STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND FIMSARTAN IN SYNTHETIC MIXTURE

Dr. Suresh Kumar*[1],
Department of Pharmaceutical Chemistry, Genezen institute of pharmacy.
Email: jdrsuresh@gmail.com

Ms. Nirma Chavda[2],
Research Scholar, Gujarat Technological University, Ahmedabad.
Email: nirmachavda@gmail.com

***Corresponding Author: Dr. Suresh Kumar[1],**
Email: jdrsuresh@gmail.com

Abstract: Stability indicating RP-HPLC method has been developed for the simultaneous estimation of HCTZ and FIM in combination. In RP-HPLC method, chromatographic separation was achieved using Hibar ODS C18 (250*4.6 mm, 5µm) as mobile phase at flow rate of 1.0 ml/min with detection wavelength of 225 nm. The linearity of both HCTZ and FIM were found in the range of 6.25-31.25 µg/ml and 30-150 µg/ml. Retention time in RP-HPLC method were found to be 8.7 min, 4.6 min for HCTZ and FIM respectively. The % recovery were found to be 99.33 ± 0.68 for HCTZ and 99.54 ± 0.59 for FIMA. The proposed method was validated as per ICH guidelines and successfully applied for the determination of drugs in pharmaceutical formulation. This evaluation supported the method's environmental friendliness regarding solvent usage, chemical substances, energy consumption, and waste generation.

Key words: Hydrochlorothaizide, Fimasartan, Validation, Stability indicating RP-HPLC.

Introduction:

Hypertension is connected with 12.8% of all deaths globally. Many countries have performed extensive programs to diagnose and manage hypertension and other chronic diseases, with diverse success. More than 1.3 billion people with hypertension globally, 82% live in low- and middle-income countries, and India alone is home to a calculated 220 million adults with hypertension. To label the burden of noncommunicable disorders, India started the National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke in 2010 (now known as the National Programme for Prevention and Control of Non-Communicable Diseases) under the National Health Mission for 100 districts across 21 states. However, few data are currently available to evaluate the success of and chances for enhanced control of high blood pressure at subnational levels.[1]

Blood pressure levels [2]

Blood Pressure Category	Systolic and Diastolic Pressure (mm Hg)
Normal	Less than 120 systolic pressure AND Less than 80 diastolic pressure
Elevated	120 to 129 systolic pressure AND Less than 80 diastolic pressure

High Blood Pressure Stage 1	130 to 139 systolic pressure OR 80 to 89 diastolic pressure
High Blood Pressure Stage 2	140 or higher systolic pressure OR 90 or higher diastolic pressure
Hypertensive Crisis	Higher than 180 systolic pressure OR Higher than 120 diastolic pressure Contact your provider immediately.

Hypertension can be classified into primary and secondary forms. Primary hypertension reports for the wide majority (≥90%) of cases, and poor diet and insufficient physical activity look to be important and clearly reversible environmental causes. A specific, sometimes remediable cause of hypertension can be identified in around 10% of adults with hypertension, called secondary hypertension. If the cause can be accurately diagnosed and treated, patients with secondary hypertension can fulfill normalization of BP or noticeable improvement in BP control, with concomitant reduction in CVD risk. The main patients with secondary hypertension have primary aldosteronism or renal parenchymal or renal vascular disease, although the rest may have more unusual endocrine disorders or drug- or alcohol-induced hypertension.[3]

Fimasartan potassium trihydrate which is chemically2- (2-butyl-4-methyl-6-oxo-1-{[2′-(1H-1,2,3,4tetrazol-5-yl)-[1,1′-biphenyl] – 4 - yl] methyl } - 1, 6 – dihydropyrimidin – 5 – yl) – N , N dimethylethanethioamide. Rosuvastatin calcium is an HMG Co A reductase inhibitor and Fimasartan is an angiotensin II receptor antagonist [4]. In blocking the AT1 receptor, fimasartan blocks vasoconstriction and supports vasodilation. At the kidney and adrenal gland, AT1 blockage and inhibition of aldosterone formation rise the excretion of water and salt by the kidneys, which lowers overall blood volume [5]. At the heart, AT1 blockage lowers contractility and the stimulatory effects of the sympathetic nervous system. Collectively, fimasartain helps to a reduction in blood pressure and relieves hypertensive symptoms. ARBs such as fimasartan have also been shown to be protective against stroke, myocardial infarction, and heart failure [6].

