STABILITY-INDICATING SPECTROFLUORIMETRIC DETERMINATION OF DOXAZOSIN MESYLATE IN RAW MATERIAL AND PHARMACEUTICAL PREPARATION.

Khalid Abdel-Salam Attia, Mohammed Wafaa Nassar, Hamed M. Abou-Seada and Samir Morshedy Mohammed Morshedy*

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Article Received on 17/12/2014                Article Revised on 11/01/2015             Article Accepted on 05/02/2015

ABSTRACT

A Simple, rapid, sensitive, accurate and precise spectrofluorimetric method has been developed for selective determination of doxazosin mesylate in presence of its alkaline degradate in bulk powder and in pharmaceutical preparation. The proposed method is based on measuring the native fluorescence of doxazosin in methanol at 403 nm after excitation at 252 nm. All variables that affect fluorescence intensity such as diluting solvents and pH were studied and optimized. The fluorescence–concentration plot was rectilinear over the range of 10-140 ng/ml with a lower detection limit (LOD) of 0.060 ng/ml and lower quantitation limit (LOQ) of 0.199 ng/ml. The proposed method can selectively analyze the drug in presence of up to 71.43% of its alkaline degradate with mean recovery of 101.37±0.72. The method was validated and successfully applied for the determination of doxazosin in its commercial preparation with an average percent recovery ± RSD% of 100.14 ± 0.89. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

KEYWORDS: Stability-indicating, doxazosin, spectrofluorimetric, excitation, emission and pharmaceutical preparation.
INTRODUCTION
Doxazosin mesylate Fig (1) is 1-(4-Amino-6,7dimethoxyquinazolin -2-yl) -4-(1,4-enzodioxan-2-ylcarbonyl)piperazine methanesulphonate.[1] It belongs to alpha blockers which are also known as alpha-adrenergic antagonists or alpha-adrenergic receptor antagonists. Doxazosin acts by inhibiting the postsynaptic alpha (1) - adrenoceptors on vascular smooth muscle. This inhibits the vasoconstrictor effect of circulating and locally released catecholamines (epinephrine and norepinephrine), resulting in peripheral vasodilatation. It is used for treatment and management of mild to moderate hypertension and urinary obstruction symptoms caused by BPH.[2] Few analytical methods have been reported for its analysis including spectrophotometric,[3-8] spectrofluorimetric,[6] electrochemical[9-14] and chromatographic methods.[15-19]

Spectrofluorimetry has been widely used in the determination of pharmaceutical compounds because it is a highly sensitive, selective, easy and economical technique.[20-25] The main task of this work is to establish a simple and accurate stability-indicating spectrofluorimetric method for the determination of doxazosin in presence of its alkaline degradate, which can be used for the routine analysis of the drug in raw material and pharmaceutical preparations.

![Figure 1: Structural Formula of Doxazosin Mesylate.](image)

MATERIALS AND METHOD
Apparatus
1. Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 Watt Xenon lamp. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. All measurements were done at medium sensitivity.
3. Hot plate, torrey pines scientific, USA.
Materials and Reagents: All chemicals and reagents used throughout the work were of analytical grade.
1. Doxazosin mesylate was kindly supplied by Pfizer company, Cairo, Egypt. Batch NO. (X711014A). The purity was assigned as 99.25%.
2. Cardura® 1 mg tablets, product of Pfizer company, Cairo, Egypt, Batch No. (MT2070512), labeled to contain 1 mg of doxazosin per tablet purchased from local pharmacies.
3. Water used throughout the procedures was freshly double distilled.
4. Acetonitrile, methanol, ethanol, 1-propanol and tetrahydrofuran, all of HPLC grades [Sigma, Germany].
5. Sodium hydroxide (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
6. Hydrochloric acid (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
7. Monobasic potassium phosphate, potassium chloride, boric acid, glacial acetic acid and sodium acetate trihydrate (El-Nasr Company, Egypt).
8. Buffers of different pH values prepared as prescribed in US pharmacopeia[26]:
   1. Acetate buffer pH range from 3 to 6.
   2. Phosphate buffer pH range from 6 to 8.
   3. Alkaline borate buffer pH range from 8 to 10.

Standard Solutions
Standard Solution of Intact Doxazosin: A standard solution of doxazosin (100 μg/ml) was prepared by dissolving 10 mg of doxazosin in 50 ml of methanol and complete to 100 ml with methanol. The working standard solution (1 μg ml⁻¹) was prepared by dilution of the stock solution with methanol. This solution was stable for one month when kept in the refrigerator.

