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Validated High Performance Thin Layer Chromatographic Estimation of Aceclofenac in Bulk and Pharmaceutical Dosage Forms

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Abstract: ***A simple, precise, accurate, and specific method is developed and validated for analysis of Aceclofenac in bulk and pharmaceutical dosage forms. The developed method used precoated silica gel 60F254 as stationary phase. The mobile phase used was a mixture of Hexane: chloroform: methanol in the ratio (6:2:2v/v). The RF value was 0.3.The detection of spots was carried out at 271nm. The calibration curve was found to be linear between 1 to 6 µg/ml with a correlation coefficient of 0.9998.The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability. The accuracy of the proposed method was determined by recovery studies and was found to be 98.35 to 100.43 %. Statistical analysis proves that the proposed method is suitable for the routine analysis of Aceclofenac in bulk and pharmaceutical formulations.***

Keywords: Aceclofenac, HPTLC, Validation, ICH guidelines

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAID's) are considered to be the first-line drugs in the symptomatic treatment of rheumatoid arthritis, osteoarthritis and spondylitis. The most common NSAIDs are aspirin, diclofenac, ibuprofen and naproxen. Aceclofenac is a newer derivative of Diclofenac with low gastrointestinal complications.[1] Aceclofenac displays a high degree of enantio-selectivity in its inhibitory effects on arachidonic *cycloxygenase* system. It was also reported to produce less GI bleeding than other NSAIDs such as indomethacin, ibuprofen or naproxen. After oral administration, aceclofenac is rapidly and completely absorbed as unchanged drug. Peak plasma concentrations are reached approximately 1.25 to 3 h following ingestion. 4-hydroxy aceclofenac is the main metabolite detected in plasma.[2]

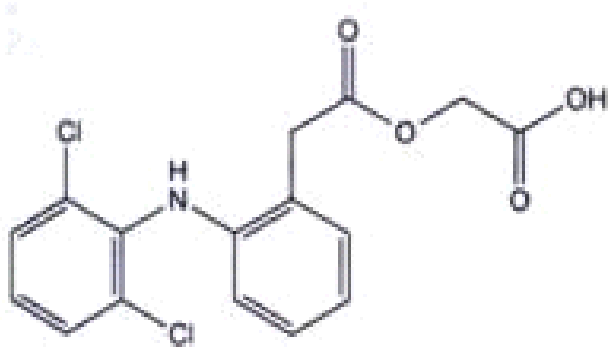


Figure 1: Structural formula of Aceclofenac

Aceclofenac is chemically 2-[(2, 6-dichlorophenylamino) phenylacetyloxy acetic acid. It has an empirical formula of $C_{16}H_{13}Cl_2NO_4$ and a molecular weight of 354.18472g/mol.[3] Literature survey revealed that various analytical methods like spectrophotometric [4-10] and HPLC [11-13] methods have been reported for the determination of Aceclofenac in pharmaceutical dosage forms. The aim of this study was to develop and validate a simple, precise, accurate and rapid HPTLC method for the estimation of Aceclofenac hydrochloride in pure and pharmaceutical dosage forms.

2. Materials and Methods

2.1 Chemicals and Equipment

Aceclofenac working standard was procured as gift sample from Nice Chemicals Ltd, Kochi. Silica gel 60F 254 TLC plates (E. Merck, Mumbai) were used as a stationary phase. The reagents used were Chloroform A. R (Ranbaxy Fine Chemicals Ltd, S.A.S. Naga.).Methanol H.P.L.C, (Thomas Baker Chemicals Ltd, Mumbai) & Hexane A.R (Nice Chemicals, Cochin). A Camag HPTLC system comprising of Camag Linnomat-V automatic sample applicator, Hamilton syringe, Camag TLC Scanner , Camag Win CATS software, Camag twin-trough chamber and ultra sonicator were used during the study.

