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Research Article

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Validated HPTLC method for simultaneous estimation of Aceclofenac and Pantoprazole in bulk and tablets dosage form

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ABSTRACT

In the present research work a new simple, precise, accurate, specific and cost effective HPTLC method was developed and validated for simultaneous estimation of Aceclofenac (ACECLO) and Pantoprazole (PANTO) in bulk and pharmaceutical dosage forms. Simultaneous estimation of ACECLO and PANTO by HPTLC was carried out using precoated silica gel 60F254 as stationary phase. The mobile phase used was a mixture of n-butanol: 1, 4-Dioxane in the ratio (6:4 v/v). The Rf values of ACECLO and PANTO was found to be 0.3 and 0.6 respectively. The detection of spots was carried out at 254 nm. The calibration curve was found to be linear between 1 to 6μg/ml for both drugs with a correlation coefficient of 0.9996 and 0.9994 for ACECLO and PANTO respectively. The proposed method was validated according to various ICH parameters such as linearity and range, specificity, precision, sensitivity and accuracy. The accuracy of the proposed method was determined by recovery studies and was found to be well within the acceptance limit. Statistical analysis proves that the proposed method is suitable for the routine analysis of ACECLO and PANTO in bulk and pharmaceutical formulations.

Keywords: Aceclofenac, Pantoprazole, HPTLC, ICH guidelines, Simultaneous estimation.

INTRODUCTION

Aceclofenac, chemically, a phenyl acetic acid derivative, has anti-inflammatory and analgesic properties. It is a potent inhibitor of cyclo-oxygenase enzyme which is involved in the production of prostaglandins [1]. ACECLO is practically insoluble in water and soluble in alcohol & methyl alcohol, freely soluble in acetone & dimethyl formamide. The chemical structure of ACECLO is (Fig.1)

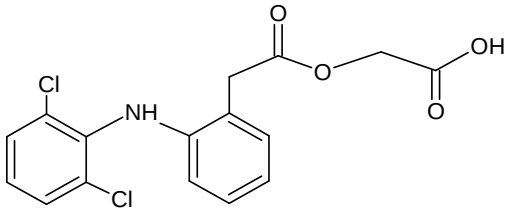

Fig 1: Chemical structure of Aceclofenac

Pantoprazole, chemically, 5-Difluoromethoxyl Benzimidazole -2-yl-3,4-dimethoxy-2-pyridyl methyl sulfoxide . PANTO is a class of substituted Benzimidazole belongs to long acting proton pump inhibitor. It acts by suppressing

gastric acid secretion through the inhibition of H^+ K^+ ATPase at the secretory surface of the parietal cells and blocks the final step of gastric acid secretion. It is well absorbed from the Gastro Intestinal Tract [2]. The chemical structure of PANTO is (Fig.2)

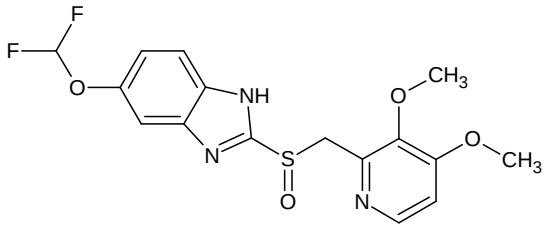

Fig 2: Chemical structure of Aceclofenac

Literature survey revealed that various methods such as UV-spectrophotometry [3,4], visible spectrophotometry [5,6], RP-HPLC [7-9], HPTLC [10-13] for ACECLO and UV-spectrophotometry [14,15], RP- HPLC [16,17],HPTLC [18] for the estimation of PANTO were reported individually and combination with other drugs. But no method was developed and validated for the simultaneous estimation of ACECLO and PANTO in combination by HPTLC.

In view of the above facts in the present study, an attempt has been made to estimate the ACECLO and PANTO simultaneously in bulk and tablet formulations using HPTLC method.

EXPERIMENTAL SECTION

Instruments and apparatus

Electronic analytical balance, A Camag HPTLC system comprising of Camag Linnomat-V automatic sample applicator, Hamilton syringe, Camag TLC Scanner, Camag Win CATS software and ultra Sonicator were used during the study.

