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Analytical Method Development and Validation for Assay of Fimasartan Potassium Trihydrate and Chlorthalidone in Tablet Dosage Form by Using RP-HPLC

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Abstract**—** *Present work was to develop and validate novel, specific, linear, accurate and precise analytical method for quantitative estimation of Fimasartan Potassium Trihydrate and Chlorthalidone in tablet dosage form using RP-HPLC. Prontosil C18 (250 mm ×4.6 mm,5µm) column utilized for this chromatographic analysis. The mobile phase of Potassium Phosphate Buffer (pH 3) and ACN in gradient mode with flow rate of 1.5 ml/min was used and the injection volume was 20 µL. The detection wavelength was 230 nm over 8-minute run time. The retention time of Fimasartan Potassium Trihydrate and Chlorthalidone was 5.0 and 2.6 respectively. Based on ICH guidelines above method were validated. The percentage Assay found was 100.6 % for Fimasartan Potassium Trihydrate and 99.5 % for Chlorthalidone which were matching the label claim of tablet of 120 mg and 25 mg Fimasartan Potassium Trihydrate and Chlorthalidone respectively. The correlation coefficient for the two drug was 0.99 which satisfied the validation acceptance criteria. The experimentally determined values for LOD and LOQ for Fimasartan Potassium Trihydrate was 1.37, 4.16 and for Chlorthalidone was 0.39, 1.21 respectively.*

Keywords**—** *Chlorthalidone; Fimasartan Potassium Trihydrate; Hypertension; Method development and Validation; RP-HPLC; Tablets.*

I. INTRODUCTION

Hypertension is a major risk factor for cardiovascular and renal disease in India. It possesses load on health care system and also reason for 57 % of all stroke death and 24 % coronary heart disease in India. High blood pressure nearly about > 180 mmHg systolic and > 120 mmHg diastolic results severe hypertension emergencies and are potentially life-threatening situations. To overcome from these situations, awareness and proper medical treatment is required [1].

Analytical Chemistry is a branch of science which revolves with set of procedure like separation, identification and quantification of the chemical components in natural and artificial materials. Analytical method development and validation play vital role in drug discovery, development and formulation of dosage form. There is great need for development of new analytical method for quality evolution of new emerging drugs [2,3].

HPLC is leading analytical method for qualitative and quantitative estimation of drug in combination, due to its accuracy and robust results [2,3].

Fimasartan Potassium Trihydrate and Chlorthalidone mainly used in treatment of hypertension. The IUPAC name of Fimasartan Potassium Trihydrate is Potassium; 2- [2-butyl-4 methyl-6-oxo-1-[[4-[2-(1,2,3-triaza-4-azanidacyclopenta-2,5-diene-5-yl)phenyl]methyl]pyrimidine-5yl]-N, N

dimethyletanethioamide; trihydrate (Fig.1) and has the molecular formula C27H36KN7O4S. Fimasartan is an Angiotensin II receptor blocker (ARB) drug used in the treatment of both heart failure and hypertension [4]. The IUPAC name of Chlorthalidone is 2-chloro-5-(1-hydroxy-3-oxo-2H-isoindol-1-yl)benzenesulfonamide and has molecular formula C14H11ClN2O4S.Chlorthalidone is a thiazide like Diuretic drug predominantly use in hypertension . This is differing chemically from thiazide by nature of the heterocyclic ring. Although its pharmacological action is indiscernible from that of thiazides [5].

Fimasartan Potassium Trihydrate and Chlorthalidone formulation belongs to Antihypertensive category, it is the new innovative combination for acts by different mechanism to get hypotensive effect.

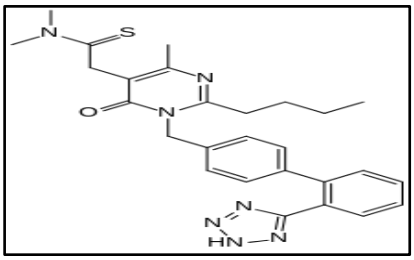

Fig. 1: Structure of Fimasartan Potassium Trihydrate

Literature survey reveals that analytical and HPLC method for the above stated drugs have been reported alone or in combination with other drugs in dosage form. Studies also shows that RP-HPLC method is not reported for the simultaneous estimation of Fimasartan Potassium Trihydrate and Chlorthalidone [6-11]. Therefor it was thought to develop novel, specific, linear, accurate and precise method for the determination of Fimasartan Potassium Trihydrate and Chlorthalidone by RP-HPLC using particular mobile phase.

