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Development and validation of spectrofluorimetric method for determination of haloperidol in pharmaceutical dosage forms

Nafisur Rahman* and Sana Siddiqui

A simple, sensitive, and accurate spectrofluorimetric method has been developed for the determination of haloperidol in pharmaceutical preparations. The present method is based on the formation of an ion-pair complex between haloperidol and alizarin red S at pH 3.4 which is extractable with chloroform. The ion-pair complex exhibits maximum fluorescence intensity at 564 nm with excitation at 466 nm. The reaction conditions were optimized to obtain maximum fluorescence intensity. The relation between the fluorescence intensity and concentration was found to be linear over the range 0.8–20 μg/mL with detection limit of 0.08 μg/mL. The method was successfully employed for quantitation of the active ingredient haloperidol in pharmaceutical preparations. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference between the methods compared in terms of accuracy and precision.

Keywords: pharmaceuticals; haloperidol; alizarin red S; spectrofluorimetry; validation

Introduction

Haloperidol is chemically known as 4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one [52-86-8] with a molecular weight of 375.86. It occurs as a white to pale yellow crystal. Its structural formula is shown in Figure 1.

Haloperidol is prescribed as a high-potency antipsychotic drug for treatment of schizophrenia, acute psychotic states, and delirium.[1] For the symptomatic management of psychotic disorder or Tourett's disorder, the usual initial dosage of haloperidol is 3 to 5 mg two or three times daily.[2] The drug is noted for its strong early and late extrapyramidal side-effects. The risk of the facial-disfiguring tardive dyskinesia is higher than with most other antipsychotic drugs. Due to the therapeutic importance, it becomes necessary to develop sensitive analytical methods for the determination of haloperidol in commercial dosage forms.

The United States Pharmacopoeia[3] recommended a potentiometric aqueous titration with perchloric acid and high performance liquid chromatography (HPLC) method for the determination of haloperidol in bulk and formulations, respectively. Haloperidol has been determined in pharmaceutical preparations and biological fluids using various techniques such as HPLC,[4–10] liquid chromatography,[11,12] liquid chromatography coupled with mass-spectrometry,[13,14] gas chromatography,[15] high performance thin layer chromatography (HPTLC),[16] square-wave adsorptive stripping voltammetry at a mercury electrode,[17] electrochemical sensor based on multi-walled carbon nanotubes,[18] NMR spectroscopy,[19] conductometric titrimetry[20] and spectrophotometry.[20–23] Spectrofluorimetry is frequently used in pharmaceutical analysis, which provides practical and significant economic advantages over other sophisticated instrumental techniques. Haloperidol in pharmaceutical preparations was determined fluorimetrically by oxidizing with acidic potassium permanganate.[24] The detection limit was found to be 0.05 μg ml^{-1}. The native fluorescence of haloperidol has been exploited to develop fluorimetric method for its quantitation.[25] The disadvantage of this method is that weak signals are obtained in most of the solvents.

The aim of this study was to develop an optimized and validated spectrofluorimetric method for the determination of haloperidol in pharmaceutical formulations. The proposed spectrofluorimetric method is based on the formation of ion-pair complex between the haloperidol and alizarin red S at pH 3.4. The ion-pair complex was extracted with chloroform which showed fluorescence at 564 nm after excitation at 466 nm. The reaction conditions are optimized and validated as per International Conference on Harmonisation (ICH) guidelines.[26]

Experimental

Apparatus

An F-2500 Hitachi fluorescence spectrophotometer (Tokyo, Japan) equipped with xenon lamp and 1-cm quartz cells was used to measure the fluorescence. Eutech instruments (Cyberscan pH 2100) pH meter was used to measure the pH. A double beam UV-Vis Spectrophotometer (Elico India Pvt. Ltd., New Delhi, India) was used for measuring absorbance for the reference method. All measurements were performed at 25 ± 1 °C.

