric flask containing few ml of methanol, the volume was made upto mark with methanol to obtained the concentration of stock solution 1000 µg/ml for each of these drugs. Take 1 mL from the Cilnidipine stock solution and 1mL from Fimasartan Potassium Trihydrate stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase to make 100 µg/ml for each of these drugs.

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 up to mark with methanol, six different concentrations for CIL prepared with ranges from 1 – 6 µg/ml and for FIMA with ranges from 6 – 36 µg/ml were prepared from their individual respective stock solutions.

Analysis of Synthetic Mixture [35]

The synthetic mixture of CIL and FIMA was prepared in ratio of 10 mg: 60 mg, respectively. Common excipients like Microcrystalline cellulose (980 mg), Magnesium Stearate (20 mg), (HPMC) Hydroxypropyl methylcellulose K100 (200 mg), Cross Carmellose Sodium (100 mg) were weighed accurately and transfer into motor pestle along with 100 mg of CIL and 600mg of FIMA which is equivalent to 10 tablets. Weight accurately equivalent to 10mg of Cilnidipine and 60mg of Fimasartanpotassium trihydrate and transfer it in 10 ml volumetric flask containing 5.0 ml of methanol and sonicated for 15 min. The solution was filtered using Whatman filter paper No.42 and collects the filtrate in another 10 ml volumetric flask and the residue was wash with 3.0 ml amount of methanol, the filtrate and residue was combined and volume was diluted to the mark with the methanol. Pipette out 1.0 ml aliquot from the above solution, transfer it in another 10 ml volumetric flask and volume made upto the mark with methanol, from above solution, Pipette out 1.0 ml aliquot and transfer it into another 10 ml volumetric flask and makeup volume upto the mark and from the above solution, Pipette out 3.0 ml aliquot from the above solution, transfer it in another 10 ml to obtain final concentration of 3 µg/ml for CIL and 18 µg/ml for FIMA respectively. The possibility of interference from other components of the synthetic mixture in the analysis was studied. It was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of CIL and FIMA were computed using regression equation.

II. RESULT AND DISCUSSION OF RP-HPLC METHOD

Selection of analytical wavelength

The solution of Cilnidipine (CIL) and Fimasartanpotassium trihydrate (FIMA) were prepared in methanol in separate volumetric flask at concentration of 10 µg/ml. It was scanned at range

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

of 200-800 nm. The wavelength of 254 nm was selected as both drugs show considerable absorbance at 254 nm which is shown in Figure 8.

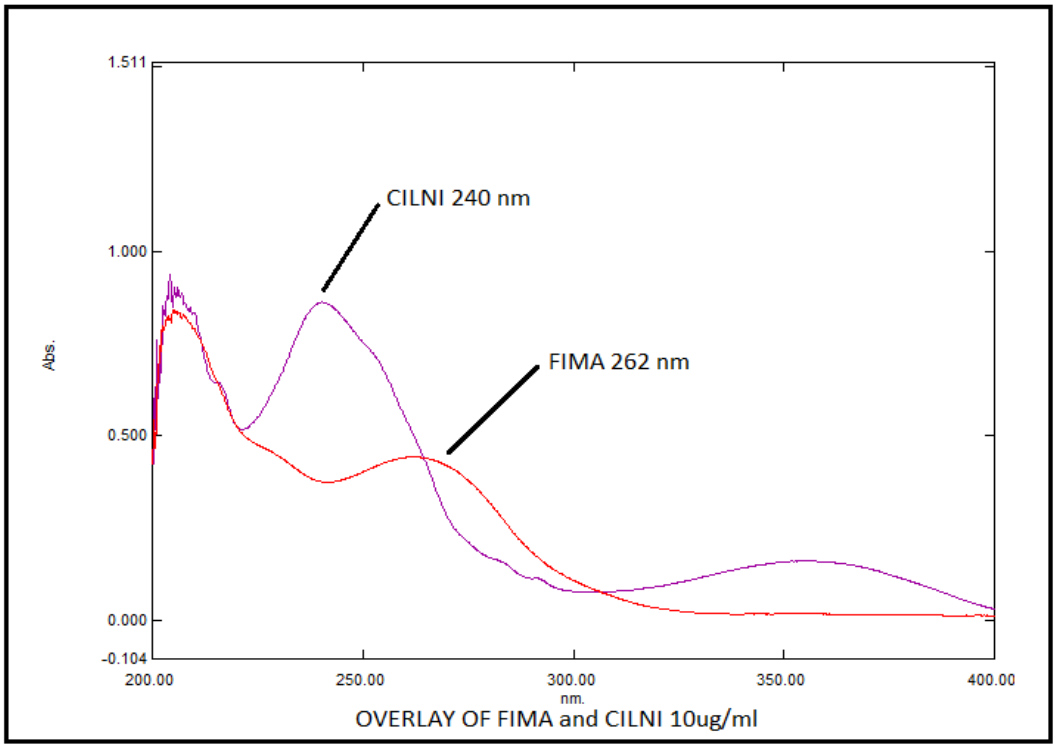

Figure 8 Overlay spectra of CIL (10 µg/ml) and FIMA (10 µg/ml) in methanol

Optimization of mobile phase

The standard solution containing 10 µg/ml of CIL and 10 µg/ml of FIMA were chromatographed using different composition of organic and aqueous solvent Methanol, Acetonitrile, Water etc. as mobile phase were tried. Optimization of mobile phase trialsaresummarizinginbelowTable 6.

Table 6 List of Mobile Phase trials for Cilnidipine and Fimasartan Potassium Trihydrate

Trial. No	Mobile Phase	Observation	Figure No.
1.	Water: Methanol (50:50)	One Peak Observed	6.2
2.	Water: Methanol (50:50)	Peak of Fimasartan Potassium Trihydrate Confirmed	6.3
3.	Water: Methanol (50:50)	Peak of Cilnidipine is not Observed	6.4
4.	Water: Methanol (30:70)	Peak of Cilnidipine Confirmed	6.5
5	Water: Methanol (10:90)	Retention time decreased but second peak did not observe	6.6
6	Water: Acetonitrile (20:80)	Retention Time Decreased	6.7
7	Water: Acetonitrile (20:80)	Second Peak Observed	6.8
8	Water: Acetonitrile (30:70)	Peak of Fimasartan Potassium Trihydrate	6.9

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

		Confirmed	
9	Phosphate Buffer (pH 5): Acetonitrile (80:20)	Resolution is not good	6.10
10	Phosphate Buffer (pH 5): Acetonitrile (70:30)	Resolution increased	6.11
11	Phosphate Buffer (pH 5): Acetonitrile (60:40)	Two pick observed asymmetry factor less than 1.5 & theoretical plate is more than 2000	6.12

Mobile phase of Phosphate Buffer: ACN: (60:40v/v) pH 5.0 adjusted using 0.1M KOH gave 2 sharp peaks of CIL and FIMA with asymmetric factor of 1.42 and 1.40 and Rt of CIL was 3.31±0.01 and Rt of FIMA was 6.26 ±0.01 respectively and hence it was selected as mobile phase for estimation of CIL and FIMA. The flow rate was maintained at 1ml/min. Detection wavelength at 254nm and injection volume was 20 µL. Individual drug peak was confirmed by injecting the CIL and FIMA in optimized chromatographic condition shown in figure 9 and figure 10.

