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DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC AND ASSAY METHOD FOR DETERMINATION OF THIOCOLCHICOSIDE IN CAPSULE

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ABSTRACT

In the present work the approach of forced degradation study was successfully applied for the development of stability-indicating assay method for determination of Thiocolchicoside in the presence of its degradation products. The RP-HPLC separation was carried out on Shimadzu® - HPLC 1100 series using a Phenomenex ODS 5µ C_{18} column (250×4.6mm) with mobile phase comprising of Acetonitrile: Phosphate Buffer (70:30) pH 3.5 v/v at flow rate of 1.0mL/min and UV detection at 260.0 nm. In stress testing a drug substance or the drug product is exposed to an environment vigorous than the normal i.e. at high temperature, high humidity over the period of time called accelerated stability conditions. The drug was subjected to Solid state analysis which includes Humidity studies (40°C/75% RH), photochemical studies (UV light and sunlight exposure) and Thermal studies to apply stress conditions. The method was validated as per ICH guidelines for accuracy, precision, linearity and range, ruggedness and robustness. The linearity of the proposed method was investigated in the range of 80-120% of label claim; the correlation coefficient for Thiocolchicoside was found to be 0.999. The proposed method was found to be simple, specific, linear and rugged and can be used for routine quality control.

Keywords: Thiocholchicoside, HPLC, Stability, dissolution, capsule

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INTRODUCTION:

Thiocolchicoside (TCD) is an anti-inflammatory analgesic agent with muscle relaxant action. Thiocolchicoside is a glucoside extracted from the seeds of Colchicum autumnale. It has muscle relaxant, anti-inflammatory, analgesic and anesthetic actions with minimal side effects and is used topically for the treatment of mascular spasms and for rheumatologic, orthopaedic and traumatologic disorders. Thiocolchicoside [1]chemically is (s)-N-[3-(B-D-glucopyranoxyloxy) – 5, 6, 7, 9- terahydro-1, 2 dimethoxy- 10- (methyl thio)-9- oxobenzo [a] heptalen-7yl] acetamide (Figure 1)

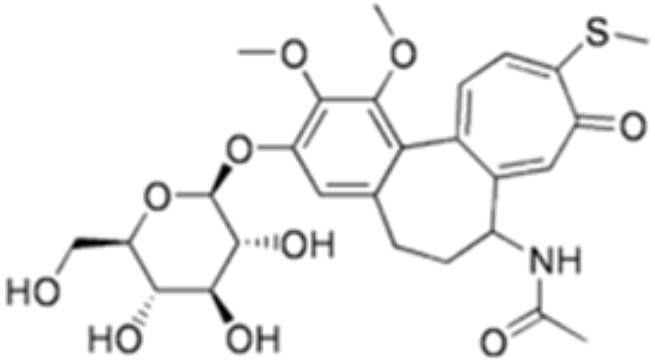

Figure 1 Structure of Thiocolchicoside

Literature survey reveals the determination of thiocolchicoside in its binary mixtures (thiocolchicoside/glafenine and thiocolchicoside/floctafenine by TLC/densitometry [2], highly specific and sensitive liquid chromatography–tandem mass spectrometry method for the determination of 3-desmethylthiocolchicine in human plasma as analyte for the assessment of bioequivalence after oral administration of thiocolchicoside [3], simultaneous estimation of Thiocolchicoside and Aceclofenac in pharmaceutical dosage form by spectrophotometric and LC method[4], Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form [5], determination of Alkaloids from the colchicine family by reversed phase high-performance liquid chromatography [6], simultaneous determination of diclofenac sodium and thiocolchicoside in fixed dose combination (tablet dosage form) by spectrophotometry[7], Quantitative estimation of thiocolchicoside in capsules by UV-Spectrophotometric [8], HPLC/UV method for quantitative analysis of Thiocolchicoside and its active metabolites in human[9]. A detailed literature survey revealed that though Thiocolchicoside have been analysed by many methods in combination with various drugs, none of the published analytical methods report degradation study of Thiocolchicoside in its dosage form.

