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Research Article

Stability-Indicating Analytical Method Development and Validation of Thiocolchicoside and Ibuprofen in Tablet Dosage Form by RP-HPLC Method

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Abstract

Objective: First time, a simple, specific, accurate and economic stability-indicating reverse phase high performance liquid chromatographic method was reported for the simultaneous estimation of THIO and IBU in tablet dosage form. **Method:** The method has shown adequate separation of THIO and IBU from their degradation products. Separation was achieved on an Inertsil, 3V ODS C18, 4.6 mm x 250 mm, 5µ column at wavelength of 248 nm, using a mobile phase Methanol: Dist. water (50:50, v/v) in a mode of isocratic elution at a flow rate of 1.0 ml/min. **Results:** This method results has minimum retention time for THIO and IBU i.e. 2.317 and 1.075 min. correspondingly, which gives fast separations of drugs furthermore both drug combinations are subjected to acidic, base, oxidation, thermal and photolytic stress environment. Thus stressed samples of these drugs are analyzed by the proposed analytical method. Quantitation was achieve with UV detection at 248 nm based on peak area with linear calibration curve at concentration range 100-600 ppm for THIO and 400-2400 ppm for IBU. The LOD's found 2.00 and 0.54 for THIO and IBU in addition to that LOQ's were found to be 6.08 and 1.63 resp. **Conclusion:** The statistical analysis proved that this novel proposed method was established to be specific, accurate, precise and stability-indicating study do not shows interfering peaks of degrades and excipient. The proposed method is therefore suitable for purpose in quality-control laboratories for quantitative analysis of THIO and IBU drugs individually and in combined dosage form, as it is performed and validated as per ICH Q2 (R1) and Q1A (R2) guideline and it meets to specific acceptance criteria.

Keywords: Thiocolchicoside (THIO), Ibuprofen (IBU), RP-HPLC, Stability-indicating, ICH.

1. INTRODUCTION:

1.1 Introduction of Drugs:

THIO is a glycoside of natural anti- inflammatory moiety which is derivative of colchicine from semi- synthetic process. It procured from the S*uperba Gloriosa* flower seeds. It gives anti-inflammatory as well as analgesic with muscle relaxant effects. It works through selective binding to the Gamma (y) Amino Butyric Acid i.e. GABA - A receptor. By activating the GABA inhibitory motor pathway it prevents muscle contractions so mainly it has major role in the cure of orthopedic and disorders of rheumatology [2- 4]. The molecular formula of THIO is $C_{27}H_{33}NO_{10}S$ and molecular weight is 563.6 g/mol. Fig.1-A shows structure of THIO.

IBU is derived from propionic acid which has a non-steroidal anti-inflammatory drug (NSAID) activity. IBU is a non-selective cyclooxygenase i.e. COX inhibitor therefore this drug inhibits the activity of both cyclooxygenase i.e. COX-1 and COX-2. The inhibition of cyclooxygenase-2 activity minimizes the overall synthesis of prostaglandins which involved in mediating inflammation, pain, fever, and swelling [5, 6]. The chemical formula for IBU is $C_{13}H_{18}O_2$ and molecular weight is 206.28 g/mol. Fig.1-B shows structure of IBU.

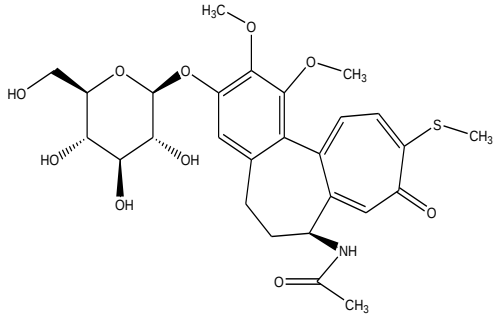

Figure 1-A: Thiocolchicoside

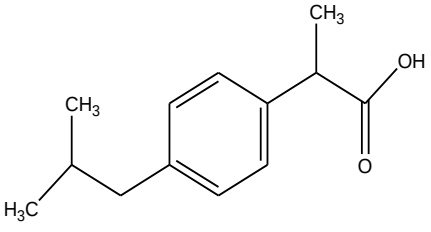

Figure 1-B: Ibuprofen

1.2 Background of study:

To the best of our knowledge, there is no reported RP-HPLC method for simultaneous estimation of THIO and IBU in pharmaceutical formulations, previous to our work. Thus, efforts were made to develop fast, selective and sensitive stability-indicating analytical method for the estimation of THIO and IBU in their tablet dosage form using Reverse Phase Chromatography i.e. RPC method. In the current work author developed a simple, accurate, reliable and reproducible stability-indicating RP-HPLC method which was duly validated by statistical parameters precision, accuracy and recovery as per ICH Q2 (R1) and Q1A (R2) guideline within all acceptance criteria.

2. MATERIALS AND METHODS:

2.1. Chemicals and reagents:

The commercial tablets Thiocolfen (Thiocolchicoside 400 mg and Ibuprofen 4 mg) were procured from the local drug market. Chemical is used as Acetonitrile (HPLC Grade), Methanol (HPLC Grade) and Dist. water (HPLC Grade) etc.

