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# Spectrophotometric determination of imipramine HCl in pure and pharmaceutical forms

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**ABSTRACT**

*Six spectrophotometric methods, A, B, C, D, E and F for the determination of Imipramine HCl have been developed, validated and applied for the assay of the drug in pharmaceuticals. Methods A, B, C and D are based on ion pair complexation of drug, in acidic buffers, with triphenylmethane dyes viz., Bromothymol blue (BTB), Bromophenol blue (BPB), Bromocresol green (BCG) and Bromocresol purple (BCP). The complexes are extracted into chloroform and absorbance is measured around 415 nm as a function of concentration of the drug. The stoichiometry of each of the complex is found be 1:1. Method E depends upon charge transfer complexation of neutralized drug with iodine which produces iodide ion whose absorbance at 366 nm is measured as function of concentration of the drug. This complex, too, has 1:1 composition as determined by Job's method. Method F is developed on the basis of oxidation of the drug with alkaline $KMnO_4$ which generates green colored manganate ion with λmax 610 nm. As the intensity of green color increased with increasing time, kinetics of the reaction is followed and calibration curves are constructed by using initial rate and fixed time methods. Excellent recovery studies with high accuracy and precision indicate that the methods can be successfully used in industries for the assay of drug in pure and pharmaceutical forms.*

**Key words:** Spectrophotometry, Imipramine HCl, Bromothymol blue, Bromophenol blue, Bromocresol purple, Bromocresol green, Iodine, Alkaline $KMnO_4$

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## INTRODUCTION

Imipramine HCl (IMP) is a dibenzazepine derivative of tricyclic antidepressant that acts by blocking the reuptake of norepinephrine and seratonin and thus making more of those substances available to act on receptors of brain.[1] It is chemically, (10, 11-Dihydro-N, N-dimethyl)-5H-dibenz [b,f] azepine-5-propanamine hydrochloride). The drug has been determined by a variety of analytical techniques such as spectrophotometry [2-11], spectrofluorimetry [12], atomic absorption spectrophotometry [13], conductimetry [14] and flow injection methods [15, 16] .However, many of these assay methods are limited in their applications or rather tedious and time consuming. Therefore it seems necessary to develop a simple and sensitive method for the determination of imipramine HCl .

In the present communication we report six quantification methods *viz.*, A, B, C.D, E and F which have been developed and validated for quantification of Imipramine HCl both in pure and pharmaceutical forms.

## MATERIALS AND METHODS

*Instruments*

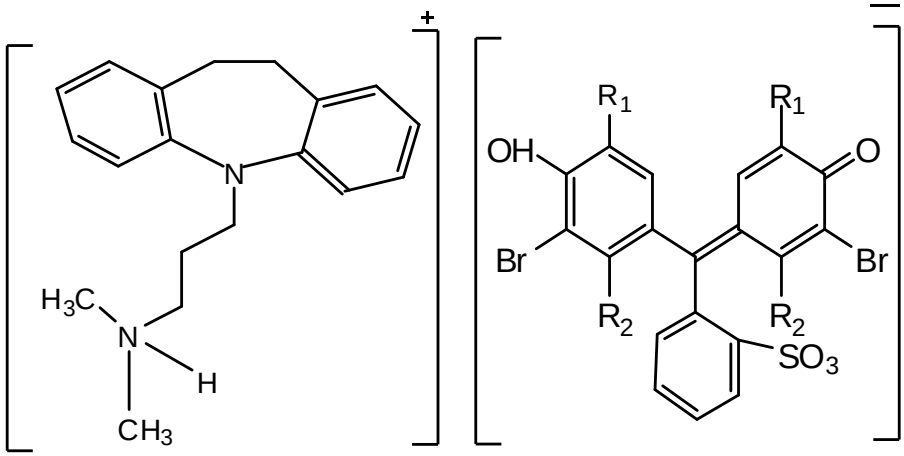
The spectra were recorded on SHIMADZU 140 double beam, Thermo Nicolet 1000 and also on ELICO 159 UV-Visible single beam spectrophotometers using quartz cells of 10 mm path length. An Elico model Li-120 *p*H meter was used for *p*H measurement.

