

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CAMYLOFIN DIHYDROCHLORIDE AND MEFENEMIC ACID IN PHARMACEUTICAL TABLET

SEETHARAMAN RATHINAM*, AND LAKSHMI K.S

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur, Chennai 603203, India. Email: seerampharm@gmail.com

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ABSTRACT

Objective: The objective of the present aims to develop new, simple, precise and accurate High Performance Thin Layer Chromatographic method for the estimation of Camylofin dihydrochloride and Mefenemic acid in pharmaceutical dosage forms.

Method: Chromatographic separation of the drugs were achieved employing merck precoated silica gel 60 F254 (0.2 mm thickness) on aluminium sheets as stationary phase with a solvent system of chloroform: methanol: ammonia in the ratio of 6:4:0.1 v/v/v, densitometric quantification of the separated bands was done at 270nm. The saturation time of the chamber and the developing distance was set at 30 minutes and 8cm respectively. The method was validated as per ICH guidelines. **Results:** The R_f values were found to 0.46 for Camylofin dihydrochloride and 0.35 for Mefenemic acid. The proposed method was found to be linear in the concentration range of 320-480 ng / band for Camylofin dihydrochloride and 1600-2400ng / band for Mefenemic acid. The average recovery was found to be 100.62% w/w and 100.02% w/w for Camylofin dihydrochloride and Mefenemic acid respectively.

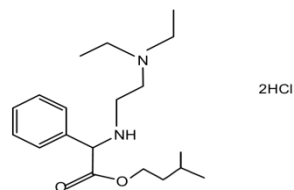
Conclusion: The novel HPTLC method developed is precise, specific and accurate. Satisfactory results were obtained from validation of the method. Hence the proposed method is suitable in the quality control of estimation Camylofin dihydrochloride and Mefenemic acid in pharmaceutical dosage forms.

Keywords: Camylofin dihydrochloride, Mefenamic acid, HPTLC, Validation, Separation science, ICH guidelines.

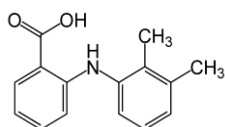
INTRODUCTION

Camylofin dihydrochloride, chemically 3-methylbutyl 2-(2-diethylaminoethylamino)-2-phenylacetate hydrochloride is a drug used as an antispasmodic[1]. Mefenamic acid is 2-[(2,3-dimethylphenyl) amino] benzoic acid. It has analgesic, anti-inflammatory and anti-pyretic properties. It works by blocking the action of a substance in the body called cyclooxygenase which is responsible for production of prostaglandins[2-3]. The literature survey revealed the lack of an analytical method for the simultaneous estimation of Camylofin dihydrochloride and mefenemic acid. However estimation of these drug molecules in combination with other drugs by UV spectrophotometric, HPLC and HPTLC were available [4-14].

The aim of the present work was to develop and validate a new simple, rapid, selective, cost effective HPTLC method for simultaneous determination of Camylofin Dihydrochloride and Mefenemic Acid in pharmaceutical formulation. The chemical structures of the drug are shown in Fig. 1.



Camylofin dihydrochloride ($C_{19}H_{32}N_2O_2 \cdot 2HCl$)



Mefenemic acid ($C_{15}H_{15}NO_2$)

Fig. 1: Chemical structure of Camylofin dihydrochloride and Mefenemic acid

MATERIAL AND METHODS

Chemicals and Reagents

Analytical pure samples of Camylofin dihydrochloride powder and Mefenemic acid powder were procured as gift samples from Ideal analytical and research institution, Puducherry, India. Commercial tablets (Bigspas - M, Mankind Pharma Ltd, AODRL022) containing Camylofin dihydrochloride (50mg) and Mefenemic acid (250mg) were used for the study. Chloroform, methanol and ammonia used were of analytical grade (E. Merck, Mumbai, India). All the other chemicals used were also of analytical grade (E. Merck, India)

Instrumentation and chromatographic condition

HPTLC plates precoated with silica gel GF254 aluminum TLC plate, (10 cm X 10 cm, 250mm thick (Merck). The plates were prewashed by methanol and activated at 105-110°C for 15 min before use in chromatography. The samples in methanol were applied

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There are no sources in the current document. As bands 6 mm wide, 10 mm from the bottom and 20 mm from the sides of the plate, under a continuous flow of nitrogen, by means of a CAMAG Linomat-5 sample applicator fitted with a 100 μ L syringe.

