A Validated Spectrofluorimetric method for the Determination of Haloperidol via Ion-pair formation with Eosin Y.
INTRODUCTION

Haloperidol is a potent antipsychotic with pharmacological profile resembling that of piperazine substituted phenothiazines [1]. It is chemically known as 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one and its molecular weight is 375.87. It is the preferred drug for the treatment of acute schizophrenia, Huntington’s disease and Giller de la Tourett’s syndrome [2]. Usual dosages for adults with moderate symptoms are from 0.5 – 2.0 mg twice daily or three times daily. For severe symptoms, the usual dosage is 3 – 5 mg twice or three times daily. Peak plasma levels of haloperidol occur within 2 to 6 hours of oral dosing and about 20 minutes after intramuscular administration. However, higher dosage may cause severe side effects such as fast heart beat, Parkinson’s like symptoms, neck spasm/involuntary muscle spasms, insomnia etc. Owing to the therapeutic importance of haloperidol, it is essentially required to develop simple and sensitive analytical method for its determination in commercial dosage forms.

Haloperidol is the subject of a monograph in the USP [3] whereby a potentiometric titration with perchloric acid and HPLC method are recommended for its determination in bulk and pharmaceutical formulations. A review of the literature revealed that several methods have been published for the determination of haloperidol in pharmaceutical preparations and biological fluids. These methods include: spectrophotometry [4-6], gas chromatography [7], HPLC [8-13], HPTLC [14], capillary electrophoresis [15], square wave-adsorptive stripping voltammetry [16], NMR spectroscopy [17] and conductometric titrimetry [4]. Haloperidol was also determined using electrochemical sensor based on multi-walled carbon
nanotubes [18]. Fluorescence spectroscopy is frequently employed to quantitate drugs in pharmaceutical preparations. In fluorimetric methods, high sensitivity and selectivity are generally expected. Uptill now there have been only two reports dealing with spectrofluorimetric determination of haloperidol [19, 20]. In the first report, the cited drug was determined by oxidizing with acidic potassium permanganate [19]. In the other report, the native fluorescence of haloperidol has been used to develop fluorimetric method for its determination [20]. The disadvantage of this method is that weak signals are obtained in most of the solvents.

This chapter describes the spectrofluorimetric method for the determination of haloperidol in commercial dosage forms. The method is based on the formation of ion-pair complex between haloperidol and eosin Y at pH 4.27. The ion-pair complex was extracted into dichloromethane which exhibited maximum fluorescence emission at 561nm after excitation at 345nm.

**EXPERIMENTAL**

**Apparatus**

Fluorescence was monitored with an F-2500 Hitachi fluorescence spectrophotometer (Tokyo, Japan) equipped with xenon arc lamp. All measurements took place in a standard quartz cells with path length of 1.0 × 1.0 cm at 25 ± 1 °C.

An Elico LI 120 pH meter (Hyderabad, India) was used for pH measurement. Absorbance measurements were made on Shimadzu UV-Visible Spectrophotometer, model No. UV- mini 1240 (Shimadzu, Japan).
Reagents and Standards

- Haloperidol (CAS: 52-86-8; M.W.: 375.87) was obtained from Sigma Chemical Company, St. Louis, USA and used as received. Two different commercial pharmaceutical formulations of haloperidol such as serenace-5 (RPG Life sciences Ltd., India), and hexidol-5 (Torrent Pharmaceuticals Ltd., India) were purchased from local drug stores.

- $2.89 \times 10^{-3}$ M Eosin Y disodium salt (CAS: 17372-87-1, M.W.: 691.85, Fluka Chemie AG, Switzerland) solution was freshly prepared in distilled water.

- Buffer solutions ranging from 3.72 – 5.57 were prepared by mixing 0.2 M acetic acid and 0.2 M sodium acetate in different volume ratios [21].

Haloperidol standard solutions

Standard solution (10 µg ml$^{-1}$) of haloperidol was prepared in methanol.