Reagents and chemicals

Aceclofenac and Pantoprazole standards were obtained as gift sample from Anglo French Drugs and Industries Ltd., Bengaluru. All the chemicals used were of AR grade and are obtained from the stores of Government College of Pharmacy, Bengaluru. ACECLO and PANTO tablet dosage form manufactured by Nov Nortis were procured from local pharmacy store.

Method Development

Selection of chromatographic mode: The reverse phase chromatographic mode was selected for the simultaneous estimation of ACECLO and PANTO using HPTLC rather than the normal phase chromatographic mode. As the stationary phase is more Non polar and mobile phase is polar or slightly polar in nature in the reverse phase method.

Preparation of mobile phase or solvent system: Measure accurately about 60 volume of n-butanol and 40 volumes of 1, 4- Dioxane (60:40v/v) transfer into TLC chamber and kept for saturation which was then used as mobile phase.

Diluent: Analytical grade methanol was used as diluent.

Preparation of standard stock solution of ACECLO and PANTO (Stock I): Accurately 10 mg of ACECLO and PANTO were weighed into a clean and dry 100 ml volumetric flask separately and dissolved with sufficient amount of methanol. The final volume was made up to the mark using methanol to get the concentration of 100µg/ml ACECLO and PANTO respectively.

Preparation of standard stock solutions of ACECLO and PANTO (Stock II): Transfer 1.0 ml of each standard stock (I) solution separately into 10 ml volumetric flasks and final volume was made up to the mark using methanol to get a concentration of 10 µg/ml ACECLO and PANTO respectively.

Determination of Rf values of Aceclofenac and Pantoprazole: Standard solution of Aceclofenac and Pantoprazole (stock II) was applied in the form of a band using CAMAG Linomat V onto a HPTLC Precoated plates Silica Gel 60F254 of dimensions 10x3 cm were employed for the spotting of standard solution. The spot was scanned by the instrument at 254 nm and Rf values was determined.

Method Validation

A HPTLC method was developed for the simultaneous estimation of ACECLO and PANTO in bulk and tablet formulations. The chromatography was carried out by using precoated silica gel TLC plates as stationary phase and the mobile phase composed of n-butanol: 1, 4-Dioxane (6:4 v/v) with UV detection at 254 nm. The Rf values of ACECLO and PANTO was found to be 0.3 and 0.6 respectively. In order to ensure the performance characteristics of the developed HPTLC method, the validation was performed using various parameters as per the ICH guidelines [19,20].

Linearity and Range: A series of dilutions were prepared from the standard stock solutions (stock II) to get a concentration of 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, 5 µg/ml 6 µg/ml ACECLO and PANTO respectively and applied in the form of bands using CAMAG Linomat V on to HPTLC precoated plates Silica Gel 60F254. 10 ml of the mobile phase n-butanol: 1, 4-Dioxane in the ratio 6:4 v/v was taken in to CAMAG twin trough chamber. The chamber was allowed to saturate and plate was then placed in the chamber for development. The developed plate was dried and scanned by using CAMAG TLC scanner at 254 nm. The six tracks were scanned and peak areas were determined.

Specificity was performed to exclude the possibilities of interference of solvent in the region of Rf values of ACECLO and PANTO. The specificity of the method was tested under the normal conditions and results of the tests proved that the components other than ACECLO and PANTO did not produce the detectable peaks at the Rf values of both the drugs.

Precision was studied to find out intra-day and inter-day variations in the test method of ACECLO and PANTO. The intra-day assay precision was found by analysis of standard drug thrice on the same day in different intervals of time and inter-day assay precision was carried out at three different days and percentage relative standard deviation (% RSD) was calculated. The % RSD should not be more than 2.0%.

Sensitivity of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ) using statistical analysis method by calibration standards. LOD and LOQ were calculated as 3.3s/S and 10s/S respectively, where S is the slope of the calibration curve and s is the standard deviation of response.

The accuracy of the developed method was determined by recovery studies at three different levels. The preanalyzed samples were spiked with 50, 100 and 150 % of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The recovery study was carried out in triplicate.