A constant application rate of 150 nL s⁻¹ was used. The plate was then placed in presaturated twin trough chamber (CAMAG; 10 \times 10 cm²) containing the mobile phase of chloroform:methanol : ammonia in the ratio of 6:4:0.1 v/v/v and ascending development was performed to a distance of 80 mm from the point of application at ambient temperature. After development, plates were air dried, observed under UV chamber and densitometric scanning was performed at 230 nm with a CAMAG TLC scanner III operated in the reflectance absorbance mode and controlled by Win CATS software version 4. The slit dimensions were 5 mm \times 0.45 mm and the scanning speed was 20 mm s⁻¹. Evaluation was by linear regression of peak area against the amount of sample per band. The statistical software used is Microsoft excel (version 7).

Preparation of Standard solution

Standard stock solutions were prepared by dissolving separately 10 mg of Camylofin dihydrochloride and Mefenemic acid each in 10 mL of methanol to obtain a concentration of 1000 μ g / mL. The standard stock solutions were suitably diluted methanol to obtain the working standard solutions of both Camylofin dihydrochloride and Mefenemic acid.

Preparation of Sample solution

To determine the amount of Camylofin dihydrochloride and Mefenemic acid in marketed tablets, twenty tablets were taken, accurately weighed, the average weight of the tablets was found out and finely powdered. An amount of powder equivalent to 50mg of Camylofin dihydrochloride (250 mg of Mefenemic acid) was weighed and transferred in to a 100mL volumetric flask; 50 mL of methanol was added. It was sonicated for 10 min and contents were diluted to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was collected. A suitable dilution of the supernatant liquid was made with methanol to obtain a concentration of 400 ng / band and 2000 ng / band of Camylofin dihydrochloride and Mefenemic acid respectively.

Method Validation

The developed method was validated as per the International Conference on Harmonization (ICH) [15-16] guidelines with respect to linearity and range, specificity, precision, accuracy and robustness.

Specificity

The specificity of an analytical method is the ability of the method to determine the analyte response in the presence of additional components such as impurities, degradation products and matrix [17]. An analytical placebo solution (containing all the excipients except Camylofin dihydrochloride and Mefenemic acid) was prepared according to the sample preparation procedure and the chromatogram was developed. To identify the interference by these excipients, a mixture of inactive ingredients, standard solutions, and the commercial pharmaceutical preparations including Camylofin dihydrochloride and Mefenemic acid were analyzed by the developed method.

Linearity and range

Calibration curves were constructed in the concentration range of and 320-480ng/band for Camylofin dihydrochloride 1600- 2400 ng / band for Mefenemic Acid. The Beer's law is obeyed over the concentration range, and the coefficient of regression for both the drugs. The stock solution with Camylofin dihydrochloride and Mefenemic acid was serially diluted to five mixed standard solutions. A volume of 2 μ L of each solution was applied on the HPTLC plate to deliver 320,360,400,440 and 480 ng / band of Camylofin

dihydrochloride and 1600, 1800, 2000, 2200 and 2400 ng / band of Mefenemic acid. This was done in triplicate. For each concentration, the applied band bands were evenly distributed across the plate to minimize possible variation along the silica layer. The results are indicated in Table 1.

Precision

Precision of the developed method was studied by performing repeatability and intermediate precision studies. The repeatability of sample application and measurement of peak area was determined by performing six replicate measurements of the same band. The concentrations of different bands were 400 ng/band of Camylofin dihydrochloride and 2000 ng/band of Mefenemic acid respectively. The intermediate precision of method was checked by repeating the study on different days.

Recovery studies

Recovery determination for Camylofin dihydrochloride and Mefenemic acid was carried out at levels of 80%, 100% and 120%. The analysed samples were spiked with extra 80%, 100% and 120% of the standard drug and the mixture was reanalysed by the proposed method. At each level of the amount, three determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

Robustness

The effect of deliberate variations in method parameters like the composition of the mobile phase, volume of the mobile phase, time from banding to development and time from development to scanning were evaluated in this study. The effect of these changes on both the R_f values and peak areas was evaluated by calculating the relative standard deviations (RSD) for each parameter.

RESULTS AND DISCUSSION

Validation of the developed method

Specificity

The chromatogram of commercial formulation showed only two peaks at R_f values of 0.46 and 0.35 for Camylofin dihydrochloride and Mefenemic acid, respectively, indicating that there is no interference of the excipients in the capsule formulations. The respective chromatogram was shown in Fig. 2 -3.