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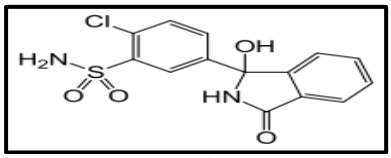

Fig. 2: Structure of Chlorthalidone

II. Materials and Methods

Chemicals and Reagents:

Fimasartan Potassium Trihydrate and Chlorthalidone reference standard having defined potency 100 and 90.2 % respectively and tablets of above drug combination consisting Fimasartan Potassium Trihydrate (120 mg) and Chlorthalidone (25 mg) were available from Alkem Laboratories Ltd. Navi Mumbai. Potassium dihydrogen phosphate (AR grade) and Orthophosphoric acid (AR grade) got from Sigma Aldrich. Acetonitrile and Methanol of HPLC grade obtained from Merch Life Science Pvt. Ltd. And HPLC grade distilled water (Milli-Q®) used. Filter paper of PVDF (0.45µ) and Nylon (0.45µ) with Millipore size were used.

Instrumentation:

HPLC system of Waters Alliance system (Waters corporation, Milford, MA, USA) With Empower software equipped with Photo Diode Array Detector -2996 were used for analysis purpose. PerkinElmer UV/VIS Spectrophotometer was used for wavelength selection. All type of weighing by Sartorius Analytical Balance and pH measurement by Phan-Lab-India pH Meter were carried out.

An aliquot of 20 µl sample was injected through column. The mobile phase consisted of Acetonitrile and 0.02 M Potassium Phosphate Buffer solution (pH 3.0) in gradient mode eluted through Prontosil C18 (250 mm ×4.6 mm, 5µm) column at a flow rate of 1.5 ml/min. The column temperature was 40ºC and detection was carried out at 230 nm over 8 min run time for simultaneous estimation of Fimasartan Potassium Trihydrate and Chlorthalidone.

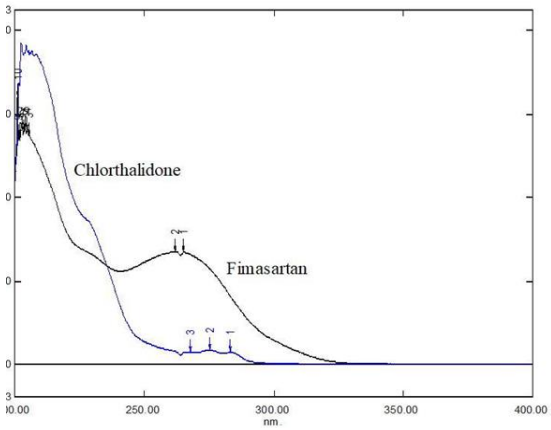

Fig. 3: UV Spectrum of Fimasartan Potassium Trihydrate and chlorthalidone

Preparations:

Preparation of Mobile phase A- Buffer pH 3.0:

2.72 g of Potassium Dihydrogen Phosphate was dissolved in 1000 ml of water and the pH was adjusted to 3.0 ± 0.1 with Orthophosphoric acid. It was then filtered through 0.45 µ membrane filter.

Preparation of Mobile phase B- Acetonitrile 100 %

Preparation of Diluent: Buffer pH 3.0 and Mrthanol in 50:50 v/v ratio.

Preparation of Standard Solution:

Chlorthalidone: Accurately about 25 mg Chlorthalidone reference standard was weighed into a 200 ml volumetric flask. 150 ml Methanol was added and sonicated to dissolved and was diluted with Methanol up to the mark.

Fimasartan Potassium Trihydrate: Accurately about 35 mg Fimasartan Potassium Trihydrate reference standard was weighed and transferred into a 50 ml volumetric flask. 30 ml Methanol was added and sonicated to dissolve and was diluted with Methanol up to mark.

