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Application of Stability - Indicating RP-HPLC Method for the Simultaneous Estimation of Thiocolchicoside and Aceclofenac in Pharmaceutical Dosage Form

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ABSTRACT

A simple, specific, sensitive, precise and stability-indicating reversed phase high performance liquid chromatographic method was developed and validated for the simultaneous determination of thiocolchicoside and aceclofenac, using a RP-18 column and a mobile phase composed of 0.1% trifluoroacetic acid: acetonitrile (45:55). The retention time of thiocolchicoside and aceclofenac were found to be 2.35 and 13.2 min, respectively. Linearity was established for both thiocolchicoside and aceclofenac in the range of 0.08-0.8 and 1-10 µg/ml. Both the drugs were subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat, and photolytic degradation. The degradation studies indicated that both thiocolchicoside and aceclofenac were susceptible to acid, alkaline and neutral hydrolysis. The degradation products of thiocolchicoside and aceclofenac were well resolved from the pure drugs with significant differences in the retention time values. This method can be successfully employed for simultaneous quantitative analysis of thiocolchicoside and aceclofenac in bulk drugs and formulations.

Keywords: Thiocolchicoside, aceclofenac, degradation products, HPLC

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INTRODUCTION

Thiocolchicoside[1] (THIO) is an antispasmodic drug. Chemically it is N-[3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo [*a*] heptalen-7-yl] acetamide. It is used as muscle relaxant, anti-inflammatory and analgesic. The antispasmodic activity is mainly due to the activation of GABA- inhibitory pathways. Thiocolchicoside is not official in any pharmacopoeia. Literature survey reveals that thiocolchicoside can be assayed by UV spectrophotometric[2], HPLC[3-6] and HPTLC[7] methods individually or in combination with other drugs.

Aceclofenac[8] (ACE) [[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy acetic acid] is a white or crystalline powder, soluble in alcohol and acetone, sparingly soluble in water. Aceclofenac is used as an effective non-steroidal anti-inflammatory drug (NSAID) derived from the phenyl acetic acid with pronounced anti-inflammatory, analgesic and antipyretic properties. It has good tolerability profile in variety of painful conditions like rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Literature survey reveals simple UV spectrophotometric[9-12], HPLC and HPTLC methods[13,14] were reported for aceclofenac and thiocolchicoside individually, in combination with other drugs, plasma as well as formulations. Extensive literature survey reveals that no stability indicating chromatographic method has been reported for simultaneous determination of thiocolchicoside and aceclofenac in tablet dosage form.

The International Conference on Harmonization (ICH) guideline entitled 'Stability testing of new drug substances and products' requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances [15-17]. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine THIO and ACE simultaneously, in presence of its degradation products is rather a challenge for pharmaceutical analyst. Therefore, it was thought necessary to study the stability of THIO and ACE under acidic, alkaline, neutral hydrolysis, oxidative, dry heat, and photolytic conditions. This paper reports validated stability-indicating HPLC method for simultaneous estimation of THIO and ACE in presence of their degradation products.

MATERIALS AND METHODS

Materials

Pharmaceutical grade of thiocolchicoside and aceclofenac (purity > 99%) were used without further purification and a commercial tablets was purchased from local market. Methanol used

was of HPLC grade and was purchased from Merck, India. The liquid chromatograph mass spectrometer Shimadzu LCMS-2010EV, which consisted of following components: a binary gradient pump, variable wavelength programmable PDA detector with auto sampler system was employed for the present study.

Instrumentation and chromatographic conditions

The chromatographic analysis was performed using Compaq Intel Core-2 DUO HP W/907 software on a pre-packed RP-18 column (250×4.6 mm, 5 µm particle size). In addition, an electronic balance (Shimadzu. Elec.balance BL-220H), a p^H meter (Eli co L127), a sonicator (Leclasonic ultrasonic cleaner), a hot air oven (Inlab equipments Ltd) were used in the study. Separation was achieved using a mobile phase consisting of 0.1%trifluroacetic acid:acetonitrile (45:55 v/v) , at a flow rate of 1ml/min and the eluent was monitored using PDA detector at 256 and 274 nm, respectively. The column was maintained at ambient temperature and injection volume of 20 µl was used.

