Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2013, 2 (4):913-921

(International Peer Reviewed Journal)

Simultaneous Estimation of Thin Layer Liquid Chromatography / Densitometry Method for Thiocolchicoside and Dexketoprofen in Bulk and in Tablet Dosage Form

Vijay K. Patil*, Jineetkumar B. Gawad and Amol J. Mhaske

*Post Graduate Department of Pharmaceutical Chemistry,R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur (Dhule) - 425 405 Maharashtra, **INDIA**.

Email: gawadjinit@yahoo.com

Received on 30th May and finalized on 15th June 2013.

ABSTRACT

The present work describes a validated normal phase high performance thin layer liquid chromatographic method for simultaneous estimation of Thiocolchicoside and Dexketoprofen in bulk and in pharmaceutical dosage form. Chromatographic separation of the drugs were performed on aluminum plates precoated with 200-µm layers of silica gel 60 F254S as the stationary phase and the solvent system consisted of toluene: ethyl acetate: methanol : glacial acetate (6 :4 :4 : 0.5)(v/v/v/v). Densitometric evaluation of the separated zones was performed at 295 nm. The two drugs were satisfactorily resolved with R_f values 0.29 ± 0.02 and 0.76 ± 0.02 for Thiocolchicoside and Dexketoprofen respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (400-2400 ng/spot for Thiocolchicoside and 2500-15000 ng/spot for Dexketoprofen), precision (intraday RSD 1.29 – 1.36 and interday % RSD 0.36– 1.17 % for Thiocolchicoside and intra-day RSD 0.89–1.71 % and inter-day RSD 0.24–1.13 % for Dexketoprofen), accuracy (99.74 ± 1.02 % for Thiocolchicoside and 100.18 ± 0.45 % for Dexketoprofen), and specificity in accordance with ICH guidelines. This novel Statistical analysis proves that the method is repeatable and selective for the analysis of Thiocolchicoside and Dexketoprofen as bulk drug and in pharmaceutical formulations without any interference from the excipients. This simultaneous estimation work with advanced High performance thin layer chromatographic technique gave the new dimensions and gives some contribution to the analytical and bio-analytical field. It was concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps and improved sensitivity made it specific, reliable and easily reproducible in any quality control set-up providing all the parameters are followed accurately for its intended use.

Keywords: Thinlayer chromatography, dexketoprofen, Thiocolchicoside.

INTRODUCTION

Chemically, Thiocolchicoside (THI) is N-[3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio)-9-oxobenzo[α]heptalen-7yl] acetamide (figure 1). 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[1,2]. Literature survey reveals that THI can

be estimated by spectrophotometry, HPLC and by HPTLC methods individually or in combination with other drugs[3].

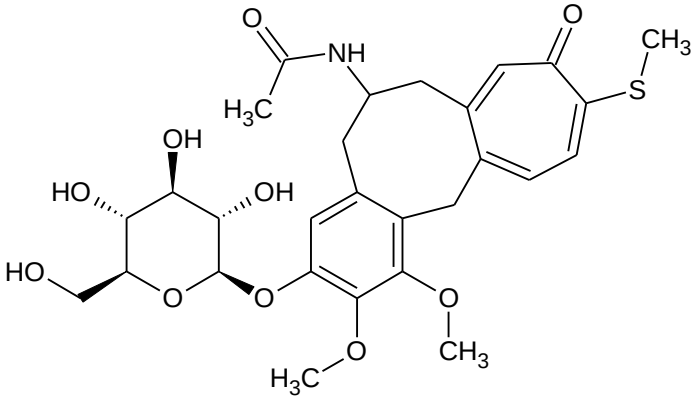

Figure 1: Chemical structures of THI

Chemically, Dexketoprofen (DEX) 2-amino-2-(hydroxymethyl) propane-1,3-diol; 2-(3-benzoylphenyl propanoic acid is a water-soluble salt of the dextrorotatory enantiomer or (*S*)-(+)-enantiomer of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen (Figure 2)[4,5]. The enantiomer is a relatively new oral NSAID with analgesic, anti-inflammatory and anti-pyretic properties and is one of the most potent in vitro inhibitors of prostaglandin synthesis[6]. Literature survey reveals that DEX can be estimated by spectrophotometry, HPLC and by HPTLC[7] methods individually or in combination with other drugs[8]. Hence, the combination of THI and DEX have synergetic action and is prescribed for symptomatic relief of low back pain, post operative pain, and rheumatic arthritis osteoarthritis, musculoskeletal injuries and chronic pain associated with cancer[9]. Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC thus reducing the analysis time and cost per analysis. Even though no single method have been proposed for analysis of THI and DEX but for the first time we are presenting simple, sensitive, accurate, precise, rapid and economic chromatographic method in bulk and in tablet dosage form[10,11].