Hydrochlorothiazide is a benzothiadiazine that is 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide substituted by a chloro group at position 6 and a sulfonamide at 7. It is diuretic used for the treatment of hypertension and congestive heart failure. It has a role as a xenobiotic, an environmental contaminant, a diuretic and an antihypertensive agent. It is a benzothiadiazine, a sulfonamide and an organochlorine compound.[7] Hydrochlorothiazide is transported from the circulation into epithelial cells of the distal convoluted tubule by the organic anion transporters OAT1, OAT3, and OAT4. From these cells, hydrochlorothiazide is transported to the lumen of the tubule by multidrug resistance associated protein 4 (MRP4).[8]

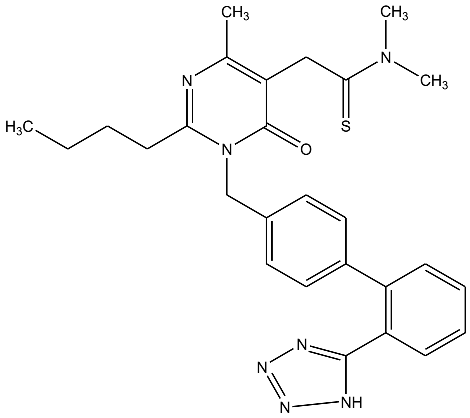

Fig.1: Structure of Fimasartan

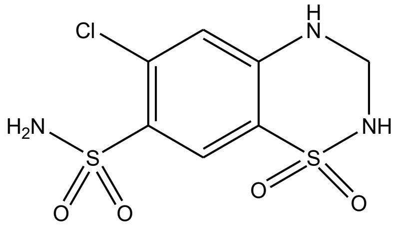

Fig.2: Structure of Hydrochlorothiazide

The combination treatment of fimasartan and HCTZ achieved better BP control than fimasartan monotherapy, and had comparable safety and tolerance to fimasartan monotherapy.[9]

Te stress testing is to be carried out to recognize the inherent stability characteristics of the active components . Te impurities from the manufacturing process and inappropriate storage or handling lead to produce degradants widely infuenced the results relating to quality, safety, and efcacy. A good stability-indicating method is capable of distinguishing the active analytes peak from degraded product [6–9]. Nowadays, the HPLC technique is used in a variety of analytical studies to estimate the drugs in pure and in pharmaceutical formulations as well as in biological fuids and their competence for stability-indicating method development. It has the advantage of being efcient, accurate, easy to use, and the ability to provide best separation of analytes; hence, it is used widely.[10=13]

A survey of the literature shows that for simultaneous estimation of TLS and RST, few HPLC methods are reported [14–25]. Te existing methods have some limitations as complex procedure of test preparation, long run time, and low resolution. Validated stabilityindicating data were available for one reported method, but stress studies were performed only on the drug substance, not on the drug product. Moreover, few robustness parameters and solution stability shall be required to be performed. Hence, this proposed study is undertaking to develop and validate stability-indicating RPHPLC method for simultaneous estimation of TLS and RST which is more rapid, simple, precise, accurate, linear, robust enough as compared to existing methods.

MATERIALS AND METHODS:

Chemicals and Materials:

Pharmaceutical grade of Hydrochlorothiazide (HCTZ) and Fimasartan (FIM) were kindly supplied as a gifts sample by Montage Laboratories Pvt Ltd and Mackur Laboratories. All solvents and chemicals used were of analytical grade or HPLC grade purchased from Merck and Aquarch. Methanol and Acetonitrile were used of HPLC Grade (Merck, Mumbai, India). All the other chemicals used were also of AR, LR and HPLC grade (Merck, India).

Sr no.	Materials	Sources
1	Hydrochlorthiazide	Montage Laboratories Pvt Ltd
2	Fimasartan	Mackur Laboratories
3	Chemicals/ Reagents:	

	Acetonitrile and Methanol	Merck, India Spectrochem, India

The pharmaceutical dosage form containing 60 mg FIM, 12.5 mg HCTZ, Kanarb Plus (**Boryung pharmaceutical, Ltd, South Korea**).