Standard preparation: 2 ml of both the above solution were taken into 25 ml volumetric flask and diluted to the mark with diluent.

Preparation of Sample Solution:

Ten tablets were weighed and transferred in 500 ml volumetric flask. 25 ml water was added and sonicated with intermittent shaking to disperse the tablets. 350 ml of Methanol was then added and sonicated for 30 min with intermittent shaking and was diluted up to the mark with Methanol. The solution was filtered 0.45 µ PVDF filter and further 2 ml was diluted to 100 ml with diluent.

Method optimization:

Mixed standard solution of Fimasartan Potassium Trihydrate and chlorthalidone was injected by using autosampler. Various trials were taken for developing a suitable method for analysis of the above stated drug. First few trails were taken with Inertsil ODS-3V column (250 mm × 4.6 mm, 5µ) with mobile phase water combined with Acetonitrile or methanol in 50:50 % v/v proportion. Flow rate for above trial was 1.0 ml/min.in result proper peak of above drugs were not detected.

Another few trials were taken with Prontosil C18 column (250 mm × 4.6 mm, 5µ) with mobile phase consisted Potassium Phosphate buffer (pH 3) as one of the mobile phases and another from Acetonitrile or methanol in in 50:50 % v/v proportion in gradient mode. Flow rate for above analysis was 1.5 ml/min. Observed peak shape was proper with good resolution and shorter retention time.

Validation of the developed method:

As per ICH guidelines above optimised method was validated for specificity system suitability, linearity, precision, accuracy (recovery), LOD, LOQ and robustness [12,13].

Specificity:

Blank (diluent), placebo and control sample solution were prepared as per developed method and injected to HPLC system. There was no interference at the retention time of above stated drugs so above method is specific for analytes.

System suitability:

Six replicate injections of mixed standard solution of Fimasartan Potassium Trihydrate and chlorthalidone was

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injected and the system suitability parameter were recorded, results are shown in Table 1 and 2.

Linearity:

The linearity of Fimasartan Potassium Trihydrate and chlorthalidone was performed using standard solution in the range of 25 to 75 µg/ml for Fimasartan Potassium Trihydrate and 5 to 10 µg/ml for Chlorthalidone. The correlation coefficients for the two drugs were greater than 0.99 which meets the validation acceptance criteria. The graph of peak area obtained verses respective concentration was plotted in terms of slope, intercept and correlation coefficient values shown in fig. 7 and 8 and results are shown in Table 3 and 4.

Precision:

System Precision: Six replicate injection of the standard solution were injected into the HPLC system. The mean, standard deviation and %RSD calculated and reported, results are shown in Table 5.

Method Precision: Six sample of a single batch were analysed as per developed method. The % assay for stated drugs in six sample was calculated and reported, results are shown in Table 6.

Intermediate Precision (ruggedness): Analysed six sample of the same batch which was used for method precision by different analysts. This was done by using different instrument, different column on different days. The % assay of stated drug in the tablets were determined. %RSD of method precision was calculated and reported. Results are shown in Table 7.

Table no:1 System suitability of Fimasartan Fimasartan Potassium Trihydrate

Sr.no	Retention time	Theoretical plates	Tailing factor
1	5.076	307112	1.612
2	5.080	317552	1.173
3	5.076	314340	1.164
4	5.072	31024	1.162
5	5.074	30827	1.163
6	5.076	30896	1.160
Limit	NMT 1%	NLT 2000	NMT 2.0

NMT: Not more than, NLT: Not less than

Table no:2 System suitability of Chlorthalidone

Sr.no	Retention time	Theoretical plates	Tailing factor
1	2.698	7898	1.235
2	2.699	7899	1.234
3	2.703	7915	1.224
4	2.698	7912	1.227
5	2.701	7852	1.209
6	2.68	7876	1.301
Limit	NMT 1%	NLT 2000	NMT 2.0

NMT: Not more than, NLT: Not less than

Table no 3: Linearity data of Fimasartan Potassium Trihydrate

Level	Concentration in µg/ml	Peak areas
50	25	808125
80	40	1311980
100	50	1665650
120	60	1955644
150	75	2520975
Slope	33971	
Intercept	-46073	
'R^2'	0.998	

Accuracy:

The placebo was spiked with known amount of Fimasartan Potassium Trihydrate and Chlorthalidone at level 50%, 100%, and 150% of test concentration of stated drugs and quantified as per developed method. At each level determination were carried out and mean recovery was calculated and reported. Results of accuracy studies at various concentration level are shown in Table 8 and 9.