Standard Solution of Degraded Sample: base-induced forced degradation was performed by adding 10 mg of doxazosin to 100 mL 0.1 M NaOH and refluxing at 80°C for approximately three hours. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 0.1 M HCL, evaporated to dryness, the residue was extracted three times with 25 ml methanol, filtered into 100 ml volumetric flask then the volume was adjusted by the same solvent. The obtained solution was claimed to contain (100 μg/ml).[3]
Procedure

Construction of the Calibration Curve (General Procedure): Different aliquots of doxazosin working standard solution (1μg/ml) ranging from (0.1–1.4) μg were transferred to a 10-ml volumetric flasks and 2 ml of acetate buffer pH 5 was added. The solutions were diluted with methanol to 10 ml and mixed well. The fluorescence intensity was measured at 403 nm (λ\text{emission}) after excitation at 252 nm (λ\text{excitation}). The measured fluorescence intensity versus the final concentration in ng/ml were plotted to get the calibration graph. Alternatively, the regression equation was derived.

Analysis of Pharmaceutical Preparation: Contents of twenty Cardura® tablets (each containing 1 mg doxazosin) were accurately weighed and finely powdered, then a quantity of the powdered tablets equivalent to 10 mg of doxazosin were transferred into a small conical flask, extract with 3 x 30 ml of methanol on three successive times. Filter the extracts into a 100 ml volumetric flask. Wash the conical flask with few milliliters of methanol. Pass the washings into the same conical flask and complete to the mark with the same solvent. Transfer aliquots covering the working concentration range into 10 ml volumetric flasks. Proceed as described under “General Procedure”. Determine the nominal content of the tablets either from the calibration curves or using the corresponding regression equations.

RESULTS AND DISCUSSION

Spectral Characteristics: Doxazosin exhibits a native fluorescence in methanol and its emission can be measured at 403 nm (λ\text{emission}) after excitation at 252 nm (λ\text{excitation}). The emission and excitation spectra of doxazosin in methanol are shown in figure (2).

![Figure 2](image-url)  

Figure (2): Excitation and Emission Spectra of Doxazosin (80 ng/ml) and its alkaline degradate (80 ng/ml) respectively, in methanol using acetate buffer pH 5.
Optimization of Experimental Conditions

(i) **Effect of Diluting Solvents:** The general procedure for the method was repeated using a fixed amount of doxazosin (800 ng) and different diluting solvents and found that; methanol is the best diluting solvent as shown in figure (3).

(ii) **Effect of pH and Buffer:** The general procedure for the method was repeated using a fixed amount of doxazosin (800 ng) and different buffers with different pH and found that; acetate buffer pH 5 gives the best result as shown in figure (4).

(iii) **Effect of Buffer Volume:** The general procedure for the method was repeated using a fixed amount of doxazosin (800 ng) and different volumes of acetate buffer pH 5 and found that; 2 ml gives the best result as shown in figure (5).

(iv) **Effect of Time:** The general procedure for the method was repeated using a fixed amount of doxazosin (800 ng) at different time interval and found that; it is stable at least for one hour as shown in figure (6).

(v) **Effect of Temperature:** The effect of temperature was studied in the range of 40–100 °C using a thermostatically controlled water bath. It was found that, increasing the temperature causes decrease of fluorescence intensity. It may be due to the collision between the excited singlet state and the solvent molecules which causes loss of energy. So, the fluorescence intensity of doxazosin was measured at room temperature (25 °C).

![Figure (3): Effect of different diluting solvents on fluorescence intensity of doxazosin (80 ng/ml)](image)
Figure (4): Effect of different buffers (2ml) on fluorescence intensity of doxazosin (80 ng/ml).

Figure (5): Effect of volume of Acetate buffer pH 5 on fluorescence intensity of (80 ng/ml) doxazosin.

Figure (6): Effect of time on fluorescence intensity of doxazosin (80 ng/ml).
Validation of the Method

(i) **Linearity and Range:** Under the described experimental conditions, the calibration graph for the method was constructed by plotting fluorescence intensity versus concentration in ng/ml. The regression plot was found to be linear over the range of 10-140 ng/ml. The linear regression equation for the graph is:

\[ \text{FI} = 6.552 \times C - 1.772 \quad (r^2 = 0.9998). \]

Where FI is the fluorescence intensity, C is the drug concentration in ng ml\(^{-1}\) and \(r^2\) is the squared correlation coefficient.

Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in Table (1).

(ii) **Limits of Detection and Quantitation:** The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH Q\(2^\) Recommendation \([27]\) from the following equations:

- LOD = \(3.3 \times S_a / \text{slope}\)
- LOQ = \(10 \times S_a / \text{slope}\)

Where \(S_a\) is the standard deviation of the intercept of regression line.

LOD was found to be 0.060 ng/ml, while LOQ was found to be 0.199 ng/ml. The small values of LOD and LOQ indicate good sensitivity.

(iii) **Accuracy and Precision:** Three replicate determinations of three different concentrations of doxazosin in pure form within linearity range were performed in the same day (intra-day) and in three successive days (inter-day). Accuracy as recovery percent (R\%) and precision as percentage relative standard deviation (RSD\%) were calculated and results are listed in Table (2). The small values of RSD\% indicates high precision of the method. Moreover, the good R\% confirms excellent accuracy.

