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Development and validation of stability-indicating RP-UPLC method for simultaneous estimation of thiocolchicoside and aceclofenac in combined dosage form

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ABSTRACT

A stability indicating RP-UPLC method was developed and validated for the simultaneous determination of Thiocolchicoside (TCC) and Aceclofenac (ACF) in tablet dosage form. The chromatographic separation was carried out by Thermo Scientific UPLC Instrument, Accela 1250 Pump, auto sampler with PDA detector, using column Thermo Scientific hypersil gold C_{18}, (50 x 2.1mm) particle size 1.9µm using 5% ammonium acetate buffer and methanol in the ratio of 40:60, pH was adjusted to 5 with ortho phosphoric acid as mobile phase at a flow rate of 250 µl/min with the detection at 276nm. The run times of the TCC and ACF were about 0.697 and 1.125 minutes, respectively. The detector response is linear from 4.8 µg/ml to 7.2 µg/ml and 63.8 µg/ml to 96 µg/ml concentrations for TCC and ACF respectively. The linear regression equation was found to be y = 20620x-677.68 (r^2 = 0.9996) for TCC and y= 50931x-319.3 (r^2 = 0.9997) for ACF. The detection limit and quantification limit was 0.076µg and 0.23µg for TCC and 0.27µg and 0.71µg for ACF. The percentage of assay of TCC and ACF were about 99.50% and 99.96% respectively. The stability indicating capability was established by forced degradation experiments. The method was satisfactorily validated as per the ICH guidelines

***Key Words*:** Thiocolchicoside, aceclofenac, stability indicating, method development, RP-UPLC, validation.

INTRODUCTION

Thiocolchicoside (TCC) is chemically named as *N*-[(7S)-3-(β-D-Glucopyranosyloxy)- 1,2-dimethoxy -10- (methylsulfanyl) -9- oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl] acetamide (figure 1). It is a semi-synthetic derivative of the natural colchicoside compound. It is an anti-inflammatory, muscle relaxant and analgesic drug and also used topically for the treatment of muscular spasms and rheumatologic disorders (O'Neil, 2006; Indian Pharmacopoeia, 2010).

Aceclofenac (ACF) [[2-[2-[(2,6-dichlorophenyl) amino]phenyl]acetyl]oxy acetic acid] (figure 2). It is used as an effective non-steroidal anti-inflammatory drug (NSAID) derived from the phenyl acetic acid with pronounced anti-inflammatory, analgesic, antipyretic activity and treatment of rheumatoid arthritis and ankylosing spondylitis (Indian Pharmacopoeia, 2007; Saraf et al, 2006).

In Literature survey, simple UV spectrophotometric (Qin et.al, 2006; Rachana and Gupta, 2010; Acharjya *et al.*, 2010), HPLC method (Rosso and Zuccaro, 1998; Sutherland *et al.*, 2002; Hilmi and Bayram, 2007; Dhaneshwar *et al.*, 2011; Suganthi *et al.*, 2013; Nyola *et al.*, 2012**)** and HPTLC methods (Ragehy *et al.*, 2003; Sunita *et al.*, 2011; Rajput *et al.*, 2013) were reported for aceclofenac and thiocolchicoside individually and in combination with other drugs. Extensive literature survey revealed that no stability indicating RP-UPLC method has been reported for simultaneous determination of thiocolchicoside and aceclofenac in combined dosage form.

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MATERIALS AND METHODS

Materials

TCC was obtained from Firstmed Therapeutics Pvt. Ltd, Pondicherry and ACF was obtained from Ideal Analytical & Research Institution, Pondicherry. Water (HPLC grade), Acetonitrile (HPLC grade), Methanol (HPLC grade) and Ammonium Acetate were of Analytical grade purchased from Merck. The commercial combination of TCC (8mg) and ACF (100mg) was purchased from drug store.

Instrumentation

A Thermo Scientific Ultra performance liquid chromatography (UPLC) Instrument, Accela 1250 Pump and auto sampler with PDA detector and Thermo hypersil gold C_{18} column (50 x 2.1mm) particle size 1.9µm. The UPLC system was operational with CHROMQUEST software for data processing. Sartorius analytical micro balance, Ultra sonicator (DC1500H MRC), pH meter (MKVI Systronics), micropipettes and micro-pore filtration set etc. were also used.

