Simultaneous analysis of propranolol HCl and hydrochlorothiazide by HPTLC

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ABSTRACT

High performance thin layer chromatographic method has been developed for the simultaneous determination of Propranolol hydrochloride and Hydrochlorothiazide from bulk and formulations. Chromatographic separation was achieved on aluminum foil plates precoated with silica gel 60F254, with chloroform: ethyl acetate: methanol 4: 4: 2 (v/v/v) as mobile phase. Detection was performed densitometrically at 274 nm. The Rf of Propranolol hydrochloride and Hydrochlorothiazide were 0.27±0.02 and 0.56±0.02, respectively. Parameters such as linearity, precision, accuracy, specificity and robustness are studied as reported in the ICH guidelines. Linearity was observed in the concentration range of 160-960 ng/band for Propranolol hydrochloride and 100-600 ng/band for Hydrochlorothiazide. The mean recoveries obtained for Propranolol hydrochloride and Hydrochlorothiazide were 99.63 % and 99.15 % respectively. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Propranolol hydrochloride and Hydrochlorothiazide.

Keywords: Propranolol hydrochloride, Hydrochlorothiazide, HPTLC, Validation

INTRODUCTION

Propranolol hydrochloride (PHCl), (+)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride, is a non-selective beta blocker mainly used in the treatment of hypertension [1-2]. Hydrochlorothiazide (HCTZ) chemically, 6-Chloro-3, 4-dihydro-2H-I, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, is a thiazide diuretic used as antihypertensive agent [3-4]. Propranolol hydrochloride and Hydrochlorothiazide are official in IP and USP [5-6].

Literature survey revealed that there are several methods available to determine PHCl and HCTZ either alone or in combination with other drugs in pharmaceutical formulations and biological fluids using various analytical techniques such as spectrophotometric techniques [7-11], several methods based on separation techniques, including HPTLC [12-14], LC–MS [15-16] and HPLC [17-22] have been also reported. There is no HPTLC method for the simultaneous determination of these drugs in combined dosage form. Hence a new HPTLC method has been developed for the estimation of PHCl and HCTZ in combined dosage form. The developed method is simple, precise, selective, and rapid and can be used for routine analysis. The structures of the drugs are shown in Fig.1.
MATERIALS AND METHODS

Chemicals and Reagents:
Reference standards of Propranolol hydrochloride and Hydrochlorothiazide were obtained as gift samples from Piramal Healthcare Ltd. Mumbai and Emcure Pharmaceuticals Ltd., Pune, India. The tablets Ciplar-H (Propranolol HCl-40 mg and Hydrochlorothiazide- 25 mg) of Cipla Ltd. is procured from the local market. Chloroform, ethyl acetate and methanol were of AR grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation and Experimental Conditions:
The Camag TLC consisted of Linomat V sample applicator (Camag, Muttenz, Switzerland). Camag TLC scanner III controlled by WinCATS software (V 3.15, Camag) was used for sample application and quantitative evaluation.

Chromatography was performed on Merck silica gel 60 F254 precoated aluminum TLC plate (10 cm × 10 cm with 250 µm thickness), with chloroform: ethyl acetate: methanol in the ratio of 4: 4: 2 v/v/v as mobile phase. Samples were applied as bands 6 mm long at 6 mm interval under a stream of nitrogen with a Camag 100 microlitre syringe (Hamilton, Bonded, Switzerland). The slit dimension was 5 mm × 0.45 mm. Ascending development to a distance of 8 cm was performed in a 20 min. presaturated 10 cm × 10 cm twin trough TLC developing chamber (Camag). Densitometric scanning was performed in absorbance mode at wavelength of 274 nm.

Preparation of standard and sample solutions
A standard mixed stock solution of Propranolol hydrochloride and Hydrochlorothiazide was prepared by accurately weighing Propranolol hydrochloride(40 mg) and Hydrochlorothiazide (25 mg) into a 25 mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume. The stock solution was further diluted with methanol to obtain a solution of PHCL (160 µg/mL or 160 ng/band) and HCTZ (100 µg/mL or 100 ng/band), respectively.

Twenty tablets of the pharmaceutical formulation Ciplar-H (40 mg Propranolol HCl and 25 mg Hydrochlorothiazide) were accurately weighed and average weight determined. They were crushed to a fine powder and an amount of the powder equivalent to 40 mg PHCl and 25 mg HCTZ was weighed and transferred into 25 mL volumetric flask and dissolved in 15 mL of methanol, sonicated for 15 min. and diluted to mark with same solvent and filtered through whatman filter paper no.1. The sample solution was further diluted with methanol. The analysis was repeated in triplicate. The possibility of excipients interference with the analysis was examined.

Analytical Method Validation:
Validation was done as per ICH guidelines [23]. The developed method was validated with respect to parameters such as linearity, LOD and LOQ, precision, accuracy and specificity.