2.2 Instrumentation:

Instruments were as follows UV-Spectrophotometer (Jasco V 530 PC), HPLC- Schimadzu Model consisting of Inertsil, 3V ODS (C18 4.6 mm x 250 mm, 5µ columns), pH Meter (Chemiline).

2.3 Preparation of standard stock solutions:

2.3.1 Ibuprofen standard stock:

100.1 mg IBU standard was weighed and dissolved in 25 ml with diluent (use mobile phase as a diluent).

2.3.2 Final standard solution:

20.1 mg of THIO drug was weighed and add 5.0 ml of IBU standard stock dissolved in 25.0 ml with diluent.

2.4 Preparation of sample solutions:

The mean weight of twenty tablets was taken and after that it crushed to fine powder; amount equal to (powder) 100 mg was kept in flask of volumetric (100 ml). The drugs ratio was 1:100. This was then dissolving in 50 ml of diluent by sonication for about 10 min. The volume is made to the mark by diluent and filtered by Whatmann filter paper (no. 41) to forms 1000 µg/ml of sol$^{n.}$ and the this sol$^{n.}$ was utilized to prepare samples of various attentiveness.

2.5 Selection of detection wavelength:

From the standard stock solution further dilutions were done using diluent and scanned over the range of 200-400 nm and the spectra were overlain. It was observed that maximum wavelength of THIO and IBU 256 nm and 228 nm resp. and these drugs showed considerable absorbance at 248 nm and their overlain spectra of is given in fig. 2.

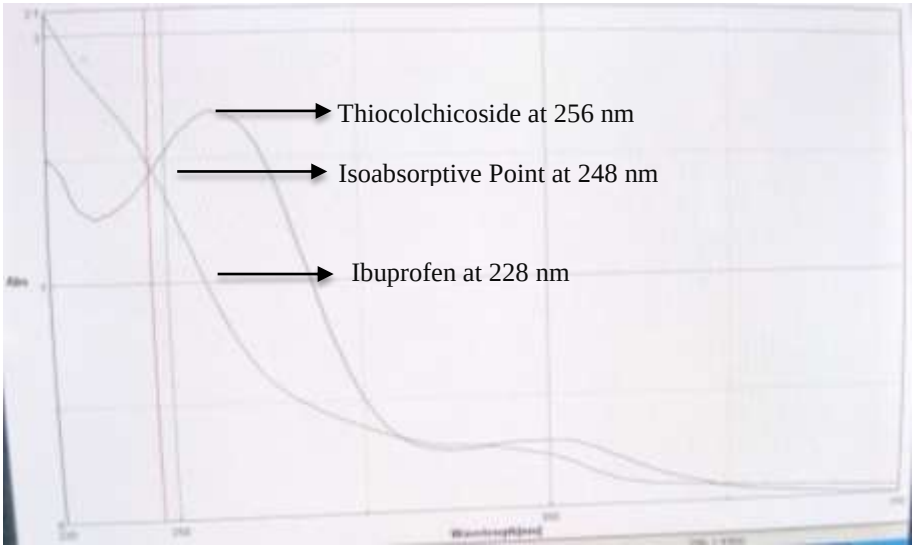

Figure 2: Overlain spectra of Thiocolchicoside & Ibuprofen

2.6 Chromatographic conditions:

HPLC experiment was carried out on a Schimadzu LC-2030 PLUS (IND) System separation module, with photodiode array detector using Auto sampler. The analytical column used for the separation was Inertsil, 3V ODS (C18 4.6 mm x 250 mm, 5µ columns). Optimized chromatographic conditions as shown in the table 1-A, Characteristic chromatogram was shown in fig. 3 and system suitability parameters of THIO and IBU shown in table 1-B.

Table 1-A: Optimized Chromatographic conditions

Sr. no.	Parameters	Method
1	Stationary phase (column)	Inertsil, 3V ODS (C18 4.6 mm x 250 mm, 5µ column)
2	Mobile phase	Methanol: Dist. water in 50:50, v/v
3	Flow rate (ml/min)	1.0
4	Column temperature (^{0}C)	30 ºC
5	Volume of injection (µl)	20
6	Detection wavelength (nm)	248 nm.
7	Run time	5 min.
8	Retention Time (min.)	Thiocolchicoside = 2.317, Ibuprofen = 1.075

