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DEVELOPMENT AND VALIDATION OF NOVEL RP- HPLC METHOD FOR RELATED SUBSTANCES IN CHLORTHALIDONE AND FIMASARTAN FORMULATIONS

Kritika Rai[1] and Dr. Nutan Rao*

Oriental College of Pharmacy, Sanpada, Navi Mumbai, Mumbai University, Maharashtra, India.

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***Corresponding Author**
Dr. Nutan Rao
Oriental College of Pharmacy, Sanpada, Navi Mumbai, Mumbai University, Maharashtra, India.

ABSTRACT

The objective of the present work was to develop and validate a novel stability-indicating method for the simultaneous estimation of chlorthalidone and fimasartan potassium trihydrate pharmaceutical tablet dosage form by reversed-phase high-performance liquid chromatography (RP- HPLC). As of now no RP- HPLC method has been reported for this combination. The method was developed using Shimadzu LC Prominence-i 2030 model with chromeleone software and the separation was done on Zodiac C18 (250*4.6mm), 5µ column with a flow rate of 1.5 ml/min and run time of 50 min. The injection volume was 20 µl and mobile phase consisted of buffer pH 3.0 and 100% methanol in the ratio of 50:50 and 230 nm was used as a detection wavelength. The retention time was found to be 12.342 min and 32.727 min for chlorthalidone and fimasartan potassium trihydrate and for known impurity Chlorthalidone Rel. Comp. A the retention time is 20.325. The calibration curve was found to be linear and r values were 0.9996 and 0.9998 for chlorthalidone and fimasartan potassium trihydrate, respectively. The wide linearity range, precision, sensitivity, accuracy, short retention time, and simple mobile phase showed that the following method is suitable for routine quantification of impurities in chlorthalidone and fimasartan potassium trihydrate formulations in pharmaceutical dosage forms with high precision and accuracy.

KEYWORDS: Fimasartan potassium trihydrate, Chlorthalidone, RP-HPLC, validation.

1. INTRODUCTION

Pharmaceutical analysis is the branch of science which deals with identification of substances and establishment of amount present in a particular sample. Pharmaceutical analysis covers the majority of the dosage forms and more recently, bulk materials, biological samples in support of bio- pharmaceutical and pharmacokinetic studies. Analysis are usually divided into areas called qualitative and quantitative analysis. Pharmaceutical products are identified using instrumental techniques.[1]

The fusion of varied techniques, permits the modern pharmaceutical analyst to exploit the virtues of every technique and, in turn, to upgrade the overall quality of analysis. Modern analytical techniques and methods offer the possibility of rising the amount of details received from particular analysis, with reduced cost, time, and sample volumes.[2]

Analytical chemistry involves separating, identifying and determining the relative amounts of the compounds making up a sample of matter. Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.[3,4,5]

Hypertension is a major individualistic risk factor for coronary artery disease, stroke, and renal failure. Reducing blood pressure (BP) below the target goal is important to prevent cardiovascular and cerebrovascular events.[7] Various kinds of antihypertensive drugs such as diuretics, calcium channel blockers, beta blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin-receptor blockers (ARBs) is usually used to lower BP effectively. A newly discovered antihypertensive drug that lowers BP by blocking the angiotensin II type 1 receptor is Fimasartan (Figure 1).[8,9,10]

Chlorthalidone (Figure 2) is a thiazide-like diuretic. Chlorthalidone is used for controlling of edema caused by conditions such as heart failure or renal impairment and for the treatment of hypertension.[11,12]

A lot of methods have been reported for the determination of fimasartan alone or in combination with other active pharmaceutical agents in dosage forms or in biological fluids. Such as fimasartan andamlodipine[13], Chlorthalidone and Atenolol[14] and combination of Amlodipine, Chlorthalidone And Olmesartan.[15] However, simultaneous determination of fimasartan and chlorthalidone has not been described previously. The purpose of present study was to develop and validate an HPLC method for simultaneous determination of fimasartan

and chlorthalidone in tablet dosage form. Moreover, stability of active ingredients was also estimated by using this method.