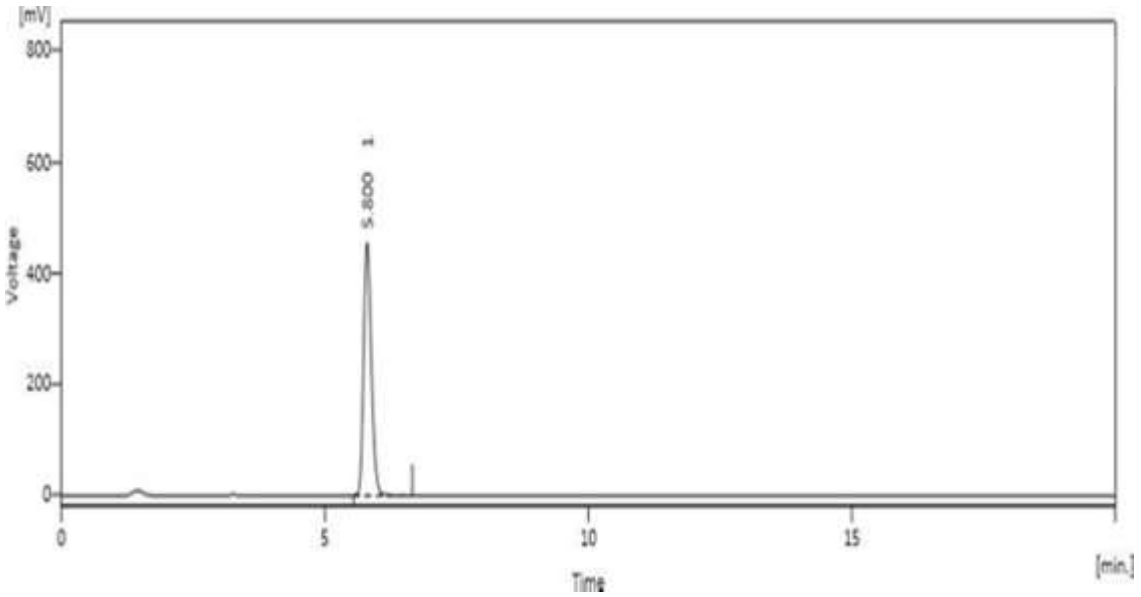

Figure 9HPLC Chromatogram of Cilnidipine and Fimasartan Potassium Trihydrate in Water: Methanol (50:50)

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

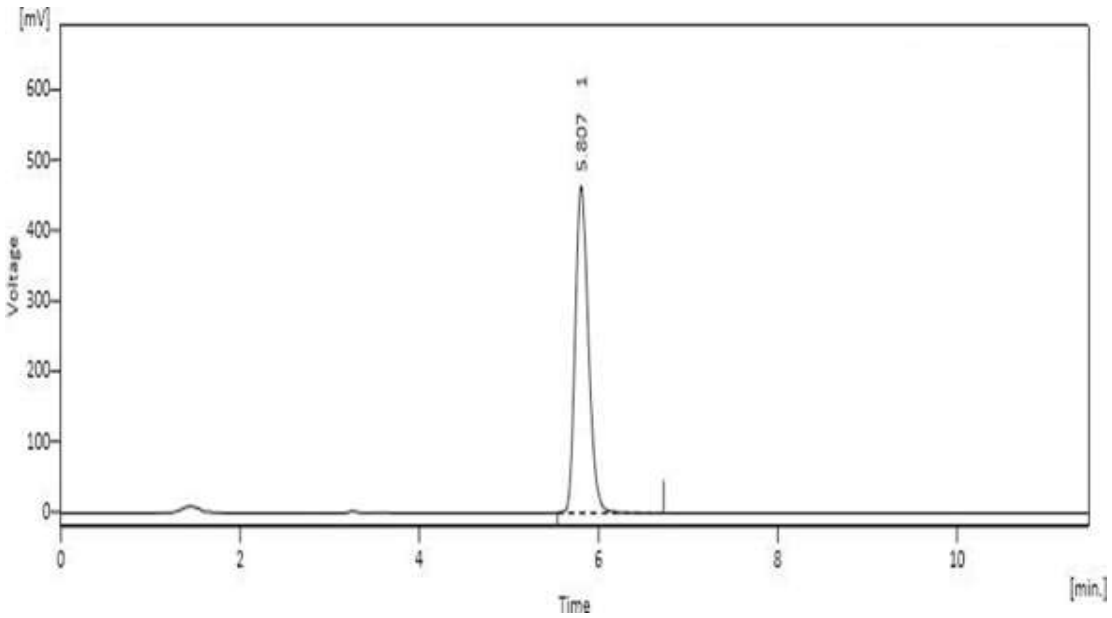

Figure 10HPLC Chromatogram of Fimasartan Potassium Trihydrate, Water: Methanol (50:50)

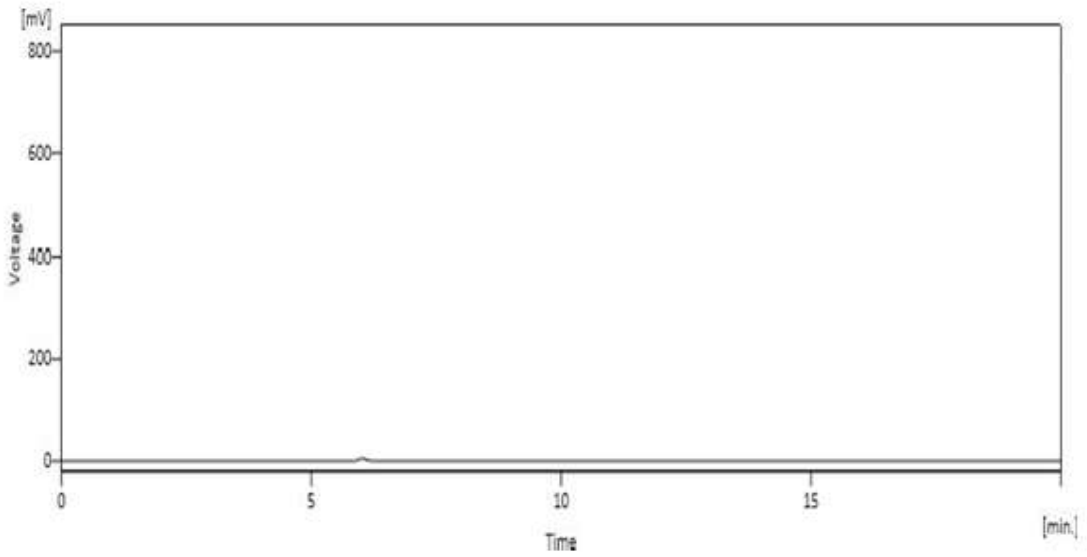

Figure 11HPLC Chromatogram of Cilnidipine in Water: Methanol (50:50)