Mobile phase selection:

Std stock soln of HCTZ: 12.5mg→100ml with methanol (125mcg/ml)

Std stock soln of FIM: 60mg→100ml with methanol (600mcg/ml)

Table-1 Optimized Chromatographic Condition:

Stationary Phase	Hibar ODS C18 (250*4.6 mm, 5µm)
Mobile Phase	Acetonitrile: Methanol: Water (45:30:25 v/v/v)
Detection wavelength	225 nm
Flow rate	1 ml/minute
Run Time	15 minutes
Retention Time	FIMA: 6.498 min, HCTZ: 8.713 min

Preparation of Standard stock solution:

Preparation of standard stock solution of FIMA:

Accurately weighed 60 mg FIMA dissolved in 100 ml methyl alcohol (600 µg.ml^{-1}). Withdraw 1.0 ml from Master Stock Solution and make up to 10 ml with methyl alcohol (60 µg.ml^{-1})

Preparation of standard stock solution of HCTZ:

Accurately weighed 12.5 mg HCTZ dissolved in 100 ml methyl alcohol (125 µg.ml^{-1}). Withdraw 1.0 ml from Master Stock Solution and make up to 10 ml with methyl alcohol (12.5 µg.ml^{-1})

Preparation of standard stock solution of mixture:

Weigh accurately about 60 mg FIMA and 12.5 mg HCTZ and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (600 µg/ml of FIMA + 125 µg/ml HCTZ). Withdraw 1.0 ml from Master Stock Solution and make up to 10 ml with methyl alcohol FIMA+HCTZ (60+12.5 µg.ml^{-1})

Assay:

Std stock soln of HCTZ: Weigh accurately about 12.5mg dissolved 100ml with methanol. (125mcg/ml). Std stock soln of FIM: Weigh accurately about 60mg dissolved 100ml with methanol (600mcg/ml)

Working Std Preparation (Combine Std Preparation):

Take 1ml from HCTZ stock, 1ml from FIM→10ml with Mobile phase (mobile phase which used for trials) (ROS-12.5mcg/ml, FIM-60mcg/ml).

Stability study:

Procedure for Stability Study:

Weigh accurately about 60 mg FIMA and 12.5 mg HCTZ and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (600 µg/ml of FIMA + 125 µg/ml HCTZ).

Sample stock solution:

Weight about sample (equivalent to 12.5mg of HCTZ/60mg of FIM) into a 100ml volumetric flask. Add 60ml methanol and put this volumetric on water bath at 60°C for 15 minutes then allow to cool at room temperature. Shake for 15 minutes. Make up volume with methanol up to 100ml. Filter this solution with whatman filter paper no-1. (HCTZ-125mcg/ml, FIM-600mcg/ml)

Working Std Preparation:

Take 1ml from sample stock solution into a 10ml volumetric flask and make up with mobile phase. (HCTZ-12.5mcg/ml and FIM-60mcg/ml)

Acid Hydrolysis Study:

1 ml filtrate of standard stock solution and sample stock solution were taken into 10 ml of volumetric flask, separately 5 ml of 1 N HCl was added in both and kept for 1 hours at room temperature. Then 5ml of 1 N NaOH was added to neutralize it and volume was made up to mark with mobile phase mixed well and injected.

Base Hydrolysis study:

1 ml filtrate of standard stock solution and sample stock solution were taken into 10 ml of volumetric flask, separately 5 ml of 1 N NaOH was added to both and kept for 1 hours at room temperature. Then 5ml of 1 N HCl was added to neutralize it and volume was made up to mark with mobile phase mixed well and injected.

Peroxide Oxidation Study:

1 ml filtrate of standard stock solution and sample stock solution were taken into 10 ml of volumetric flask, separately 5 ml of 6% H_2O_2 was added to both and kept for 2 hours at room temperature. Then volume was made up to mark with mobile phase mixed well and injected.

Thermal Stress Study:

ROS and FIM std degradation:

Weigh accurately about 60 mg of FIM and 12.5 mg of HCTZ. Transferred into petri dish and was kept in preheated oven at 80^0C for 2 hours. Transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. Transfer 1 mL of above Solution into10Ml Volumetric flask to the mark with Methanol. Inject 20 µL the above solution under optimized chromatographic condition.

RESULTS AND DISCUSSION:

Selection of Analytical Wavelength:

A Working standard of FIMA (60 $\mu g.ml^{-1}$) and HCTZ (12.5 $\mu g.ml^{-1}$) using methanol as a solvent, were scanned in UV 200-400 nm region and overlapped.

