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VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF THIOCOLCHICOSIDE AND ACECLOFENAC IN BULK DRUG AND FORMULATION

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ABSTRACT

This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of Thiocolchicoside and Aceclofenac as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of toluene: ethyl acetate: methanol: glacial acetic acid (4: 6: 2: 0.5 v/v/v/v). Densitometric evaluation of the separated zones was performed at 255 nm. The two drugs were satisfactorily resolved with R_F values 0.16 and 0.79 for Thiocolchicoside and Aceclofenac, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (6–21 ng/spot for Thiocolchicoside and 10-35 ng/spot for Aceclofenac), precision (intra-day RSD 0.31–1.01 % and inter-day RSD 0.51–0.72 % for Thiocolchicoside, and intra-day RSD 0.66–0.94 % and inter-day RSD 0.79–1.04 % for Aceclofenac), accuracy (100.30 ± 0.35 % for Thiocolchicoside and 99.48 ± 0.41 % for Aceclofenac), and specificity, in accordance with ICH guidelines.

KEYWORDS

Thin layer chromatography, densitometry, validation and quantification, Thiocolchicoside, Aceclofenac

INTRODUCTION

Thiocolchicoside is *N*-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide **(Figure 1).** It is a muscle relaxant with anti-inflammatory and analgesic actions. Thiocolchicoside, displaces both [3H] gamma-aminobutyric acid (3H) (GABA) and [3H] strychnine binding, suggesting an interaction with both GABA and strychnine-sensitive glycine receptors. It is used topically for the treatment of muscular spasms and for rheumatologic, orthopedic, and traumatologic disorders [1-3].

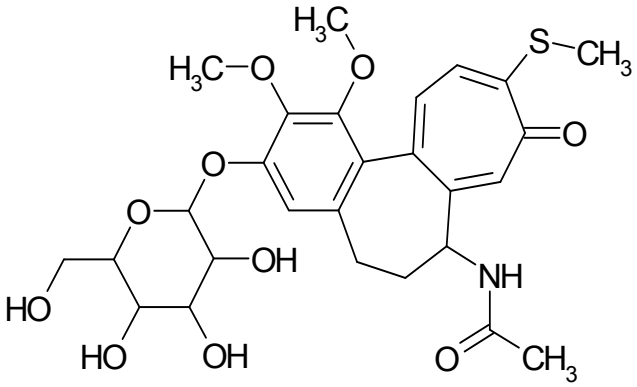

Figure 1
Structure of Thiocolchicoside

Aceclofenac chemically is 2-[[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl] oxy]acetic acid **(Figure 2).** It is used as analgesic and anti-inflammatory. Aceclofenac plays an important role in symptomatic management of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis and other acute pain conditions. The mode of action of Aceclofenac is largely based on the inhibition of prostaglandin synthesis. Aceclofenac is potent inhibitor of the enzyme cyclooxygenase, which is involved in the production of prostaglandins [4-5].

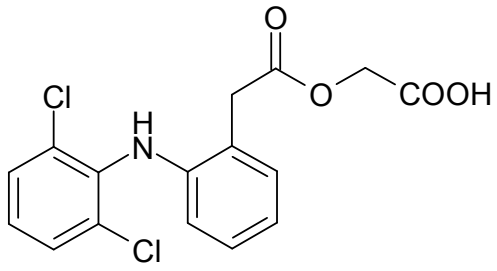

Figure 2
Structure of Aceclofenac

Literature review reveals that methods have been reported for analysis of Thiocolchicoside and Aceclofenac by UV spectrophotometry either alone or in combination with other drugs [6-8], simultaneous estimation of Thiocolchicoside and Aceclofenac in pharmaceutical dosage form by spectrophotometric and LC method [9], simultaneous estimation of Thiocolchicoside and Aceclofenac by HPTLC method either alone or in combination with other drugs [10-13] and stability-indicating HPTLC method for simultaneous determination of Drotaverine and Aceclofenac in tablet formulation [14].

To date, there have been no published reports about the simultaneous estimation of Thiocolchicoside and Aceclofenac by TLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous estimation of Thiocolchicoside and Aceclofenac by TLC in bulk drug and in pharmaceutical dosage forms.