Table no 4: Linearity data of Chlorthalidone

Level	Concentration in µg/ml	Peak areas
50	5	26953
80	8	44453
100	10	55612
120	12	65853
150	15	82753
Slope	5548	
Intercept	-358	
'R^2'	0.9996	

Table no:5 System Precision study

	Area of standard	
Sr.no	Fimasartan Potassium Trihydrate	Chlorthalidone
1	1608523	552240
2	1633468	556942
3	1643492	547820
4	1644573	567930
5	1633083	558217
6	1652482	555847
Mean	1635936	556499
SD	13971.1	6160
%RSD	0.85	1.10
Limit	% RSD NMT 2.0	

SD=Standard Deviation, %RSD= Percentage Relative Standard Deviation
NMT= Not More Than

Table no:6 Method Precision study

	% Assay	
Sr.no	Fimasartan Potassium Trihydrate	Chlorthalidone
1	99.6	100.6
2	100.2	100.7
3	98.4	100.2
4	100.6	100.6
5	99.8	100.1
6	99.7	100.0
Mean	99.7	100.7
SD	0.71	0.63
%RSD	99.6	100.6
Limit	% RSD NMT 2.0	

SD=Standard Deviation, %RSD= Percentage Relative Standard Deviation
NMT= Not More Than

Table no:7 Intermediate Precision (Ruggedness)

	% Assay			
Sr.no	Fimasartan Potassium Trihydrate		Chlorthalidone	
	Analyst 1	Analyst 1	Analyst 1	Analyst 1
1	101.2	98.8	100.1	99.3
2	100.9	98.9	101.4	99.1
3	100.8	100.4	100.3	100.1
4	100.2	100.7	100.5	100.4
5	101.2	100.6	100.2	100.2
6	100.8	100.8	100.1	99.6
Mean	100.8	100.0	100.7	100.0
SD	0.68	0.83	0.63	1.0
%RSD	0.68	0.83	0.63	1.0
Limit	% RSD NMT 2.0			

SD=Standard Deviation, %RSD= Percentage Relative Standard Deviation
NMT= Not More Than

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Table no 8: Accuracy data of Fimasartan Potassium Trihydrate

% Level	Actual amount(mg)	Amount found (mg)	% Recovery	Mean % recovery
50	15.79	15.78	99.93	100.07
100	31.72	31.78	100.1	
150	47.37	47.48	100.2	

Table no 9: Accuracy data of Chlorthalidone

% Level	Actual amount(mg)	Amount found (mg)	% Recovery	Mean % recovery
50	12.5	12.5	100	99.96
100	25.2	25.16	99.84	
150	37.6	37.62	100.05	

Table no 10: Results of Sensitivity

Drug	LOD (ppm)	LOQ (ppm)
Fimasartan Potassium Trihydrate	0.39	1.21
Chlorthalidone	1.37	4.16

