

A New Spectrofluorimetric Approach for the Quantitation of Imipramine HCl in Commercial Dosage Forms

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Abstract

A spectrofluorimetric method has been developed for the determination of imipramine HCl in bulk and commercial dosage forms. The method was based on measuring the fluorescence emission intensity of imipramine-eosin Y ion pair complex ($\lambda_{em} = 558$ and $\lambda_{ex} = 319$) in dichloroethane at buffer solution (sodium acetate and acetic acid) of pH 4.8. The stoichiometric ratio between imipramine and eosin Y was studied by Job's method of continuous variations and found to be 2:1. Formation constant (K_f) and Gibb's free energy change (ΔG) were calculated and pointed towards the spontaneous nature of the reaction. A series of variables were studied to optimize the reaction conditions. The proposed method was validated as per ICH guidelines and successfully applied for the determination of active imipramine HCl in commercial dosage forms with high degree of accuracy and precision.

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Keywords: : imipramine HCl; spectrofluorimetry, validation; ICH guideline, commercial dosage forms

Received Jul 20,2017; **Accepted** Sep 02,2017; **Published** : Sep 04 ,2017

Imipramine hydrochloride is chemically known as 5-3-(dimethylamino)propyl-10,11-dihydro-5H-dibenz[b,f]-azepine monohydrochloride (Chemical Abstract Service: 113-52-0; Molecular Weight: 316.88). The drug is a dibenzazepine derivative of tricyclic antidepressant, competitively blocks the reuptake of norepinephrine and serotonin in synapses in brain [1]. The drug is prescribed in the treatment of psychiatric patients suffering from depression. The drug has many variety of side effects which include drowsiness, convulsions, respiratory disorders, ophthalmoplegia, and finally coma [2]. Therefore, lower dosages of imipramine hydrochloride are recommended. The inactive ingredients of imipramine are calcium phosphate, cellulose compounds, docusate sodium, iron oxides, magnesium stearate, polyethylene glycol, povidone, sodium starch glycolate, sucrose, talc, and titanium dioxide. The drug is always administered orally. It is advisable to initiate treatment at a dose of 10-25 mg daily. Toxic-effects can be induced especially when overdosed and/or high-dose drugs is combined with alcohol [3]. With increasing regulatory strictness, the quality, quantity and safety of imipramine can be maintained for obtaining optimum therapeutic concentration and for quality assurance in pharmaceutical formulations. The importance of analytical techniques involved in the quality control analysis of active drug in pharmaceutical formulations has been discussed in published papers [4-6].

Imipramine HCl is officially listed in British Pharmacopoeia [7] which describes a liquid chromatographic method for its assay in bulk and tablet forms. Several other analytical methods have been reported based on high performance thin layer chromatography [8], high performance liquid

chromatography [9,10], electrochemical analysis [11-13] and spectrophotometry [14-19]. Imipramine HCl is weakly fluorescent in nature and reagents such as erythrosine B in chloroform [20] and rhodamine B in ethylene glycol-distilled water medium [21] have been utilized for quantitative determination of active drug in pharmaceutical formulations by fluorescence spectrophotometry. Eosin Y is a xanthene polyprotic fluorescent acid dye which contains 2 acidic protons with pK_a of 2 and 3.8 in water [22]. Eosin Y is an interesting reagent for determination purposes because of the fluorescent and dianionic nature of the dye. Eosin Y has been used for determination of doxepin [23] and citalopram HBr [24]. In this study, eosin Y acted as a fluorescent probe and formed fluorescent ion-pair complex with imipramine in the presence of sodium acetate-acetic acid buffer solution of pH 4.8. The complex was extracted in dichloroethane provided fluorescence emission intensity at 558 nm after excitation at 319 nm. As per literature survey and gathered information, there is no spectrofluorimetric method based on the extraction of complex in dichloroethane for quantitation of imipramine. Extraction in dichloroethane improved sensitivity and selectivity of the method. The reactions conditions are optimized and validated as per International Conference on Harmonisation guidelines (USA) [25].

Materials and methods

Apparatus

Fluorescence intensity and spectra were recorded on Thermo Scientific Agilent's Cary Eclipse Fluorescence Spectrophotometer (Australia) equipped with a xenon 150 W arc lamp and 1-cm quartz cells. Excitation and emission wavelengths were set with slit widths of 5 nm. pH values were measured using Hanna pH meter (USA).

Materials

All reagents and solvents used were of analytical reagent grade. 0.03% eosin Y disodium salt (CAS: 17372-87-1, M.W.: 691.85, Fluka Chemie AG, Switzerland) solution was prepared by dissolving 0.03 g of eosin Y in 100 mL standard volumetric flask and diluted up to the mark with distilled water.

Walpole sodium acetate-acetic acid buffer solution of different pH (3.72-5.57) were prepared using different volumes of 0.2M sodium acetate and 0.2M acetic acid in a total volume of 10 mL [26].

Imipramine hydrochloride was purchased from Sigma-Aldrich (USA). 0.02% imipramine HCl solution was prepared by dissolving 0.02 g drug in 100 mL distilled water. Imipramine 25 (Actavis, UK) and imipramine HCl 25 (SGH, Singapore) were procured from SQU hospital (Oman) and Ibn Sina Hospital (Oman), respectively.

Procedure for the Determination of Imipramine Hydrochloride by Proposed Method

0.15-1.0 mL of 0.02% imipramine HCl and 2 ml of sodium acetate-acetic acid buffer solution of pH 4.8 were added with 1.6 ml of 0.03% eosin Y into 10 mL standard volumetric flask. The contents of the flask were diluted up to the mark with distilled water at $25\pm 1^\circ\text{C}$ and transferred into a 100 mL separating funnel. 10 mL of dichloroethane was poured in the separating funnel and the contents of the funnel were thoroughly mixed for 2 min. 2 layers were formed and the dichloroethane layer was enriched with fluorescent ion-pair complex. The dichloroethane layer was separated and treated with anhydrous sodium sulphate (4g). The said layer was now subjected for recording fluorescence emission intensity at 558 nm after keeping excitation wavelength

at 319 nm. The calibration graph was plotted and the linear regression equation was developed for the estimation of imipramine HCl in commercial tablets.