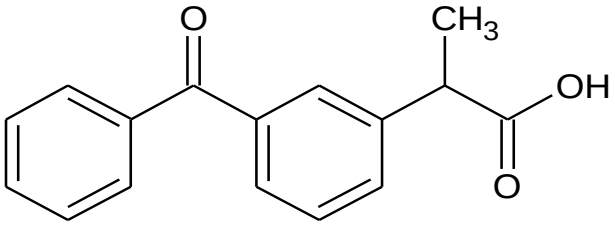

Figure 2: Chemical structure of DEX

This paper presents HPTLC method for determination of Thiocolchicoside and Dexketoprofen in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines

MATERIALS AND METHODS

Thiocolchicoside and Dexketoprofen were provided as a gift sample by Emcure Pharmaceutical Ltd (Pune). All other reagents and solvents utilized were of analytical (AR) grade and are from Merck Chemicals Ltd, Mumbai (India).

Instrumentation and chromatographic condition : The chromatography was performed on 20 cm ×10 cm aluminium-backed TLC plates coated with 200-µm layers of silica gel 60 F254S were used. Before chromatography the plates were prewashed with methanol and activated at 105^0C for 5 min in oven. The samples were applied as 6 mm wide bands with the help of Linomat 5 sample applicator (Muttenz, Switzerland) fitted with a 100-µl sample syringe (Hamilton, Bonaduz, Switzerland). The plate was developed in a pre-saturated Camag twin trough glass chamber (20 cm × 10 cm). Toluene: ethyl acetate: methanol: glacial acetate (6: 4: 4: 0.5) were used as mobile phase and chamber saturation time was 15 min. The plates were developed to a distance of 8.0 cm and scanned densitometrically using Camag TLC Scanner 3 equipped with win CATS software version 1.3.0 at 295nm for both method. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200- 400 nm. Evaluation was performed using peak area with linear regression.

Preparation of standard and sample solutions : Independent stock solution of 1000 µg mL^{-1} of THI and 10000 µg mL^{-1} of DEX were prepared in methanol.

Preparation of calibration curves : From each stock standard solution, 0.4 – 2.4 mL of THI and 2.5 - 15 µL of DEX were transferred into six 10 µL volumetric flasks separately and volume was made up to the mark with methanol From each volumetric flask a volume 1 µL was applied on TLC plate to obtain series of concentration 400- 2400 ng/band of THI and 2500 - 15000 ng/band of DEX. The plates were developed and scanned as described. Each standard in six replicates was analyzed and peak areas were recorded calibration curves of THI and DEX were plotted separately of peak area *vs.* respective concentration.

Optimization of HPTLC method : Firstly, single solvents were selected on the basis of their polarity to separate the spots. Then the mixtures of solvents are used for separation purpose of THI and DEX. The spots were developed in mixtures of Toluene: ethyl acetate: methanol in the ratio of 6:4:4 *v/v*. The R_f value obtained was good but slight tailing was observed. Hence, to reduce the tailing, glacial acetate was added in the solvent system. Thus, the final mobile phase consisted of toluene: ethyl acetate: methanol: glacial acetate in the ratio (6: 4: 4: 0.5) (*v/v/v/v*). The chamber saturation time was 30 min. The R_f for THI and DEX were found to be 0.29± 0.02 and 0.76 ± 0.02, respectively (figure 3).