Materials and reagents

- Haloperidol was procured from Sigma Chemical Company (St Louis, MO, USA). The following dosage forms containing

* *Correspondence to: Nafisur Rahman, Department of Chemistry, Aligarh Muslim University, Aligarh, U.P-202002, India. E-mail: cht17nr_amu@yahoo.com*

Department of Chemistry, Aligarh Muslim University, Aligarh, U.P-202002, India

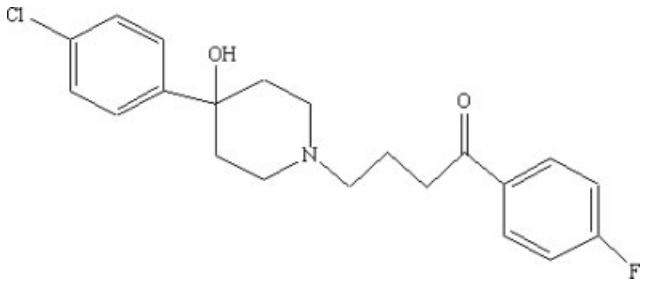

Figure 1. Structure of Haloperidol.

haloperidol were purchased from local commercial sources: (1) Trancodol tablet equivalent to 10 mg haloperidol (Intas Pharmaceuticals Pvt. Ltd., Ahmedabad India); and (2) Senorm tablet equivalent to 10 mg haloperidol (Sun Pharmaceutical Industries Ltd., Mumbai India).

- 5.84×10^{-3} M Alizarin Red S (CAS: 130-22-3), M.W.: 342.26, Fluka Chemie AG, Buchs SG Switzerland) solution was freshly prepared in distilled water.
- Buffer solutions ranging from pH 2.2 to 3.8 were prepared by mixing 50 ml of 0.1 M potassium biphthalate with appropriate volumes of 0.1 M HCl and diluting each solution to 100 ml with distilled water.

Standard test solution

Standard solution of haloperidol (100 µg ml^{-1}) was prepared by dissolving 10 mg of drug in 100 ml methanol.

Analytical procedure

Appropriate volumes of standard solution of haloperidol (100 µg ml^{-1}) corresponding to 4.0–100 µg were transferred into a series of 25 ml separating funnels. To each separating funnel, 2.0 ml of 5.84×10^{-3} M alizarin red S solution and 1 ml of buffer solution (pH–3.4) were added. The contents of the separating funnel were shaken vigorously with 5 ml of chloroform for 2 min and then allowed to separate into the two layers. The fluorescence intensity of the organic layer was observed at 564 nm with excitation at 466 nm and the calibration graph was constructed.

Assay of the drug in dosage forms

Five tablets were powdered and haloperidol was extracted by shaking with 20 ml of methanol followed by another two extractions, each with 10 ml of methanol. The extracts were filtered through Whatmann No. 44 filter paper (Whatmann International Limited, Kent, UK) into a 50 ml volumetric flask and then diluted to volume with methanol. The stock solution of drug was further diluted with methanol, and analyzed following the proposed procedure.

Procedure for Reference Method [27]

Aliquots of 0.1% standard methanolic solution of haloperidol corresponding to 50–100 µg ml^{-1} of haloperidol were pipette into a series of 10 ml volumetric flasks. To each flask, 2.0 ml of p-chloranilic acid solution (0.2%) was added and diluted to the mark with the methanol. The contents of each flask were mixed well and the absorbance at each concentration of haloperidol was recorded at 540 nm against the reagent blank prepared similarly without the drug. The amount of the drug in a given sample can be estimated either from the calibration graph or the corresponding linear regression equation.

