Ion-pair Formation for the Determination of Mianserin Using Fast Sulphon Black F

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Abstract

Aim: The objective of the current study is to develop a colorimetric method for the determination of mianserin, an antidepressant drug. Materials and Methods: Fast Sulphon Black F, an acidic dye was used to develop a soluble colored ion-pair complex. The complex was extracted into an organic solvent and absorbance was measured. Results: Reaction conditions were optimized to obtain a sensitive and stable chromophore (λ_max 554 nm) in dichloromethane. Good linearity was observed for the calibration curve plotted in the studied concentration range (4–14 μg/mL) with regression analysis (r > 0.9997). High percentage recovery values (98.25–101.40) show that the method is accurate. Reproducibility of the method is evident from lower relative standard deviation (<2%) for both intra- and inter-day precision studies. Conclusions: The proposed method is validated as per the existing ICH guidelines. This method is simple as it does not require any pre-treatment process.

Key words: Assay, Fast Sulphon Black F, ion-pair complex, method development, mianserin, validation

INTRODUCTION

Mianserin is used to get relief from depression by working on nerve cells of brain. It is metabolized in liver by enzyme cytochrome P450 2D6 through a sequence of reactions such as N-oxidation, aromatic hydroxylation, and N-demethylation. It is a tetracyclic piperazinoazepine with molecular formula C_{20}H_{20}N_{2} [Figure.1].¹,² Hindering the role of L-DOPA antiparkinsonian limits its prospective clinical usage, though it is active in relieving from PD psychosis as well dyskinesia.³ Mianserin modulates (a) the decrease in levels of interleukin-6 and tumor necrosis factor-alpha and (b) regulation of cytokine amounts in stressed animals.⁴ A thorough literature survey shows that spectrophotometric,⁵-⁹ high-performance liquid chromatography,¹⁰-¹³ capillary gas chromatography and electrophoresis,¹⁴-¹⁶ and gas chromatography¹⁷,¹⁸ based analytical methods were published for quantitative determination of it. In view of high cost of the above stated instruments, Fast Sulphon Black F (FSBF) was used as a chromogen for color development to determine mianserin spectrophotometrically in bulk drug as well as tablet dosage forms.

MATERIALS AND METHODS

TECHOMP (UV 2310) double-beam UV-visible spectrophotometer with HITACHI software version 2.0 was used to measure the absorbance. Quartz cuvettes (10 mm path length) were used for the analysis. Digital pH meter (Elico LI-120) and balance (Shimadzu AUX-220) were used to weigh the samples and to measure pH, respectively. Spectroscopic measurements were conducted at room temperature (25 ± 5°C). All chemicals used in the present study were AR grade. In the entire process, used water was double distilled.

Preparation of reagents

FSBF solution

About 300 mg of FSBF is dissolved in 100 mL of distilled water. Then, the solution was washed with chloroform to remove soluble impurities in the organic solvent.

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Preparation of standard drug solution

The standard mianserin (25 mg) was weighed accurately and transferred to 25 mL volumetric flask. It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg/mL (stock solution). 2.0 mL from the stock solution was further diluted to 10.0 mL to get a standard stock solution having 200 µg/mL of mianserin.

RESULTS AND DISCUSSION

Out of the available different techniques, ion association involved colored complex formation is a widespread approach in the quantitative determination of pharmaceutical drugs. This method can be adopted to all those drugs consisting a heteroatom (for example, nitrogen) which bears lone pair of electrons. Hence, they undergo protonation by accepting proton(s) and form a cation. Dyes capable of attaining anionic form can cultivate an ion-pair complex with the above formed cation from a drug. Organic solvent is used to extract this complex and its absorbance is measured by visible spectrophotometry.\(^{[19]}\) Applicability to determine the precise compound even in the presence of different constituents of formulations is the additional benefit of this method. Prompted by the above gains, the current study explains the establishment of a process which is based on the development of a soluble ion-pair complex in the presence of an acidic chromogenic dye like FSBF. The developed chromophore has shown an absorption maximum at 554 nm [Figure 2].