Procedure for the determination of haloperidol

Aliquots of the standard solution (10 µg ml$^{-1}$) corresponding to 0.5 – 16.0 µg were transferred into 25 mL separating funnels. Then 1.7 ml of eosin Y ($2.89 \times 10^{-3}$M) solution was added followed by 1.0 ml of sodium acetate-acetic acid buffer solution of pH-4.27 and was mixed well. The contents of separating funnel were shaken vigorously with 5 ml dichloromethane for 2.5 min and then allowed to stand for clear separation of aqueous and organic layers. The fluorescence intensity of the organic layer was measured at 561 nm after excitation at 345 nm. The calibration graph was obtained by plotting the fluorescence intensity against the concentration of haloperidol.
Procedure for the determination of haloperidol in commercial dosage forms

Ten tablets were accurately weighed and finely powdered. An amount of the powder equivalent to 25 mg haloperidol was shaken with a sufficient volume of methanol. The residue was filtered using Whatmann No. 42 filter paper (Whatmann International Limited, Kent, UK) and the filtrate was diluted to 50 mL with methanol. This solution was further diluted and analyzed following the recommended procedure.

RESULTS AND DISCUSSION

Spectral studies and reaction mechanism

Haloperidol is a derivative of butyrophenone with piperidyl group possessing tertiary nitrogen atom in its moiety. Its methanolic solution fluoresces at 544 nm after excitation at 312 nm. In the literature a spectrophotometric method for its determination has been reported based on the ion-pair complex formation between haloperidol and sodium 1, 2-napthaquinone-4-sulfonate [22]. In the present study it was found that the methanolic solution of haloperidol containing tertiary amine group reacts with aqueous solution of eosin Y at pH 4.27 resulting in the formation of fluorescent ion-pair complex. The ion-pair complex formed is light pink in colour and was extracted in dichloromethane which exhibited emission at 561 nm after excitation at 345 nm (Fig. 5.1). The blank solution remained colourless as the eosin Y was not extractable into dichloromethane at pH 4.27.

The Job's method of continuous variation [23] was employed for the estimation of stoichiometry of the reaction between haloperidol and eosin Y at pH 4.27. Master equimolar (2.66 × 10⁻⁴M) solutions of haloperidol and eosin Y were prepared. Now, different volumes, that is, 0, 0.4, 1.2, 1.8, 2.0, 2.2, 2.6,
Fig. 5.1. Excitation and emission spectra of haloperidol and eosin Y ion-pair complex extracted in dichloromethane.
2.65, 2.67, 3.2, 3.8, and 4.0 ml of haloperidol were mixed with 4.0, 3.6, 2.8, 2.2, 2.0, 1.8, 1.4, 1.35, 1.33, 1.3, 0.8, 0.2 and 0 ml of eosin Y into a series of 25 mL separating funnels and were further manipulated as described under the general recommended procedure. The fluorescence intensity was measured at 561 nm and plotted against the mole fraction of drug (Fig. 5.2). As can be seen from fig. 5.2 that the combining molar ratio between haloperidol and eosin Y is 1:1. The apparent formation constant and Gibbs free energy ($\Delta G'$) were calculated and found to be $3.214 \times 10^6$ and $-37.14$ kJ mol$^{-1}$, respectively. A schematic reaction proposal of the reaction pathway is given in Scheme 5.1.

**Optimization of the experimental parameters**

The spectrofluorimetric properties of the coloured ion-pair complex as well as the different experimental parameters affecting the colour development and its stability were carefully studied and optimized. The experimental factors include pH, volume of buffer solution, concentration of eosin Y, reaction time, shaking time for extraction of the ion-pair complex and solvent. Such factors were changed individually while the others were kept constant.

**Effect of reaction time**

The effect of the reaction time on the formation of ion-pair complex was studied. The ion pair complex got stabilized immediately at 25 ± 1°C and remained stable for at least 1 hour.

**Effect of the volume of eosin Y**

The effect of the concentration of eosin Y on the fluorescence intensity of the ion pair complex was studied using different volumes of $2.89 \times 10^{-3}$M solution of the reagent in the presence of acetate buffer of pH 4.27. Increasing the
Fig. 5.2. Job's plot to establish the stoichiometry of the reaction.
Scheme 5.1. Mechanism of the proposed method
volume of the reagent produced a proportional increase in the fluorescence intensity of the ion-pair complex upto 1.5 ml and remained constant upto 2.3 ml. Therefore, 1.7 ml of $2.89 \times 10^{-3}$M solution of eosin Y was chosen as the optimum volume of the reagent (Fig. 5.3).