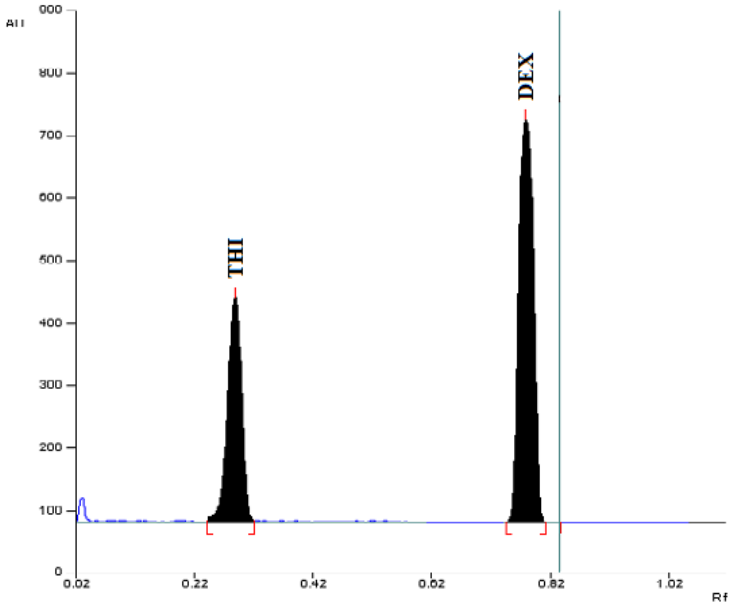

Figure 3: Densitogram of standard THI and DEX showing R_f 0.29 and 0.76 respectively.

Validation : The method was validated by establishing linearity, accuracy, inter - day and intra - day precision of measurement of sample application. The limit of detection and limit of quantification were also determined.

Linearity : Linearity was studied in the concentration range from 400 – 2400 ng/band for THI and 2500 – 15000 ng/band for DEX for normal phase. The drugs showed good linearity in the tested range. The regression co-efficient values for THI and DEX were found to be r^2 = 0.999 and 0.999 (table 1).

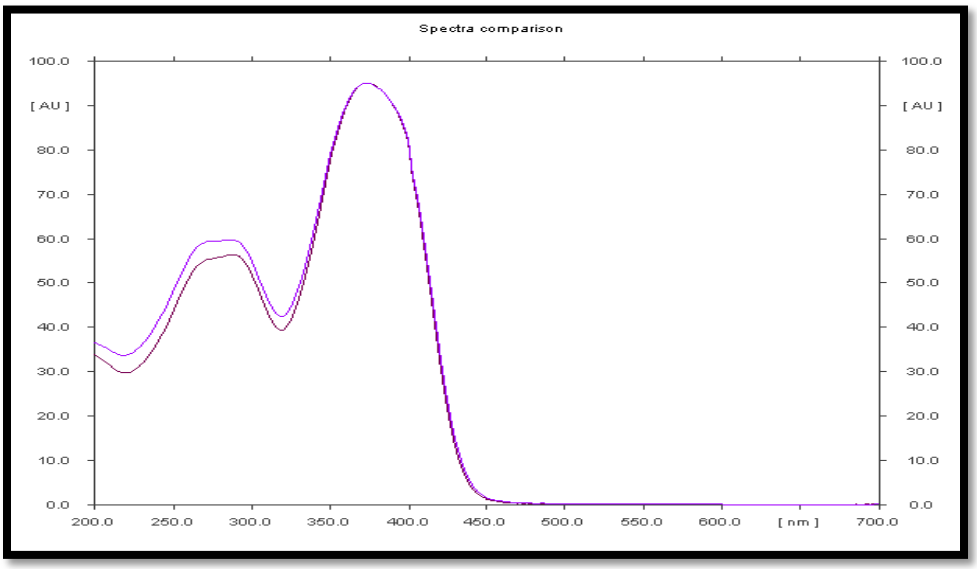

Figure 4: Peak purity spectra of standard THI, sample extracted from Thiocolchicoside - Dexketoprofen tablet, scanned at the peak - start, peak - apex and peak - end positions of the band (Correlation > 0.99)