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

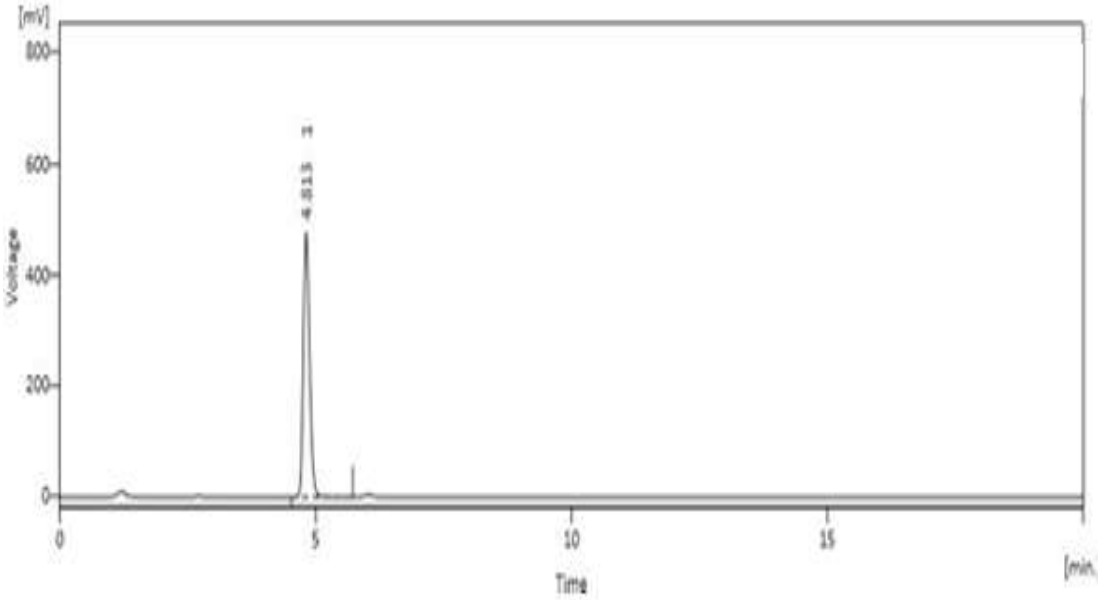

Figure 12HPLC Chromatogram of Cilnidipine 25ppm in Water: Methanol (30:70)

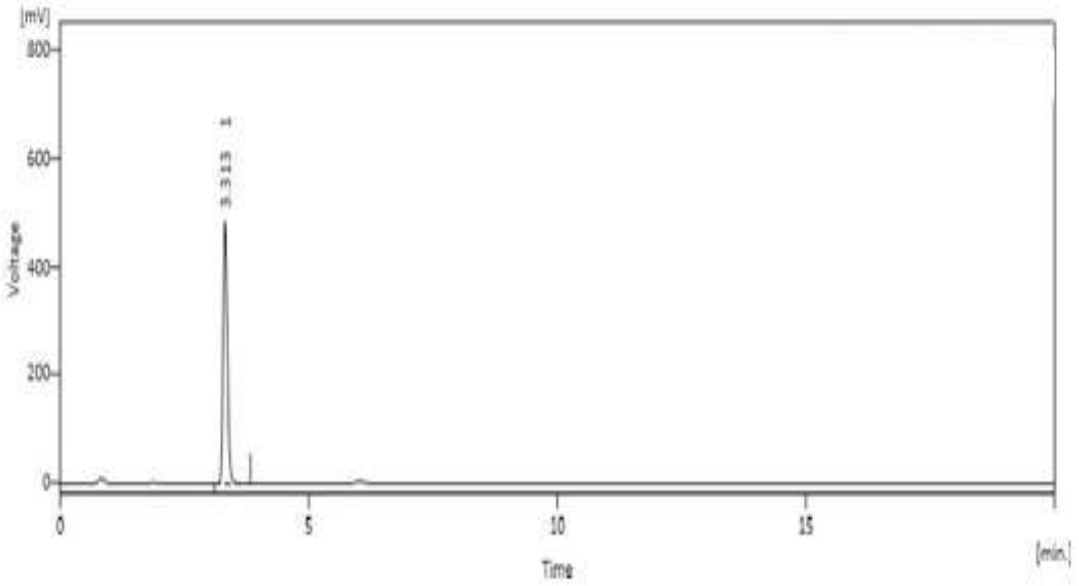

Figure 13HPLC Chromatogram of Cilnidipine and Fimasartan Potassium Trihydrate in Water: Methanol (10:90)

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

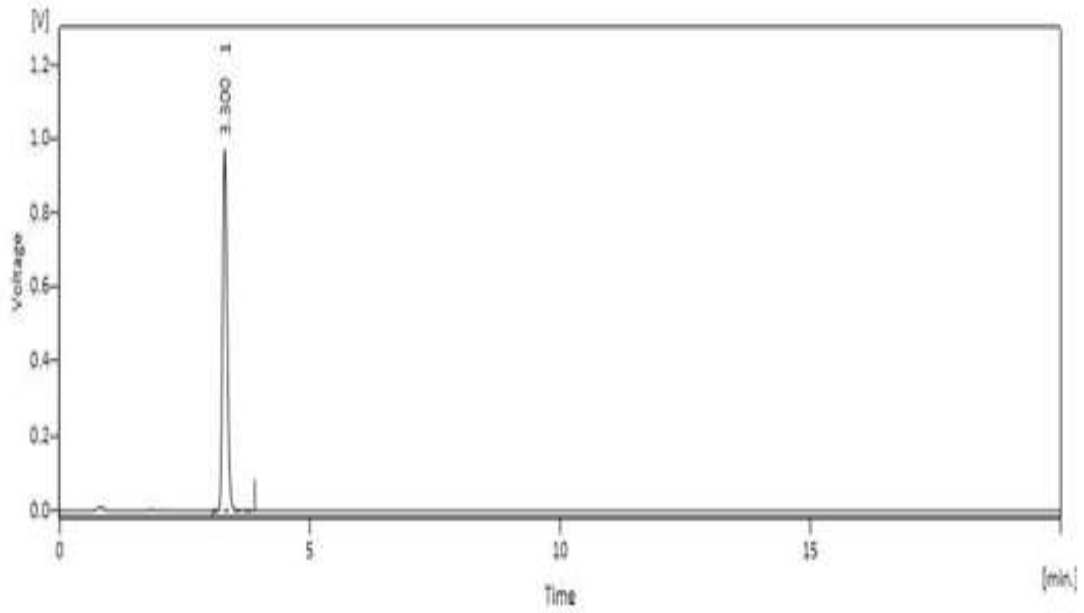

Figure 14HPLC Chromatogram of Cilnidipine and Fimasartan Potassium Trihydrate in Water: Acetonitrile (20:80)

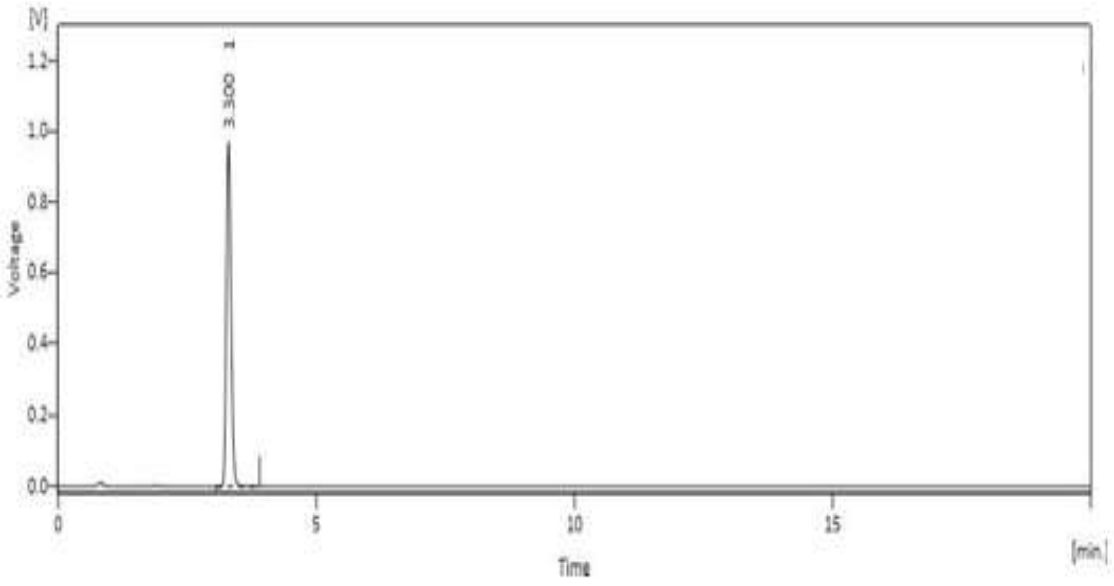

Figure 15HPLC Chromatogram of Cilnidipine and Fimasartan Potassium Trihydrate in Water: Acetonitrile (20:80)