*Materials*

HPLC grade chloroform and Analytical grade (AR) $KMnO_4$,HCl, Sodium acetate, Sodium hydroxide and dyes *viz*., a) BTB b) BPB c) BCG d) BCP are supplied by Sd Fine Chemicals, Mumbai were used in the study. Iodine (BDH, Poole, UK) was twice sublimed and preserved in vacuum desiccators (mp 113.6 -3$^{o}$C). Iodine in 1, 2 dichloroethane (DCE) was freshly prepared (daily) by dissolving 254mg solute in 50ml of solvent ($4.0\times10^{-3}$ *M* ). The Imipramine HCl was procured from HETERO drugs private limited, Hyderabad, as gift sample.

*Methods A, B, C and D*

The methods A, B, C and D are based on the interaction of the drug with Bromothymol blue (BTB), Bromophenol blue (BPB) Bromocresol green (BCG) and Bromocresol purple (BCP) respectively, to form chloroform extractable ion pair complexes (Scheme 1) which absorb around 415 nm (Fig 1) The absorbance of this band increases with increasing the concentration of the drug and formed a basis for the quantification of the drug. The dyestuffs were used as 0.025% solutions in doubly distilled water. Sodium acetate-hydrochloric acid buffers of *p*H 2.8, 2.5, 3.5 and 2.5 were prepared by mixing 50ml of 1.0M sodium acetate solution with 49.50 ml, 50.50ml, 46.25ml and 50.50 ml of 1.0 M HCl solution respectively and diluted to 250 ml with doubly distilled water. The *p*H of each solution was adjusted to an appropriate value with the aid of a *p*H meter.




Bromothymol blue : R1 = isopropyl, R2 = CH3
Bromophenol blue : R1=Br, R2= H
Bromocresol purple: R1=.CH3, R2= H
Bromocresol green:R1=Br, R2 = CH3

Scheme -1 Imipramine HCl - dye complex

*Method E*

The method depends up on the interaction of drug with Iodine that generates iodide ion having an absorption band at 366 nm. (Fig 2) The absorbance of this band increases with increasing the concentration of the drug and formed a basis for the quantification of the drug. Mixing the solution of iodine prepared in DCE with IMP resulted in a change of violet color of iodine into light brown to pale yellow , and as a consequence, absorption spectra exhibited a band of 366nm. This is attributed due to $I_3^-$ ion formed by the interaction of iodine with drugs and the same is shown in (Scheme 2).