Preparation of standard and sample solutions

Standard Preparation

Standard stock solutions containing 100 µg /ml of THIO and ACE were prepared in separate 100 ml volumetric flasks using methanol. A stock solution containing mixture of THIO and ACE in the ratio of 0.08:1 was also prepared using methanol. Working solutions were prepared by diluting the stock solutions with mobile phase to contain 0.08-0.8 and 1-10 µg/ml for THIO and ACE respectively. These solutions were used to generate the calibration graph by plotting peak area versus concentrations, and regression equations were computed for both the drugs.

Sample preparation

Twenty tablets, each containing 8 mg of THIO and 100 mg of ACE were weighed, and powder equivalent to 8 mg of THIO was weighed accurately and taken into 100 ml volumetric flask. The drugs were extracted into methanol; volume was adjusted to 100 ml, vortexed, and then filtered through a 0.22 µm nylon filter. From this solution, further dilutions were made using mobile phase to get a final concentration of 0.8 µg/ml of THIO and 10 µg/ml of ACE. Twenty microlitres of solution was injected into HPLC system to obtain chromatogram for standard drug solution (6 replicates) and sample solution (6 replicates). Concentrations of THIO and ACE in the formulation were calculated by comparing the peak area of sample with that of standard.

Forced Degradation Studies

The drug substances and the drug products under went forced degradation through thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. After achieving good separation,

the drugs were subjected to various stress conditions like hydrolysis, oxidation, dry heat and photolysis. For each study four samples were prepared; the blank solution stored under normal condition, the blank subjected to stress in the same manner as the drug solution, zero time sample containing the drug which was stored under normal conditions and the drug solution subjected to stress treatment. First, the study was conducted separately for THIO and ACE of concentration 0.8 and 10 µg/ml respectively. Secondly, it was conducted for bulk drugs in combination. Lastly the study was applied to the formulation. For formulation study, the tablet powder equivalent to 10 mg of ACE was transferred to 100 ml round bottom flasks and treated under acidic, alkaline, neutral, oxidizing, thermal and photolytic stress conditions. When degradation was complete, the solutions were left to equilibrate to room temperature and an aliquot of sample was withdrawn and diluted with mobile phase to get the concentration equivalent to 0.8µg/ml of THIO and 10 µg/ml of ACE.

Acid hydrolysis was performed by heating the drugs under reflux with 0.1M hydrochloric acid for two hours. For alkaline hydrolysis, the drug solution was heated under reflux with 0.01M NaOH for half an hour. To study neutral degradation, the drug was dissolved in acetonitrile and heated under reflux with water for 12 hours. Degradation with hydrogen peroxide was performed by treating the drug with 10% H_2O_2 (v/v) for 24 hour at ambient temperature. For thermal degradation, solid drugs were placed in temperature-controlled oven at 80°C for 12 hours. Photolytic stress was performed by exposing the drug to sunlight for 12 hours. After degradation, sample solutions were diluted with mobile phase to obtain final concentration of 0.8 µg/ml of THIO and 10µg/ml of ACE. Then 20 µl solution was injected into the HPLC system and analyzed by the previously described chromatographic condition.

RESULTS AND DISCUSSIONS

The present experiment and data reported in literature showed that methanol and acetonitrile were the common solvents which could dissolve THIO and ACE. Both THIO and ACE have more solubility in methanol than acetonitrile. Therefore methanol was chosen as a suitable solvent to extract THIO and ACE from pharmaceutical formulation. Using the initial chromatographic conditions, THIO and ACE were not well resolved from degradants (R_t = 2.2 and 9.3 minutes). So various mobile phase ratio were tried in order to get optimum separation. The mobile phase consisting of 0.1% TFA: acetonitrile (45: 55, v/v), with the flow rate optimized to 1ml/min gave two sharp well resolved peaks for the analytes and were well separated from their degradants. The retention times for THIO and ACE were 2.35 and 13.2

minutes respectively (Figure. 1). UV overlain spectra of both THIO and ACE showed the maximum absorbance at 256 and 274 nm, so these wavelength were selected as the detection wavelengths. The peak purity index for both the analytes and degradation products were found to be close to 1, which proved the selectivity and specificity of the method.

