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Stability Indicating HPLC Method Development and Validation for Simultaneous Estimation of Ketoprofen and Thiocolchicoside in Combined Solid Oral Dosage Form

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ABSTRACT:

A new stability-indicating reversed-phase high-performance liquid chromatographic method for the analysis of Ketoprofen and Thiocolchicoside was developed and validated. The column used was Phenomex - C18 (150 × 4.6 mm, 5µ) with flow rate of 1.0ml/min using PDA detector at 330 nm. Methanol was used as a solvent. The chromatograms were developed using 1% Ammonium acetate and 1% acetic acid: Acetonitrile (80:20 %v/v) as a mobile phase. The described method was linear over a concentration range of 4000-6000 ppm and 320-480 ppm for the assay of Ketoprofen and Thiocolchicoside respectively. The retention times of Ketoprofen and Thiocolchicoside were found to be 16.104 min and 5.853 min respectively. The % recovery of Ketoprofen and Thiocolchicoside was found to be 101 % and 98.85 % respectively. The limit of detection and limit of quantification were found to be 16.37 µg/ml and 49.62 µg/ml for Ketoprofen and for Thiocolchicoside were found to be 13.60 µg/ml and 41.23 µg/ml respectively. The % RSD of Ketoprofen and Thiocolchicoside were found to be 0.09 % and 0.39 % respectively. The method developed is robust. The drug was exposed to acidic, basic, oxidative, photolytic and thermal degradation. The peaks of degradation products were well-resolved from the peak of the standard drug with significantly different values. Results showed that the developed method is simple, specific, accurate and robust for the determination of Ketoprofen and Thiocolchicoside in its formulation. The method can effectively separate the drug from its degradation products and it can be considered as stability-indicating assay.

Key words: Ketoprofen, Thiocolchicoside, Acetonitrile, Method Development, Validation, Stability study, Methanol.

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INTRODUCTION:

Ketoprofen (KET) is chemically known as 2-(4-isobutylphenyl) propionic acid, as shown in fig.1. It is a nonsteroidal anti- inflammatory and analgesic agent. Ketoprofen is used for its antipyretic, analgesic, and anti-inflammatory properties by inhibiting cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes reversibly, which decreases production of proinflammatory prostaglandin precursors. [1] Ketoprofen is official in IP 2014. [2]

Thiocolchicoside (THC) is chemically, N-[3-(B-D-glucopyranoxyloxy) - 5, 6, 7, 9-tetrahydro-1, 2-methoxy-10-(methylation) -9-oxobenzo[a]heptalen-7yl] acetamide, as shown in fig.2. It has selective affinity for γ-amino- butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA- inhibitory pathways thereby acting as a potent muscle relaxant. Its mode of action includes modulation of chemokine and prostanoid production and inhibition of neurophil and endothelial cell adhesion

molecules by which it interferes with the initiation and amplification of the joint inflammation. [3] Thiocolchicoside is official in IP 2014. [4]

Combination of Ketoprofen and Thiocolchicoside is approved by CDSCO in 2010. The literature survey reveals that only few selected RP-HPLC, UV methods were reported for estimation of Ketoprofen [5, 6] and Thiocolchicoside [7-9] in their individual dosage form and in combination with other drugs. On detailed Literature survey it has been found that stability indicating RP-HPLC method is not reported till date for simultaneous estimation of Ketoprofen and Thiocolchicoside. The method was validated as per ICH guidelines.

MATERIALS AND METHODS

The High performance liquid chromatography used was a Water-600 controller, UV-Visible Spectrophotometer Analytical used was a Uv-VIS spectro, Water bath: PEI-6B, pH meter: Hanna HI22/J, Ultra sonicator: PEI- UC-300 was used.

Chemicals and reagents used

Active pharmaceutical ingredient of Ketoprofen is gifted as a sample by Uma microns, Ranoli and Thiocolchicoside was obtained from Swiss parentrals, Ahmedabad. Marketed formulation containing Ketoprofen and Thiocolchicoside (50:4 %w/w) was procured from local pharmacy. Methanol was used as a solvent throughout the experiment and ammonium acetate, acetic acid and acetonitrile was used as mobile phase.

