Stability indicating HPTLC determination of pimozide in bulk and pharmaceutical dosage form

A. S. Manjula Devi* and T. K. Ravi

Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore

ABSTRACT

A simple, selective, precise and stability indicating high-performance liquid chromatographic method was developed and validated for the determination of pimozide in bulk and pharmaceutical dosage form. The method employed aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene:acetone:ammonia (5:5:0.1 v/v/v). Compact spot was observed with Rf value 0.55±0.0052. Densitometric analysis of pimozide was carried out in the reflectance mode at 285 nm. Good linear relationship was obtained in the concentration range 80 to 360 ng/spot. The method was validated for specificity, accuracy, precision and robustness. The limit of detection and quantification were 8.855 ng/spot and 26.834 ng/ spot, respectively. Pimozide was subjected to acid and alkali hydrolysis, oxidation, photolysis and thermal degradation. The drug undergoes degradation under acid and base. The proposed HPTLC method can be applied for identification and quantitative determination of pimozide in bulk and tablet dosage form.

Key Words: Pimozide, stability indicating, degradation, ICH, validation

INTRODUCTION

Pimozide is a highly potent, long acting specific neuroleptic drug, belonging to the diphenylbutylamine series. Chemically it is 1-[1-[4,4-Bis(p-fluorophenyl)butyl]-4-piperidyl]-2-benzimidazolione. Pimozide is used for the treatment of schizophrenia and other psychiatric diseases. The drug is administered orally in daily doses of 2–8 mg. Pimozide is an alternative to haloperidol approved by United States Food and Drug Administration [1,2]. A Spectrofluorimetric method [3] for the quantitation of pimozide in oral preparations and HPLC method for the determination of pimozide in human plasma [4] have been reported in the literature. But there is no analytical method for the estimation of pimozide in bulk and dosage form by HPTLC. Moreover, none of the reported methods are stability indicating in nature. The International Conference on Harmonization (ICH) entitled ‘stability testing of new drug substances and products’ requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance [5]. An ideal stability indicating method is the one that quantifies the analyte and also resolves its degradation products. HPTLC is becoming a routine analytical technique due to several advantages. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering time and cost per analysis. HPTLC facilitates automated application and scanning in situ. It also facilitates repeated scanning with same or different parameters. Simultaneous assay of several components from a multicomponent formulation is possible [6, 7, 8]. The aim of the present study is to develop and validate an accurate and specific stability indicating HPTLC method for the quantification of pimozide in the presence of its degradation products.
MATERIALS AND METHODS

Materials
A reference standard of pimozide with assigned purity greater than 99% was used in the study. All chemicals and reagents were of analytical grade. Pimozide tablets (label claim of 2 mg of pimozide) were procured from local pharmacy.

Instrumentation
The samples were spotted in the form of bands of 6mm width by means of CAMAG (Muttenz, Switzerland) Linomat 5 sample applicator equipped with a 100 µL syringe (Hamilton, Bonaduz, Switzerland) on a 10 x 10 cm aluminum sheet pre-coated with silica gel 60F254 of 250 µm thickness (E. Merck, Darmstadt, Germany). The mobile phase consisted of toluene:acetone:ammonia (5:5:0.1 v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The chamber saturation time for the mobile phase was optimized to 10 min to ensure a concentrated zone of the component and hence better resolution. Approximate development distance was 80 mm. Subsequent to development, the TLC plate was air dried and densitometric scanning was performed on Camag TLC scanner 3 in the 285 nm in reflectance mode. The source of radiation used was the deuterium lamp.

Optimization of variants in TLC and fixing of initial chromatographic conditions
Mobile phase composition, chamber saturation time, plate equilibration time, band width of the spot and solvent front were varied and their effect on the $R_f$ value of the drugs were evaluated. Solubility of pimozide and polarity of solvent system were taken into consideration in this stage. Following the development of chromatogram, the bands were scanned in the range of 200-400nm and detection wavelength was selected. Chamber saturation time of 10 to 20 minutes was tried. Plate equilibration time also has a major effect in obtaining reproducible results in HPTLC. The mobile phase was taken in one side of a twin trough chamber and spotted plates on the other side. The plates were kept for equilibrium with the vapour phase for 25 minutes. In order to determine the effect of solvent front, the plates were developed with a distance of solvent front ranging between 7.0 to 9.0 cm. Band width was optimized through several trials to obtain good peak shape and reproducibility of $R_f$ values. The effect was also studied at lowest and highest volume spotted (0.5-2µL). After development, densitometric evaluations were carried out in order to understand the effect of the above mentioned variables over the peak shape and $R_f$ values of pimozide and thus to fix the initial chromatographic conditions.