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

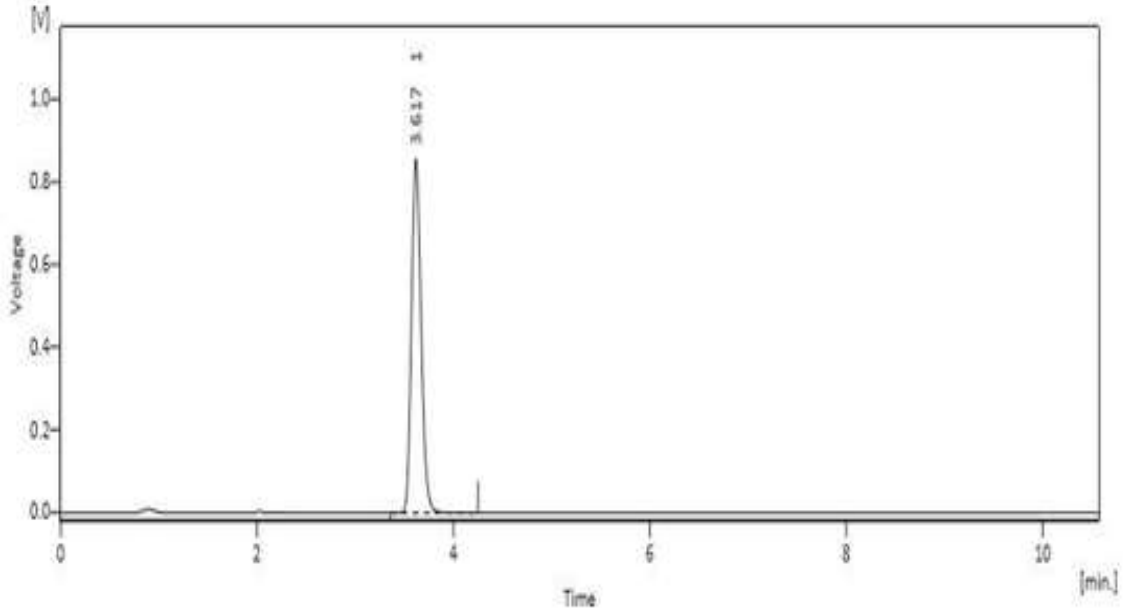

Figure 16HPLC Chromatogram of Cilnidipine in Water: Acetonitrile (30:70)

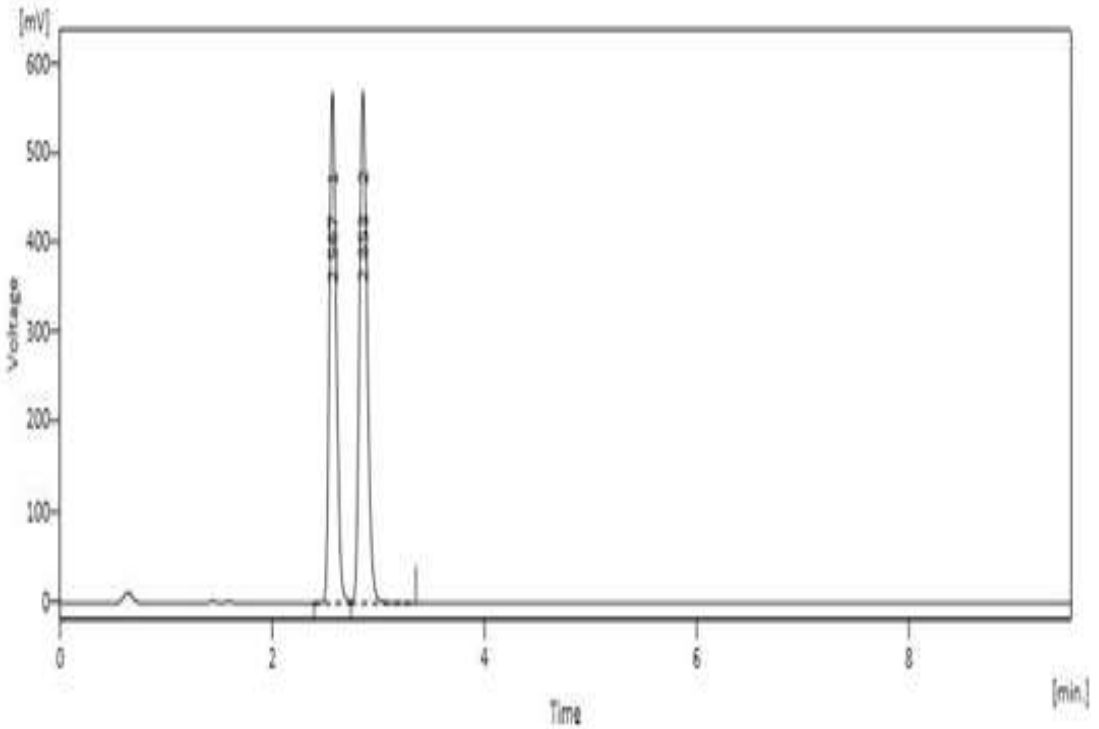

Figure 17HPLC Chromatogram of Cilnidipine and Fimasartan Potassium Trihydrate in Buffer, (pH 5.0): Acetonitrile (80:20)

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

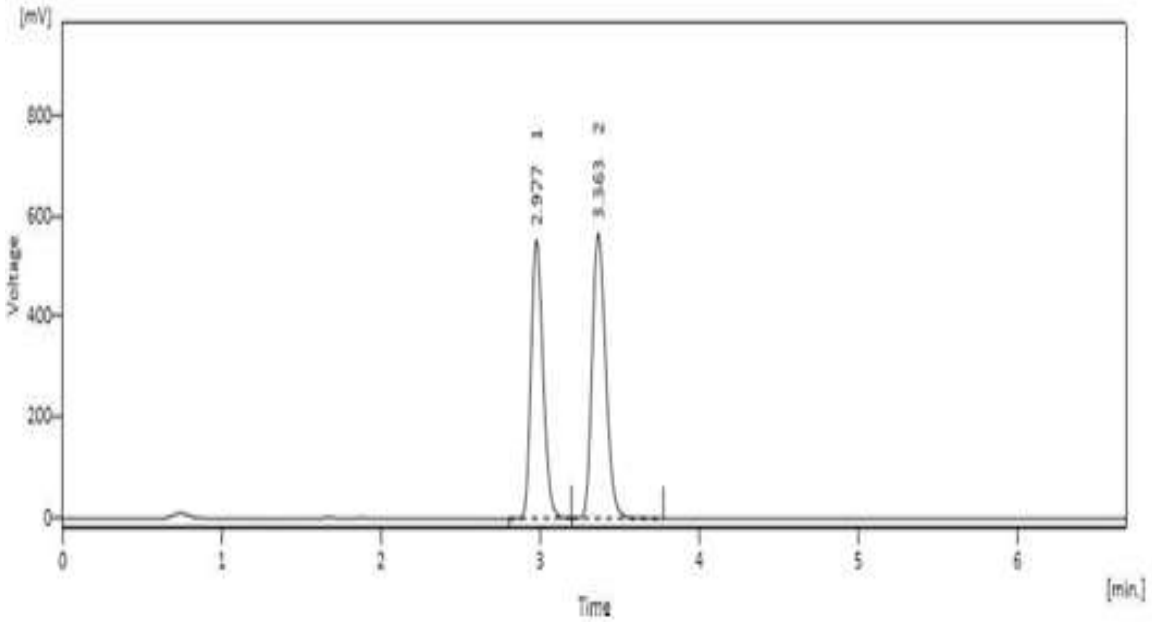

Figure 18HPLC Chromatogram of Cilnidipine and Fimasartan Potassium Trihydrate in Buffer, (pH 5.0): Acetonitrile (70:30)