When both UV spectrum were overlapped, iso-absorptive point was observed at 236 & 260 nm, and both the drugs showed adequate absorbance too. 260 nm was selected as detection wavelength.

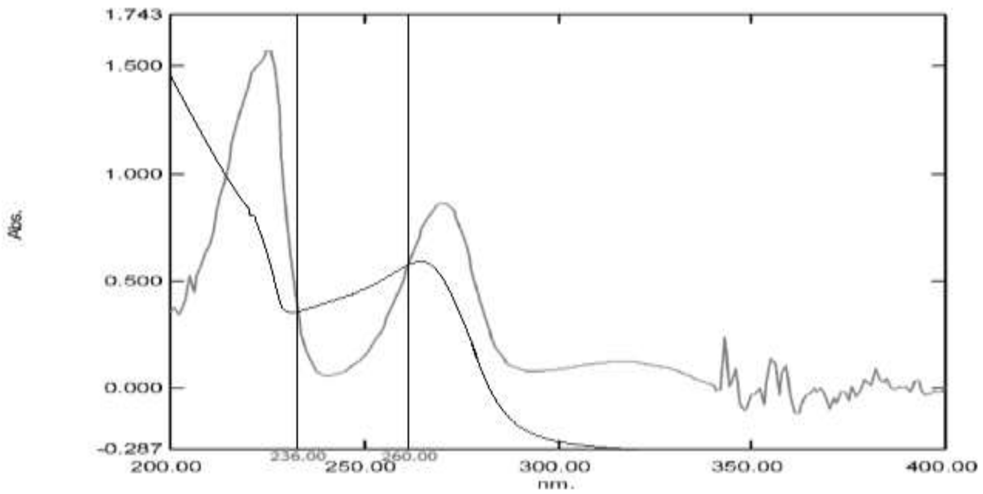

Fig.3: Overlay spectra of Hydrochlorothiazide and Fimasartan

Linearity and Range:

The linearity study was carried out for both drugs at different concentration levels. The linearity of FIMA and HCTZ was in the range of 30-150µg/ml and 6.25-31.25 µg/ml. % RSD of all results were less than 2%.

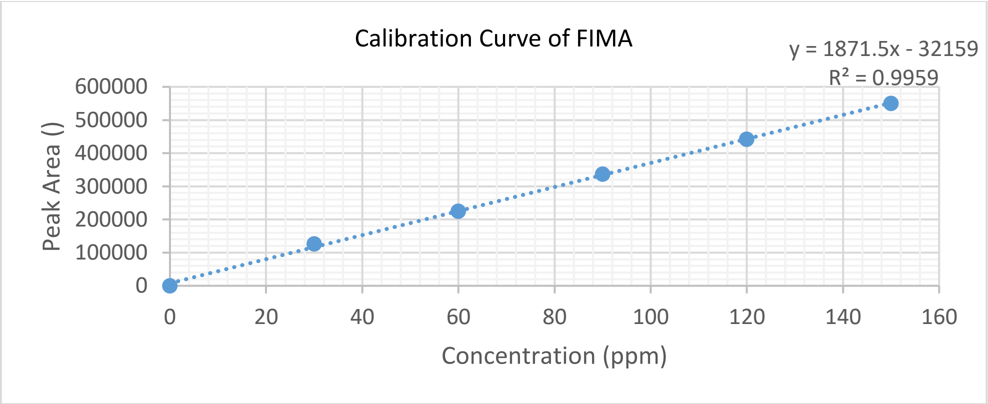

Fig.4: Calibration curve of Fimasartan

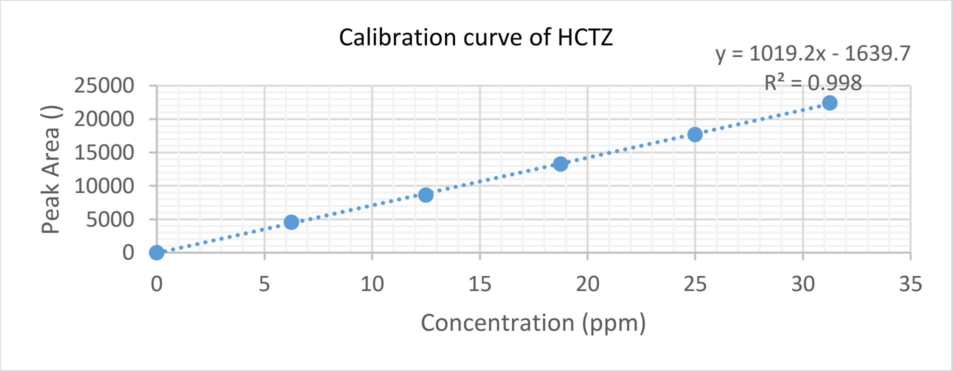

Fig.5: Calibration curve of Hydrochlorothiazide

Table-2 Linearity data for ROS and FIM in RP-HPLC:

Conc(mcg/ml)		**Area**	
HCTZ	**FIM**	**HCTZ**	**FIM**

6.25	30	4573.8	126069
12.5	60	8633	224474.6
18.75	90	13276.6	336579.2
25	120	17679.2	441869.4
31.25	150	22440.6	549570.4
correlation coefficient		0.995	0.998

Analysis of variance (ANOVA) of peak's area determined in the obtaining of the calibration curve of HCTZ using HPLC method						
Source of variation	***df***	***Sum of square***	***MS***	***F calculated***	***Significance F***	
Regression	1	20052304	20052304.8	4599.675	7.06	
Residual	3	130785.1	43595.05	-	-	
Total	4	20065383	-	-	-	

Analysis of variance (ANOVA) of peak's area determined in the obtaining of the calibration curve of FIMA using HPLC method					
Source of variation	***df***	***Sum of square***	***MS***	***F calculated***	***Significance F***
Regression	1	113294225	1132942250	9633.213	2.33
Residual	3	35282379	11760793	-	-
Total	4	1133295074	-	-	-

Table-3 Result of LOD and LOQ in RP-HPLC:

PARAMETERS	**HCTZ**	**FIM**
LOD	0.28 µg/ml	0.87 µg/ml
LOQ	0.85 µg/ml	2.65 µg/ml

Accuracy:

Table-4 Accuracy study of HPLC method:

Drugs	**Amount of drugs (µg/ml)**	**% Of std added**	**Total amount added**	**Amount found (µg/ml)**	**% Recovery (Mean ± SD)**	**% RSD**
HCTZ	12.5 (n=3)	50%	6.25	6.18	98.93 ±0.49	0.49
		100%	12.5	12.47	99.79± 0.68	0.68
		150%	18.75	18.61	99.27 ± 0.89	0.89

FIM	60 (n=3)	50%	30	29.55	98.49 ± 1.02	1.03
		100%	60	59.96	99.94 ± 0.37	0.37
		150%	90	89.27	99.19 ± 0.38	0.38

Precision:

Table-5 Intraday precision of HCTZ and FIM in HPLC:

HCTZ				**FIMA**		
Precision	**Conc (µg/ml)**	**(Mean ± SD) (n=3)**	**% RSD**	**Conc (µg/ml)**	**(Mean ± SD) (n=3)**	**% RSD**
Intraday	6.25	4571 ± 56.03	1.23	30	125723± 1340.8	1.07
	18.75	13270± 149.36	1.13	90	334465± 2771.79	0.83
	31.25	22477±211.46	0.94	150	546750±3841.52	0.70
Interday	6.25	4587.67± 66.52	1.45	30	125783.33±1411.10	1.12
	18.75	13231.67±172.02	1.30	90	334092.0±3380.72	0.34
	31.25	22496.67±248.40	1.10	150	546704.67±4467.96	0.82

Robustness:

Table-5 Robustness study of HPLC method:

Parameter	**Level of change**	**FIM**	**HCTZ**
Flowrate	0.9 mL/min	221314	8561
	1.0 mL/min	222436	8694
	1.1 mL/min	225234	8711
	Mean	222994.67	8655.33
	SD	2018.83	82.14
	RSD	0.91	0.95
Mobile Phase Composition	47-28-25	220537	8532
	45-30-25	222436	8694
	45-32-23	222937	8611
	43-30-27	226581	8761
	Mean	223122.75	8649.50
	SD	2526.64	99.50
	RSD	1.13	1.15

Analysis of variance (ANOVA) of FIMA for Robustness:

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8202.67	1	8202.67	65535	-	-
Within Groups	0	0	65535	-	-	-
Total	8202.67	1	-	-	-	-

Analysis of variance (ANOVA) of HCTZ for Robustness:

