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A Validated Reversed-Phase High Performance Liquid Chromatographic Method forSimultaneous Estimation ofThiocolchicoside and Dexketoprofen in Bulk and in Tablet Dosage Form

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ABSTRACT

The present work describes a rapid, precise and specific validated Reverse phase high performance liquid chromatographic method for simultaneous estimation of Thiocolchicoside and Dexketoprofen in bulk and in pharmaceutical dosage form. Chromatographic separations was achieved on Waters Younglin system C-18 (5µm, 250×4.6 mm) HPLC column within a short runtime of 10 min. HPLC system having isocratic mode, with mobile phase containing Acetonitrile: water (pH 3) (70:30% v/v) and flow rate maintained at 1.0 mL min^{-1} was used. Effluents were monitored at 260 nm. Retention time of Thiocolchicoside and Dexketoprofen was found to be 4.8 and 2.7 min respectively. Linearity was studied in the concentration range of 2 - 12 µg mL^{-1} and 12 - 72 µg/mL for Thiocolchicoside and Dexketoprofen respectively, with a correlation coefficient of 0.999 for both the drugs. The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. 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: Dexketoprofen, Liquid chromatography, Specificity, Thiocolchicoside.

INTRODUCTION

Chemically, Thiocolchicoside (THI) is N-[3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio)-9-oxobenzo[a]heptalen-7yl] acetamide. It has selective affinity for γ-amino-butyric acid (GABA) receptors and acts on the muscular contracture by activating theGABA- inhibitory pathways thereby acting as a potent muscle relaxant [1]. Literature surveyreveals that THI can be estimated by spectrophotometry [2-4], HPLC [5, 9] and by HPTLC [10] methods individually or in combination with other drugs.

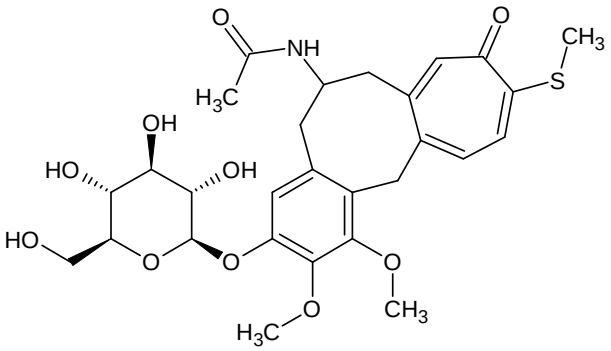

Figure 1 a:Chemical structures of THI

Chemically, Dexketoprofen (DEX) 2-amino-2-(hydroxymethyl) propane-1,3-diol; 2-(3-benzoylphenyl propanoicacid is a water-soluble salt of the dextrorotatory enantiomer or (*S*)-(+)-enantiomer of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen. The enantiomer is a relatively neworal NSAID with analgesic, anti-inflammatory and anti-pyretic properties and is one of themost potent in vitro inhibitors of prostaglandin synthesis [11]. Literature survey reveals that DEX can be estimated by spectrophotometry [12], HPLC [13] and by HPTLC [14] methods individually or in combination with other drugs.

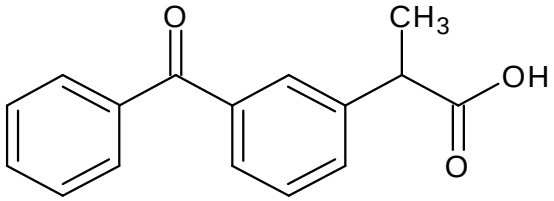

Figure 1b: Chemical structure of DEX

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. Today HPLC 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 HPLC 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.

This paper presents HPLC 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

HPLC grade Acetonitrile and analytical grade ortho-phosphoric acid were procured from Merck® India Ltd. (Mumbai). Pure standards of Thiocolchicoside (99.36 %) and Dexketoprofen (99.89 %) were obtained as gift samples from Emcure Pharmaceutical Ltd. Pune (India). Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a membrane filter (Millipore Millex® FH, filter units, Durapore-PVDF, Polyethylene, 0.45 µm pore size) and degassed before use.

Instrumentation: Analysis was performed on WatersYounglin HPLC separation module within built UV-detector. Chromatographic software Empower 2 was used for data collection and processing. The analytical column was Phenomenex Gemini C 18 (5 µm, 250 mm X 4.6 mm).

Chromatographic Conditions: The samples were introduced through a Rheodyne injection valve with 20 µL sample loop. Simultaneous separation and quantification of THI and DEX were performed by use of an isocratic mobile phase prepared from 70:30 (*v/v*) acetonitrile: water, pH 3 (adjusted with ortho phosphoric acid) giving well resolved, sharp peak for THI and DEX with a retention time (RT) 4.8, 2.7 respectivelyfigure 2. The flow rate was maintained at 1.0 mL min^{-1}, UV detection was performed at 260 nm and ambient temperature (25^0 C) for column oven was found to be the best for analysis.

