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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF ACECLOFENAC AND THIOCOLCHICOSIDE IN BULK AND TABLETS DOSAGE FORMS

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ABSTRACT

A simple, selective, precise high-performance thin-layer chromatographic method for simultaneous determination of Aceclofenac and Thiocolchicoside in bulk and pharmaceutical combined dosage form was developed and validated. The method employed HPTLC aluminum plates precoated with silica gel 60F-254 (10×10) as the stationary phase. The solvent system consisted of Ethyl acetate: Methanol: 1% Glacial acetic acid (80:20:1% v/v). The system was found to give a compact spot for Aceclofenac (Rf = 0.41± 0.02) and Thiocolchicoside (Rf = 0.20±0.02). Densitometric analysis of aceclofenac and Thiocolchicoside was carried out in the absorbance mode at 276 nm. Linear regression analysis data for the calibration plots showed good linear relationship with *r*2 = 0.9912 with respect to peak area in the concentration range 40-160 ng per spot for aceclofenac and *r*2 = 0.9976 with respect to peak area in the concentration range 40–160 ng per spot for Thiocolchicoside. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 20.00 and 40 ng per spot for aceclofenac and 10 and 20 ng per spot for thiocolcoside, respectively. Statistical analysis proved that the method is selective, precise and accurate for the estimation of aceclofenac and Thiocolchicoside.

Key words: Aceclofenac, HPTLC, Thiocolcoside, pharmaceutical formulation.

INTRODUCTION

Aceclofenac (ACE, fig. 1), chemically, 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl] oxyacetic acid[1]. Aceclofenac is a Non-steroidal anti-inflammatory drug (NSAID) used for relief of pain and inflammation in osteoarthritis, rheumatoid arthritis[2-4]. Thiocolchicoside (TCH, fig. 1), is chemically N-[(7s)--3-(beta-D-glucopyranosylony)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9- tetrahydro benzo(a)heptalen-7-yl] acetamide. It is a Muscle relaxant, it is used in the symptomatic treatment of pain full muscle spasm[5].

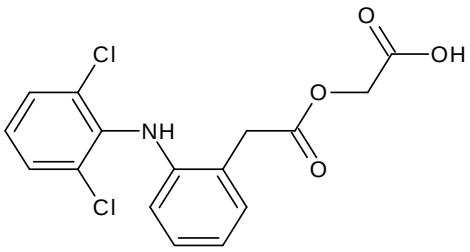


Fig.1 chemical structure of Aceclofenac.

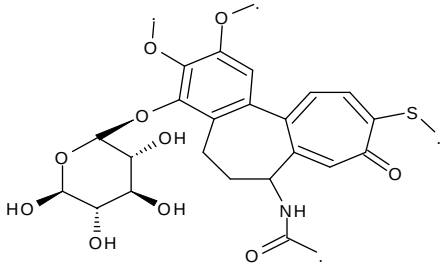


Fig.2 chemical structure of Thiocolchicoside.

For estimating ACE, methods have been reported using HPLC, HPTLC and UV spectro photometry alone or in combination with other drugs[6-20]. Various methods have been reported for the analysis of THIO in bulk and in pharmaceutical formulation such as those using HPLC, ultra performance liquid chromatography (UPLC) with different column materials and mobile phase systems[21-29]. This method developed has chosen over the reported HPTLC method owing to a better mobile phase composition of the method reported. Literature review revealed that no HPTLC method has been reported for estimation of ACE and THIO as single components or as a mixture. The present study reports development and validation of a simple, accurate, economical and reproducible method for the analysis of ACE and THIO using HPTLC at 254 nm either as bulk drug mixture or in combined tablet dosage form.

MATERIAL AND METHODS

Aceclofenac and Thiocolchicoside were obtained as a souvenir samples from Shine Pharmaceuticals Limited Pvt. Ltd., Chennai. Toluene, methanol, ethyl acetate and triethylamine were used as solvents to prepare the mobile phase. All chemicals used were of HPLC grade (S. D. Fine Chem. Ltd., Mumbai, India) used without further purification.

Instrumentation and HPTLC conditions

The samples were spotted in the form of bands of width 6 mm with 100 µl sample syringe on precoated silica gel aluminium plate 60 F254 (10×10 cm, E Merck, Darmstadt, Germany) using a Camag Linomat 5 (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110° for 5 min, prior to chromatography. A constant application rate of 150 nl/sec was employed and space between two bands was maintained at 14 mm. The slit dimension was kept at 6×0.45 mm. The mobile phase consists of Ethyl acetate: Methanol: 1% Glacial acetic acid (80:20:1% v/v). Linear ascending development was carried out in 10×10 cm twin trough glass chamber. The optimized chamber saturation time for mobile phase was 30 min, at temperature (25±2°) and relative humidity (60±5%); the length of chromatogram run was 8 cm and TLC plates were air‑.dried. Densitometric scanning was performed on a Camag TLC Scanner 3 equipped with winCATS software version 1.3.0 at 254 nm. The source of radiation utilized was deuterium lamp. Evaluation was performed using peak area with linear regression.