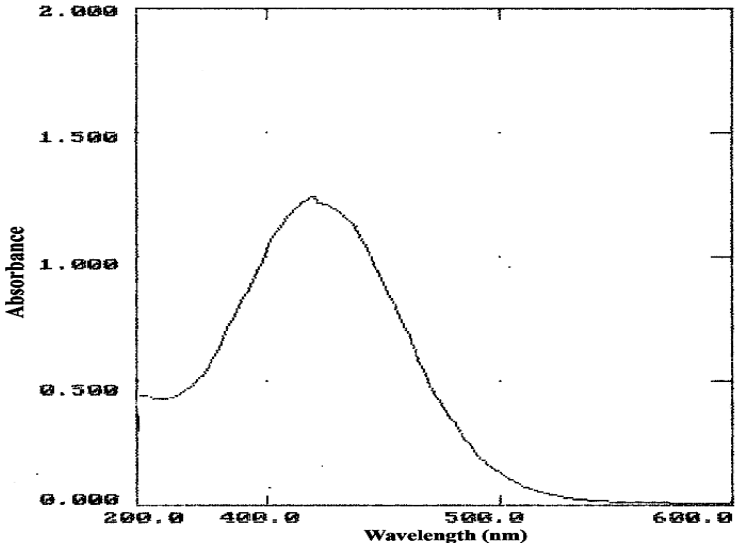



**Fig. 1 Absorption spectra of Imipramine HCl- BPB ion-pair complex**

D + I2 ⇌ DI2 (Outer complex) fast ……1

DI2 (Outer complex) ⇌ ( DI+) I- (inner complex ) slow……..……2

( DI+) I- (inner complex ) + I2 ⇌ ( DI+) + I3- fast ……..……3

**Scheme 2**

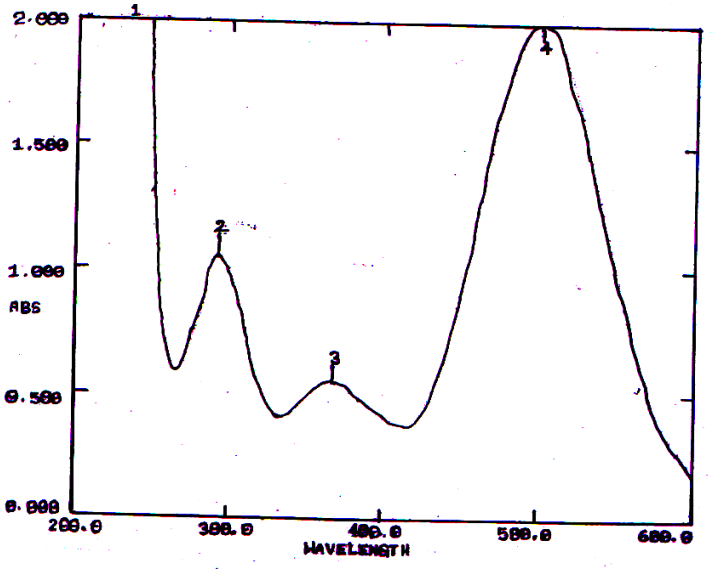



**Fig. 2 Absorption spectra of Imipramine HCl – iodine ion-pair complex**

*Method F*

The method depends on the oxidation of the drug with alkaline $KMnO_4$ ($1x10^{-2}$ M) to produce Manganate ion which

absorbs at 610 nm (Fig 3) and formed a basis for quantification of drug. A solution of 0.45M NaOH is used to produce required alkalinity. Mixing the solutions of permanganate and the drug slowly developed green colour and hence kinetics of the reaction was followed spectrophotometrically with a view to develop a method for the quantitative determination of the drug. The initial rate and fixed time methods are followed for the determination of IMP.