METHOD DEVELOPMENT

The method was developed by taking different trials by using different Columns Phenomex - C_8, C_{18} (150 × 4.6 mm, 5µ), Detector: PDA Detector was used for detection and analytical wavelength was obtained at 330 nm, injection volume used was 10 µl and flow rate applied was 1.0 ml/min. Run Time obtained was 16 minutes by using mobile phase 1% Ammonium acetate and 1% acetic acid: Acetonitrile (80:20 %v/v).

Preparation of solutions of KET and THC

Accurately weighed 500 mg of std.ketoprofen was transferred into a 100 ml volumetric flask and diluted with methanol (5000 ppm) and accurately weighed 40mg of std. Thiocolchicoside was transferred into a 100 ml volumetric flask and diluted with methanol. (400 ppm). Take 0.5ml from each of stock solution of Ketoprofen and Thiocolchicoside and make up the volume up to the marked level in 10 ml volumetric flask with methanol. (100 ppm).

Selection of analytical wavelength

The standard solutions of Ketoprofen and Thiocolchicoside in methanol were scanned in the UV range 200-400 nm using methanol as blank and the overlain spectra were recorded.330 nm analytical wavelength was selected for estimation of Ketoprofen and Thiocolchicoside as shown in fig.3

METHOD VALIDATION

Linearity

From standard stock solution, aliquots of 4.0,4.5,5.0,5.5 and 6.0 ml were transferred to 10 ml volumetric flask and volume was made up to the mark with methanol to obtain concentration of KET 5-10 µg/ml and THC 5-10 µg/ml. The calibration curve of area Vs concentration was recorded for both drugs as shown in fig.6 and 7.

Precision

Precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, reproducibility (Interday) precision, repeatability.

Intermediate precision

Solution containing 15 µg/ml of KET and 12 µg/ml of THC were analyzed six times. %RSD was calculated as shown in table 3.

Repeatability

Solution containing 15 µg/ml of KET and 12 µg/ml of THC were analyzed six times. %RSD was calculated as shown in table 4.

Limit of detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

LOD =3.3 *(σ/S)

Where, σ = standard deviation of the Y intercept of calibration curve.

S = mean slope of corresponding calibration curve.

Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ =10 *(σ/S)

Where, σ = standard deviation of the Y intercept of calibration curve.

S = mean slope of corresponding calibration curve.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guidelines at three different concentration levels 80%, 100%, 120% and the values were measured at all wavelengths for Ketoprofen and Thiocolchicoside. This performance was done in triplicate. The amount of Ketoprofen and Thiocolchicoside were calculated at each level and % recoveries were calculated by measuring the absorbance and fitting the values in equation as shown in table 5.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Here, the flow rate, pH and mobile phase composition were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. Here flow rate changes are studied as shown in table 6.

The standard solution of Ketoprofen and Thiocolchicoside in methanol was scanned in the UV range 200-400 nm using methanol as blank and the overlain spectra were recorded and 330 nm analytical wavelength was selected for estimation of Ketoprofen and Thiocolchicoside.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity chromatograms are as shown in fig.

Analysis of the marketed formulation

To determine the content of commercial formulation, 20 tablets (KET 50 mg: THC 4mg) were weighed, their mean weight was determined and finely powdered. An accurately weighed quantity of tablet powder equivalent to 1015.55 mg of KET was taken and diluted to 50 ml up to marked volume with methanol, sonicated for 15 min and diluted to 10 ml with methanol. The analysis was repeated in triplicate and % Recovery was studied as shown in table 7.

Forced Degradation Study

To develop stability indicating method, stress testing, in the form of forced degradation and a photo stability study, should be carried out on an early stage so that impurities and degradation products can be identified and characterized. The forced degradation Study was carried out to check acidic, basic, oxidative, thermal and photolytic degradation. The forced degradation was performed in the dark to exclude the possible degradation effect of light and controls of the respective solution was made at each stage of degradation to eliminate possible changes due to heat and light. It should be carried out according to ICH Q2A (R1) guideline.