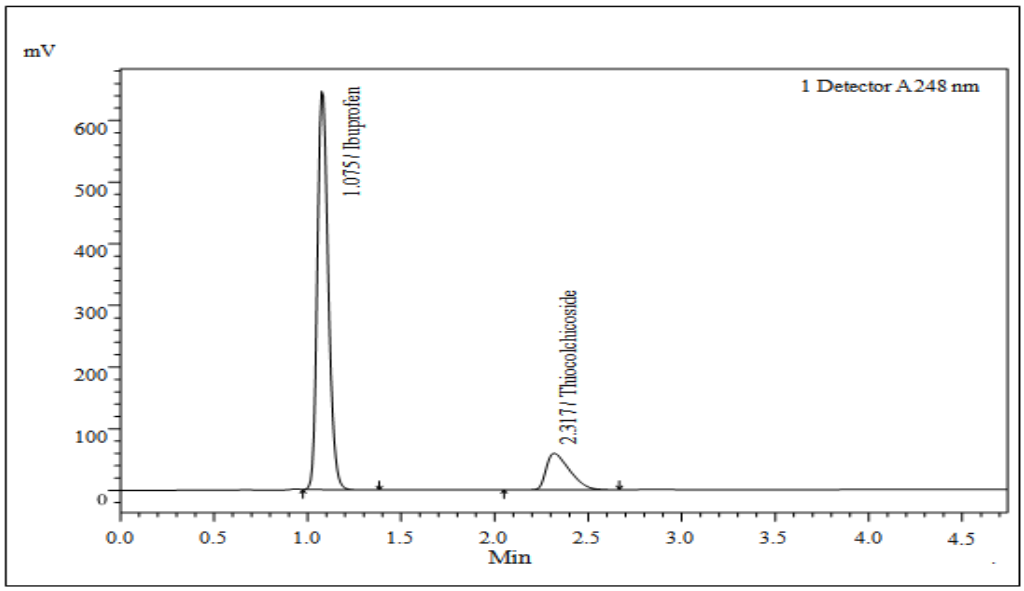

Figure 3: The chromatogram of optimized standard mixture

Table 1-B: System suitability parameters of optimized standard mixture

Sr. no.	Parameter	Retention Time	Resolution	Peak Area %	Theoretical Plates	Tailing Factor
1	Ibuprofen	1.075	--	83.633	8771	1.304
2	Thiocolchicoside	2.317	6.909	16.367	9866	1.703

3. METHOD DEVELOPMENT:

The objective of this experiment was to achieve good separation with good resolution peaks between all the components by trying different proportions of solvents like Methanol, Acetonitrile and Dist. water testing. For that we have tried different mobile phases and we obtained optimized chromatogram by using Methanol (MeOH) and Dist. water (H_2O) in 50:50, v/v ratios at 1 ml/min flow rate with 20 µl injection volumes in 5 min. run time and it was detected at 248 nm wavelengths. This trial gives more asymmetry & sharp peaks with good resolutions which is shown in fig. 3 and table 1-B.

4. METHOD VALIDATION:

This method was validated according to ICH validation parameters which gives acceptable results [16- 19];

5. FORCED DEGRADATION STUDIES:

Sample is stressed by different conditions to assess stability indicating aspect of the method. The degraded sample is analyzed using a HPLC [23, 24];

Table 2: Sample and percentage impurity detection in forced degradation studies

Sr. no.	Sample	% Impurity
1	As such sample	Not Detected
2	Acid Sample (2 mL of 2M HCl, heat for 10 minutes)	Detected
3	Base Sample (2 mL of 2M NaOH, heat for 10 minutes)	Detected
4	Oxidation Sample (2 mL of 2M H2O2, heat for 10 minutes)	Detected
5	Thermal Sample (Heat for 30 minutes at 50°C)	Detected
6	Photo-stability Sample (At 254 nm)	Detected
7	Photo-stability Sample (At 366 nm)	Detected

6. RESULTS AND DISCUSSION:

6.1 Accuracy:

Accuracy study was performed; in that drug Assay was conducted in duplicate as per desired test method with equivalent amount of THIO & Ibuprofen into flask of volumetric each for level of spike to become the mass of THIO & IBU equivalent to 25 %, 50 %, 75 %, 100 %, 125 %and 150 %for amount labelled as per desired method of test. The mean % recovery of the THIO & IBU at each apex level is not less than 90.0% and not more than 110.0%. Result of recovery study shown in table 3-A and 3-B.

Table 3-A: Data of Accuracy for THIO

Concentration % of spiked level	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	% Recovery (Mean)
25 % Sample 1	1233203	1250	1258	100.68	100.35
25 % Sample 2	1225136	1250	1250	100.02	
50 % Sample 1	1464534	1500	1497	99.78	99.97
50 % Sample 2	1470057	1500	1502	100.15	
75 % Sample 1	1704966	1750	1737	99.24	99.21
75 % Sample 2	1704038	1750	1736	99.19	
100 % Sample 1	1944899	2000	1982	99.10	99.07
100 % Sample 2	1943776	2000	1981	99.04	
125 % Sample 1	2197581	2250	2241	99.62	99.64
125 % Sample 2	2198298	2250	2242	99.65	
150 % Sample 1	2445173	2500	2492	99.67	99.63
150 % Sample 2	2443682	2500	2490	99.60	

