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SIMULTANEOUS ESTIMATION OF DICLOFENAC SODIUM AND FAMOTIDINE BY REVERSED-PHASE THIN LAYER LIQUID CHROMATOGRAPHY / DENSITOMETRY METHOD IN BULK AND IN TABLET DOSAGE FORM

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ABSTRACT: The present work describes a validated reverse phase high performance thin layer liquid chromatographic method for simultaneous estimation of Diclofenac sodium and Famotidine in bulk and in pharmaceutical dosage form. Chromatographic separation of the drugs were performed on aluminum plates precoated with 200-µm layers of silica gel 60 RP-18 F254S as the stationary phase and the solvent system consisted of methanol: water: triethylamine (7.5:3.5:0.5) (v/v/v). Densitometric evaluation of the separated zones was performed at 291 nm. The two drugs were satisfactorily resolved with R_f values 0.54 ± 0.02 and 0.69 ± 0.02 for Diclofenac Sodium and Famotidine, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity over the range of 1500 ng/band to 9000 ng/ band for Diclofenac Sodium and over the range of 500 ng/ band to 3000 ng/ band for Famotidine respectively.

INTRODUCTION: Diclofenac sodium is chemically known as {2-[2-(2, 6-dichlorophenylamino) phenyl] acetic acid} (**Figure 1a**). Diclofenac sodium (DICLO) is responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis. Detailed literature survey reveals various methods for its determination such as stress induced HPLC method [1-6], HPTLC [7-11], UV-spectrometric method [8-10], simultaneous determination of FAM and DICLO in pharmaceutical formulation.

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Famotidine (FAM) {3-[[[[2-(diaminomethylene) amino] thiazole-4-yl] methyl] sulphanyl]-N'sulphamoyl propanimidamide} (**Figure 1b**) is a histamine H2-receptor antagonist that inhibits stomach acid production and is commonly used in the treatment of peptic ulcer disease and gastro esophageal reflux disease.

Combination of famotidine and diclofenac sodium is available as over the counter in various countries. It is beneficial as it prevents the gastric ulcers, acid indigestion, heart burns which results during the single diclofenac potassium therapy for a long term for osteoarthritis or rheumatoid arthritis, painful menstrual periods, and general pain etc. Even though various methods have been proposed for analysis of FAM and DICLO like HPLC [11], HPTLC [12-14,15] and UV- spectrometric method [13], but for the first time we are presenting simple, sensitive, accurate, precise, rapid and economic chromatographic method in bulk

and in tablet dosage form. No work has been reported for the determination of the FAM and DICLO in combined dosage form by HPTLC method. This paper presents HPTLC method for determination of Famotidine and Diclofenac sodium in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

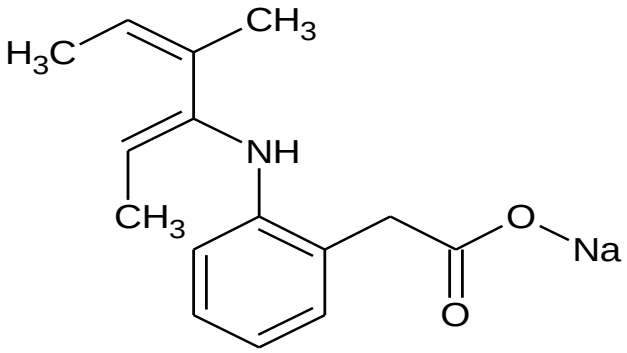

FIG. 1A: CHEMICAL STRUCTURE OF DICLOFENAC

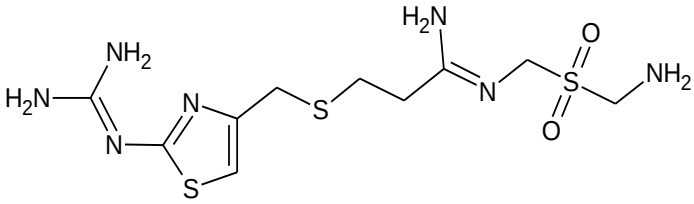

FIG. 1B: CHEMICAL STRUCTURE OF FAMOTIDINE

EXPERIMENTAL:

Materials and Reagents: Diclofenac sodium and Famotidine were provided as a gift sample by Cadila HealthCare Ltd (Vadodara, Gujarat). All other reagents and solvents utilized were of analytical (AR) grade and are from Merck Chemicals Ltd, Mumbai (India).

