ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FIMASARTAN POTASSIUM TRIHYDRATE AND CILNIDIPINE IN SYNTHETIC MIXTURE BY HPLC METHOD FOR THE TREATMENT OF HYPERTENSION

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Abstract

This study aimed to develop and validate an analytical method for the simultaneous estimation of Fimasartan Potassium Trihydrate and Cilnidipine in a synthetic mixture using the HPLC method for the treatment of hypertension. A specific, accurate, precise, robust, and cost-effective HPLC method was developed and validated for the quantitative analysis of Fimasartan Potassium Trihydrate and Cilnidipine in a fixed-dose combination. The isocratic elution was performed using a Kromasil C18 column (250 mm × 4.6 mm ID, 5 µm) at 40°C. The mobile phase consisted of Methanol: 0.1% OPA in water (80:20% v/v) at a flow rate of 1.0 mL/min. The injection volume was 20 µL, and UV detection was carried out at 223 nm with a total run time of 12 minutes. Fimasartan Potassium Trihydrate and Cilnidipine were eluted at retention times of 3.57 min and 7.99 min, respectively. The method was validated as per ICH guideline Q2(R1). Calibration plots were linear over the concentration ranges of 6-90 µg/mL for Fimasartan Potassium Trihydrate and 1-15 µg/mL for Cilnidipine, with correlation coefficients of 0.99998 and 0.99999, respectively. Accuracy results ranged from 99.61-100.00% for Fimasartan Potassium Trihydrate and 99.61-99.18% for Cilnidipine. The LOD values were 0.708 µg/mL and 0.081 µg/mL, and the LOQ values were 2.146 µg/mL and 0.244 µg/mL for Fimasartan Potassium Trihydrate and Cilnidipine, respectively. The results demonstrated that the developed HPLC method is specific, accurate, precise, and reliable for the routine analysis of Fimasartan Potassium Trihydrate and Cilnidipine in synthetic mixtures.

Keywords: HPLC, Cilnidipine, Fimasartan Potassium Trihydrate, Method Development, Validation

1. INTRODUCTION

High-Performance Liquid Chromatography (HPLC), also known as High-Pressure Liquid Chromatography, is one of the most popular analytical techniques used for the separation, identification, and quantification of individual components within a mixture. HPLC is an advanced form of column liquid chromatography [1,2]. Unlike traditional liquid chromatography, where solvents flow through the column under gravity, HPLC employs high pressures—up to 400 atmospheres—to force the solvent through the column. This facilitates the separation of components in a sample based on their differences in relative affinities to the stationary and mobile phases [3-5].

In HPLC, pressurized liquid solvent containing the sample mixture is passed through a column packed with a solid adsorbent material. Separation occurs due to the varying degrees of interaction between the analytes and the stationary phase, enabling precise quantification and analysis [6,7]. An analytical method is developed to test specific characteristics of a drug substance or product against established acceptance criteria [8-10]. The choice of instrumentation and methodology during the development of an analytical procedure is guided by the intended purpose and scope of the method [11-13].

Method validation is a critical process that establishes documented evidence, ensuring the reliability, accuracy, and precision of an analytical method for its intended use. This process provides a high level of confidence that the analytical results meet predefined quality and performance standards [14,15].

Hypertension, commonly known as high blood pressure, is defined as a blood pressure measurement ≥140/90 mmHg. It is a major risk factor for stroke, cardiovascular diseases, chronic kidney disease, and premature mortality worldwide. Normal systolic blood pressure ranges between 130-140 mmHg, and diastolic blood pressure between 80-90 mmHg [16,17]. Effective management of hypertension often requires a combination of antihypertensive agents to achieve optimal blood pressure control.

Fimasartan Potassium Trihydrate is a novel angiotensin II receptor blocker (ARB) with potent and selective activity against AT1 receptors. It is used for the treatment of mild to moderate essential hypertension. Chemically, it is potassium 5-[4'-({2-butyl-5[(dimethylcarbamothioyl)methyl]-4-methyl-6-oxo-1,6-dihydropyrimidin-1-yl}methyl)-[1,1'-biphenyl]-2-yl]-1H-1,2,3,4-tetrazole-1-ide trihydrate [18]. Its molecular structure is presented in Figure 1.