Preparation of standard solution

An accurately weighed quantity (10 mg) of ACE and TCH were transferred to 10 ml volumetric flask containing 4 ml methanol and volume was adjusted to mark with methanol to obtain a concentration of 1000 ng/µl of ACE and TCH. Dilutions were prepared from the stock solution of ACE and TCH. The linearity range employed was 40-160 ng/l for ACE and TCH.

Analysis of tablets

Twenty **BAKFLEX-A8** (100 mg ACE + 8 mg TCH) tablets were weighed and powdered in a glass mortar. An amount of powder equivalent to 25 mg of ACE was transferred to 25 ml volumetric flask, extracted with methanol for 20 min by shaking mechanically. The solution was diluted to volume with the same solvent and filtered. A sample solution of 10 µl was spotted on TLC plate followed by development and scanning as described in instrumentation and HPTLC condition section. The concentration of drugs was determined from linear regression equations and % label claim was calculated. The developed method was validated in terms of linearity, specificity, precision, accuracy, robustness and ruggedness.

RESULTS AND DISCUSSION

In this study, quantitative determination of ACE and TCH in tablets was performed by a HPTLC method. The HPTLC developed was found to be simple, rapid and sensitive, which did not require any pretreatment procedure. Typical overlain spectra of ACE and TCH were shown in fig. 3**.** Also the typical HPTLC Chromatogram obtained from the analysis of standard ACE (Rf = 0.41) and TCH (Rf = 0.20) was shown in fig. 4. The peak purity of ACE and TCH were found to be 0.999 and 0.998, respectively indicating that no impurities or degradation products were found along with the peaks of standard drug solutions, hence making the method specific. Regression analysis for the HPTLC method was carried out results were shown in Tables. 1. Quantitative determination of ACE and TCH in tablets using this HPTLC method indicated good agreement with the labeled amount of ACE and TCH (Table 2). Closeness of the amount found to the amount taken and the low coefficient of variation value showed that the proposed method was accurate and precise. Recovery study conducted by the HPTLC method was performed to ensure the reliability of the method, mixing a known quantity of standard drug with the preanalyzed sample formulation carried out recovery studies and contents were analysed by the proposed method. The percentage recovery was found to be as shown in Table 3.

The method was found to be precise based on the results obtained in the intra-day and inter-day precision evaluation study these results were shown in table 4. These results were expressed in terms of % RSD that was found to be less than 2. High recovery values followed by low % RSD value (<2) coupled with low standard deviation makes the proposed method highly suitable for accurate and precise determination of ACE and TCH in combined tablet dosage forms.

Table.1.Optical characteristics of Aceclofenac and Thiocolchicoside by HPTLC method

S.NO	PARAMETERS	ACECLOFENAC	THIOCOLCHICOSIDE
1	λ max (nm)	276	276
2	Beer's law limit (µg/ml)	40-160	40-160
3	Correlation Coefficient (r)	0.9912	0.9976
4	Regression Equation (y=mx+c)	y =12.032x+396.639	y =13.074x+ 166.667
5	Slope (m)	12.032	13.074
6	Intercept (c)	396.039	309.825
7	LOD (ng/ml)	20	10
8	LOQ (ng/ml)	40	20
9	Standard Deviation	3.74	1.87

Table.2. Quantification Of Formulation (Bakflex-A8) By Hptlc Method

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage Obtained	Average (%) ± S.D	% R.S.D.	S.E.
ACE	1	100	100.28	100.28	100.09 ± 0.4025	0.4021	0.0111
	2	100	100.34	100.34			
	3	100	100.04	100.04			
	4	100	99.68	99.68			
	5	100	99.58	99.58			
	6	100	100.62	100.62			
THI0	1	8	7.98	99.75	98.74 ± 1.1968	1.2120	0.0332
	2	8	8.10	100.10			
	3	8	7.78	97.25			
	4	8	7.86	98.25			
	5	8	7.96	99.50			
	6	8	7.81	97.62			

Table.3. Recovery Analysis Of Formulation (Bakflex-A8) By Hptlc Method

Drug	Sample No.	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated (µg/ml)	Amount recovered (µg/ml)	%Recovery	± S.D	% R.S.D	S.E.
ACE	1	50.04	40	39.92	39.88	99.70	±0.0416	0.0417	0.0046
	2	50.04	50	99.92	49.98	99.76			
	3	50.04	60	109.85	59.81	99.68			
					Mean	99.71			
THIO	1	3.90	3.2	7.19	3.29	102.81	±0.5093	0.4982	0.0565
	2	3.90	4.0	8.07	4.08	102.12			
	3	3.90	4.8	8.79	4.89	101.87			
					Mean	102.22			

Table.4. Intra day and inter day analysis of formulation (bakflex-a8) by hptlc method

Drug	Sample No.	Labelled amount (mg/tab)	Percentage obtained*		± S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
ACE	1	100	100.54	100.44	±0.2913	±0.3429	0.2908	0.3422
	2	100	100.44	100.13				
	3	100	100.05	100.46				
	4	100	100.13	100.03				
	5	100	100.05	100.54				
	6	100	99.74	99.64				
Mean			99.84	100.20				