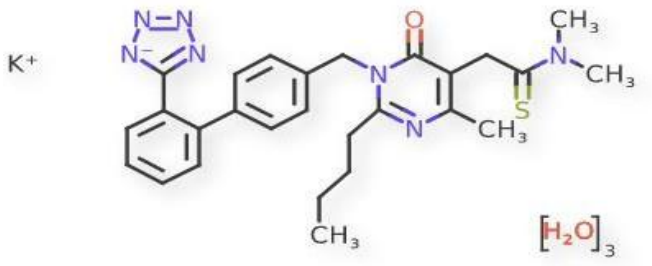

Figure 1: Structure of Fimasartan potassium trihydrate.

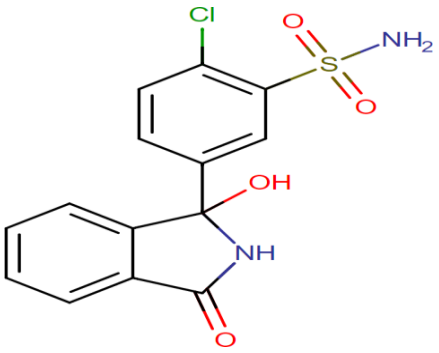

Figure 2: Structure of Chlorthalidone.

2. MATERIALS AND METHODS

2.1.Materials

Table 1: List of working standard and sample.

Working Standard and Sample	Potency
Chlorthalidone	100
Fimasartan potassium trihydrate	90.2
Sample	120mg and 25mg respectively

2.2.Instrumentation

Instruments used in the method development are listed in Table 2.

Table 2: List of instruments.

Instrument	Make and Model
HPLC	Shimadzu HPLC
HPLC	Agilent HPLC
Weighing balance	Sartorius
pH meter	Phan-Lab India
HPLC Columns	Hypersil 5 ODS C18 (250*4.6mm), 5µ
	Zodiac C18 (250*4.6mm), 5µ
	Prontosil (250*4.6mm), 5µ

2.3. Chromatographic conditions

Fimasartan and chlorthalidone, were freely soluble in selected analytical solvents like water, acetonitrile (ACN) and methanol (MeOH). The chromatographic conditions were developed by different means (using different columns, different buffers and different organic phases). Early chromatographic work was performed with different brands of C8 and C18 columns as stationary phase and various combinations of buffered (pH 3) organic phases (ACN and/or methanol). The flow rate of mobile phase was varied within the range of 1.0–1.5 mL/min. Wavelength for monitoring the eluent was selected by scanning standard solution of drug within 200–400 nm using Shimadzu LC Prominence-i 2030 model.

All noted measurements were performed with an injection volume of 20 mL and UV detection at 230 nm of samples dissolved in a diluent; Mobile phase-A [Buffer pH 3.0]: Mobile phase-B [Methanol 100%] in 50:50 (v/v).

2.4. Reagents used

Potassium dihydrogen phosphate (AR Grade), Orthophosphoric Acid (AR Grade), Water (Milli Q-Grade), Acetonitrile (HPLC Grade), Methanol (HPLC Grade).

Preparation of solutions Preparation of buffer pH 3.0

2.72 gm potassium dihydrogen phosphate was dissolved 1000 ml water. pH was adjusted 3.0± 0.05 with orthophosphoric acid and solution was filtered through 0.45 µ membrane filter.

Preparation of diluent

Mixture of buffer pH 3.0 and methanol was prepared the ratio of 50:50 and degased.

Preparation of Mobile Phase A

Buffer pH 3.0.

Preparation of Mobile Phase B

Methanol 100%.

Preparation of Chlorthalidone Standard Stock Solution

25mg of Chlorthalidone working standard was weighed into a 200 ml volumetric flask, add 150 ml methanol, sonicate to dissolve and dilute to the mark with methanol.