Chromatographic Conditions

The analysis was carried out on Thermo Scientific Hypersil gold C_{18} Column (50 x 2.1mm, particle size 1.9µm), the mobile phase containing 5% ammonium acetate buffer and methanol (40:60), pH adjusted to 5 with ortho phosphoric acid was found to resolve TCC and ACF. The mobile phase was filtered on 0.22 micron membrane filter and sonicated for 15 min. The injection volume was 10 µl. The flow rate was set to be 250µl/min. The wavelength was selected at 276 nm with PDA detector for analysis. All determinations were performed at constant column temperature (25 ± 2°C). The total run time of the analysis was 3 min. The retention time of the TCC and ACF was 0.697min and 1.125min respectively and the chromatogram was given in figure 3.

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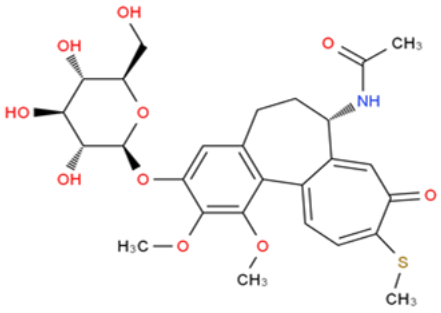


Figure 1: Chemical Structure of Thiocolchicoside.

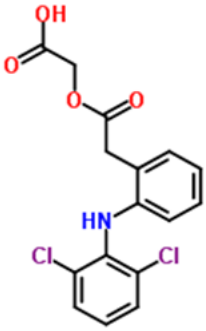
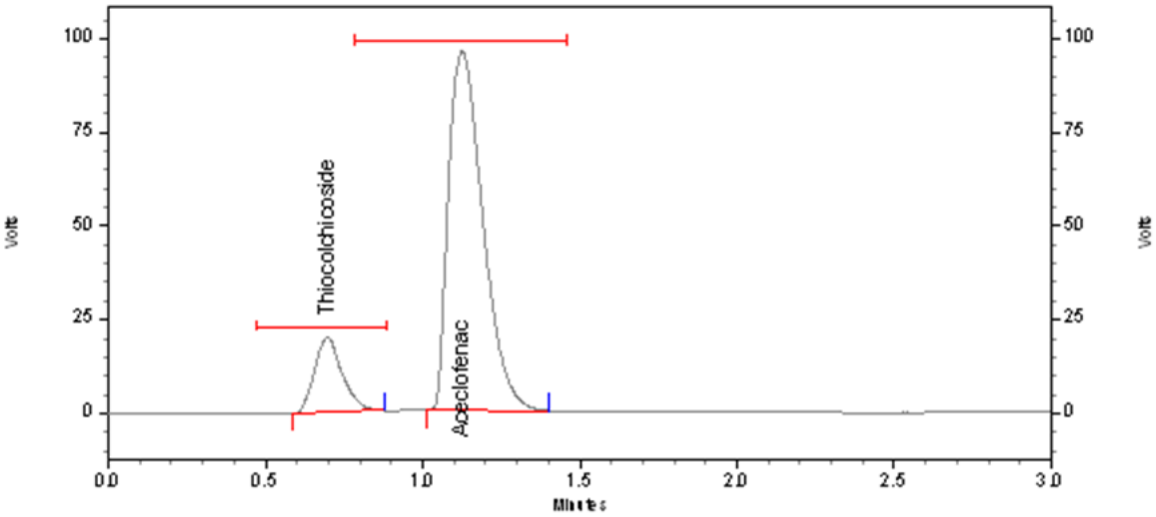


Figure 2: Chemical Structure of Aceclofenac.



Name	Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
Thiocolchicoside	0.697	121892	14.31	2299	0.00	1.23
Aceclofenac	1.125	729978	85.69	3509	2.38	1.57
Totals		851870	100.00			

Figure 3: UPLC - Chromatogram of TCC and ACF.

Preparation of standard solution

The standard stock solutions were prepared by transferring 16 mg of TCC and 200 mg of ACF working standards in 25ml volumetric flask and dissolved with methanol upto the mark. From the standard stock solution 1ml was taken into 100ml flask, further diluted with mobile phase to get the final concentration of 6.4 µg/ml of TCC and 80 µg/ml of ACF.

