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Original Article

Simultaneous Determination of Fluoxetine hydrochloride and Alprazolam in Its Multicomponent Dosage Form by UV Spectrophotometry

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Abstract

A simple, accurate, precise and rapid UV spectrophotometric method has been developed for the simultaneous estimation of Fluoxetine hydrochloride (FLX) and Alprazolam (APZ) in its bulk and pharmaceutical formulation. The absorbance maximum of both drugs was found out at 252 nm and 285 nm. The overlaid spectra showed maximum absorbance at 280 nm. Both the drugs obey Beer’s Law in the concentration ranges of 2-10 µg/ml (r² =0.9998) and 10-50 µg/ml (r²=0.9995) for Fluoxetine hydrochloride and Alprazolam in acetonitrile as the solvent system. The results of analysis were validated statistically and by doing recovery studies. The present method is found to be simple, precise and accurate and can be easily applied for routine estimation of both the drugs in combined form.

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Keywords: Fluoxetine hydrochloride, Alprazolam, Regression, UV spectrophotometer

1. Introduction

Alprazolam is a short acting anxiolytic of the benzodiazepine class of psychoactive drugs. Chemically it is 8-chloro-1-methyl, 1-6-phenyl-4H-[1, 2, 4] triazolo [4, 3-a][1,4] benzodiazepine. (Figure-1). Alprazolam binds to specific sites on the other GABA, gamma-amino-butyric acid receptor. Alprazolam is used for the medical treatment of panic disorder and anxiety disorders [1, 2, 3]. Fluoxetine hydrochloride (FLX) chemically is (RS)-N-methyl-3-phenyl-3-[4-trifluoromethyl] propan-1-amine. (Figure-2) It is an antidepressant of the selective serotonin receptor inhibitor (SSRI) class. Fluoxetine is used in the treatment of major depression (including pediatric depression), panic disorders and premenstrual dysphonic disorder. It also been used for cataplexy, obesity and depression), panic disorders and premenstrual dysphonic disorder. It also been used for cataplexy, obesity and premenstrual dysphonic disorder.

2. Materials and Methods

2.1 Instrument

UV-Visible Spectrophotometer 1700(model), Schimadzu, connected to the digital system with UV Probe Software was used for the study.

2.2 Reagent and Materials

Working standards of FLX and APZ were procured from Psycorem; Ludhiana. Other chemicals used were of AR grade. Combination of Fluoxetine hydrochloride and Alprazolam formulation was procured from local market.

2.3 Selection of solvent system

FLX and APZ were dissolved separately in acetonitrile and the final volume was made up with the same solvent. The absorbances at respective wavelengths of FLX and APZ were found out.

2.4 Selection of wavelength (λmax)

Standard solutions were scanned in the range of 200-400nm. Acetonitrile was used as the solvent system. FLX (Figure-3) and APZ (Figure-4) showed absorbance maxima at 252nm and 285nm respectively.

2.5 Preparation of FLX and APZ standard solution

Stock solution of 1000µg/ml of FLX and APZ were prepared separately in acetonitrile. The standard solutions were prepared by dilution of the stock solution with acetonitrile in a concentration range of 2-10µg/ml and 10-50µg/ml for FLX and APZ respectively. Acetonitrile was used as a blank solution.

3. Method Validation

3.1 Linearity

Stock solution of 1000µg/ml of FLX and APZ were prepared separately in acetonitrile. The standard stock solutions were prepared by dilution of the stock solution
with acetonitrile in a concentration range of 2-10µg/ml and 10-50µg/ml for FLX and APZ respectively.

**Figure 1:** Alprazolam - Chemical Structure

**Figure 2:** Fluoxetine - Chemical Structure

**Figure 3:** UV Spectrum of APZ

### 3.2 Sample preparation

Twenty tablets were weighed accurately and average weight was determined. Tablets were crushed to get fine powder. A composite portion of the tablet powder equivalent to 20 mg of FLX and 0.25 mg of APZ was weighed and transferred to a volumetric flask and volume was made up to 100 µg/ml with acetonitrile. The solution was filtered through Whatman filter paper no. 41 and sonicated for 45 minutes. Appropriate dilutions from stock solution were made to obtain a concentration of 10µg/ml of FLX and 20µg/ml of APZ. The volume was made up with acetonitrile for UV spectrophotometric measurements. (Figure-5)

### 3.3 Precision

The interday-intraday studies were carried out by analyzing six times, the assay of the sample containing 2, 6, 10 µg/ml for FLX and 10, 30, 50 µg/ml for ATZ.

**Figure 4:** UV Spectrum of FLX

**Figure 5:** Overlain Spectra of APZ and FLX

### 3.4 Specificity

Specificity of the method was established by analyzing standard drug, pharmaceutical dosage form and placebo. It was observed that there is no interference of the placebo with the principle peak of Fluoxetine hydrochloride and Alprazolam.