Instrumentation and chromatographic condition: Reverse phase chromatography was performed on 20 cm ×10 cm aluminium-backed RP-TLC plates coated with 200-µm layers of silica gel 60 RP-18 F254S were used. Before chromatography the plates were prewashed with methanol and activated at 105^0C for 5 min in oven. The samples were applied as 6 mm wide bands with the help of Linomat 5 sample applicator (Muttenz, Switzerland) fitted with a 100-µl sample syringe (Hamilton, Bonaduz, Switzerland). The plate was developed in a pre-saturated Camag twin trough glass chamber (20 cm × 10 cm). Methanol: Water: Triethylamine (7.5: 3.5: 0.5 v/v) were used as mobile phase and chamber saturation time was 30 min.

The plates were developed to a distance of 8.0 cm and scanned densitometrically using Camag TLC Scanner 3 equipped with win CATS software version 1.3.0 at 254 nm for both method. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200- 400 nm. Evaluation was performed using peak area with linear regression.

Preparation of standard and sample solutions: Independent stock solution of 1000 µg/mL of each DICLO and FAMO were prepared in methanol.

Preparation of calibration curves: From each stock standard solution, 1.5 – 9.0 ml of DICLO and 0.5 - 3.0 ml of FAMO were transferred into six 10 ml volumetric flasks separately and volume was made up to the mark with methanol. From each volumetric flask a volume 1 µl was applied on TLC plate to obtain series of concentration 1500- 9000 ng/band of DICLO and 500 - 3000 ng/band of FAMO. The plates were developed and scanned as described. Each standard in six replicates was analyzed and peak areas were recorded Calibration curves of DICLO and FAMO were plotted separately of peak area *vs.* respective concentration.

Optimization of HPTLC method: Firstly, single solvents were selected on the basis of their polarity to separate the spots. Then the mixtures of solvents are used for separation purpose of DICLO and FAMO. The spots were developed in mixtures of methanol and water in the ratio of 7.5:3.5 *v/v*. The R_f value obtained was good but slight tailing was observed. Hence, to reduce the tailing, triethylamine was added in the solvent system. Thus, the final mobile phase consisted of methanol: water: triethylamine in the ratio (7.5:3.5:0.5 *v/v*). The chamber saturation time was 30 min. The R_f for DICLO and FAMO were found to be 0.54± 0.02 and 0.69 ± 0.02, respectively (**Figure 2**).

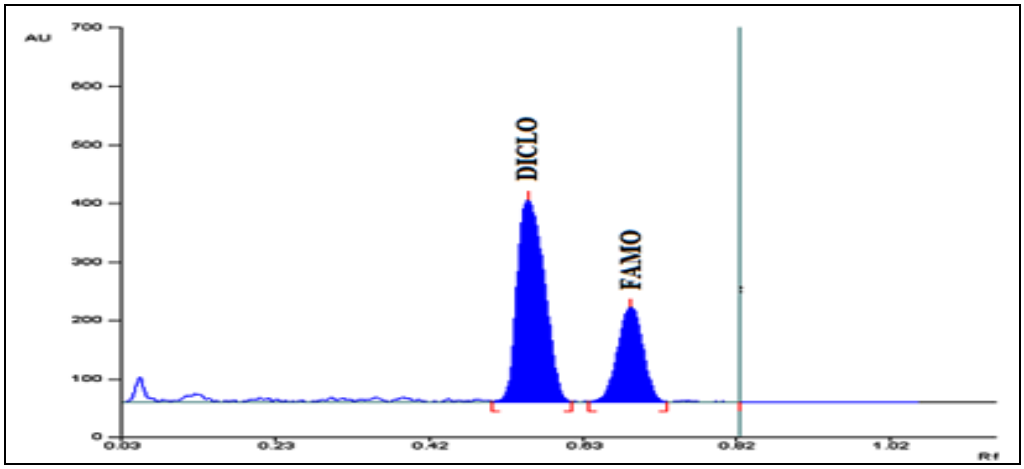

FIGURE 2: DENSITOGRAM OF STANDARD DICLO (R_f 0.54±0.02) AND FAMO (R_f 0.69±0.02), IN RATIO OF (1:3) MEASURED AT 291 nm.