Procedure for the Determination of Imipramine Hydrochloride by Reference Method

Into a series of 10 mL standard volumetric flask, 0.2-0.5 mL of 0.02% imipramine HCl solution was taken and the solution was diluted up to the mark with distilled water at room temperature. The fluorescence emission intensity at 407 nm was measured after keeping excitation wavelength at 259 nm against distilled water as a blank. The calibration graph was constructed by plotting fluorescence emission intensity against the initial concentration of imipramine HCl. The linear regression equation was generated using Origin Pro6.1 software and utilized for quantitation of active imipramine in pharmaceutical formulations.

Procedure for the Determination of Active Imipramine Hydrochloride in Pharmaceutical Formulations by Proposed and Reference Methods

Ten tablets of Imipramine 25 (Actavis, UK) and imipramine HCl 25 (SGH, Singapore) were weighed and finely powdered using agate mortar and pestle. An amount of powdered tablets equivalent to 20 mg imipramine HCl was transferred in 100 mL beaker and dissolved in 50 mL of distilled water. The solution was passed through a filter funnel equipped with Whatmann No 42 filter paper in 100 mL standard volumetric flask. The residue on filter paper was washed with 4×10 mL portions of distilled water. The filtrate was diluted up to the mark with distilled water in 100 mL volumetric flask and finally cleaned using $0.45\mu\text{m}$ polyethersulfone membrane (Filter-Lab) using 5mL syringe. The recommended procedures were followed for the

determination of active imipramine HCl in commercial dosage forms.

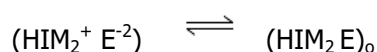
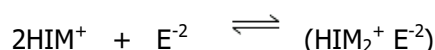
Results and Discussion

In the literature, it was reported that doxepin HCl [23] and citalopram HBr [24] possessed tertiary amine group. 2 acidic protons of carboxylic and phenolic group sites of eosin Y were ionized [22] forming dianionic eosin Y with strong green fluorescence ($\lambda_{\text{excitation}} = 257.96 \text{ nm}$ and $\lambda_{\text{emission}} = 544.02 \text{ nm}$). There was no extraction of eosin Y in organic solvent in the presence of buffer solution alone. Hence, 2 molecules of said protonated drugs were considered binding with carboxylic and phenolic group sites of eosin Y and extracted in chloroform/dichloromethane. Similarly, here in acidic condition (pH 4.8 buffer solution of sodium acetate-acetic acid), eosin Y ionized 2 acidic protons which was protonated to 2 molecules of imipramine through nitrogen as centre. Hence, dianionic eosin Y interacted with protonated imipramine and formed fluorescent ion pair complex ($\lambda_{\text{em}} = 558 \text{ nm}$ and $\lambda_{\text{ex}} = 319 \text{ nm}$). At the same time, dianionic eosin Y with acidic buffer solution (without imipramine) was not extracted in dichloroethane. The aqueous solution of imipramine HCl showed an emission wavelength of 407 nm after keeping excitation wavelength constant at 259 nm. The spectrofluorimetric spectra of aqueous imipramine, aqueous eosin Y and ion-pair complex were given in Fig 1.

The selectivity offered by fluorescence measurements is invaluable because of distinct excitation and fluorescence spectra and wavelengths available for each fluorophore [27]. A further extremely important group of selective methods is based on the phenomenon of energy transfer. Self-quenching of aqueous eosin Y and even in sodium acetate-acetic acid buffer solution of pH

4.8 was not observed. Stokes shift was observed on the interaction of eosin Y with imipramine in the presence of sodium acetate-acetic acid buffer solution of pH 4.8 (Fig. 1). This resulted in the resonance energy transfer from eosin Y to imipramine. The transition occurred from the interaction of the transition dipoles of eosin Y and imipramine groups.

The extraction equilibria can be represented as follows:



where HIM^+ and E^{2-} are protonated imipramine and dianionic eosin Y, respectively, and the subscript o refers to the dichloroethane layer. The reaction sequence is shown in Fig. 2.

The stoichiometric ratio between imipramine and eosin Y for the ion pair complex was established by Job's method of continuous variations [28]. In this method, identical molar concentration ($6.31 \times 10^{-4} \text{ M}$) of imipramine and eosin Y were mixed in varying volume ratios but the total volume of each mixture was same. The fluorescence emission intensity of each solution was recorded and plotted against the mole fraction of imipramine, $[\text{imipramine}] / [\text{imipramine}] + [\text{eosin Y}]$. The results obtained with this method resulted in the stoichiometric ratio between imipramine and eosin Y as 2:1. The formation constant (K_f) was calculated using the following expression [23, 24, 29]:

$$K_f = \frac{(F_{\text{obs}}/F_{\text{extp}})C}{[C_{\text{IM}} - 2\left(\frac{F_{\text{obs}}}{F_{\text{extp}}}\right)C]^2 [C_{\text{E}} - (F_{\text{obs}}/F_{\text{extp}})C]} \quad \text{Eq.1}$$

Where F_{obs} and F_{extp} are observed and extrapolated fluorescence emission intensities of the complex. C_{IM} , C_{E}