Preparation of Fimasartan potassium trihydrate

35 mg Fimasartan potassium trihydrate working standard was weighed accurately flask into a 100 ml volumetric flask, 60 ml methanol was added, sonicated the solution to dissolve and dilute to the mark with methanol.

Standard preparation

2 ml of Chlorthalidone standard stock solution and 4 ml of Fimasartan potassium trihydrate stock solution was added to 100 ml volumetric flask and dilute to the mark with the diluent.

Placebo preparation

Placebo (equivalent of about 600 mg Fimasartan potassium) was weighed and transferred into 250 ml volumetric flask, add about 25 ml water was added, solution was sonicated with intermittent shaking to disperse the tablets, 150 ml of methanol was added and sonicated for about 30 minutes with intermittent shaking and the volume was made upto the mark with methanol. Solution was filtered through 0.45µ PVDF filter. Further 5 ml of this solution was diluted to 10 ml with the diluent.

Sample Preparation

5 tablets (equivalent of about 600 mg Fimasartan potassium) was weighed and transferred into 250 ml volumetric flask, add about 25 ml water was added, solution was sonicated with intermittent shaking to disperse the tablets, add 150ml of methanol was added and sonicated with intermittent shaking to disperse the tablets, add 150 ml of methanol was added and sonicated for about 30 minutes with intermittent shaking and make the volume to the mark with methanol. Filter through 0.45µ PVDF filter. Further 5 ml of this solution was diluted to 10 ml with the diluent.

2.5 Results and discussion Method Validation System suitability

20 µL of standard solution was injected into HPLC column six times and the chromatogram was recorded, % RSD of paracetamol, ibuprofen and its impurities peaks area was within the limit of 5.0% and resolution between Fimasartan potassium trihydrate, Chlorthalidone and Chlorthalidone Rel. Comp. A was not more than 2.0 for the entire activity. The details are given in below Table 3-5 and Figure 3-7.

Table 3: Result of System suitability for Chlorthalidone.

Sr. no	Retention time	Theoretical plates	Tailing factor
1	12.462	11541	1.1
2	12.460	11540	1.1
3	12.501	11550	1.0
4	12.342	11560	1.0
5	12.465	11455	1.2
6	12.510	11440	1.2

Table 4: Result of System suitability for Fimasartan potassium trihydrate.

Sr. no	Retention time	Theoretical plates	Tailing factor
1	32.827	102316	1.1
2	32.852	102456	1.0
3	32.868	102459	1.2
4	32.892	102379	1.0
5	32.841	102482	1.2
6	32.874	102357	1.1

Table 5: Result of System suitability for Chlorthalidone Rel. Comp. A.

Sr. no	Retention time	Theoretical plates	Tailing factor
1	20.320	61232	1.1
2	20.325	60343	1.2
3	20.327	60133	1.0
4	20.325	60545	1.2
5	20.320	61221	1.3
6	20.331	61354	1.3