Optimization of reactions conditions

Reaction conditions were optimized at 30 ± 1°C (ambient temperature). At initial volumes of 0.1 N HCl, absorption increased with an increase of acid volume up to 4 mL. Further, increase in acid volume resulted in a decrease of the absorbance due to reversal of FSBF hydrolysis which results in lowering the availability of the number of FSBF anions [Figure 3a]. 1.5 mL of FSBF (0.2% w/v) was the optimized conditions for dye solution [Figure 3b]. Instantaneous development of color after mixing of reactants and persistence of color intensity for long hours was witnessed. Dichloromethane was recognized as the best solvent for extraction among the tried solvents (\(\text{CH}_2\text{Cl}_2\), \(\text{CHCl}_3\), \(\text{C}_6\text{H}_5\text{Cl}\), \(\text{C}_6\text{H}_5\text{NO}_2\), and \(\text{C}_6\text{H}_5\text{NH}_2\)) [Figure 4]. Persistent higher absorbance was resulted for the addition of 10 mL dichloromethane (organic solvent) to 15 mL of aqueous layer. Hence, contact time was fixed as 2 min.
between the optimized volumes of organic and aqueous phases (2:3 v/v). Operative successive addition mode of reagents was mianserin, acid and dye solution. Development of 3:1 ion-pair complex between protonated mianserin and FSBF anion was established from Job’s continuation method. Scheme 1 demonstrates the formation of colored ion-pair complex between mianserin cation (MH+) and FSBF\(^{-3}\) anion.

**Optimized method procedure**

Appropriate aliquots of the standard solution of mianserin (200 µg/mL) were transferred into an array of separating funnels of 125 mL volume each. Sequential addition of HCl (4.0 mL of 0.1 N conc.) and FSBF solution (1.5 mL of 0.3% concentration) was followed by it. The total volume of aqueous layer was made to 15 mL by the addition of distilled water. After addition of 10 mL of dichloromethane, the contents were shaken for 2 min. Separated the organic layer from aqueous and absorbance values of organic layers was measured.

**Chromophore formation and chemistry**

The three sulfonic acid groups present in FSBF undergo hydrolysis in aqueous medium to form a tribasic anion.\(^{[21]}\) Formation of an ion-pair complex with a stoichiometry of 1:1 explains that only one nitrogen of mianserin is protonated out of the available two nitrogen on it. Perhaps, the lone pair of electrons existing on the other nitrogen (of azepine) is engaged in resonance with the neighboring benzene ring. Therefore, lone pair of electrons present on the second nitrogen is not available for protonation. Hence, the second nitrogen is not protonated. Hence, tribasic charged FSBF anion attracts three monoprotonated mianserin cations (formed in acid medium). Electrostatic attraction between these oppositely charged ions helps them to keep together and acts as a single unit. The chemical reactions involved in the formation of colored ion-pair complex are shown in Figure 5.

**Validation of method**

**Linearity and range**

Development of color was carried out with mianserin in a concentration range of 4–14 µg mL\(^{-1}\) by adopting the above developed procedure. Thrice measured the absorbance for each concentration of mianserin and their mean value was noted [Table 1]. A linear calibration curve was obtained by plotting mean absorbance values versus concentrations of mianserin [Figure 6]. Linear regression of the data resulted the equation \( y = 0.0755x - 0.0147 \) with a correlation coefficient >0.9997. Hence, linearity of the proposed analytical method was tested. Table 2 represents key parameters of method development and validation.

**Accuracy**

The proposed method’s accuracy was tested by studying percentage recovery studies and the obtained results were noted in Table 3. To perform this, various quantities of mianserin (range of 50–150%) were supplemented to constant amount of drug to maintain total concentration within linearity range. The obtained recovery percentages are in the range of 98.25–101.40. The proposed method is found to be accurate because SD as well as percentage relative standard deviation (RSD) values are <1%.

**Precision**

Both precisions of this method were checked by choosing three different concentrations in the linearity range
Scheme 1: Colored ion-pair complex formation

Figure 5: Reaction of mianserin with Fast Sulphon Black F
A sequence of six independent analyses was performed for each concentration on 6 concurrent days [Table 4]. Precision studies of the current method were found to be satisfied because percentage RSD values for interday and intraday were in the range of 1.88–1.97 and 1.57–1.98, respectively.

Ruggeness

Under the optimized conditions, the current proposed method’s ruggedness was appraised by carrying out mianserin assay by two different analysts on different days at three different quantities (4, 16, and 24 μg/mL). The obtained results are reproducible as there is no significant difference in the values produced by different analysts [Table 5]. Hence, the ruggedness of this method was confirmed.