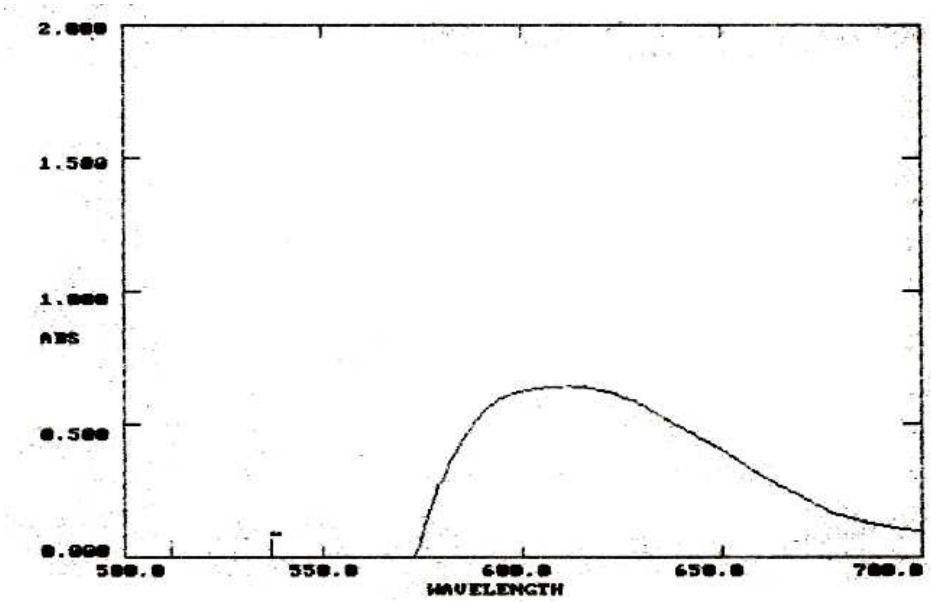



**Fig. 3 Absorption spectrum of imipramine HCl with alkaline $KMnO_4$**

*Calibration curves for method A, B, C and D*

Different aliquots of drug solution were transferred into 125 ml separating funnel. To this 5 ml of buffer, 5 ml of dye were added and total volume was made up to 20 ml with water. 10 ml of chloroform was added and the contents were shaken for 5 min. The two layers were allowed to separate for 5 min. The organic layer was separated and absorbance of yellow colored solution which is stable at least for 3 hrs is measured at 415 nm against blank similarly prepared. The same procedure of analysis is followed either for assay of pure drug or for dosage form. The calibration graphs are linear for all the dyes analysed using these methods.(Fig 4)

*Calibration curve for method E*

Into separate 10ml of volumetric flasks different aliquots of IMP solution was transferred followed by the addition of 1ml of iodine solution prepared in 1, 2-dichloroethane ($4.0\times10^{-3}$ *M* ). The volume was completed using the same solvent and the absorbance was measured against reagent blank at 366nm.

(Table 1) summarizes the values for Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries.

*Calibration curve for method F*

*Initial rate method*

Aliquots of 1 to 8 ml of Imipramine HCl solution containing 2.5 mg ml $^{-1}$ of drug were pipetted into a series of 10 ml standard flasks. To each flask, 1.0 ml of 0.45 M NaOH and 1 ml of 1x□$10^{-2}$M potassium permanganate were added successively and then diluted with distilled water at 25 ±□10C. The contents of each flask were mixed well, and the increase in absorbance as a function of time was measured at 610 nm. The initial rate of the reaction (ν) at different concentrations was evaluated by measuring the slope of the tangent to the absorbance-time plot. The calibration graphs were obtained by plotting the initial rate of reaction (ν) versus the molar concentration of the Imipramine HCl

*Fixed time method*

A fixed time of 25 min was selected for the fixed time method. At this time the absorbance of reaction mixture was

measured at 610 nm against a reagent blank prepared similarly without the drug.

The calibration curve was obtained by plotting the absorbance against the initial concentration of Imipramine HCl