Cilnidipine is a dihydropyridine calcium channel blocker used for the treatment of hypertension. It acts on both L-type and N-type calcium channels, contributing to its dual mechanism of action and unique pharmacological profile. Chemically, it is 3-(2-methoxyethyl) 5-(2E)-3-phenylprop-2-en-1-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [19,20]. Its molecular structure is shown in Figure 2.

This study aims to develop and validate a specific, accurate, precise, robust, and cost-effective HPLC method for the simultaneous estimation of Fimasartan Potassium Trihydrate and Cilnidipine in a synthetic mixture.

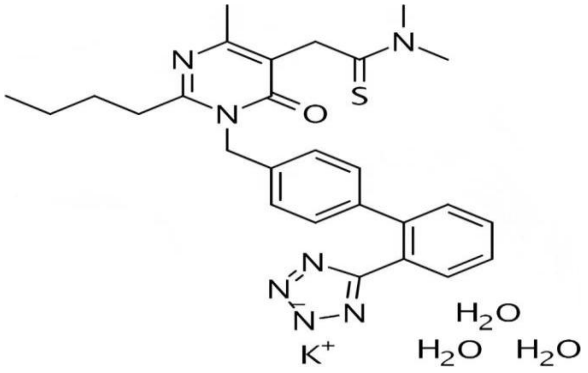

Figure 1. Chemical structure of Fimasartan Potassium Trihydrate

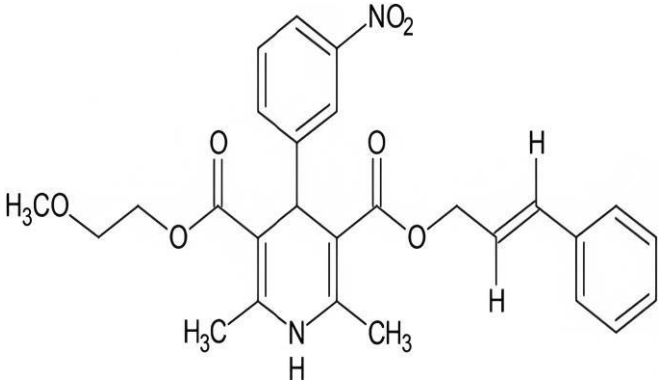

Figure 2. Chemical structure of Cilnidipine

2. MATERIALS AND METHODS

2.1. Materials and Instrumentation

Fimasartan Potassium Trihydrate was obtained from Vidisha Analytical Laboratories, Nashik. Cilnidipine provided by reputed company. HPLC grade Methanol, Water and Ortho-phosphoric Acid (OPA) were used. All other chemicals were analytical reagent grade. Chromatographic analysis was carried out using an Agilent Technologies HPLC, UV detector, and Openlab EZ Chrome software for data acquisition. A 4.45 µm PVDF filter was used for filtration

2.2. Chromatographic condition

The isocratic elution was accomplished by Kromasil C18, 250 mm X 4.6 mm ID, 5 µm at 400C. Mobile phase composition is Methanol: 0.1% OPA in water (80:20 %v/v) at a flow rate of 1.0 mL/min, injection volume 20 µL with UV detection at 223 nm, with run time 12 minutes.

2.3. Preparation of Standard Stock Solution

A standard stock solution of 100 µg/ml for Fimasartan Potassium Trihydrate was prepared by using methanol as diluent.

2.4. Preparation of working solution of the mixture of Fimasartan Potassium Trihydrate and Cilnidipine

A working solution of 60 µg/ml for Fimasartan Potassium Trihydrate and 10 µg/ml for Cilnidipine were prepared by appropriate dilution.