The present paper describes the validation of stability-indicating gradient RP-HPLC method for the determination of TCD in presence of their degradation products. The method was validated according to ICH guidelines [10]. For the establishment of stability-indicating nature of method [11, 12], forced degradation of drug substances and drug products was performed under stress conditions and then the samples were analysed by the established method. The proposed RP-HPLC method was able to separate both drugs from degradation products generated during forced degradation studies.

MATERIALS AND METHOD

Materials:

Thiocolchicoside API and capsule formulation were supplied by Sanofi Aventis and Sun Pharma Pvt. Ltd. Label claim of TCD in formulation is 4mg.

Chemicals and Reagents:

a) Acetonitrile - HPLC grade
b) Potassium dihydrogen phosphate - AR grade
c) Orthophosphoric acid - AR grade
d) Double Distilled Water
e) Methanol - HPLC grade

Chromatographic system:

The HPLC system Shimadzu® HPLC 1100 series chromatograph equipped with binary pump LC- 10ADvp, UV- Visible detector with manual injector 7725 I (Rheodyne) with 20 µl loop and a reversed phase 5 µ Phenomenex ODS C18 column (250 x 4.6 mm) with pore size of 100 A^0 was used for chromatographic studies. The column was maintained at 28-30°C and injection volume of 20 µl was used. The mobile phase used for the studies consist of Phosphate Buffer: Acetonitrile (30:70) v/v and the pH adjusted to 3.5 with Orthophosphoric acid. The mobile phase was filtered through 0.45µ membrane filter and degassed by sonication. The flow rate was adjusted to 1.0 mL/min and detection was carried out on wavelength 260 nm. The mobile phase was used for the preparation of stock and diluted solutions of standard and test samples.

Preparation of Stock and Standard Solutions:

Standard stock solution of TCD (Concentration: 100 µg/mL, TCD) was prepared in the mobile phase. A 1.0 mL portion of the above standard solution was transferred using A-grade single mark pipette and was diluted up to 10.0 mL with mobile phase in volumetric flask to get the concentration of 10µg/mL TCD. The Standard Chromatogram for TCD is shown in Figure 2.

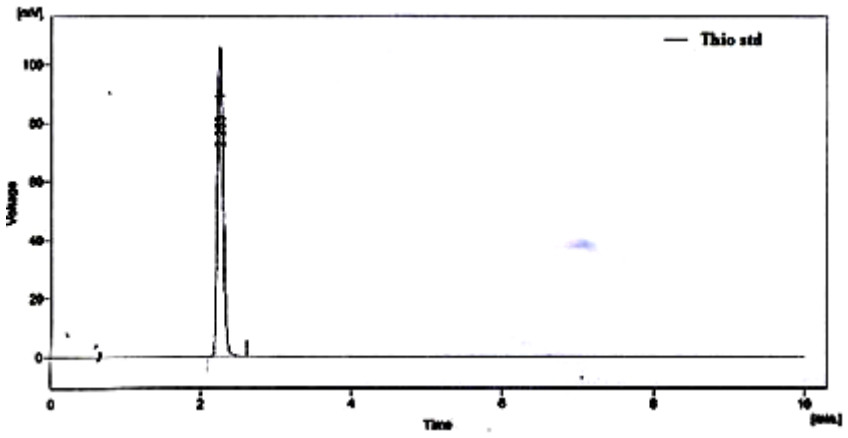

Figure 2. Standard Chromatogram of Thiocolchicoside

Preparation of Sample solution for assay:

Twenty filled capsules were weighed. Capsule shells were removed, weighed and the weight of empty capsule shell was deducted from the total weight to obtain the content of capsule powder (average weight 0.21593 g). An accurately weighed quantity of capsule powder equivalent to average weight of capsule was transferred to 50.0 mL volumetric flask to it sufficient quantity of mobile phase was added, sonicated for 30 min and then volume was made up to mark with mobile phase. The content of the flask was filtered through Whatmann filter paper (no.41). A 2.5 mL portion of the filtrate was further diluted to 10.0 mL with mobile phase to get concentration of 20.0µg/mL (on label claim basis). Results are shown in Table 1.

Preparation of the Sample and Standard solutions for Stress degradation studies:

A) Preparation of Standard solution (exposed): Accurately weighed TCD (~10.0 mg) was transferred to 50.0 mL volumetric flask and volume was made upto mark with mobile phase. A 2.5 mL portion was further diluted with mobile phase up to 25.0 mL to get concentration 20.0µg/mL.