**Effect of pH**

The influence of pH on the fluorescence intensity of the reaction product was evaluated using sodium acetate-acetic acid buffer solution in the range of 3.72 – 5.57. The maximum fluorescence intensity of the complex was obtained at pH 4.27 (Fig. 5.4). Therefore, all fluorescence intensity measurements were made at pH 4.27 in the determination process.

**Effect of volume of pH 4.27 buffer solution**

The influence of the volume of pH 4.27 buffer solution on the fluorescence intensity of the complex was studied in the range of 0.5 – 1.5 ml. Increasing the volume of the buffer solution resulted in the increase of the fluorescent signal upto 0.9 ml, after which further increase did not alter the fluorescence intensity of the complex (Fig. 5.5). Therefore, 1.0 ml of pH 4.27 buffer solution was chosen as an optimum volume for measuring fluorescence intensity in the determination procedure.

**Effect of shaking time**

The dependence of the fluorescence intensity on the shaking time for the extraction of the ion pair complex was studied in the range of 1.0 – 3.5 min. The maximum fluorescence intensity of the complex was obtained at 2.0 min and above this up to 3.5 min, the fluorescence intensity remained constant (Fig. 5.6). Therefore, 2.5 min was used as an optimum shaking time throughout
Fig. 5.3. Effect of the volume of $2.89 \times 10^{-3}$ eosin Y on the fluorescence intensity of ion-pair complex.
Fig. 5.4. Effect of different pH on fluorescence intensity of the reaction product.
Fig. 5.5. Effect of the volume of acetate buffer solution of pH 4.27 on the fluorescence intensity of the reaction product.
Fig. 5.6. Effect of shaking time for the extraction of the ion pair complex.
the determination process. The ion-pair complex was quantitatively recovered in one extraction only and was stable for at least 1 h.

**Choice of organic solvent**

Different organic solvents such as chloroform, carbon tetrachloride, dichloromethane, dichloroethane, and ethyl acetate were tested for extraction of the ion pair complex in order to provide an applicable extraction procedure and maximum fluorescence intensity. The highest fluorescence intensity of the complex was found when extraction was carried with dichloromethane. It can be seen from the Fig. 5.7 that no extraction was possible with carbon tetrachloride. Therefore, dichloromethane was selected as the best solvent for extraction of the ion-pair complex.

**Method validation**

The validity of the proposed method was tested regarding linearity, specificity, accuracy, precision and robustness according to ICH Q2B recommendations [24].

**Linearity, limits of detection and quantitation**

Under the optimum reaction conditions, the fluorescence intensity - concentration plot for the proposed method was found to be rectilinear over the range 0.2 - 3.2 µg ml⁻¹. Linear regression analysis of the calibration data gave the following equation:

\[ F = 0.443 + 170.288C; \ r = 0.9999; \ n = 15 \]

where \( F \) is the fluorescence intensity and \( C \) is the concentration of haloperidol in µg ml⁻¹. The high value of the correlation coefficient (\( r = 0.9999 \)) and low value of intercept (0.443) indicate good linearity of the calibration graph. The limits of detection (LOD) and quantitation (LOQ) were calculated according
Fig. 5.7. Effect of the solvent on the fluorescence intensity of the ion-pair complex.
to ICH Q2B [24] and were found to be 0.05 and 0.16 μg ml⁻¹, respectively.

**Accuracy and precision**

The accuracy and precision of the proposed method was determined at three concentration levels (lower, middle, & upper) of haloperidol within the same day (intra-day) and on five consecutive days (inter-day). The relative standard deviations and % relative error are summarized in Table 5.1. The relative standard deviation did not exceed 0.8 % which indicated the high reproducibility of the results and precision of the method. This level of precision is suitable for quality control analysis of haloperidol in commercial dosage forms.

**Recovery**

The recovery experiment was performed using standard addition method.
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**Recovery**

The recovery experiment was performed using standard addition method. Recoveries were calculated using the appropriate regression equations and the results are summarized in Table 5.2 and Fig. 5.8. The obtained recovery values ranged from 99.92 % - 100.09 % (Table 5.2). Also the regression line of standard addition method was good which indicates that the proposed method is precise as well as accurate.