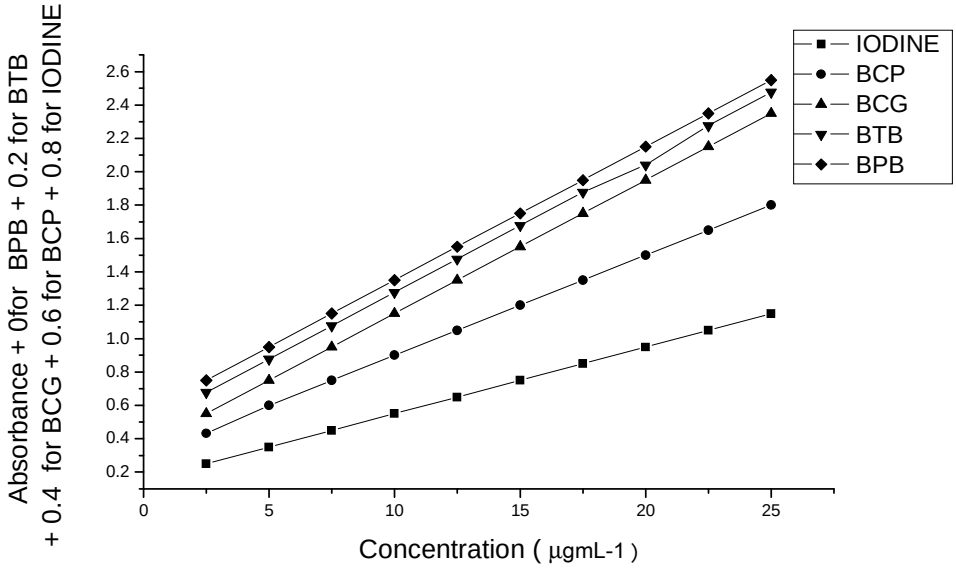



**Fig. 4 Calibration curves**

**Table 1 Optical and analytical parameters of the methods**
Y= aX +b, Where X is the concentration in $\mu gmL^{-1}$

| **Parameters** | **BTB** | **BPB** | **BCP** | **BCG** | **$I_2$** | **KMnO4** |
|---|---|---|---|---|---|---|
| $\lambda_{max}$ (nm) | 415 | 415 | 415 | 415 | 366 | 610 |
| Beer's law limit ($\mu g\ mL^{-1}$) | 2.5-25 | 3.0-25 | 2.5-25 | 2.5 - 25 | 2.0-25 | 3.0 - 25 |
| Sandell sensitivity ($\mu g\ cm^{-2}$) | 0.0145 | 0.0159 | 0.0142 | 0.0136 | 0.0141 | 0.033 |
| Molar absorbvity ($Lmol^{-1}\ cm^{-1}$) | 19335.30 | 17553.90 | 20497.13 | 19901.70 | 20501.5 | 21050.3 |
| Formation constant , K, $M^{-1}$ | $2.31 \times 10^5$ | $1.37 \times 10^5$ | $4.05 \times 10^5$ | $2.37 \times 10^5$ | $3.15 \times 10^5$ | $2.61 \times 10^5$ |
| Slope (specific absorptivity), b | 0.0689 | 0.0626 | 0.0709 | 0.0731 | 0.0710 | 0.0298 |
| Intercept (a) | 0.0004 | 0.1750 | -0.0250 | -0.0400 | 0.0107 | 0.1750 |
| Correlation coefficient (r) | 0.9974 | 0.9964 | 0.9977 | 0.9982 | 0.9987 | 0.9990 |
| Standard deviation of intercepts (% n=6) | 0.0023 | 0.0203 | 0.0156 | 0.0199 | 0.0500 | 0.0100 |
| Limit of detection, $\mu gmL^{-1}$ | 1.08 | 1.05 | 0.72 | 0.90 | 2.30 | 1.13 |
| Limit of quantification, $\mu gmL^{-1}$ | 3.24 | 3.15 | 2.17 | 2.60 | 6.90 | 3.39 |
| Regression equation | Y= .0689*X+0.0004 | Y= 0.0626*X+0.175 | Y= 0.0709*X -0.025 | Y= 0.0731*X+0.043 | Y= 0.0298*X+0.1750 | Y= 0.0298*X+0.1750 |

*Procedure for the assay of pure drug*

Four different solutions of pure drug in the range of calibration curve were selected and the recovery experiments were performed. The recoveries and their relative standard deviation is tabulated in (Table 2).

