



## DEVELOPMENT OF ION-ASSOCIATION METHODS FOR SPECTROPHOTOMETRIC ASSAY OF IMIPRAMINE HYDROCHLORIDE

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### ABSTRACT

Three simple and sensitive spectrophotometric methods (M<sub>1</sub>-M<sub>3</sub>) for the assay of imipramine in pure and dosage forms based on the formation of chloroform soluble ion-associates under specified experimental conditions are described. Three acidic dyes, namely, azocarmine G (ACG, M<sub>1</sub>), naphthalene blue 12BR (NB 12BR, M<sub>2</sub>) and woolfast blue BL (WFB BL, M<sub>3</sub>) are utilized. The extracts of the ion-associates exhibit absorption maxima at 550, 620 and 590 nm for methods M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub>, respectively. Regression analysis of Beer's law plots showed good correlation in the concentration ranges 2.0-12.0, 4.0-16.0 and 1.0-12.0 µg/mL for methods M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub>, respectively. These methods are found to be suitable for the assay of imipramine in pharmaceutical formulations. All the variables have been optimized and the reaction mechanisms presented. The concentration measurements are reproducible within a relative standard deviation of 1.0%.

**Key words:** Spectroscopy, Imipramine, Azocarmine G, Naphthalene blue 12BR, Wool fast blue BL.

### INTRODUCTION

Imipramine (IMP)<sup>1</sup> is an original tricyclic antidepressant for oral, administration and it is chemically known as 5-3-(dimethylamino)propyl-10,11-dihydro-5H-dibenz[*b,f*]azepine. Literature survey reveals that spectrophotometric<sup>1-10</sup>, HPLC<sup>11-32</sup> and LC-MS<sup>33,34</sup> methods were reported for the determination of IMP in its formulation and in biological fluids. During the course of our efforts to develop sensitive visible spectrophotometric methods, it

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was observed that the analytically useful tertiary amino group in IMP has not been properly exploited. Hence, there is a need to develop some new methods with either sensitivity or selectivity by exploiting the tertiary amino group in IMP. As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the determination of drugs, this technique was therefore utilized in the present work for the assay of IMP. IMP, being basic in nature, forms an ion-association complex with the acidic dye, namely ACG, NB 12BR or NBB, which is extractable into chloroform. The protonated aliphatic tertiary nitrogen (positive charge) of the IMP in acid medium is expected to attract the oppositely charged part (negative charge) of the dye ( $\text{SO}_3^-$ ) and behaves as a single unit, being held together by electrostatic attraction. The results are statistically validated.

## EXPERIMENTAL

### Instruments

A Milton Roy Spectronic 1201 with 1 cm matched quartz cells was used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

All reagents and chemicals used were of analytical grade and doubly distilled water was used throughout. Aqueous solutions of ACG (BDH, Mumbai, India, 0.05%) NB 12BR (BDH, Mumbai, India, 0.2%) and WFB BL (BDH, Mumbai, India, 0.2%) were prepared by dissolving the required amount in doubly distilled water. The solutions were washed with chloroform to remove the chloroform-soluble impurities. The glycine-HCl buffer solution (pH 1.5) was prepared for methods  $M_1$ - $M_3$ .

### Preparation of standard drug solution

A one mg/mL solution was prepared by dissolving 50 mg of pure IMP in 50 mL of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solution of concentrations 40  $\mu\text{g/mL}$  for  $M_1$ ,  $M_2$  and  $M_3$ .

### Recommended procedures

To each of the aliquots of standard IMP solution, 0.5-2.5 mL, 40  $\mu\text{g/mL}$  ( $M_1$ ), 1.0-3.0 mL, 40  $\mu\text{g/mL}$  ( $M_2$ ) and 0.5-2.5 mL, 40  $\mu\text{g/mL}$  ( $M_3$ ) the buffer solution (pH 1.5, 6.0 mL) and ACG ( $M_1$ ), NB12 BR ( $M_2$ ), WFB BL ( $M_3$ ), solutions (2.0 mL) were added and the total volume of aqueous phase was adjusted to 15.0 mL with distilled water. Then chloroform (10 mL) was added to it and shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 550 nm ( $M_1$ ), 620 nm ( $M_2$ )

and 590 nm ( $M_3$ ) against the reagent blank. The amount of IMP was calculated from the calibration plot.