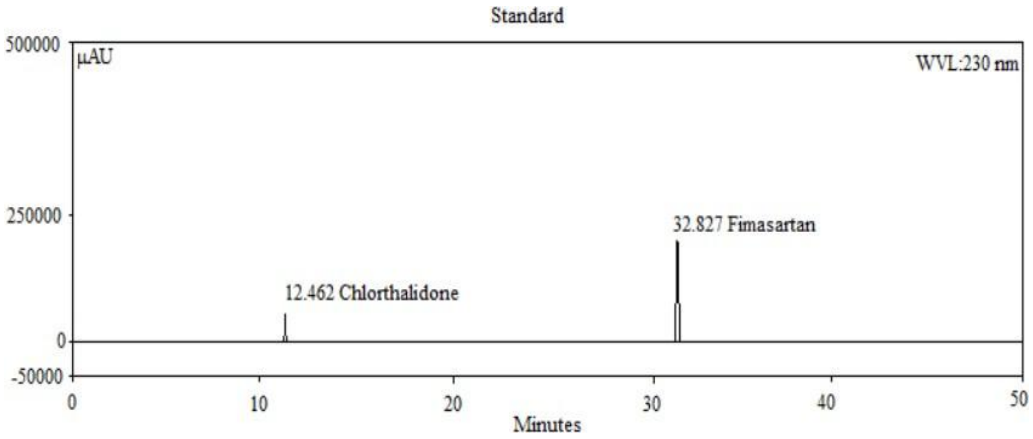

Figure 3: Chromatogram of the Standard.

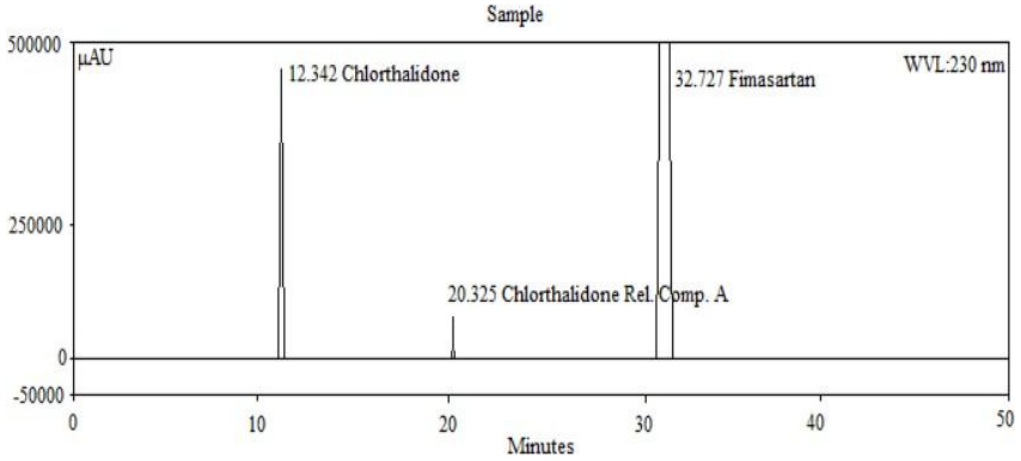

Figure 4: Chromatogram of the Sample.

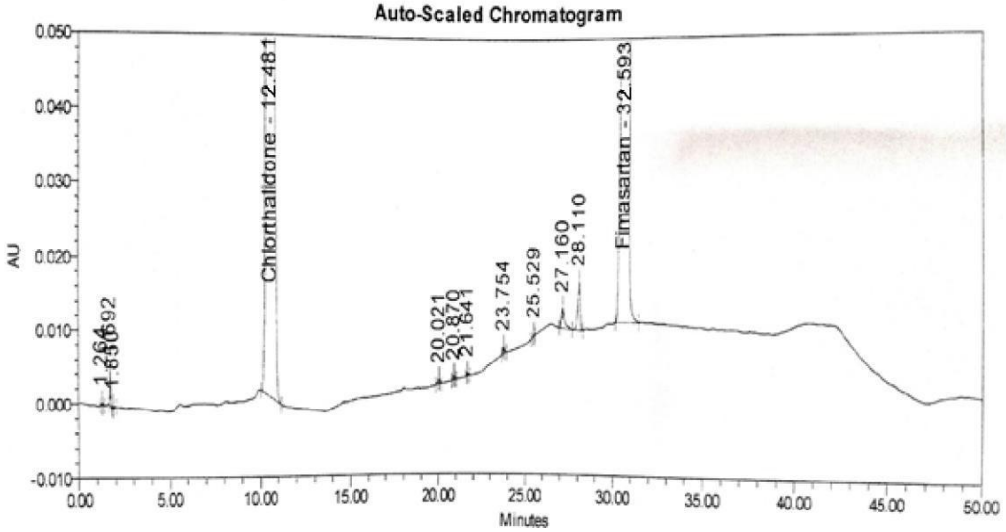

Figure 5: Chromatogram of the peak purity.