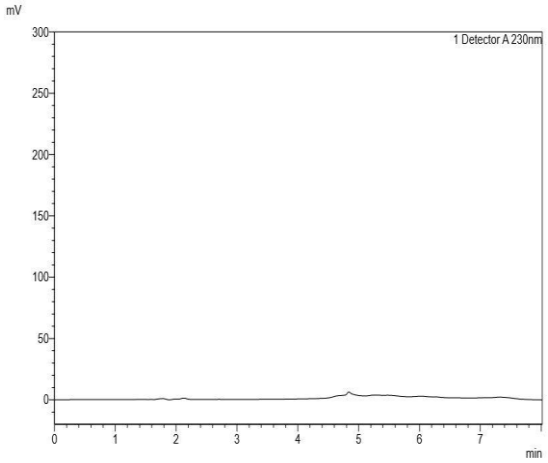

Fig. 4: Chromatogram of blank solution of the optimized method

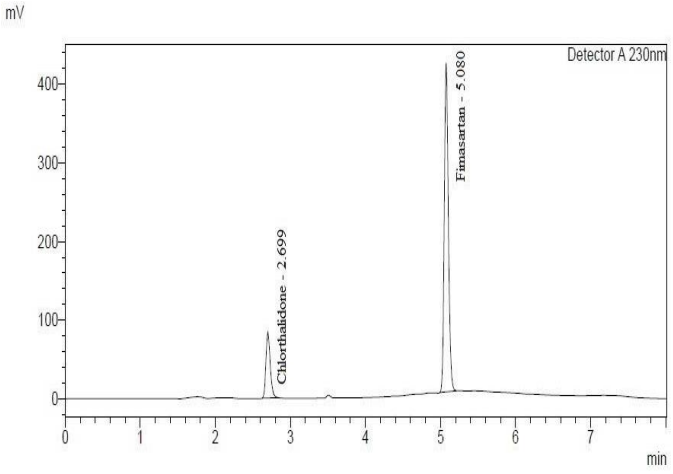

Fig. 5: Chromatogram of mixed standard solution

LOD and LOQ (Sensitivity):

The sensitivity of stated drugs was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ). These two parameters were calculated using the equation LOD = 3.3 σ/S and LOQ = 10 σ/S

Where, σ is the standard deviation of the intercept of calibration plots and S is the average of individual slopes of the corresponding calibration plot. The results are summarized in Table 10

Robustness:

Small deliberate changes were made in the method by varying certain condition like changing the wavelength (±2 nm), changing the flow rate (±0.1 ml/min) and changing the temperature (±2 ºC). Mean, SD, %RSD of % assay calculated and reported, results are shown in Table 11 and 12.

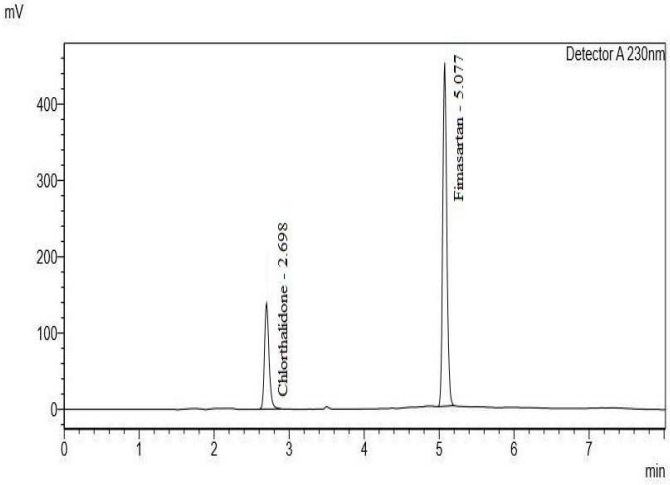

Fig. 6: Chromatogram of sample solution

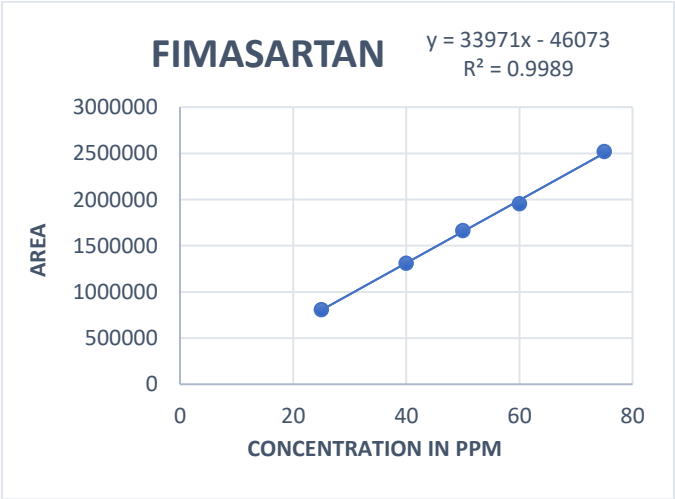

Fig. 7: Linearity graph of Fimasartan Potassium Trihydrate

Assay of tablet:

20 µl volume of standard and sample solution of Fimasartan Potassium Trihydrate and chlorthalidone in triplicates were injected into HPLC system for performing assay of above tablet. Mean, SD and % RSD of sample peak area and % assay was calculated and reported. The results are shown in table and Chromatogram of sample solutions are shown in fig.9,10 and 11. Results are shown in Table 13.