## RESULTS AND DISCUSSION

The optimum conditions for the color development in each method were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

### Optimum conditions fixation

Conditions, under which the reaction of IMP with each dye fulfills the essential analytical requirements, were investigated. All the experimental conditions studied were optimized at room temperature ( $25 \pm 3^\circ\text{C}$ ) and were established by varying one parameter at a time (10) and observing its effect on the absorbance of the colored species.

In the preliminary experiments, in view of developing methods of analysis suitable for assaying small quantities of IMP, seven acidic dyes (Table 1) like tropaeolin 00, alizarin red S, bromocresol green, azocarmine G, naphthalene blue 12BR, wool fast blue BL, naphthalene blue black and erichrome black T were tested at various pH ranges as the color producing agents by a dye salt partition technique. Different organic solvents such as benzene, toluene, nitrobenzene, carbon tetrachloride, 1,2-dichloromethane, chloroform, ethyl acetate and isobutyl ketone were tested for the extraction of the ion-association complex formed between the IMP and each dye. The criterion for the best dye was the highest absorbance value of the complex in the organic phase at the wavelength of maximum absorbance. The above studies reveal that three dyes namely ACG (CI No.50085), NB12 BR (CI No.20500) and WFB BL (CI No.50315) gave better results than the other dyes. These dyes also gave low absorbance for the reagent blank. Chloroform was suggested as the solvent of choice for the extraction of the colored complex with respect to maximum stability.

**Table 1:  $\lambda_{\text{max}}$  and  $\epsilon_{\text{max}}$  values of IMP-dye complexes**

Dye	Category	$\lambda_{\text{max}}$ (nm)	$\epsilon_{\text{max}}$ ( $\text{L mol}^{-1}\text{cm}^{-1}$ )
NBB*	Azo dye	580	$2.43 \times 10^3$
NB12BR*	Azo dye	620	$1.29 \times 10^4$
TPOO	Azo dye	430	$1.10 \times 10^4$

Cont...

Dye	Category	$\lambda_{\max}$ (nm)	$\epsilon_{\max}$ (L mol <sup>-1</sup> cm <sup>-1</sup> )
EBT	Azo dye	520	6.21 x 10 <sup>3</sup>
ACG*	Phenazine dye	540	2.06 x 10 <sup>4</sup>
WFB BL	Azo dye	590	2.48 x 10 <sup>4</sup>
ARS	Anhraquinone dye	430	5.87 x 10 <sup>3</sup>

\*Chosen for further investigations

In order to establish the optimum pH range (for M<sub>1</sub>-M<sub>3</sub>), the IMP was allowed to react with the respective dye in aqueous solution buffered between pH 1.0-10.0 and the complex formed was extracted into chloroform for absorbance measurements. The results show that a quantitative extraction was produced between pH 1.1-1.5 (for M<sub>1</sub>-M<sub>3</sub>). All subsequent studies were carried out at pH 1.5 (for M<sub>1</sub>-M<sub>3</sub>). The pH was adjusted using a glycine-HCl buffer solution (this buffer was chosen on account of its elevated complexing ability, which could be of use in overcoming interferences). The volume of this buffer added (4-10 mL) had no effect in methods for M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub>. A 6.0 mL portion of buffer was found to be optimal in methods M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub>. The minimum shaking time was determined by varying the shaking time from 1-10 min, although 1 min was sufficient; prolonged shaking had no adverse effect on the extraction and 2 min was selected for this study. A ratio of 2 : 3 of organic to aqueous phases was required for efficient extraction of the colored species and lower reagent blank reading. It was found that better reproducibility and lower reagent blank were achieved, if the dye was purified by extraction with chloroform initially. The color products were stable upto 30 min. The stoichiometric ratio of the IMP to dye was found as 1 : 1 with ACG or 2 : 1 with NB 12BR or WFB BL through slope analysis method.

### Interference studies

The interference studies in the determination of IMP in pharmaceutical formulation revealed that the normally existing excipients and additives like starch, lactose, gelatin, talc, magnesium stearate, aluminum hydroxide, sorbitol, calcium silicate and glycerin do not interfere even when present in excess than the anticipated amount. However, a preliminary clean up procedure with chloroform is necessary to avoid interference due to the presence of reducing sugars like lactose if present, prior to the estimation of IMP in formulations for method M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub>.

