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**Research Article**

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# High Performance Thin Layer Chromatographic Determination of Dexketoprofen and Thiocolchicoside in Combined Tablet Dosage Form

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**ABSTRACT**

*A simple, accurate High Performance Thin Layer Chromatographic (HPTLC) method for determination of Dexketoprofen and Thiocolchicoside in combined tablet dosage form has been developed and validated. The mobile phase selected was Toluene: Methanol: Ethyl Acetate (6: 2.5: 0.5, v/v/v) with UV detection at 280 nm. The retention factor for Thiocolchicoside and Dexketoprofen were found to be 0.33 ± 0.011 and 0.61 ± 0.007, respecti vely. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonisation (ICH) guidelines. Results found to be linear in the concentration range of 100-800 ng/band for Thiocolchicoside and 600-4800 ng/band for Dexketoprofen. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 100 .07 ± 0.872 for Thiocolchicoside and 99.97 ± 0.731 for Dexketop rofen. The method can be used for routine analysis of these drugs in combined tablet dosage forms in quality-control laboratories.*

**Keywords:** Dexketoprofen, Thiocolchicoside, HPTLC, Tablet dosage form.

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## INTRODUCTION

Dexketoprofen (DEXKETO), chemically, (2S)-2-[3-(benzoyl) phenyl] propanoic acid is non-steroidal anti-inflammatory drug and is used for the management of mild to moderate pain [1]. Thiocolchicoside (THIO), N-[(7S)-3-(beta-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9-oxo-5, 6, 7, 9-tetrahydrobenzo[a]heptalen-7-yl]acetamide and is used as muscle relaxant with anti-inflammatory and analgesic effects [2].

Literature survey reveals high-performance liquid chromatographic (HPLC) [3-5] and High Performance thin layer chromatographic (HPTLC) [6] methods for the determination of DEXKETO either as a single or in combination with other drugs. Analytical methods reported for THIO includes HPLC [7-10], HPTLC [11, 12] and spectrophotometry [13-15] either as single or in combination with other drugs.

To our best knowledge no reports were found for the simultaneous estimation of DEXKETO and THIO in combined dosage form by HPTLC method. This paper describes a simple, accurate, and validated high-performance thin layer chromatographic method for the simultaneous quantification of these compounds as a bulk drug and in tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

## EXPERIMENTAL SECTION

### Chemicals and Reagents

Working standards of pharmaceutical grade DEXKETO and THIO were obtained as generous gifts from Emcure Pharmaceuticals Ltd, Pune, India. The pharmaceutical dosage form used in this study was Infen MR Tablets (Emcure Pharmaceuticals Ltd, Pune, India) labelled to contain 25 mg of DEXKETO and 4 mg of THIO were procured from the local market. Toluene (AR grade) was purchased from Thomas Baker Pvt. Ltd. (Mumbai, India). Ethyl Acetate was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India) and Methanol (AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

### Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width of 6 mm with space between bands of 8.8 mm, with a 100 µl sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 $F_{254}$ (10 ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Methanol: Ethyl Acetate (6: 2.5: 0.5, v/v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 280 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 solution THIO was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1 mg/ml from which 1 ml was further diluted to 10 ml to get solution having concentration 100 ng/µl. Standard stock solution of DEXKETO was prepared by dissolving 10 mg of drug in 10 ml of methanol to get final concentration of 1000 ng/µl.

### Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 280 nm. So, 280 nm was selected as the wavelength for detection as shown in Figure 1.