**Table 2. Precision and accuracy studies of proposed methods for the determination of IMP in pure form**

| IMP | BTB | BPB | BCG | BCP | $I_2$ | $KMnO_4$ |
|---|---|---|---|---|---|---|
| **Taken(µgmL$^{-1}$)** | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 |
| | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 |
| | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| **Found (µgmL$^{-1}$)** | 5.10 | 5.09 | 5.20 | 5.15 | 5.20 | 4.99 |
| | 7.75 | 7.45 | 7.68 | 7.57 | 7.55 | 7.45 |
| | 10.20 | 10.15 | 10.25 | 10.18 | 9.98 | 10.05 |
| | 12.56 | 12.60 | 12.67 | 12.59 | 12.60 | 12.59 |
| | 15.10 | 15.20 | 15.25 | 15.10 | 14.98 | 15.07 |
| **Recovery( %)** | 102.00 | 101.80 | 104.00 | 103.00 | 104.00 | 99.80 |
| | 103.33 | 99.33 | 102.40 | 100.93 | 100.67 | 99.33 |
| | 102.00 | 101.50 | 101.80 | 101.80 | 99.80 | 100.50 |
| | 100.48 | 100.80 | 101.36 | 100.72 | 100.80 | 100.72 |
| | 100.67 | 101.33 | 101.60 | 100.67 | 99.87 | 100.47 |
| **Mean** | 101.60 | 100.94 | 102.20 | 101.50 | 101.03 | 100.16 |
| **RSD(%)** | 0.86 | 0.67 | 0.95 | 0.68 | 1.71 | 0.59 |
| **SD** | 0.85 | 0.68 | 0.93 | 0.67 | 1.72 | 0.58 |
| **Referance method, Mean ± SD (2)** | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 |
| **t-test(2.3534)*** | 1.12 | 0.42 | 2.30 | 1.06 | 0.09 | 2.72 |
| **F-test(5.05)*** | 2.77 | 1.77 | 3.30 | 1.72 | 2.96 | 0.34 |

*Each result is the average of five separate determinations. *Values between paranthesis are the tabulated t and F values respectively, at p=0.05 [17]*

*Procedure for the assay of dosage forms*

Five tablets of antidep 25mg are powdered and dissolved in doubly distilled water and stirred thoroughly, filtered through a Whatman No. 42 filter paper. This solution was transferred into 100 ml standard volumetric flask and diluted with doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assay was estimated using the calibration curve. The results of the recovery experiments are tabulated in (Table 3).

**Table 3 Application of proposed methods for the determination of IMP in pharmaceutical preparation**

| IMP | BTB | BPB | BCG | BCP | $I_2$ | $KMnO_4$ |
|---|---|---|---|---|---|---|
| **Taken(µgmL$^{-1}$)** | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| **Antidep (25mg/Tablet)** | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 |
| | 16.00 | 16.00 | 16.00 | 16.00 | 16.00 | 16.00 |
| | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| **Found (µgmL$^{-1}$)** | 4.09 | 4.10 | 4.11 | 4.13 | 3.98 | 4.05 |
| | 8.11 | 8.10 | 8.17 | 8.18 | 7.95 | 8.09 |
| | 12.07 | 12.10 | 12.13 | 12.06 | 11.79 | 12.04 |
| | 16.30 | 16.10 | 16.18 | 16.08 | 15.99 | 15.95 |
| | 20.05 | 20.20 | 20.50 | 20.10 | 20.01 | 19.90 |
| **Recovery( %)** | 102.25 | 102.50 | 102.50 | 103.25 | 99.50 | 101.25 |
| | 101.37 | 101.25 | 102.12 | 102.25 | 99.38 | 101.13 |
| | 100.58 | 100.80 | 101.00 | 101.50 | 98.25 | 100.33 |
| | 101.87 | 100.62 | 101.12 | 100.50 | 99.94 | 99.69 |
| | 100.50 | 101.00 | 100.25 | 100.50 | 100.05 | 99.50 |
| **Mean** | 100.31 | 101.23 | 101.43 | 101.40 | 99.42 | 100.38 |
| **SD** | 0.55 | 0.53 | 0.69 | 0.88 | 0.71 | 0.80 |
| **RSD(%)** | 0.56 | 0.54 | 0.69 | 0.89 | 0.72 | 0.81 |
| **Referance method,mean±SD(2)** | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 | 101.1 ± 0.51 |
| **t-test(2.3534)*** | 2.30 | 0.39 | 0.86 | 0.72 | 2.25 | 1.68 |
| **F-test(5.05)*** | 1.16 | 1.07 | 1.83 | 2.90 | 0.54 | 0.66 |