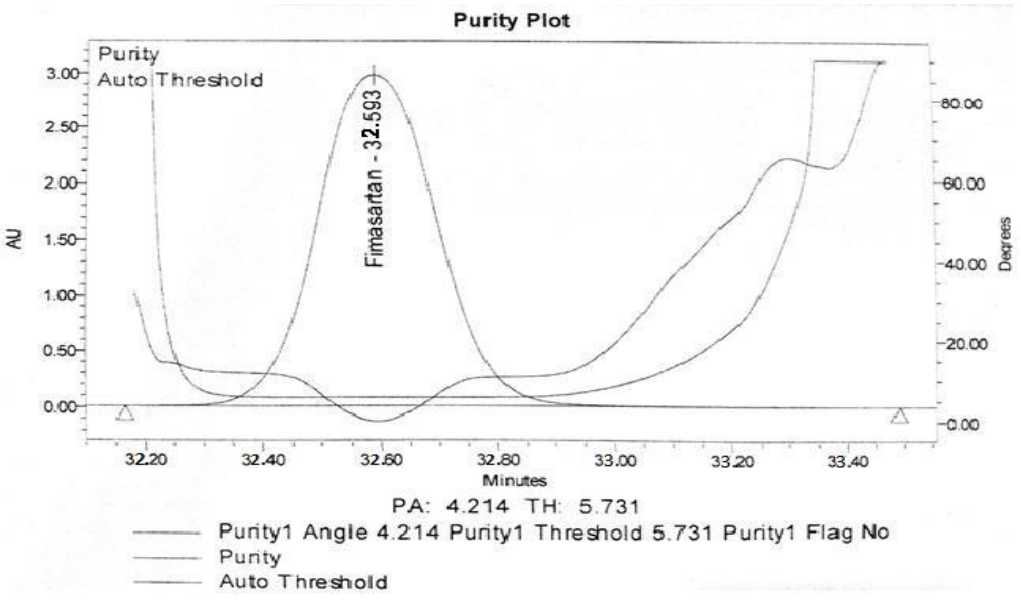

Figure 6: Chromatogram of the purity plot of Fimasartan.