(iv) **Specificity:** The specificity of the proposed method was assured by applying the laboratory prepared mixtures of the intact drug together with its degradation product. The proposed method was adopted for the specific determination of intact doxazosin in presence of up to 71.43\% of its corresponding degradate. The percentage recovery ± RSD % was 101.37 ± 0.72 Table (3).
Pharmaceutical Applications: The proposed method was applied to the determination of the studied drug in *Cardura® 1 mg tablets*. The results were validated by comparison to a previously reported method [6]. No significant difference was found by applying t-test and F-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form table (4).

Table (1): Spectral Data for Determination of Doxazosin By The Proposed Method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proposed Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{emission}$ (nm)</td>
<td>403</td>
</tr>
<tr>
<td>$\lambda_{excitation}$ (nm)</td>
<td>252</td>
</tr>
<tr>
<td>Linearity range (ngml$^{-1}$)</td>
<td>10—140</td>
</tr>
<tr>
<td>LOD (ngml$^{-1}$)</td>
<td>0.060</td>
</tr>
<tr>
<td>LOQ (ngml$^{-1}$)</td>
<td>0.199</td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
</tr>
<tr>
<td>Slope ($b$)</td>
<td>6.552</td>
</tr>
<tr>
<td>Intercept ($a$)</td>
<td>1.772</td>
</tr>
<tr>
<td>Correlation Coefficient ($r^2$)</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

* $y = a + bx$ where $y$ is the fluorescence intensity and $x$ is the concentration in ng ml$^{-1}$.

Table (2): Intraday And Interday Accuracy And Precision For The Determination Of Doxazosin By The Proposed Method

<table>
<thead>
<tr>
<th>Taken conc. (ng ml$^{-1}$)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found Conc. ±SD</td>
<td>Accuracy</td>
<td>Precision</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>(R %)</td>
<td>RSD %</td>
</tr>
<tr>
<td>60</td>
<td>59.54 ± 0.577</td>
<td>99.23</td>
</tr>
<tr>
<td>80</td>
<td>79.48 ± 1</td>
<td>99.35</td>
</tr>
<tr>
<td>100</td>
<td>100.09 ± 1</td>
<td>100.09</td>
</tr>
</tbody>
</table>

Table (3): Determination of Intact Doxazosin In Mixtures With Its Alkaline Degradate By The Proposed Method

<table>
<thead>
<tr>
<th>Intact (ng ml$^{-1}$)</th>
<th>Degradate (ng ml$^{-1}$)</th>
<th>Degradate %</th>
<th>Intact found (ng ml$^{-1}$)</th>
<th>Recovery % of Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>20</td>
<td>14.29</td>
<td>120.39</td>
<td>100.33</td>
</tr>
<tr>
<td>100</td>
<td>40</td>
<td>28.57</td>
<td>101.43</td>
<td>101.43</td>
</tr>
<tr>
<td>80</td>
<td>60</td>
<td>42.86</td>
<td>81.60</td>
<td>102.00</td>
</tr>
<tr>
<td>60</td>
<td>80</td>
<td>57.14</td>
<td>61.02</td>
<td>101.70</td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>71.43</td>
<td>40.02</td>
<td>100.05</td>
</tr>
<tr>
<td>Mean ± RSD%</td>
<td></td>
<td></td>
<td>101.37 ± 0.72</td>
<td></td>
</tr>
</tbody>
</table>
Table (4): Determination of Doxazosin In Cardura® Tablets (1 Mg) By The Proposed And Reported Methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cardura® 1 mg tablets</th>
<th>Proposed method</th>
<th>Reported method&lt;sup&gt;(6)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N*</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>100.66</td>
<td>100.21</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.870</td>
<td>0.530</td>
<td></td>
</tr>
<tr>
<td>RSD%</td>
<td>0.863</td>
<td>0.530</td>
<td></td>
</tr>
<tr>
<td>t**</td>
<td>1.197 (1.782)</td>
<td>———</td>
<td></td>
</tr>
<tr>
<td>F**</td>
<td>0.371 (4.88)</td>
<td>———</td>
<td></td>
</tr>
</tbody>
</table>

* No. of experimental.
** The values in the parenthesis are tabulated values of t and F at (p= 0.05).

CONCLUSION

The proposed method is simple, rapid, accurate, selective and inexpensive. It permits the determination of doxazosin mesylate in its pure form and pharmaceutical preparations. The method proved its ability to be used for stability-indication of the drug. Therefore, it can be used for purity testing, stability studies, quality control and routine analysis of the drug.

ACKNOWLEDGMENT

I am deeply thankful to ALLAH, by the grace of whom this work was realized. I wish to express my indebtedness and gratitude to staff members of Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt for their valuable supervision, continuous guidance and encouragement throughout the whole work.

REFERENCES