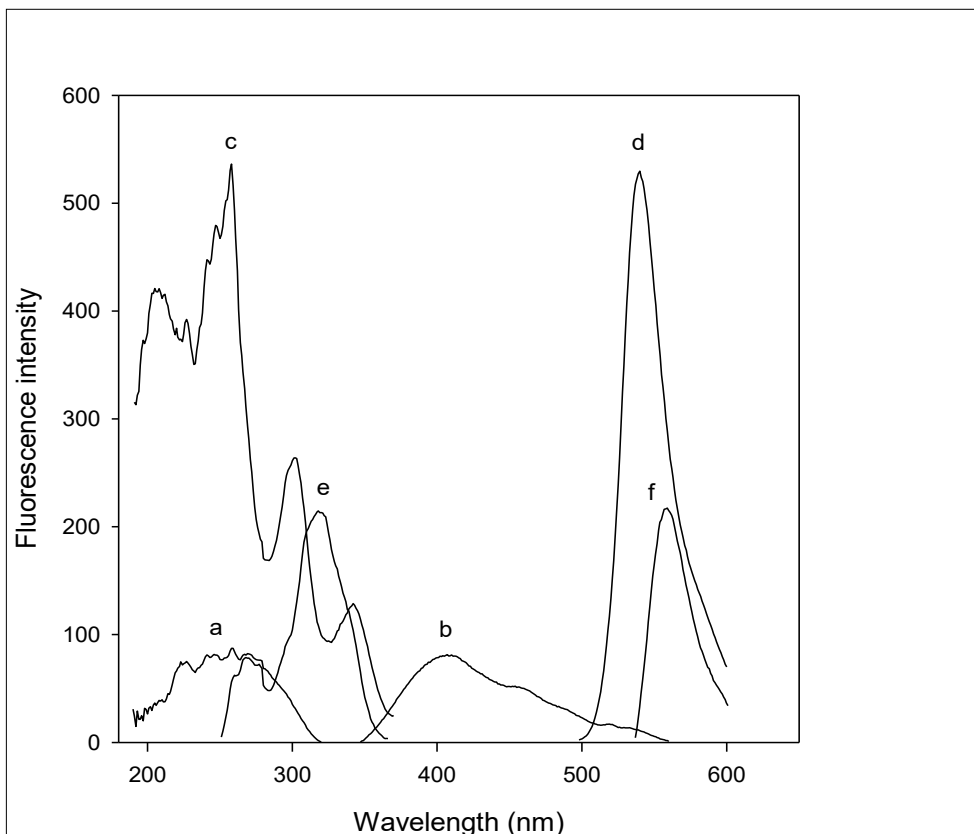


Fig. 1. Spectrofluorimetric spectra: 0.5 mL of 0.02% imipramine HCl in 10 mL volumetric flask and diluted up to mark with distilled water (a) $\lambda_{\text{excitation}}=259$ nm and (b) $\lambda_{\text{emission}}=407$ nm; 0.5 ml of 0.03% eosin Y in 10 mL volumetric flask and diluted up to mark with distilled water (c) $\lambda_{\text{excitation}}=257.96$ nm and (d) $\lambda_{\text{emission}}=544.02$ nm; 1 ml of 0.02% imipramine HCl + 1 mL of sodium acetate-acetic acid buffer solution of pH 4.8 + 1.6 ml of 0.03% of eosin Y in 10 mL volumetric flask and diluted up to mark with distilled water, then extracted in 10 mL dichloroethane (e) $\lambda_{\text{excitation}}=319$ nm and (f) $\lambda_{\text{emission}}=558$ nm. Excitation wavelengths used to measure emission spectra and emission wavelengths used to measure excitation spectra.

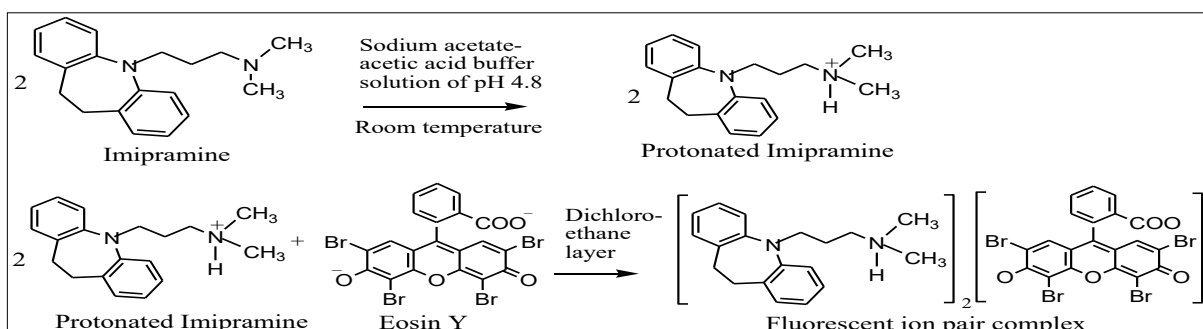


Fig. 2. Reaction sequence of the proposed method.

and \bar{c} are initial concentration of imipramine, eosin Y and limiting concentration ($=C_E$) in mol L^{-1} , respectively. The K_f of the associated complex was found to be 4.36×10^{12} . The apparent Gibbs free energy (ΔG°) was calculated using $\Delta G^\circ = -2.303 RT \log K_f$ and found to be $-72.09 \text{ kJ mol}^{-1}$. The high negative value of ΔG° provided strong evidence for the feasibility of the reaction.

time for extraction of complex were optimized with 20.0 mg mL^{-1} imipramine hydrochloride.

The effect of the reaction time was investigated. The maximum fluorescence emission intensity of the complex was achieved immediately and stable up to 1 h at $25 \pm 1^\circ\text{C}$. Therefore, the analysis can be performed within 1 h.