THIO	1	8	99.25	100.66	±0.0558	±0.6868	0.5577	0.6853
	2	8	100.50	99.33				
	3	8	100.50	100.66				
	4	8	100.50	100.66				
	5	8	100.50	100.66				
	6	8	99.25	99.33				
Mean			100.08	100.21				

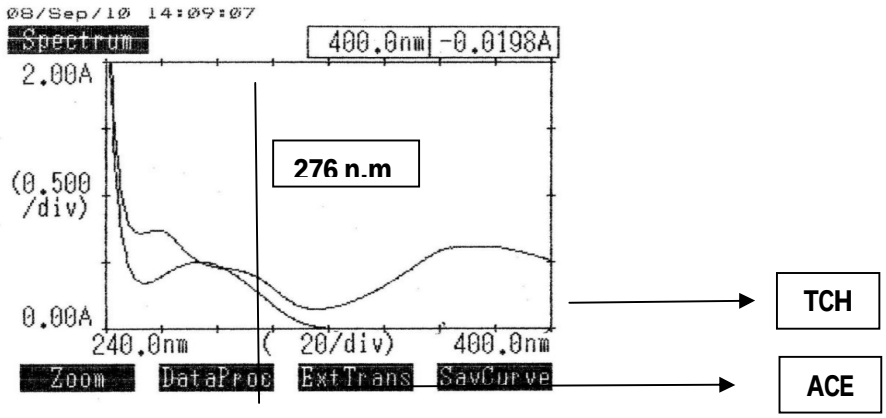


Fig.3. Over lane spectra of samples

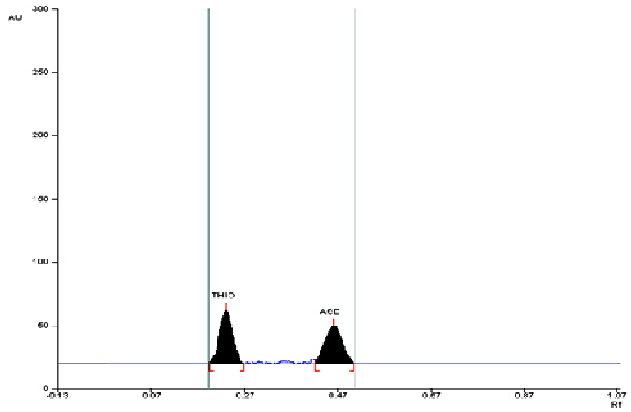
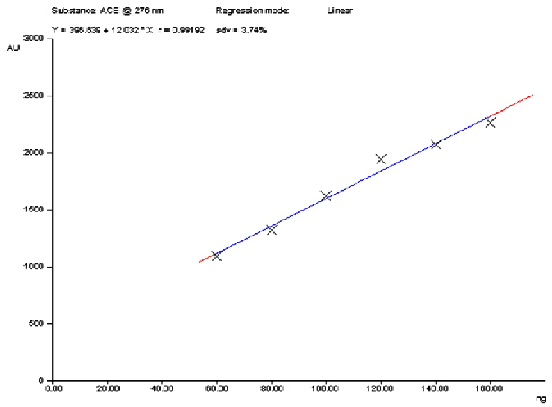


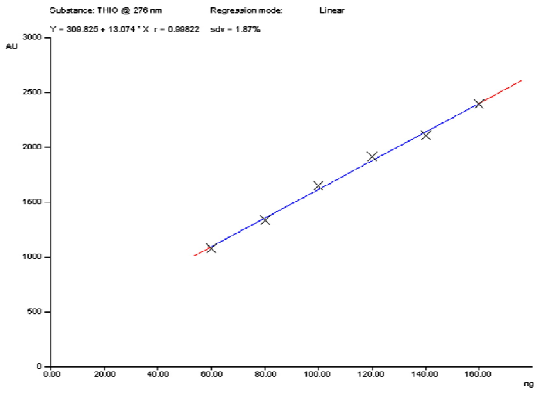
Fig. 4: Typical HPTLC chromatograms of ACE and TCH Typical HPTLC chromatograms of ACE (aceclofenac, Rf=0.41) and TCH (thiocolchicoside, Rf=0.20) in mobile phase consisting of Ethyl acetate: Methanol: 1% Glacial acetic acid (80:20:1% v/v at 276 nm.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.20	2.1	0.23	41.6	56.87	0.26	0.3	977.9	47.16	THIO
2	0.42	1.8	0.46	31.5	43.13	0.51	2.0	1095.5	52.82	ACE

Fig.5. Calibration Curve Of Aceclofenac & Thiocolchicoside At 276 nm By HPTLC Method

ACECLOFENAC **THIOCOLCHICOSIDE**





CONCLUSION

The developed HPTLC technique is found to be precise, specific, accurate and stability indicating. The developed method was validated based on ICH guidelines. Statistical analysis indicated that the method is repeatable and selective for the analysis of ACE and TCH both in bulk drug mixture and in tablets. The developed method appears to be useful for determining purity of these drugs available from various sources. In conclusion, the proposed HPTLC method is suitable for the analysis of Aceclofenac and thicolchicoside in commercial tablets.

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