Quantification and detection limits

To estimate the present method’s sensitivity, limit of detection (LOD) and limit of quantification (LOQ) values were calculated as per the ICH guidelines (2005) using formulae (3.3 × σ/S) and (10 × σ/S), respectively,[22,23] where S (calibration curve slope) and σ (SD of the response). The resultant obtained values are given below.

LOD = 0.60 μg/mL and

LOQ = 1.8 μg/mL

Analysis of pharmaceutical formulations

Chromophore was generated with the extracts of mianserin tablets (Deipnon®) by following the above developed procedure and measured the absorbance values to determine API quantity in the tablet formulation by considering average weight as basis [Table 6]. The above developed method can be extended successfully to determine the mianserin amount present in of Deipnon, tablet formulation due to excellent recovery values of API. It shows the non-interference of common excipients in this method. Spectrophotometry is the best selected analytical technique in quality control laboratories of developing and underdeveloped countries.[24-31]

Therefore, the above developed method involving ion-pair formation by mianserin using a chromogen (FSBF) can be extended to determine its quantity in pure and tablet formulations.

CONCLUSIONS

The proposed method comprising FSBF as an ion-pair forming agent is simple due to no requirement to maintain intricate reaction conditions (such as elaborate procedure for sample treatment and maintenance of critical optimum pH). Moreover, it is not necessary of using high-end costly instruments. These profits inspire the adaptation of this method in quality control divisions for mianserin routine analysis in tablet formulation as well as bulk drug.
Table 3: Recovery of mianserin

<table>
<thead>
<tr>
<th>Level of recovery (%)</th>
<th>Amount of drug recovered (µg/mL) (practical)</th>
<th>Statistical evaluation</th>
<th>% Recovery = Practical x 100 / Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>5.97</td>
<td>Mean 5.92</td>
<td>99.50</td>
</tr>
<tr>
<td></td>
<td>5.87</td>
<td>SD 0.041</td>
<td>97.83</td>
</tr>
<tr>
<td></td>
<td>5.93</td>
<td>% RSD 0.694</td>
<td>98.83</td>
</tr>
<tr>
<td>100</td>
<td>7.86</td>
<td>Mean 7.88</td>
<td>98.25</td>
</tr>
<tr>
<td></td>
<td>7.88</td>
<td>SD 0.020</td>
<td>98.50</td>
</tr>
<tr>
<td></td>
<td>7.91</td>
<td>% RSD 0.260</td>
<td>98.87</td>
</tr>
<tr>
<td>150</td>
<td>10.14</td>
<td>Mean 10.05</td>
<td>101.40</td>
</tr>
<tr>
<td></td>
<td>10.05</td>
<td>SD 0.073</td>
<td>100.50</td>
</tr>
<tr>
<td></td>
<td>9.96</td>
<td>% RSD 0.731</td>
<td>99.60</td>
</tr>
</tbody>
</table>

Nominal concentration used (a): 4 µg/mL. Amount of drug added (b): 2, 4, and 6 µg/mL, respectively, for 50%, 100%, and 150% recovery levels. Theoretical amount: Total amount of drug (a+b) = 6, 8, and 10 µg/mL, respectively, for 50%, 100%, and 150% recovery levels.

Table 4: Intraday and interday precision readings

<table>
<thead>
<tr>
<th>Concentration of mianserin (µg/mL)</th>
<th>Concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intraday Mean±SD (µg/mL)</td>
</tr>
<tr>
<td>4</td>
<td>3.956±0.075</td>
</tr>
<tr>
<td>10</td>
<td>9.916±0.196</td>
</tr>
<tr>
<td>14</td>
<td>13.877±0.261</td>
</tr>
</tbody>
</table>

* Average of six determinations

Table 5: Ruggedness data of mianserin

<table>
<thead>
<tr>
<th>Test concentration of mianserin (µg/mL)</th>
<th>Concentration* analyst change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD (µg/mL)</td>
</tr>
<tr>
<td>4</td>
<td>3.969±0.062</td>
</tr>
<tr>
<td>10</td>
<td>9.916±0.196</td>
</tr>
<tr>
<td>14</td>
<td>13.890±0.234</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table 6: Estimation of mianserin from its formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled amount (mg)</th>
<th>Amount found* (mg)</th>
<th>% Drug recovered</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deipnon®</td>
<td>30</td>
<td>29.709±0.184</td>
<td>99.03</td>
<td>0.62</td>
</tr>
</tbody>
</table>

*Average of three determinations

REFERENCES


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