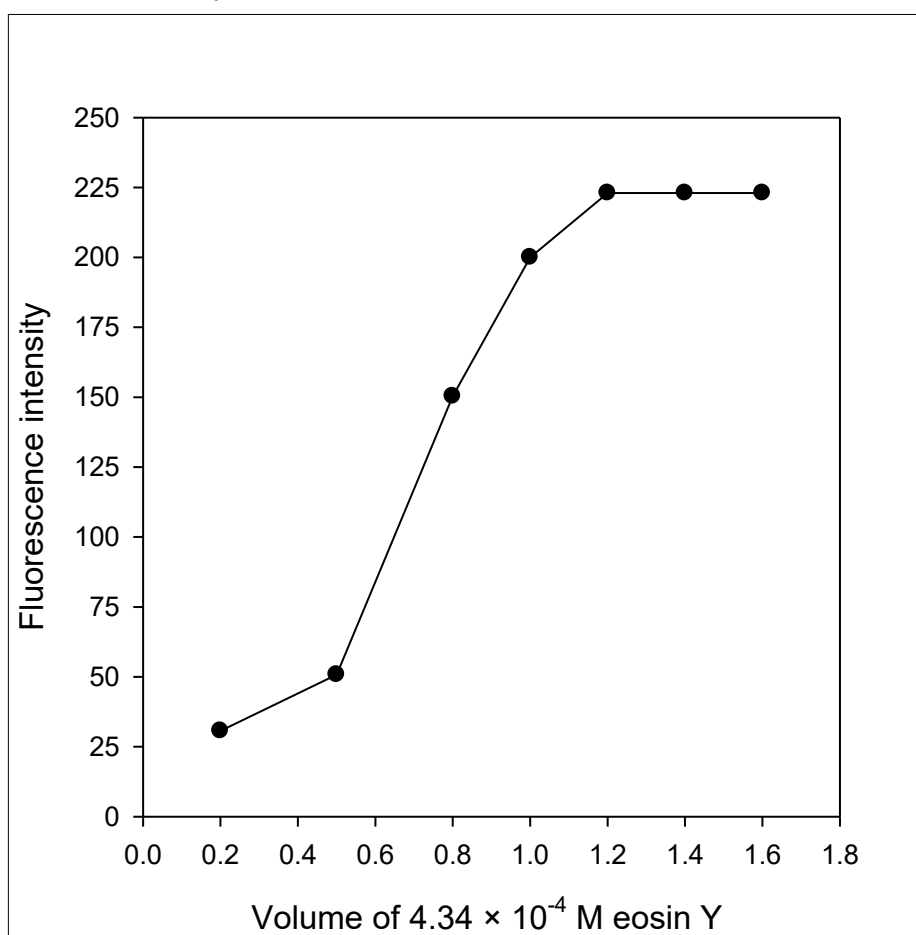


Fig. 3. Effect of the volume of $4.34 \times 10^{-4} \text{ M}$ eosin Y on the fluorescence intensity of the imipramine-eosin Y complex.

Method Optimization and Validation

For the development and optimization of analytical method, certain validation parameters and experimental variables were investigated separately.

Optimization of Variables

The experimental variables such as reaction time, concentration of eosin Y, sodium acetate-acetic acid buffer solutions of different pH, volume of buffer solution at particular pH, extracting solvents and shaking

The influence of the volumes of $4.34 \times 10^{-4} \text{ M}$ eosin Y (0.03%) was studied. The maximum fluorescence emission intensity was obtained with 1.2 mL of $4.34 \times 10^{-4} \text{ M}$ eosin Y and remained constant up to 1.6 mL of $4.34 \times 10^{-4} \text{ M}$ eosin Y (Fig. 3). Therefore, 1.4 mL of $4.34 \times 10^{-4} \text{ M}$ eosin Y was used as optimum volume of eosin Y for the analysis of active imipramine in commercial dosage forms.

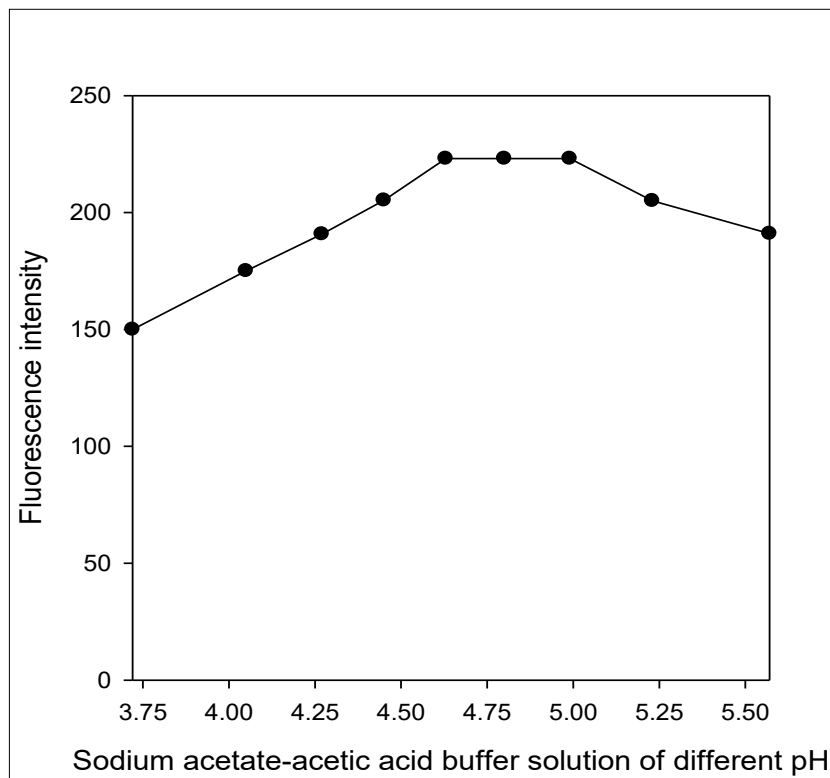


Fig. 4. Effect of sodium acetate-acetic acid buffer solution of different pH on the fluorescence intensity of the imipramine-eosin Y complex.