Linearity
Standard solutions of PHCl and HCTZ(1-6 µL) were applied on TLC plate to obtain concentrations 160 - 960 ng/band for PHCl and 100 - 600 ng/band for HCTZ, respectively. Chromatograms were recorded. Calibration plots were constructed by plotting peak area against the corresponding amount of each drug.

Limit of detection and limit of quantitation:
LOD and LOQ were experimentally verified by spotting a series of dilute standard solutions of known concentrations of PHCl and HCTZ until the average responses were approximately 3 or 10 times the standard deviation of the responses for three replicate determinations.
Precision:
The precision of the method was assessed by intraday and interday studies. Intraday variations were performed by analysis of three different concentrations (320, 640, and 960 ng/band for PHCl and 200, 400, and 600 ng/band for HCTZ) of the drugs three times on the same day. The interday variations were similarly evaluated over a period of 3 days.

Repeatability of measurement:
1 µL of the standard solution PHCl (160 ng/band) and HCTZ (100 ng/band) were spotted on a TLC plate, developed, dried and the spots were scanned seven times without changing the plate position and % RSD for measurement of peak area for both the drugs were calculated to determine the instrumental precision.

Repeatability of sample application:
1 µL of the standard solution PHCl (160 ng/band) and HCTZ (100 ng/band) were applied six times on a TLC plate, developed, dried and the spots were scanned and % RSD for measurement of peak area for both the drugs were calculated to determine the repeatability.

Robustness:
Robustness of the method was performed by making small deliberate changes in chromatographic conditions. A study was performed by changing the mobile phase composition, amount of mobile phase, development distance, duration of saturation time of chamber and the time from spotting to chromatography and from chromatography to scanning. Standard solution prepared as per test method and chromatograms were run.

Accuracy:
Accuracy was done in terms of recovery studies. Recovery studies were carried out by standard addition method. The analysed samples were spiked with extra 80%, 100% and 120% of standard drugs. The mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate.

Specificity:
The specificity of the method was ascertained by analyzing standard drugs and samples extracted from formulations. The spots for PHCl and HCTZ were confirmed by comparing the Rf of the sample with those of the standard.

RESULTS AND DISCUSSION

HPTLC method development:
HPTLC method was optimized with a view to develop a simultaneous determination for PHCl and HCTZ from tablet formulations. The mixed standard stock solution (160 ng/band of PHCl and 100 ng/band of HCTZ) were taken and applied on to TLC plates and run in different solvent systems. After many trials it was found that chloroform: ethyl acetate: methanol in the ratio of 4: 4: 2 (v/v/v) gave a good resolution, sharp and symmetrical peaks with Rf of 0.27±0.02 for PHCl and 0.56±0.02 for HCTZ, respectively. Wavelength of 274 nm was selected for densitometric evaluation, since both the drugs show good absorption.

Validation:
Using optimised chromatographic conditions the HPTLC method developed was validated in terms of specificity, linearity, LOD and LOQ, precision, robustness and accuracy.

Specificity:
The method is specific for PHCl and HCTZ, since it resolved the peak of PHCl (Rf = 0.27±0.02) and HCTZ (Rf = 0.56±0.02) in presence of other excipients in the formulation (Fig. 2).
Linearity:
Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 160–960 ng/band for PHCl and 100–600 ng/band for HCTZ. The linear regression equations were $Y = 7.252X + 2805$ ($r^2 = 0.9998$) for PHCl and $Y = 12.38X + 1628$ ($r^2 = 0.9998$) for HCTZ. The residuals plot shows a random pattern, indicating a good fit for a linear model. The calibration curves and residuals plots are given in Fig. 3 for PHCl and Fig. 4 for HCTZ, respectively.

![Fig. 2. Typical densitogram of PHCl (peak 1) and HCTZ (peak 2)](image)

![Fig. 3. Calibration curve and residual plot for PHCl](image)
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Fig. 4. Calibration curve and residual plot for HCTZ

Limits of Detection and Quantitation:
The results of the LOD and LOQ were found to be 80 ng/band and 135 ng/band for PHCl and 40 ng/band and 60 ng/band for HCTZ, respectively.

Precision:
The precision of the developed method was demonstrated by intraday and interday precision studies. This was done by three replicate analysis of the composite sample. The precision of the method was expressed as relative standard deviation (RSD, %).

The values less than 2.0 indicate that there were no significant variations in the analysis of PHCl and HCTZ at the given concentration levels (Table 1).