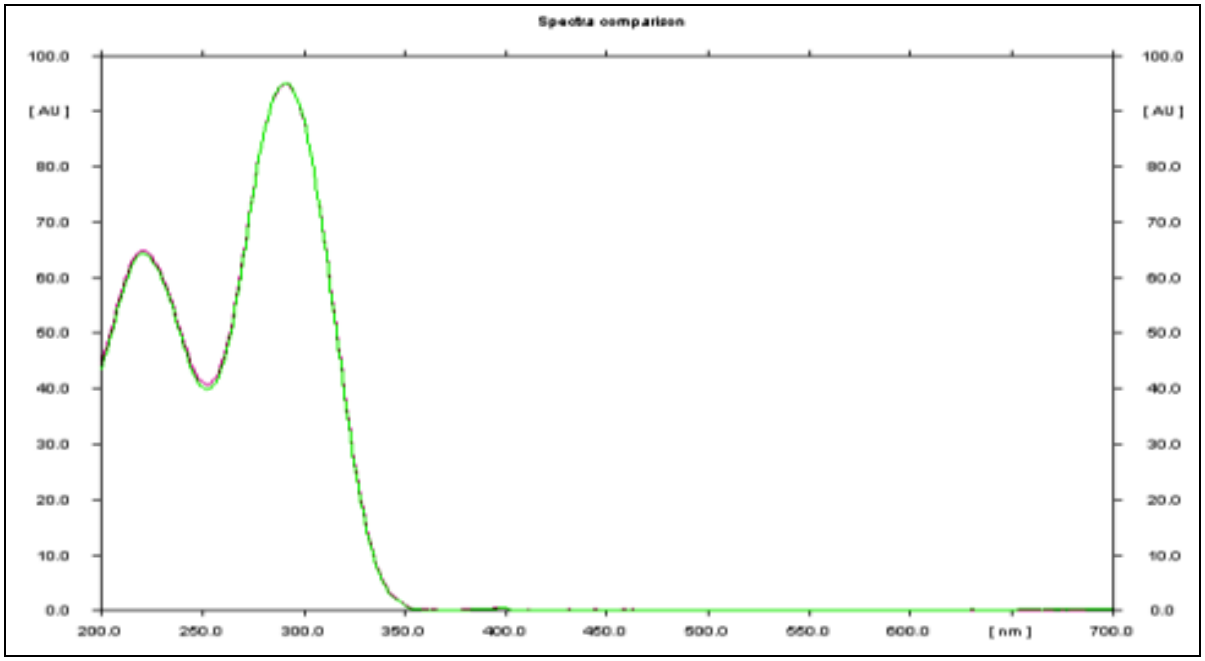

FIGURE 3: PEAK PURITY SPECTRA OF STANDARD DICLO, EXTRACTED FROM DICLOFENAC - FAMOTIDINE TABLET, SCANNED AT THE PEAK - START, PEAK - APEX AND PEAK - END POSITIONS OF THE BAND (CORRELATION > 0.99)