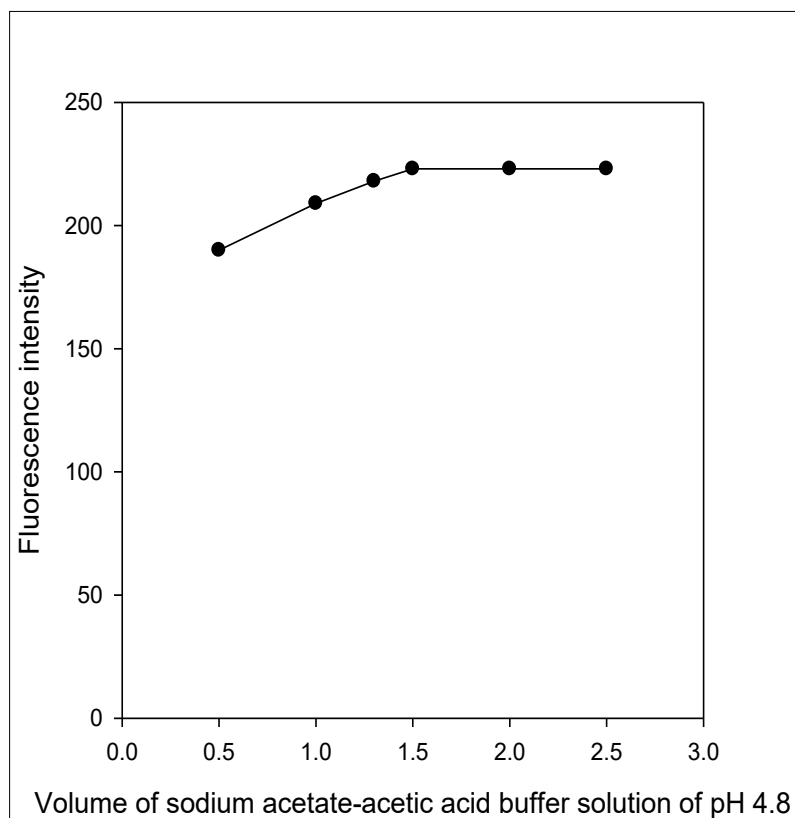


Fig. 5. Effect of the volume of sodium acetate-acetic acid buffer solution of pH 4.8 on the fluorescence intensity of the imipramine-eosin Y complex.

The effect of the pH of the aqueous phase on fluorescent ion-pair extraction was studied using sodium acetate-acetic acid buffer solutions over the pH range 3.72-5.57. The fluorescence intensity of dichloroethane

selected as an optimum volume for further measurement of fluorescence emission intensity in the determination process.

The effect of the extracting solvent such as dichloroethane, dichloromethane, chloroform, hexane,

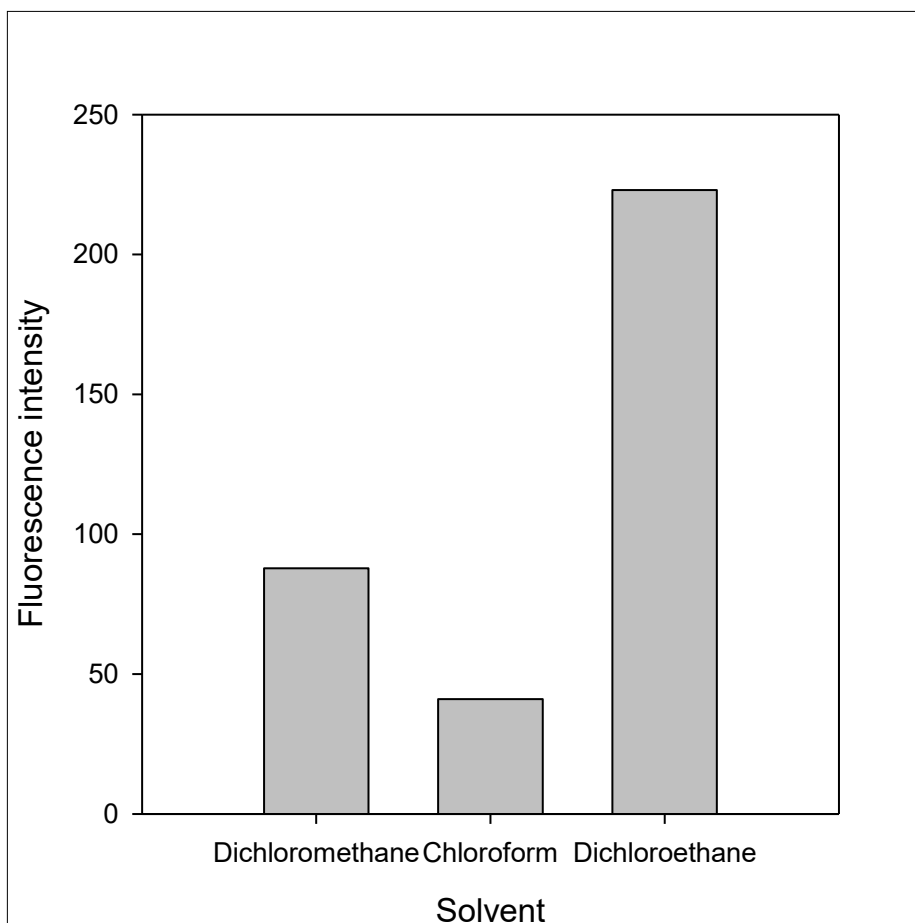


Fig. 6. Effect of the solvent Y on the fluorescence intensity of the imipramine-eosin Y complex

extract was maximum and constant in the pH range 4.63-4.99. Above pH 4.99, the fluorescence intensity was decreased (Fig 4). Therefore, all fluorescence intensity measurements were made at pH 4.8 in the determination process.

The effect of volume of sodium acetate-acetic acid buffer solution of pH 4.8 was examined in the range of 0.5-2.5 mL. The highest fluorescence intensity was obtained with 1.5 mL, later on remained constant (Fig. 5). Therefore, 2.0 ml of pH 4.8 buffer solution was

ethyl acetate, and benzene was tested. The polarity of a solvent affects both the extraction efficiency and fluorescence intensity. There was no fluorescence intensity recorded in ethyl acetate, benzene and hexane. The fluorescence emission intensity of the blank solution was also recorded and found negligible in the solvent. The maximum fluorescence emission intensity was obtained using dichloroethane as extracting solvent (Fig. 6). Therefore, dichloroethane was selected as the best extracting solvent for the determination procedure.

Table 1. Optical, statistical and regression characteristics of the proposed and reference

Parameters	Analytical methods	
	Proposed spectrofluorimetric method	Reference spectrofluorimetric method
Maximum wavelength (nm)	$\lambda_{em}=558; \lambda_{ex}=319$	$\lambda_{em}=407; \lambda_{ex}=259$
Linear dynamic rang ($\mu\text{g mL}^{-1}$)	3-20	4-10
No. of concentration levels	8	7
Linear regression equation	$A= 0.36 + 11.13C$	$A= 0.29 + 4.39C$
Standard deviation of intercept, S_a	0.199 ($v=6$)	0.163 ($v=5$)
Confidence limit of the intercept, $\pm tS_a$	0.486	0.418
Standard deviation of slope, S_b	0.0154	0.0118
Confidence limit of the slope, $\pm tS_b$	0.038 ($v=6$)	0.030 ($v=5$)
Correlation coefficient (r)	0.999 ($n=8$)	0.999 ($n=7$)
Variance (S_o^2)	0.071	0.031
Standard deviation of calibration line (S_o)	0.267	0.176
Limit of detection, LOD ($\mu\text{g mL}^{-1}$)	0.079	0.132
Limit of quantification, LOQ ($\mu\text{g mL}^{-1}$)	0.240	0.400

Table 2. Selectivity and specificity: tolerance amount of excipients at $20 \mu\text{g mL}^{-1}$ imipramine HCl.

