



RESEARCH ARTICLE

APPLICATION OF HPTLC IN THE SIMULTANEOUS ESTIMATION OF THIOCOLCHICOSIDE AND DICLOFENAC IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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A simple, precise and accurate HPTLC method was developed for the simultaneous estimation of thiocolchicoside (THIO) and diclofenac potassium (DICLO) as the bulk drug and in capsule dosage form. Chromatographic separation was performed on silica gel 60 F₂₅₄ as the stationary phase and the toluene: acetone: methanol: formic acid (5:2:2:0.01 v/v/v/v) as mobile phase. Densitometric evaluation of the separated zones was performed at 280 nm. The two drugs were satisfactorily resolved with R_f values of 0.29±0.02 and 0.71±0.02 for THIO and DICLO, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (160-800 ng spot⁻¹ for THIO and 1000-5000 ng spot⁻¹ for DICLO), precision (repeatability RSD 0.658-0.788% and intermediate RSD 0.579-1.012% for THIO, and repeatability RSD 0.340-1.092% and intermediate RSD 0.429-1.007% for DICLO), accuracy (100.97±0.921% for THIO and 99.22±0.022% for DICLO) and specificity, in accordance with ICH guidelines.

Key words: Thiocolchicoside, Diclofenac potassium, HPTLC, Validation, ICH guidelines.

INTRODUCTION

Thiocolchicoside, a thiocolchine analog with chemical name *N*-[3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide is a muscle relaxant drug with anti-inflammatory, analgesic action and used topically for the treatment of musculoskeletal disorders. Thiocolchicoside (THIO) allosterically inhibits strychnine sensitive glycine receptor in brain stem and spinal cord, may provide a possible mechanism for myorelaxant activity (Cimino *et al* 1996; Balduini *et al* 1999). Diclofenac, as the potassium salt, is a benzene acetic acid derivative, designated chemically as 2-[(2,6-dichlorophenyl)amino] benzene acetic acid, monopotassium salt. The mechanism of action of diclofenac potassium (DICLO), like that of other NSAIDs, is not completely understood but may

be related to prostaglandin synthetase inhibition (BP, 2003).

In literature, analytical methods are described for determination of THIO and other drugs in pharmaceuticals, including the UV spectrophotometry (Patil *et al* 2011; Shukla *et al* 2011; Shah *et al* 2011), HPLC (Rosso and Zuccaro, 1998; Vargas *et al* 2001; Prasanthi *et al* 2011), LC-MS methods for their quantitative estimation in human plasma [Ferrari *et al* 2001; Sutherland *et al* 2002] and HPTLC method for quantification (El-Ragehy *et al* 2003).

To date, there are no published reports about the simultaneous quantification of THIO and DICLO by TLC in bulk drug and in pharmaceutical dosage forms. The present study reports for the first time simultaneous quantification of THIO and DICLO by TLC in bulk drug and in pharmaceutical dosage forms. The

proposed method was validated as per ICH guidelines (ICH, 2005).

EXPERIMENTAL

Materials

Working standards of pharmaceutical grade THIO (Batch No. 077101805008) and DICLO (Batch No. 160-2008) were obtained as generous gifts from Alchem International Ltd. (Haryana, India) and Brihons Laboratories, Pune (Maharashtra, India) respectively. It was used without further purification and certified to contain 99.9% (w/w) and 99.30% (w/w) on dry weight basis of THIO and DICLO, respectively. Fixed dose combination capsule (Thioact D 8) containing 8 mg THIO and 50 mg DICLO were procured from Sun Pharmaceuticals Industries, Dadra, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Chromatographic conditions

An attempt was made to develop simultaneous assay method for the combination of THIO and DICLO. THIO was polar whereas DICLO was non-polar in nature. The mixed standard stock solution containing THIO and DICLO was spotted on to HPTLC plates and run in different solvent systems. Out of various solvent systems tried, the mobile phase consisting of toluene: acetone: methanol: formic acid (5:2:2:0.01 v/v/v/v) was found to be optimum. The optimized chamber saturation time for the mobile phase was 30 min at room temperature ($25^{\circ}\text{C}\pm 2$) at relative humidity of $60\%\pm 5$. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed at 280 nm using a Camag TLC scanner III in the reflectance-absorbance mode and operated by CATS software (V 3.15, Camag).

Preparation of standard solution and construction of calibration plots

Standard solution was prepared by dissolving 10 mg of THIO and DICLO in 10 ml methanol ($1000\ \mu\text{g ml}^{-1}$). The working standard solutions were prepared by dilution of the stock solution with methanol to obtain concentration range of 160-800 ng spot⁻¹ and 1000-5000 ng spot⁻¹ for THIO and DICLO respectively. Each concentration was applied six times to the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves. The drug response was linear over the concentration range between

160-800 ng spot⁻¹ and 1000-5000 ng spot⁻¹ for THIO and DICLO respectively.

