A FLOURESCENCE QUENCHING BASED METHOD FOR THE DETERMINATION OF RISPERIDONE IN BULK AND TABLETS

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ABSTRACT

Objective: The objective of the method was to develop a new, simple, rapid and accurate spectrofluorimetric method for the determination of risperidone in bulk and tablets. Method: Quenchofluorimetric method; is based on the study of quantitative quenching effect of risperidone on native fluorescence of eosin-y in acidic medium due to the formation of ionpair complex. The fluorescence intensity was measured at 555 nm after excitation at 260 nm. Results: Under the optimum conditions, the quenched fluorescence intensity was linear with the concentration of risperidone in the range of 0.5 – 7 μg/ml (R²= 0.9998) with a detection limit of 0.015 μg/ml. The proposed method was successfully applied to the analysis of risperidone in pure form and tablets with good precision and accuracy compared to the reported method as revealed by t- and F- tests. Conclusion: The developed method was simple, fast, sensitive, accurate and precise. It is the first time to develop spectrofluorimetric method for determination of risperidone in bulk and tablets. It could be applied for routine quality control analysis of risperidone in its pure form and in tablets.

KEYWORDS: Fluorescence quenching; risperidone; spectrofluorimetric method.

INTRODUCTION

Risperidone (Fig.1) is 4-[(2-[4-(6-fluorobenzo[d]isoazol-3-yl)-1-piperidyl]ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one, belongs to the chemical class of benzisoxazole derivatives. It is an atypical antipsychotic agent and acts through selective antagonism of serotonin 5HT₂, dopamine D2 receptors[1]. Clinically, it is used in the treatment of...
schizophrenia and other psychoses\[2\]. The therapeutic importance of the drug has promoted the development of several analytical methods for its quantitative determination. The British Pharmacopeia adopts a non-aqueous titrimetric method for the determination of risperidone\[3\]. Other analytical techniques include several spectrophotometric\[4-10\], polarographic\[11\] and chromatographic\[12-18\] methods for determination of risperidone in pure form, pharmaceutical preparations and/or biological fluids have been reported.

![Fig. 1: Structural formula of risperidone](image)

Eosin–y was utilized in the literature with metal ions for the spectrophotometric and fluorimetric determination of many drugs via ternary complex formation\[19-22\].

The main purpose of this study was to establish simple spectrofluorimetric method for the determination of risperidone in pure form as well as its pharmaceutical dosage form without prior extraction. The method was developed and was simply based on the fluorescence quenching effect by a binary ion–pair complex formation between the studied drug and one of xanthene dyes, eosin-y.

**MATERIALS AND METHODS**

**Apparatus**
- Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 Watt Xenon lamp, holographic gratting excitation and emission monochromators for all measurements. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used.
- Jenway, 3510 pH meter (Jenway, USA).
• **Materials and reagents**

**Pure sample**
Risperidone; was kindly supplied by multi-apex company, Egypt, B. No. (RN0030612).

**Pharmaceutical preparation**
Sigmadone® tablets: product of Sigma company, Egypt, Batch No. (40708), labeled to contain 3 mg of risperidone per tablet purchased from local pharmacies.

**Reagents and solvents**
All chemicals and reagents used throughout the work were of analytical grade.
Water used throughout the procedures was freshly double distilled.
- methanol, of HPLC grades [Sigma, Germany].
- Acetate buffer pH range from 3 to 5, as prescribed in United State (US) Pharmacopeia [23].
- Eosin-y (2 x 10$^{-4}$ M) and (2.4 x 10$^{-5}$ M) aqueous solution.

**Standard solution**
- Stock solution of risperidone (0.1 mg ml$^{-1}$) was prepared by dissolving 10 mg of risperidone in 100 ml methanol and this is diluted 10 times with methanol to obtain the working standard solution (10 ug/ml).