Excipients	Concentration			Tolerating volume (mL)	Fluorescence intensity	Tolerance amount (mg mL^{-1})
	M	%				
Sucrose ^a	0.01	-		1.0	223	3.42
Povidone	-	0.2		0.25	224	0.05
Methyl cellulose	-	0.2		0.25	222	0.05
Starch	-	0.1		0.25	221	0.025
Poly ethylene glycol	-	0.1		0.25	223	0.025

Table 3. Intraday and interday precisions of the proposed method

Actual concentration ($\mu\text{g mL}^{-1}$) ¹⁾	Intraday assay and interday precisions					
	Measured concentration \pm SD ($\mu\text{g mL}^{-1}$)		RSD ^a %		Recovery, %	
	Intraday	Interday	Intra-day	Inter-day	Intraday	Interday
4	3.95 \pm 0.32	3.94 \pm 0.26	0.73	0.59	98.75	98.5
10	10.02 \pm 0.16	10.03 \pm 0.11	0.14	0.10	100.2	100.3
18	17.96 \pm 0.24	17.97 \pm 0.31	0.12	0.15	99.78	99.83

^a5 independent analyses

The effect of the shaking time from 0.5 to 3 min was investigated. The maximum fluorescence intensity of the complex was obtained at 1.5 min. The time acquired above did not produce any increase in fluorescence intensity. Therefore, the shaking time of 2.0 min was sufficient for the extraction of ion-pair complex into dichloroethane.

Validation

The fluorescence emission intensity of proposed method (or reference method) at 558 nm (or 407 nm) after keeping excitation wavelength constant at 319 nm (or 259 nm) of drug-eosin complex (or drug solution) was recorded and the linear regression equation was generated using OriginPro 6.1 software. The linear dynamic range of the proposed and reference methods were established and found to be in the range of 3.0 to 20.0 $\mu\text{g mL}^{-1}$ (proposed method, $n=8$) and 4 to 10 $\mu\text{g mL}^{-1}$ (reference method, $n=7$). The calibration data was treated with OriginPro 6.1 software to generate slope, intercept, standard deviation of intercept and slope, correlation coefficient, standard deviation of calibration line (S_0). Optical characteristics, linear regression equation and statistical data along with detection limit and quantitation limit are summarized in Table 1. The linearity of the regression line of the proposed was validated by high value of correlation coefficient (0.9999). Test of significance of intercepts, a , of regression lines (proposed and reference methods) showed that these did not differ significantly from the theoretical value, 0. For this, the quantity t - was calculated using the following equation [30]:

$$t = a/s_a$$

Eq. 2

The value of t - were found to be 1.81 and 1.78 for

proposed and reference methods, which were less than the tabulated t -value (2.447, $V=6$, proposed method) and (2.571, $V=5$, reference method) at 95% confidence level. It confirmed that the calculated intercepts for the proposed and reference methods are not significantly different from 0. Hence, a significant relationship between the experimental fluorescence emission intensity and concentration of imipramine were existed for both methods. The proposed method's procedure are free from procedural error and as much as effective as the reference method. The proposed method was suitable for the determination of active imipramine in commercial dosage forms.

Ideally the true relation between found and added concentration will provide straight line passing through the origin (intercept 0) with a slope equal to unity [31]. Here, in the proposed method, the found concentration of imipramine (mg mL^{-1}) differs slightly from added concentration of imipramine because of random fluctuations. The intercept and slope obtained by the proposed method were near to zero (5.4×10^{-4}) and unity (1.0001). Hence the statistical theory of fitting straight line confirmed the previous conclusions for intercept. Therefore, the proposed method is free from determinate errors and suitable for the determination of active imipramine in commercial dosage forms.

Limits of detection (LOD) and quantitation (LOQ) were calculated according to the International conference on Harmonization guidelines [25]. The low value of LOD ($0.079 \mu\text{g mL}^{-1}$) and variance indicated good sensitivity of the proposed method.

The statistical analysis of the calibration data was tested for calculating the error (S_c) in the determination

of a given concentration of imipramine HCl using the following expression [32]:

$$S_c = \frac{S_0}{b} \left[1 + \frac{1}{n} + \frac{(F-\bar{F})^2}{b^2 \sum (C-\bar{C})^2} \right]^{\frac{1}{2}}$$

Eq. 3

Where \bar{C} and \bar{F} are average imipramine concentration and fluorescence emission intensity, respectively, for n standard solutions. The uncertainty in the determination of imipramine HCl over the linear concentration range was taken. The least error was observed at 11.41 mg mL⁻¹ imipramine HCl.

The precision of the proposed method was tested in terms of intraday (over a single day, $n=5$) and interday (over 5 consecutive days in a week, $n=5$) precisions at 3 concentration levels i.e. 4, 10 and 18 mg mL⁻¹ imipramine HCl. The results are summarized in Table 2. The RSD values were in the ranges of 0.12-0.72 % for intraday and 0.15-0.59 % for inter day precisions. Satisfactory mean % recoveries (99.4 -100.25%) and low % RSD values for intraday and interday precisions at different concentrations confirmed high precision of the proposed method. Hence, the proposed method can be used to analyze imipramine HCl in commercial dosage forms.