2.2 Preparation of standard solution of Aceclofenac

Accurately weiged 100mg of Aceclofenac and transferred to a 100 ml standard flask, dissolved and made up to mark with methanol. The solution had a concentration of 1mg/ml.This solutions was used for application on HPTLC plate.

2.3 Development of Solvent System

For the initial studies, conventional TLC plates (10x3cm) were employed. Silica Gel GF was made into smooth slurry with water and poured on to the plates and allowed to air dry. The plates were then activated by keeping in an oven at 110°C for 30 minutes. The mobile phase was selected based on the polarity of aceclofenac and the absorption property of silica gel plates. The mobile phase was found out by trial and error method.

Hexane:chloroform:methanol mobile phase in the ratio of 3:1:1 was chosen as it gave a good Rf value of about 0.3 without any tailing.

2.4 Determination of Rf value and λmax of Aceclofenac hydrochloride

Standard solution of Aceclofenac hydrocloride(as prepared in Section 1)was applied in the form of a band using CAMAG Linomat.HPTLC Precoated plates Silica Gel 60F 254 of dimensions 10x3 cm were employed for the spotting of standard solution.

The spot was scanned by the instrument, Rf value found to be 0.3. The wavelength of the integrated spot was scanned from 200-350nm and the wavelength of max absorbance was found to be 271nm.

2.5 Determination of linearity range of Aceclofenac

Standard solution was prepared in section I was taken in a micro syringe previously rinsed with methanol 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 hexane: chloroform: methanol was taken in the ratio 6:2:2 in to 10x10 cm 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 with CATS soft ware. The six tracks were scanned and integrated and peak height and peak area were found out.

2.6 Calibration data for Aceclofenac

Table 1: Calibration data for Aceclofenac

Volume (µl)	Concentration (µg/ µl)	Peak Area
1	1	6156
2	2	9439
3	3	12578
4	4	15717
5	5	18763
6	6	22135

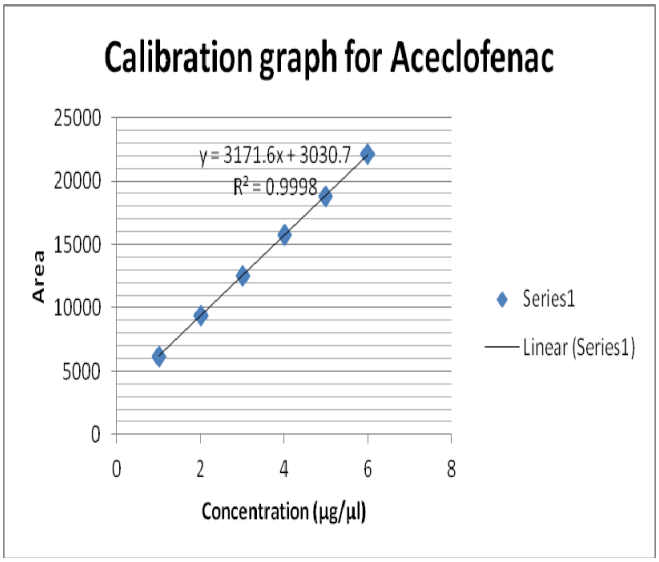
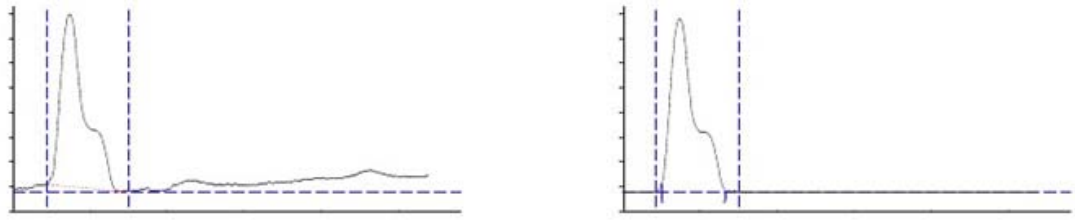
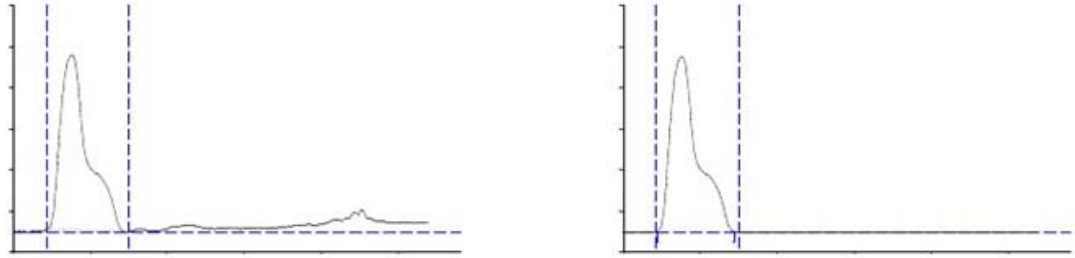


