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A simple and sensitive RP-HPLC method for simultaneous estimation of dexketoprofen and thiocolchicoside in combined tablet dosage form

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ABSTRACT

This paper describes a simple, sensitive, accurate, and validated reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous quantification of these compounds as the bulk drug and in tablet dosage forms. Separation was carried out on Jasco HPLC system equipped with HiQ sil C_{18} HS column (250 × 4.6 mm i.d.) and PDA detector using Methanol: Sodium acetate buffer (70:30, v/v) with pH adjusted to 5 with Glacial acetic acid as the mobile phase and detection was carried out at 265 nm. Results were linear in the range of 5-30 µg mL^{-1} for Dexketoprofen and 1 –10 µg mL^{-1} for Thiocolchicoside. The method was successfully applied for the analysis of drugs in pharmaceutical formulation. Results of the analysis were validated statistically and by recovery studies.

Keywords: Dexketoprofen, Thiocolchicoside, RP-HPLC, 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 RP-HPLC method. This paper describes a simple, sensitive, accurate, and validated reverse-phase high-performance liquid 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].

MATERIALS AND METHODS

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. Methanol (HPLC grade) purchased from Merck specialties Pvt. Ltd, Sodium acetate (AR grade) purchased from S. D. Fine. Chem Ltd. (Mumbai, India) and double distilled water were used in analysis.

Instrumentation and Chromatographic Conditions

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pump and MD 2010 PDA detector and JASCO Borwin- PDA software (version 1.5) was used for analysis. Separation was carried out on HiQ sil $C_{18\ HS}$ (250 x 4.6 mm i.d.) column using Methanol: Sodium acetate buffer (70:30, v/v) as mobile phase at flow rate of 1 mL min^{-1}. Samples were injected using Rheodyne injector with 50 µL loop and detection was carried out at 265 nm.

Preparation of Standard Stock Solutions

Standard stock solution of DEXKETO was prepared separately by dissolving 10 mg of drug in 10 mL Methanol to get concentration of 1000 µg mL^{-1} from which 1 mL of solution was further diluted with methanol to get a working standard solution having concentration 100 µg mL^{-1}. Standard stock solution of THIO was prepared by dissolving 10 mg of drug in 10 mL methanol to get concentration of 1000 µg mL^{-1} from which 1 mL of solution was further diluted with methanol to get a working standard solution having concentration 100 µg mL^{-1}.

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 12.5 mg of DEXKETO (2 mg of THIO) was weighed and transferred to 10 mL volumetric flask containing about 6 mL of mobile Phase and ultrasonicated for 10 min and volume was made upto the mark with the mobile phase. The solution was filtered through Whatman paper No. 41. One mililitre of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the mobile phase to get solution of concentration 125 µg $mL^{-1}$for DEXKETO and 20 µg $mL^{-1}$for THIO. Further one mililitre of above solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the mobile phase to get solution of concentration 12.5 µg mL^{-1} for DEXKETO and 2 µg mL^{-1} for THIO. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

System Suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg mL^{-1} of both the drugs. The resolution, peak asymmetry, number of theoretical plates, and HETP were calculated as represented in Table 1.

Table 1: System suitability parameters for RP-HPLC method

Sr. No.	Parameters	DEXKETO	THIO
1	Theoretical Plates	4884.36	3934.26
2	HETP (cm)	0.00511	0.00635
3	Resolution [a]	--	11.27
4	Asymmetry Factor	1.22	1.19

[a] *With respect to previous peak.*

The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. Mean retention time and standard deviation was found to be 3.0133 ± 0.019 for THIO and 6.013 ± 0.019 min for DEXKETO respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

Method Validation

The method was validated for linearity, accuracy and intra-day and inter-day precision, repeatability of measurement of peak area, and repeatability of sample application, in accordance with ICH guidelines [16].

Linearity

Aliquots 0.5, 1, 1.5, 2, 2.5 and 3 mL of working standard solution of DEXKETO (100 µg/ml) and 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL of THIO (100 µg/ml) were transferred in a series of 10 mL volumetric flasks and the volume was made up to the mark with mobile phase. Six replicates per concentration were injected and chromatograms were recorded. The peak areas were recorded and calibration curve was plotted of peak area against concentration of drug.

Precision

One set of three different concentrations of mixed standard solutions of DEXKETO and THIO were prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. For Inter day variations

study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses.