Preparation of sample Solution

Accurately weighed 10 tablets were triturated with a mortar and pestle. An amount equivalent to 16 mg of TCC and 200 mg of ACF sample was transferred to a 25 ml clean volumetric flask, diluted with 10 ml methanol and sonicate to dissolve it completely and made the volume with the same solvent. Further 1ml of the TCC and ACF of above stock solution was taken into a 100ml volumetric flask, diluted with mobile phase and analyzed under optimized chromatographic conditions. The chromatogram was eluted. The assay results were shown in Table.1

Forced Degradation Studies

It is a process in which the natural degradation rate of a pharmaceutical formulation is increased by applying the additional stress. UPLC method is used to separate, detect, and quantify the various drug related degradation substances. The stock solution of the sample was treated with various degradation conditions (Blessy *et al.*, 2013; Ngwa, 2010) such as acidic (0.1N HCL, 60°C, 24 hours), alkaline (1N NaOH, 60°C, 24 hours), oxidization (3% H_2O_2, 60°C, 24 hours), thermal (60°C, 24 hours) and sunlight (24 hours). Both TCC and ACF were found to be highly sensitive to acidic, alkali and oxidative degradation. The peak area and assay value were dropped in all the above mentioned conditions. The results of forced degradation studies were given in table 2 and shown in figure 4-8. However no major degradation was found in thermal condition and under sunlight.

Method Validation

The developed method was validation as per ICH guidelines (ICH-Guidelines, 1996). The validation parameters are

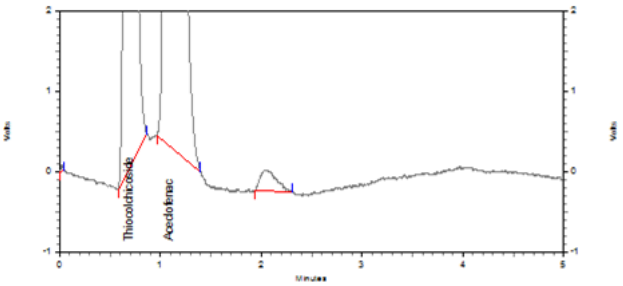


Figure 4: Acid degradation.

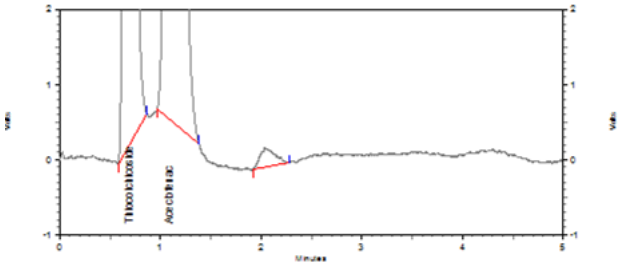


Figure 5: Alkali degradation.

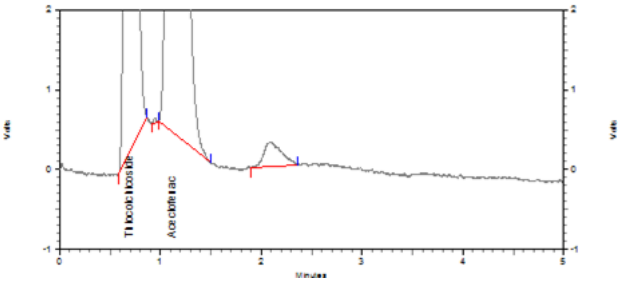


Figure 6: Peroxide degradation.

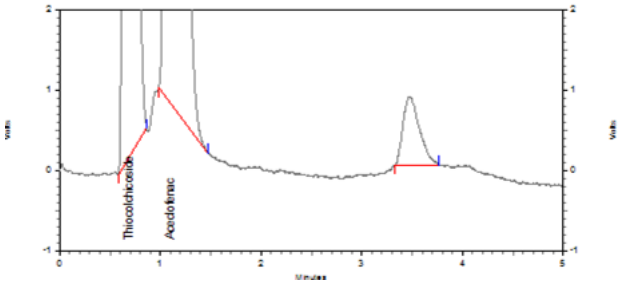


Figure 7: Thermal degradation.