The specificity and selectivity of the proposed method was investigated. The influence of foreign substances (sucrose, methyl cellulose, povidone, starch and polyethylene glycol) that can commonly accompany imipramine in pharmaceutical preparations was studied. Solution of imipramine (20 mg mL⁻¹) and each said compound were mixed to obtain samples. The tolerance limit of each interfering substances was calculated as the maximum concentration yielding a relative error of $\pm 2\%$ at a concentration of imipramine HCl in the analytical signal. The tolerated amount of excipients at

20 mg mL⁻¹ imipramine HCl was calculated using the following expressions:

$$\text{Mass/Volume (g L}^{-1} \text{ or mg mL}^{-1}) = \frac{\text{Molar concentration} \times \text{MW}}{\text{Eq. 4}}$$

where MW is the molecular weight of excipients.

$$\text{Mass/Volume (g L}^{-1} \text{ or mg mL}^{-1}) = \frac{\text{Volume taken (mL)} \times \% \text{ concentration}}{\text{Eq. 5}}$$

The results are summarized in Table 2. The method was tolerated variety of excipients, hence the proposed method is specific and selective, and can be used to determine active imipramine HCl in pharmaceutical preparations. This study also suggested that co-formulation substances are inactive ingredients and did not interfere in the determination of active imipramine in tablets.

The robustness of the proposed method was tested by deliberately changing the reaction conditions and studying the effect on the fluorescence intensity. The effect of varying volumes (1.4 \pm 0.2 mL) of 4.34 \times 10⁻⁴ M eosin Y, buffer solution of pH 4.63, 4.8 and 4.99, volumes (2 \pm 0.5 mL) of buffer solution of pH 4.8, shaking time (2 \pm 0.5 min) showed that these changes did not affect the percentage recovery of the drug. Results of variation in the experimental parameters were acceptable at room temperature, hence proved that the proposed method is robust.

The accuracy of the proposed method was investigated by standard addition technique. A series of known amount of pure imipramine was spiked with constant amount of Tofranil solution and the fluorescence emission intensity of the associated complex was recorded. Standard addition plot was constructed using fluorescence emission intensity at y-axis and initial concentration of imipramine at x-axis (Fig. 7). The regression line was generated with

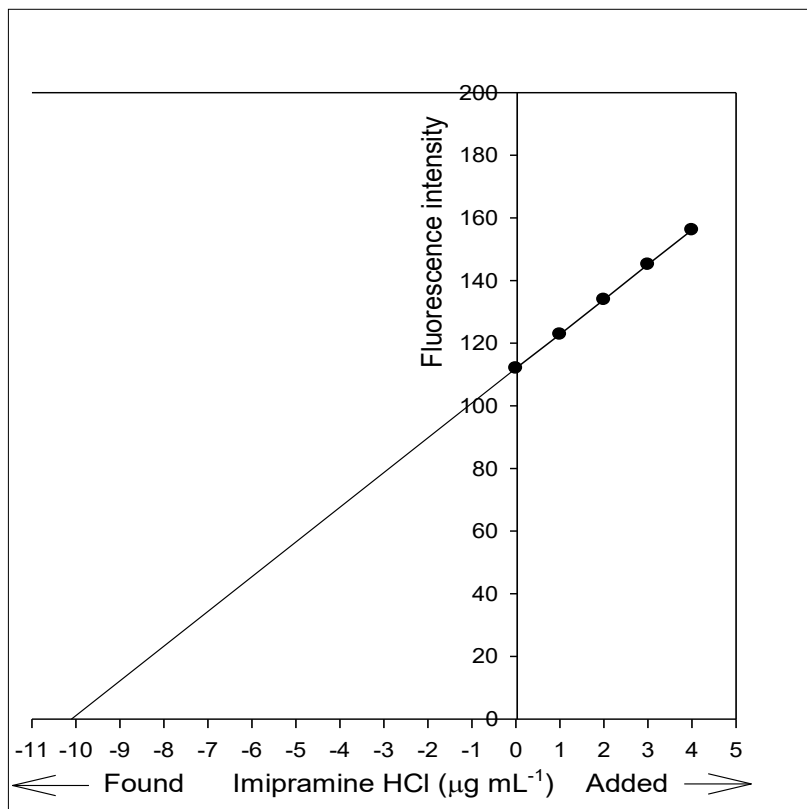


Fig. 7. Standard addition plot: 0.5 mL of 0.02% Tofranil solution was spiked with 0, 0.05, 0.1, 0.15 and 0.2 mL standard solution of 0.02% pure imipramine HCl.

Table 4. Significance of testing: Point and interval hypothesis tests for the determination of active imipramine HCl in tablets at 95% confidence level.

Dosage forms	Proposed method		Reference method		t-value ^b	F-value ^b	θ_L^c	θ_U^c
	Recovery (%)	RSD ^a (%)	Recovery (%)	RSD ^a (%)				
Imipramine HCl 25 (SGH)	100.11	0.36	100.16	0.23	1.09	2.28	0.989	1.004
Imipramine HCl 25 (Actavis, UK)	99.96	0.235	99.94	0.50	1.37	1.75	0.99	1.008

- 5 independent analyses.
- Theoretical t ($V = 10$) and F -values ($V = 5, 5$) at 95% confidence level are 2.306 and 6.39, respectively.
- A bias, based on recovery experiments, of $\pm 2\%$ is acceptable.

intercept and slope of 111.866 and 11.061, respectively. The amount of imipramine in Tofranil tablet was estimated either by dividing the intercept with slope or through extrapolation of the same line of best fit. The amount of imipramine in Tofranil tablet was found to be 10.114 $\mu\text{g mL}^{-1}$. The statistical analysis of the calibration data was tested for calculating the error (S_c) in the determination of a given concentration of imipramine HCl by standard addition method using the following expression [33]:

$$S_c = \frac{S_0}{b} \sqrt{\frac{1}{n} + \frac{F^2}{b^2 \sum_i (C_i - C)^2}}$$

Eq. 6

The value of S_c was found to be 0.044 $\mu\text{g mL}^{-1}$. The confidence limit for the concentration of imipramine in Tofranil tablet was calculated by $C_i \pm tS_c$ [34] at $n - 2$ degrees of freedom and found to be $10.114 \pm 0.140 \mu\text{g mL}^{-1}$. The error is quite low, hence the method is accurate with acceptable recovery and precision.