III. Result and Discussion

Assay method developed and validated for simultaneous

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determination of Fimasartan Potassium Trihydrate and Chlorthalidone in tablet dosage form by RP-HPLC. In optimized method the peaks of Fimasartan Potassium Trihydrate and Chlorthalidone were well resolved and were found at 5.0 and 2.6 respectively. There was no interference observed from the bank and placebo at retention time of Fimasartan Potassium Trihydrate and Chlorthalidone peaks. Hence above method is specific for the analytes. The correlation coefficients for the two drugs were greater than 0.99 which state that developed method is linear. % RSD of six injection of standard mixture and working concentration showed value less than 2 and limit of mean recovery are 98% to 102% which is within acceptable limit affirming above method is precise by repeatability and accurate. The results of robustness studies show that applied method is robust at small but deliberate change. The experimental determined values of LOD and LOQ indicate that the method is sensitive for analysis of pharmaceutical formulations.

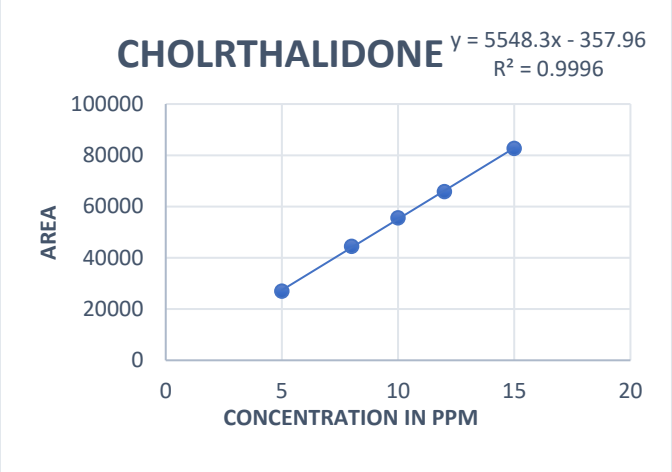

Fig. 8: Linearity graph of Chlorthalidone

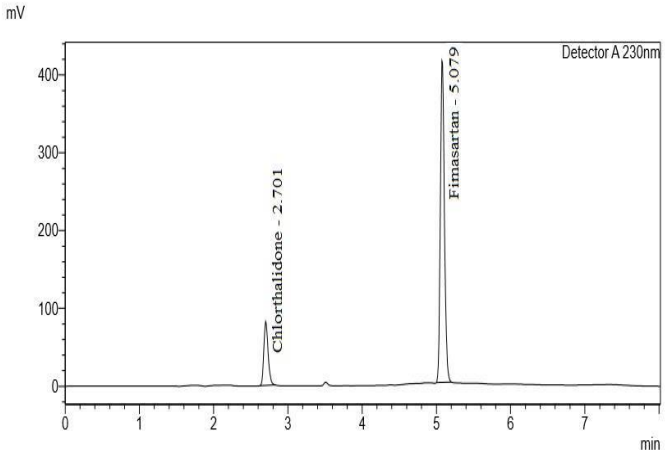

Fig. 9: Chromatogram of sample solution 1

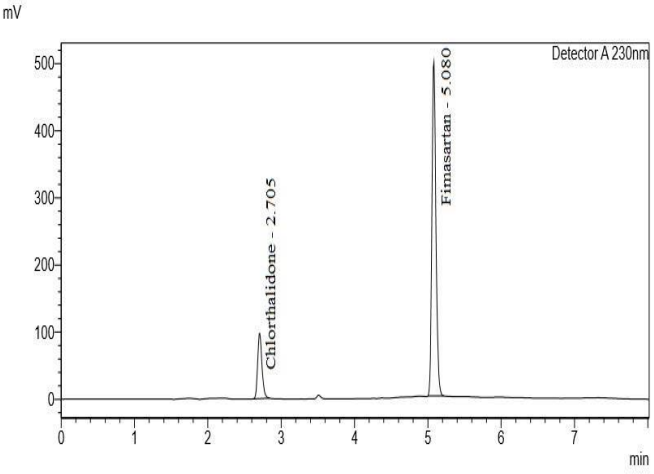

Fig. 10: Chromatogram of sample solution 2

Table no 11: Robustness data of Fimasartan Potassium Trihydrate

Parameter	Change in Parameter	% Assay	Mean	SD	%RSD	Limit
Flow rate (± 2%)	1.2 ml/min	99.2	99.4	0.205	0.206	% RSD NMT 2 %
	1.4 ml/min	99.7				
	1.6 ml/min	99.5				
Column oven temperature (± 2%)	38 ºC	100.2	100.7	0.754	0.748	
	40 ºC	100.2				
	42 ºC	101.8				
Wavelength (± 2%)	228 nm	100.2	100.6	0.410	0.408	