Figure 2: The calibration curve was found to be linear in the range of 1-6µg/µl.

Track Aceclofenac



Peak	Start Rf	Max Rf	End Rf	Area	Assigned substance
1	0.1	0.31	0.45	6156	Aceclofenac



Peak	Start Rf	Max Rf	End Rf	Area	Assigned substance
1	0.13	0.30	0.50	9439	Aceclofenac

Estimation of Aceclofenac hydrochloride in dosage forms

a. Preparation of sample solutions from Aceclo and Aceroc tablets

Twenty tablets each of Aceclo and Acero were accurately weighed,powdered and average weight was calculated. Weight equivalent to 100mg each of Aceclofenac was transferred to two conical flasks and dissolved in methanol. The solution was sonicated for 15 min. The extracts were filtered through Whatmann filter paper no.41 and the residue was washed with methanol.The extracts and washings were pooled and transferred to a 100 ml volumetric flask and volume was made with methanol to obtain a concentration of 1µg/µl.

b. Spotting and development of spots

Standard solution as prepared in section 1 was taken in a microsyringe and concentrations of 1,2,3,4 µg/µl were spotted and then 3 µl of the sample solution of Aceclo and 3 µl of the sample solution of Aceroc were spotted. The plate was then allowed to develop in a twin trough chamber previously saturated with hexane: chloroform: methanol in the ratio 6:2:2.The developed plate was dried and then scanned by using CAMAG V scanner with CATS software.

c. Scanning and integration of the chromatogram.

All the chromatograms were scanned and integrated. All the spots showed an Rf value of 0.3 and maximum value of absorbance was found to be 271nm.

d. Calibration and quantification of Acecloc and Aceroc tablets

The areas of the chromatograms were calibrated and were used to quantify the amount of Aceclofenac in each of the tablets as shown in Table 2.

Table 2

Substance	Peak Area	% Label claim (area wise)	Amount of active substance
Aceclofenac	12578		
Acecloc	11997	95.38	95.38mg
Aceroc	12102	96.21	96.21mg

Method Validation

The method was validated as per ICH[14] guidelines with respect to linearity, range, sensitivity, specificity, precision, accuracy, and robustness.

Linearity and Range

Areas under curve (AUC) of six different concentrations were determined and a calibration plot was obtained by plotting AUC area verses concentration equation. Linearity range was found to be in the range of 1-6 µg/ spot with a correlation coefficient of 0.9998.

Sensitivity

The sensitivity of the method was estimated in terms of Limit of Quantification and Limit of Detection.LOD and LOQ were determined by applying decreasing amount of the drug in triplicate on the plate. The LOD & LOQ was found to be 10 & 20ng/spot respectively.

Specificity

No interference was observed during analysis between drugs and excipients in tablet. Hence the method was found to be specific.

Precision

Precision of the method was tested by performing intra-day and inter-day studies.

