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Simultaneous estimation of Thiocolchicoside and Aceclofenac in pharmaceutical dosage form by spectrophotometric and LC method

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Abstract

A simple, accurate, and reproducible UV spectrophotometric and HPLC method for simultaneous estimation of thiocolchicoside (THC) and aceclofenac (ACE) in combined tablet dosage form have been developed. The first developed method is Area under curve method, wavelength range selected are 264.5-254.5 nm for thiocolchicoside and 279.0-269.0 nm for aceclofenac respectively. Linearity was observed in concentration range of 4-36 µg/ml for thiocolchicoside as well as for aceclofenac. Second developed method is RP- HPLC method using Thermo C18 column (4.6 mm i.d. × 250 mm) and acetonitrile: water: 0.025M pot. dihydrogen orthophosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) in the ratio of 70:10:20 % v/v/v as mobile phase. For HPLC method, linearity was observed in concentration range of 1-6 µg/ml for thiocolchicoside and 25-150 µg/ml for aceclofenac. Results of analysis were validated statistically and by recovery studies

Keywords: Thiocolchicoside, Aceclofenac, UV- spectrophotometry, HPLC.

INTRODUCTION

Chemically, thiocolchicoside (THC) is N-[3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio)-9-oxobenzo[a]heptalen-7yl] acetamide. 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 [1]. Literature survey reveals that thiocolchicoside can be estimated by spectrophotometry [2], HPLC [3, 4] and by HPTLC [5] methods individually or in combination with other drugs. Chemically, aceclofenac (ACE) is 2-[2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid. Aceclofenac is used as anti-inflammatory drug [6]. Aceclofenac alone or in combination with the other drugs is

reported to be estimated by TLC-densitometry, differential spectrophotometry [7, 8, 9] HPLC [10, 11] and fluorimetry [12].

Since no spectrophotometric & HPLC methods are reported for the simultaneous estimation of thiocolchicoside and aceclofenac in combination therefore in the present work, a successful attempt has been made to estimate both these drugs simultaneously by a simple UV-spectrophotometric method (AUC) & RP-HPLC method [13]. The proposed methods were optimized & validated as per ICH guidelines [14].

MATERIALS AND METHODS

2.1. Materials

Pure drugs thiocolchicoside and aceclofenac were supplied as a gift sample by Sanofi-Synthelabo Ltd., Bangalore and Glenmark Pharmaceuticals Pvt. Ltd., Mumbai, respectively. Combined dose tablet formulation containing thiocolchicoside and aceclofenac (BAKFLEX-A, 4 mg of thiocolchicoside and 100 mg of aceclofenac, Marketed by INTAS PHARMA), were purchased from local market. All chemicals used are of HPLC/AR grade and were purchased from Thermo Fisher Scientific Limited, Mumbai.

2.2. UV- spectrophotometry

2.2.1 Instrument: A double-beam Shimadzu UV- Visible spectrophotometer, 1700 Pharmaspec, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of solution.

2.2.2 Solvent used: Methanol- AR grade

2.2.3 Preparation of stock solution

Accurately weighed quantity of thiocolchicoside (5 mg) and aceclofenac (5 mg) were transferred to two separate 50.0 ml volumetric flask. Thiocolchicoside and aceclofenac were dissolved in 10 ml methanol. Then both the drug solutions were diluted up to the mark with the methanol (Stock solution 100 µg/ml).

2.2.4 Area under curve method

From the overlain spectra of both drugs, area under the curve in the range of 264.5-254.5 nm (for thiocolchicoside) and 279.0-269.0 nm (for aceclofenac) were selected for the analysis (Fig. no.1). The calibration curves for thiocolchicoside and aceclofenac were plotted in the concentration range of 4-36 µg/ml. The ‘X’ values for both the drugs were determined at the selected AUC range. The ‘X’value is the ratio of area under the curve at selected wavelength ranges with the concentration of component in g/lit. These ‘X’ values were the mean of six determinations. A set of two simultaneous equations obtained by using mean ‘X’ values are given below.

$$A_1 = (286.2)\, C_1 + (242.2)\, C_2 \quad (\text{at } \lambda_{279.0\text{-}269.0\text{nm}}) \qquad (1)$$

$$A_2 = (210.1)\, C_1 + (338.2)\, C_2 \quad (\text{at } \lambda_{264.5\text{-}254.5\text{ nm}}) \qquad (2)$$

Where A_1 and A_2 were area under curve of sample at the wavelength range 279.0-269.0 nm and 264.5-254.5 nm, respectively.