Table 3-B: Data of Accuracy for IBU

Concentration % of spiked level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	% Recovery (Mean)
25 % Sample 1	6092161	5000	4962	99.25	99.01
25 % Sample 2	6063332	5000	4939	98.78	
50 % Sample 1	7198356	6000	5872	97.86	98.06
50 % Sample 2	7220608	6000	5895	98.25	
75 % Sample 1	8434814	7000	6858	97.97	97.96
75 % Sample 2	8432762	7000	6856	97.95	
100 % Sample 1	9778632	8000	7954	99.43	99.47
100 % Sample 2	9787977	8000	7962	99.52	
125 % Sample 1	11005046	9000	8960	99.55	99.95
125 % Sample 2	11093755	9000	9032	100.35	
150 % Sample 1	12302362	10000	10006	100.06	100.01
150 % Sample 2	12357775	10000	9996	99.96	

6.2 Precision:

The assays of THIO & IBU are not less than 90.0 % and not more than 110.0 %. The test results of combination gives that the method for test is precise. Result of recovery study for method and system precision are shown in table 4-A and 4-B.

Table 4-A: Method precision studies of THIO and IBU

Injection: 1000 ppm Concentration	Peak area of THIO	Average % Assay of THIO	Injection: 4000 ppm Concentration	Peak area of IBU	Average % Assay of IBU
1	990063	100.51	1	4953888	100.36
	989855			4946376	
2	989271	100.72	2	4934145	100.23
	989901			4928472	
3	996177	101.82	3	4872786	99.62
	996022			4887965	
4	995031	101.04	4	4896768	99.26
	995876			4897076	
5	992971	101.11	5	4897116	99.15
	996082			4869558	
6	995118	100.91	6	4895233	99.30
	991696			4895704	
Mean	993172	101.02	**Mean**	4906257	99.65
SD	2844.127	0.449	**SD**	27702.968	0.523
% RSD	0.29	0.44	**% RSD**	0.56	0.53

Table 4-B: System precision studies of THIO and IBU

Injection: 1000 ppm Concentration	RT of THIO	Peak area of THIO	Injection: 4000 ppm Concentration	RT of IBU	Peak area of IBU
1	2.317	991488	1	1.075	4943891
2	2.317	991189	2	1.075	4942174
3	2.317	991743	3	1.075	4947109
4	2.317	991287	4	1.075	4947168
5	2.317	993227	5	1.075	4948164
Mean	2.317	991787	**Mean**	1.075	4945701
SD	0.000	832.547	**SD**	0.000	2545.425
% RSD	0.00	0.08	**% RSD**	0.00	0.05

6.3 Linearity:

A series of solutions are prepare using IBU & THIO working standard at concentration levels for IBU from 400 ppm to 2400 ppm of target concentration & concentration levels for THIO from 100 ppm to 600 ppm of target concentration. The line are fit of the system was illustrated graphically. The calibration curve (Concentration V/s. Response) of THIO and IBU are shown in fig. 4-A and 4-B resp. The result of linearity study shown in table 5-A, and optical characteristics are shown in 5-B.

Table 5-A: Linearity studies of THIO and IBU

Sample	THIO		IBU	
Sr. no.	Concentration (ppm)	Area	Concentration (ppm)	Area
1	0	0	0	0
2	100	109328	400	558882
3	200	218650	800	1087640
4	300	310794	1200	1610268
5	400	422276	1600	2186391
6	500	514384	2000	2633475
7	600	619903	2400	3111865