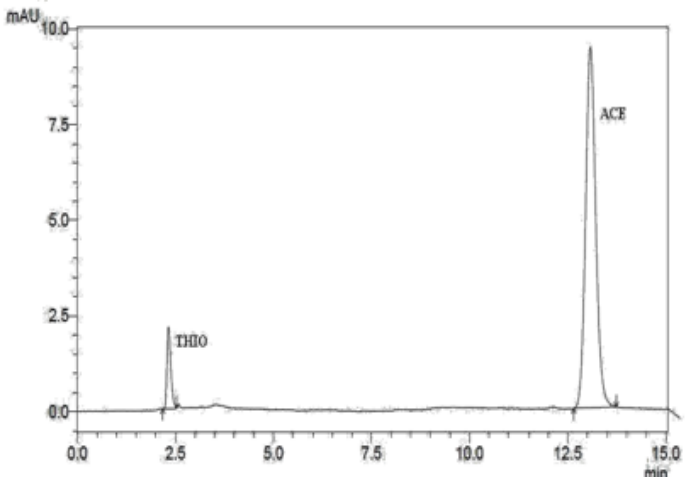


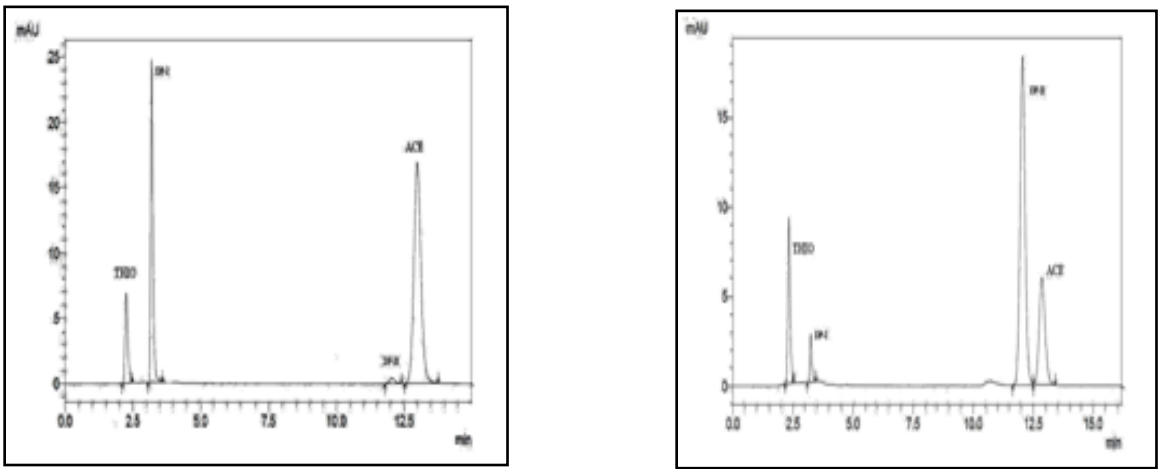
Figure 1:Standard chromatogram of Thio and ACE

Forced degradation studies of both the drugs were carried out under conditions of hydrolysis, dry heat, oxidation, and photolysis. The degradation study indicated that THIO was susceptible to acid hydrolysis more than alkaline and neutral hydrolysis whereas ACE was susceptible to alkaline hydrolysis more than acid hydrolysis and stable to H_2O_2, thermal and direct sunlight. THIO and ACE showed one degradation peak for each, after addition of 0.1M HCl and 0.01M sodium hydroxide along with the retention time of 3.26 and 12.2 minutes (Figure. 2a and b). In oxidation and photolytic degradation, the drugs degrade as shown by the decreased peak area when compared to peak area of the same concentration of the non degraded peaks. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of both the drugs under normal condition. Summary of degradation studies of both the drugs is given in table 1.