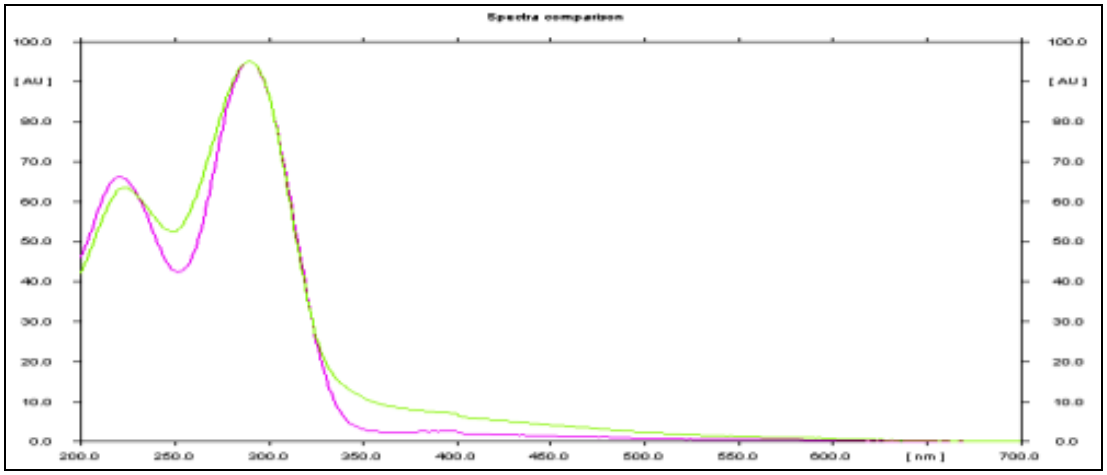

FIGURE 4: PEAK PURITY SPECTRA OF STANDARD FAMO, EXTRACTED FROM DICLOFENAC - FAMOTIDINE TABLET, SCANNED AT THE PEAK - START, PEAK - APEX AND PEAK - END POSITIONS OF THE BAND (CORRELATION > 0.99)

Validation: The method was validated by establishing linearity, accuracy, inter - day and intra - day precision of measurement of sample application. The limit of detection and limit of quantification were also determined.

1. **Linearity:** Linearity was studied in the concentration range from 1500 – 9000 ng/band for DICLO and 500 – 3000 ng/band for FAMO for reverse phase. The drugs showed good linearity in the tested range. The regression co-efficient values for DICLO and FAMO were found to be r^2 = 0.999 and 0.999 **(Table 1).**

2. **Precision:** In order to validate and prove the applicability of the method, a laboratory mixture of DICLO and FAMO was prepared from the stock solutions in the ratio corresponding to amounts in the dosage form. For quantitative estimation of the mixture, three series (3000, 4500, 6000 ng/band and 1000, 1500, 2000 µg/mL for DICLO and FAMO respectively) were prepared **(Table 2).**

TABLE 1: LINEARITY OF DICLO AND FAMO FOR PROPOSED METHOD (n=6)

Parameters	DICLO	FAMO
Linearity range (ng/band)	1500-9000	500-3000
Slop	7.698	3.2
Intercept	3695	28.13
Correlation Coefficient (r^2)	0.999	0.999

TABLE 2: PRECISION OF DICLO AND FAMO FOR PROPOSED METHOD (n=3)

Drugs	Conc. [ng/band]	Intra-day precision		Inter-day precision	
		Mean ± S.D.	% *RSD* [*n* = 3]	Mean ± S.D.	% *RSD* [*n* = 3]
DICLO	3000	8350 ± 54.61	0.65	8378 ± 23.51	0.20
	4500	10747 ± 17.95	0.16	10818.67 ± 101.79	0.94
	6000	13101.62 ± 62.85	0.47	13162 ± 55.67	0.42
FAMO	1000	3246.33 ± 46.60	1.43	8378 ± 23.51	0.28
	1500	4840 ± 44.64	0.92	10818.67 ± 101.79	0.94
	2000	6515.66± 42.77	0.65	13162 ± 55.67	0.42

FAMO: Famotidine; DICLO: Diclofenac

3. **Accuracy:** The accuracy of the experiment was established by spiking pre-analyzed sample with known amounts of the corresponding drugs at three different concentration levels i.e. 80, 100 and 120 % of the drug in the tablet. The spiked samples were then analyzed for three times. The mean recovery is within acceptable limits, indicating the method is accurate for both methods (**Table 3).**

TABLE 3: RECOVERY STUDIES

Drugs	Initial amount (ng per band)	Amount added (%)	% recovery	%RSD [n=3]
DICLO	3000	80	101.88	1.07
	3000	100	99.99	0.59
	3000	120	99.58	0.47
FAMO	1000	80	100.57	0.90
	1000	100	99.58	0.43
	1000	120	100.36	1.08

4. **Analysis of tablet formulation:** Twenty tablets (Dicka-F50) (each contained 20 mg FAMO and 50 mg DICLO) were accurately weighed and finely powdered. A quantity of the powder containing weight equivalent to 20 mg FAMO and 50 mg DICLO was transferred to a 50 mL volumetric flask and methanol (35 mL) was added followed by ultrasonication for 10 min, volume was adjusted to mark and filtered using 0.45 µm filter (Mill filter, Milford, MA) and 1 ml of filtrate was further diluted to 10 ml with methanol. Appropriate volume 10 µl, was spotted for assay of FAMO and 30 µl DICLO. The plates were developed and scanned as described in above chromatographic conditions in reverse phase. In this methods good separation and well resolved spots were obtained which indicate that there are no interferences of excipients commonly present in the tablet formulation and result as shown in **(Table 4).**