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16.99445	1	16.99445	65535	-	-
Within Groups	0	0	65535	-	-	-
Total	16.99445	1	-	-	-	-

Repeatability:

Table-7 Repeatability study of HPLC method:

HCTZ		**FIM**	
Mean ± SD (n=6)	**%RSD**	**Mean ± SD (n=6)**	**%RSD**
13320.64± 146.11	1.09	335712.32± 2334	0.69

Table-8 System suitability parameters:

System suitability test parameters	**HCTZ**	**FIM**
Retention time (min)	8.71	6.50
%RSD	1.17	1.44
Resolution (Rs)	4.93	
Tailing factor	1.31	1.06
Theoratical plates	8440	11711

Table-9 Analysis of Physical mixture:

Drugs	**Amount taken**	**%Amount of drug found**	**%RSD**
HCTZ (n=3)	12.5 µg/ml	99.44%	0.45
FIM (n=3)	60 µg/ml	98.78%	0.57

Stability study data:

1. **Acid degradation study (1 N HCl, 1 hr)**

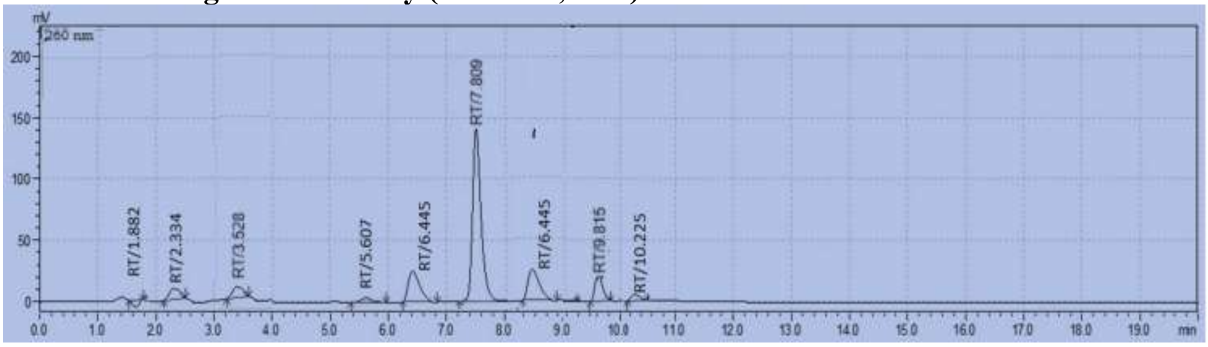

Fig.6: Chromatogram of acid hydrolysis (Performed with 1N HCl and refluxed at 60°C for 1 Hr)

2. **Base Degradation study (1 N NaOH, 1 hr)**

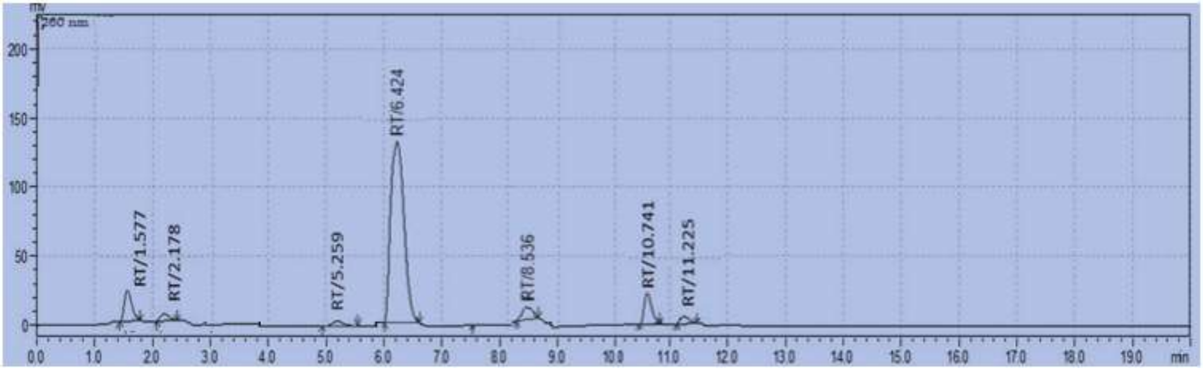

Fig.7: Chromatogram of base hydrolysis (Performed with 1N NaOH and refluxed at 60°C for 1 Hr)