EXPERIMENTAL

Materials

Working standards of pharmaceutical grade Aceclofenac (Batch no. 16043/01) was obtained from

Cipla Pharmaceuticals Ltd. Mumbai, India and Thiocolchicoside (Batch no. 2148/009) was obtained from Zydus Cadila, Ahmedabad, Gujarat, India. It was used without further purification and certified to contain 99.98 % (w/w) on dry weight basis for Aceclofenac and 99.36 % (w/w) on dry weight basis for Thiocolchicoside. All drugs were used without further purification. Fixed dose combination tablets (BAKFLEX-A) containing 4 mg Thiocolchicoside and 100 mg Aceclofenac were procured from local market**.** All chemicals and reagents were of analytical grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 $_{F-254}$ plates, [20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 ^{o}C for 5 min prior to chromatography. A constant application rate of 0.1 µL/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: ethyl acetate: methanol: glacial acetic acid (4: 6: 2: 0.5) (v/v/v/v) and 12.5 mL of mobile phase was used per chromatographic run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ^{o}C ± 2) at relative humidity of 60 % ± 5. The length of each chromatogram run was 8 cm. Following the development the TLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 255 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression.

Preparation of Standard Stock Solutions

Standard stock solutions of concentration

1000 µg/mL of Thiocolchicoside and 1000 µg/mL of Aceclofenac were prepared separately using methanol. From the standard stock solution, the mixed standard solution was prepared using the methanol to contain 40 µg/mL of Thiocolchicoside and 1000 µg/mL of Aceclofenac. The stock solution was stored at 2-8 °C protected from light.

Optimization of the HPTLC method

The TLC procedure was optimized with a view to develop a simultaneous assay method for Thiocolchicoside and Aceclofenac respectively. The mixed standard stock solution of 40 µg/mL of Thiocolchicoside and 1000 µg/mL of Aceclofenac was prepared and 1 µL spot was applied on to TLC plates and run in different solvent systems. Initially, toluene, ethyl acetate and methanol was tried in the ratio of 4: 6: 2 v/v/v but R_f was found to be very less, both the peaks merged and peak shape of individual drugs was not good. To increase the R_f and to improve the peak shape glacial acetic acid was added to the above mobile phase. Finally, the mobile phase consisting of toluene: ethyl acetate: methanol: glacial acetic acid (4: 6: 2: 0.5 v/v/v/v) was found optimum with R_f values 0.16 and 0.79 for Thiocolchicoside and Aceclofenac with acceptable resolution and peak shape **(Figure 3).** In order to reduce the neckless effect TLC chamber was saturated for 20 min using saturation pads. The mobile phase was run upto a distance of 8 cm; which takes approximately 20 min for complete development of the TLC plate.