Procedure for preparation of solutions for forced degradation study

Preparation of Std. stock solution

The std.solution was prepared by taking 40.7 mg Thiocolchicoside and was diluted with methanol in 100 ml volumetric flask and 500.4 mg of Ketoprofen and was diluted with methanol in 100 ml volumetric flask and this solution is further used as std.stock solution in stability studies.

Acid Degradation

Procedure to prepare blank solution for acid degradation

10 ml 1N HCl was kept in dark for 24 hours and after that the pH of acid was neutralized to 7.0 with 1N NaOH. It is

transferred to 100 ml volumetric flask and was diluted with methanol up to the marked level and then vial filled with preparation was injected to HPLC to check out acidic degradation.

Procedure to prepare solutions of KET and THC for acidic degradation

10 ml std.stock solution of mixture and 10 ml HCl were mixed and kept in dark for 24 hours. The pH of acid was being neutralized to 7.0 with 1 N NaOH and was transferred to 100ml volumetric flask and it is diluted with methanol up to the marked level and then vial filled with preparation was injected to HPLC to check out acidic degradation. Chromatogram is shown in fig.11.

Basic Degradation

Procedure to prepare blank solution for basic degradation

10 ml 1N NaOH was kept in dark for 24 hours and after that the pH of acid was neutralized to 7.0 with 1N HCl. It is transferred to 100 ml volumetric flask and was diluted with methanol up to the marked level and then vial filled with preparation was injected to HPLC to check out basic degradation.

Procedure to prepare solutions of KET and THC for basic degradation

10 ml std.stock solution of mixture and 10 ml NaOH were mixed and kept in dark for 24 hours. The pH of acid was being neutralized to 7.0 with 1 N HCl and was transferred to 100 ml volumetric flask and it is diluted with methanol up to the marked level and then vial filled with preparation was injected to HPLC to check out basic degradation. Chromatogram is shown in fig.12.

Oxidative Degradation

Procedure to prepare blank solution for oxidative degradation

10 ml 3% H_2O_2 was kept in dark for 24 hours and after that it was transferred to 100 ml volumetric flask and was diluted with methanol up to the marked level and then vial filled with preparation was injected to hplc to check out oxidative degradation.

Procedure to prepare solutions of KET and THC for oxidative degradation

10 ml std.stock solution of mixture and 10 ml 3% H_2O_2 were mixed and kept in dark for 24 hours. The solution is then transferred to 100 ml volumetric flask and it is diluted with methanol up to the marked level and then vial filled with preparation was injected to HPLC to check out oxidative degradation.

Thermal Degradation

Procedure to prepare blank solution for thermal degradation

10 ml mobile phase 1%ammonium acetate and 1%acetic acid: Acetonitrile (80:20v/v) was taken and exposed to 60^0C temperature for 24 hours in an oven. Then it was diluted with mobile phase up to 100ml. The resulting solution was injected in HPLC system and the chromatogram was recorded.

Procedure to prepare solutions of KET and THC for thermal degradation

10 ml Std.stock solution and 10ml mobile phase 1%ammonium acetate and 1%acetic acid: Acetonitrile (80:20v/v) was taken and exposed to 60^0C temperature for 24 hours in an oven. The solution was then diluted with mobile phase up to 100ml.The resulting solution was injected in HPLC system and the chromatogram was recorded.

Photolytic Degradation

Procedure to prepare blank solution for Photolytic degradation

10 ml mobile phase 1%ammonium acetate and 1%acetic acid: Acetonitrile (80:20v/v) was taken as test and methanol was taken as blank and was scanned in UV in the range of 200-400 ml.The chromatogram was then recorded.

Procedure to prepare solutions of KET and THC for photolytic degradation

10 ml Std.stock solution and 10ml mobile phase 1%ammonium acetate and 1%acetic acid: Acetonitrile (80:20 v/v) was used as test and methanol was taken as blank and was scanned in UV in the range of 200-400 ml.The chromatogram was then recorded.