2.5. Calibration Standards

Six standard concentrations with 6, 30, 60, 75 and 90 µg/mL were prepared from the stock solution of Fimasartan Potassium Trihydrate drug powder (pure form). Similarly, six standard strengths with 1, 5, 10, 12.50 and 15 µg/mL were prepared from the stock solution of Cilnidipine drug powder (pure form).

2.6. Preparation of Mobile Phase

A mixture of Methanol and 0.1% OPA in water in the volume ratio 80:20%v/v for method optimization prepared, mixed well and sonicated to degas the mixture.

2.7. System suitability

The typical values for evaluating system suitability of a chromatographic procedure include the RSD < 1%, Asymmetry factor < 2, and Theoretical plates > 2000. The determination of system suitability of the analytical method was accomplished by assaying three samples of the same strength as Fimasartan Potassium Trihydrate and Cilnidipine. The sample concentration of Fimasartan and Cilnidipine used in this analysis was 60 µg/mL and 10 µg/mL respectively. The retention time, peak area, theoretical plates, and asymmetry factor were evaluated for system suitability.

2.8. Linearity

The linearity response was determined by analyzing 05 independent levels of calibration curve in the range from 10% to 150% (10%, 50%, 100%, 125% & 150%) of working concentration (6, 30, 60, 75 & 90 µg/ml) for Fimasartan and (1, 5, 10, 12.50 & 15 µg/ml) for Cilnidipine. Each level injected in triplicate and mean area calculated. Calibration curve was plotted graphically as a function of analyte concentration in µg/ml on X-axis Vs mean area on y-Axis as given in results. Correlation coefficient and regression line equations for Fimasartan and Cilnidipine were calculated .

2.9. Accuracy

The accuracy of the method was performed in triplicate at three different concentration levels of 50, 100 and 150% of the targeted concentration of drugs. The accuracy of the method was evaluated by calculating percentage recovery.

2.10. Precision

Repeatability was performed under 6 replicates at the assay concentration of Fimasartan and Cilnidipine. Intra-day and inter-day variations of Fimasartan and Cilnidipine were performed in triplicate at three different concentration levels of 50, 100, and 150%. Results are expressed in the form of RSD.

2.11. Specificity

Specificity was performed by injecting diluent, placebo and sample solution to check the interference of excipients.

2.12. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by formula as given in ICH (Q2R1). LOD = 3.3 × Standard deviation/Slope LOQ = 10 × Standard deviation/Slope

2.13. Robustness

The robustness of the method was established by introducing small deliberate change in experimental conditions like flow rate, pH of Mobile phase and Mobile phase composition. The changes made in flow rate ± 0.1 mL/min, for pH of Mobile phase ± 0.2 and mobile phase composition ± 2%.

2.14. Assay

The combination of Fimasartan Potassium Trihydrate (60 mg) and Cilnidipine (10 mg) (Film Coated Tablet) is approved by CDSCO for Phase-III clinical trial. So, there is no any single marketed pharmaceutical formulation is available. The sample (synthetic mixture) was analyzed for assay containing 60µg/mL of Fimasartan Potassium Trihydrate and 10µg/mL of Cilnidipine by optimized HPLC method. (Conc. or dose ratio is suggested by CDSCO).

3. RESULT

3.1. Optimized condition for method development

Various mobile phase compositions, different columns, pH, flow rate were investigated and the best result was achieved on Symmetry C18 (150 mm × 4.6 mm, 5 µm) column with the mobile phase composition was Methanol: 0.1% OPA in Water (80:20 v/v). The detection was carried out at 223 nm with 1.0 mL/min flow rate. The retention time of Fimasartan and Cilnidipine is 3.57 and 7.99 min respectively with a total run time of 10 min. The optimized chromatogram and conditions are mentioned in Figure 3 and Table 1.

