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Research Article

A SIMPLE AND SENSITIVE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ETODOLAC AND THIOCOLCHICOSIDE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive, accurate reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of Etodolac and Thiocolchicoside has been developed and validated. Separation was carried out on Jasco HPLC system equipped with HiQ sil C_{18} HS column (250 × 4.6 mm i.d.) and UV/VIS detector using Acetonitrile: 20 mM potassium dihydrogen phosphate buffer (65:35, v/v) as the mobile phase, and detection was carried out at 257 nm. Results were linear in the range of 25–150 µg mL^{-1} for Etodolac and 0.5 –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: Etodolac, Thiocolchicoside, RP-HPLC, Tablet dosage form.

INTRODUCTION

Etodoalc (ETO), chemically, (RS)-2-(1, 8-Diethyl-4, 9-dihydro-3H-pyrano [3, 4-b] indol-1-yl) acetic acid used for the management of mild to moderate pain, fever, and inflammation.[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 is used as muscle relaxant with anti-inflammatory and analgesic effects[2].

Literature survey reveals high-performance liquid chromatographic (HPLC) [3-4], Liquid chromatography - Mass Spectroscopy (LC-MS) [5] and spectrophotometric[6-7] methods for the determination of ETO in human plasma and in pharmaceutical formulations as a single and in combination with other drugs.Analytical methods have been reported for the determination of THIO includes HPLC[8-11], densitometric[12-13] and spectrophotometric[14-15] as single component or in combination with other drugs.

To the best of our knowledge no HPLC method of analysis has been reported for the simultaneous estimation of ETO and THIO in combined tablet dosage form. 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 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 ETO and THIO were obtained as generous gifts from Emcure Pharmaceuticals Ltd, Pune, India. The pharmaceutical dosage form used in this study was Proxym MR Tablets (Emcure Pharmaceuticals Ltd, Pune, India) labelled to contain 200 mg of Etodolac and 4 mg of Thiocolchicoside were procured from the local market. Acetonitrile (HPLC grade), Potassium dihydrogen phosphate (AR grade) purchased from Merck specialties Pvt. 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 UV-2075 plus UV/VIS detector and JASCO Borwin 1.50.8.0 version software was used for analysis. Separation was carried out on HiQ sil C_{18} HS (250 x 4.6 mm i.d.) column using Acetonitrile: 20 mM potassium dihydrogen phosphate buffer (65:35, 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 257 nm. All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of Standard Stock Solutions

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

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 50 mg of Etodolac (1 mg of Thiocolchicoside) 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 mL 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 50 µg mL^{-1} for ETO and 1 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} and 10 µg mL^{-1} of ETO and THIO respectively. 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

S. No.	Parameters	THIO	ETO
1	Theoretical Plates	2244	10951
2	HETP (cm)	0.0111	0.00228
3	Resolution [a]	--	20.82
4	Asymmetry Factor	1.31	1.04

[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 2.240 ± 0.0185 for THIO and 7.141 ± 0.0049 min for ETO respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