RESULTS AND DISCUSSION

Solutions of standard drug were run in different mobile phases containing Acetonitrile and different buffer in different ratios. Different columns (e.g. C_8, C_{18}) with different dimensions were used. The retention times were found to be 16.104 min and 5.853 min and tailing factor was found to be 1.10 and 1.14 for Ketoprofen and Thiocolchicoside respectively. Phenomex, C_{18}, 150*4.6, 5µ analytical column was selected which gives a sharp and symmetrical peak with minimum tailing. Calibration curve was found to be linear at range of 5-10 µg/ml for both drugs. The correlation of coefficient (R^2) obtained was found to be 0.997 and 0.996 for Ketoprofen and Thiocolchicoside respectively. It was observed that the concentration range showed a good relationship. The LOD and the LOQ were found to be 16.37 µg/ml and 49.62 µg/ml for Ketoprofen and 13.60 µg/ml and 41.23 µg/ml for Ketoprofen and Thiocolchicoside respectively. The % RSD of Ketoprofen and Thiocolchicoside were found to be 0.09 % and 0.39 % respectively. The % assay of Ketoprofen and Thiocolchicoside was found to be 100.01 % and 100.05% respectively. The average % recovery for Ketoprofen and Thiocolchicoside was found to be 100.13% and 99.28% respectively.

CONCLUSION

A simple, accurate, precise, specific and robust, stability indicating RP-HPLC method is developed and validated for simultaneous determination of Ketoprofen and Thiocolchicoside in solid oral dosage form. Stability study was carried out to check out acidic, basic, oxidative, thermal and photolytic degradation which shows that the combination of drug is stable in nature and is free from interferences and excipients.

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TABLES

Table 1 System Suitability Parameters

Parameters	KET	THC	Acceptance criteria
Retention Time	16.104	5.853	<20
Theoretical plate	114587	1934	>2000
Tailing factor	1.10	1.14	< 2
Resolution	27.47	-	>2
Area	4307119	6087923	-

Flow rate	1.0 ml/min	1.0 ml/min	-

Table 2 Linearity Data of KET and THC

KET (n=5)		THC (n=5)	
Concentration (µg/ml)	**Mean peak area**	**Concentration (µg/ml)**	**Mean peak area**
4000	3925897	320	5018561
4500	4307119	360	6087923
5000	4757504	400	7053119
5500	5241088	440	8271795
6000	5725985	480	9056740
Correlation coefficient	0.997	Correlation coefficient	0.996

Limit: Correlation coefficient NLT 0.999, n = number of replicate injection.

Table 3 Intermediate Precision

Sr. no.	Peak area(n=6)		Retention Time(min.)		Tailing factor	
	KET	**THC**	**KET**	**THC**	**KET**	**THC**
1	23846561	2974932	12.72	5.24	1.85	1.38
2	23846661	2975492	12.70	5.36	1.92	1.40
3	23897771	2978685	12.61	5.21	1.89	1.38
4	23886672	2988431	12.60	5.18	1.88	1.38
5	23846581	2975492	12.60	5.19	1.81	1.38
6	23879862	2974892	12.69	5.20	1.99	1.41
Mean	**23867351.33**	**2977987.33**				
SD	**23438.83**	**5308.98**				
%RSD	0.09%	0.39%				

Limit: %RSD for area NMT 2.0%, n=number of replicate injection

Table 4 Repeatability data of KET and THC

Sr. no.	Peak area(n=6)		Retention time(min)		Tailing factor	
	KET	**THC**	**KET**	**THC**	**KET**	**THC**
1	232422	28478	16.098	5.906	1.10	1.17
2	232597	28978	16.096	5.904	1.09	1.19
3	232497	29501	16.096	5.927	1.10	1.20
4	231521	29504	16.103	5.942	1.09	1.22
5		28574				1.22
	230211		16.096	5.963	1.07	
6	231451	29501	16.097	5.982	1.08	1.25
Mean	**100.11**	**100.27**				
SD	919.8657	482.1990				
%RSD	0.40%	1.82%				

Limit: %RSD for area NMT 2.0%, n=number of replicate injection.