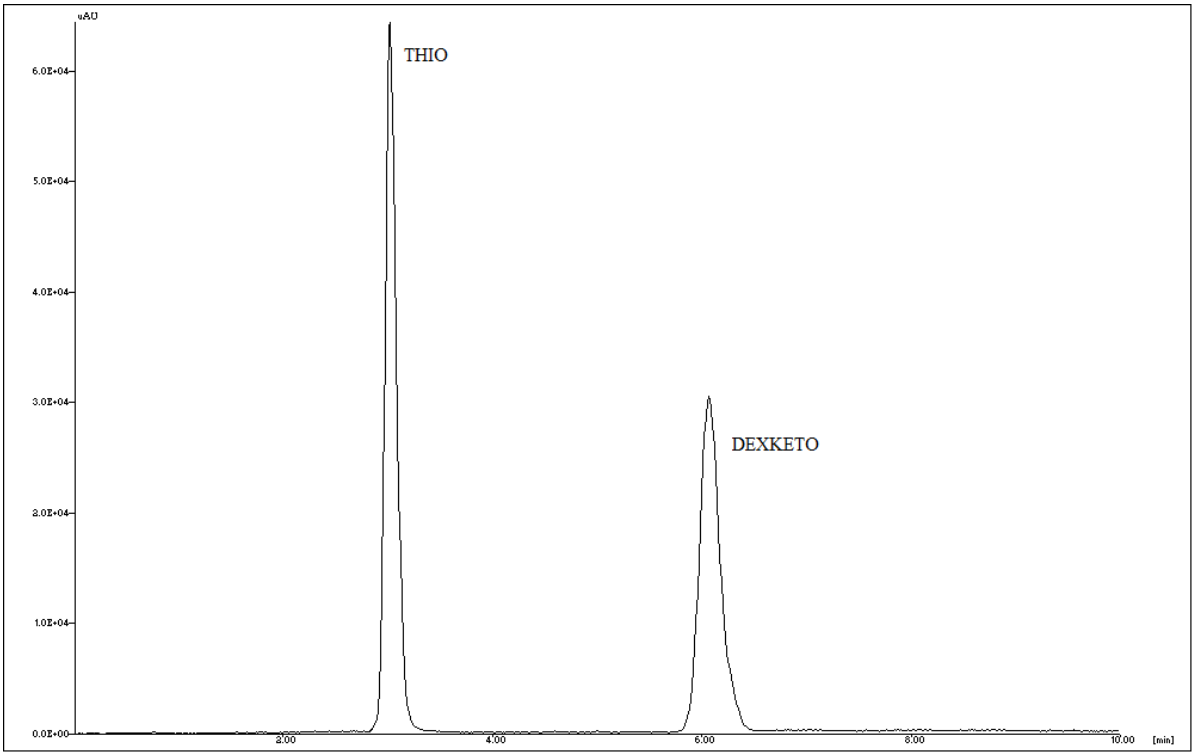

Figure 1: Representative chromatogram obtained for standard mixture of THIO
(10 µg/ ml, 3.0133 min), DEXKETO (10 µg/ml, 6.013 min)

Accuracy

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 %. The percentages of recoveries were calculated, the results of which are represented in Table 2.

Table 2: Recovery studies of DEXKETO and THIO

Drug	Amount taken (µg mL^{-1})	Amount added (µg mL^{-1})	Total amount found (µg mL^{-1})	% Recovery[a]	% RSD[a]
DEXKETO	12.5	5	17.53	100.20	0.64
	12.5	10	22.65	100.68	0.07
	12.5	15	27.67	100.33	0.83
THIO	2	1	03.04	101.0	0.45
	2	2	04.02	100.21	0.72
	2	3	05.01	100.36	0.73

[a] *Average of three determinations; RSD is the relative standard deviation.*

Limit of detection and Limit of quantitation

Limit of detection and Limit of quantitation 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

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase (1 ± 0.05 mL min^{-1}), a wavelength at which the drugs were recorded (265 ± 1 nm). One factor at the time was changed to estimate the effect. The solutions containing 15 µg mL^{-1} of DEXKETO and 4 µg mL^{-1} of THIO were applied onto the column. A number of replicate analyses (n = 3) were conducted at 3 levels of the factor (-, 0, +). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

Results were found to be linear in the concentration range of 5-30 µg mL^{-1} for DEXKETO and 1-10 µg mL^{-1} for THIO with high correlation coefficient. The proposed method was also evaluated by the assay of commercially available tablets containing DEXKETO and THIO. The % assay was found to be 100.23 ± 0.84 for DEXKETO and 100.54 ± 0.95 for THIO (mean ± S.D., n = 6). The % recovery was found to be 100.20 to 100.68 for DEXKETO and 100.36 to100.57 for THIO. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatograms (RSD <2), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of proposed HPLC method is given in Table 3.

Table 3: Summary of validation parameters of proposed RP-HPLC method

Parameters	DEXKETO	THIO
Linearity range (µg mL^{-1})	5 – 30	1-10
Correlation co-efficient	0.998	0.995
Slope (m)	41205	46417
Intercept (c)	37180	26554
LOD[a] (µg mL^{-1})	1.01	0.127
LOQ[b] (µg mL^{-1})	3.06	0.38
Accuracy (% Recovery)	100.20-100.68	100.21-101.00
Precision (% R.S.D.)[c]		
Intra day (n[d] = 3)	0.71-1.34	1.01-1.22
Inter day (n = 3)	0.58-1.38	0.48-1.17

[a]*LOD = Limit of detection.* [c]*R.S.D. = Relative standard deviation.*
[b]*LOQ =Limit of quantitation.* [d]*n = Number of determination.*

CONCLUSION

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

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