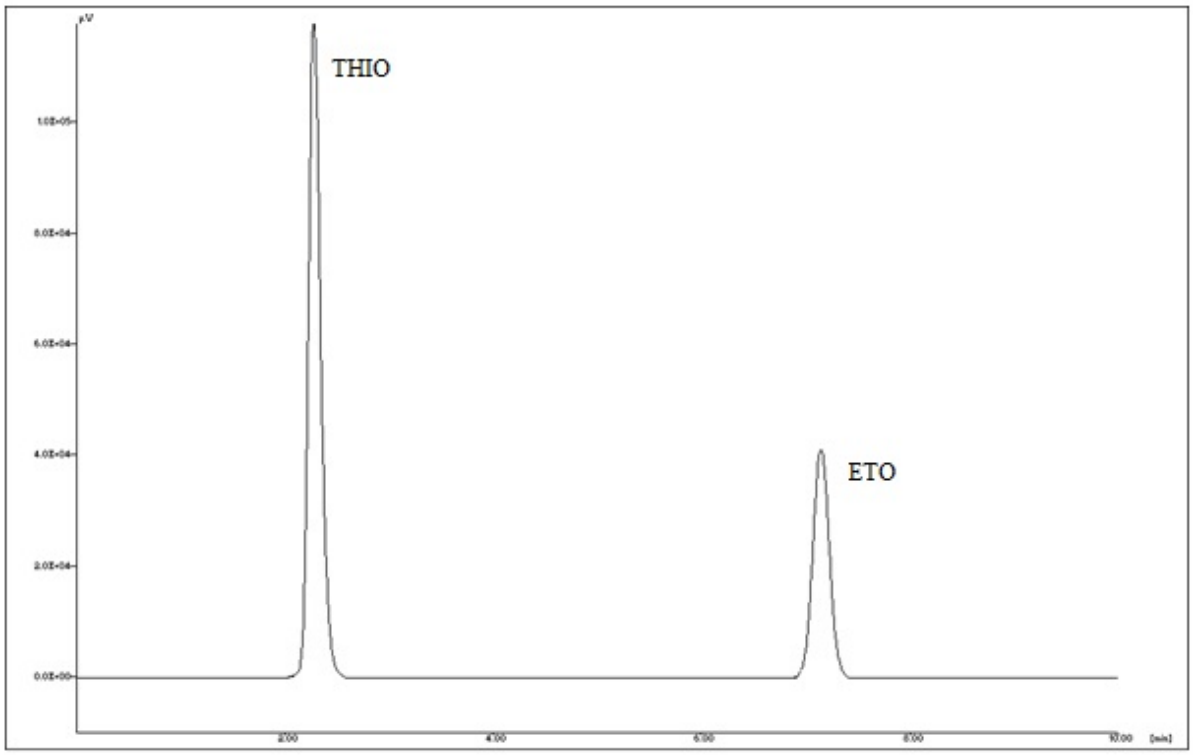

Fig. 1: Representative chromatogram obtained for standard mixture of THIO (10 µg mL^{-1}, 2.240 min), ETO (10 µg mL^{-1}, 7.141 min)

Method Validation

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

Linearity

Aliquots 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mL of working standard solution of ETO (1000 µg mL^{-1}) and 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL of THIO (10 µg mL^{-1}) 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 ETO 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.

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 80, 100 and 120 %. The percentages of recoveries were calculated, the results of which are represented in Table 2.

Table 2: Recovery studies of ETO and THIO

Drug	Amount taken (µg mL^{-1})	Amount added (µg mL^{-1})	Total amount found (µg mL^{-1})	% Recovery[a]	% RSD[a]
	50	40	90.06	100.06	0.51
ETO	50	50	100.66	100.66	0.19
	50	60	110.78	100.71	0.31
	1	0.8	01.79	99.94	0.58
THIO	1	1	01.99	99.86	0.62
	1	1.2	02.19	99.96	0.53

[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 (257 ± 1 nm). One factor at the time was changed to estimate the effect. The solutions containing 75 µg mL^{-1}of ETO 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 25-150 µg mL^{-1} for ETO and 0.5-10 µg mL^{-1} for THIO with high correlation coefficient. The proposed method was also evaluated by the assay of commercially available tablets containing ETO and THIO. The % assay was found to be 99.981 ± 0.756 for ETO and 99.695 ± 0.494 for THIO (mean ± S.D., n = 6). For ETO, the recovery study results ranged from 100.6 to 100.71 % with % RSD values ranging from 0.19 to 0.51. For THIO, the recovery results ranged from 99.86 to 99.96 % with % RSD values ranging from 0.53 to 0.62. 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	ETO	THIO
Linearity range (µg mL^{-1})	25 - 150	0.5-10
Correlation co-efficient	0.994	0.999
Slope (m)	46360	107309
Intercept (c)	6084	3112
LOD[a] (µg mL^{-1})	0.433	0.095
LOQ[b] (µg mL^{-1})	1.31	0.29
Accuracy (% Recovery)	100.06-100.71	99.86-99.96
Precision (% R.S.D.)[c]		
Intra day (n[d] = 3)	0.52-1.38	0.25-0.72
Inter day (n = 3)	0.24-1.00	0.40-0.72

[a]LOD = Limit of detection ; [b]LOQ =Limit of quantitation ; [c]R.S.D. = Relative standard deviation ; [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 ETO and THIO in combined tablet dosage form.