### Analytical data

The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity for the method are given in Table 2. The precision of the method was found by measuring absorbances of six replicate samples containing known amounts of drug. Regression analysis using the method of least squares was made to evaluate the parameters. The accuracy of the methods was ascertained by comparing the results by the reference method (Table 3). This comparison shows that there is no significant difference between the results of studied methods and those of the reference one.

**Table 2: Optical characteristics, precision and accuracy of the proposed methods for imipramine**

Parameters	Method M <sub>1</sub>	Method M <sub>2</sub>	Method M <sub>3</sub>
	ACG	NB 12 BR	WFB BL
$\lambda_{\max}$ (nm)	550	620	590
Beer's law limits ( $\mu\text{g/mL}$ )	2-12	4-16	1-12
Molar absorptivity ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	$2.063 \times 10^4$	$1.296 \times 10^4$	$2.475 \times 10^4$
Sandell's sensitivity ( $\mu\text{g/cm}^2/0.001$ absorbance unit)	0.015	0.024	0.013
Regression equation ( $y = a + bc$ ) Slope (b)	0.0653	0.0511	0.0783
Standard deviation on slope ( $S_b$ )	$3.9 \times 10^{-4}$	$3.0 \times 10^{-4}$	$3.8 \times 10^{-4}$
Intercept (a)	-0.0013	-0.1020	-0.0012
Standard deviation on intercept ( $S_a$ )	$2.56 \times 10^{-3}$	$2.53 \times 10^{-3}$	$2.54 \times 10^{-3}$
Standard error of estimation ( $S_e$ )	$2.44 \times 10^{-3}$	$1.89 \times 10^{-3}$	$2.42 \times 10^{-3}$
Correlation coefficient (r)	0.9999	0.9999	0.9999
Relative standard deviation*	0.369	0.412	0.248
% error in bulk sample (95% confidence limit)**	-0.096	-0.122	-0.14

\*Average of six determinations considered. \*\*Average of three determinations

**Table 3: Assay of imipramine in pharmaceutical formulations**

Sample	Labeled amount (mg)	Amount found by proposed methods *			Ref. method	% Recovery by proposed methods **		
		M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>		M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
<b>Tab III</b>	25	24.82 ±	25.00 ±	24.94 ±	25.03 ±	99.26 ±	100.0 ±	99.76 ±
		0.08	0.08	0.05	0.05	0.32	0.33	0.22
		F=2.12	F=2.2	F=1.07				
		t=2.81	T=0.31	T=1.82				
<b>Tab IV</b>	25	24.77 ±	24.92 ±	24.81 ±	24.98 ±	99.11 ±	99.65 ±	99.24 ±
		0.20	0.09	0.10	0.11	0.81	0.38	0.42
		F=3.25	F=1.35	F=1.09				
		T=1.12	T=1.57	T=1.72				
<b>Tab I</b>	50	50.1 ±	49.9 ±	49.8 ±	49.8 ±	99.8 ±	99.6 ±	100.1 ±
		0.35	1.47	0.31	0.202	0.30	0.61	0.66
		F=2.93	F=1.90	F=2.30				
		t=1.27	t=0.63	t=0.68				
<b>Tab II</b>	50	50.1 ±	49.9 ±	49.7 ±	49.9 ±	100.1 ±	99.9 ±	99.5 ±
		0.27	0.33	0.27	0.06	0.55	0.67	0.54
		F=1.83	F=2.73	F=1.78				
		t=1.10	t=0.35	t=0.92				

Average ( $\pm$  RSD) of six determinations; the t and F values refer to comparison of the proposed method with the reference method; theoretical values at 95% confidence limits, t = 2.57, F = 5.05

## CONCLUSION

A significant advantage of an extractive spectrophotometric determination is that it can be applied to the determination of individual compounds in a multicomponent mixture. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibilities in the assay of a particular component in a complex dosage formulation. In the present study, IMP was determined successfully as a pure compound as well as a component in representative dosage formulation. The proposed methods are simple, selective and can be used in the routine determination of IMP in bulk samples and formulations with reasonable precision and accuracy.

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