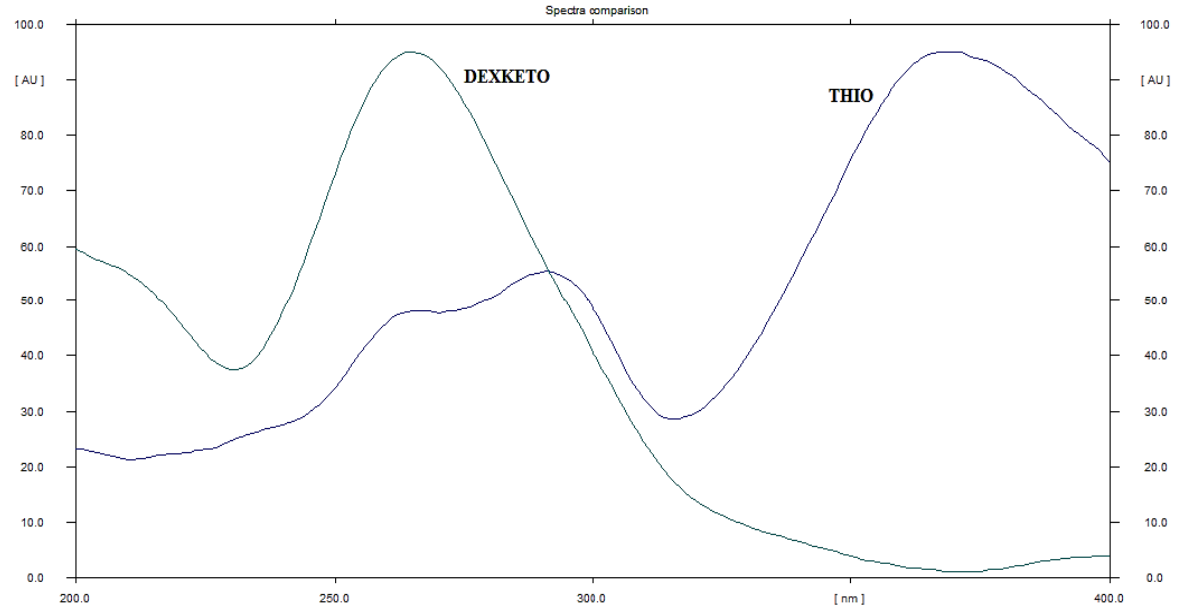


**Figure 1: Overlain spectra of THIO and DEXKETO**

### Analysis of Tablet formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 2 mg of THIO (12.5 mg DEXKETO) was weighed and dissolved in 10 ml of methanol in volumetric flask to get concentration of 1

mg/ml from which 1 ml was further diluted to 10 ml to get solution having concentration 200 ng/µl of THIO and 1250 ng/µl of DEXKETO. The solution was filtered using Whatman paper No. 41. One microlitre volume of this solution was applied on different TLC plate to obtain final concentration of 200 ng/band for THIO and 1250 ng/band for DEXKETO. After chromatographic development peak areas of the bands were measured at 280 nm and the amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

**Method Validation**

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines [16].

**Preparation of Calibration Curve**

The standard stock solutions of THIO (100 ng/µl) and DEXKETO (1000 ng/µl) were applied by overspotting on TLC plate in range of 1, 2, 3, 4, 5, 6, 7, 8 µl and 0.6, 1.2, 1.8, 2.4, 3, 3.6, 4.2, 4.8 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 six replicates was analyzed and peak areas were recorded. Calibration curves of THIO and DEXKETO were plotted separately of peak area versus respective concentration of THIO and DEXKETO.

**Precision**

Set of three different concentrations in three replicates of mixed standard solutions of THIO and DEXKETO were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. For Inter day variation study, three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days.

**Limit of detection (LOD) and Limit of quantitation (LOQ)**

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

**Robustness Studies**

In the robustness study, the influence of small, deliberate variations of the analytical parameters on peak area of the drugs was examined. Factors varied were mobile phase composition (± 2 %), mobile phase saturation (± 10 %), time from application to development (0, 10, 20 and 30 min) and from development to scanning (0, 30, 60 and 90 min) and development distance (± 10 %). One factor at a time was changed to estimate the effect. Robustness of the method was checked at a concentration level of 300 ng/ band for THIO and 1800 ng/band for DEXKETO.

**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 %. Chromatogram was developed and the peak areas were noted. At each level of the amount, three determinations were carried out. The results of recovery studies were expressed as percent recovery and are shown in Table 1.

**Table 1: Recovery Studies of THIO and DEXKETO**
*[a]Average of three determinations*

| Drug | Amount taken (n (ng/band) | Amount added (ng/band) | Total amount found (ng/band) | % Recovery | % RSD[a] |
|---|---|---|---|---|---|
| THIO | 200 | 100 | 302.64 | 100.88 | 0.311 |
| | 200 | 200 | 402.70 | 100.67 | 0.426 |
| | 200 | 300 | 502.89 | 100.57 | 0.339 |
| DEXKETO | 1250 | 600 | 1861.52 | 100.62 | 0.955 |
| | 1250 | 1200 | 2454.80 | 100.19 | 0.864 |
| | 1250 | 1800 | 3068.06 | 100.59 | 0.975 |

## RESULTS AND DISCUSSION

Different mobile phases containing various ratios of Ethyl acetate, Methanol, Chloroform and Toluene were examined (data not shown). Finally the mobile phase containing Toluene: Methanol: Ethyl Acetate (6: 2.5: 0.5, v/v/v) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 280 nm. The retention factors for THIO and DEXKETO were found to be 0.33 ± 0.011

and 0.61 ± 0.007 respectively. Representative densitogram of mixed standard solution of THIO and DEXETO is shown in Figure 2.