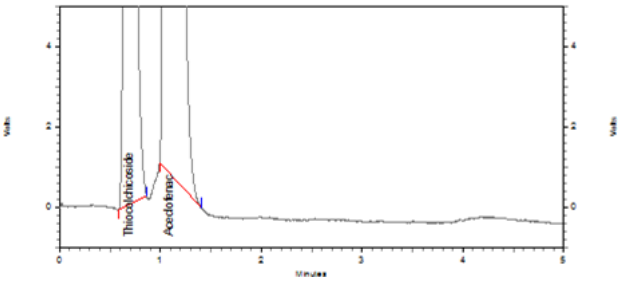


Figure 8: Sunlight degradation.

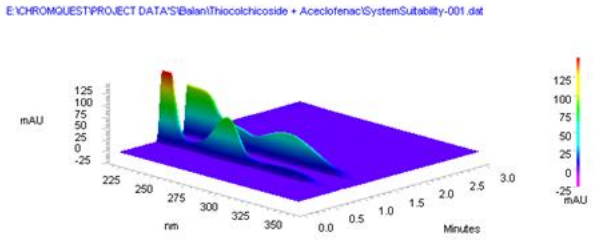


Figure 9: System Suitability 3D Picture of TCC and ACF.

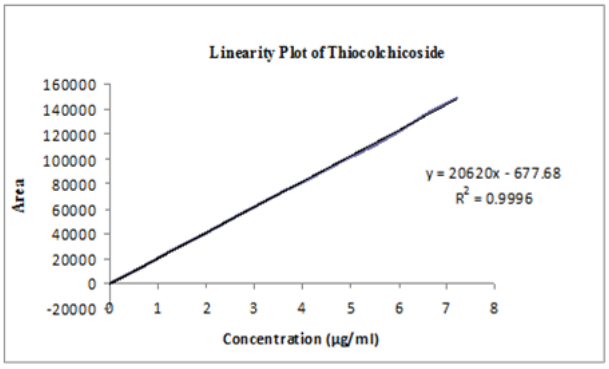


Figure 10: Linearity Curve for Thiocolchicoside.

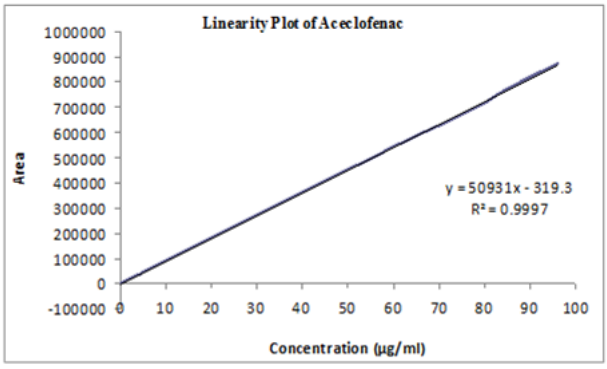


Figure 11: Linearity Curve for Aceclofenac.

Table 1: Assay of TCC and ACF.

Formulation	Drug	Label Claim(mg)	Purity (%)
Tablet	Thiocolchicoside	8	99.50
	Aceclofenac	100	99.96

Table 3: System Suitability Parameters.

Parameters	TCC	ACF
Resolution Factor	---	2.38
Theoretical Plates	2299	3509
Asymmetric Factor	1.23	1.57
Retention Time	0.697	1.125
Standard Deviation	1360	3419
RSD	1.13	0.47
DL	0.076µg	0.27µg
QL	0.23µg	0.71µg

Table 2: Forced Degradation Studies.

Degradation Parameters	Degradation Time (hr)	Standard Peak Area		Peak Area Product		% of Recovery		% of Degradation	
		TCC	ACF	TCC	ACF	TCC	ACF	TCC	ACF
Acid Degradation (0.1N HCl, 60°C)	24	120807	725077	102255	590482	85.16	81.95	14.84	18.05
Alkali Degradation (0.1N NaOH, 60°C)	24	120807	725077	106146	630717	88.4	87.53	11.6	12.47
Peroxide Degradation (3% H_2O_2, 60°C)	24	120807	725077	100650	580365	83.83	80.54	16.17	19.46
Thermal Degradation (Oven, 60°C)	24	120807	725077	115647	674391	96.32	93.59	3.68	6.41
Sunlight	24	120807	725077	117648	672256	97.98	93.3	2.02	6.7

Table 4: Linearity.

Level	TCC		ACF	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
80%	4.8	97361	63.8	575039
90%	5.4	109148	72	644985
100%	6	122625	79.9	715732
110%	6.6	136417	87.9	804480
120%	7.2	148974	96	872634

Table 5: Accuracy (Recovery) Data.

