INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
Available online http://www.irjponline.com
Research Article

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF DICLOFENAC SODIUM AND THIOCOLCHICOSIDE IN FIXED DOSE COMBINATION

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Article Received on: 12/11/10 Revised on: 30/11/10 Approved for publication: 03/12/10

ABSTRACT

A simple, specific, accurate, and precise high-performance thin-layer chromatographic method for analysis of Diclofenac Sodium and Thiocolchicoside in fixed dose combination has been developed and validated. The method use aluminium plates precoated with silica gel 60 F_{254} as stationary phase and Toluene: Ethyl acetate: Methanol (5:3:2, v/v/v) as mobile phase. Densitometric evaluation of the separated bands was performed at 285 nm. The two drugs were satisfactorily resolved with Rf values 0.53 ± 0.005 and 0.17 ± 0.002 for Diclofenac Sodium and Thiocolchicoside, respectively. Results were found to be linear over the concentration range 50–300 ng/ band for both the drugs. Intra-day variation, as RSD (%), was 0.468 for Diclofenac Sodium and 0.357 for Thiocolchicoside. Interday variation, as RSD (%) was 0.949 for Diclofenac Sodium and 0.739 for Thiocolchicoside. This method has been successfully validated and applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 100.521 ± 0.833 for Diclofenac Sodium and 101.132 ± 0.612 for Thiocolchicoside.

KEYWORDS: High performance thin layer chromatography, Thiocolchicoside, Diclofenac Sodium

INTRODUCTION

Diclofenac sodium (DIC), [Sodium (o- {(2, 6-dichlorophenyl) amino} phenyl) acetate] is a synthetic nonsteroidal anti-inflammatory drug (NSAID), has been proved to be safe and efficacious drug in the treatment of a variety of inflammatory and rheumatoid disorders[1]. Thiocolchicoside (THIO), chemically, is (s)-N-[3-(B-D-glucopyranoxyloxy)-5,6,7,9-tetrahydro-1,2-dimethoxy-10-(methylthio)-9-oxobenzo [a] heptalen-7yl] acetamide. Thiocolchicoside is a muscle relaxant which has been claimed to possess GABA mimetic and glycinergic actions. It is used in the symptomatic treatment of painful muscle spasm[2].

Literature survey reveals spectrophotometric[3] and HPTLC[4 5] determination of DIC in combination with other drugs. HPLC[6] and bioanalytical chromatographic methods[7] for quantification of DIC are also reported. For simultaneous determination of THIO with other drugs spectrophotometric[8], HPTLC[9] and HPLC methods[10,11] are reported.

No reports were found for determination of DIC and THIO by HPTLC method in fixed dose combination. Aim of present work was to develop simple, economical, rapid, accurate and precise HPTLC method for determination of these drugs in fixed dose combination. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines[12].

MATERIALS AND METHODS

Reagents and Chemicals

Analytically pure samples of DIC and THIO were kindly supplied by Cipla Pvt. Ltd. (Mumbai, MH) and Aventis Pharma Pvt. Ltd (Goa, India), respectively and were used as such without further purification. The pharmaceutical dosage form used in this study was a THIOACT-D4 (Sun Pharmaceuticals Industries Ltd, Mumbai, India) capsule labeled to contain 4 mg of Thiocolchicoside and 50 mg of Diclofenac sodium enteric coated tablet, per capsule were procured from local market.

Instrumentation and Chromatographic Conditions

The samples were applied in the form of bands of width 6 mm with space between bands of 5 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The plates were prewashed with methanol and activated at 110 ^{0}C for 5 minute, prior to chromatography. The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec were employed in analysis.

The linear ascending development was carried out in 10 cm ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using mobile phase Toluene: Ethylacetate: Methanol (5:3:2, v/v/v). The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 30 min. HPTLC plates were dried in a current of air with the help of a hair dryer. Densitometric scanning was performed on CAMAG thin layer chromatography scanner 3 at 285 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard Stock Solutions

Standard stock solutions of DIC and THIO were prepared by dissolving 50 mg of drug in 50 mL methanol separately to get concentration of 1 mg/mL from which 0.5 mL was further diluted to 10 mL to get working standard stock solution of 50 ng/µL for both the drugs.

Selection of Detection Wavelength

After chromatographic development spots were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 285 nm. So, 285 nm was selected as the detection wavelength as shown in Figure 1.

Preparation of Calibration Curve

The standard stock solutions of DIC and THIO (50 ng/µL each) were applied by overspotting on HPTLC plate in range of 2-10 µL with the help of CAMAG 100 µL sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under above established chromatographic conditions. Each standard in five replicates was analyzed and peak areas were recorded. Calibration curves of DIC and THIO were plotted separately of peak area Vs respective concentration of DIC and THIO.