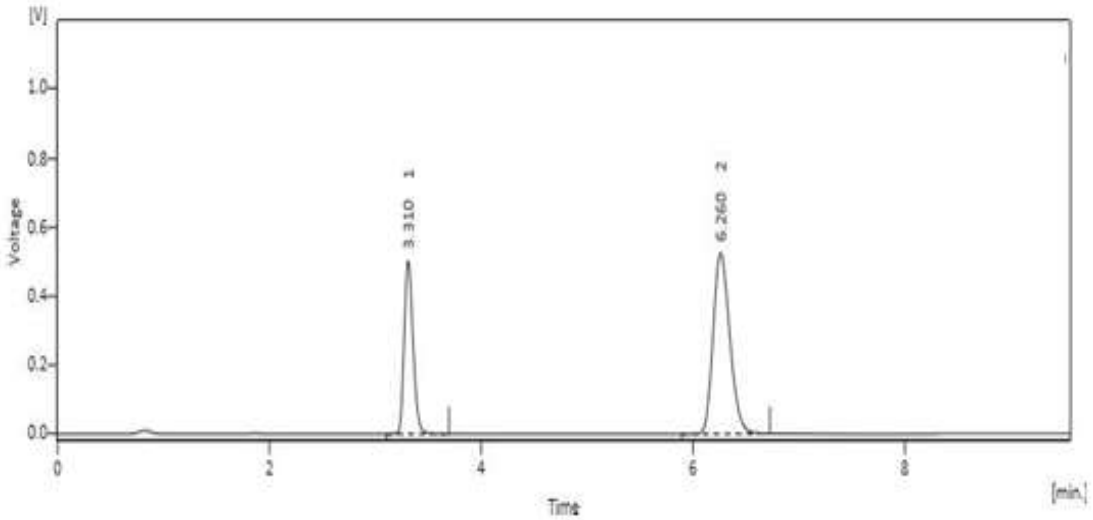

Figure 19HPLC Chromatogram of Cilnidipine and Fimasartan Potassium Trihydrate in Phosphate Buffer, (pH 5.0): Acetonitrile (60:40)

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

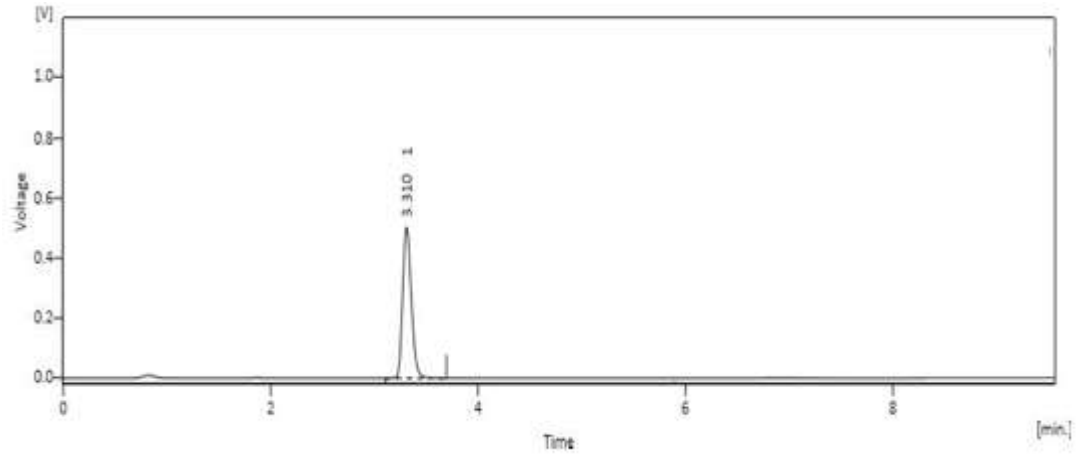

Figure 20HPLC Chromatogram of Cilnidipine in Phosphate Buffer, (pH 5.0): Acetonitrile (60:40)

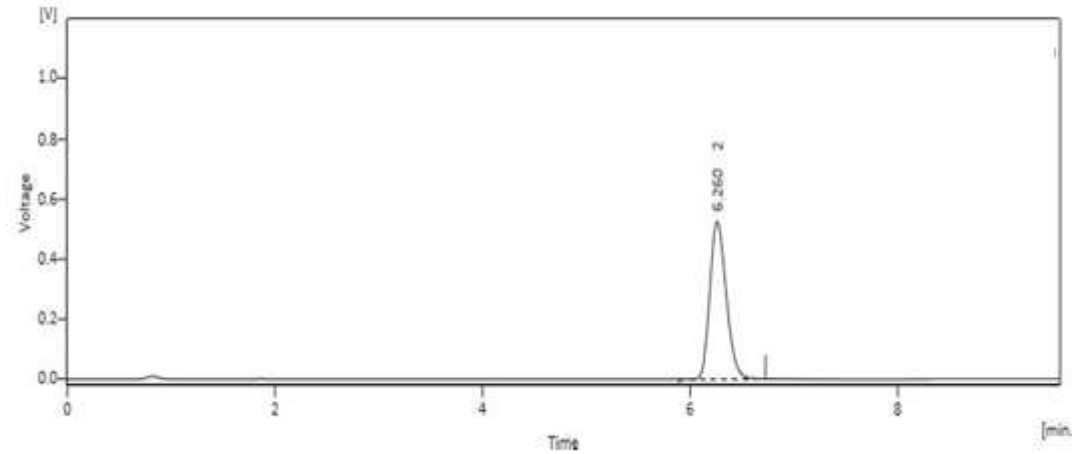

Figure 21HPLC Chromatogram of Fimasartan Potassium Trihydrate in Phosphate Buffer, (pH 5.0): Acetonitrile (60:40)

SYSTEM SUITABILITY TEST

The system suitability test was carried out on freshly prepared working standard stock solution of CIL (2 µg/ml) and FIMA (12 µg/ml), respectively. The system suitability parameters like resolution, theoretical plates and asymmetric factor were calculated and compared with standard values shown in Table7.