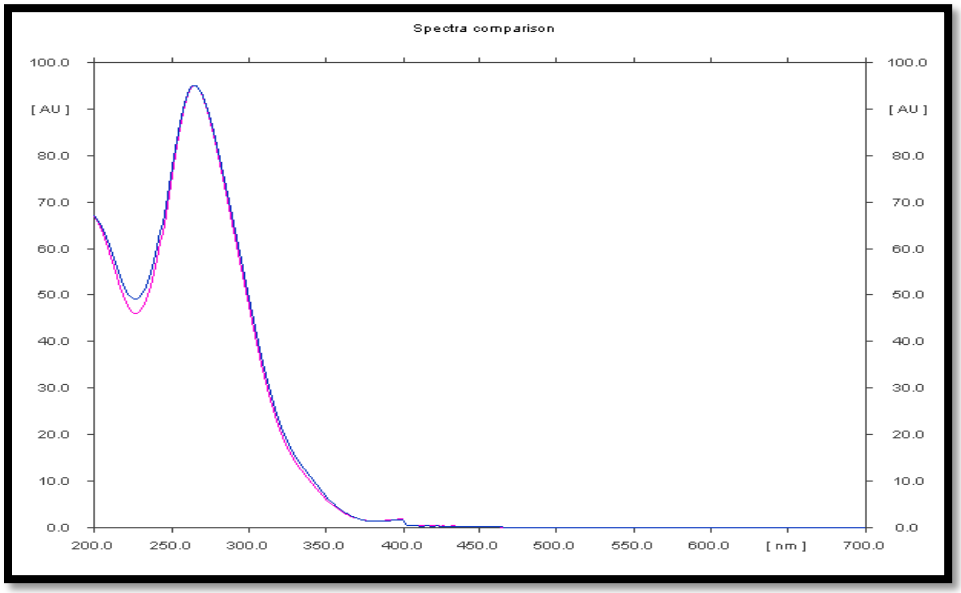

Figure 5: Peak purity spectra of standard DEX, sample extracted from Thiocolchicoside - Dexketoprofen tablet, scanned at the peak - start, peak - apex and peak - end positions of the band (Correlation > 0.99)

Table 1 Linearity of DEX and THI for proposed method (n=6)

Parameters	THI	DEX
Linearity range (ng/band)	400 – 2400	2500 – 15000
Slop	5.167	0.775
Intercept	1013	9476
Correlation Coefficient (r^2)	0.999	0.999

Precision : In order to validate and prove the applicability of the method, a laboratory mixture of THI and DEX was prepared from the stock solutions in the ratio corresponding to amounts in the dosage form. For quantitative estimation of the mixture, three series (800, 1200, 1600 ng/band and 5000, 7500, 10000 µg/mL for THI and DEX respectively) were prepared (table 2).

Table 2 Precision of DEX and THI for proposed method (n=3)

Drugs	Conc. [ng/spot]	Intra-day precision		Inter-day precision	
		Mean ± S.D.	% *RSD* [n = 3]	Mean ± S.D.	% *RSD* [*n* = 3]
THI	800	5113.02 ± 66.00	1.29	5206.33 ± 46.47	0.89
	1200	7263.00 ± 99.23	1.36	7229.66 ± 65.42	0.90
	1600	9168.33 ± 114.42	1.24	9235.00 ± 158.30	1.71
DEX	5000	13323.33 ± 156.31	1.17	13390.01 151.99	1.13
	7500	15256.67 ± 102.47	0.67	15323.33 ± 153.26	1.00
	10000	17265.67± 63.12	0.36	17315.67 ± 42.23	0.24

Accuracy: The accuracy of the experiment was established by spiking pre-analyzed sample with known amounts of the corresponding drugs at three different concentration levels i.e. 80, 100 and 120 % of the drug in the tablet. The spiked samples were then analyzed for three times. The mean recovery is within acceptable limits, indicating the method is accurate for both methods(Table 3).

Table 3 .Recovery studies

Drugs	Initial amount (ng per band)	Amount added (%)	% Recovery	%RSD [n=3]
THI	800	80	101.07	1.29
	800	100	98.72	0.43
	800	120	98.66	1.35
DEX	5000	80	98.67	0.76
	5000	100	100.51	0.36
	5000	120	101.38	0.24

Analysis of tablet formulation : Twenty tablets (Infen-MR) (each contained 4 mg THI and 25 mg DEX) were accurately weighed and finely powdered. A quantity of the powder containing weight equivalent to 8 mg THI and 50 mg DEX was transferred to a 50 mL volumetric flask and methanol (35 mL) was added followed by ultrasonication for 10 min, volume was adjusted to mark and filtered using 0.45 µm filter (Mill filter, Milford, MA) and 1 mL of filtrate was further diluted to 10 mL with methanol. Appropriate volume 8 µL, was spotted for assay of THI and 50 µL DEX. The plates were developed and scanned as described in above chromatographic conditions. In this methods good separation and well resolved spots were obtained which indicate that there are no interferences of excipients commonly present in the tablet formulation and result as shown in (table 4).