*Each result is the average of five separate determinations. *Values between paranthesis are the tabulated t and F values respectively, at p=0.05[17]*

## RESULTS AND DISCUSSION

*Methods A, B, C and D*

IMP forms ion-pair complexes in acidic buffer with dyestuffs *viz.*, BTB, BPB, BCG and BCP. These complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with dyes absorbed maximally at 415 nm (Fig 1). The reagent blank under similar conditions showed no absorption. IMP contains two tertiary nitrogen atoms, one with in the ring system and other in the side chain.

Hence we propose the protonation of side chain containing tertiary nitrogen in acidic medium, while sulphonic acid group is present in any of the dyes that is the only group undergoing dissociation in the *p*H range 1-5.

The colour of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally the protonated IMP forms ion-pairs with the dyestuff which is quantitatively extracted into chloroform.

*Stoichiometry*

In order to establish molar ratio between IMP and dyestuffs used, the Job's method of continuous variation has been applied [18]. In this method, solutions of drug and dyestuff with identical molar concentrations [8 x $10^{-5}$M] were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug, [drug]/[drug]+[dyestuff] . This measurement showed that 1:1 complex was formed.

The Job's method of stoichiometry is also applied for Iodine with IMP which indicated the charge transfer complex formed is of 1:1 composition (Fig 5).

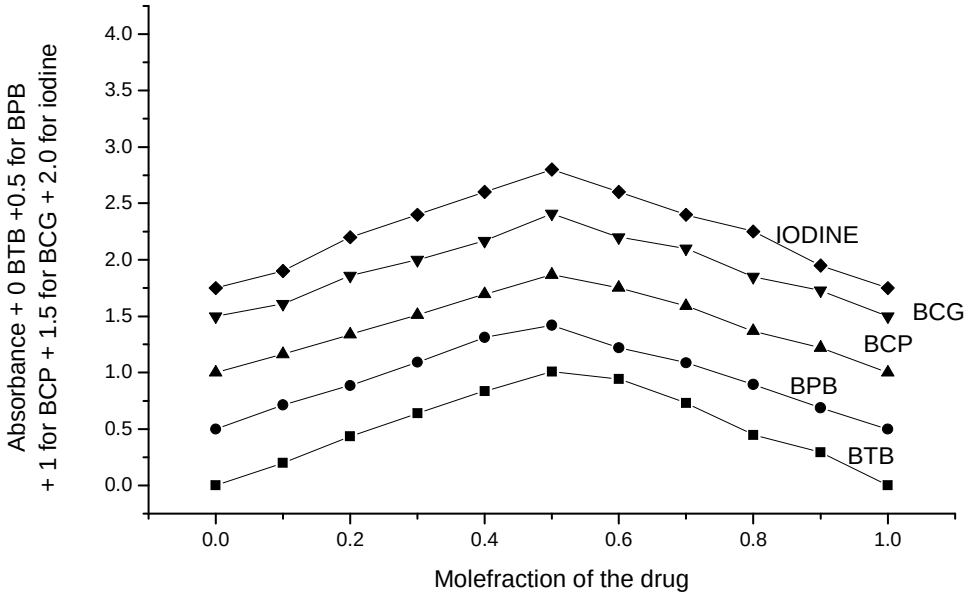



Figure 5 Jobs Continuous variation plots

The stoichiometric ratio between Imipramine HCl and potassium permanganate was evaluated by limiting logarithmic method [19].In this method two sets of experiments were performed. In the first set the concentration of IMP was varied keeping a constant concentration of $KMnO_4$, while in the second set, the concentration of IMP was

kept constant and the $KMnO_4$ concentration was varied. Log absorbance versus log [IMP] or [$KMnO_4$] was plotted to evaluate the slopes of the respective lines. The slope was found to be unity in each case thus indicating the molar combining ratio of 1:1 between Imipramine HCl and potassium permanganate. The literature survey reveals that Imipramine HCl undergoes oxidation at N,N-Dimethyl nitrogen and gives rise to N,N-Dimethyl nitrogen N-oxide [20].(Fig 6)