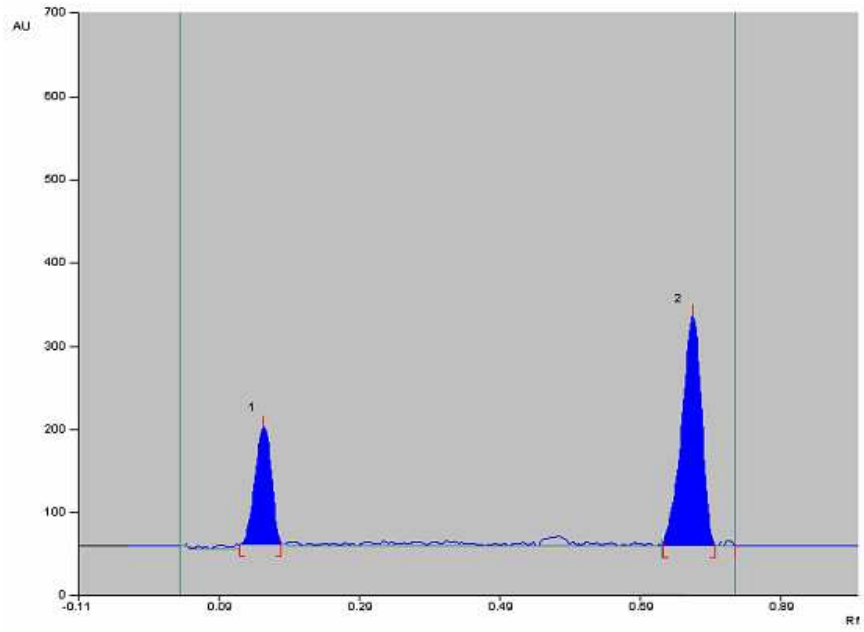

Figure 3
HPTLC densitogram of standard

Peak 1: Thiocolchicoside R_f (0.13) and Peak 2: Aceclofenac R_f (0.77).
Mobile phase: Toluene: ethyl acetate: methanol: glacial acetic acid (4: 6: 2: 0.5, v/v/v/v)
Concentration of drugs: 40 µg/mL for Thiocolchicoside and 1000 µg/mL for Aceclofenac
Application volume: 1 µL

Validation of the method

Validation of the optimized TLC method was carried out with respect to the following parameters.

Linearity and range

From the mixed standard stock solution 3 µg/mL of Thiocolchicoside and 5 µg/mL of Aceclofenac, 2 to 7 µL solution spotted on TLC plate to obtain final concentration 6-21 ng/spot for Thiocolchicoside and 10-35 ng/spot for Aceclofenac. Linearity of the method was studied by injecting six concentrations of the drug each concentration was applied three times to the TLC plates. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (6, 12, 18 ng/spot for Thiocolchicoside and 10, 20, 30 ng/spot for Aceclofenac) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitaiton

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for Thiocolchicoside and Aceclofenac by spotting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Thiocolchicoside and Aceclofenac were made from the standard stock solution in the range of 0.1–10 ng/spot. The samples were applied to TLC plate and the chromatograms were run and measured signal from the samples was compared with those of blank samples.

Robustness of the method

Following the introduction of small changes in the mobile phase composition (± 0.1 mL for each component), the effects on the results were examined. Mobile phases having different compositions, e.g. toluene: ethyl acetate: methanol: glacial acetic acid (4.1: 6: 2: 0.5 v/v/v/v), (4: 6.1: 2: 0.5 v/v/v/v), (4: 6: 2.1: 0.5 v/v/v/v), (4: 6: 2: 0.6 v/v/v/v), were tried and chromatograms were run. The amount of mobile phase was varied over the range of ± 5 %. The plates were prewashed with methanol and activated at 60 °C for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied from + 10 min. The robustness of the method was determined at three different concentration levels 6, 12, 18 ng/spot for Thiocolchicoside and 10, 20, 30 ng/spot for Aceclofenac.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for Thiocolchicoside and Aceclofenac in the samples was confirmed by comparing the R_f and spectrum of the spot with that of a standard. The peak purity of Thiocolchicoside and Aceclofenac was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (Thiocolchicoside and Aceclofenac combination tablet) to which know amount of Thiocolchicoside and Aceclofenac standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of Thiocolchicoside and Aceclofenac in conventional tablet (Brand name: BAKFLEX-A Label claim: 4 mg Thiocolchicoside and 100 mg Aceclofenac per tablet), ten tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 4 mg of Thiocolchicoside and 100 mg Aceclofenac was transferred into a 50 mL volumetric flask containing 30-35 mL methanol, sonicated for 30 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (80 and 2000 µg/mL for Thiocolchicoside and Aceclofenac respectively). Then 5 mL of the above filtered solution was diluted to produce a concentration of 40 µg/mL for Thiocolchicoside and 1000 µg/mL for Aceclofenac respectively and 1 µL of this solution (40 and 1000 ng/spot for Thiocolchicoside and Aceclofenac respectively) was applied to a TLC plate which was developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for Thiocolchicoside and Aceclofenac in the current study involving toluene: ethyl acetate: methanol: glacial acetic acid (4: 6: 2: 0.5, v/v/v/v) as the mobile phase for TLC are given below.