242.2 and 338.2 were 'X 'values of thiocolchicoside at wavelength range 279.0-269.0 nm and 264.5-254.5 nm respectively. Similarly 286.2 and 210.1 were 'X 'values of aceclofenac at wavelength range 279.0-269.0 nm and 264.5- 254.5 nm, respectively.

The concentration of thiocolchicoside and aceclofenac in sample was determined by using the equations (1) and (2).

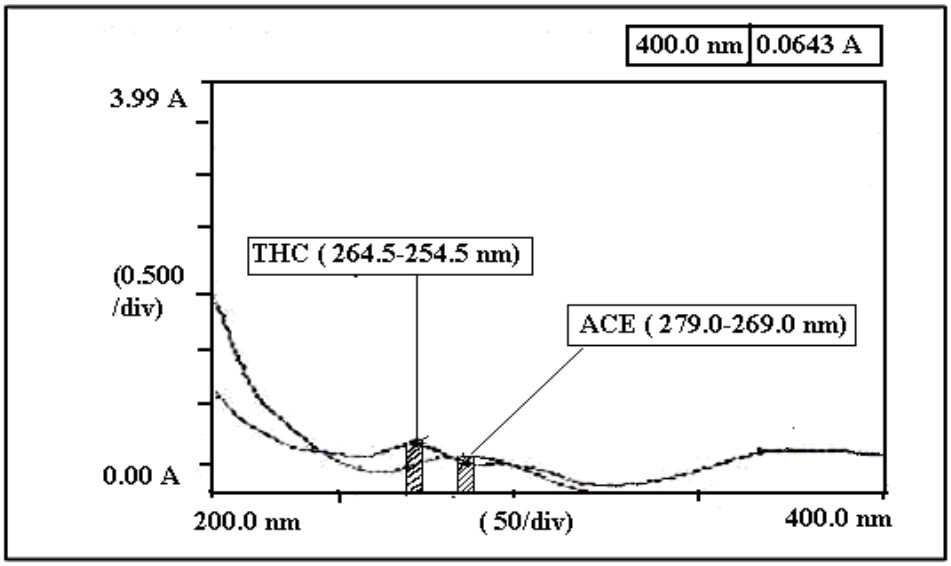

Fig. no. 1 - Overlain spectra of Thiocolchicoside (THC) and Aceclofenac (ACE)

2.2.5 Assay of tablet formulation by AUC method

For the estimation of drugs in the commercial formulations, twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 4 mg thiocolchicoside and 100 mg of aceclofenac was transferred to 100 ml volumetric flask. Then 96 mg of standard thiocolchicoside was weighed & added in the same volumetric flask; 35 ml methanol was added and sonicated for 20 min.

Table No. 1 - Result of marketed formulation analysis

Method	Drug	Label Claim (mg/tablet)	Amount of drug estimated (mg/tablet)	% of label claim estimated ± S.D*
UV spectrophotometry	THC	04	3.96	100.72 ± 0.33
	ACE	100	99.09	99.09 ± 0.43
HPLC	THC	04	4.01	100.47 ± 0.83
	ACE	100	100.70	100.70 ± 0.37

** Mean of six estimation. THC =Thiocolchicoside, ACE= Aceclofenac.*

The volume was then made up to the mark with methanol. The resulting solution was filtered through Whatmann filter paper and filtrate was appropriately diluted with distill water to get

approximate concentration of 16 µg/ml of thiocolchicoside as well as of aceclofenac. The concentration of both thiocolchicoside and aceclofenac were determined by measuring area under curve in the range of 264.5-254.5 nm and 279-269 nm and values were substituted in equations (1) and (2) to obtain concentration of both the drugs. Results of tablet analysis are shown in table no. 1.

2.3. HPLC method:

2.3.1 Instrumentation

HPLC system (Merck Hitachi) consisting of quaternary gradient pump, auto sampler, column oven, and UV detector (L – 7400) was employed for analysis. Chromatographic data was acquired using Winchrome software.