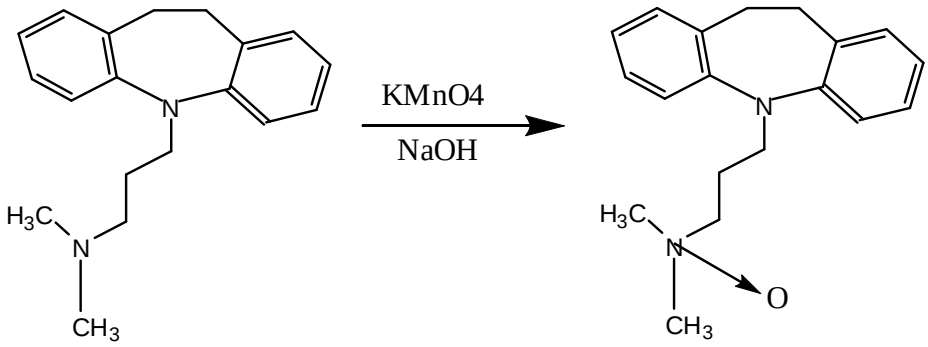



Figure 6 oxidation of IMP by alkaline KMnO4

*Formation constants*

The formation constant was also estimated from Job's plot by following method described by Likussa and Boltz [21] and Momoki etal [22].The method involves drawing the tangents at the origin of Job's plot from both side and the absorbance at intersection point is taken for 100% complexation. The absorbance at peak height of Job's plot is taken for (100-x)% where x is the % degree of dissociation of the complex.

The instability constant, K' = Cx/(100x) is calculated, where C is concentration of drug used for Job's method. The reciprocal of K' is the required stability constant K.

*Formation constant for method E*

Formation constant (K) has been evaluated by using Benesi-Hildebrand equation [23]

$[A_0]/d = I/K[D_0]e + 1/e$

Where d is absorbance, e is molar absorptivity, $A_0$ and $D_0$ are initial concentrations of acceptor [$I_2$] and donor[drug] respectively. A plot of $[A_0]/d$ Vs $1/[D_0]$ yields a straight line whose slope and intercept gives the value of K.

*Optimization of the factors affecting the absorbance.*

The factors effecting the absorbance of ion pair complexes like *p*H and volume of the dye, in methods A, B, C and D, have been optimized. 1.8 ml of BTB and buffer of *p*H 2.8, 1.6 ml of BPB and buffer of *p*H 2.5, 2.0 ml of BCG and buffer of *p*H 3.5 and 1.7 ml of BCP and buffer of *p*H 2.5 are found to optimal for methods A.B,C and D respectively. However 5 ml of each dye is used, at optimal *p*H, in the study to ensure complete extraction of the drug. Similarly the 1 ml of iodine for method E and 1 ml of $KMnO_4$, 1 ml of 0.45 NaOH are found to be optimal and hence are used in the study.

*Validation of the proposed methods*

The proposed method have been validated in terms of guideline proposed by ICH [24] *viz*., selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. To test the reproducibility of the proposed methods, six replicate determinations of 10.0µg ml of IMP were made. The coefficient of variation was found to be less than 1.2% for all the procedures. The student t-test and variance F-test have been performed (Table 2, 3) in comparison with a reference method.

The proposed methods have been successfully applied to the determination of IMP in pharmaceutical preparations.

The results obtained and shown in (Table 1) were compared to those obtained by a reference method [2] by means of *t*-test at 95% confidence level. In all cases, the average results obtained by proposed methods and reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level.

The proposed methods are simple, sensitive and reproducible and can be used for routine analysis of IMP in pure form and in formulation.

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