Linearity

The drug response was linear (r^2 = 0.9944 for Thiocolchicoside and 0.9956 for Aceclofenac) over the concentration range between 6-21 ng/spot for Thiocolchicoside and 10-35 ng/spot for Aceclofenac. The mean (± RSD) values of the slope and intercept for Thiocolchicoside and Aceclofenac were 15.724 (± 0.763), 60.229 (± 1.02) and 0.9989 (± 1.42) and 28 (± 0.932), 333.33 (± 0.782) respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in **Table 1.**

The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

Table 1
Precision study for Thiocolchicoside and Aceclofenac

Concentration (ng/spot)	Intraday precision (n=6)			Inter day precision (n=6)		
	Measured conc.	(%) RSD	Recovery (%)	Measured conc. ±SD	(%)RSD	Recovery (%)
Thiocolchicoside						
6	5.88	0.31	98.00	5.96	0.51	99.33
12	12.11	0.82	100.91	11.97	0.72	99.75
18	17.66	1.01	98.11	18.09	0.60	100.50
Aceclofenac						
10	10.07	0.94	100.70	9.98	1.04	99.80
20	19.92	0.66	99.60	20.05	0.79	100.25
30	30.04	0.75	100.13	29.86	0.90	99.53

LOD and LOQ

Signal-to-noise ratios of 3: 1 and 10: 1 were obtained for the LOD and LOQ respectively. The LOD

and LOQ were found to be 2 ng/spot and 3 ng/spot for Thiocolchicoside and 4 ng/spot and 5 ng/spot for Aceclofenac, respectively.

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in **Table 2** indicated robustness of the method.

Table 2
Robustness Testing of Thiocolchicoside and Aceclofenac (n=3)

Parameter	% RSD for **Thiocolchicoside**	% RSD for **Aceclofenac**
Mobile phase composition (± 0.1 ml)	0.94	0.74
Amount of mobile phase (± 5 %)	0.53	0.91
Time from spotting to chromatography (+ 10 min)	1.01	0.57
Time from chromatography to scanning (+ 10 min)	0.74	0.88

Specificity

The peak purity of Thiocolchicoside and Aceclofenac was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9983 and r (M, E) = 0.9991. A good correlation (r = 0.9994) was also obtained between the standard and sample spectra of Thiocolchicoside and Aceclofenac respectively.

Recovery Studies

As shown from the data in **Table 3** good recoveries of the Thiocolchicoside and Aceclofenac in the range from 98.16 to 101.66 % were obtained at various added concentrations. The average recovery of three levels (nine determinations) for Thiocolchicoside and Aceclofenac were 100.30 % and 99.48 % respectively.

Table 3
Recovery studies of Thiocolchicoside and Aceclofenac

Lable Claim (mg/ tablet)	Amount added %	Total amount (mg)	Amount recovered (mg ± % RSD)	Recovery %
Thiocolchicoside 4 mg	80 (3.2 mg)	7.2	7.28 ± 0.43	101.12
	100 (4.0 mg)	8.0	7.94 ± 0.87	99.31
	120 (4.8 mg)	8.8	8.84 ± 1.11	100.47
Aceclofenac 100 mg	80 (80 mg)	180	177.51 ± 0.99	98.62
	100 (100 mg)	200	196.32 ± 1.02	98.16
	120 (120 mg)	220	223.65 ± 0.46	101.66

Analysis of a formulation

Experimental results of the amount of Thiocolchicoside and Aceclofenac in tablets, expressed as a percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients, which are normally present in tablets. The average drug content

was found to be 99.35 % and 99.01 % for Thiocolchicoside and Aceclofenac, respectively. Two different lots of Thiocolchicoside and Aceclofenac combination tablets were analyzed using the proposed procedures and the results are summarized in **Table 4.**

Table 4
Analysis of commercial formulation

Bakflex-A Thiocolchicoside (4 mg)	Thiocolchicoside found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	3.96 ± 2.33	99.17
2nd Lot	3.98 ± 2.98	99.53
Aceclofenac (100 mg)	Aceclofenac found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	98.80 ± 2.18	98.80
2nd Lot	99.23 ± 2.21	99.23

CONCLUSION

The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of Thiocolchicoside and Aceclofenac as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Thiocolchicoside and Aceclofenac and also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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