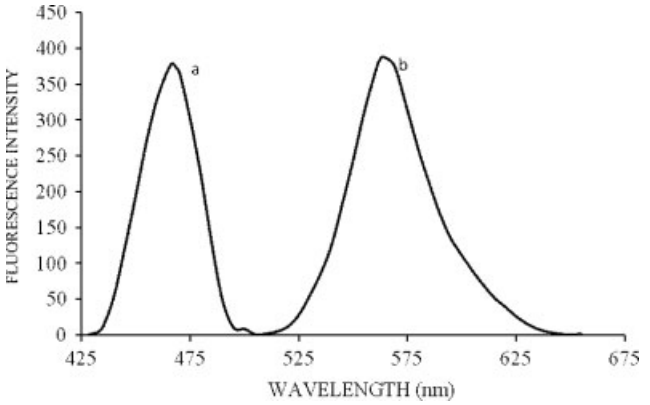

Figure 2. Excitation and emission spectra of ion pair complex: 10µg ml^{-1} haloperidol with 2.0 ml of alizarin red S (0.2%) and 1.0 ml buffer solution (pH 3.4). The complex was extracted in 5 ml chloroform.

Results and Discussion

Haloperidol was found to react with alizarin red S at pH 3.4 resulting in the formation of an ion-pair complex which was extracted into chloroform. The ion-pair complex exhibited fluorescence at $\lambda_{em} = 564$ nm with excitation at $\lambda_{ex} = 466$ nm. The excitation and emission spectra are shown in Figure 2. The ion-pair complex was stable at least for 2 h. The fluorescence nature of the ion-pair complex was exploited to develop a new spectrofluorimetric method for determination of haloperidol in commercial dosage forms.

Stoichiometry and Reaction Mechanism

The molar combining ratio between haloperidol and alizarin red S was evaluated by Job's method of continuous variations.[28] The results are shown in Figure 3. It is apparent from the figure that

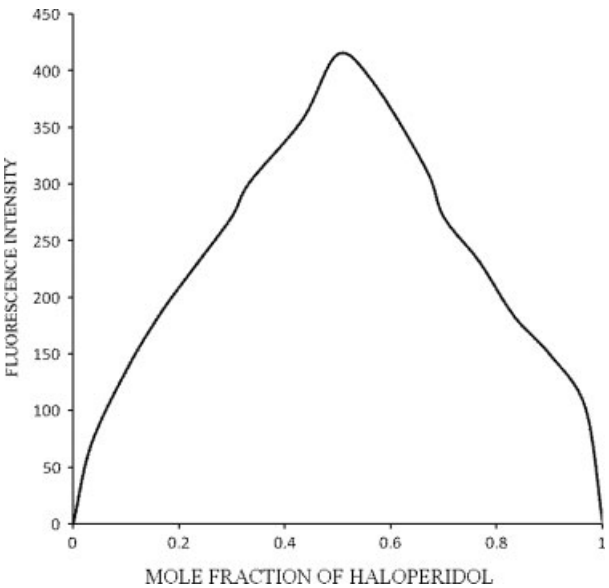

Figure 3. Job's plot for haloperidol-alizarin red S ion-pair complex (each 2.66×10^{-4} M).