TABLE 4 ANALYSIS OF TABLET FORMULATION

Drug	Label Claim (mg)	%Amount Found	%RSD (n = 6)
DICLO	50	101.67	0.91
FAMO	20	99.08	1.44

5. **Specificity:** The mobile phase designed for the method resolved both the drugs very efficiently. The Rf value of DICLO and FAMO was found to be 0.54 and 0.69, respectively. The peak purity of DICLO extracted from tablet and standard DICLO was tested at the peak - start (S), peak - apex (A) and at the peak - end (E) positions (**Figure 3**). The peak purity of FAMO was tested by correlating the spectra's of FAMO at the peak - start (S), peak - apex (A) and at the peak - end (E) positions (**Figure 4**).

6. **Ruggedness and Robustness:** Ruggedness of the both method was performed for DICLO and FAMO by two different analysts maintaining similar experimental and environmental conditions. Robustness of the method was performed by introducing various changes in the previous chromatographic conditions; effects on the results were examined for both method.

7. **Sensitivity:** The sensitivity of measurements of DICLO and FAMO by the use of the proposed method was estimated in terms of the Limit of Quantitation (LOQ) and the lowest concentration detected under the chromatographic conditions as the Limit of Detection (LOD).

   LOQ and LOD were calculated by the use equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where 'N' is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. The results were recorded for both the methods. Different validation parameters for the both methods for determining DICLO and FAMO content were summarized in **(Table 5).**

TABLE 5 LOD AND LOQ OF THE METHOD

Drugs	LOD	LOQ
DICLO	151.64	451.82
FAMO	51.11	153.33

TABLE 6: VALIDATION PARAMETERS

Parameters	DICLO	FAMO
Linearity (correlation coefficient)	0.999	0.999
Slope	7.698	3.2
Intercept	3695	28.13
Ruggedness [% RSD]		
Analyst I [n=6]	1.42	0.19
Analyst II [n=6]	1.37	0.10
Robustness [% RSD] [n=6]		
Mobile phase composition	1.38	0.60
Mobile phase volume	1.49	0.67
Development distance	1.57	0.61
Sensitivity		
Limit of Detection (ng)	151.64	51.11
Limit of Quantitation (ng)	451.82	153.33
Intra-day [n = 3]	0.47 – 0.65	0.65–1.18
Inter-day [n = 3]	0.28–0.94	0.65–1.43
Repeatability [n = 6]	1.26	0.48

RESULTS AND DISCUSSION: An RP-HPTLC method was optimized with a view to develop an accurate and reproducible method so as to resolve drugs. Optimization of method was done by altering almost all the chromatographic conditions and the effect on R_f and peak shape were monitored for the drugs selected i.e. DICLO and FAMO. The final chromatographic conditions was performed on 20 cm × 10 cm aluminum-backed RP-TLC plates coated with 200-µm layers of silica gel 60 RP-18 F_{254} S (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India).

The plates were prewashed by methanol and activated at 105 – 110°C for 15 min prior to chromatography. The samples were applied on the plates as bands, under continuous flow of nitrogen, by means of a CAMAG (Muttenz, Switzerland) Linomat-5 sample applicator fitted with a 100-µL syringe. Methanol: water: Triethylamine in the ratio (7.5:3.5:0.5 *v/v*) showed well- defined and resolved peaks when the chamber was saturated with mobile phase for 30 min at room temperature. Both the peaks were well resolved and no telling observed when plate was scanned at 291 nm. The R_f for DICLO and FAMO were found to be 0.54± 0.02 and 0.69 ± 0.02, respectively.

The results of specificity studies indicated no interference from excipients, impurities which assured that the peak response was due to a single component and it passes peak purity criteria**.**

CONCLUSION: The modalities adopted in experiment were successfully validated as per ICH guidelines. The proposed RP –HPTLC method was validated by preliminary analysis of standard sample and by recovery studies for the determination of DICLO and FAMO in bulk and in tablet dosage form and the percentage of average recoveries for DICLO and FAMO was obtained 100.56 and 100.17 respectively. Summary of all validation parameter are shown in **Table 6.** The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of DICLO and FAMO in combined tablet dosage form.

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