Assay of capsule formation

To determine the contents of THIO and DICLO in conventional capsules [Brand name: Thioact D 8, label claim: 8 mg and 50 mg THIO and DICLO respectively per capsule], twenty capsules were emptied and content was weighed, their mean weight determined and finely powdered. The weight of the content equivalent to 8 and 50 mg of THIO and DICLO respectively were transferred into a 50 ml volumetric flask containing 25 ml methanol, sonicated for 30 min and diluted to 50 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 micron filter (Millipore, Milford, MA).

The above stock solution was further diluted to obtain sample solution at three different concentration levels of 240, 320, 400 ng spot⁻¹ and 1500, 2000, 2500 ng spot⁻¹ for THIO and DICLO respectively. The plate was developed in the previously described chromatographic conditions. The peak areas were measured at 280 nm, concentrations in the samples were determined using multilevel calibration developed on the same HPTLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION

HPTLC method development and optimization

The mixed standard stock solution of THIO and DICLO was spotted on to HPTLC plates and run in different solvent systems. The mobile phase consisting of toluene: acetone: methanol: formic acid in the ratio of 5:2:2:0.01 v/v/v/v was found to be optimum. Densitometric scanning was done at 280 nm as both drugs showed maximum response at that wavelength. Under the optimum chromatographic conditions, the R_f values obtained for THIO and DICLO were 0.29 and 0.71 respectively (**Figure 1**).

Validation of the developed method

Validation of the optimized TLC method was carried out with respect to the following parameters:

Linearity

The linear regression data (n=6) showed a good linear relationship over a concentration range of 160-800 ng spot⁻¹ ($r^2 \pm \text{S.D.} = 0.9904 \pm 0.961$) and

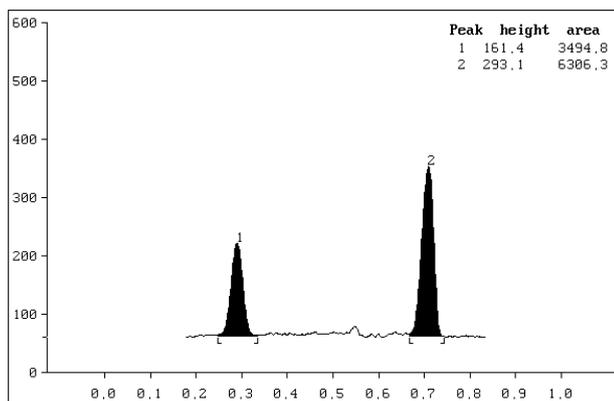


Fig. 1. Densitogram of THIO and DICLO

1000-5000 ng spot⁻¹ ($r^2 \pm S.D. = 0.9983 \pm 0.694$) for THIO and DICLO, respectively. The drug response was linear over the concentration range between 160-800 ng spot⁻¹ and 1000-5000 ng spot⁻¹ for THIO and DICLO, respectively.

Accuracy

These studies were carried out at three levels *i.e.* multiple level recovery studies. Sample stock solution from capsule formulation of each drug

was prepared. To the sample solutions, 50%, 100% and 150% of the standard drug solutions were added. Dilutions were made and recovery studies were performed. % recovery was found to be within the limit as listed in **Table 1**.

Precision

The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guidelines. Separation of the drug was found to be similar when analyses were performed using chromatographic system on different days. The results of the repeatability and intermediate precision experiments are shown in **Table 2**.

LOD and LOQ

The signal to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The limit of detection (LOD) and the limit of quantitation (LOQ) were found to be 10 ng spot⁻¹ and 33 ng spot⁻¹ for THIO and 76.77 ng spot⁻¹ and 255.90 ng spot⁻¹ for DICLO respectively.

Table 1. Recovery studies of THIO and DICLO by HPTLC

Label claim per capsule	Amount added (%)	Total amount (mg)	Amount recovered (mg) \pm % RSD	Recovery* (%)
THIO (8 mg)	50	12	12.22 \pm 0.028	101.83
	100	16	15.96 \pm 0.159	99.75
	150	20	19.94 \pm 0.102	99.70
DICLO (50 mg)	50	75	76.5 \pm 0.091	102.00
	100	100	100.55 \pm 0.166	100.55
	150	125	125.23 \pm 0.864	100.18

* n = 6

Table 2. Precision studies of proposed HPTLC method

Drugs	Conc. ($\mu\text{g ml}^{-1}$)	Repeatability*		Intermediate precision*	
		Found conc. \pm SD	RSD (%)	Found conc. \pm SD	RSD (%)
THIO	160	164.07 \pm 1.294	0.788	159.95 \pm 0.927	0.579
	480	477.51 \pm 3.146	0.658	485.03 \pm 4.913	1.012
	800	809.37 \pm 5.568	0.687	801.53 \pm 6.845	0.853
DICLO	1000	998.50 \pm 7.408	0.741	1009.04 \pm 4.338	0.429
	3000	3035.91 \pm 33.180	1.092	2989.31 \pm 30.130	1.007
	5000	4955.98 \pm 16.890	0.340	4911.08 \pm 16.250	0.330

* n = 6

Specificity

The peak purity of THIO and DICLO was assessed by comparing their respective spectra at the peak start, apex and peak end positions of

the spot *i.e.* $r(S, M) = 0.9975$, $r(M, E) = 0.9969$ and $r(S, M) = 0.9984$, $r(M, E) = 0.9974$, respectively. A good correlation ($r^2 = 0.9904$ and $r^2 = 0.9983$) was also obtained between the

standard and sample spectra of THIO and DICLO.