Application of developed HPTLC method for simultaneous estimation of ACECLO and PANTO

Preparation of sample solution: 20 tablets, each containing 100 mg of ACECLO and 40 mg of PANTO were weighed and powdered. A quantity of powder equivalent to 100 mg of ACECLO and 40 mg of PANTO was accurately weighed and transferred to the 50 ml volumetric flask, about 25 ml of methanol was added and shaken thoroughly for 10 min and then sonicated for 5 min. The volume was adjusted to the mark with methanol. The solution was filtered and filtrate was diluted to obtain the concentration of 1 µg/ml of ACECLO and 1 µg/ml of PANTO.

RESULTS AND DISCUSSION

A new HPTLC method was developed and validated for simultaneous estimation of ACECLO and PANTO. A satisfactory separation of both the drugs was carried out using precoated silica gel 60F254 as stationary phase. The mobile phase used was a mixture of n-butanol: 1, 4-Dioxane in the ratio (6:4 v/v). The Rf values of ACECLO and PANTO was found to be 0.3 and 0.6 respectively. The detection of spots was carried out at 254 nm. The developed method specification was presented in **Table 1**.The developed HPTLC method was validated as per ICH guidelines in terms of linearity and range, specificity, precision, sensitivity and accuracy. The results of validation parameter found to be well within the acceptance limit.

The linearity response of ACECLO and PANTO was observed in the concentration range of 1 to 6 µg/ml for both the drugs respectively and statistical data such as regression equation and correlation coefficient was found well within the acceptance criteria limit. The results were presented in **Table 2,3.** The HPTLC chromatograms and standard calibration curve were presented in **Figure 3,4**,**5.**

The developed HPTLC method was found to be specific as the diluents and mobile phase and excipients of tablet formulation showing no peaks at the Rf values of Aceclofenac and Pantoprazole and not showing any interference in the analysis.

The % RSD value of concentration obtained for six replicates of injections of ACECLO and PANTO was found to be less than 2%. Hence developed method was found to be precise and precision studied data were presented in **Table 4.**

The method was found to be sensitive and data were presented in **Table 5.**

The developed HPTLC method was found to be accurate as the accuracy study data of ACECLO and PANTO showed excellent % recovery values at three different levels. The results of accuracy study were presented in **Table 6,7.**

The results of % assay shows that there is no interference of excipients and no impurities were observed in sample for the developed HPTLC method. The results of assay were presented in **Table 8.**

Table 1: Developed HPTLC method specification

Instrument and Specification	
Applicator	CAMAG Linomat 5
Developing chamber	Camag twin trough glass chamber
Solvent system	n-butabol: 1,4-Dioxane
Ratio of Solvent	6:4 v/v.
Integration software	Wincats
Wavelength of Detection	254 nm
Injection Volume	2µl
Rf Value of ACECLO	0.3
Rf Value of PANTO	0.6

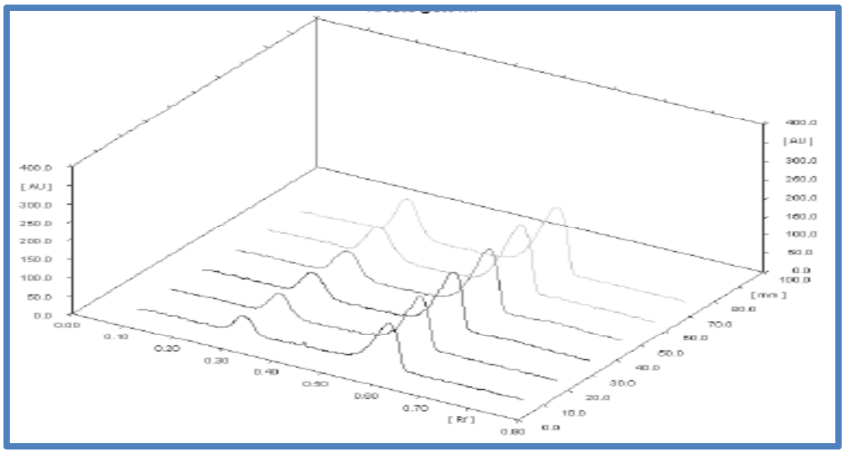

Fig 3: HPTLC Chromatogram of Aceclofenac and Pantoprazole

Table 2: Linearity range data of Aceclofenac and Pantoprazole

Sr. No.	Concentration µg/ml	Peak area for ACECLO	Peak area for PANTO
1	1µg/ml	189	1581
2	2 µg/ml	370	3175
3	3 µg/ml	563	4758
4	4 µg/ml	757	6330
5	5 µg/ml	936	7909
6	6 µg/ml	1105	9215