**Procedure**

**Construction of the calibration curve (General procedure)**
Aliquots of drug solution of (10 ug ml$^{-1}$) containing (0.5 – 7 ug) were transferred into a series of 10 ml volumetric flasks. Eosin-y, 1.25 ml of (2 x 10$^{-4}$ M) solution was then added and the mixture was mixed well before addition of 1.5 ml of acetate buffer pH 4. The mixture was adjusted to the volume with water then the difference in fluorescence intensity between the reagent blank and each experiment was measured at $\lambda_{em}$ 555 nm after excitation at $\lambda_{ex}$ 260 nm.

**Analysis of pharmaceutical preparation**
Ten Sigmadone® 3 mg tablets were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of risperidone was shaken three times with 25 ml methanol 10 minutes then filtered into 100 ml volumetric flask and the volume was adjusted to the mark with methanol to obtain a concentration of (0.1 mg ml$^{-1}$). Proceed as described under “General Procedure”.
RESULTS AND DISCUSSION

The purpose of the present study was to develop simple and sensitive spectrofluorimetric method for the determination of risperidone in pure form and in its pharmaceutical formulation. In the current study, risperidone was found to form an ion pair red complex with eosin-y at pH4. The formed complex is mainly due to the electrostatic interaction between risperidone and anionic functional group of eosin-y under acidic pH \[24\]. The formed ion pair complex is not fluorescent; therefore, the decrease in the fluorescence of eosin upon the addition of the drug was the basis for the spectrofluorimetric measurement at 555 nm after excitation at 260 nm, (Fig.2).

![Excitation and emission spectra of the reaction product of risperidone with eosin-y.](image)

Optimization of experimental conditions

Effect of PH

Accurately measured aliquot of the drug equivalent to 30 µg was transferred into a series of 10 ml volumetric flasks and 1 ml of (4 x 10^{-4} M) eosin-y solution was added and the mixture was mixed well before the addition of 0.5 ml of different acetate buffers ranging from (pH 3 - 5). The mixtures were completed to volume with water then the difference in fluorescence intensity between the reagent blank and each experiment was measured at \(\lambda_{em}\) 555 nm after excitation at \(\lambda_{ex}\) 260 nm. For the highest difference in the relative fluorescence intensities and maximum precision, the buffer solution should be added after mixing the drug-dye solution at neutral pH. It was found that, maximum difference in the relative fluorescence intensities was obtained upon using acetate buffer pH 4, (Fig.3)
Fig. 3: Effect of PH on the fluorescence of risperidone (3 µg/ml⁻¹) reaction product with eosin-y at λₑₘ 555 nm.

Effect of buffer volume
The procedure under "Effect of pH" was repeated using different volumes (0.25- 2 ml) of acetate buffer pH 4. It was revealed that 0.5 ml of the buffer solution was sufficient to give (Fig.4).

Fig. 4: Effect of buffer volume on the fluorescence of risperidone (3 µg/ml⁻¹) reaction product with eosin-y at λₑₘ 555 nm.
Effect of eosin-y volume

The procedure detailed under "Effect of pH" was followed using 0.5 ml of acetate buffer pH 4 and different volumes of eosin-y (2 x 10^{-4} M) ranging from (0.5 - 2 ml). It was revealed that 1 ml of the dye was sufficient to give maximum difference in the relative fluorescence intensities, (Fig.5).

![Graph showing the effect of eosin-y volume on the fluorescence of risperidone (3 µg ml^{-1}) reaction product with eosin-y at λ_{em} 555 nm.](image)

**Fig. 5:** Effect of eosin-y volume on the fluorescence of risperidone (3 µg ml^{-1}) reaction product with eosin-y at λ_{em} 555 nm.

**Determination of the stoichiometry of the reaction**

**Continuous variation (job's method)\textsuperscript{25}**

In a series of 10 ml volumetric flasks (0.5,1,1.5……..,4.5 ml) of (2.4 x 10^{-5} M) risperidone were transferred, then (4.5,4,3.5……..,0.5 ml) of eosin-Y solution (2.4 x 10^{-5} M) were added (the sum of risperidone and reagent equals to 5 ml), 0.5 ml of acetate buffer pH 4 was added then volumes were completed to the mark with water. The difference in fluorescence intensity of the formed ion pair complexes with eosin-y were measured at λ_{em} 555 nm after excitation at λ_{ex} 260 nm against the appropriate reagent blank, the drug: reagent ratio was found to be 1:1, (Fig.6).
Fig. 6: Stoichiometry of the reaction of risperidone (2.4 x 10^{-5} M) with eosin–y by continuous variation (Job’s) method at λ em 555 nm.