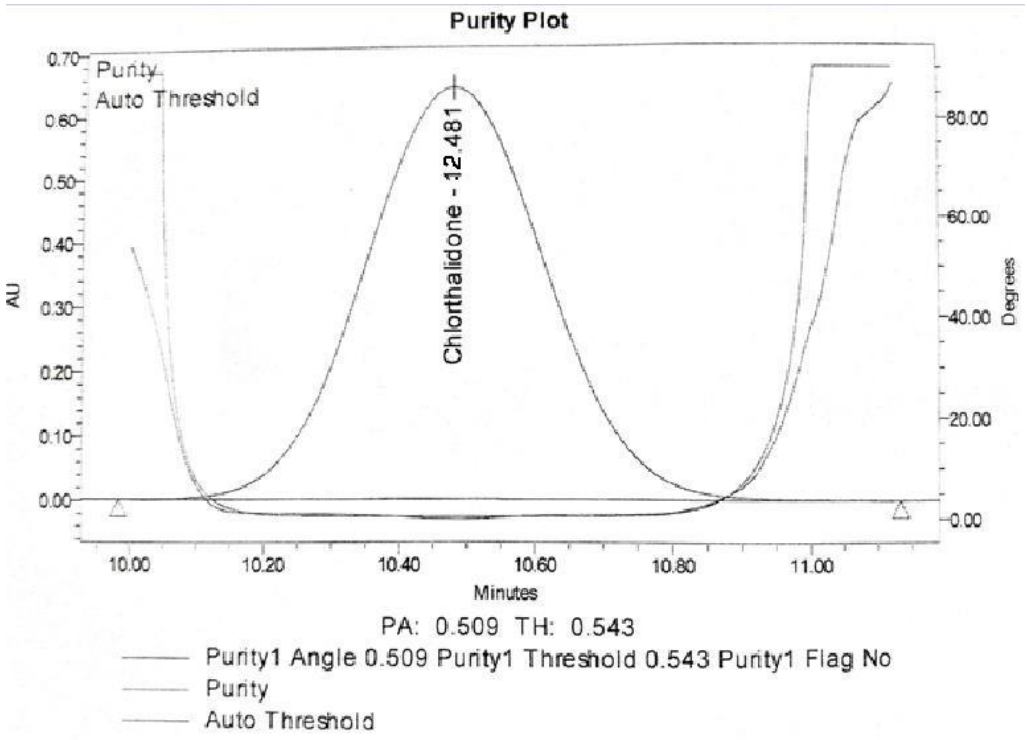

Figure 7: Chromatogram of the purity plot of Chlorthalidone.

PRECISION

System precision

The precision of method was carried out by injecting six preparations of Fimasartan potassium trihydrate and Chlorthalidone and Chlorthalidone Rel. Comp. A in the presence of 0.15% all impurities with respect to authentic concentration. The content of % RSD was calculated for all impurities. The results are tabulated in Table 6.

Table 6: Result of System precision.

Injection	Area of Standard		
	Chlorthalidone	Fimasartan potassium trihydrate	Chlorthalidone Rel Comp. A
1	119526	493924	5361
2	118891	497344	5352
3	119198	481189	5350
4	118524	481215	5348
5	119013	495992	5355
6	119228	497386	5360
Mean	119063	491175	5354
SD	341.30	7827.07	5
%RSD	0.29	1.59	0.09

Method Precision

Here the %RSD of the working concentrations of six replicates showed value less than 2 which

is the acceptable limits. This concludes that the developed method is precise by repeatability and hence can give consistence reproducible results as shown in Table 7.

Table 7: Result of method precision.

Injection	Chlorthalidone Rel Comp. A
	% w/w
1	0.087
2	0.086
3	0.087
4	0.087
5	0.087
6	0.087
Mean	0.087
SD	0.0004
%RSD	0.46

Intermediate precision (Ruggedness)

The ruggedness of the method was performed in the same laboratory with different analyst, different column and different instrument. The precision and intermediate precision %RSD results were compared and found within the acceptance criteria.

Ruggedness results were tabulated in Table 8.

Table 8: Result of ruggedness.

Injection	Chlorthalidone Rel Comp. A	
	Method precision	Ruggedness
1	0.087	0.086
2	0.086	0.086
3	0.087	0.087
4	0.087	0.086
5	0.087	0.086
6	0.087	0.087
Mean	0.087	0.086
SD	0.0004	0.0005
%RSD	0.46	0.58
over all Mean	0.087	
over all SD	0.0005	
over % RSD	0.57	

Linearity

The correlation coefficients for the two drugs was greater than 0.99 which meets the validation acceptance criteria. This concludes that the developed method is linear. Results were tabulated in Table 9 and Figure 8-10.

Table 9: Linearity data of Fimasartan potassium trihydrate, Chlorthalidone and Chlorthalidone Rel. Comp. A.

spike level in %	Fimasartan		Chlorthalidone	
	Concentrations (mcg/ml)	area	Concentrations (mcg/ml)	area
50	0.500	255201	0.500	62586
80	0.800	403229	0.800	102221
100	1.000	501224	1.000	127483
120	1.200	608021	1.200	149855
150	1.500	749973	1.500	186983
	Slope	497146	Slope	123664
	Intercept	6384	Intercept	2161
	r value	0.99987	r value	0.99961
			RF	4.02

spike level in %	Chlorthalidone Rel.Comp. A	
	Concentrations (mcg/ml)	area
50	0.500	3227
80	0.800	5089
100	1.000	6361
120	1.200	7538
150	1.500	9526
	Slope	6275
	Intercept	74
	r value	0.99986