Analysis of Capsules

Content of twenty capsules were weighed accurately and finely powdered. Two different stock solutions were prepared for DIC and THIO. A quantity of powder equivalent to 10 mg DIC was weighed and transferred to a 10 mL volumetric flask containing approximately 5 mL methanol. The mixture was ultrasonicated for 5 min and diluted to volume with methanol. The solution was filtered using Whatman no. 41 paper. From stock solution 0.5 mL was taken and diluted to 10 mL with methanol. 2 µL of this solution was applied to a TLC plate to furnish 100 ng /band for DIC. Similarly a quantity of powder equivalent to 10 mg THIO was weighed and transferred to a 10 mL volumetric flask containing approximately 5 mL methanol. The mixture was ultrasonicated for 5 min then diluted to volume with methanol. The solution was filtered using Whatman no. 41 paper. 2 µL of this stock solution was applied to a TLC plate to furnish 100 ng / band for THIO. After chromatographic development the peak areas of the bands were measured at 285 nm and the amount of each drug in each tablet was determined from the respective calibration plots. The analytical procedure was repeated six times for the homogenous powder sample.

Recovery Studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %.

Precision

To study intra-day variation, six mixed standard solutions containing DIC (100 ng/band) and THIO (100 ng/band) were prepared and analyzed on the same day to record any intra-day variation in the results. To study inter-day variation, analysis of three mixed standard solutions of the same concentration was performed on three different days.

Specificity and Selectivity

The specificity of the method was ascertained by analyzing standard drug and sample. The spots for both drugs were confirmed by comparing the Rf and spectra of the sample spots with that of standard drugs.

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness Studies

The robustness of the method was studied, during method development, by small but deliberate variations in chamber saturation period (± 10 %), development distance (± 10 %), time from application to development (0, 10, 20, 30 min), time from development to scanning (0, 30, 60, 90 min). One factor at a time was changed to study the effect on the peak area of the drugs (Concentration level 100 ng/band for DIC and 100 ng/band for THIO).

RESULTS AND DISCUSSION

Different mobile phases containing various ratios of Toluene, Methanol, n- Hexane, Ethyl acetate and Isopropyl alcohol were examined (data not shown). Finally the mobile phase containing Toluene–ethyl acetate-methanol (5:3:2, v/v/v) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 285 nm. The retention factors for DIC and THIO were found to be 0.53 ± 0.005 and 0.17 ± 0.002, respectively. Densitogram of mixed standard solution of DIC and THIO is shown in Figure 2.

The standard calibration curves were found to be linear over a concentration range of 50-300 ng/band for both drugs with correlation coefficients of 0.992 ± 0.172 for DIC and 0.995 ± 0.376 for THIO. For DIC, the recovery study results ranged from 100.615 to 101.422 % with % RSD values ranging from 0.415 to 0.719. For THIO, the recovery results ranged from 100.782 to 101.088 % with % RSD values ranging from 0.413 to 0.761. Results of recovery studies are reported in Table 1.

The proposed method was also evaluated by the assay of commercially available tablets containing DIC and THIO. Six replicate determinations were performed on the accurately weighed amounts of tablets. The % assay was found to be 101.126 ± 0.439 and 100.915 ± 0.155 for DIC and THIO (mean ± % RSD) respectively. Intra-day variation, as RSD (%), was found to be 0.468 for DIC and 0.357 for THIO, respectively. Inter-day variation, as RSD (%), was found to be 0.949 for DIC and 0.739 for THIO, respectively. The spectra acquired for DIC and THIO extracted from the tablet were also compared with those acquired from DIC and THIO standards; correlation was good indicates specificity of the method. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes in the operational parameters (% RSD < 2). Summary of validation parameters of proposed RP-HPLC method is given in Table 2.

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of DIC and THIO in combined tablet dosage form.

ACKNOWLEDGEMENTS

The authors express their gratitude to Cipla Pharmaceuticals Pvt. Ltd. (Mumbai, MH) and Aventis Pharmaceuticals Ltd (Goa, India) for the gift samples of pure drug. Special thanks to Dr. (Mrs.) A. R. Madgulkar, Principal, AISSMS College of Pharmacy for providing facilities and his constant support.