The applicability of proposed method for the determination of active imipramine HCl in Tofranil and Imipramine HCl tablets has been tested. Percentage recovery of active drug in tablets was estimated. The results of the proposed method were statistically compared with those obtained by the reference method and summarized in Table 4. It is clear from the table that the calculated t - and F values are less than the theoretical ones at 95% confidence level, indicated no significant difference between the methods compared.

Interval hypothesis test was also utilized to calculate bias and found to be within the acceptable range of $\pm 2\%$ [35] using the following quadratic equation.

$$\theta^2 \left(x_1^2 - S_p^2 t_{tab}^2 / n_1 \right) + \theta \times -2x_1x_2 + \left(x_2^2 - S_p^2 t_{tab}^2 / n_2 \right)$$

Eq. 7

This quadratic equation in θ has two roots (θ_L and θ_U) provided θ_L and θ_U of 0.989 and 1.004, respectively in SGH imipramine tablets and 0.99 and 1.008, respectively in Actavis imipramine tablets. The results are acceptable and showed the compliance with regulatory guidelines [36].

Conclusions

A comparison of the proposed method with those of published reported methods was presented in Table 5. It is clear from the table that so many analytical methods have been published but required analysis time of more than 2 min (analysis time of proposed method). HPLC technique is high enough but their related method's linear dynamic range were broader [9,10] as compared to proposed method. Hence discussed HPLC methods are less sensitive for trace analysis of active imipramine. Electroanalytical methods were time consuming required high pre-processing time [11-13]. It can be seen from the table that so many published spectrophotometric methods are sensitive but suffered disadvantages of using oxidant + H_2SO_4 [16], H_2SO_4 [18], oxidant + H_3PO_4 [18], oxidant + NaOH [19]. The proposed method is simple, selective and economical with advantage of using commonly available fluorescent dye i.e. eosin Y. Therefore, the proposed method can be used as an alternative method in academic institutions, hospitals and pharmaceutical industries for routine quality control analysis of active imipramine HCl in pharmaceutical formulations and biological fluids.

Acknowledgement

The authors are thankful to Dean, Heads of Applied Sciences and Chemistry Section, Higher College of Technology, Muscat, Oman for the facilities. The authors are grateful to to the higher-up of the Ministry of

Table 5. Comparison of the proposed spectrofluorimetric method with other published methods for the determination of imipramine HCl.

S.No.	Reagents/Mobile phase/Electrode	λ_{\max} (nm)	Linear range ($\mu\text{g mL}^{-1}$)	Analysis time (min)	References
HPTLC					
1	Toluene: ethyl acetate: ethanol: diethanolamine (70: 15: 4: 1 v/v/v/v)	288	5-9	30	[8]
HPLC					
2	Mobile phase: Phosphate buffer (pH 3.4)-acetonitrile (55:45) Flow rate: 1.0 mL/min	250	12.5-125	2.2	[9]
3	Mobile phase: water (pH 6)-methanol-triethylamine (70:30:0.1 % v/v/v) Flow rate: 1.0 mL/min	216 nm	50-150	5.05	[10]
Electroanalytical methods					
4	Amberlite-titanium dioxide nanoparticles modified glassy carbon paste electrode at pH 6 phosphate buffer (0.1M)	-	0.0004-1.97	Preprocessing time, approx. 24 h	[11]
5	Carbon nanocomposite electrode designed by montmorillonite nanoclay into a carbon ionic liquid electrode	-	0.63-12.68	Preprocessing time, approx. 3 h	[12]
6	Graphite-polyurethane composite electrode	-	0.10-0.73	Preprocessing time, approx. 24 h	[13]
Flow injection spectrophotometry					
7	Methyl orange in water-dichloroethane medium + universal buffer (pH)	425	0.79-25.3	Preprocessing time, approx. 3 h	[14]
Spectrophotometry					
8	Drug was alkalized with ammonia and extracted in chloroform. The drug solution was heated at 70°C using water bath for removing chloroform. The residue was dissolved in acetonitrile and reacted with 2,3-dichloro-5,6-dicyano-p-benzoquinone	460	10-60	Preprocessing time 1h	[15]
8	Drug + ammonium metavanadate + 10M sulphuric acid	620	0.6-40	30 min	[16]

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10	Drug + eriochrome cyanine R. Extracted in n-butanol	520	10-80	5 min	[17]
11. (a)	Drug + ammonium peroxodisulfate + 10M phosphoric acid	658	10-110	35 min	[18]
(b)	Drug + niobium(V) thiocyanate + 10M sulphuric acid. Extracted in n-butanol-chloroform (9:1) medium	350	0.8-8	7 min	[18]
12. (a)	Drug + bromothymol blue + sodium acetate-HCl buffer solution of pH 2.8. Extracted in chloroform	415	2.5-25	5	[19]
(b)	Drug + bromophenol blue + sodium acetate-HCl buffer solution of pH 2.5. Extracted in chloroform	415	3.0-25	5	[19]
(c)	Drug + bromocresol green + sodium acetate-HCl buffer solution of pH 3.5. Extracted in chloroform	415	2.5-25	5	[19]
(d)	Drug + bromocresol purple + sodium acetate-HCl buffer solution of pH 2.5. Extracted in chloroform	415	2.5-25	5	[19]
(e)	Drug + I ₂ , reacted in dichloroethane	366	2.5-25	5	[19]
(f)	Drug + KMnO ₄ + 0.45 M NaOH	610	3.0-25	5	[19]
Spectrofluorimetry					
13	Erythrosine B in water-chloroform medium + acetate buffer (pH 5)	$\lambda_{ex} = 544$ $\lambda_{em} = 560$	0.12-2.8	Preprocessing time, approx. 3 h	[20]
14	Rhodamine B in ethylene glycol-distilled water medium	-	0.1-20.0	10	[21]
This work	Drug + Eosin Y + sodium acetate-acetic acid buffer solution of pH 4.8. Extracted in dichloroethane	$\lambda_{em} = 558$ and $\lambda_{ex} = 319$)	3.0-20	2	Proposed method

ManPower (Higher College of Technology) Muscat, Sultanate of Oman for support to carry out this work.

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