B) Preparation of Sample solution (exposed): On the sampling day, accurately weighed capsule powder (~4.0 mg of TCD) was transferred to 50.0 mL volumetric flask to it 25.0 mL of mobile phase was added. The flask was sonicated for 30 min. and then volume was made up to the mark with mobile phase in volumetric flask of 50.0 mL. The contents of the flask were filtered through whatmann filter paper (no.41). A 2.5 mL portion of the each filtrate was further diluted with mobile phase up to 10.0 mL to get concentration 20.0 µg/mL of TCD (on label claim basis).

Stress Studies:

Stress studies are carried out under the conditions of humidity, photolysis (UV and visible light exposure) and thermal on standard drug and marketed formulation.

Humidity study was carried out on the standard and marketed formulation. The standard drug and commercial packed marketed formulation (strip) was kept in stability chamber set at condition 40°C/75%RH for a period of two months. The exposed standard and marketed formulation were withdrawn at an interval of 7 days (1st, 7th, 14th, 21st and 28th day). On the sampling days, the standard and sample solutions were prepared by the following the procedure described earlier. The overlain chromatogram so recorded for standard drug solution and sample solutions are shown in Figure 3 and 4 respectively.

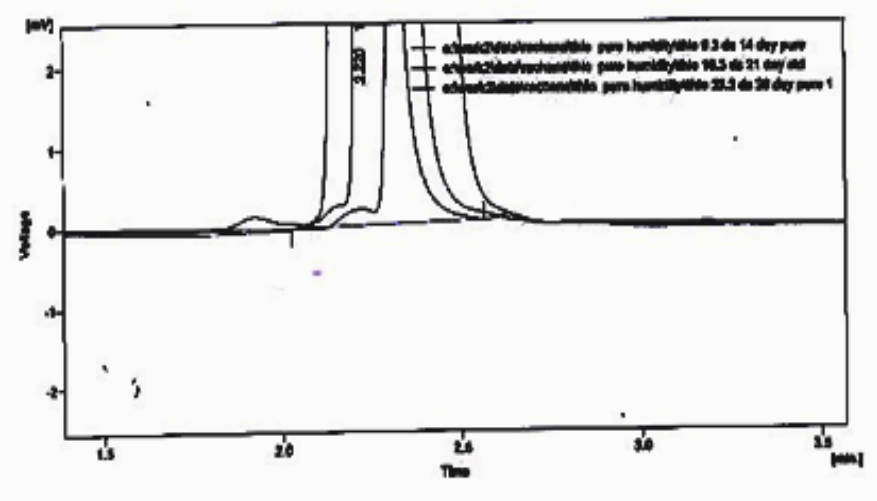

Figure 3. Overlain chromatogram of standard solution (exposed) to humidity study

For Photochemical studies (UV light exposure), according to the ICH Guidelines samples should be exposed to light providing an overall illumination of not less than 200 watt hours / square meter. Accordingly the study was carried out for 7 days. TCD standard drug and capsule were placed in two different Petri dishes and kept in the stability chamber under UV Light exposure as per ICH guidelines. The samples were withdrawn on 1st, 3rd and 7th day for analysis. The overlain chromatogram so recorded for standard drug solutions and sample solutions are shown in Figure 5 and 6 respectively. For Photochemical studies (Visible light exposure), the samples are exposed for 1.2 million Lux hours in photo stability chamber. The samples were withdrawn on 1st and 4th day for analysis. On the sampling days, the standard and sample solutions were prepared by the following the procedure described earlier. The overlain chromatogram so recorded for standard drug solutions and sample solutions are shown in Figure 7 and 8 respectively.

A thermal study was carried out on the standard and marketed formulation, placing the samples (solid state, commercial packing while solution state using capsule powder in 0.1N HCL) were kept at 40°C for a period of 2 h, withdrawing the samples every 30 min. The standard and

sample solutions (exposed to Solid/solution state stability) so prepared were chromatographed separately using optimized conditions. On the sampling days, the standard and sample solutions were prepared by the following the procedure described earlier. The overlain chromatogram so recorded for standard drug solutions and sample solutions (exposed to solid/solution state stability) are shown in Figure 9 and 10/11 respectively.