Table 1: Summary of degradation studies for THIO and ACE

Experimental Condition	% Estimated	
	THIO	ACE
Acid, 0.1 M HCl (reflux at 70°c),2h	56.37	80.58
Base , 0.01 M NaOH (reflux at 70°c),½h	70.78	48.62
Neutral , water (reflux at 70°c) ,12h	93.54	90.47
Oxidative, 10%v/v H_2O_2(ambient, in dark),24h	95.48	97.39
Dry heat (80°c) , 12h	98.89	99.41
Direct sun light(photolysis), 12h	95.17	97.10

*Average of 6 determination



a) **b)**

Figure 2: Chromatogram of THIO and ACE in presence of a) 0.1M HCl b) 0.01M NaOH

Specificity of the analytical method indicates ability of the developed method to measure the analyte response in the presence of potential interferences. Forced degradation studies were conducted to establish the selectivity and stability indicating power of the proposed RP-HPLC method. The specificity of the method was determined by exposing the solutions of THIO and ACE to stress conditions, i.e. 0.1 M HCl and 0.01 M NaOH. There was one degradant peak each for THIO and ACE in the presence of 0.1 M HCl or 0.01 M NaOH, which were significantly different from the retention time of THIO and ACE. The peak purity of THIO and ACE were assessed by comparing the shape of spectra of standard drugs and degraded samples at the upslope, the apex, and at the down slope of the peak. Peaks were found to be spectrally pure. No other co-eluting peak was found with the main peaks, suggesting the specificity of the method for the simultaneous estimation of THIO and ACE in presence of degradation products and impurities.

Linearity was demonstrated by the calibration graph of THIO and ACE which was linear over the range of 0.08-0.8 µg/ml and 1-10 µg/ml respectively. The proposed method was successfully applied to the determination of THIO and ACE in their combined tablet dosage form. The results for the combination were comparable with the corresponding labeled amounts. The developed method was also found to be specific, since it was able to separate other degradants present in the tablet from the two drugs. To prove the precision of the method, the intraday and interday precision studies were carried out and the %RSD was calculated for each study (Table 2). Low values of % RSD indicate that the method is precise. Accuracy was assessed using six determinations over three concentration levels covering the linear range. The amount of both the drugs estimated, % recoveries of THIO and ACE at each level, and each replicate was determined and %RSD was calculated.

Table 2: Summary of validation parameter

Parameter (units)	THIO	ACE
Linearity range (µg/ml)	0.08-0.8	1-10
Correlation Coefficient(±SD*)	0.9996±0.00034	0.9995±0.00032
LOD (ng/ml)	1.2	4.499
LOQ (µg/ml)	0.08	1.0
Recovery (%±RSD*)	100.33±1.1205	100.32±0.5749
Interday precision (%RSD*)	0.7359	0.2241
Intraday Precision (%RSD*)	0.7345	0.2392

*mean of six determination

The LOD & LOQ for THIO and ACE were found to be 1.2 & 4.499 ng/ml and 0.08 &1 µg/ml, respectively. For robustness evaluation, influence of small changes in flow rate, percentage of methanol in mobile phase, and pH of mobile phase were studied. Table 3 shows the robustness evaluation of the method. Each factor selected was changed at three levels with respect to the optimized parameters. Robustness of the method was done at the concentration of 0.4 and 5 µg/ml for both THIO and ACE. The method was found to be robust since no significant effect was observed on the system suitability parameters and the % RSD values were less than 2. The drug was stable when stored for 24 hours at laboratory temperature (32 ± 1°C) and for 48 hours under refrigeration (8 ± 0.5°C) in 0.1%trifluoro acetic acid: acetonitrile (45:55 v/v) and reanalyzed. Results of other system suitability parameters such as α, N, tailing factor, *k* and asymmetric factor are presented in table 4. As given in this data, acceptable system suitability parameters were obtained. For the assay of commercial formulations, the concentration was found from the standard graph prepared and the amount was calculated using the dilution factor. The % label claim and % RSD are shown in table 5. For the stability studies, the prepared solutions were injected at regular interval of time under fixed chromatographic conditions. The amount found at each time was calculated as 100%. It was found to be stable for 6 hours. The % amount of drugs and %RSD were calculated. All parameters were found to be within the limit.