Linearity

The calibration plot was found to be linear in the concentration range of 320-480 ng / band and 1600-2400 ng / band for Camylofin dihydrochloride and Mefenemic acid respectively. The linearity was validated by the high values of the correlation coefficient. The results are tabulated in Table 1, the calibration curve plots and the residual plots are shown in Fig. 4-7.

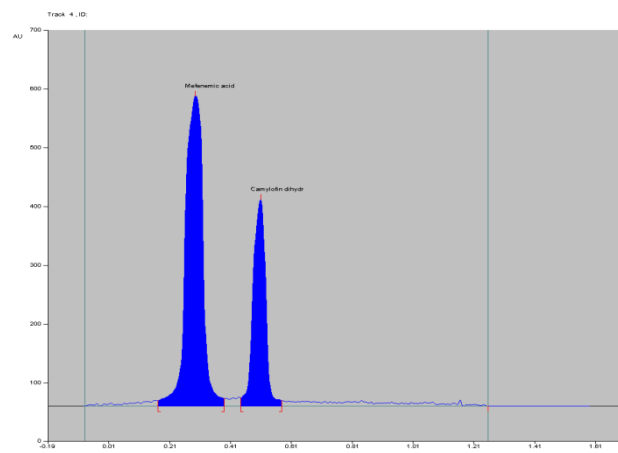
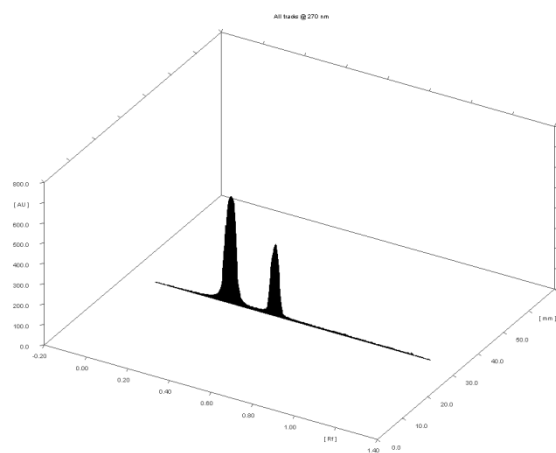


Fig. 2: Chromatogram of Camylofin dihydrochloride and Mefenemic acid

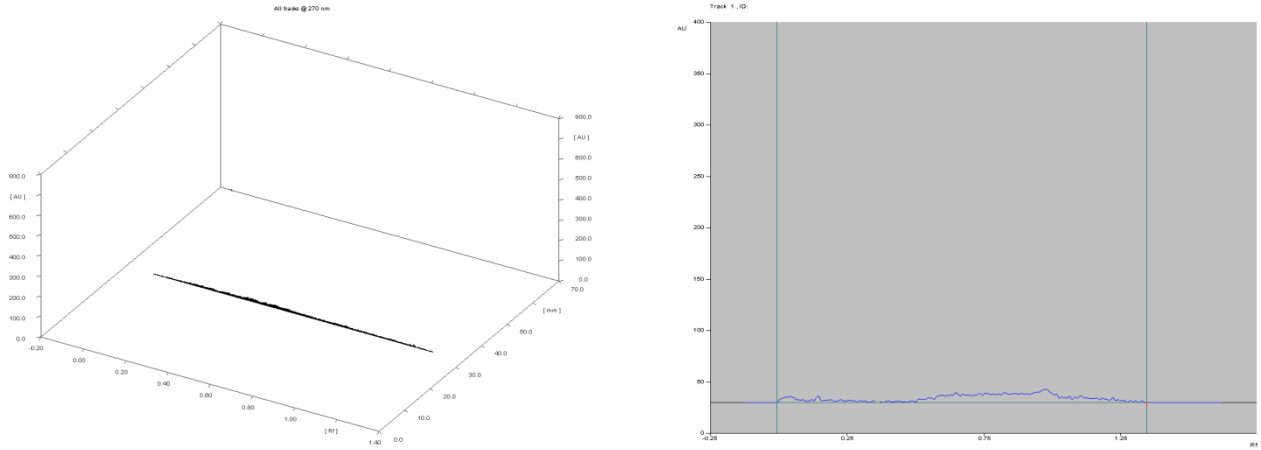


Fig. 3: Chromatogram of placebo

Table 1: Summary of linear regression data

Parameters	Camylofin Dihydrochloride	Mefenemic acid
Linearity Range	320-480 ng / band	1600-2400 ng / band
Linear regression equation	$y = 5.72x - 30.2$	$y = 11.43 + 862.2$
Slope+SD	5.7+0.412	11.435+0.381
Intercept+SD	30.2 + 0.258	862.2+1.478
Correlation coefficient (r ²)	0.9993	0.9989