RESULTS AND DISCUSSION

The present research pursuit aims at the development and validation of stability- indicating assay method for determination of thiocolchicoside in capsule dosage form. During development of the method, number of mobile phases in different compositions were attempted to elute TCD. Method development was started with acetonitrile and phosphate buffer pH 3.5 used in different proportions and with different pH. The ratio of buffer and acetonitrile was selected on the basis of elution of TCD with sharp well defined peak with reasonable retention time. A mobile phase containing Phosphate Buffer pH 3.5: Acetonitrile (30:70) v/**v** was selected which gave reasonable retention time of 2.247. The system suitability studies were carried out which showed that all the parameters were found to be within range which passes the system suitability test and can be used for further analysis.

The developed method was also applied for routine analysis and stability studies of capsule formulation.

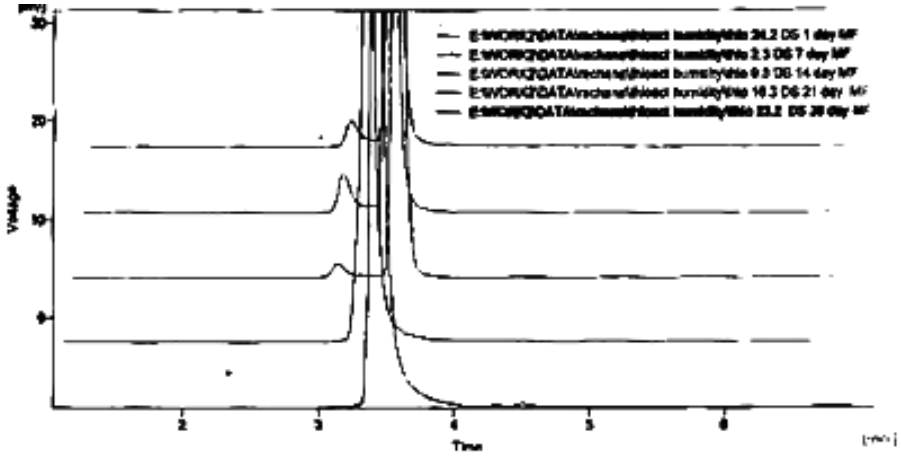

Figure 4. Overlain chromatogram of sample solution (exposed) to humidity study

Stability studies:

The humidity study was carried out at 40°C/75% RH on the standard drug (TCD) and the marketed formulation for a period up to two months. The study reveals that standard drug when exposed to 40°C/75% RH showed slight fronting in the peak but no additional peak(s) was observed. Similarly, sample on exposure to 40°C/75% RH showed the formation of additional

peak (Figure 4) which goes on increasing up to 21st day. This generation of additional peak in sample could be attributed to the formation of degradation products due to drug excipient interaction and not due to the drug, since chromatogram of standard drug (exposed) to similar condition showed no presence of additional peaks.

The photolytic degradation study was carried out for the period of 7 days. The study of chromatograms for UV light exposure (254.0 nm) reveals (Figure 5 and 6) decrease in % un degraded drug in both standard and sample on exposure to UV light, indicating that the drug is susceptible to UV light. The study of overlain chromatograms (on visible light exposure) for Standard (exposed) and sample (Figure 7 and 8) revealed that the drug was degraded to around 10% in both standard (exposed) and sample. The formation of additional peak in sample on 1st day showed that the degradation in sample is accelerated due to photochemical reaction between the drug and excipient leading to slow formation of additional peak, which does not occurred in standard drug (exposed).