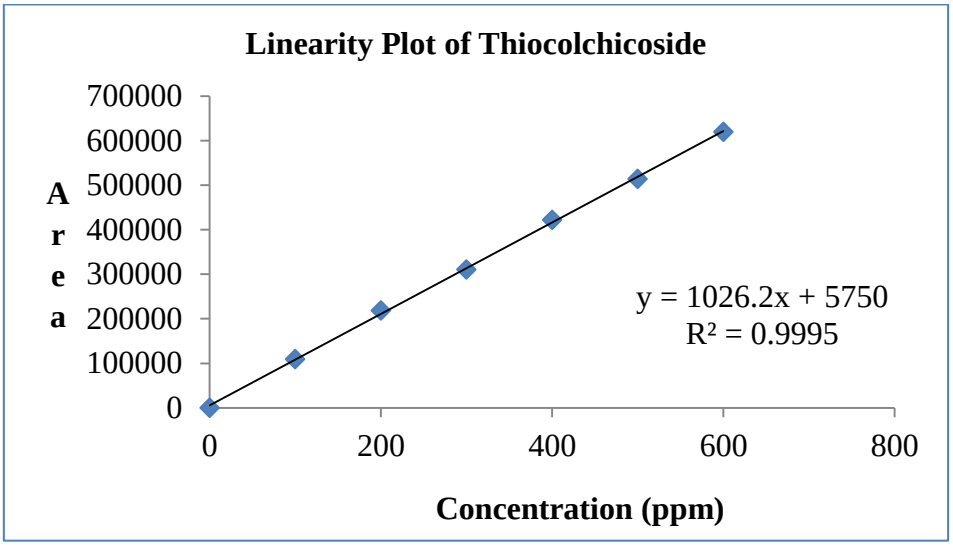

Figure 4-A: Linearity Plot of THIO

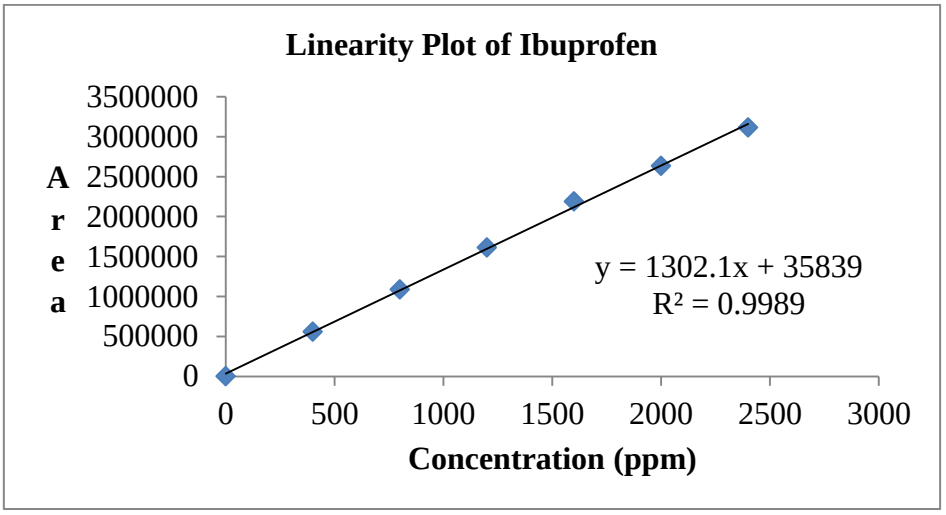

Figure 4-B: Linearity Plot of IBU

6.4 Limit of Detection (LOD) and Limit of Quantification (LOQ) parameters:

From the linearity data, the limit of detection and quantitation are calculated using the formula is as follow;

6.4.1 From the linearity plot the LOD and LOQ are calculated for IBU:

LOD = 3.3 × SD / Slope = 3.3 × 5843.029/ 35839 = 0.54

LOQ = 10 × SD / Slope = 10 × 5843.029/ 35839 = 1.63

6.4.2 From the linearity plot the LOD and LOQ are calculated for THIO:

LOD = 3.3 × SD / Slope = 3.3 × 3493.175/ 5749.7 = 2.00

LOQ = 10 × SD / Slope = 10 × 3493.175/ 5749.7 = 6.08

Table 5-B: Optical characteristics of THIO and IBU

Sr. No.	Parameter	THIO	IBU
1	Calibration range (ppm)	100-600	400-2400
2	Correlation coefficient (R^2)	0.9995	0.9989
3	Slope (m)	1026.2	1302.1
4	Intercept (c)	5749.7	35839
5	Limit of detection (µg/ml)	2.00	0.54
6	Limit of Quantitation (µg/ml)	6.08	1.63

6.5 Robustness:

To check robustness, we made alteration in rate of flow and mobile phase composition.

6.5.1 Effect of alteration in rate of flow:

THIO & IBU was resolved from peaks of all other and there retention times were comparable with peaks obtained for mobile phase having flow rates 1.0 ml/min. The factor of symmetry for THIO & IBU for alteration in rate of flow was within the limits which are reported in table 6-A & 6-B.

6.5.2 Effect of alteration in mobile phase composition:

THIO & IBU was resolved from all other peaks and there retention times were comparable with obtained for mobile phase having composition MeOH: H_2O (50:50 v/v). The factor of symmetry for THIO & IBU for alteration in mobile phase composition was within the limits which are reported in table 6-C & 6-D.

Table 6-A: Data for effect of alteration in rate of flow for THIO

	Std. Area	Tailing factor		Std. Area	Tailing factor		Std. Area	Tailing factor
Flow 0.9 ml	1104842	2.023	**Flow 1.0 ml**	989210	2.130	**Flow 1.1 ml**	906727	1.974
	1107341	2.058		983274	2.086		905476	1.975
	1107006	2.041		983699	2.163		906573	1.965
	1101161	2.099		990644	2.072		905461	1.988
	1103825	2.142		989493	2.010		910654	1.992
Avg.	1104835	2.073	**Avg.**	987264	2.092	**Avg.**	906978	1.979
SD	2525.456		**SD**	3493.175		**SD**	2138.767	
%RSD	0.23		**%RSD**	0.35		**%RSD**	0.24	