Table 5 Accuracy study of KET and THC

Drug	% Level	Amount of sample taken(mg)	Area (n=3)	Amount of standard. recovered (mg)	% Recovery	Mean	%RSD
KET	80	400.4	3714586	401.17	100.19	99.63	1.05
	100	500.3	4678591	505.32	101.00	100.13	0.78
	120	600.2	5547812	599.16	99.83	100.40	1.55
THC	80	40.2	7194989	39.83	99.08	99.68	0.53
	100	40.3	7195959	39.84	98.85	99.28	0.60
	120	39.8	7248565	40.13	100.82	99.68	1.01

TABLE 6 Robustness data of KET and THC

Variation	KET		THC	
	RT	**Peak area**	**RT**	**Peak area**
Flow rate (1 ml/min.)	16.180	5128022	6.150	
- 0.2	15.440		8132966	
+ 0.2	4659717		5.69	
% RSD	0.14		7499857	
			0.02	

Limit: %RSD for area NMT 2.0 %, RT = retention time

Table 7 Analysis of marketed formulation

Sr.no.	Actual conc.		Conc. Found		%Assay	
	KET(mg/ml)	**THC(mg/ml)**	**KET(mg/ml)**	**THC(mg/ml)**	**KET (%)**	**THC (%)**
1	500	40	500.4	40.2	100.08	100.5
2	500	40	500.2	40.1	100.04	100.25
3	500	40	499.6	39.8	99.92	99.5
Mean					100.01	100.5
SD					0.083	0.520
%RSD (Limit: NMT 2%)					0.08	0.51

Table 8 Regression analysis of KET and THC

Parameters	Ketoprofen	Thiocolchicoside
Linearity range(µg/ml)	4000-6000	320-480
Slope	906.8	25651
Intercept	25737	2106
Regression coefficient	0.997	0.996
Limit of detection(µg/ml)	16.37	13.60
Limit of quantification(µg/ml)	49.62	41.23
Retention time(min.)	16.104	5.853
Tailing factor	1.10	1.14
Resolution factor	27.47	-
Theoretical plate	114587	1934

Table 9 Results of forced degradation of KET

Stress condition	Time (hours)	Retention time (min.)	Area	% Area	Degradation (% Area)
1N HCl	24	12.907	5144415	60.02	20
1N NaOH	24	12.907	5744815	55.65	22.90
3% H_2O_2	24	12.901	6243216	75.27	24.89
Thermal	24	12.914	8243816	68.89	32.86
Photolytic	24	12.908	6943516	70.80	58.68

Table 10 Results of forced degradation of THC

Stress condition	Time (hours)	Retention time (min.)	Area		
1N HCl	24	2.451	6087591		
1N NaOH	24	2.443	7092461		
3% H_2O_2	24	2.445	7562487		
Thermal	24	2.448	8143261	71.56	37.17
Photolytic	24	2.448	9324561	83.59	42.56