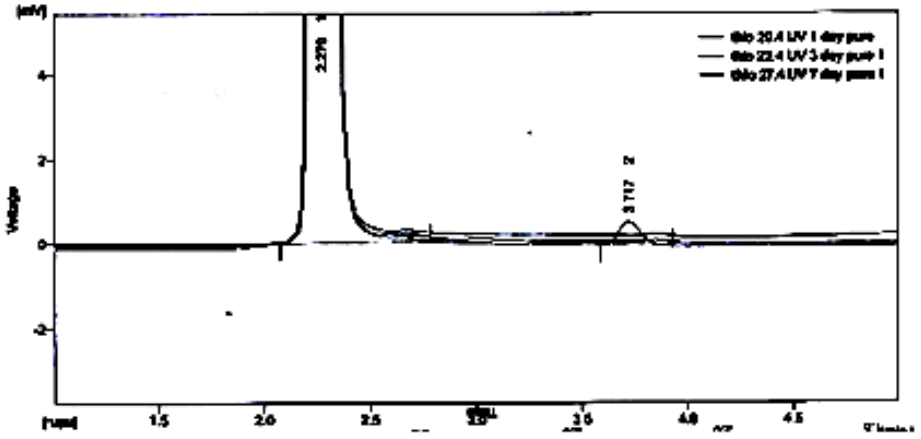

Figure 5. Overlain chromatogram of Standard under UV light exposure

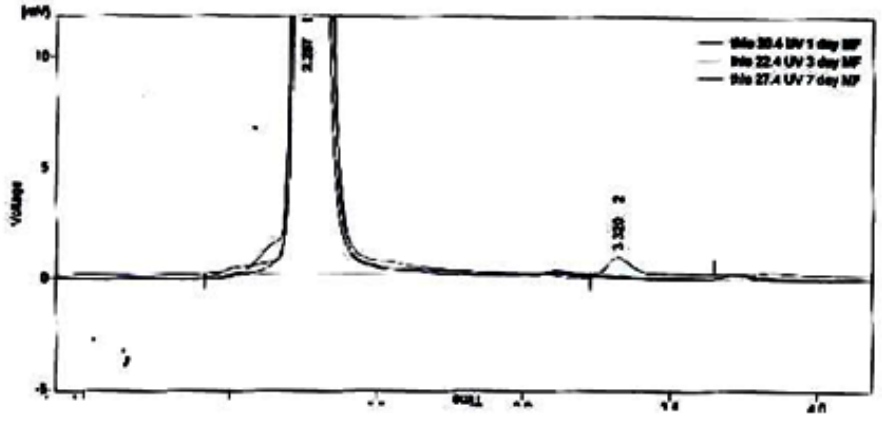

Figure 6. Overlain chromatogram of Sample under UV light exposure

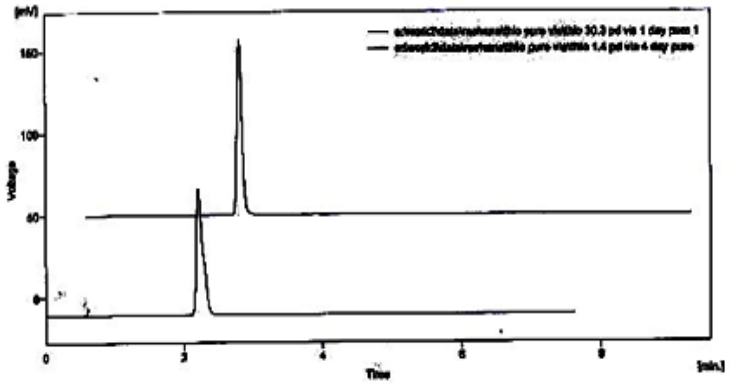

Figure 7. Overlain chromatogram of Standard at visible light exposure

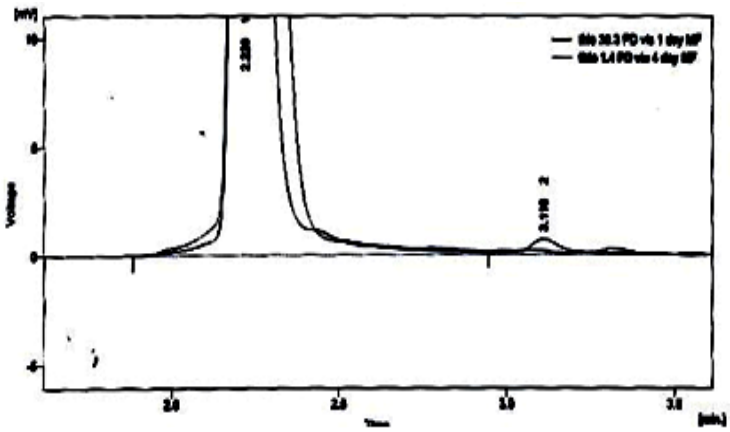

Figure 8. Overlain chromatogram of Sample at visible light exposure

Thermal degradation study revealed the slight degradation of standard drug with no generation of additional peak (Figure 9) while sample showed more amount of degradation in solution state analysis (40°C/0.1N HCl) with additional peak on 30th min of exposure (Figure 10) as compared to solid state analysis (Figure 11) on exposure to 40°C showed presence of additional peak around 60th min of exposure.