	230 nm	100.6				
	232 nm	101.2				

SD=Standard Deviation, %RSD= Percentage Relative Standard Deviation, NMT= Not More Than

Table no 12: Robustness data of Chlorthalidone

Parameter	Change in Parameter	% Assay	Mean	SD	%RSD	Limit
Flow rate (± 2%)	1.2 ml/min	101.8	101.9	0.294	0.288	% RSD NMT 2 %
	1.4 ml/min	102.3				
	1.6 ml/min	101.6				
Column oven temperature (± 2%)	38 ºC	100.8	100.3	0.408	0.407	
	40 ºC	100.3				
	42 ºC	99.8				
Wavelength (± 2%)	228 nm	101.2	101.2	0.313	0.309	
	230 nm	100.7				
	232 nm	101.8				

SD=Standard Deviation, %RSD= Percentage Relative Standard Deviation, NMT= Not More Than

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Table no 13: Assay result of Fimasartan Potassium Trihydrate (A) and Chlorthalidone (B)

Sample.no.	Weight of standard "A" (mg)	Sample weight (mg)	Mean area of standard	Area of Sample	% Assay
1	120.72	331.86	1930482	1780952	99.5
2		330.36		1808226	101.1
3		331.46		1795972	100.4
Average	331.2	Limit % RSD NMT 2 %	Mean	1795050	100.3
			SD	11153.6	0.654896
			% RSD	0.621	0.65272
Sample.no.	Weight of standard "B" (mg)	Sample weight (mg)	Mean area of standard	Area of Sample	% Assay
1	25.86	331.86	477100	473410	99.8
2		330.36		482220	101.3
3		331.46		479648	101.7
Average	331.2	Limit % RSD NMT 2 %	Mean	478426	100.9
			SD	3699	0.817
			% RSD	0.773	0.810

SD=Standard Deviation, %RSD= Percentage Relative Standard Deviation, NMT= Not More Than

The % assay Fimasartan Potassium Trihydrate and Chlorthalidone in tablet dosage form was found as 100.3 % and 100.9 % respectively. Here the % RSD of the sample solution showed valued less than 2 which is within the acceptable limits. The proposed validated method was successfully applied for the routine simultaneous estimation of above stated drugs in tablet dosage form.