Mobile phase composition and flow rate were decided on the basis of tailing factor, peak shape, peak asymmetry, resolution, baseline drift, and time needed for analysis. The solvent system selected was 0.1% trifluoroacetic acid: acetonitrile (40:60 v/v) at pH 2.4 which gave good resolution peak shape and acceptable resolution for THIO and ACE. Purity of THIO was not good and R_t was very short when water: acetonitrile (40:60) was tried initially. When pH of water: acetonitrile (50:50) was adjusted to 2.5 using OPA, R_t of ACE was >20 at a flow rate of 1ml/min. Water: acetonitrile (50:50) at pH 3.5 furnished impure peak for THIO at 1.9 min. 0.1%TFA: acetonitrile in the ratio of 55:45 resulted in peak tailing with a purity of 0.652

Methanol-water (50:50 %v/v) did not furnish a sharp, well-defined peak, but affected a high tailing factor (1.82). Other mobile phases tried resulted either in much lower sensitivity, delayed retention time, or poor peak shapes, and so these were not considered. Mobile phase composition and flow rate were decided on the basis of tailing factor, peak shape, peak asymmetry, resolution, baseline drift, and time needed for analysis. Extensive degradation was shown by both THIO and ACE in 0.1M NaOH. Therefore, the strength of NaOH was reduced to 0.01M in the degradation studies. Sufficient degradation was attained within 30 minutes. In THIO, decomposition reaction involves rupture of C-O linkage when refluxed with water.

Table 3: Robustness evaluation of the method

Factor	Level	Asymmetric Factor		No. of theoretical plate		%RSD*			
						THIO	ACE	THIO	ACE
Flow Rate (ml/min)									
0.9	-0.1	1.28	1.08	4209	12436	1.5873	0.9259	0.2008	1.1758
1	0	1.26	1.09	4294	12683				
1.1	+0.1	1.24	1.07	4211	12421				
%B of mobile phase									
54	-1	1.25	1.07	4199	12509	0.3978	0.9259	1.1239	0.8495
55	0	1.26	1.09	4294	12683				
56	+1	1.26	1.08	4239	12489				
Ionic strength of TFA									
0.05	-0.05	1.27	1.08	4265	12547	0.3959	0.9259	0.3389	0.8299
0.1	0	1.26	1.09	4294	12683				
0.15	+0.05	1.26	1.07	4280	12478				

*mean of six determination

Table 4: System Suitability Parameters for THIO-ACE

PARAMETER	THIO	ACE	DP-I	DP – II
Retention time (t_R)	2.35	13.2	3.26	12.2
Peak purity index	1.0000	1.0000	0.9999	0.9999
Asymmetry (As)	1.26	1.09	1.11	1.01
Tailing factor (T)	1.28	1.10	1.13	1.03
Resolution (Rs)	---	1.25	3.64	19.87
Capacity factor (k')	1.6	13.67	2.6	12.56
Selectivity (α)	---	1.08	1.63	3.74
Theoretical plates (N)	3876	9914	7243	14180

Table 5: Assay of THIO-ACE in formulation

Drug	Labeled amount (mg/tab)	Amount found (mg)	Assay(%)±RSD*
THIO	8	7.9816	99.77±0.3212
ACE	100	100.02	100.02±0.1599

*Average of 6 determination

Assay of THIO and ACE from its tablet dosage form

The assay results of THIO and ACE in tablet dosage forms were comparable with the values of labeled claim. The results presented in table 5 indicate the suitability of the method for routine analysis of THIO and ACE from their combined tablet dosage form.

CONCLUSION

The study presents a simple stability-indicating HPLC method for the simultaneous estimation of THIO and ACE in presence of their degradation products and validated as per ICH guidelines. Statistical analysis proved that the method developed was accurate, precise and repeatable. The method was successfully used for the estimation of drugs in pharmaceutical formulation. Recovery results for combined dosage form using proposed method showed 100.33±1.1205 and 100.32±0.5749, for THIO and ACE respectively. There was no interference observed due to excipients or other components present in the tablet dosage form. The results indicated the suitability of the method to study stability of THIO and ACE under various forced degradation condition viz. acid, base, neutral, oxidative, dry heat and photolytic degradation. It can be concluded that the developed method is accurate, precise, and specific and possesses the advantage of determining both drugs simultaneously and in presence of degradants. Hence the suggested method was successfully applied to the analysis of the cited drugs in pharmaceutical formulations.

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