All the results of Stress degradation studies are summarised in Table 2.

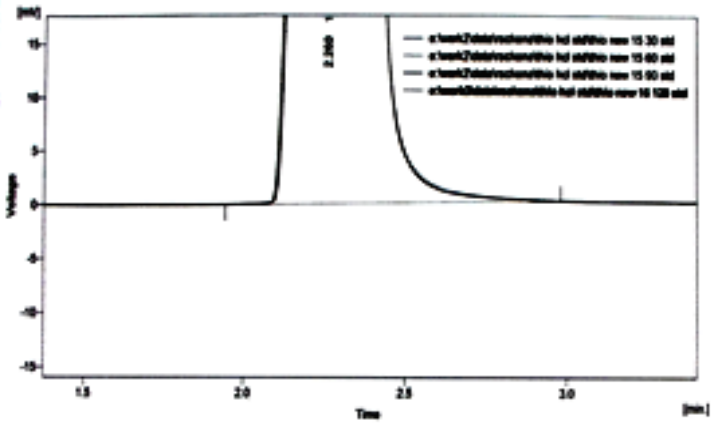

Figure 9. Overlain chromatogram of Standard under thermal studies

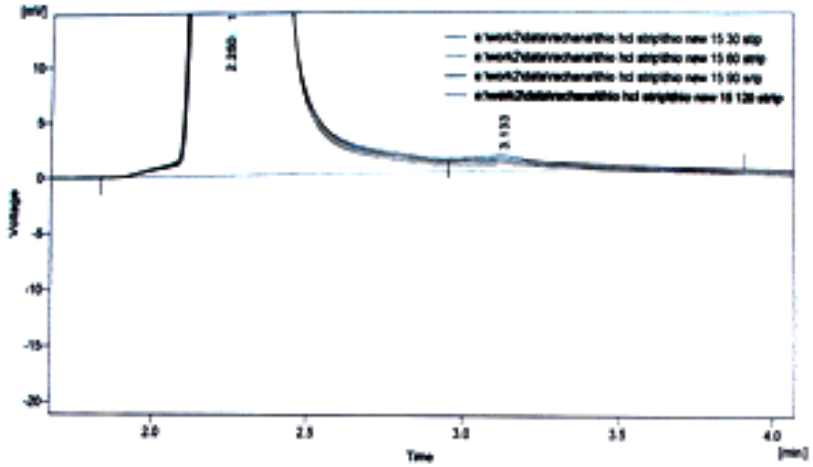

Figure 10. Overlain chromatogram of Sample under thermal studies

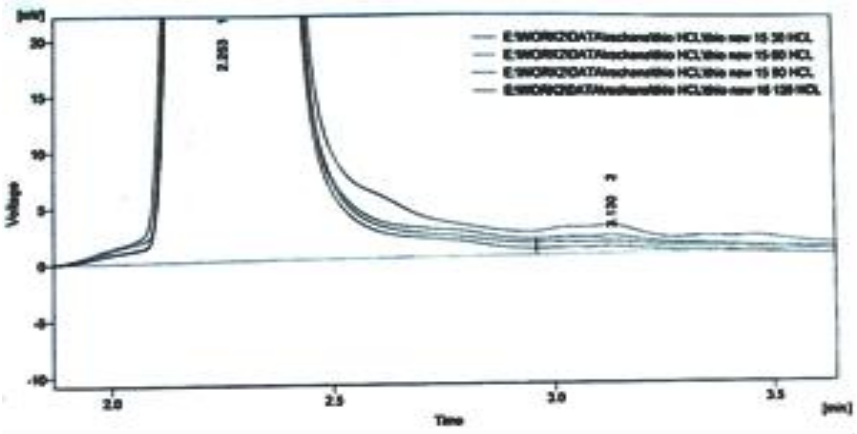

Figure 11. Overlain chromatogram of sample under thermal studies (Solution state stability study)