Table 1: Precision studies of PHCl and HCTZ

<table>
<thead>
<tr>
<th>Conc. (ng/spot)</th>
<th>Intra-day precision (n=3)</th>
<th>Inter-day precision (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured Conc. ± SD</td>
<td>(%) RSD</td>
</tr>
<tr>
<td>Propranolol hydrochloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>318.56 ± 3.50</td>
<td>1.10</td>
</tr>
<tr>
<td>640</td>
<td>638.10 ± 7.00</td>
<td>1.09</td>
</tr>
<tr>
<td>960</td>
<td>959.02 ± 10.84</td>
<td>1.13</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>198.24 ± 2.24</td>
<td>1.13</td>
</tr>
<tr>
<td>400</td>
<td>398.76 ± 4.90</td>
<td>1.23</td>
</tr>
<tr>
<td>600</td>
<td>598.56 ± 6.82</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Instrumental precision:
The % RSD for 160 ng/band of PHCl and 100 ng/band of HCTZ (n=6) was found to be 0.941 and 0.872, respectively.

Repeatability of sample application:
The mean % RSD for peak area was found to be 0.641 for PHCl and 0.982 for HCTZ, respectively.

Robustness:
The robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions and there were no significant changes in the retention times of PHCl and HCTZ. The low values of the % RSD indicate the robustness of the method, as shown in Table 2.
Table 2: Robustness evaluation of PHCI and HCTZ (n=3)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PHCI</th>
<th>HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD of the peak area</td>
<td>% RSD</td>
</tr>
<tr>
<td>A: Mobile phase composition (± 0.1 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform: ethyl acetate: methanol (4.1: 4.1: 2 v/v/v)</td>
<td>15.033</td>
<td>1.16</td>
</tr>
<tr>
<td>Chloroform: ethyl acetate: methanol (3.9: 3.9: 2 v/v/v)</td>
<td>20.82</td>
<td>1.25</td>
</tr>
<tr>
<td>B: Mobile phase volume (± 1 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.0 mL</td>
<td>7.12</td>
<td>1.01</td>
</tr>
<tr>
<td>12.0 mL</td>
<td>10.11</td>
<td>1.03</td>
</tr>
<tr>
<td>C: Development distance (± 0.5 cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5 cm</td>
<td>9.28</td>
<td>1.24</td>
</tr>
<tr>
<td>8.5 cm</td>
<td>11.39</td>
<td>1.04</td>
</tr>
<tr>
<td>D: Duration of saturation (± 5 min.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 min</td>
<td>10.21</td>
<td>1.26</td>
</tr>
<tr>
<td>30 min</td>
<td>12.08</td>
<td>1.16</td>
</tr>
<tr>
<td>Time from application to chromatography (+10 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.69</td>
<td>1.14</td>
<td>12.83</td>
</tr>
<tr>
<td>Time from chromatography to scanning (+10 min)</td>
<td>13.10</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Accuracy:
Accuracy of the method was evaluated by carrying out recovery study. A known concentration of the standard drug was added to a preanalysed tablet sample at three different levels namely 80 %, 100 % and 120 %. Each level was repeated three times. Total amount of the drug was determined by the proposed method. The percentage recovery was calculated. (Table 3).

Table 3: Recovery study of PHCL and HCTZ (n=3)

<table>
<thead>
<tr>
<th>Label claim (mg/tablet)</th>
<th>Amount Added (%)</th>
<th>Total amount (mg)</th>
<th>Amount recovered (mg)</th>
<th>Recovery (%)</th>
<th>Mean (% Recovery (± SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHCl 40</td>
<td>80</td>
<td>72</td>
<td>71.63</td>
<td>99.49</td>
<td>99.63 ± 0.127</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>80</td>
<td>79.79</td>
<td>99.74</td>
<td>99.74 ± 0.027</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>88</td>
<td>87.69</td>
<td>99.65</td>
<td>99.65 ± 0.027</td>
</tr>
<tr>
<td>HCTZ 25</td>
<td>80</td>
<td>45</td>
<td>44.58</td>
<td>99.07</td>
<td>99.15 ± 0.116</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>50</td>
<td>49.64</td>
<td>99.28</td>
<td>99.28 ± 0.087</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>55</td>
<td>54.30</td>
<td>99.09</td>
<td>99.09 ± 0.087</td>
</tr>
</tbody>
</table>

Analysis of marketed formulation:
Two spots at Rf of 0.27 PHCl and 0.56 HCTZ were observed in the chromatogram of the drug samples extracted from conventional tablets. There was no interference from the excipients commonly present in the conventional tablets. The % drug content found for PHCl and HCTZ were 99.65 % and 99.60%, respectively. This indicates that there is no degradation of PHCl and HCTZ in the marketed formulations that were analyzed by this method.

CONCLUSION
In the present study a HPTLC method has been developed for simultaneous estimation of propranolol hydrochloride and hydrochlorothiazide in bulk and its formulation. The proposed method was sufficiently sensitive and reproducible for the analysis of the PHCl and HCTZ tablet dosage form. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with the determination of PHCl and HCTZ. The method is simple, precise, specific, and accurate and can be used for the routine simultaneous analysis of the PHCl and HCTZ in pharmaceutical preparations.

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