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

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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 270 nm. The saturation time of the chamber and the developing distance was set at 30 minutes and 8 cm respectively. The method was validated as per ICH guidelines. Results: The Rf values found to be 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 1600-2400 ng / 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-diethylaminothiopropylamino) - 2-phenyl-acetate hydrochloride is a drug used an antispasmodic[1]. Mefenamic acid is 2-[[2, 3-dimethyphenyl] 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 Mefenamic 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 Mefenamic Acid in pharmaceutical formulation. The chemical structures of the drug are shown in Fig. 1.

MATERIAL AND METHODS

Chemicals and Reagents

Analytical pure samples of Camylofin dihydrochloride powder and Mefenamic acid powder were procured as gift samples from Ideal pharmaceuticals and Mefenamic acid powder were procured as gift samples from Ideal pharmaceuticals and research institution, Puducherry, India. Commercial tablets (Bigspas - M, Mankind Pharma Ltd, AODRL022) containing Camylofin dihydrochloride (50mg) and Mefenamic 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 aluminium 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 side of the plate, under a continuous flow of nitrogen, by means of a CAMAG Linomat-5 sample applicator fitted with a 100μL syringe.

A constant application rate of 150 Nl. s-1 was used. The plate was then placed in pressaturated twin trough chamber (CAMAG; 10 x 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 x 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).

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

Camylofin dihydrochloride (C19H24N2O5.2HCl)

Mefenamic acid (C13H13NO5)
Preparation of Standard solution

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

Preparation of Sample solution

To determine the amount of Camylofin dihydrochloride and Mefenamic 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 50 mg of Camylofin dihydrochloride [250 mg of Mefenamic acid] was weighed and transferred into a 100 mL 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 Mefenamic 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 Mefenamic 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 Mefenamic acid were analyzed by the developed method.

Linearity and range

Calibration curves were constructed in the concentration range of 320-4800 ng/band for Camylofin dihydrochloride 1600–2400 ng/band for Mefenamic 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 Mefenamic acid was serially diluted to five mixed standard solutions. A volume of 2 µL 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 Mefenamic 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 Mefenamic acid respectively. The intermediate precision of method was checked by repeating the study on different days.

Recovery studies

Recovery determination for Camylofin dihydrochloride and Mefenamic 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 Rf 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 Rf values of 0.46 and 0.35 for Camylofin dihydrochloride and Mefenamic 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 Mefenamic 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.
Table 1: Summary of linear regression data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Camylofin Dihydrochloride</th>
<th>Mefenemic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range</td>
<td>320-480 ng / band</td>
<td>1600-2400 ng / band</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>( y = 5.72x - 30.2 )</td>
<td>( y = 11.43 + 862.2 )</td>
</tr>
<tr>
<td>Slope + SD</td>
<td>5.7 ± 0.412</td>
<td>11.43 ± 0.381</td>
</tr>
<tr>
<td>Intercept + SD</td>
<td>30.2 ± 0.258</td>
<td>862.2 ± 1.478</td>
</tr>
<tr>
<td>Correlation coefficient ( r^2 )</td>
<td>0.9993</td>
<td>0.9989</td>
</tr>
</tbody>
</table>

Fig. 3: Chromatogram of placebo

Fig. 4: Calibration curve plot for Camylofin dihydrochloride

Fig. 5: Residual plot of camylofin dihydrochloride
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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Camylofin dihydrochloride</th>
<th>Mefenemic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Label claim, Intra Assay</td>
<td>% Label claim, Intra Assay</td>
</tr>
<tr>
<td>1.</td>
<td>100.17</td>
<td>101.33</td>
</tr>
<tr>
<td>2.</td>
<td>100.89</td>
<td>101.66</td>
</tr>
<tr>
<td>3.</td>
<td>100.79</td>
<td>100.23</td>
</tr>
<tr>
<td>4.</td>
<td>100.93</td>
<td>100.34</td>
</tr>
<tr>
<td>5.</td>
<td>100.38</td>
<td>101.76</td>
</tr>
<tr>
<td>6.</td>
<td>100.78</td>
<td>100.67</td>
</tr>
<tr>
<td>Mean</td>
<td>100.65</td>
<td>100.99</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.3063</td>
<td>0.6655</td>
</tr>
<tr>
<td>Grand Mean</td>
<td>100.62</td>
<td>101.95</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.2768</td>
<td>0.8469</td>
</tr>
<tr>
<td>S.E</td>
<td>0.1136</td>
<td>0.3490</td>
</tr>
<tr>
<td>95% CI</td>
<td>±0.222</td>
<td>±0.684</td>
</tr>
</tbody>
</table>

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

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

* Denotes average of three estimations of each level of recovery
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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REFERENCE