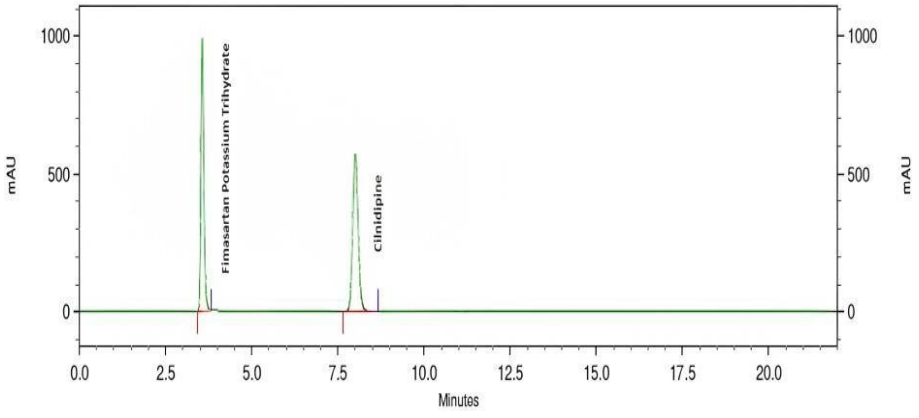

Figure 3: Optimized chromatogram

Table 1: Optimized Chromatographic Conditions

Parameter	Description
Mode	Isocratic
Column Name	Kromasil C18, 250 mm X 4.6 mm ID, 5 µm id.
Mobile Phase	Methanol: 0.1% OPA in water (80:20%V/V)
Flow Rate	1.0 ml/min
Column Oven Temp	40ºC
Injection Volume	20 µl
Wavelength	223 nm
Detector	UV Detector
Diluent	Mobile Phase
Run Time	12 Minutes
Retension Time	Fimasartan 3.57 & Cilnidipine 7.99

3.2 System suitability parameter

The optimized method is acceptable as system suitability parameters are valid as it passed the criteria of acceptability, as shown in Table 2.

Table 2: System suitability parameters

Name	Retention time	Area	Asymmetry	Theoretical plate (USP)	Resolution (USP)
Fimasartan	3.57	55610791	1.20	8571	0.00
Cilnidipine	7.99	7859103	1.14	12331	20.03
Total		62469894			

3.2 Method Validation

3.3.1 Linearity

The calibration curve obtained for Fimasartan and Cilnidipine in the range of 6-90 µg/mL and 1-15 µg/mL and the correlation coefficient were found to be 0.99998 and 0.99999 respectively. Linearity graph of both the drugs is revealed in Figure 4 & 5.