2.3.2 Chromatographic conditions

Thermo C18 column (4.6 mm i.d. × 250 mm) was used as stationary phase. Acetonitrile: water: 0.025 M potassium dihydrogen orthophosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) in the ratio of 70:10:20 % v/v/v was used as mobile phase and was filtered before use through 0.45 µ membrane filter. A constant flow of 1.0 ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 260 nm.

2.3.3 Preparation of solutions

Standard stock solution

Standard stock solution of thiocolchicoside and aceclofenac were prepared by transferring 4 mg of thiocolchicoside and 100 mg aceclofenac in 100ml volumetric flask. Sufficient amount of mobile phase was added, sonicated and remaining volume was made up to the mark with mobile phase.

Sample solution

Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 4 mg thiocolchicoside and 100 mg of aceclofenac was transferred to 100 ml volumetric flask; 50 ml portion of mobile phase was added and sonicated for 20 min. and then volume was made up to the mark with mobile phase. The resulting solution was mixed and filtered through Whatmann filter paper and filtrate was appropriately diluted to get approximate concentration of 4 µg/ml of thiocolchicoside and 100 µg/ml of aceclofenac. The diluted solutions were filtered through 0.20 µ filter.

2.3.4 Chromatography

Twenty microlitres of standard and sample solutions were injected and chromatographed under above mentioned chromatographic conditions. The calibration curves for thiocolchicoside and aceclofenac were prepared in the concentration range of 1 - 6 µg/ml and 25 - 150 µg/ml, respectively at 260 nm by plotting concentration against peak area. The concentrations of both thiocolchicoside and aceclofenac in the sample solutions were determined by comparing peak area of sample with that of standard at 260 nm. Respective peak areas, dilution factors, sample and standard weights were taken into account to quantitate the amounts of thiocolchicoside and aceclofenac in mg per tablet. The results of tablet analysis are shown in table no. 1.

2.4 Method Validation

The accuracy studies of UV (AUC) and HPLC method were carried out by adding known amount of each drug corresponding to three concentration levels 80%, 100% and 120% of the label claim along with the excipients in triplicate.

Precision of these methods was checked by analyzing the samples at three different time intervals of the same day (intraday precision) as well as on different days (interday precision). Robustness for HPLC method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.9 mL/min and 1.1 mL/min while ratio of the mobile phase was changed by ± 1%.

LOD and LOQ are calculated by using the values of slopes and intercepts of the calibration curves for both the drugs.

RESULTS AND DISCUSSION

Both, UV spectrophotometric and HPLC methods were found to be simple, accurate and rapid for routine simultaneous estimation of thiocolchicoside and aceclofenac, in tablet dosage forms.

3.1 UV spectrophotometry (AUC method)

For AUC method linearity was observed in the concentration range of 4-36 µg/ml for both drugs. Commercial formulations containing thiocolchicoside and aceclofenac were analyzed by the proposed method. Six replicate analysis of formulation were carried out and the mean assay values were found close to 100 %. The proposed method was validated as per the ICH guidelines. The accuracy of the proposed method was determined by recovery studies. It was confirmed from results that the method is highly accurate (table no.2). Precision was calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for intraday and interday precision for thiocolchicoside were 0.5098 % and 0.3304 % and that for aceclofenac were 0.6285 % and 0.4112 % respectively which are well within the acceptable limit of 2 %.

3.2 HPLC method

For HPLC method linearity was observed in the concentration range of 1-6 µg/ml for thiocolchicoside and 25-150 µg/ml for aceclofenac respectively. Commercial formulations containing thiocolchicoside and aceclofenac were analyzed by the proposed method. A typical chromatogram of marketed formulation is shown in fig. no. 2. Six replicate analysis of formulation were carried out and the mean assay values were found close to 100 %. The tailing factors were <2.0 for both the peaks. The elution order was thiocolchicoside (RT = 2.70 min) and aceclofenac (RT = 4.76 min), at a flow rate of 1.0 mL/min. The chromatogram was recorded at 260 nm as the overlain UV spectrum of thiocolchicoside and aceclofenac showed maximum response at this wavelength. The accuracy of the proposed method was determined by recovery studies. It was confirmed from results that the method is highly accurate (table no.2). Precision was calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for intraday and interday precision for thiocolchicoside were 0.5538 % and 0.4077 % and that for aceclofenac were 0.4021 % and 0.2076 % respectively which are well within the acceptable limit of 2 %. For robustness studies in all deliberately varied conditions, the RSD of

contents of thiocolchicoside and aceclofenac were found to be well within the acceptable limit of 2%. System suitability was established by injecting standard solution and results are shown in table no.3.