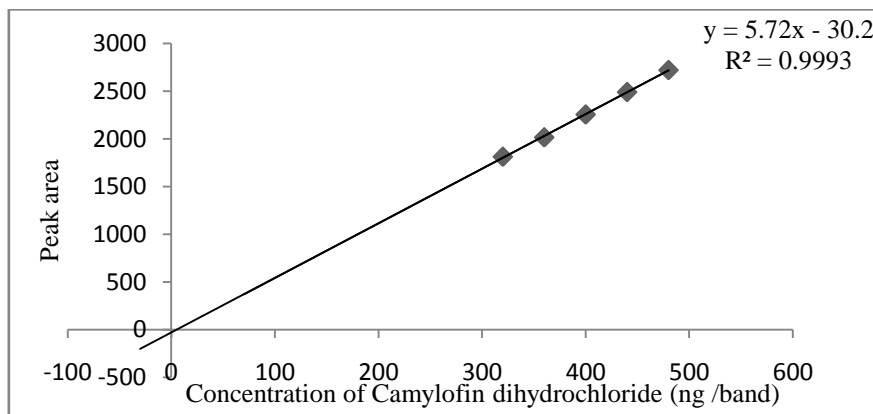


Fig. 4: Calibration curve plot for Camylofin dihydrochloride

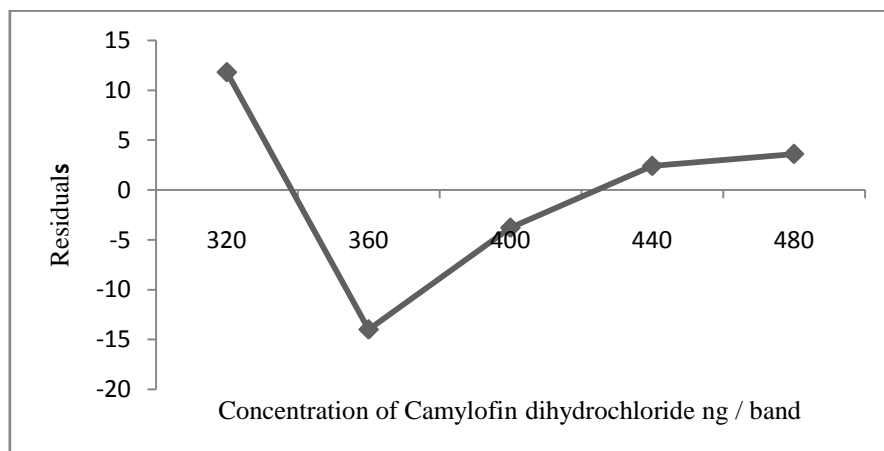


Fig. 5: Residual plot of camylofin dihydrochloride

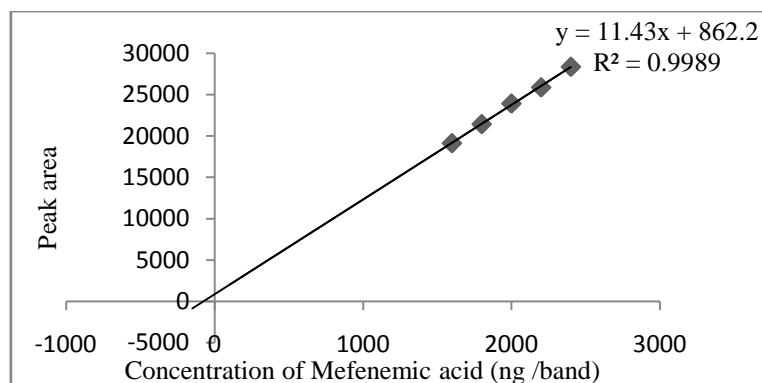


Fig. 6: Calibration curve plot for Mefenemic acid

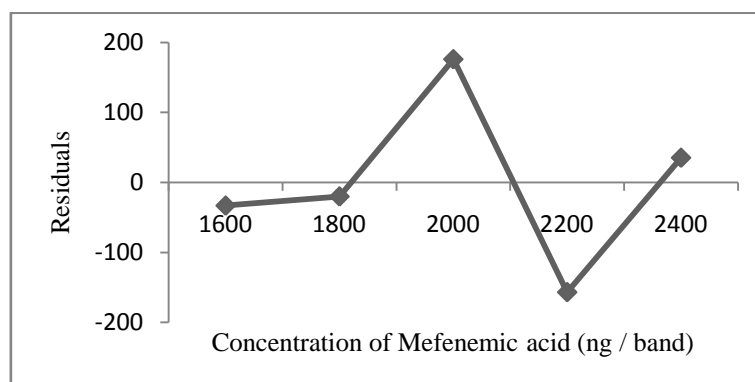


Fig. 7: Residual plot of Mefenemic acid

Precision studies

The repeatability (intra-day precision) and intermediate precision (inter-day precision) of sample application and measurement of peak area were expressed in terms of % R.S.D and was found to be less than 2% as depicted in Table 2.

Recovery studies

The recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The results of the recovery studies and its statistical validation are given in Table 3.

Table 2: Precision data

S. No.	Camylofin dihydrochloride % Label claim		Mefenemic acid % Label claim	
	Intra Assay	Inter Assay	Intra Assay	Inter Assay
1.	100.17	100.33	101.33	100.12
2.	100.89	100.41	101.66	99.53
3.	100.79	100.37	100.23	101.89
4.	100.93	100.85	100.34	101.91
5.	100.38	100.89	101.76	100.36
6.	100.78	100.65	100.67	101.68
Mean	100.65	100.58	100.99	100.91
%RSD	0.3063	0.2472	0.6655	1.03
Grand Mean	100.62		101.95	
%RSD	0.2768		0.8469	
S.E	0.1136		0.3490	
95% CI	±0.222		±0.684	

Table 3: Recovery study report of Camylofin dihydrochloride and Mefenemic acid

Drug	Recovery level (%)	Initial amount (ng / band)	Amount added (ng / band)	% recovery*	% RSD*
Camylofin dihydrochloride	80	200	120	98.92	0.325
	100	200	200	100.12	0.258
	120	200	280	100.06	0.359
Mefenemic acid	80	1000	600	99.89	0.412
	100	1000	1000	99.62	0.587
	120	1000	1400	99.95	0.581

* Denotes average of three estimations of each level of recovery

Robustness

To evaluate the robustness of the method, selected parameter was varied at different levels. The results presented in Table-4 indicate that R_f and % drug content were unaffected by small variations in the selected method parameters, also the low values of % R.S.D. (< 2) of % drug content obtained after introducing small changes in mobile phase composition and volume of mobile phase were indicative of the robustness of the method as presented in Table 4.