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Table 1: Recovery studies of DIC and THIO

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% RSD[a]
DIC	100	50	152.010	101.341	0.531
	100	100	201.230	100.615	0.418
	100	150	253.554	101.422	0.729
THIO	100	50	151.173	100.782	0.495
	100	100	202.176	101.088	0.418
	100	150	252.190	100.876	0.768

[a]Average of three determinations

Table 2: Summary of validation parameters of RP-HPLC method

Parameter	DIC	THIO
Detection Wavelength (nm)	285	
Beer's Law Limit (ng/band)	50-300	50-300
Correlation Coefficient (r)	0.992	0.995
Linear Regression Equation[a] (y = mx + c)		
Intercept (c)	302.3	324.6
Slope (m)	10.95	12.13
LOD (ng/band)	11.673	7.089
LOQ (ng/band)	38.52	23.393

[a]With respect to y = mx + c, where y is the peak area and x is the concentration (ng/band)

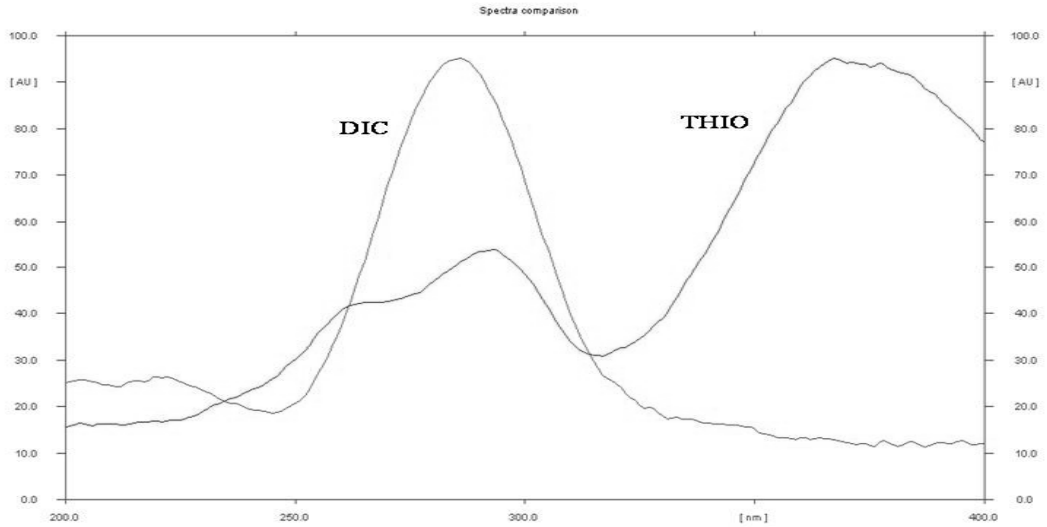

Figure 1: In situ overlain spectrum of DIC and THIO measured from 200 to 400 nm.

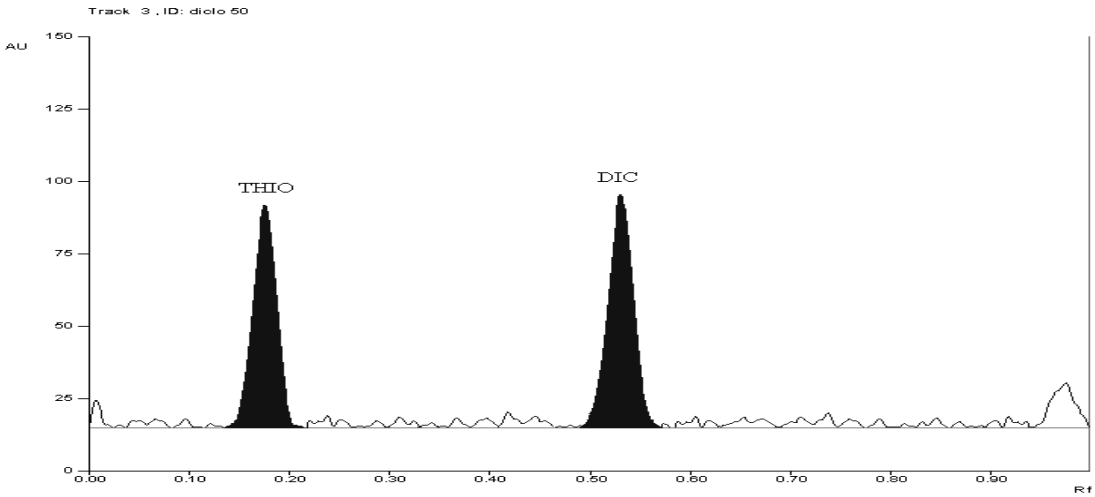

Figure 2: Representative densitogram obtained fro mixed standard solution of DIC (50 ng/band, Rf = 0.53 ± 0.005) and THIO (50 ng/band, Rf = 0.17 ± 0.002)

Source of support: Nil, Conflict of interest: None Declared