Table 2. Results of Degradation studies of sample using the developed HPLC method

Stress conditions	Thiocolchicoside			
	Standard drug		Sample	
	% Assay	% Degradation	% Assay	% Degradation
Humidity study (40^0C/75% RH)	89.20	10.80	89.74	10.26
Photo degradation (UV light exposure)	89.22	10.78	89.59	10.41
Photo degradation (Visible light exposure)	86.63	12.37	85.09	14.91
Thermal study (Solid State)	67.4	32.6	69.2	30.8
(Solution state)			66.3	33.7

Method Validation [13, 14]:

The developed chromatographic method was validated using ICH guidelines before implementation with respect to various parameters which include linearity, limit of detection and quantitation, specificity, robustness, accuracy and repeatability.

Accuracy:

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. To accurately weighed capsule powder known quantities of reference standard TCD was added at four different levels and was analyzed for recovery of the added drug. The recovery range for each of the analytes was found to be 97.38% to 100.60% and the relative standard deviation was found to be 1.40 (Table 3).

Table 3. Data for the Recovery study of Thiocolchicoside

Sr.No.	Total amt of drug estimated (mg)	Amt. of Drug recovered (mg)	% Recovery
1.	4.04	2.01	100.60
2.	4.97	2.90	97.38
3.	5.86	3.91	98.31
4.	6.76	4.80	97.97
		Mean	98.57
		% RSD	1.49

Precision:

Precision of any analytical method is expressed as SD and RSD of series of measurements. Precision of estimation of TCD by proposed method was ascertained by replicate analysis of six homogeneous samples of capsule powder on the same day. The data related to precision are given in Table 1.

Table 1. Percent label claim of Thiocolchicoside in capsule

Wt. of cap powder taken (mg)	Peak area of sample (mV)	% Label Claim
206.6	745.378	100.08
216.0	786.852	101.05
218.4	801.187	102.03
215.7	793.371	101.72
216.0	792.009	102.02
	Mean	101.33
	% RSD	0.78

Linearity:

Accurately weighed quantities of capsule powder equivalent to 80, 90, 100, 110 and 120% of label claim of TCD were taken and dilutions were made as described under marketed formulation. The peak areas obtained against each concentration of the analytes were used to build a linear regression equation and to determine value of correlation coefficient. Good linearity was observed over the above mentioned range with linear regression equation Y=4.960X-0.506 (*X* is concentration of analyte in µg/mL and *Y* is peak area). The value of correlation coefficient was found to be 0.999.

Solution stability:

The solution stability period for samples was determined by Intraday and Interday studies. After 2, 3 and 4th hour (Intraday study) and on 5th and 7th day (Interday study) the sample solutions were analyzed. No significant changes were observed in intraday study, the slight change is observed in the chromatographic response on 7th day. Data related to solution stability are summarised in Table 4.

Table 4. Data indicating solution stability

Sample solution stability			
Time (h) (Intraday)	**% Assay**	**Time (days) (Interday)**	**% Assay**
2	100.7	5	103.7
3	101.4	7	104.7
4	101.1		

Robustness:

Robustness of the method was evaluated by changing the flow rate and mobile phase ratio. Data related to robustness are summarized in Table 5.

Table 5. Data indicating robustness of the method

Sr.no.	Deliberate changes	RT	Asymmetry	Theoretical plates/meters
1.	Standard conditions	2.250	1.205	47350
2.	Changes in flow rate (mL/min)			
	0.8	2.787	1.114	46570
	1.2	1.857	1.201	47560
3.	Changes in mobile phase ratio (buffer: ACN)			
	25:75	2.267	1.250	47566
	35:65	2.230	1.094	46576

Applications

Determination of Thiocolchicoside in capsule dosage form:

The developed UV spectrophotometric method was applied for the analysis of Thiocolchicoside in capsules and was compared with the HPLC method described above. The comparison of linearity and precision of both the methods was shown in Table 6.