Table 3: Linearity report of Aceclofenac and Pantoprazole

Parameters	ACECLO	PANTO
Linearity Range	1-6 µg/ml	1-6 µg/ml
Regression equation	Y=185.61x+3.0357	Y=1552x+53.679
Correlation Coefficient	0.9996	0.9994
Percentage Curve Fitting	99.96	99.94
Slope	189	1581

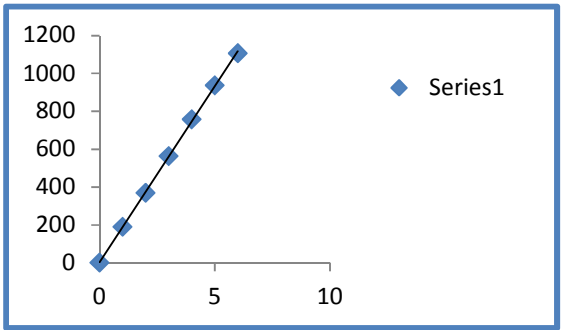

Fig 4: Standard calibration curve of Aceclofenac

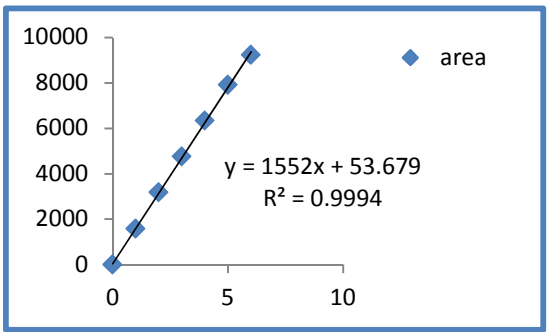

Fig 5: Standard calibration curve of Pantoprazole

Table 4: Precision studies data of Aceclofenac and Pantoprazole

Precision	ACECLO	PANTO
Intra-Day Precision	1.90 %	1.53 %
Inter-Day Precision	1.52 %	0.59 %

Table 5: Sensitivity data of Aceclofenac and Pantoprazole

SENSITIVITY		
Drugs Name	**LOD**	**LOQ**
ACECLO	0.08 µg/ml	0.25 µg/ml
PANTO	0.01 µg/ml	0.04 µg/ml

Table 6: Accuracy study data of Aceclofenac

Sample No.	Spike Levels	% Recovery	Mean % Recovery
1	50	100.46	100.05 %
	50	99.91	
	50	99.78	
2	100	102.45	101.27 %
	100	100.19	
	100	102.66	
3	150	100.90	99.71 %
	150	99.11	
	150	99.11	

Table 7: Accuracy study data of Pantoprazole

Sample No.	Spike Levels	% Recovery	Mean % Recovery
1	50	99.80	100.38 %
	50	101.42	
	50	99.92	
2	100	99.58	99.59 %
	100	99.58	
	100	99.62	
3	150	101.00	100.28 %
	150	99.94	
	150	99.90	

Table 8: Assay results of Aceclofenac and Pantoprazole

Drug	Brand Name	Labeled amount	Amount found	% assay
ACECLO	HIFENAC	100 mg	98.41	98.41%
PANTO	PAN 40	40 mg	39.72	99.30%

CONCLUSION

The developed HPTLC method was found to be simple, precise, sensitive, specific and economic for simultaneous estimation of ACECLO and PANTO in bulk and tablet dosage form with good accuracy and precision. The proposed method utilizes inexpensive solvents and the percentage recovery data shows that the method is free from interference of the excipients used in formulation and hence can be used for routine analysis in quality control laboratories.

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