Assay of capsule formulation

The content of THIO and DICLO were found to be $100.97 \pm 0.93\%$ and $99.02 \pm 1.02\%$ with %RSD of

0.921 and 0.022, respectively. The low %RSD value indicated the suitability of this method for routine analysis of THIO and DICLO in pharmaceutical dosage form. The result of analysis are reported in **Table 3**.

Table 3. Determination of THIO and DICLO in capsule formulation

Parameters	THIO	DICLO
Label claim (mg) found	8	50
Drug content (%) \pm SD	100.97 ± 0.930	99.22 ± 0.022
%RSD	0.921	0.022

CONCLUSION

Over the past decade, HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents and bio-macromolecules. Review of literature reveals that no HPTLC method has been reported so far for simultaneous estimation of thiocolchicoside and diclofenac, therefore, densitometric method (HPTLC) was developed and validated according to ICH guidelines, for the combinations of simultaneous estimation of thiocolchicoside and diclofenac. The results meet with the requirements of ICH guidelines. The proposed

method was found to be rapid, specific, precise and accurate which could be used as effective quality control tool for routine analysis of above compounds as bulk drugs and in dosage formulations.

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REFERENCES

- Balduini W, Cimino M, Depoortere H, Cattabeni F. Characterization of [^3H]thiocolchicoside binding sites in rat spinal cord and cerebral cortex. *Eur. J. Pharmacol.* 1999;376(1-2):149-157. [DOI: 10.1016/S0014-2999(99)00371-4]
- British Pharmacopoeia, Volume II, British Pharmacopoeial Commission, Her Majesty Stationery Office: London, 2003; 582-3.
- Cimino M, Marini P, Cattabeni F. Interaction of thiocolchicoside with [^3H]strychnine binding sites in rat spinal cord and brainstem. *Eur. J. Pharmacol.* 1996; 318(1):201-4. [DOI: 10.1016/S0014-2999(96)00884-9]
- El-Ragehy NA, Ellaithy MM, El-Ghobashy MA. Determination of thiocolchicoside in its binary mixtures (thiocolchicoside-glafenine and thiocolchicoside-floctafenine) by TLC-densitometry. *Farmaco* 2003;58(6): 463-8.
- Ferrari MP, Gatti G, Fattore C, Fedele G, Novellini R. Comparative bioavailability and tolerability study of two intramuscular formulations of thiocolchicoside in healthy volunteers. *Eur. J. Drug Metab. Pharmacokinet.* 2001;26 (4):257-62.
- ICH, Q2 (R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, Geneva, 2005.
- Patil MG, Banerjee SK, Bonde CG, Chhabra GS. UV spectrophotometric method development for the determination of desvenlafaxine succinate in tablet formulation. *Bull. Pharm. Res.* 2011;1(1):40-3.
- Prasanthi V, Mary K, Narasimha Raju CH, Basaveswara Rao MV. Development and validation of new RP-HPLC method for determination of acetyl sulfisoxazole in bulk and pharmaceutical dosage forms. *Bull. Pharm. Res.* 2011;1(1):47-53.
- Rosso A, Zuccaro S. Determination of alkaloids from the colchicine family by reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* 1998;825(1):96-101. [DOI: 10.1016/S0021-9673(98)00595-0]
- Shah J, Banerjee SK, Chhabra GS. UV spectrophotometric method development and validation for entacapone in bulk and formulation. *Bull. Pharm. Res.* 2011;1(2):7-9.
- Shukla R, Shivkumar R, Shivan KN. Development of a UV-spectrophotometric method for the simultaneous determination of tramadol hydrochloride and paracetamol in bulk and marketed product. *Bull. Pharm. Res.* 2011;1(1):62-6.
- Sutherland FCW, Smit MJ, Herbst L, Els J, Hundt HKL, Swart KJ, Hundt AF. Highly specific and sensitive liquid chromatography-tandem mass spectrometry method for the determination of 3-desmethylthiocolchicine in human plasma as analyte for the assessment of bioequivalence after oral administration of thiocolchicoside. *J. Chromatogr. A* 2002;949(1-2):71-7. [DOI: 10.1016/S0021-9673(02)00008-0]
- Vargas F, Mendez H, Fuentes A, Sequera J, Fraile G, Velasquez M, Caceres G, Cuello K. Photosensitizing activity of thiocolchicoside: photochemical and in vitro phototoxicity studies. *Pharmazie* 2001;56(1):83-8.