Table 6: Linearity and Precision of spectrophotometric method and HPLC

	UV-spectrophotometric	HPLC
Slope	0.040	4.906
Intercept	-0.006	-0.506
Correlation coefficient (r^2)	0.999	0.999
Intraday (n=3)	1.12	0.65
Interday (n=3)	1.19	1.43
LOD (µg/mL)	1.49	0.36
LOQ (µg/mL)	4.51	1.09

Dissolution test of TCD capsules:

The release rate of API from the drug product determines the drug absorption from solid dosage form after oral administration. In vitro dissolution test can be used to predict the release and the dissolution of the drug, hence predict the in vivo performance of the drug. Since Thiocolchicoside is not official in Pharmacopoeia, we have developed the dissolution testing conditions for this drug. The developed UV- spectrophotometric method was used for the analysis in dissolution testing. The dissolution test was performed on a dissolution apparatus (USP model Apparatus 1 by dissolving Thiocolchicoside capsule in a vessel consisting of 500 mL of dissolution medium. The dissolution medium used were 0.1N HCL, 0.01N HCL, phosphate buffer 2.0 and phosphate buffer 6.8. The dissolution testing was carried out in all four dissolution medium to select best dissolution medium for TCD capsule formulation A and B. The temperature of the medium was controlled at 37°C±2°C and the basket was rotated at a speed of 75 rpm. The dissolution profile was illustrated in Figure 12 and 13.

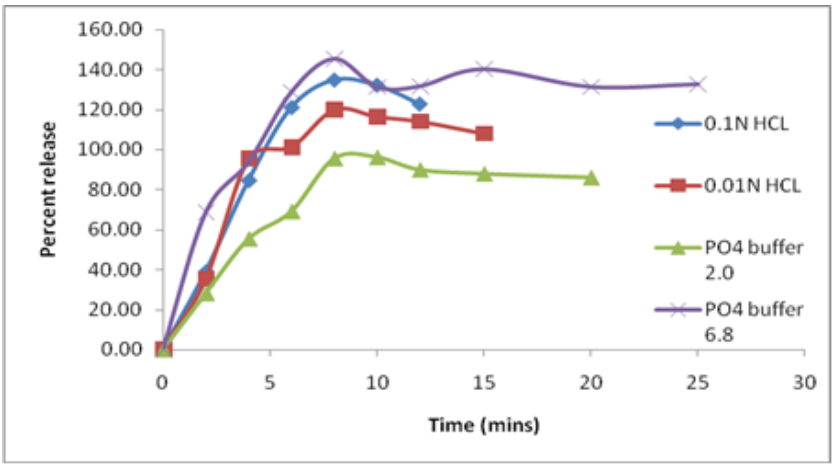

Figure 12. Dissolution profile of Formulation A

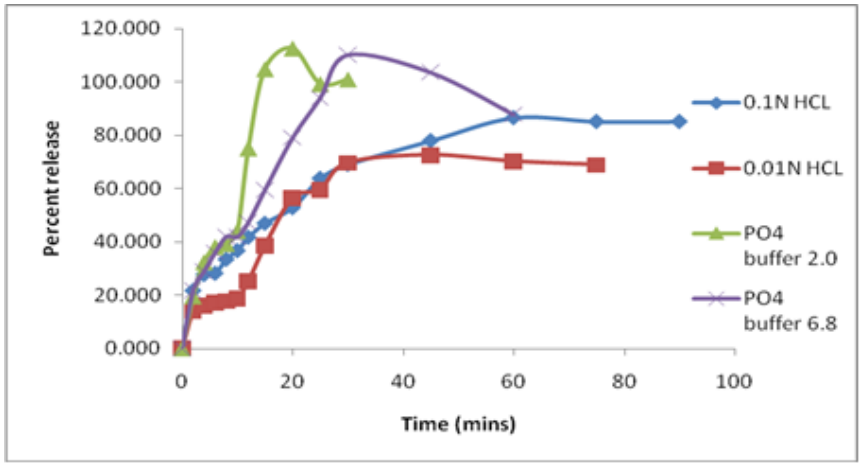

Figure 13. Dissolution profile of Formulation A

The study of dissolution profile of both the formulation (Figure 12 and 13) revealed that there is difference in the dissolution behaviour of two formulations in selected mediums and the % drug release were also significantly different. This might be due to the excipient in the formulation which can be varied among two brands.

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