Table 7System suitability parameter

Parameters	**Cilnidipine**	**Fimasartan Potassium Trihydrate**
RetentionTime	3.310	6.260
TheoreticalPlates	8903	9364
Asymmetry	1.42	1.40
Resolution	-	13.019

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

METHOD VALIDATION

Linearity and Calibration curve:

Calibration curve for CIL was found to be 1-6 µg/ml with regression coefficient of 0.998 showed in (Figure 22) and For FIMA it was 6-36 µg/ml with correlation coefficient of 0.9983 (Figure 23). Figure 24 shows the overlay chromatogram of CIL (1-6 µg/ml) and FIMA (6-36 µg/ml) at 254 nm. The calibration range was prepared in such a way that the ratio of combination was maintained throughout simultaneous estimation of both drugs in bulk and synthetic mixture. The result of calibration curve and regression analysis of calibration curve are shown in **Table 8, Table 9 & Table 10.**

Table 8Result of calibration curve for CIL at 254 nm

Concentration (µg/ml)	Area ± SD (n=6)	%RSD
1	33463.65 ± 339.74	1.02
2	54683.38 ± 611.53	1.12
3	80543.28 ± 426.05	0.53
4	102481.00 ± 896.58	0.87
5	127887.77 ± 2095.94	1.64
6	156548.57 ± 2555.42	1.66

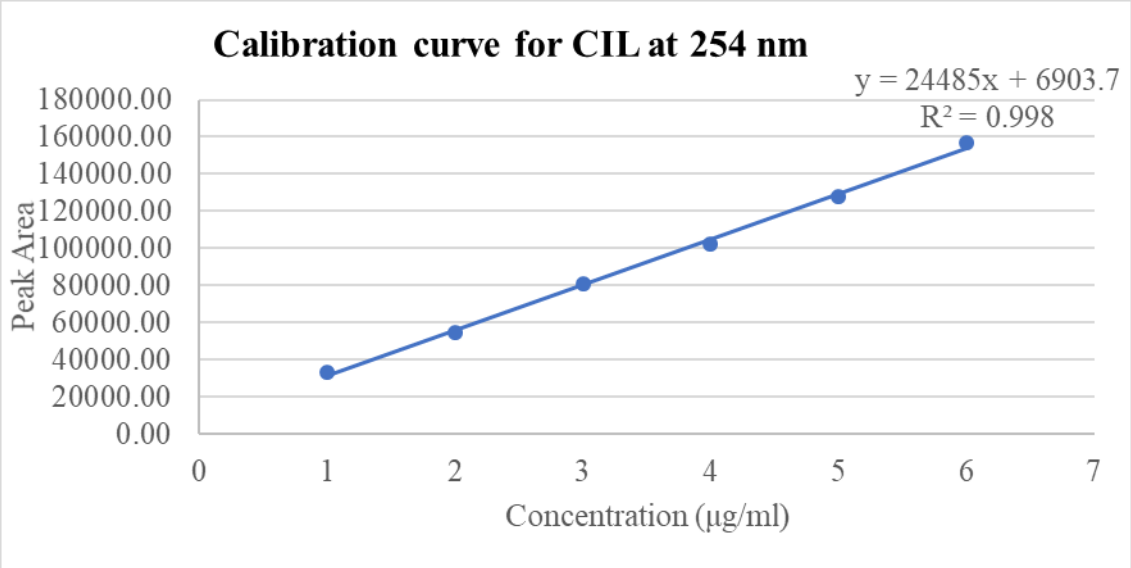

Figure 22Calibration curve for CIL at 254 nm

Table 9Result of calibration curve for FIMA at 254 nm

Concentration (µg/ml)	Area ± SD (n=6)	%RSD
6	72600.55	0.53
12	135371.53	0.47
18	212651.82	0.93
24	293231.32	0.64
30	376143.23	0.76
36	441727.33	1.78

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

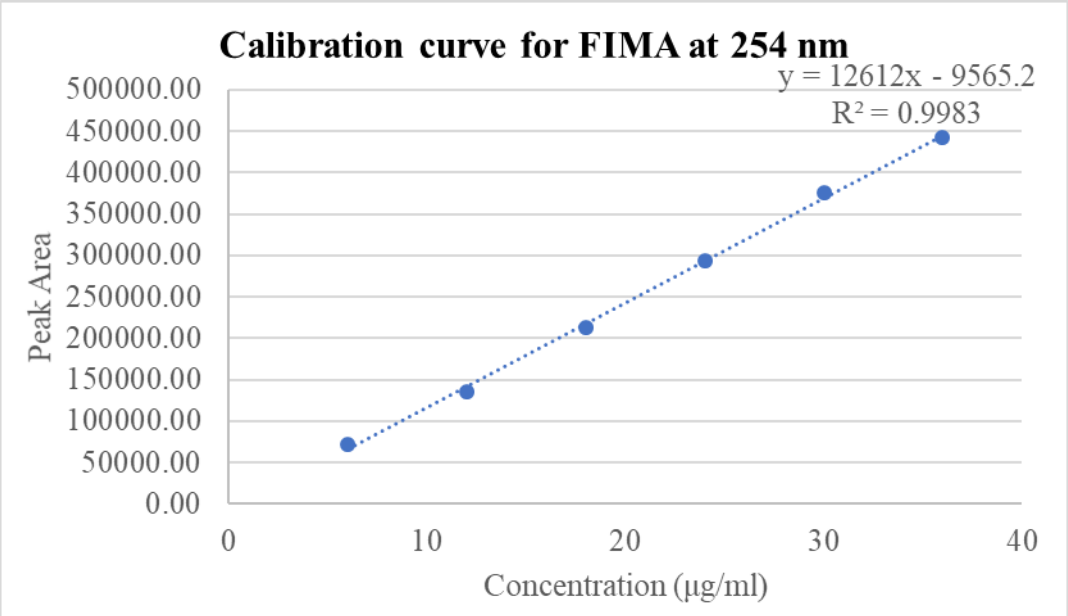

Figure 23Calibration curve for FIMA at 254 nm

Table 10Linear regression parameters for estimation of CIL and FIMA

Parameters	**CIL**	**FIMA**
Calibration range (µg/ml)[a]	1-6	6-36
Regressionequation	y = 24485x + 6903.7	y = 12612x - 9565.2
Standarddeviation Ofslope	541.03	162.13
Standarddeviation Ofintercept	1400.148	2173.41
Correlation coefficient(r^2)	0.998	0.9983

[a]=5replicates,Confidence[b]intervalat95%confidencelevel

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

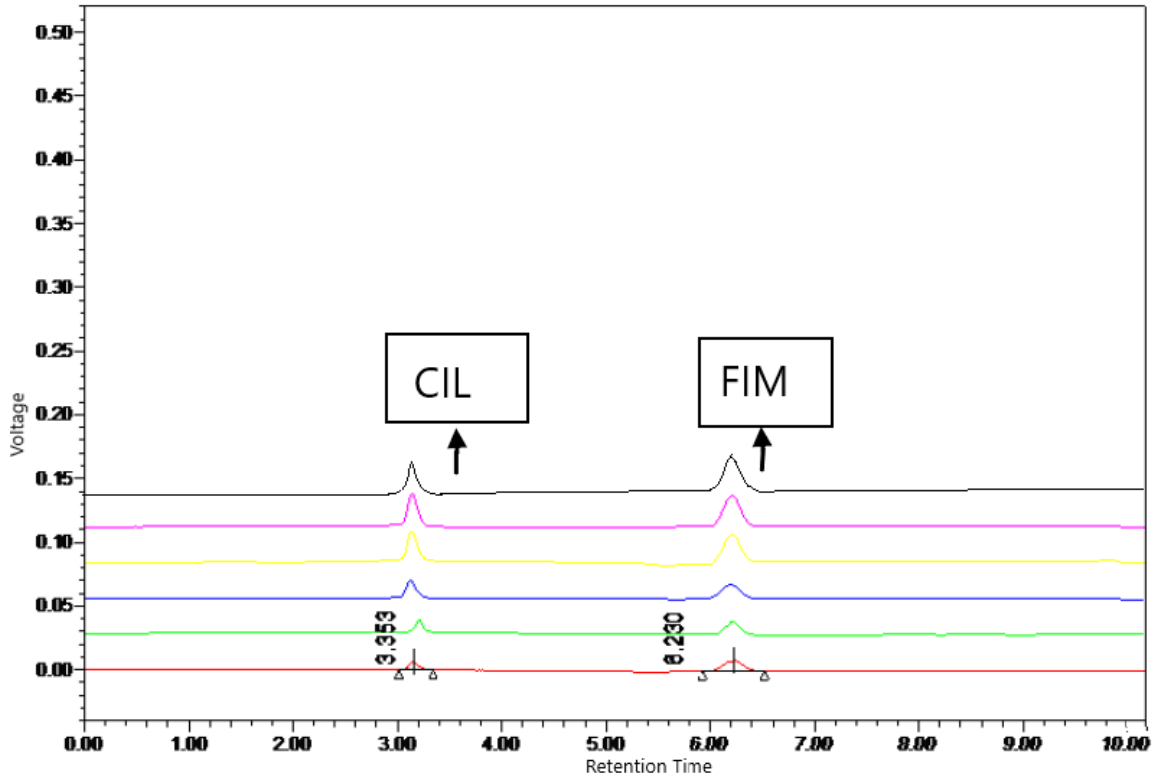

Figure 24shows the overlay chromatogram of CIL (1-6 µg/ml) and FIMA (6-36 µg/ml) at 254 nm