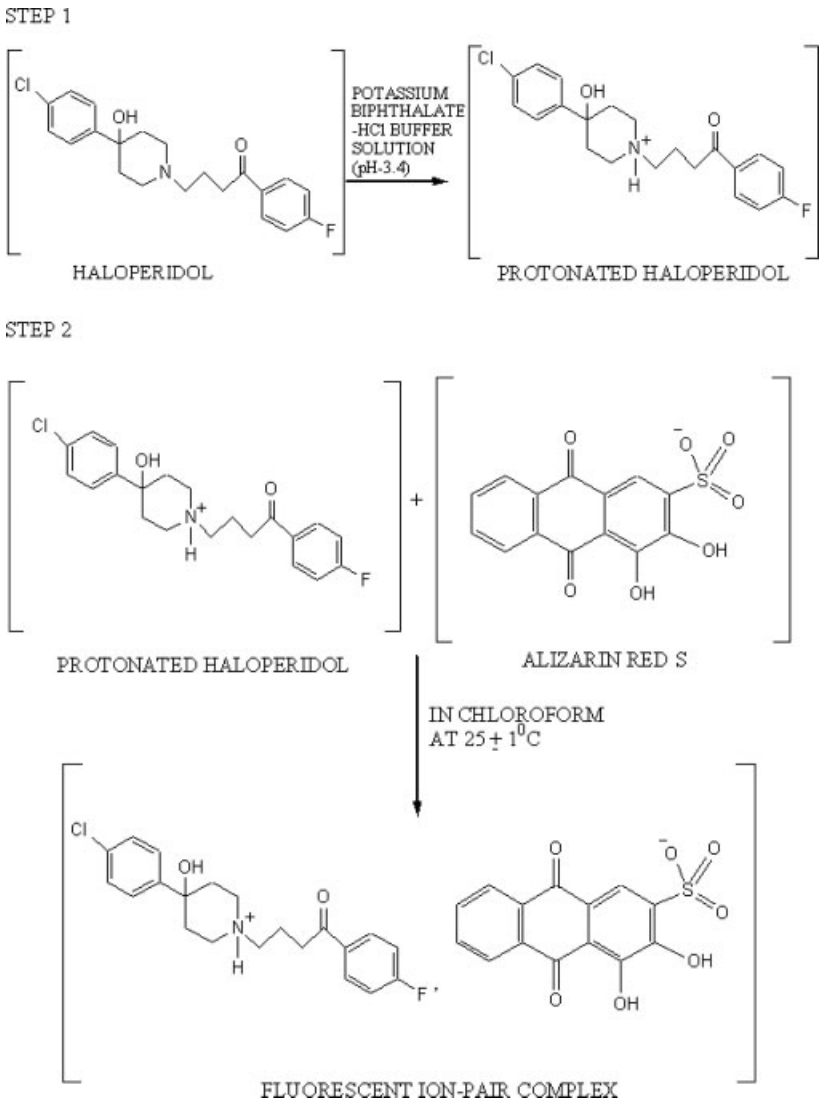

Figure 4. Reaction mechanism of the proposed method.

the combining molar ratio between haloperidol and alizarin red S is 1 : 1. The apparent formation constant and standard Gibbs free energy (ΔG°) were calculated and found to be 2.715×10^6 and -36.71 kJ mol^{-1}, respectively.

Haloperidol contains a piperidyl group which has a tertiary nitrogen atom in its structure. In acidic solution (pH 3.4), the nitrogen atom of piperidyl group is protonated and consequently a positive center is created in the drug molecule. Alizarin red S is sodium salt of 3, 4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid which ionizes in aqueous solution; a negative charge is developed on it. Thus, one mole of protonated haloperidol reacts with one mole of alizarin red S resulting in the formation of ion-pair complex. The complex formed is conveniently extracted in chloroform. Based on the literature background and our experimental findings, the reaction sequence of the proposed method is given in Figure 4.

Optimization of Reaction Conditions

Investigations were carried out to establish the optimum reaction conditions with respect to solvent, shaking time for extraction, reagent concentration and pH.

Solvent effect

The extraction efficiency of various organic solvents such as chloroform, carbon tetrachloride, dichloromethane, dichloroethane, and ethyl acetate was examined. The fluorescence intensity of the ion-pair complex extracted in different solvents was measured and results are presented in Figure 5. As can be seen from Figure 5, complete extraction was attained in chloroform whereas no extraction occurred in ethyl acetate. Therefore, the ion-pair complex was extracted in chloroform for determination process.

Effect of shaking time for extraction

To optimize the shaking time for extraction of fluorophore into chloroform, experiments were performed for the periods ranging from 1–3 min (Figure 6). It can be seen from the figure that maximum and constant fluorescence intensity was obtained from 1.5 min of shaking and therefore, 2 min shaking time was recommended for the extraction of fluorophore.