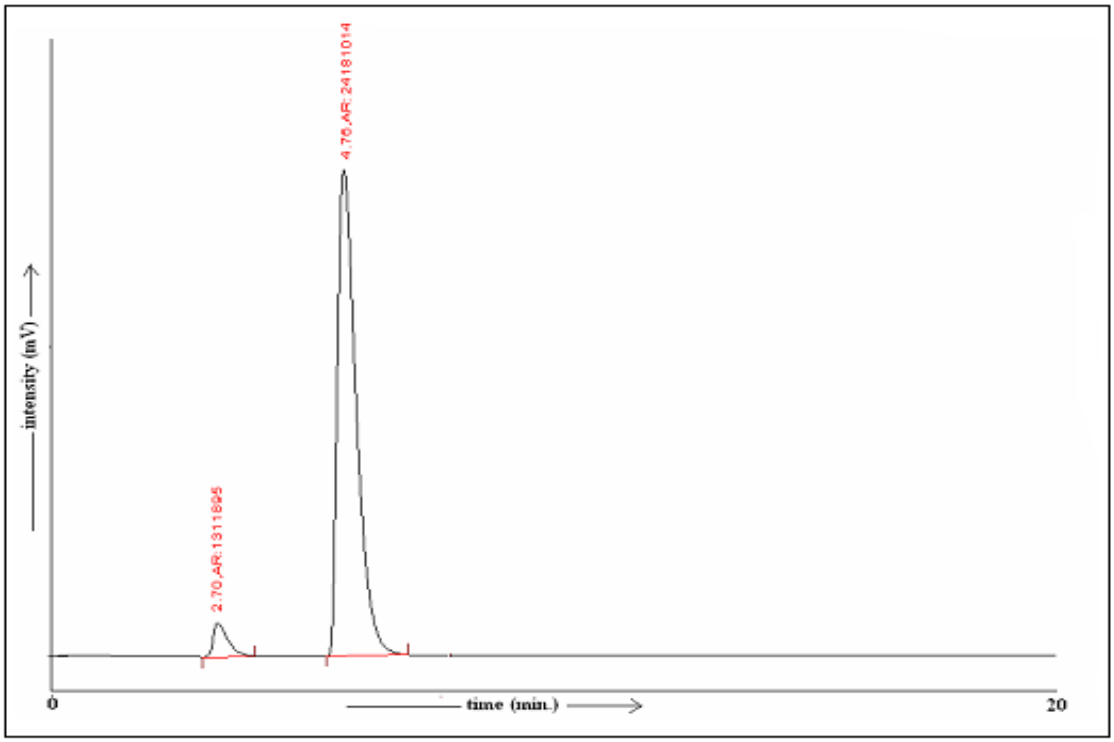

Fig. no. 2 - Chromatogram of marketed formulation of Thiocolchicoside (RT: 2.70) and Aceclofenac (RT: 4.76)

Table No. 2 - Recovery studies

Level of recovery	Drug	UV spectrophotometry		HPLC	
		Recovery (%)*	%R.S.D.	Recovery (%)*	%R.S.D.
80	THC	100.32	0.5218	99.08	0.2993
	ACE	99.54	0.7454	98.90	0.2572
100	THC	100.42	0.2927	99.31	0.1287
	ACE	99.44	0.1714	100.25	0.1533
120	THC	100.39	0.1112	99.20	0.1704
	ACE	99.35	0.1464	99.53	0.2739

**Mean of three estimation. THC =Thiocolchicoside, ACE= Aceclofenac*

Table no. 3: System Suitability Parameters for HPLC method

Component	Retention time	Resolution	Tailing Factor	LOD * (µg/ml)	LOQ * (µg/ml)
THC	2.70	2.5283	1.50	0.00921	0.02791
ACE	4.76	--	1.583	0.1962	0.5944

** Mean of six estimation. THC =Thiocolchicoside, ACE= Aceclofenac.*

CONCLUSION

Both the proposed methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods.

Hence, it can be concluded that the developed spectrophotometric & HPLC method is accurate, precise and selective and can be employed successfully for the estimation of thiocolchicoside and aceclofenac in marketed formulation.

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