3. **Peroxide study (6% H_2O_2 , 2 hr)**

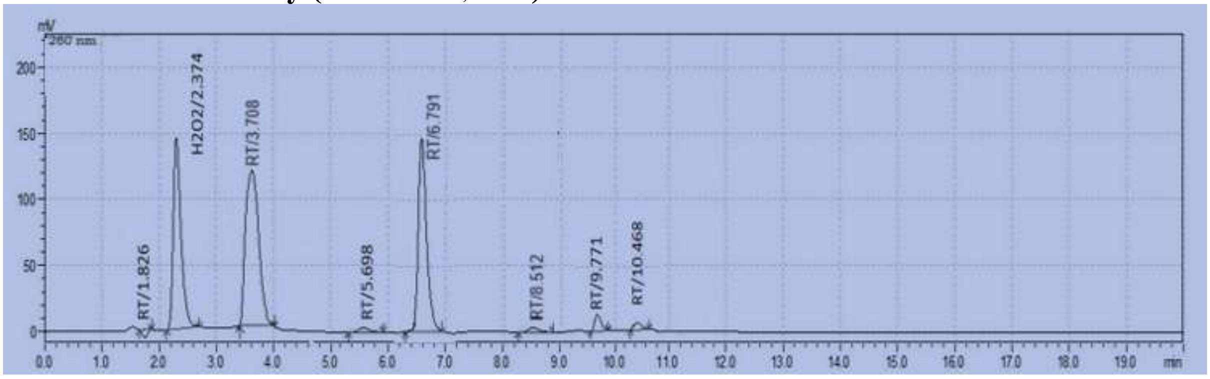

Fig.8: Chromatogram of Peroxide study (Performed with 6% H_2O_2 at 2 hr)

4. **Thermal degradation study (2 hours at 80 ºC temperature)**

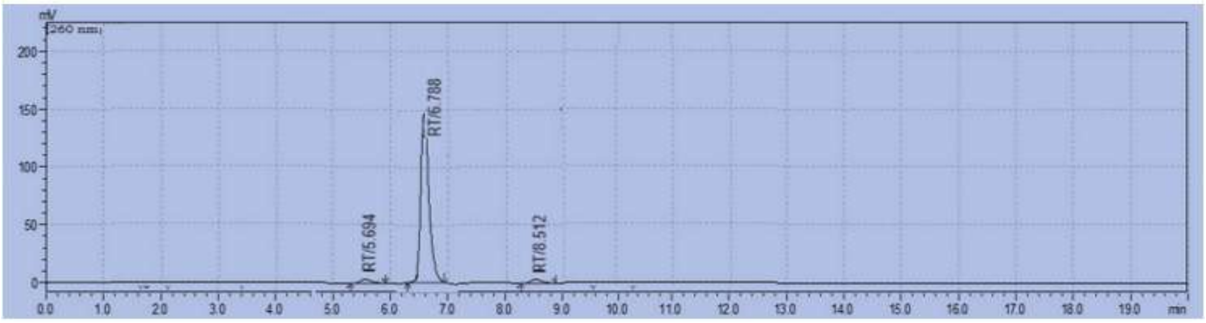

Fig.9: Chromatogram of thermal degradation study (Performed 2 hours at 80 ºC temperature)

Table-10 Summary of stress degradation condition:

Stress Condition	Area	FIMA	HCTZ	% Degradation	
				FIMA	HCTZ
Acid Hydrolysis	0-hr sample Area	218745	8589	18.75	20.08
	Treated Sample Area	177730	6864		
Base Hydrolysis	0-hr sample Area	216531	8521	21.52	19.31
	Treated Sample Area	169934	6876		
Oxidation Stress	0-hr sample Area	219745	8611	17.89	15.86
	Treated Sample Area	180433	7245		
Thermal Stress	0-hr sample Area	221554	8715	18.23	13.77
	Treated Sample Area	181165	7515		

CONCLUSIONS:

Proposed study describes a new stability indicating RP-HPLC method for the estimation HCTZ and FIM in combination using simple mobile phase. The method gives good resolution between the compounds along with its degradation products with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise and stability indicating. The run time is short which enables rapid quantitation of many samples in routine analysis. No interference from the excipients was observed. The results demonstrated that the method would have a great value when applied in quality control and stability studies for this drugs. So the developed method can be used conveniently for analysis of HCTZ and FIM in its combined pharmaceutical dosage form.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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