Parameters	TCC		ACF	
	% Recovery	% RSD	% Recovery	% RSD
80%	100.55	0.9	100.8	0.42
100%	100.19	0.82	99.8	0.52
120%	100.38	0.71	100.12	0.47

Table 6: Precision data.

Drug	Intraday assay		Inter day assay	
	% Obtained	% RSD	% Obtained	% RSD
TCC	100.6	0.42	100.41	0.81
ACF	100.3	0.16	99.91	0.22

Table 7: Robustness data.

Factors	Level	Retention time		Area (µv²sec)	
		TCC	ACF	TCC	ACF
Standard	250µl / min	0.697	1.23	120807	725077
Flow rate	225µl / min	0.728	1.145	111163	640224
	275µl / min	0.651	1.06	111043	682621
Wavelength	274nm	0.7	1.12	116383	712689
	278nm	0.7	1.12	122745	732359
Analyst	I	0.698	1.25	120912	725062
	II	0.697	1.24	120876	725152

linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

System Suitability

The six replicated injections were made in the standard solutions of both TCC and ACF and system suitability parameters such as theoretical plates (USP), resolution (USP) and asymmetry factor were evaluated. The results of the system suitability parameters were given in table 3. The 3D chromatogram was given in figure 9.

Linearity

Linearity was demonstrated from five different concentration levels for both TCC and ACF, which were found to be linear in the range of 4.8 µg/ml to 7.2 µg/ml and 63.8µg/ml to 96 µg/ml, respectively. The values were given in Table 4. Correlation coefficient for TCC and ACF was 0.9996 and 0.9997 respectively. The calibration curves were shown in the figure10 and 11.

Accuracy (Recovery Studies)

To check the degree of accuracy of the method, the recovery studies were performed by standard addition method at

80%, 100% and 120%. Known amounts of standard mixture of TCC and ACF were added to pre-analyzed samples and were subjected to the proposed UPLC method. Results of recovery studies were shown in table 5.

Precision

Intraday precision and inter-day precision were evaluated by carrying out six independent sample preparations (6.4 µg/ml of TCC and 80 µg/ml of ACF) from a single lot formulation. Percentage relative standard deviation (%RSD) was calculated. The results for precision were given in table 6.

Robustness

To evaluate the robustness of the developed RP-UPLC, small deliberate variations in the optimized method parameters were done. The effect of ±2 % change in flow rate, ±2 nm wavelength and 2 different analysts on the retention time and area were studied. The results of robustness were tabulated in table 7.

Detection Limit (DL) and Quantitation Limit (QL)

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected. The quantification limit (QL) is defined as the lowest concentration of the substance that can be quantified with acceptable precision and accuracy. The detection limit and Quantitation limit were calculated as DL = 3.3 x Syx /slope and QL = 10.0 x Syx / Slope, here Syx is residual variance due to regression. The DL and QL values were given in table 3.

RESULTS AND DISCUSSION

To develop a suitable UPLC method for analysis of the drugs in pharmaceutical formulation, initially tests were carried out to select optimum conditions. After quite a lot of trials were tested by using various proportions of solvents including buffer and acetonitrile. The goal of this study was to develop a rapid UPLC method for the analysis of TCC and ACF in a finished combined tablet dosage form using a ammonium acetate buffer and methanol in the ratio of 40:60 as mobile phase at a flow rate of 250 µl/min, hypersil C_{18} (50 x 2.1 mm), particle size 1.9µm column with the PDA detection at 276 nm. The retention time was found to be 0.697 and 1.125 min for TCC and ACF, respectively. The linear regression equations were y = 20620x-677.68 (r^2 = 0.9996) for TCC, y= 50931x-319.3 (r^2 = 0.9997) for ACF. The developed method found to be accurate and precise; the RSD values are less than 1. The stability indicating capability was established by forced degradation experiments. The percentage of assay for TCC and ACF were about 99.50% and 99.96%, respectively.

CONCLUSION

The developed method was simple, rapid, and accurate for simultaneous determination of thiocolchicoside and aceclofenac in combined tablet dosage form. The mobile phase is simple and easy to prepare and also economical. This proposed method can be easily adopted for routine analysis of Thiocolchicoside and Aceclofenac in combined dosage form.

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