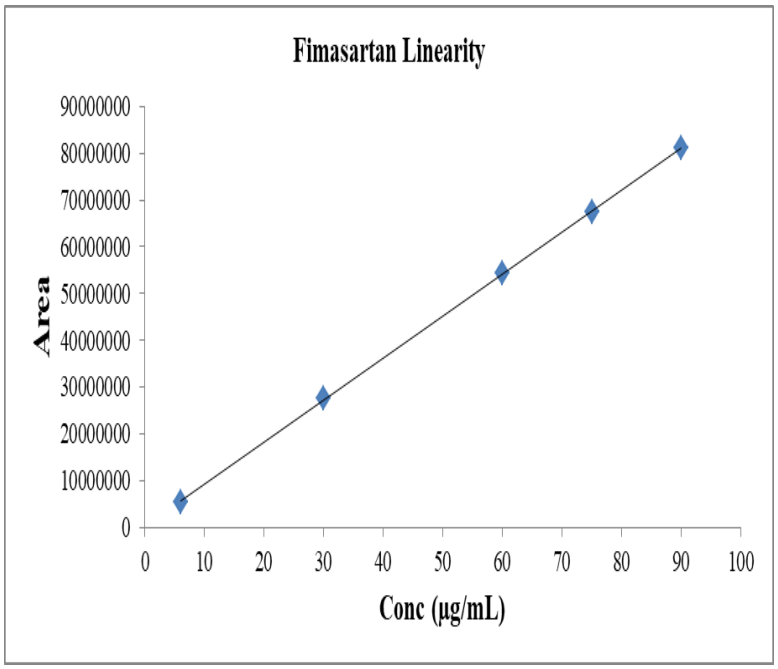

Figure 4: Calibration curve of Fimasartan

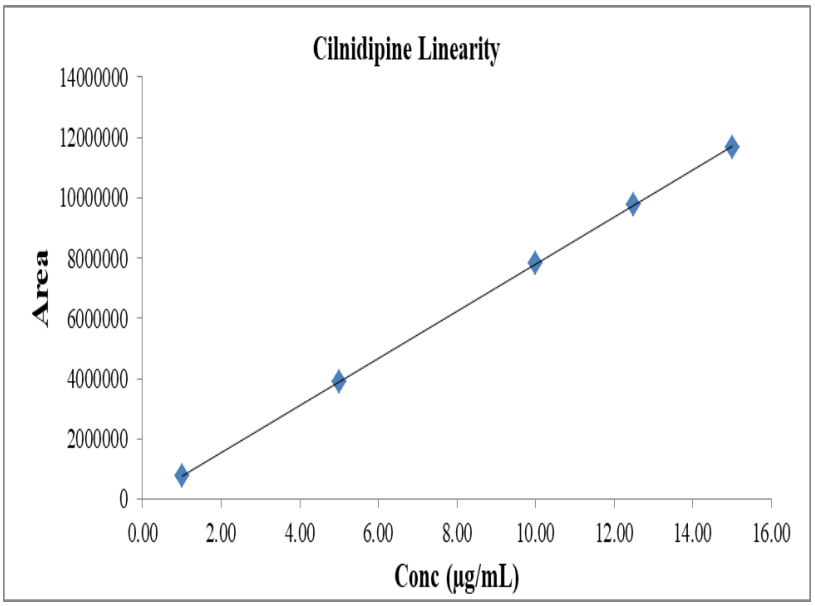

Figure 5: Calibration curve of Cilnidipine

3.3.2 Accuracy

This method is accurate as % recovery was found in the range of 99.16-100.00% for Fimasartan, while for Cilnidipine it was found to be in the range of 99.05-99.61% are shown in Table 3 and 4.

Table 3: Result and statistical data of Accuracy of Fimasartan

Level (%)	Area	Fimasartan Recovered conc (µg/mL)	Fimasartan Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	26792502	29.88	30.20	98.94	99.61	0.658
	27146850	30.28	30.20	100.25		
	26893250	29.99	30.10	99.65		
100	53249720	59.39	60.10	98.82	99.16	0.318
	53649100	59.83	60.30	99.23		
	54121975	60.36	60.70	99.44		
150	81006470	90.34	90.20	100.16	100.00	1.079
	80297553	89.55	90.60	98.85		
	81590204	91.00	90.10	100.99		

Table 4: Result and statistical data of Accuracy of Cilnidipine

Level (%)	Area	Cilnidipine Recovered conc (µg/mL)	Cilnidipine Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4019632	5.18	5.20	99.64	99.61	0.613
	3964450	5.11	5.10	100.20		
	4069682	5.25	5.30	98.98		
100	7763072	10.01	10.20	98.10	99.05	0.841
	7809654	10.07	10.10	99.67		
	7786001	10.04	10.10	99.37		
150	11694034	15.07	15.30	98.52	99.18	1.304
	11597316	14.95	15.20	98.35		
	11949720	15.40	15.30	100.67		

3.3.3 Precision

Repeatability and intermediate precision express in term of RSD. As RSD was found to be < 2 indicating the presented method is precise. The results are summarized for repeatability study and intermediate precision in Table 5 & 6 for fimasartan and cilnidipine respectively.