Analysis of marketed formulation

The chromatograms of the drug extracted from commercial formulation, exhibited two peaks at R_f value of 0.46 and 0.35 for Camylofin dihydrochloride and Mefenemic acid, respectively. The mean drug content was found to be 39.75 mg and 150.14 mg for Camylofin dihydrochloride and Mefenemic acid with a % R.S.D of 0.33 and 0.28 respectively. The results of the analysis of marketed formulation are given in Table 5.

Table 4: Robustness study for the developed method

Parameters	Drug	% SD	% RSD
Mobile Phase composition	Camylofin dihydrochloride	0.318	0.319
	Mefenemic acid	0.658	0.635
Amount of mobile phase	Camylofin dihydrochloride	0.654	0.684
	Mefenemic acid	0.464	0.860
Time from banding to chromatography (+ 10 min)	Camylofin dihydrochloride	0.486	0.499
	Mefenemic acid	0.358	0.358
Time from banding to chromatography (+ 10min)	Camylofin dihydrochloride	0.362	0.371
	Mefenemic acid	0.694	0.686

*Denotes average of three estimation at each level

Table 5: Results of sample analysis for the proposed method

Brand	Analyte	Label claim / tablet (mg)	% Found (mean+SD)	% RSD
Bigspas M	Camylofin dihydrochloride	50	99.68+0.31	0.3301
	Mefenemic acid	250	99.84+0.25	0.2841

CONCLUSION

HPTLC determination of Camylofin dihydrochloride and Mefenemic acid from pharmaceutical capsule dosage form revealed no interference between two drugs and excipients of the marketed capsule contents. The method is rapid, allowing a high sample throughput necessary for routine analysis with an added advantage of low solvent consumption. Also the method is simple, rapid, specific and well suited for quantitative estimation of both drugs individually from bulk drug and from pharmaceutical preparations.

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