Intraday Precision

Intra-day precision was determined by analyzing standard solutions of Aceclofenac at three different concentrations in the linearity range of the drug, for three times on the same day. The intra-day relative standard deviations were in the range of 0.07 – 0.3% as shown in the table 3 below. These low values indicated that the method is precise.

Table 3

Volume applied(µl)	Peak Area	Average	Standard Deviation	% RSD
2	9436 9439 9448	9441	6.24	0.07
4	15717 15735 15703	15718.3	16.04	0.1
6	22135 22034 22157	22108	65.39	0.3

Interday Precision

Interday Precision was found out by carrying analysis of the standard drug at three concentration three times for three different days and the % RSD was calculated. The inter-day relative standard deviations were in the range of 0.06 – 0.3% as shown in the table 4 below. These low values indicated that the method is precise.

Table 4

Volume applied(µl)	Peak Area		
	1st day	2nd day	3rd day
2	9456	9467	9481
	9439	9449	9434
	9471	9453	9469
Average	9455.3	9456	9461.3
Standard deviation	16.01	9.45	24.41
% RSD	**0.17**	**0.10**	**0.26**
4	15732	15741	15789
	15715	15775	15751
	15780	15723	15717
Average	15742.3	15746.3	15752.3
Standard deviation	33.7	26.4	36.018
% RSD	**0.21**	**0.17**	**0.23**
6	22101	22143	22135
	22152	22125	22148
	22132	22117	22162
Average	22128	22128	22148
Standard deviation	25.69	13.31	13.5
% RSD	**0.12**	**0.06**	**0.06**

Accuracy

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug (**Aceclofenac**) in the pre analyzed tablet sample. This parameter was evaluated by the recovery studies at concentration levels of 80%, 100%, and 120% of **Aceclofenac.** Result of recovery studies are given in **Table 5.** Percentage recovery was found to be within limits, as listed in Table.5.

Table 5

Accuracy level	Amount added (µg)	Amount recovered (µg)	%recovery	SD	%RSD
80%	80	78.75	98.43		
	80	79.88	99.85	1.37	1.39
	80	77.69	97.11		
100%	100	99.89	99.89		
	100	98.35	98.35	0.93	0.93
	100	100.03	100.03		
120%	120	120.51	100.43		
	120	121.73	101.44	1.005	1.00
	120	119.32	99.43		

3. Results and Discussion

A simple, fast, sensitive and accurate HPTLC method was developed. HPTLC precoated silica gel 60F 254 plates were used for spotting Aceclofenac solution. Different mobile phases were tried out of which Hexane: Chloroform: Methanol in the ratio of 6:2:2 was used when a sufficient Rf of 0.3±.05 was obtained.

The method was validated in accordance with ICH guidelines. The method was linear in the range 1 to 6 µg per band of Aceclofenac (with correlation coefficient 0.9998, n = 6). The intra-day relative standard deviations were in the range of 0.07 – 0.3% and the inter-day relative standard deviations were in the range of 0.06 – 0.3%. These low values indicated that the method is precise.

The accuracy of the analysis was evaluated by determination of recovery at three different concentrations of the drug in the dosage form. The results indicated that the method enables accurate estimation of the drugs in the tablet dosage form. The assay values for the marketed formulations were found to be within the limits. The low RSD value indicated the suitability of the method for routine analysis of Aceclofenac in pharmaceutical dosage forms. Thus the method can be applied for the routine analysis of Aceclofenac in pharmaceutical formulations.

4. Conclusion

The developed HPTLC method is simple, precise, specific and accurate. Statistical analysis indicates that the method is reproducible and selective for the routine analysis of Aceclofenac in bulk drug and pharmaceutical formulations without interference from excipients. Hence this method can be easily and conveniently adopted for the routine analysis of Aceclofenac in bulk and its pharmaceutical dosage forms.

5. Future Scope

The developed HPTLC method can be further utilized for analyzing combination dosage forms of Aceclofenac.

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