Table 6-B: Data for effect of alteration in rate of flow for IBU

	Std. Area	Tailing factor		Std. Area	Tailing factor		Std. Area	Tailing factor
Flow 0.9 ml	5503740	1.220	**Flow 1.0 ml**	4926593	1.248	**Flow 1.1 ml**	4521134	1.263
	5511045	1.260		4916621	1.300		4519566	1.262
	5505373	1.229		4930215	1.301		4522586	1.263
	5489310	1.194		4930645	1.306		4530553	1.290
	5498586	1.245		4929355	1.316		4531022	1.276
Avg.	5501611	1.230	**Avg.**	4926686	1.294	**Avg.**	4524972	1.271
SD	8187.656		**SD**	5843.029		**SD**	5417.521	
%RSD	0.15		**%RSD**	0.12		**%RSD**	0.12	

Table 6-C: Data for effect of alteration in mobile phase composition for THIO

	Std. Area	Tailing factor		Std. Area	Tailing factor		Std. Area	Tailing factor
MeOH: H_2O (40:60)	1290619	2.227	**MeOH: H_2O (50:50)**	989210	2.130	**MeOH: H_2O (60:40)**	839408	1.896
	1290262	2.182		983274	2.086		840286	1.855
	1289891	2.155		983699	2.163		841051	1.872
	1292215	2.152		990644	2.072		841117	1.836
	1291154	2.138		989493	2.010		841439	1.835
Avg.	1290828	2.171	**Avg.**	987264	2.092	**Avg.**	840660	1.859
SD	904.441		**SD**	3493.175		**SD**	817.917	
%RSD	0.07		**%RSD**	0.35		**%RSD**	0.10	

Table 6-D: Data for effect of alteration in mobile phase composition for IBU

	Std. Area	Tailing factor		Std. Area	Tailing factor		Std. Area	Tailing factor
MeOH: H_2O (40:60)	6406521	1.202	MeOH: H_2O (50:50)	4926593	1.248	MeOH: H_2O (60:40)	4217310	1.286
	6403788	1.203		4916621	1.300		4219308	1.268
	6404702	1.205		4930215	1.301		4222650	1.294
	6407753	1.209		4930645	1.306		4219250	1.295
	6407060	1.209		4929355	1.316		4227131	1.289
Avg.	6405965	1.206	**Avg.**	4926686	1.294	**Avg.**	4221130	1.286
SD	1661.295		**SD**	5843.029		**SD**	3865.517	
%RSD	0.03		**%RSD**	0.12		**%RSD**	0.09	

6.6 Ruggedness:

To check ruggedness, we made system-system variability as a system 1 to system 2 and study has performed on different HPLC systems for THIO & IBU under same circumstances at different times. Samples of six were made for drugs and each was studies as per method of test. Results of differentiation for both the obtained on 2 dissimilar systems of HPLC, proves that the given method for test assay are robust for variability of system-system for this combination. % RSD was within the limit for the systems. For result of system 1 refer table 4 and result of system 2 are given in table 7.

Table 7: Data of system to system variability (sample) System-2 for THIO and IBU

Injection: 500 ppm Concentration	Peak area of THIO	Average % Assay of THIO	Injection: 2 ppm Concentration	Peak area of IBU	Average % Assay of IBU
1	199392	101.06	1	965410	98.31
	200103			964230	
2	198897	101.71	2	957256	98.44
	199545			957660	
3	199928	101.53	3	956764	98.85
	196060			957645	
4	204903	100.97	4	974540	97.36
	202053			973943	
5	213950	101.27	5	1027003	98.01
	214199			1030645	
6	201204	101.84	6	970641	98.29
	203743			970123	
Mean	202831	101.40	**Mean**	975488	98.21
SD	5729.173	0.353	**SD**	25759.667	0.484
% RSD	2.82	0.35	**% RSD**	2.64	0.49

6.7 Forced Degradation Studies:

Analysis of statistics proved that the given method for THIO and IBU gives result values as per guidelines of ICH. All the stability studies results are shown in table 2 and Fig. 5.