Table 4 Analysis of Tablet formulation

Drug	Label Claim (mg)	%Amount Found	%RSD (n = 6)
THI	4	100.70	0.54
DEX	25	100.92	0.26

Specificity: The mobile phase designed for the method resolved both the drugs very efficiently. The Rf value of THI and DEX were found to be 0.29 and 0.76, respectively. The peak purity of THI extracted from tablet and standard THI was tested at the peak - start (S), peak - apex (A) and at the peak - end (E) positions. (Figure 4) The peak purity of DEX was tested by correlating the spectra's of DEX at the peak - start (S), peak - apex (A) and at the peak - end (E) positions (figure. 5).

Ruggedness and Robustness : Ruggedness of the both method was performed for THI and DEX by two different analysts maintaining similar experimental and environmental conditions. Robustness of the method was performed by introducing various changes in the previous chromatographic conditions; effects on the results were examined for both method.

Sensitivity : The sensitivity of measurements of THI and DEX by the use of the proposed method was estimated in terms of the Limit of Quantitation (LOQ) and the lowest concentration detected under the chromatographic conditions as the Limit of Detection (LOD).

LOQ and LOD were calculated by the use equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where 'N' is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. The results were recorded for both the methods. Different validation parameters for the both methods for determining THI and DEX content were summarized in (table 5).

Table 5 LOD and LOQ of the method

Drugs	LOD	LOQ
THI	38.11	114.36
DEX	249.03	747.09

RESULTS AND DISCUSSION

An HPTLC method was optimized with a view to develop an accurate and reproducible method so as to resolve drugs. Optimization of method was done by altering almost all the chromatographic conditions and the effect on R_f and peak shape were monitored for the drugs selected. Toluene: ethyl acetate: methanol: glacial acetate in the ratio (6: 4: 4: 0.5 *v/v/v/v*) showed well- defined and resolved peaks when the chamber was saturated with mobile phase for 15 min at room temperature. Both the peaks were well resolved and no telling observed when plate was scanned at 295 nm. The RF for THI and DEX were found to be 0.29± 0.02 and 0.76 ± 0.02, respectively. The results of specificity studies indicated no interference from

excipients, impurities which assured that the peak response was due to a single component and it passes peak purity criteria**.**

APPLICATIONS

The present validated HPTLC method proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of THI and DEX in combined tablet dosage form.

CONCLUSIONS

The modalities adopted in experiment were successfully validated as per ICH guidelines. The proposed HPTLC method was validated by preliminary analysis of standard sample and by recovery studies for the determination of THI and DEX in bulk and in tablet dosage form and the percentage of average recoveries for THI and DEX was obtained 99.74 and 100.18 respectively. Summary of all validation parameter as shown in (table 6). The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of THI and DEX in combined tablet dosage form.

Table 6 Validation parameters

Parameters	THI	DEX
Linearity	400 – 2400	2500 – 15000
Correlation coefficient (r^2)	0.999	0.999
Slope	5.167	0.775
Intercept	1013	9476
Ruggedness [% RSD]		
Analyst I [n=6]	0.39	0.38
Analyst II [n=6]	0.62	0.46
Robustness [% RSD] [n=6]		
Mobile phase composition	0.73	0.36

Duration of saturation time	0.86	0.56
Mobile phase volume	1.04	0.42
Development distance	1.01	0.38
Sensitivity		
Limit of Detection (ng)	38.11	249.03
Limit of Quantitation (ng)	114.36	747.09
Precision [%RSD]		
Intra-day [n = 3]	1.24 – 1.36	0.36–1.17
Inter-day [n = 3]	0.89 – 1.71	0.24–1.13
Repeatability [n = 6]	0.74	0.36

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