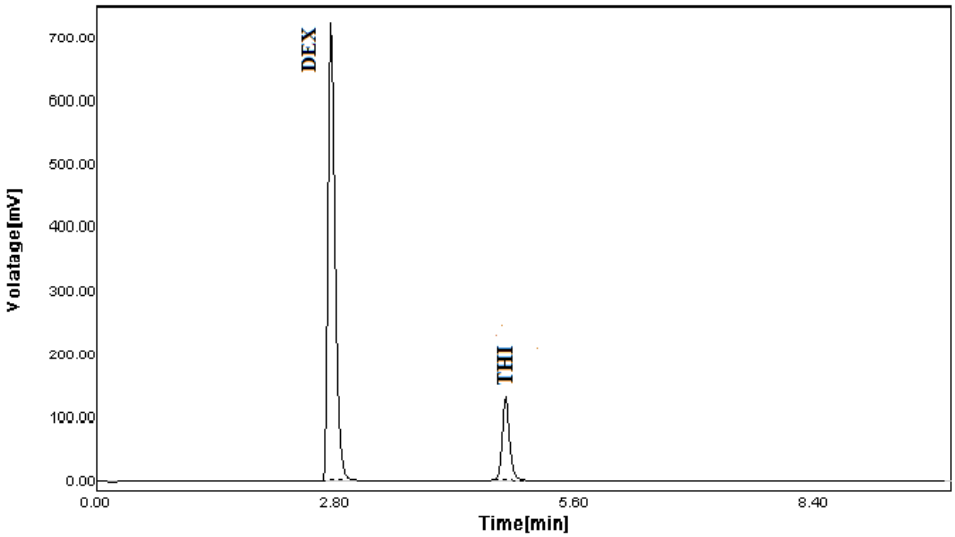

Figure 2: Typical chromatogram of THI and DEX showing R_t 4.8 and 2.7 respectively

Standard Stock Solutions: Standard solutions were prepared by dissolving the drugs in the diluent and diluting them to the desired concentration. Diluent used for the standard and sample preparation was composed of acetonitrile and water pH 3 in the ratio of 70:30 (v/v).
THI Standard Stock Solutions: A 10 mg sample of THI was accurately weight and transfer in to the 100 mL volumetric flask and diluted to volume with diluent.
DEX Standard Stock Solutions: A 10 mg sample of DEX was accurately weight and transfer in to the 100 mL volumetric flask and diluted to volume with diluent.

Method of Validation

Linearity: For constructing calibration plots, a series of five dilutions in the concentration range 2-12 µg mL^{-1} for THI and 12-72µg mL^{-1} of DEX was taken.
Precision: The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample.
The first solution was prepared by dissolving 10 mg of THI in 100 ml of diluent further diluted to get concentration of 4, 6, 8 µg mL^{-1}. Similarly the DEX stock solution were prepared and diluted to get concentration of 24, 36, 48 µg mL^{-1} solution.
Recovery: Accuracy indicates the deviation between the mean value found and the true value. lt is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at

three levels, 80, 100 and 120% of THI and DEX standard concentration. Three samples were prepared for each recovery level.

Repeatability: Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day.

Tablet assay: Twenty tablets, Infen - MR, (Emcure pharmaceutical Ltd.) 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 of THI and 50 mg of DEX was transferred to volumetric flask. The drug was diluted using diluent to get a concentration of 4 µg /mL for THI and 25 µg/mL for DEX and was used for further analysis. The contents were mixed thoroughly and filtered through a 0.45 µ filter, further used for HPLC analysis.

Limit of detection (LOD) and Limit of quantification (LOQ): This is the lowest concentration in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. The limit of detection is important for impurity tests and the assays of dosages containing low drug levels and placebos.

LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

Robustness: To verify the robustness of proposed method, the analysis was done under variable flow rates and mobile phase composition. The flow rate has been purposely changed by ± 0.1 mL min^{-1}, while small deliberate change of ± 5.0% has been made in mobile phase composition.

RESULTS AND DISCUSSION

Literature survey revealed number of reported methods for individual determination of THI and DEX but no single method was reported for THI and DEX in combine dosage form. To develop accurate, precise and specific RP-HPLC method for simultaneous estimation of THI and DEX using various mobile phases with different composition and flow rate were tried. After several trials above method has been optimized. Satisfactory separation of THI and DEX with good peak symmetry and steady baseline was obtained with the mobile phase acetonitrile: water (70:30 *v/v*), pH 3.0 at a flow rate of 1.0 mL min^{-1}. Complete resolution of the peak with clear base line giving well resolved peaks for THI and DEX with a retention time (RT) of 4.8 and 2.7 min respectively at 260 nm as shown in figure 2.

Linearity: The standard curves for THI and DEX were linear over the investigated concentration range 2-12 µg/mL and 12-72 µg mL^{-1} respectively with a percent relative standard deviation (RSD) of not more than 2.0 based on five successive readings and a correlation coefficient of 0.999 and 0.999 and result are tabulated in table 1.