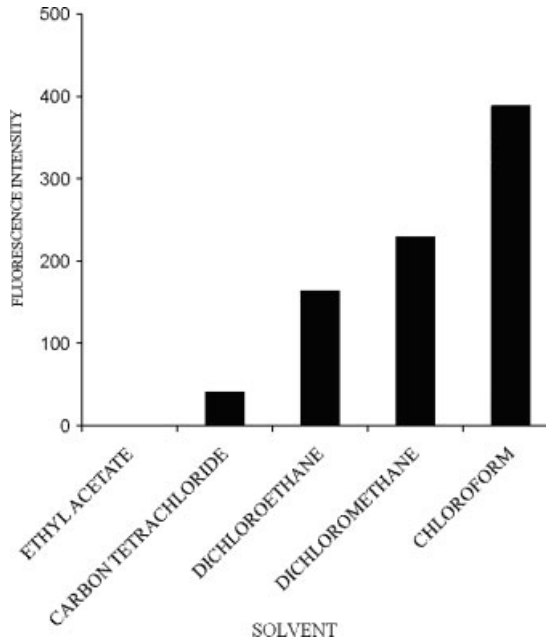

Figure 5. Effect of the solvent on the extraction of ion-pair complex.

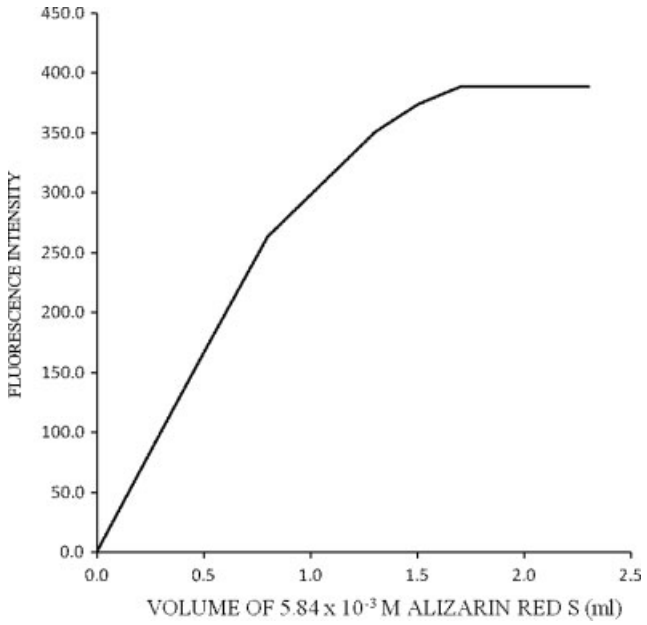

Figure 7. Effect of the volume of 5.84×10^{-3} M alizarin red S on the fluorescence intensity of the ion-pair complex (20 μg ml^{-1} haloperiodol).

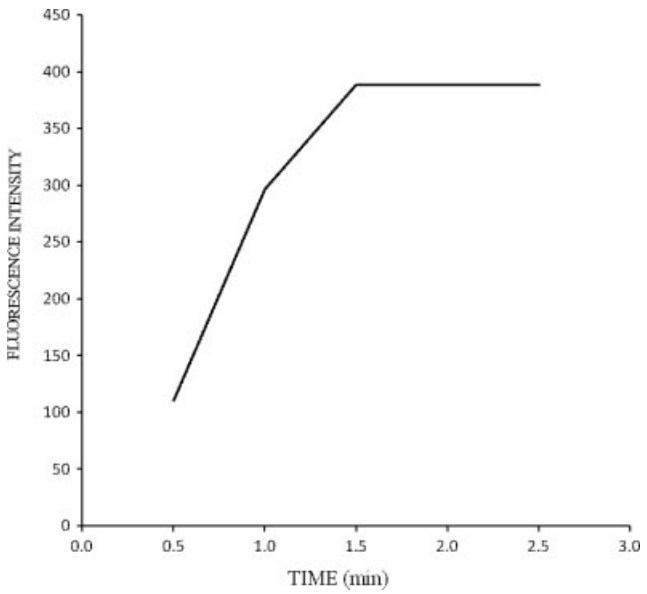

Figure 6. Effect of shaking time for the extraction of the ion pair complex.

