

Validated HPTLC method for simultaneous estimation of Etodolac and Thiocolchicoside 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 etodolac and thiocolchicoside as the bulk drug and in tablet dosage form. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F₂₅₄ as the stationary phase and the solvent system consisted of chloroform : methanol : toluene (6 : 2 : 3 v/v/v). Densitometric evaluation of the separated zones was performed at 238 nm. The two drugs were satisfactorily resolved with R_F values of 0.20 and 0.50 for thiocolchicoside and etodolac, respectively.

Key Words: etodolac, thiocolchicoside, HPTLC

Introduction:

Etodolac is chemically 1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid, it belongs to class of nonsteroidal anti-inflammatory drugs (NSAIDs) and is used as anti-inflammatory and analgesic agent. The drug is official in Indian Pharmacopoeia, United States Pharmacopoeia [14] and British Pharmacopoeia.

Thiocolchicoside is chemically N-[(7S)-3-(beta-D-glucopyranosyl oxy)-1,2-dimethoxy-10-(methylsulfonyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl] acetamide, an anti-inflammatory, analgesic agent with muscle relaxant action implicated in the treatment of musculoskeletal disorders [3-4]. The drug is official in Indian Pharmacopoeia.

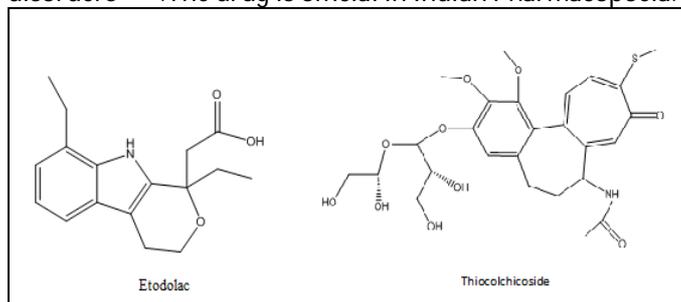


Figure 1. Structure of Etodolac and Thiocolchicoside

To the best of our knowledge no HPTLC method of analysis has yet been reported for simultaneous analysis of Etodolac and Thiocolchicoside in combination. This paper describes simple, accurate and precise HPTLC method for simultaneous determination of etodolac and thiocolchicoside in combined tablet dosage form. The method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

Materials and Methods:

Materials

Working standards of pharmaceutical grade etodolac (Batch No. 4325602) and Thiocolchicoside (Batch NO. 2409/10) were obtained as generous gifts from Cadilla Pharmaceuticals Ltd Ankleshwar and Sanofi Aventis Goa. They were used without further purification and certified to contain 99.60 % (w/w) and 99.80 % (w/w) for etodolac and thiocolchicoside respectively on dry weight basis. Fixed dose combination tablets. (Brand Name: Proxym-MR) containing 200 mg of Etodolac and 4 mg of Thiocolchicoside were procured from Emcure Pharmaceuticals, India.) All chemicals and reagents of analytical grade 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 syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated aluminum 60 F₂₅₄ 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 °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 chloroform: methanol: toluene (6: 2 : 3 v/v/v). 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 40 min at room temperature ($25\text{ }^{\circ}\text{C} \pm 2$) at relative humidity of $60\% \pm 5$. The saturation time was kept 30 min for each chromatographic run. Each chromatogram was developed over a distance of 8 cm. Following the development, the TLC plates were dried in a stream 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 238 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 light. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

Preparation of Standard Stock Solutions:

Standard stock solutions with a concentration of 1000 $\mu\text{g/mL}$ were prepared in methanol for Etodolac and Thiocolchicoside. From the standard stock solutions, diluted mixed standard solutions were prepared containing 100 $\mu\text{g/mL}$ for Etodolac and 100 $\mu\text{g/mL}$ for Thiocolchicoside respectively. The stock solution was stored at $2-8\text{ }^{\circ}\text{C}$ protected from light.

Selection of detection wavelength:

Solutions of 10 $\mu\text{g/mL}$ etodolac and thiocolchicoside were prepared in methanol and scanned in UV range. Maximum absorbance for etodolac was found at 224 nm and thiocolchicoside showed maximum absorbance at 257 nm. Overlain Spectra of both drugs in UV region indicates that 238 nm was an ideal wavelength for simultaneous determination by HPTLC analysis. (Figure 2).

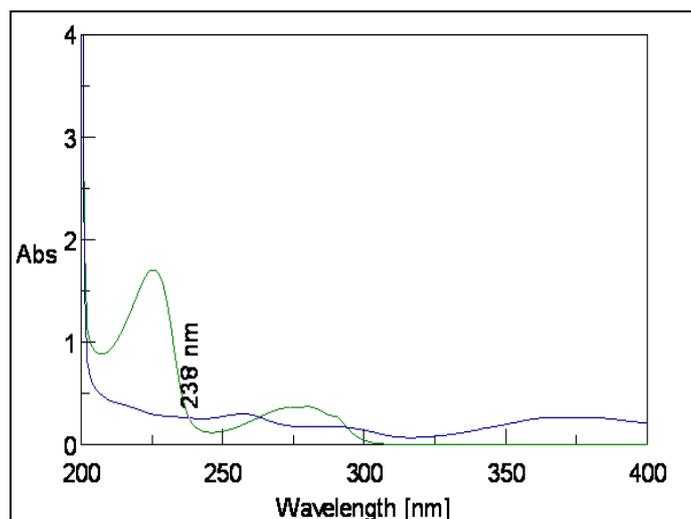


Figure 2. Overlain Spectra of Etodolac and Thiocolchicoside Over range of 200-400 nm

Optimization of HPTLC method

The TLC procedure was optimized in view to develop a simultaneous assay method for etodolac and thiocolchicoside. Etodolac and thiocolchicoside were spotted on TLC plates and run in different solvent systems.