Precision

In RP-HPLC method, repetablity has been carried out by injection repetablity. Injection repetablity was carried out by analysing the samples of CIL and FIMA samples in which CIL(3 µg/ml) and FIMA(18 µg/ml) six times and peak area was measured which was shown in Table 11

Table 11Repeatability data for CIL (3 µg/ml) and FIMA (18 µg/ml)

Sr. No.	CIL (3µg/ml) Peak Area (n=6)	Rt of CIL	FIMA (18µg/ml) Peak Area (n=6)	Rt of FIMA
1	80585.2	3.31	210478.2	6.26
2	81135.8	3.29	211252.5	6.24
3	80747.5	3.32	212795.5	6.25
4	80563.2	3.3	211663.6	6.27
5	80382.4	3.31	213785.8	6.26
6	79845.6	3.33	215935.3	6.26
Average	80543.28333	3.31	212651.82	6.26
SD	426.05	0.01	1987.31	0.01
% RSD	0.53	0.43	0.93	0.17

The precision of method was determined by carring out intraday and interday precision. Intraday precision was determined by analysing sample solution of CIL (1 µg/ml, 3 µg/ml and 6 µg/ml) and for FIMA (6 µg/ml, 18 µg/ml, 36 µg/ml) which covers lower, medium and high concentrations of the calibration curve three times on the same day. Intraday precision was determined by analysing sample solution of CIL and FIMA with same concentration as intraday at three levels covering lower, middle and high concentration over the 3 different successive days. The peak areas obtained were used to calculate mean and % RSD values shown in Table 12. The percentage (%) RSD was found to be less than 2 % which indicate method is precise.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

Table 12 Precision data for CIL and FIMA

Drug	Conc. (µg/ml)	Intraday (n=3) ± SD	% RSD Intraday	Interday (n=3) ± SD	% RSD Interday
CIL	1	33438.57 ± 411.79	1.23	33488.73 ± 342.18	1.02
	3	80822.83 ± 282.92	0.35	80263.73 ± 373.23	0.47
	6	156688.04 ± 1402.24	0.89	158006.84 ± 2177.93	1.38
FIMA	6	72467.83 ± 465.92	0.64	72630.97 ± 720.10	0.99
	18	211903.87 ± 799.07	0.38	213237.20 ± 3958.57	1.86
	30	376966.80 ± 3559.25	0.94	375917.93 ± 4480.83	1.19

Accuracy:

Table 13Accuracy study for CIL and FIMA

Drug	% Level of spike	Amount of drug taken (µg/ml)	Amount of drug spiked	Mean Area^n ± SD	Amount of drug found (µg/ml)	% Recovery
CIL	0	2	0	54999.77 ± 437.31	1.96	98.22
	80	2	1.6	94048.83 ± 277.92	3.56	98.86
	100	2	2	103781.87 ± 1279.26	3.96	98.92
	120	2	2.4	116496.73 ± 2146.15	4.48	101.73
FIMA	0	12	0	139506.60 ± 1477.72	11.82	98.50
	80	12	9.6	267237.20 ± 3736.96	21.95	101.61
	100	12	12	292996.77 ± 2475.97	23.99	99.96
	120	12	14.4	318699.41 ± 2524.30	26.03	98.59

n= Replicate of 3

The accuracy of method was determined by calculating % recovery of drug by standard spiking of 80 %, 100 % and 120 % of standard in pre quantified sample solution from synthetic mixture. For CIL (1.6 µg/ml, 2 µg/ml, 2.4 µg/ml) were spiked, and for FIMA (9.6 µg/ml,12 µg/ml, 14.4 µg/ml) were spiked in prequantified sample solution which were prepared from synthetic mixture. Method was accurate with % recovery of 98.22-101.73 % for CIL and 98.50-101.61 % for FIMA shown in Table 13.

LOD and LOQ:

LODand LOQof CILand FIMA were determined by equation according to ICH guideline. LOD for CIL and FIMA was found to be 0.17 and 0.51µg/ml respectively.LOQ for CIL and FIMA was found to be 0.57 and 1.72µg/ml respectively as shown in Table14 indicating sensitivity of the method.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 222-244 www.ijprajournal.com ISSN: 2249-7781

Table 14LOD and LOQ for HPLC method

Parameters	CIL (µg/ml)	FIMA (µg/ml)
LOD	0.17	0.51
LOQ	0.57	1.72

Robustness

The small deliberate variations in liquid chromatography conditions were used toevaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The results of analysis of robustness study are as shown in Table15, where %RSD less than 2 indicate that the method is robust, and is not affected by small changes in routine use.

Table 15 Robustness study for HPLC method

EFFECT OF CHANGE IN VOLUME OF PHOSPHATE BUFFER									
55 ml				**60 ml**			**65 ml**		
	Peak Area	**%RSD**	**Rt(min)**	**Peak Area**	**%RSD**	**Rt(min)**	**Peak Area**	**%RSD**	**Rt(min)**
CIL	54704.5	0.25	3.68	54941.3	0.444	3.32	54425.2	0.864	3.01
FIMA	135995.5	1.236	6.97	135948.5	0.906	6.26	135575.8	0.122	5.89
EFFECT OF CHANGE IN FLOWRATE									
0.9 ml/min				**1 ml/min**			**1.1 ml/min**		
	Peak Area	**%RSD**	**Rt(min)**	**Peak Area**	**%RSD**	**Rt(min)**	**Peak Area**	**%RSD**	**Rt(min)**
CIL	54738.6	0.25	3.53	54941.3	0.444	3.32	53850.1	0.864	3.2
FIMA	133345.9	1.236	6.56	135948.5	0.906	6.26	135095.3	0.122	6.12
EFFECT OF CHANGE IN DETECTION									
252 nm				**254 nm**			**256 nm**		
	Peak Area	**%RSD**	**Rt(min)**	**Peak Area**	**%RSD**	**Rt(min)**	**Peak Area**	**%RSD**	**Rt(min)**
CIL	54298.7	0.25	3.31	54941.3	0.444	3.32	53450.7	0.864	3.33
FIMA	135345.7	1.236	6.27	135948.5	0.906	6.26	133052.1	0.122	6.26

Specificity

There was no interfering peak at Rt of CIL and FIMA from the excipient added in preparation of synthetic mixture, thereby confirming the specificity of method. Figure 24 shows the chromatogram of mobile phase using Phosphate Buffer: ACN (60:40 v/v) pH 5.0 adjusted using 0.1M KOH.

III. CONCLUSION

A simple, sensitive and accurate RP-HPLC method was developed for simultaneous estimation Cilnidipine and Fimasartanpotassium

trihydrate. In RP-HPLC method, good resolution and separation of two drugs was achieved. The Phosphate Buffer: ACN (60:40 v/v) pH 5.0 adjusted using 0.1M KOH was used as mobile phase and detection wavelength was 254nm. Retention time of Cilnidipine and Fimasartanpotassium trihydrate were found to be 3.310 min and 6.260 min respectively with a flow rate of 1 ml/min.