Table 1. Linearity of THI and DEX for proposed method*

Parameters	THI	DEX
Linear range (µg/mL)	2 - 12	12 – 72
SD	5.08	13.49
%RSD	1.35	1.03
Slope	53.08	35.58
Intercept	8.066	102.3
Correlation Coefficient (r^2)	0.999	0.999

*mean of five determinations

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. Precision studies were determined by intra-day and intermediate precision and %RSD was found less than 2 indicating the proposed method is more precise and result are tabulated in table 2**.**

Table 2. Precision of the RP–HPLC method

Drugs	Conc. [µg/mL]	Intraday Amount found [µg]		Interday Amount found [µg]	
		Mean ± S.D.	% R.S.D*	Mean ± S.D.	% R.S.D*
THI	4	3.92 ± 3.0	1.41	4.01 ± 3.4	1.56
	6	5.98 ± 3.2	0.98	6.02 ± 5.0	1.53
	8	8.05 ± 7.3	1.69	8.10 ± 2.6	0.60
DEX	24	23.98 ± 14.46	1.51	24.04 ± 11.37	1.18
	36	36.18 ± 25.00	1.79	36.38 ± 14.00	1.00
	48	48.33 ± 17.43	0.95	48.61 ± 17.43	0.95

*mean of three determinations

Recovery: The mean recovery data of THI in real sample were within the range of 99.69 and 101.61 % and for DEX in real sample were within the range of 99.28 and 100.90 %. Mean % R.S.D. was 1.49 and 0.49 for THI and DEX respectively that satisfying the acceptance criteria for the study. It proved that there is no interference due to excipients used in tablet formulation and result are tabulated in table 3.

Tablet assay: From that six solutions were prepared affording final concentrations of 4 µg/mL for THI and 25 µg/mL for DEX and % amount found in tablet assay for THI is 100.32 %and for DEX is 99.77 % and result as shown in table 4**.**

Table 3. Recovery studies

Drugs	Initial amount [µg/mL]	Amount added [µg mL^{-1}]	Amount recovered* ± S.D. [µg mL^{-1}]	% Recovered	% R.S.D.
THI	4	3.2	7.17 ± 5.56	99.69	1.43
	4	4	8.08 ± 7.00	101.02	1.60
	4	4.8	8.94 ± 7.02	101.61	1.45
DEX	24	16	39.71 ± 8.02	99.28	0.52
	24	20	44.39 ± 8.00	100.90	0.47
	24	24	48.33 ± 9.16	100.69	0.50

*mean of three determinations

Table 4. Tablet Assay

Drugs	Label claim [mg/Tab]	% Label claim*	% R.S.D.
THI	4	100.32	1.56
DEX	24	99.77	1.19

*mean of six determinations

Limit of detection (LOD) and Limit of quantification (LOQ): The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × N/B and LOQ = 10 × N/B where 'N' is the standard deviation of the peak areas of the drug (n=3), taken as the measure of the noise, and 'B' is the slope of the corresponding calibration plot. The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 and LOQ regarded as the amount for which the signal to noise ratio was 10:1 Results are shown in table 5.

Table 5.LOD and LOQ of the method

Drugs	LOD	LOQ
THI	0.21	0.62
DEX	1.15	3.45

Robustness: In robustness studies small and deliberate change in flow arte and mobile phase composition and result as shown in table 6and final chromatographic condition as shown intable 7**.**

Table 6 Robustness of the method

Parameters	Retention time (R_t)		Tailing factor (T_f)	
	THI	**DEX**	**THI**	**DEX**
A: Flow rate (ml/min)				
0.90	4.9	3.1	0.90	1.21
1.00	4.8	2.7	1.30	1.10
1.10	4.4	2.5	1.20	1.24
Mean	**4.7 ± 0.26**	**2.76 ± 0.30**	**1.13 ± 0.20**	**1.18 ± 0.07**
B: Percentage methanol in mobile phase (v/v) (± 5%)				
75	4.4	2.4	1.56	1.24
70	4.8	2.7	1.31	1.33
65	5.3	3.1	1.42	1.57
Mean	**4.83 ± 0.45**	**2.73 ± 0.35**	**1.43 ± 0.12**	**1.38 ± 0.17**

Table 7. Final Chromatographic Conditions

Chromatographic Mode	Chromatographic Condition
Standard solution (µg/mL)	100 of THI and DEX in methanol
HPLC System	Younglin 1100 Series HPLC system
Pump	Gradient pump

Detector	UV Detector
Degasser	G1322A
Data processor	Ezechrome Elite Chromatographic data system
Weighing Balance	Shimandzu AUX 120
Digital pH Meter	Systronicsμ pH System 362
Ultrasonicator	ENERTECH Electronics Pvt. Ltd.
Filters	Millipore (0.45 μm)
Stationary phase	Phenomenex Gemini C18 (5 μm, 250 mm X 4.6 mm i.d.)
Mobile phase	acetonitrile: water (pH 3) (70:30% *v/v*)
Detection wavelength	260 nm
Flow rate	1 mL/min
Sample size	20 μL

APPLICATIONS

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

CONCLUSIONS

The isocratic RP-HPLC method developed for analysis of binary mixture of THI and DEX in their pharmaceutical preparations is simple, rapid, accurate, precise, and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested.

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