Initially, toluene and ethyl acetate and methanol were tried. Finally, the optimum mobile phase with good resolution selected was chloroform: methanol: toluene (6: 2: 3 v/v/v). The mobile phase was run up to a distance of 8 cm; which takes approximately 30 min for development of TLC plate.

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, 4000 $\mu\text{g/mL}$ of etodolac and 80 $\mu\text{g/mL}$ of thiocolchicoside was taken, 0.5 to 2.5 μL solutions were spotted on TLC plate to obtain final concentration 4000-20000 ng/spot for etodolac and 80-400 ng/spot for thiocolchicoside. Linearity of the method was studied by applying five 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 (4000 ng/spot, 12000 ng/spot and 20000 ng/spot for Etodolac and 80 ng/spot, 240 ng/spot and 400 ng/spot for Thiocolchicoside respectively) 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 Quantitation:

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 etodolac and thiocolchicoside 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 etodolac and thiocolchicoside were prepared from the standard stock solution in the range of 20-1800 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.

Robustness of the method:

Following the introduction of small changes in the mobile phase composition ($\pm 0.1\text{ mL}$ for each component), the effects on the results was examined. Mobile phases having different compositions, chloroform : methanol : toluene (6 : 1.9 : 3 v/v/v), (5.9 : 2 : 3 v/v/v), (6 : 2 : 2.9 v/v/v) were tried and chromatograms were run. The amount of mobile phase was varied over the range of $\pm 5\%$. The plates were prewashed with methanol and activated at $110\text{ }^{\circ}\text{C}$ for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied by 10 min. The

robustness of the method was determined at three different concentration levels (4000 ng/spot, 12000 ng/spot and 20000 ng/spot for etodolac and 80 ng/spot, 240 ng/spot and 400 ng/spot for thiocolchicoside respectively).

Specificity:

The specificity of the method was determined by analyzing standard drug and test samples. The spot for etodolac and thiocolchicoside in the samples was confirmed by comparing the R_f and spectrum of the spot with that of standard. The peak purity of etodolac and thiocolchicoside 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).

Recovery studies:

Accuracy of the method was checked by applying the method to drug sample (etodolac and thiocolchicoside combination tablet) to which known amount of etodolac and thiocolchicoside 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.

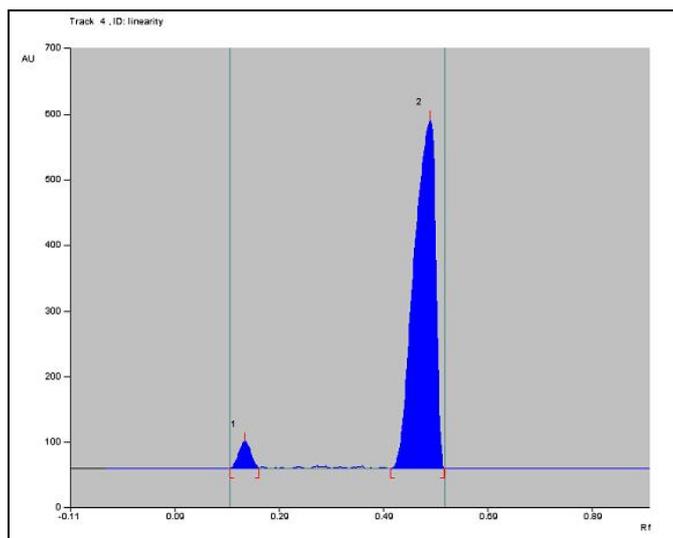


Figure 3.HPTLC densitogram of Standard.

Analysis of a marketed formulation:

To determine the content of etodolac and thiocolchicoside in conventional tablet [(Brand Name: Proxym-MR) containing 200 mg of etodolac and 4 mg of thiocolchicoside were procured from Emcure Pharmaceuticals, India.]. Twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 200 mg of etodolac and thiocolchicoside of 4 mg was transferred into a 25 mL volumetric flask containing 20 mL methanol, sonicated for 30 min with occasional shaking and diluted to 25 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined. 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 of the method developed for etodolac and thiocolchicoside in the current study using as the mobile phase chloroform : methanol : toluene (6 : 2 : 3 v/v/v) for TLC are given below.

Linearity:

The drug response was linear ($r^2 = 0.998$ for etodolac and 0.999 for thiocolchicoside) (Figure 4 and 5) over the concentration range between 4000-20000 ng/spot for etodolac and 80-400 ng/spot for thiocolchicoside (Table 1).