The proposed RP-HPLC method was specific, accurate, precise and robust. Therefore, proposed method can be used for routine analysis of Cilnidipine and Fimasartanpotassium trihydrate in bulk as well as synthetic mixture.

Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity was tested. Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust.

REFERENCE

[1]. Bryant K., Kingwood D., "A Determination of liquid chromatographic peak purity by electro spray ionization mass spectrometry", J. of Chromato.,**1996,**721,41-51.

[2]. Cameron G., Jackson P., Gorenstein M., " A new approach to peak purity assessment using photodiode array detection",ChemAus.,**1993**,288-9.

[3]. Carr P. & Wahlich J., " A practical approach to method validation in pharmaceutical analysis", J Pharm Biomed Anal.,**1990**,86,613-8.

[4]. Donald D. & Mumtaz S. "Development and validation of HPLC Stability indicating Assays", **2008**,332.

[5]. Shabir G.,"Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities Between Validation Requirements of FDA, the US Pharmacopeia and the ICH", J. Chromatogr.,**2003**,987(1),57-66.

[6]. Georg N., " Forced degradation studies as a integral part of HPLC stability indicating method developme nt", Drug delivery technology. **2010.**

[7]. Huynh K., " Development of Stability indicating methods.Handbook of Stability Testing in Pharmaceutical Development",**2009**,154.

[8]. Michael J., " Regulatory Considerations for Stability Indicating Analytical Methods in Drug Substance and Drug Product Testing", American Pharm. Review. **2005**,8(3),51-4.

[9]. Monika B. & Saranjit S., "Development of validated stability-indicating assay methods-critical review", J. Pharm. Biomed. Anal., **2002**,28(6),1011-40.

[10]. Reynolds D., Facchine K., Mullaney J., Alsante K., "Available guidance and best practices for conducting forced degradation studies", Pharm Tech.,**2002,**48-56.

[11]. Ruan J., Tattersall P., Lozano R., Shah P., "The role of forced degradation studies in stability indicating HPLC method development", Am Pharm Rev.,**2006,**9,46-53.

[12]. Stepensky D., Chorny M., Dabour Z., "Schumacher I. Long-term stability study of Ladrenaline injections: kinetics of sulfonation and racemization pathways of drug degradation", J Pharm Sci.,**2004**,93,969-80.

[13]. Swartz M. & Krull I., "Developing and Validating Stability- Indicating Methods", LCGC North America.,**2005**,23(6),586- 593.

[14]. www.wikipedia.com

[15]. www.drugbank.com

[16]. A. Lakshmana Rao, Mukkanti Eswarudu and and K. Vijay, Bioanalytical method development and validation for simultaneous determination of chlorthalidone and cilnidipine drugs in human plasma by rp-hplc, International Journal of Research In Pharmacy And Chemistry, 2019, 9(1), 33-44.

[17]. Buchiya F., Hasumati R. , Vineet J., Mihir B., and Kaushik P., Method development and validation of RP-HPLC for simultaneous estimation of cilnidipine and valsartan in synthetic mixture, International Journal of Pharmaceutical Chemistry and Analysis 2020;7(3):119–124.

[18]. Hyeon W, Abid M., Kwan H. and Han C. (2014), A Evaluation of stability and simultaneous determination of Fimasartan and amlodipine by a HPLC method in combination tablets, Asian journal of pharmaceutical sciences, 9 (2014), 123-128.

[19]. Dr. Nutan Rao and Kritika Rai, development and validation of novel rp- hplc method for related substances in chlorthalidone and Fimasartan formulations, World Journal of Pharmaceutical Research, 2020, 9 (4), 828-841.

[20]. Sankar R. * , V. Swathi and P. Srinivasa Babu, DEVELOPMENT AND VALIDATION OF NOVEL UV AND RP-HPLC METHODS FOR DETERMINATION OF CILNIDIPINE (A NEW GENERATION Ca CHANNEL BLOCKER) IN PHARMACEUTICAL DOSAGE FORM, IJPSR, 2019; Vol. 10(4): 1886-1894.

[21]. Pranavkumar S. and Bhavin D. (2020), Related Impurities High-performance Liquid Chromatography Method Development and Validation for drug combinations: Olmesartan Medoxomil, Chlorthalidone and Cilnidipine, International Journal of Pharmaceutical Sciences and Drug Research, 2020, 12(1): 1-10.

[22]. Haarika B. and Prabhakar V., " A Rp-Hplc Method Development For The Estimation Of Rizatriptan Benzoate From Formulated Fast Disintegrating Sublingual Tablets", IJPSR, 2014, 5(9): 3784-3789.

[23]. Pawanjeet J. and Apparao K., "Devolpment And Validation Of Simple Stability Indicating Rp-Hplc Method For Analysis Of Saxagliptin And Its Forced Degradation Impurities In Bulk Drug And Pharmaceutical Dosage Form", Int. J. Res. Dev. Pharm. L. Sci.,2014, 3(3), 993-1003.

[24]. Neeraj V. and Sangeeta P., "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Nebivolol and Indapamide in Pharmaceutical Dosage Form", Asian J. Pharm. Ana. 2014,. 4,(3),98-102.

[25]. Aruna P. and Uma R., "Analytical method development and validation of simultaneous estimation of dosulepin and Methylcobalamin in tablet dosage form by RP-HPLC", Int J Pharm 2014, 4(3), 267-274.

[26]. Dhara B., "Relative Degradation Study of Local Vs Multinational Brand of Diltiazem by Means of UV Spectrophotometer", AJPP, 2014, 2(1), 10-18.

[27]. Shashe P and Rao v., "Rp-Hplc Method Development and Validation for Simultaneous Estimation of Gabapentin and Methylcobalamin in Tablet Dosage Forms", IJPRA, 2014, 7(4), 388-392.

[28]. Bhatt K. and Ashwin M., "Simultaneous Estimation of Pregabalin and Methylcobalamin in Pharmaceutical Formulation by RP-HPLC Method", J Anal Bioanal Techniques 2013, 4(1),1-4.

[29]. Nandini R. and Seema S., "A simple and validated RP-HPLC method for the estimation of methylcobalamin and Alphalipoic acid in soft gelatine capsule dosage form", Der Pharmacia Sinica, 2013, 4(5):46-53.

[30]. C Prabhakar., et al. "Method Development and Validation of Azelnidipine by RP-HPLC". International Journal of Chem Tech Research 10.10 (2017): 418-423.

[31]. Amin AA., et al. "Simultaneous Determination of Azelnidipine and Olmesartan Medoxomil in Pharmaceutical Dosage Forms by UFLC Method". Journal of Pharma SciTech (2016): 69-74. 14.

[32]. Gore MG and Dabhade PS. "Rp-Hplc Method Development and Validation of Azelnidipine". IJPSR (2016): 5111-5114.

[33]. Muralidharan S., et al. "Simple Validation of Azelnidipine By Rp-HPLC Method". Rapports De Pharmacie 1.1 (2015): 43-45.

[34]. Ganduri RB., et al. "Stability Indicating Liquid Chromatographic Method for the Simultaneous Determination of Olmesartan Medoxomil and Azelnidipine in Combined Tablet Dosage Form". IJPSR (2014): 275-282.

[35]. Patel NK and Patel JK. "Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Azelnidipine and Olmesartan in Their Combined Dosage Form". Scientia Pharmaceutica 82 (2014): 541-554.