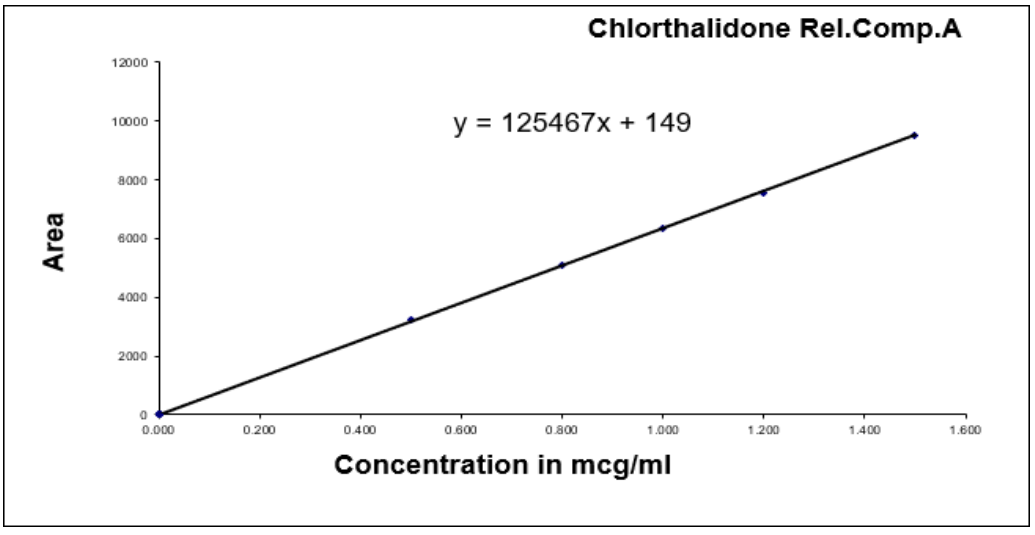

Figure 8: Calibration curve of Chlorthalidone.

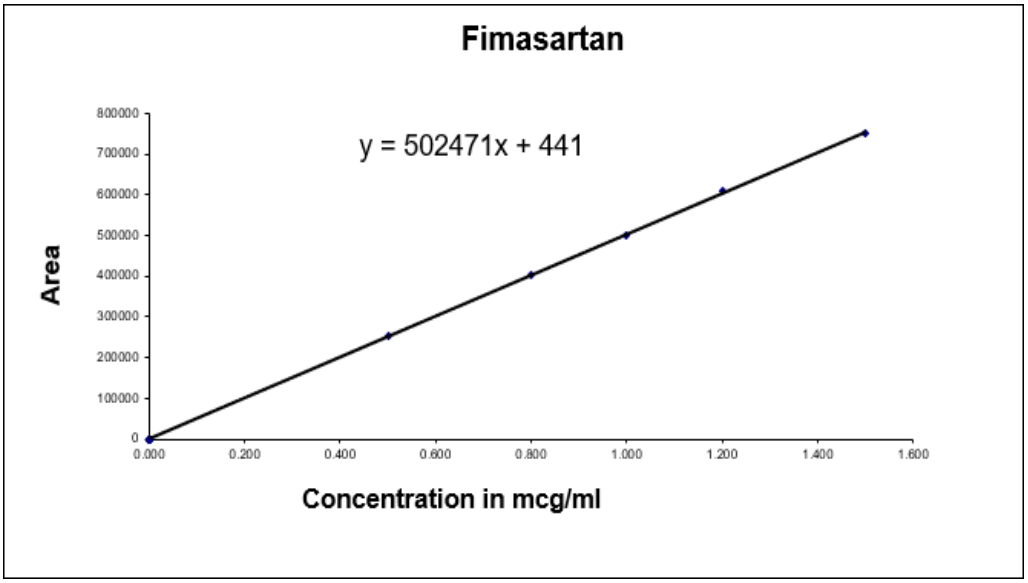

Figure 9: Calibration curve of Fimasartan potassium trihydrate.