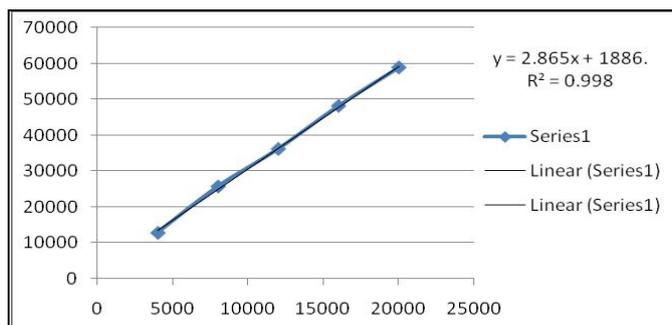


Figure 4. Calibration Curve of Etodolac

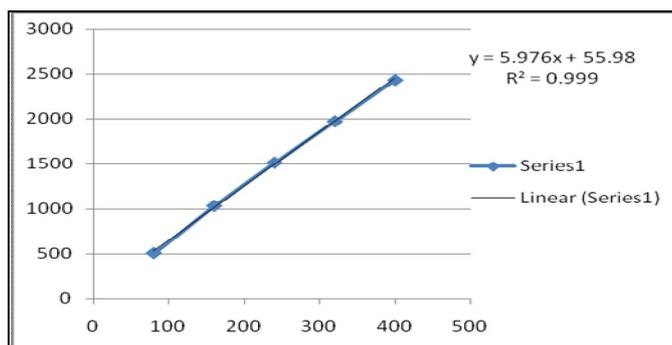


Figure 5. Calibration curve of Thiocolchicoside

Table 1. Linear regression data for the calibration curves

Compound	Linearity (ng.spot ⁻¹)	y = A + Bx		r ²
		A	B	
Thio	80-400	55.98	5.976	0.999
Eto	4000-20000	1886	2.865	0.998

n = 6; r²=coefficient of correlation

Precision:

The results of the repeatability and intermediate precision experiments are shown in table 2. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, as recommended by ICH guidelines (Table 2).

Table 2. Intra and inter day precision of HPTLC Method

Compound	Intraday precision		Interday precision	
	S.D of areas.	% R.S.D.	S.D of areas.	% R.S.D.
Thio	325.10	0.048	535.28	0.09
Eto	1293.1	1.21	864.42	0.36

LOD and LOQ:

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

to be 100 ng/spot and 130 ng/spot for thiocolchicoside, 120 ng/spot and 300 ng/spot for etodolac, 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 3 indicated the robustness of the method.

Table 3. Robustness Testing

Parameter	Eto		Thio	
	SD of peak area	% RSD	SD of peak area	% RSD
Mobile phase composition (± 0.1 ml)	535	1.01	470	0.27
Amount of mobile phase (± 5 %)	355	1.89	631	0.81
Time from spotting to chromatography (± 2 min)	493	0.91	286	0.33
Time from chromatography to scanning (± 20 min)	592	0.05	361	0.09

^a n = 6

Specificity:

The peak purity of etodolac and thiocolchicoside was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., $r(S, M) = 0.9903$ and $r(M, E) = 0.9911$. A good correlation ($r = 0.9990$) was also obtained between the standard and sample spectra of etodolac and thiocolchicoside respectively.

Recovery studies:

As shown from the data in table 4, good recoveries of the etodolac and thiocolchicoside in the range from 98.90 % w/w to 99.91 % w/w were obtained at various added concentrations.

Table 4. Recovery studies ^a

Label claim	Amount of drug added (%)	Total amount of drug present (ng/spot)	Amount found	% Recovery
Thio 4	80	7.2	7.19	99.83
	100	8	7.94	99.29
	120	8.8	8.79	99.91
Eto 200	80	360	356.04	98.90
	100	400	398.28	99.57
	120	440	437.54	99.44

Analysis of a formulation:

Experimental results of the amount of etodolac and thiocolchicoside in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients that are normally present in tablets. Two different lots of etodolac and thiocolchicoside combination tablets were analyzed using the proposed procedures (Table 5).

Table 5. Applicability of the HPTLC method for the analysis of the pharmaceutical formulations

Sample	Label claim (mg)	Drug content (%)	% R.S.D.
Thio	4	99.89	1.11
Eto	200	101.95	1.62

Conflict of interest: - Not declared

Source of funding:-None.

Table 6. Summary of validation parameters

Parameter	Thio	Eto
Linearity range (ng.spot ⁻¹)	80-400	4000-20000
Correlation coefficient	0.999	0.998
Limit of detection (ng. spot ⁻¹)	100	120
Limit of quantitation (ng. spot ⁻¹)	130	300
Recovery (n = 6)	99.67	99.30
Precision (% R.S.D.)		
Repeatability	0.048	0.09
Inter day	1.21	0.36
Robustness	Robust	Robust
Specificity	Specific	Specific

Conclusion:

The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of etodolac and thiocolchicoside as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of etodolac and thiocolchicoside 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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