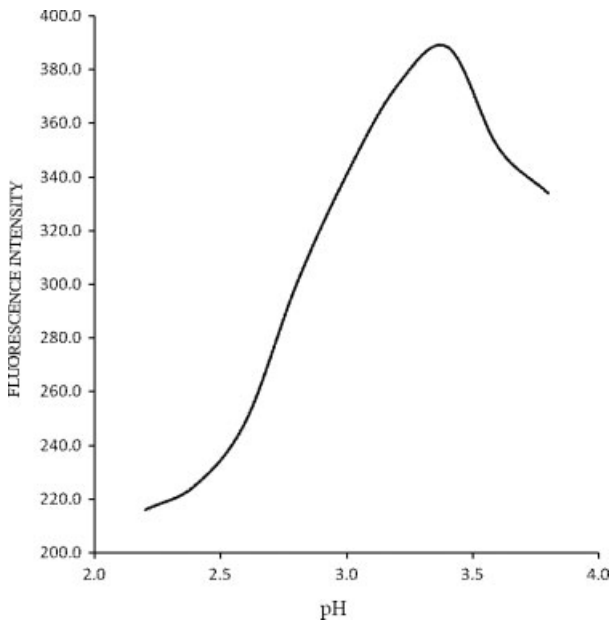

Figure 8. Effect of different pH on the fluorescence intensity of the ion-pair complex (20 μg ml^{-1} haloperidol; potassium biphthalate-HCL buffer solution).

Effect of alizarin red S concentration

The effect of volume of 5.84×10^{-3} M alizarin red S on the fluorescence intensity was studied in the range of 0.3–2.3 ml; keeping the amount of the drug (100 μg) constant (Figure 7). It can be seen that the fluorescence intensity increased linearly with increasing the volume of 5.84×10^{-3} M alizarin red S from 0.3 to 1.7 ml and became constant over the range 1.7 to 2.3 ml. Thus, a volume of 2.0 ml of 5.84×10^{-3} M alizarin red S was chosen as an optimum volume for all measurements.

Effect of pH

The influence of pH on the fluorescence intensity has been studied using potassium biphthalate-HCl buffer solution in the range of pH 2.2–pH 3.8. It is evident (Figure 8) that the maximum fluorescence intensity was found at pH 3.4. Therefore, all fluorescence intensity measurements were made at pH 3.4 in the determination process.

Effect of volume of pH 3.4 buffer solution

The effect of volume of pH 3.4 buffer solution on the fluorescence intensity was investigated in the range of 0.1–1.2 ml. The maximum and constant fluorescence intensity was obtained in the range of 0.8–1.2 ml (Figure 9). Therefore, 1.0 ml of pH 3.4 buffer

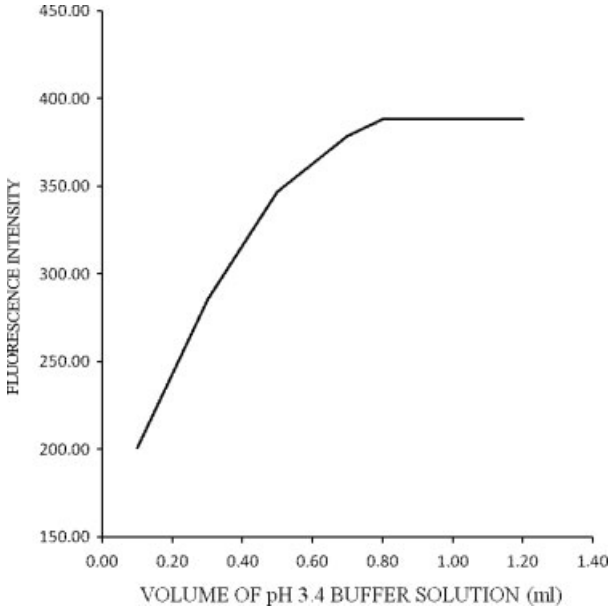

Figure 9. Effect of the volume of pH 3.4 buffer solution on the fluorescence intensity (20 μg ml^{-1} haloperidol).

solution was adopted as an optimum volume for fluorescence measurement.