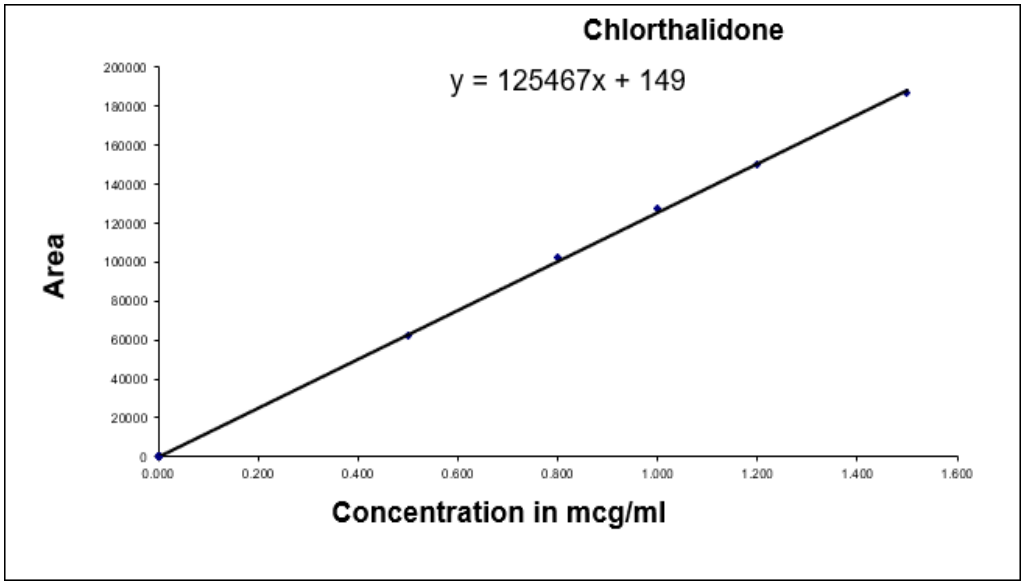

Figure 10: Calibration curve of Chlorthalidone Rel.Comp. A.

Accuracy

The known amounts of impurities were added in to sample calculated recovery at LOQ to 150% level. The accuracy studies for all impurities were carried out in triplicate at all levels (LOQ to 150%) at target analyte concentration. The results were consisting recoveries at LOQ level to 150% ranging from 97.7 to 103.3%. The method was found to be accurate at LOQ level to 150%. and the results are tabulated in table 10.

Table 10: Result of Accuracy for Chlorthalidone Rel Comp. A

Spike level in %	Actual amount of Chlorthalidone Rel Comp. A	Recovery
50%prep-1	0.075	100.3
50%prep-2	0.074	98.5
50%prep-3	0.074	97.9
100%prep-1	0.1506	100.1
100%prep-2	0.1498	99.5
100%prep-3	0.1502	99.8
150%prep-1	0.2252	99.7
150%prep-2	0.2214	98.1
150%prep-3	0.2207	97.7

CONCLUSION

This HPLC method is novel, sensitive, accurate, precise, reproducible, specific, and linear. The proposed method was found to be simple and linear in the concentration range. This method was found to be accurate and precise as indicated by the recovery studies and relative standard deviation. The Relative Response factor for impurities determined from validation can be used for quantification of impurities in Fimasartan potassium trihydrate, Chlorthalidone in routine testing, by which there is no need to use impurities standard. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, simplified extraction procedure, low retention time (Rt) and no internal standard. Hence this method suitable for quantification of impurities in combination in pharmaceutical dosage forms without interference and can be successfully used for routine analysis. It can therefore be concluded that it can be used in small laboratories with very high accuracy and a wide linear range.

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