### 3.5 Accuracy

The accuracy of the experiment was assessed by recovery studies at three different levels. The standard drug solution was added to the pre-analyzed sample solution at three different levels 80%, 100% and 120%. The percentage recovery was calculated from the amount of drug present in the solution.
3.6 Robustness of the method

The robustness of the method was determined by small deliberate changes in the wavelength (±5nm), and the results were examined.

4. Results and Discussion

4.1 Linearity

Linear regression was obtained between absorbance and concentration of FLX and APZ in the range of 2-10µg/ml and 10-50 µg/ml. Data of regression analysis was summarized in Table: 1.

Table 1 Validation Parameters for Fluoxetine hydrochloride and Alprazolam

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>Fluoxetine Hydrochloride(FLX)</th>
<th>Alprazolam(APZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength(nm)</td>
<td>252</td>
<td>285</td>
</tr>
<tr>
<td>Beer’s Law</td>
<td>2-10</td>
<td>10-50</td>
</tr>
<tr>
<td>Regression coefficient(°2)</td>
<td>0.9998</td>
<td>0.9995</td>
</tr>
<tr>
<td>LOQ(µg/ml)</td>
<td>0.084</td>
<td>0.151</td>
</tr>
<tr>
<td>LOQ(µg/ml)</td>
<td>0.271</td>
<td>0.329</td>
</tr>
<tr>
<td>Interday Precision(%RSD)</td>
<td>0.90</td>
<td>0.09</td>
</tr>
<tr>
<td>Intraday Precision(%RSD)</td>
<td>1.52</td>
<td>0.84</td>
</tr>
</tbody>
</table>

LOD-Limit of detection
LOQ-Limit of quantification

4.2 Precision

To determine the precision of the method, FLX and APZ solutions of three different concentrations (2, 6, 10 µg/ml for FLX) and (10, 30, 50 µg/ml of APZ) were analyzed six times. Interday –intraday studies are given in Table: 1.

4.3 Robustness and Ruggedness

The robustness of the developed methods was tested by changing parameters such as degree of deviation, wavelength range, and N value and the optimum parameters were chosen for this study. The result showed no statistical differences suggesting that the developed methods were robust and rugged. The results are given in Table: 2.

Table 2 Results of analysis of FLX and APZ by different analysts

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interday Precision</th>
<th>Intraday Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLX</td>
<td>S.D</td>
<td>%RSD</td>
</tr>
<tr>
<td>APZ</td>
<td>2.42</td>
<td>1.52</td>
</tr>
</tbody>
</table>

4.4 Specificity

The specificity of the method was confirmed by comparing the \( \lambda_{max} \) of the standard drugs with that of FLX and APZ in the pharmaceutical dosage form. There are no interfering peaks due to excipients present in the tablets. Hence the developed method is specific and selective.

4.5 Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equation.

\[
\text{LOD}=3\sigma/S \quad \text{and} \quad \text{LOQ}=10\sigma/S,
\]

Where, \( \sigma \) = Standard deviation of the slope of (y-intercept) 

S = Slope of the calibration curve.

4.6 Recovery study

To check the accuracy of the method, recovery studies were carried out by the standard addition method. The standard drug solution was added to the preanalyzed sample solution at three different levels 80%, 100% and 120%. The amount of drug recovered was calculated from calibration curves. At each level of the amount, three determinations were performed. The results are shown in Table: 3.

Table 3 Accuracy

<table>
<thead>
<tr>
<th>Amount added (%)</th>
<th>FLX % Recovery</th>
<th>RSD</th>
<th>APZ % Recovery</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>100.3</td>
<td>1.32</td>
<td>100.0</td>
<td>0.54</td>
</tr>
<tr>
<td>100</td>
<td>99.8</td>
<td>1.2</td>
<td>101.8</td>
<td>0.60</td>
</tr>
<tr>
<td>120</td>
<td>98.8</td>
<td>0.97</td>
<td>100.1</td>
<td>0.54</td>
</tr>
</tbody>
</table>

4.7 Analysis of marketed formulation

There was no interference from the excipients present in the tablet. The low % R.S.D value indicates suitability of the method for the routine analysis of FLX and APZ in pharmaceutical dosage form. The result of analysis was depicted in Table: 4.

Table 4 Analysis of Tablet formulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FLZ</th>
<th>APZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label Claim(mg)</td>
<td>20</td>
<td>0.25</td>
</tr>
<tr>
<td>Drug Content(%±S.D)</td>
<td>100.24±0.89</td>
<td>99.81±0.26</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.80±1.08</td>
<td>1.31±1.81</td>
</tr>
</tbody>
</table>

5. Conclusion

The developed UV-Spectrophotometric method for the simultaneous estimation of Fluoxetine hydrochloride and Alprazolam is simple, precise, and rapid. As System suitability parameters are concern the percentage of each parameter lies below the limit of 2% as per the norms of ICH. The low % RSD indicates the suitability of the method. Hence the present method can be used for the routine analysis of components in the combined dosage form.

6. Acknowledgement

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7. References

3. The United State Pharmacopoeia, U.S.Pharmacopoeial Convention, Inc; Rockville MD, 28th Revision, (2005), 853.

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