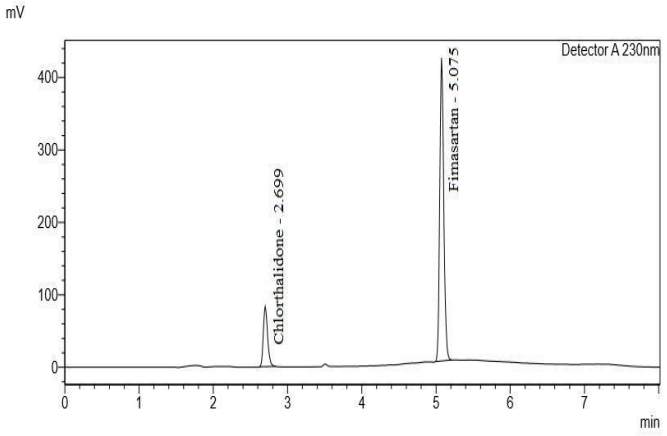

Fig. 11: Chromatogram of sample solution 3

IV. Conclusion

The sole purpose of the research work was to develop a novel RP-HPLC analytical method for the estimation of Fimasartan Potassium Trihydrate and Chlorthalidone in the pharmaceutical dosage form. Various trials were undertaken by changing the mobile phase, its proportion, flow rate, wavelength, HPLC columns etc. Peaks were not resolved properly on isocratic mode of elution and hence gradient mode was applied. The method was optimized after conducting several trials and validated according to ICH guidelines. The results obtained after validation were within limits. So above RP-HPLC method was found to be novel, specific, linear, precise, accurate, and robust method for the determination of Fimasartan Potassium Trihydrate and Chlorthalidone. The optimized method was successfully applied for routine analysis of tablets containing Fimasartan Potassium Trihydrate and Chlorthalidone.

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References

[1] R. Achala, N. Khannuri, H. Pant, H. Khan, "Hypertension in India: A systematic review and metanalysis of prevalence awareness and control of hypertension," Journal of hypertension, vol.32, Issue 6, pp. 1170-1177, 2014.

[2] S. Bhardwaj, K. Dwivedi, D. Agarwal, "A review: HPLC method development and validation," International Journal of analytical and bioanalytical chemistry, IISN-2231-5012, vol.5, Issue 4, pp. 76-81, 2015.

[3] J.A. Adamovics, "Chromatographic analysis of Pharmaceutical," *Plastics*, 2nd ed., vol. 74, J. Cazes, Ed. New York, pp. 62–64, 1996.

[4] National library of Medicine (US), National Center for Biotechnology Information:2014. PubChem Compound Summery for CID 46929800, Fimasartan Potassium Trihydrate, [cited 2022 March 6]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Fimasartan-Potassium-Trihydrate

[5] National library of Medicine (US), National Center for Biotechnology Information:2014. PubChem Compound Summery for CID 2732, Chlorthalidone, [cited 2022 March 6]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Chlorthalidone.

[6] B. Singh, D. Patel, S. Ghosh, "A Reversed- Phase High Performance Liquid Chromatographic Method for Determination of Chlorthalidone in Pharmaceutical Formulation," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 1 Issue 2,2009.

[7] M. Elgawish, S. Mostafa, A. Elshanawane," "A. Simple and Rapid HPLC Method for Simultaneous Determination of Atenolol and Chlorthalidone in Spiked Human Plasma," Saudi Pharm J, vol. 19, Issue 1, pp. 43-49, 2011.

[8] V. Sawale, P. Dangre, D. Dhabarde," Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Olmesartan Minoxidil and Chlorthalidone in Tablet Dosage Form," Int. J. Pharm, Pharm. Sci, vol. 7, Issue 5, pp 266-69, 2015.

[9] Y. SeoSeo, O. Seul, S. Kim," Validated LC–MS/MS Assay for the Quantitative Determination of Fimasartan in Human Plasma: Application to Pharmacokinetic Studies," J. Chromatogr. Sci, vol. 53, Issue 8, pp.

1250–1256,2015.
[10] M. WooWoo, Y. Hyeon, "Evaluation of Stability and Simultaneous Determination of Fimasartan and Amlodipine by a HPLC Method in Combination Tablets," Asian J. Pharm. Sci, vol. 9, pp. 1016-1023,2014
[11] H. Patel, D. Damahe, S. Narkhede, "Development and validation of RP-HPLC method for Amlodipine, Chlorthalidone and Olmesartan in solid dosage form, Indo American journal of pharmaceutical research, Vol. 10, Issue 7, pp. 597-605, 2017.
[12] L. R. Snyder, J. L. Glajch, J. J. Kirkland, "Practical HPLC Method Development, 2nd ed., John Willey and Sons, Inc. Hoboken, 1997.
[13] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, November 2005. Available from: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pd