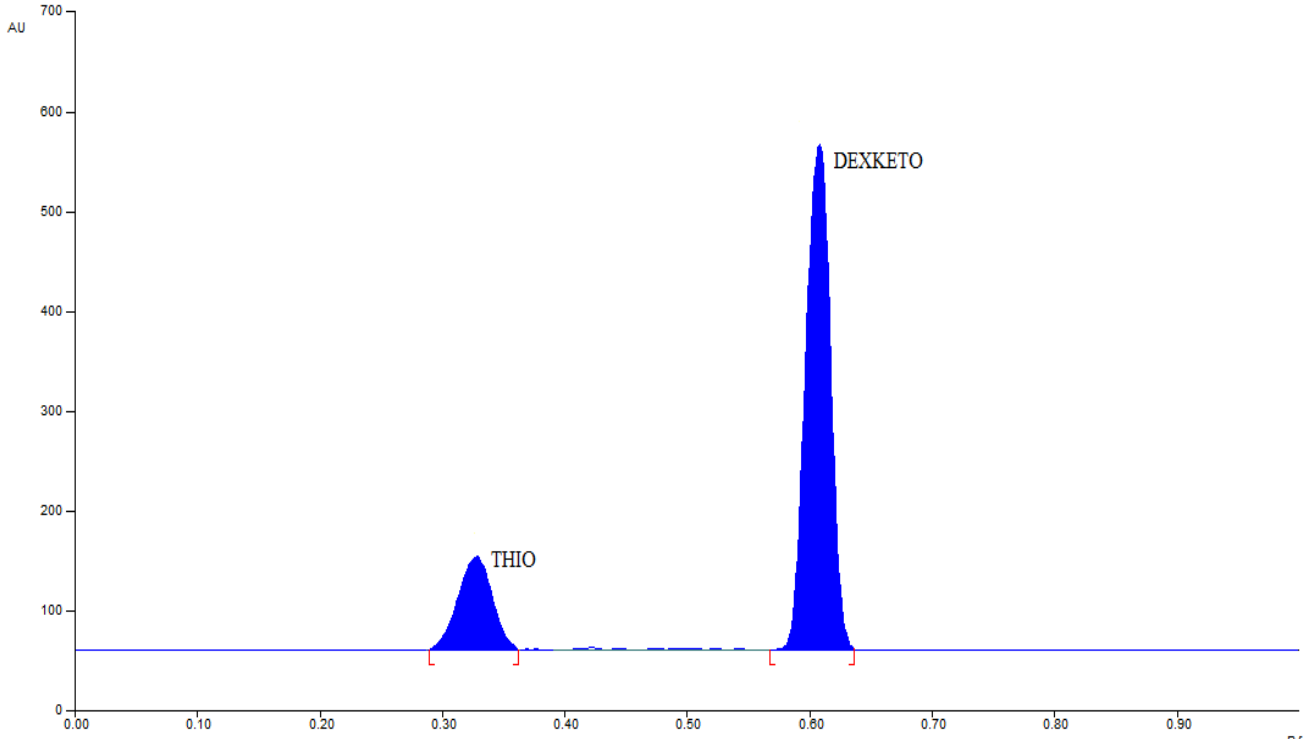


**Figure 2: Representative chromatogram of mixed standard solution of THIO (200ng/band,**
*$R_f$ = 0.33±0.011) and DEXKETO (1200ng/band, $R_f$= 0.61±0.007)*

Straight-line calibration graphs were obtained for THIO and DEXKETO in the concentration range 100-1800 ng/band and 600-4800 ng/band respectively with high correlation coefficient. The proposed method was also evaluated by the assay of commercially available tablets containing THIO and DEXKETO. The % assay (Mean ± S.D.) was found to be 100.07 ± 0.87for THIO and 99.97 ± 0.73 for DEXKETO. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2).

For THIO, the recovery study results ranged from 100.57 to 100.88 % with % RSD values ranging from 0.311 to 0.426. For DEXKETO, the recovery results ranged from 100.19 to 100.62 % with % RSD values ranging from 0.864 to 0.975. The method was found to be accurate and precise, as indicated by recovery studies as recoveries were close to 100 % and % RSD not more than 2. Intra-day variation, as RSD (%), was found to be in the range of 0.62–0.87 for THIO and 0.71–0.92 for DEXKETO. Interday variation, as RSD (%) was found to be in the range of 0.67–0.95 for THIO and 0.16–0.77 for DEXKETO. The summary of validation parameters of proposed method are given in Table 3.

**Table 2: Summary of validation parameters of proposed method**

| **Parameters** | **THIO** | **DEXKETO** |
|---|---|---|
| Linearity range ( ng/band) | 100 - 800 | 600 -4800 |
| Correlation coefficient (r) | 0.998 | 0.998 |
| LOD[a] (ng/band) | 20.47 | 67.79 |
| LOQ[b] (ng/band) | 62.05 | 205.44 |
| Accuracy (% Recovery) | 100.57-100.88 | 100.19-100.62 |
| Precision (% RSD)[c] | | |
| Intra day (n[d] = 3) | 0.62–0.87 | 0.71–0.92 |
| Inter day (n = 3) | 0.67–0.95 | 0.16–0.77 |

[a]*LOD* = *Limit of detection.*
[b]*LOQ* = *Limit of quantitation.*
[c]*RSD* = *Relative standard deviation.*
[d]*n* = *Number of determinations*

## CONCLUSION

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

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