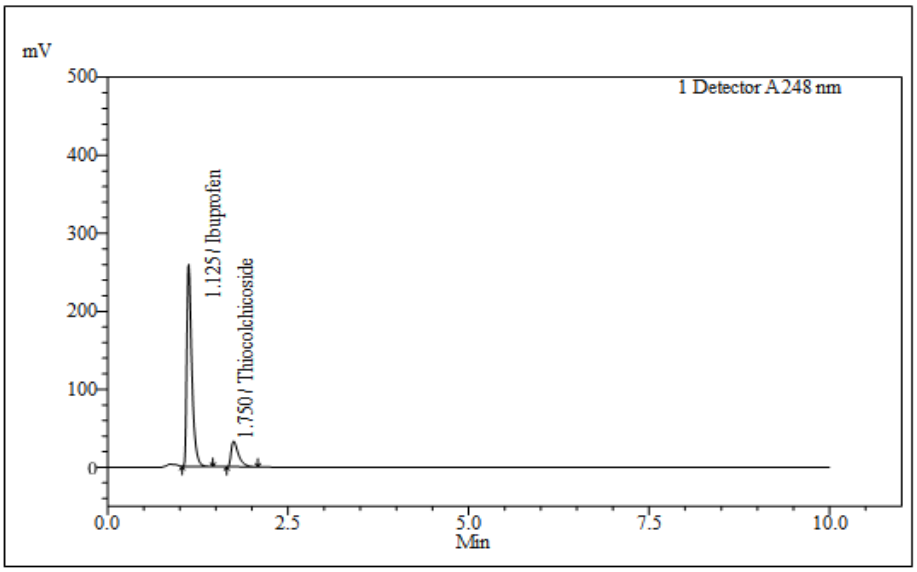

Figure 5-A: Chromatograms of Acid degradation

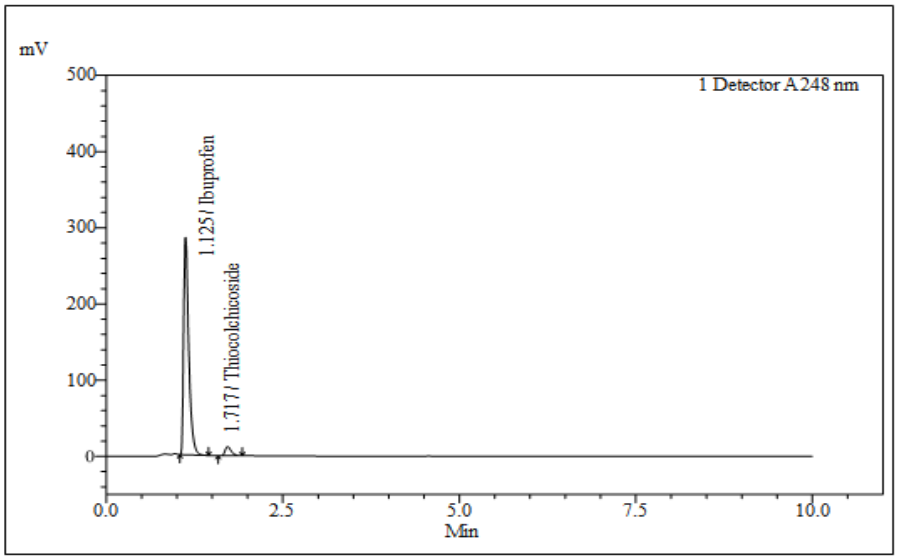

Figure 5-B: Chromatograms of Base degradation

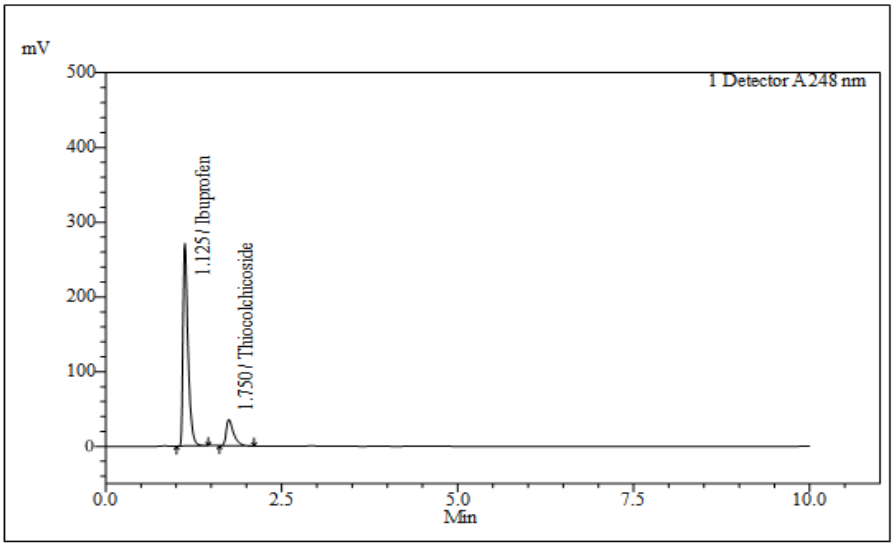

Figure 5-C: Chromatograms of Peroxide degradation

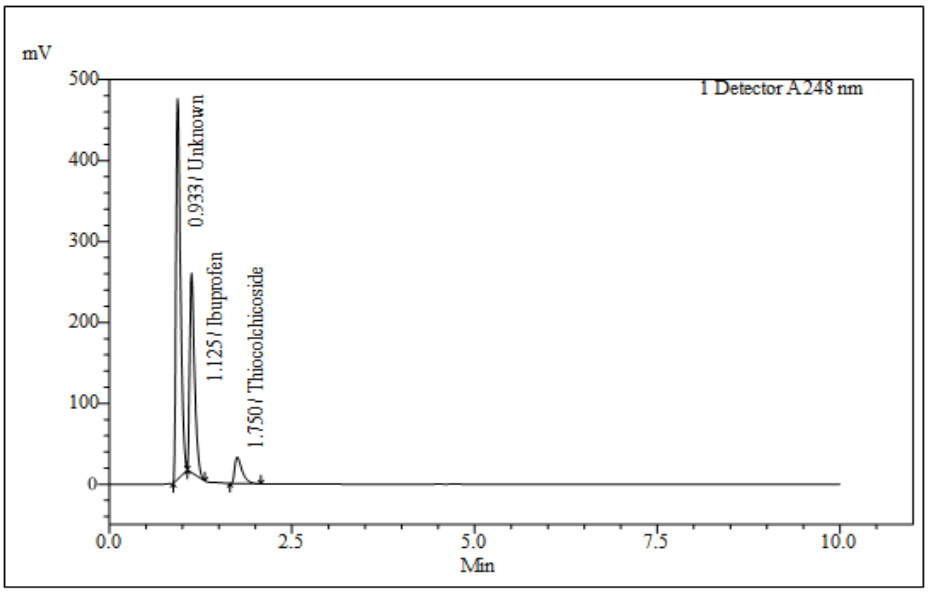

Figure 5-D: Chromatograms of Thermal degradation

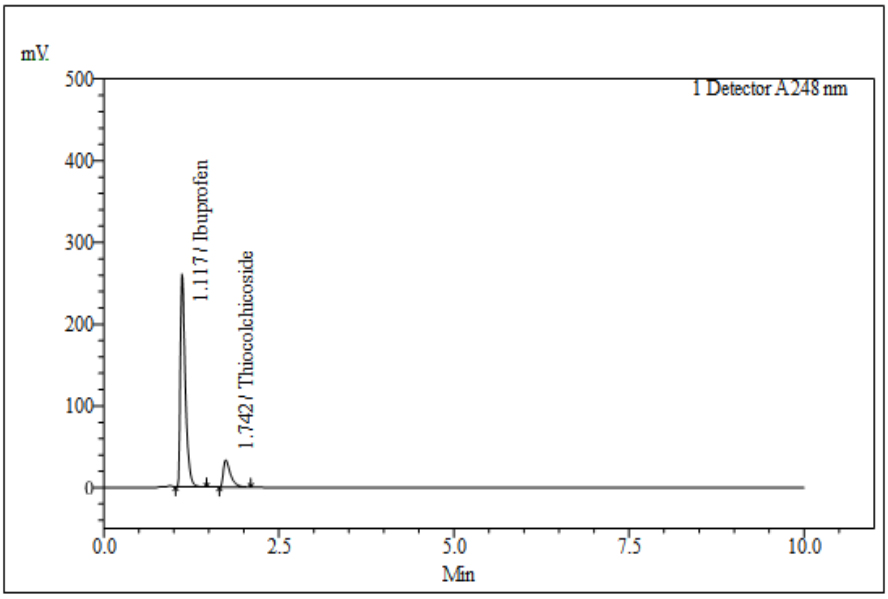

Figure 5-E: Chromatograms of Photostability at 256 nm

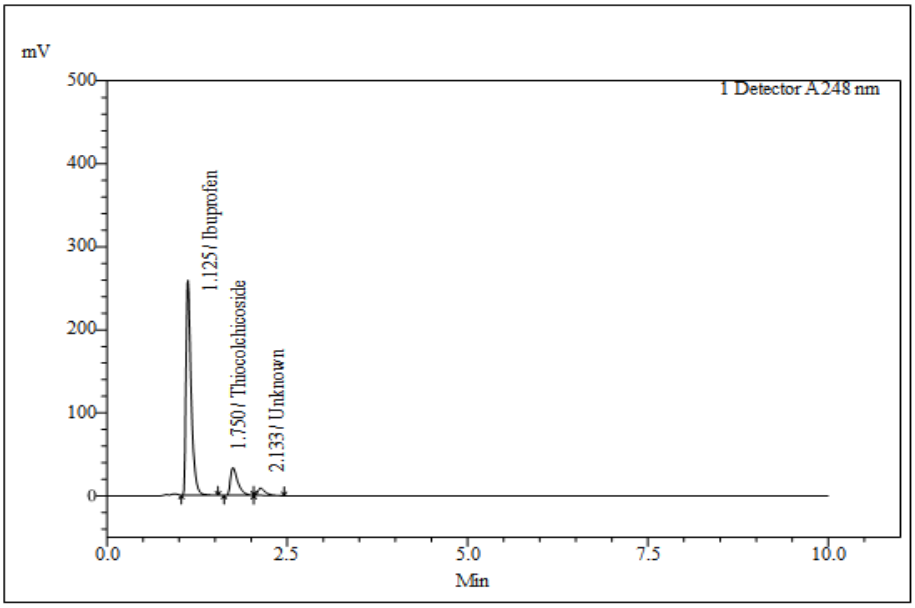

Figure 5-F: Chromatograms of Photostability at 228 nm

7. CONCLUSION:

In this a novel RP- HPLC (Stability- indicating) method has developed successfully & certified for the analysis of THIO and IBU in tablet dosage first time as stated by guidelines of ICH. This performed method was satisfyingly separated all the two compounds (drug) with degradants, estimate the active contents in forced degradation which is significant part of drug development stage and the pharmaceutical industry has a lots of attraction in this area.

CONFLICT OF INTEREST:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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