Analytical Performance of the Proposed Method

Linearity

The linearity of the proposed method was evaluated by analyzing a series of different concentrations of haloperidol. Linear correlation was found between fluorescence intensity and haloperidol concentration and is described by the regression equation:

$$I_F = 0.4455 + 19.4394C, \quad r = 0.99999, \quad n = 9 \qquad (1)$$

where I_F is the fluorescence intensity and C is the concentration in μg ml^{-1}; r is the correlation coefficient and n is the number of measurement levels. The linearity was obtained in the concentration range of 0.80–20.0 μg ml^{-1}. The variance of the calibration line was found to be 0.23 μg ml^{-1}. Regression analysis showed good linearity of the calibration line as indicated from the correlation coefficient value (>0.9999).

Detection and quantitation limits

According to ICH guidelines,[26] the limits of detection (LOD) and quantitation (LOQ) are calculated using the following equations:

$$LOD = 3.3 \times S_o/b \qquad (2)$$

$$LOQ = 10.0 \times S_o/b \qquad (3)$$

where S_o and b are the standard deviation and slope of calibration line.

The LOD and LOQ values were found to be 0.08 μg ml^{-1} and 0.24 μg ml^{-1}, respectively.

Precision and accuracy

To evaluate intra-day and inter-day precisions, analysis of standard haloperidol at three concentration levels (2, 6 and 16 μg ml^{-1}) of the calibration curve was carried out by performing five experiments on the same day using the same analyte standard solution and over five consecutive days using different solutions. The results of these assays are reported in Table 1. The intra-day and inter-day RSD values ranged from 0.10–0.64% and 0.29–0.58% respectively, reflecting the usefulness of the method in routine use. The percent relative error was −0.10–0.35% and 0.16–0.55% for intra-day and inter-day assay respectively.

Table 1. Test of accuracy and precision of the proposed method

Parameters	Intra-day assay			Inter-day assay		
Concentration taken (μg mL^{-1})	2.00	6.00	16.00	2.00	6.00	16.00
Concentration found (μg mL^{-1})	2.01	5.99	15.98	2.01	6.01	16.03
Standard deviation[a] (μg mL^{-1})	0.01	0.02	0.02	0.01	0.02	0.05
Relative standard deviation (%)	0.64	0.40	0.1	0.58	0.30	0.29
Error (%)	0.35	−0.10	−0.11	0.55	0.23	0.16

[a] Mean for 5 independent determinations.
[b] Confidence limit at 95% confidence level and four degrees of freedom (t = 2.776).

Table 2. Recovery of haloperidol from Trancodol tablet by standard addition technique

Concentration (μg mL^{-1})			Coefficients of linear regression equation of standard addition			
Trancodol tablet	Standard added	Found	Intercept	slope	r^a	Recovery[b] (%)
2.0	0, 1.0, 2.0, 3.0, 4.0	2.003	39.074	19.51	0.99996	100.14
4.0	0, 1.0, 2.0, 3.0, 4.0	4.001	78.228	19.551	0.99991	100.03

[a] Coefficient of correlation.
[b] Mean for 5independent analyses.

Recovery

The recovery experiments were performed on haloperidol tablets at two concentration levels, i.e. 2.0 and 4.0 μg ml^{-1} using standard addition method. Each concentration was spiked with four different concentrations of pure drug. Figure 10 shows the plot of fluorescence intensity *vs* concentration of haloperidol added. The recovery was evaluated either by dividing the intercept by the slope value of the regression line or by extrapolating the same regression line. The recoveries ranged from 100.03–100.14% (Table 2).