FIGURES

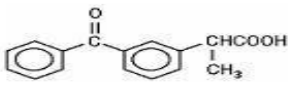

Fig. 1 Chemical structure of Ketoprofen

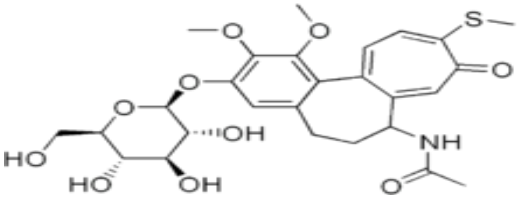

Fig. 2 Chemical structure of Thiocolchicoside

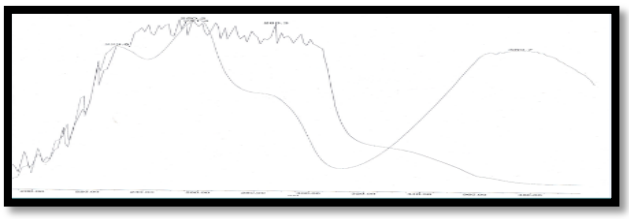

Fig.3 Overlain spectra of KET and THC

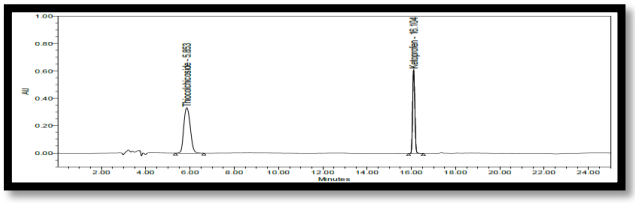

Fig.4 Optimized chromatogram of Ketoprofen and Thiocolchicoside

Linearity

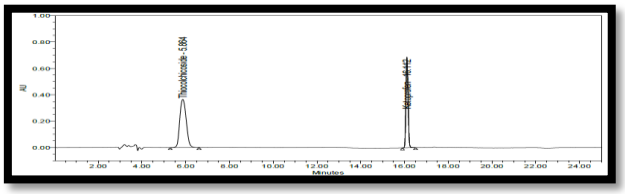

Fig.5 Overlay chromatogram of KET and THC in combination for linearity

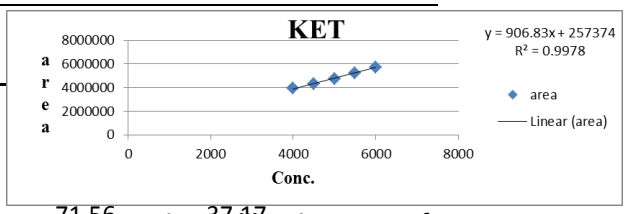

Fig.6 Calibration curve of KET

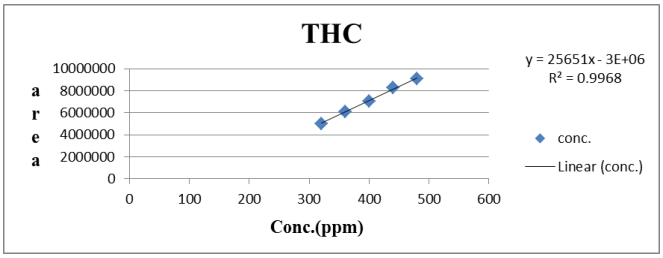

Fig.7 Calibration curve of THC

Specificity

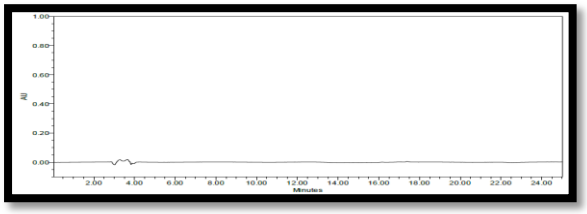

Fig.8 Blank Chromatogram

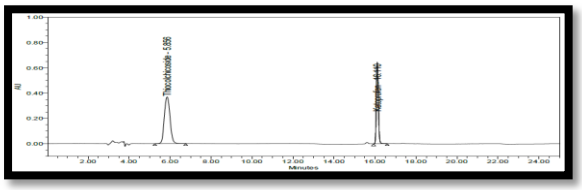

Fig.9 Chromatogram of KET and THC for marketed formulation.

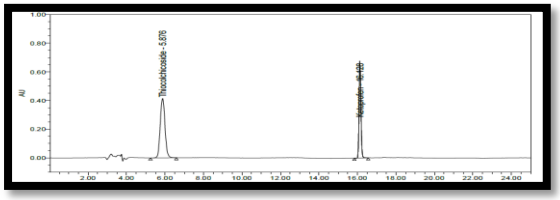

Fig.10 Chromatogram of KET and THC in combination for specificity.

Forced degradation study

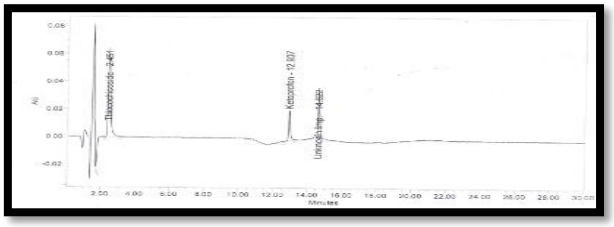

Fig.11 Chromatogram of Acid Degradation

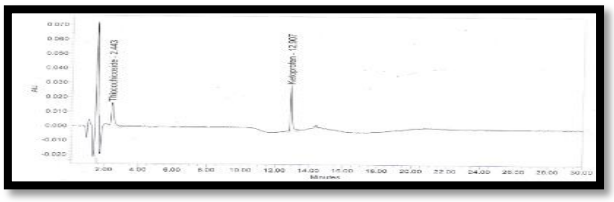

Fig.12 Chromatogram of Basic Degradation