Validation of the method

Linearity

Under the described experimental conditions, the calibration graph was constructed by plotting the difference in the fluorescence intensity (ΔF) versus concentration. The correlation coefficient was 0.9998 indicating good linearity, in the concentration range of 0.5 – 7 μg/ml. The intercept, slope, limit of detection (LOD), and limit of quantitation (LOQ) are summarized in table 1.

The linear regression equation was

$\Delta F_{555} = 30.5198 \, C + 0.8983 \quad (r^2 = 0.9998)$

LOD and LOQ values were calculated according to ICH Q2B[26] using the following equations: LOQ = 10 σ/S LOD = 3.3 σ/S Where σ is the standard deviation of intercept of the regression line and S is the slope of the calibration curve (table 1).

Table 1: Selected spectral data for the determination of risperidone by the proposed eosin-y procedure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ_{ex} and λ_{em}</td>
<td>260 and 555 nm</td>
</tr>
<tr>
<td>Linearity range (μg ml^{-1})</td>
<td>0.5–7</td>
</tr>
<tr>
<td>LOD (μg ml^{-1})</td>
<td>0.015</td>
</tr>
<tr>
<td>LOQ (μg ml^{-1})</td>
<td>0.050</td>
</tr>
<tr>
<td>- Regression Equation</td>
<td>$\Delta F = 30.5198C + 0.8983$</td>
</tr>
<tr>
<td>- Slope ± S.D</td>
<td>30.5198 ± 0.021</td>
</tr>
</tbody>
</table>
ΔF* is fluorescence intensity difference.

C** is concentration in $\mu$g ml$^{-1}$.

**Accuracy and precision**

According to the ICH guidelines$^{[26]}$, three replicate determinations of three different concentrations of the studied drugs in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method. 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 methods. Moreover, the good R% confirms excellent accuracy.

**Table 2: Intraday and interday accuracy and precision for the determination of risperidone by the proposed method**

<table>
<thead>
<tr>
<th>Conc. (µg ml$^{-1}$)</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found Conc. ± SD</td>
<td>Accuracy (R %)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.49±0.003</td>
<td>98.96</td>
</tr>
<tr>
<td>2</td>
<td>2.02±0.016</td>
<td>100.92</td>
</tr>
<tr>
<td>5</td>
<td>4.95±0.033</td>
<td>99.02</td>
</tr>
</tbody>
</table>

**Pharmaceutical Applications**

The proposed methods were applied to the determination of the studied drug in Sigmadone® 3 tablets. The results were validated by comparison to a previously reported method$^{[10]}$. No significant differences were found by applying t-test and F-test at 95% confidence level$^{[27]}$, indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their pharmaceutical dosage form, Table (3).

**Table 3: Statistical analysis of the results obtained by the proposed and reported method$^{[10]}$ for the determination of sigmadone® tablets**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sigmadone® 3 mg tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proposed Method</td>
</tr>
<tr>
<td>N*</td>
<td>7</td>
</tr>
<tr>
<td>X**</td>
<td>99.65</td>
</tr>
<tr>
<td>SD</td>
<td>0.638</td>
</tr>
<tr>
<td>RSD%</td>
<td>0.640</td>
</tr>
<tr>
<td>t***</td>
<td>1.445</td>
</tr>
</tbody>
</table>
CONCLUSION

The proposed method is simple, rapid, accurate and precise and can be used for the determination of risperidone in pure form and in pharmaceutical dosage form. The method developed was fully validated, and therefore, can be applied for the routine analysis of risperidone in quality control laboratories.

REFERENCES