Table 5: Results of Intra-day and Inter-day Precision for test sample assay of Fimasartan

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	300.3	53891364	100.07
	Sample 2	300.2	53406190	99.20
	Sample 3	299.8	53201846	98.96
	Sample 4	300.1	53156479	98.77
	Sample 5	299.9	52798715	98.17
	Sample 6	300.4	53978103	100.20
	Mean			**99.23**
	STD DEV			**0.7809**
	% RSD			**0.787**
Intermediate Precision (Inter-Day)	Sample 1	300.1	53791042	99.95
	Sample 2	300.2	53000397	98.45
	Sample 3	299.9	53006744	98.56
	Sample 4	300.1	53209764	98.87
	Sample 5	299.8	53469701	99.46
	Sample 6	300.3	53974003	100.23
	Mean			**99.25**
	STD DEV			**0.7408**
	% RSD			**0.746**
Repeatability Plus	**Mean**			**99.242**
Inter-day	**STD DEV**			**0.7258**
	% RSD			**0.731**

Table 6: Result of Intra- day and Inter- Day Precision for Cilnidipine test sample assay

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	**Sample 1**	300.3	7741679	99.69
	Sample 2	300.2	7610314	98.03
	Sample 3	299.8	7703476	99.36
	Sample 4	300.1	7611031	98.07
	Sample 5	299.9	7691079	99.17
	Sample 6	300.4	7610395	97.97
	Mean			98.72
	STD DEV			0.776978
	% RSD			0.787
Intermediate precision (Inter-Day)	**Sample 1**	300.1	7619862	98.19
	Sample 2	300.2	7758123	99.94
	Sample 3	299.9	7603645	98.04
	Sample 4	300.1	7802623	100.54
	Sample 5	299.8	7726943	99.67
	Sample 6	300.3	7672980	98.81
	Mean			99.20
				1.007832
	% RSD			1.016
Repeatability Plus Inter-day	**Mean**			98.957
	STD DEV			0.89391
	% RSD			0.903

3.3.4 Specificity

Excipients interference is not observed at the working wavelength of 223 nm, as % Interference was found less than 0.6% for both drugs. It is proven by comparing chromatogram of blank, Placebo and sample preparation solution; there was no interference of excipients with the peak of Fimasartan Potassium Trihydrate and Cilnidipine. Thus, the method is Specific. The results are shown in Figure 6, 7 & Table 7, 8.

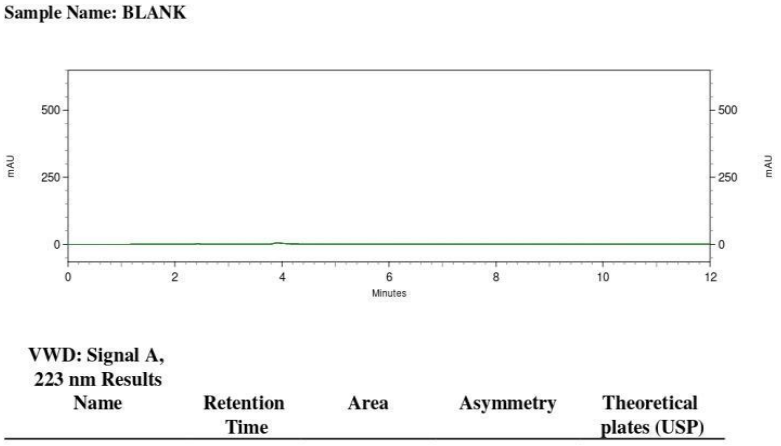

Figure 6: Typical chromatogram of blank solution

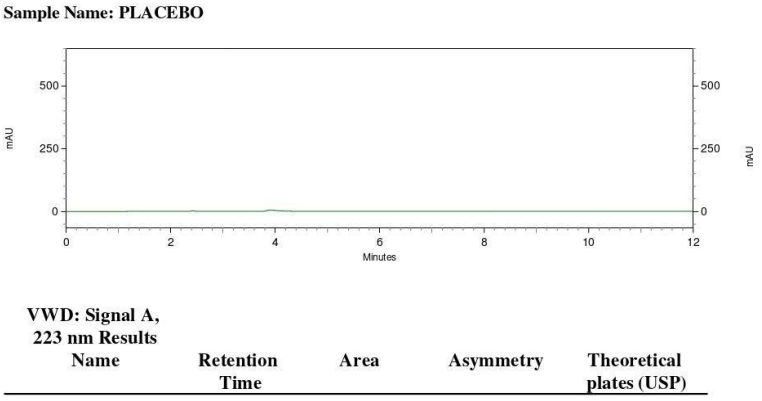

Figure 7: Typical chromatogram of placebo solution

Table 7: Result of specificity study (Fimasartan)