Method validation
The developed method was validated as per ICH recommendations [9] in terms of linearity and range, specificity, accuracy, precision, limit of detection and limit of quantification.

Linearity
Stock solution of pimozide was prepared by dissolving 10 mg of standard pimozide in 50 ml methanol. Different volumes of stock solution viz., 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 µL were spotted on TLC plates to obtain concentrations of 80, 120, 160, 200, 240, 280, 320 and 360 ng/spot, developed and evaluated densitometrically. The data of peak area versus concentration was treated by linear least-square regression.

LOD and LOQ: The LOD and LOQ were determined using the calibration curve using the following equations. 
LOD = 3.3xD/S and LOQ = 10 x D/S, where, D is the standard deviation of $y$-intercept of regression line and S is the slope of the calibration curve.

Accuracy
The analyzed samples were spiked with extra 50, 100 and 150% of the standard pimozide and the mixtures were analyzed by the proposed method. At each level, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

Precision
Repeatability of sample application and measurement of peak area was carried out using six replicates of the same spot (120 ng/spot of pimozide). The intra- and inter-day variation for the determination of pimozide was carried out at three different concentration levels (80, 200 and 360 ng/spot).
Robustness
By introducing small changes in the mobile phase composition, its volume, chamber saturation time and solvent migration distance, their effects on the results were analyzed. Robustness of the method was evaluated at 200 ng/spot and the %RSD of the peak area was calculated.

Stability in sample solution: Stability was evaluated after storing the stock solutions at room temperature for 24 hours and under refrigeration for 5 days. A concentration of 360 ng/spot was employed for densitometric analysis

Assay of marketed formulations
To determine the content of pimozide in formulation, the tablets were powdered and powder equivalent to 10 mg of pimozide was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 20 min and volume was made up to 50 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. Then 0.5 µL of the filtered solution (100 ng/spot) was applied on TLC plate followed by development and scanning as described earlier. The analysis was repeated in six replicates. The possibility of excipient interference in the analysis was studied.

Forced degradation studies
Four samples were used in the experiment, viz., the blank solution stored under normal condition, the blank subjected to stress in the same manner as the drug solution, zero time sample containing the drug which was stored under normal conditions and the drug solution subjected to stress treatment. The study was conducted separately for pimozide in bulk followed by formulation.

Acid and base induced degradation
Both acid and base induced degradation studies were carried out by separately dissolving 10 mg of pimozide in 50 ml methanolic solution of 0.01 M HCl and 0.01M NaOH. These solutions were refluxed at 70°C for 3.5 hrs and 1 hour respectively. The resultant solutions were applied on TLC plates (360 ng/spot) and chromatograms were run as described earlier.

Hydrogen peroxide-induced degradation:
10 mg of pimozide was dissolved in 50 ml of methanolic solution of hydrogen peroxide (3%v/v) and kept at room temperature for 24 hours. Appropriate volume of the above solution was spotted on TLC plate, developed and analyzed densitometrically at 285 nm.

Thermal hydrolysis
The standard drug was kept in oven at 70°C for 8 hours, dissolved in methanol, and analyzed densitometrically at 285 nm. Peak area of pimozide after application of 360 ng/spot was obtained.

Photolysis
Photochemical stability of pimozide was studied by exposing the stock solution to direct sunlight for 5 hours. Appropriate volume was spotted and chromatograms were run as mentioned before.

RESULTS AND DISCUSSION
Maximum absorption of pimozide was found to be at 285 nm. The spot of pimozide in sample was confirmed by comparing the $R_f$ values and spectra of the spot with that of standard. Well-defined spots were obtained with toluene:acetone:ammonia (5:5:0.1 v/v/v) as the mobile phase when the chamber was saturated with the mobile phase for 10 min at room temperature. Among the various mobile phase tried, the $R_f$ value was less than 0.1 with toluene:dioxane (5:5 v/v), toluene:acetone (7:3 v/v), toluene:ethyl acetate (7:3 v/v) while it was more than 0.9 with methanol:dioxane (5:5 v/v). Compact spot with desired $R_f$ at 0.55 was given by a combination of toluene:acetone (5:5 v/v), but the peak was fronting. Therefore, a drop of ammonia was added which resulted in symmetrical peak of pimozide.
Table 1: Summary of validation parameters