The selectivity of the proposed method was evaluated by analyzing a standard solution of haloperidol in the presence of tablet excipients such as lactose, starch, glucose, fructose, and magnesium stearate. It was observed that these excipients

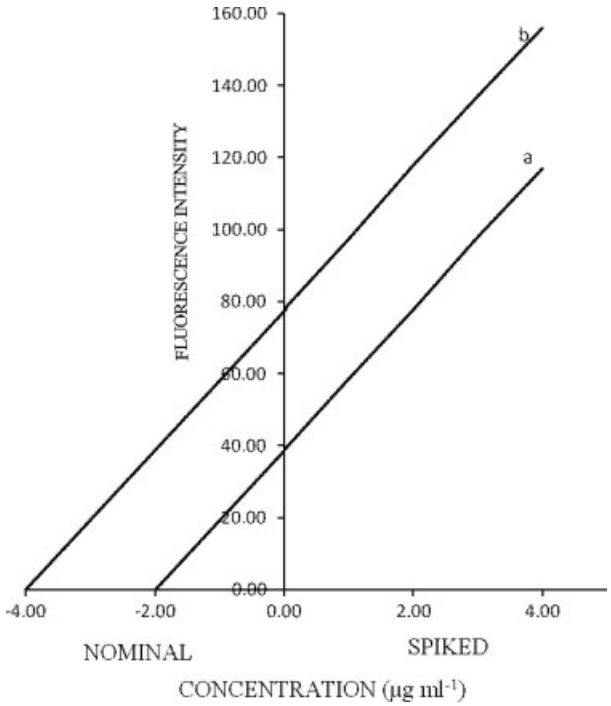

Figure 10. Recovery of haloperidol from trancodol tablet by standard addition-technique: (a) 2.0 and (b) 4.0 μg ml^{-1}.

Table 3. Comparision of the proposed method with the reference method.[27]

Pharmaceutical formulations	Proposed method		Reference method		Paired t- & F values[b]	θ_L[c]	θ_U[c]
	Recovery (%)	RSD, (%)	Recovery (%)	RSD[a] (%)			
Trancodol tablet	100.01	0.012	99.989	0.02	t = 0.233 F = 2.115	0.997	1.002
Senorm tablet	100.02	0.013	100.04	0.02	t = 0.188 F = 2.546	0.996	1.003

[a] Mean for 5 independent analyses.
[b] Theoretical *t* ($\nu = 8$) and *F*-values ($\nu = 4, 4$) at 95% confidence level are 2.306 and 6.39, respectively.
[c] A bias, based on recovery experiments, of ±2% is acceptable.

did not interfere with the proposed method. The results of the recovery experiment also indicated that neither the accuracy nor the precision of the proposed method is affected by the co-formulated substances.

Robustness

According to ICH,[26] robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations. To test the robustness of the proposed procedure, the following parameters were challenged:

- reaction temperature, 25 ± 2 °C;
- volume of 5.84 × 10^{-3} M alizarin red S solution, 2.0 ± 0.3 ml;
- volume of pH 3.4 buffer solution, 1.0 ± 0.2 ml; and
- shaking time, 2.0 ± 0.5 min.

Under the above conditions, the haloperidol sample (Trancodol tablet) solution containing 20 μg ml^{-1} was determined by the proposed method. It was found that there is no significant effect on the result of analysis owing to the small and deliberate changes in experimental conditions, indicating the robustness of the proposed method.

Application to Dosage Forms

The proposed method was applied to the analysis of haloperidol in commercial tablets such as Trancodol and Senorm and the results were statistically compared with those obtained by reported spectrophotometric method[27] using point and interval hypothesis tests (Table 3). The assay results showed that student's *t*- and F- values did not exceed the theoretical ones at 95% confidence level. In all cases, the values of lower and upper limits based on recovery experiments were found to be within ±2%. This indicated the compliance of the regulatory authority.[29]

Conclusions

The proposed method for the determination of haloperidol in pharmaceutical formulations is based on the formation of an ion-pair complex which displays fluorescence at 564 nm (λ_{ex} =

466 nm). The proposed method was optimized and validated for precision and accuracy. The present work has the advantage of having simple operation, high sensitivity, repeatability, and reproducibility. In addition, the proposed method has a linear dynamic range of 0.8–20.0 μg ml^{-1} with a detection limit of 0.08 μg ml^{-1}. Hence, the proposed method can be used for routine quality control analysis of haloperidol in industries, research laboratories, and hospitals.

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