Description	Observation
Blank	No interference at R.T. of Fimasartan due to blank
Placebo	No interference at R.T. of Fimasartan due to placebo

Table 8: Result of Specificity study (Cilnidipine)

Description	Observation
Blank	No interference at R.T. of Cilnidipine due to blank
Placebo	No interference at R.T. of Cilnidipine due to placebo

3.3.5 LOD and LOQ

LOD and LOQ were found to be 0.708µg/mL and 2.146µg/mL for Fimasartan and 0.081µg/mL and 0.244µg/mL for Cilnidipine respectively.

3.3.6 Robustness

Making a deliberate change in wavelength, flow rate, column oven temperatures were taken place and RSD was found to be less than 2, specify that the method is robust. Results, presented in Table 9 and 10 indicate that the selected factors remained unaffected by a small variation of these parameters.

Table 9: Result of Robustness study (Fimasartan)

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (226 NM)	3.57	51507175	1.20	8624
Wavelength by -3 NM (220 NM)	3.57	57806854	1.20	8603
Flow rate by +10% (1.1mL/min)	3.24	46845852	1.21	7952
Flow rate by -10% (0.9mL/min)	3.96	57451587	1.21	9278
Column oven temp by +2ºC (42 ºC)	3.58	55975236	1.23	8622
Column oven temp by -2ºC (38 ºC)	3.57	53945036	1.21	8491

Table 10: Result of Robustness study (Cilnidipine)

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates	Resolution
Wavelength by +3 NM (226 NM)	7.97	8412081	1.13	12422	2.0.03
Wavelength by -3 NM (220 NM)	7.98	8090843	1.13	12364	20.02
Flow rate by +10% (1.1mL/min)	7.25	7644163	1.14	11598	19.35

Flow rate by -10% (0.9mL/min)	8.87	9371317	1.14	13140	20.73
Column oven temp by +2ºC (42 ºC)	7.98	7729645	1.17	1218	20.09
Column oven temp by -2ºC (38 ºC)	7.99	7801795	1.18	12296	20.03

4 DISCUSSION

The present method was developed using methanol: 0.1% OPA in water 80:20%v/v. The method was developed with the minimum or reduced amounts of organic solvents as the mobile phase which results in a more sensitive and cost-effective method. In the present method, Fimasartan Potassium Trihydrate and Cilnidipine were eluted with retention time 3.57 min and 7.99 min respectively. On the basis of literature survey, it was found that there is no sensitive and cost-effective method available for estimation of Fimasartan Potassium Trihydrate and Cilnidipine.

5 CONCLUSION

The developed method of Cilnidipine and Fimasartan is simple, precise, accurate and suitable RP-HPLC method. Rapid and robust HPLC method was developed for simultaneous estimation of Cilnidipine and Fimasaratan in the synthetic mixture, provide good resolution between both drugs. In developed method of HPLC, the analyte was resolve by using isocratic programmed and mobile phase is methanol:0.1% OPA in water (80:20%v/v) on HPLC system containing UV- visible detector with EZ- Chrome software and kromasil C18 column (250 mm× 4.6mm, 5µm) and the detection was carried out at 223 nm. The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r2) for each analyte is not less than 0.99 which shows good linearity. The % accuracy or recovery was in the acceptable range in lab mix. The %RSD was also less than 2% showing high degree of precision. The developed method was validated by testing its accuracy, precision, robustness, specificity, solution stability, system suitability and result were found to be acceptance limit as per ICH (Q2 R1) guideline. The statistical analysis proves that method is suitable for simultaneous determination of both drugs in pharmaceutical analysis and routine quality control without any interference.

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