**Selectivity**

The proposed method has the advantage of that all measurements are made at 561 nm after excitation at 345 nm, as mostly the pharmaceutical additives and adjuvants are non-fluorescent that might be coextracted from haloperidol containing dosage forms. Potential interference by excipients in the dosage forms was studied. Samples were prepared by mixing known amount of haloperidol with various amounts of the common excipients such as starch, glucose, lactose, acacia and magnesium stearate.
### TABLE 5.1: Test of accuracy and precision.

<table>
<thead>
<tr>
<th>Proposed Method</th>
<th>Concentration (µg/ml)</th>
<th>Taken</th>
<th>Found ± SD</th>
<th>RSD (%)</th>
<th>Error (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Intraday assay</td>
<td>0.30</td>
<td>0.299± 0.002</td>
<td>0.795</td>
<td>-0.333</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.90</td>
<td>1.902± 0.145</td>
<td>0.145</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.10</td>
<td>3.101± 0.002</td>
<td>0.709</td>
<td>0.032</td>
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</tr>
<tr>
<td>Interday assay</td>
<td>0.30</td>
<td>0.298 ± 0.002</td>
<td>0.006</td>
<td>-0.667</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.90</td>
<td>1.902 ± 0.002</td>
<td>0.001</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.10</td>
<td>3.092 ± 0.002</td>
<td>0.002</td>
<td>-0.258</td>
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</table>
Fig. 5.8. Recovery of haloperidol from serenate-5 tablet by standard addition technique: (a) 0.400 and (b) 0.799 µg ml⁻¹.
<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Coefficients of linear regression equation of standard addition</th>
<th>Recovery&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Slope</td>
</tr>
<tr>
<td>Serenace-5&lt;br&gt;(Torrent Pharma.)</td>
<td>0.400</td>
<td>0, 0.2, 0.4, 0.6, 0.8</td>
</tr>
<tr>
<td></td>
<td>0.800</td>
<td>0, 0.2, 0.4, 0.6, 0.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Coefficient of correlation.

<sup>b</sup>Mean for five independent analyses.
The results of analysis revealed that no interference was observed from any of these excipients.

**Robustness**

The robustness of the proposed method is demonstrated by the constancy of the fluorescence intensity with deliberate minor changes in experimental parameters such as:

- volume of buffer solution of pH 4.27, 1.0 ± 0.2 ml,
- volume of $2.89 \times 10^{-3}$ M eosin Y, 1.7 ± 0.1 ml,
- reaction time, 25 ± 3°C,
- effect of shaking time, 2.5 ± 0.5 min,

During experimental operation, these minor changes may take place which did not influence the fluorescence intensity of the ion-pair complex. Also the regression line of standard addition method was good which indicates that the proposed method is precise as well as accurate.

**Application of the proposed method to analysis of haloperidol in tablets**

It is evident from the above mentioned results that the proposed methods gave satisfactory results which can be suitable for the quality control analysis of haloperidol. The proposed and reported methods [25] were applied to the determination of haloperidol in its dosage forms. The results obtained by the proposed method were compared statistically with those obtained by the reported method. The recovery of the labeled amount was 99.90 - 99.97% (Table 5.3). The results of t- and F- tests revealed that no significant differences were found at 95% confidence level with regard to accuracy and precision. In addition, interval hypothesis test [26] was also performed which revealed that the true bias of all the samples lie in the range of 0.98 and 1.02
<table>
<thead>
<tr>
<th>Pharmaceutical Formulations</th>
<th>Proposed method</th>
<th>Reference method</th>
<th>$t_{cal}$</th>
<th>$F_{cal}$</th>
<th>$\theta_L$</th>
<th>$\theta_U$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
<td></td>
<td></td>
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<tr>
<td>Serenace-5</td>
<td>99.90</td>
<td>0.240</td>
<td>99.87</td>
<td>0.235</td>
<td>0.199</td>
<td>1.043</td>
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<tr>
<td>Hexidol-5</td>
<td>99.97</td>
<td>0.154</td>
<td>99.91</td>
<td>0.237</td>
<td>0.449</td>
<td>1.420</td>
</tr>
</tbody>
</table>

\(^a\)Mean for 5 independent analyses.

\(^b\)Theoretical $t$ ($v = 8$) and $F$-values ($v = 4$) at 95 % confidence level are 2.306 and 6.39, respectively.

\(^c\)A bias, based on recovery experiments, of $\pm 2\%$ is acceptable.
(θ_L to θ_U). This also confirmed that no difference exists between the methods compared.

REFERENCES