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>Data obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (ng/spot)</td>
<td>80 – 360 ng/spot</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9993±0.00054</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99.87±0.6477</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
</tr>
<tr>
<td>Intra-day</td>
<td>0.9952</td>
</tr>
<tr>
<td>Inter-day</td>
<td>0.5527</td>
</tr>
<tr>
<td>Repeatability of sample</td>
<td></td>
</tr>
<tr>
<td>application</td>
<td>1.3055</td>
</tr>
<tr>
<td>Repeatability of sample</td>
<td></td>
</tr>
<tr>
<td>measurement</td>
<td>0.1101</td>
</tr>
<tr>
<td>Robustness</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Summary of degradation studies of pimozide

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>%Drug remaining</th>
<th>( R_f )</th>
<th>( D_p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01M HCL, reflux at 70°C for 3.5 hrs</td>
<td>78.709</td>
<td>0.58</td>
<td>0.33</td>
</tr>
<tr>
<td>Base, 0.01 M NaOH, reflux at 70°C for 1 hr</td>
<td>88.395</td>
<td>0.58</td>
<td>0.33</td>
</tr>
<tr>
<td>Neutral, reflux at 80°C for 8 hours</td>
<td>98.92</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Oxidative degradation, 30% ( H_2O_2 ) for 24 hours</td>
<td>98.04</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Dry heat, 70°C for 4 hrs</td>
<td>99.01</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Photolysis, direct sunlight for 12 hrs</td>
<td>98.33</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Densitogram of 0.01M HCl treated pimozide

The linear regression data for the calibration curves showed good linear relationship over the concentration range 80 to 360 ng per spot with respect to peak area. The regression equation and correlation coefficient \( r^2 \) obtained was 4.157X+17.4363 and 0.9993±0.00054 respectively. The detection limit and quantification limit of pimozide by the current method was 8.855 ng/spot and 26.834 ng/spot respectively. The proposed HPTLC method, when used for the extraction and subsequent quantification of pimozide from pharmaceutical dosage forms after spiking with 50, 100 and 150% of additional drug offered recovery of 99.39 to 99.9005%. The repeatability of sample application and measurement of peak area were expressed in terms of % R.S.D and found to be 1.3055 and 0.11014 respectively. The intra- and inter-day variations of pimozide at three different concentration levels were less than 0.9952 and 1.0935 respectively. The %RSD was found to be less than 2 for each parameter studied which indicated the robustness of the method. A single spot of \( R_f \) 0.55 was observed in the chromatogram of pimozide samples extracted from tablets. There was no interference from the excipients commonly present in tablet formulations. Pimozide content was found to be 100.097% with %RSD of 1.1738. Low %RSD values indicated the suitability of the present method for routine analysis in pharmaceutical dosage form. Results of validation are summarized in table 1. The drug was found to be stable in solution state during the HPTLC analysis for 24 hours in room temperature.
There was no indication of compound instability in solution for 5 days under refrigeration. The chromatograms of pimozide samples degraded with 0.01M HCl and 0.01M NaOH showed well-separated peaks of pimozide and degradant peak at $R_f$ value 0.33 (figure 1 and 2). Figure and 3 and 4 shows the overlay spectra of drug with acid and base induced degradant. Under mild acidic conditions, 21.29% of pimozide has undergone degradation while it was found to be 11.6% in alkaline environment. The amount of pimozide remained after forced degradation were calculated and listed in Table 2. The drug was found to be stable to neutral hydrolysis, oxidation, dry heat, and sunlight.

Figure 2: Densitogram of 0.01M NaOH treated pimozide

Figure 3: Overlay spectrum of pimozide and acid degradant
CONCLUSION

The developed HPTLC method is highly selective, sensitive, repeatable and stability indicating. The proposed methods were applied for the analysis pimozide in tablets. Statistical analysis proved that the method is precise and reproducible. The system being economical can be employed for the routine estimation of the drug in pharmaceutical formulations as well as in bulk drug analysis. The developed method may be extended to study the degradation kinetics of pimozide.

Acknowledgement

The authors would like to thank the management, SNR Sons Charitable Trust, Coimbatore for providing samples and reagents from industries to carry out the study.

REFERENCES