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REFERENCES

1. http://en.wikipedia.org/wiki/Etodolac (accessed on 21/02/2012)
2. http://en.wikipedia.org/wiki/Thiocolchicoside (accessed on 21/02/2012)
3. Cosyns L, Spain M, Kraml M. Sensitive high-performance liquid chromatographic method for the determination of etodolac in serum. J Pharm. Sci. 2006; 72: 257-277.
4. Dongre V, Shah G, Sweta B, Gunaji S, Phadke M, Jadhav V. Simultaneous Determination of etodolac and acetaminophen in tablet dosage form by RP-LC. Chromatographia. 2009; 69: 1019-1023.
5. SooLee H, Kang IM, Lee HW, HyungSeo J, HeeRyu J, Choi SJ. Development and validation of a high performance liquid chromatography–tandem mass spectrometry for the determination of etodolac in human plasma. J. Chromatogr. B 2008; 863: 158-162.
6. Kousy N.M. Spectrophotometric and spectrofluorimetric determination of etodolac and aceclofenac. J. Pharm. Biomed. Anal. 1999; 20: 185-194.
7. Patidar R, Baghel US, Patel S, Singhal M, Patidara N. Simultaneous spectrophotometric estimation of paracetamol and etodolac in tablet dosage forms. Journal of Global Pharma Technology 2009; 1: 225-229.
8. Umarkar AR, Rewatkar NS, Chaple DR, Thote LT. Stability indicating RP-HPLC method for estimation of thiocolchicoside in capsule dosage forms. RJPBCS 2011; 2: 750-756.
9. Chitlange SS, Shinde PS, Pawbake GR, Wankhede SB. Simultaneous estimation of thiocolchicoside and aceclofenac in pharmaceutical dosage form by spectrophotometric and LC method. Der Pharmacia Lettrers. 2010; 2: 86-93.
10. Kuchekar BS et al. Development and validation of a RP-HPLC-PDA method for simultaneous determination of lornoxicam and thiocolchicoside in pharmaceutical dosage form and its application for dissolution study. Int. J. Res. Pharm. Sci. 2011; 2: 1-7.
11. Dhaneshwar SR, Raut K, Bhusari V. Validated HPLC method for simultaneous estimation of paracetamol, aceclofenac and thiocolchicoside in bulk drug and formulation. RJPBCS 2011; 2: 435-445.
12. Gandhi SV, Deshpande PB, Sengar M. High performance thin layer chromatographic determination of diclofenac sodium and thiocolchicoside in fixed dose combination. Int. Res. J. Pharm. 2010; 1: 220-224.
13. Rajmane V, Gandhi SV, Patil U, Sengar M. High-performance thin-layer chromatographic determination of etoricoxib and thiocolchicoside in combined tablet dosage form. J. Aoac Int. 2010; 93: 783-786.
14. Acharjya SK, Mallick P, Panda P, Annapurna MM. Spectrophotometric methods for the determination of thiocolchicoside in bulk and pharmaceutical dosage forms. J Pharm. Edu. Res. 2010; 1: 51-57.
15. Sengar MR, Gandhi SV, Patil UP, Rajmane VS. Simultaneous determination of diclofenac sodium and thiocolchicoside in fixed dose combination by spectrophotometry. Asian Journal of Pharmaceutical and Clinical Research 2010; 3: 89-91.
16. International Conference on Harmonization (2005) ICH harmonised tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, Nov (2005).