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Extractive Spectrophotometric Determination of Clomipramine

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Three simple and sensitive spectrophotometric methods (M_1–M_3) for the assay of clomipramine 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_1), naphthalene blue 12BR (NB 12BR, M_2) and naphthalene blue black (NBB, M_3) are utilized. The extracts of the ion-associates exhibit absorption maxima at 540, 620 and 580 nm for methods M_1, M_2 and M_3, respectively. Regression analysis of Beer's law plots showed good correlation in the concentration ranges 1.0–5.0, 2.0–10 and 2.0–10 μg/mL for methods M_1, M_2 and M_3, respectively. These methods are found to be suitable for the assay of clomipramine in pharmaceutical formulations. All of 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, Clomipramine, Azocarmine G, Naphthalene Blue 12BR, Naphthalene Blue Black.

INTRODUCTION

Clomipramine (CLP) is a synthetic antidepressant for oral administration and chemically known as 3-chloro-1,10,11-dihydro-N,N-dimethyl-5H-dibenz[b]-fazepine-5-propanamine. Literature survey revealed that only a few spectrophotometric methods[1–10] were reported for its quantitative determination in bulk drug and pharmaceutical formulations. During the course of our efforts to develop sensitive visible spectrophotometric methods, it was observed that the analytically useful tertiary amino group in CLP 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 CLP. As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the determination of drugs, the technique was therefore utilized in the present work for the assay of CLP. CLP 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 CLP in acid medium is expected to attract the oppositely charged part (negative charge) of the dye

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(SO_3^{2-}) and behaves as a single unit being held together by electrostatic attraction. The results are statistically validated.

EXPERIMENTAL

Systronics 166 digital UV spectrophotometer with 1 cm matched quartz cells was used for the spectral and absorbance measurements. A Systronics digital pH-meter 361 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 NBB (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

1 mg/mL solution was prepared by dissolving 50 mg of pure CLP in 50 mL of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solution of concentrations 20 μg/mL for method M_1 and 40 μg/mL for methods M_2 and M_3, respectively.

Recommended procedure

To each of the aliquots of standard CLP solution 0.5–2.5 mL, 20 μg/mL (M_1), 0.5–2.5, 40 μg/mL (M_2), 0.5–2.5, 40 μg/mL (M_3), the buffer solution (pH 1.5, 6.0 mL) and ACG (M_1), NB 12 BR (M_2), NBB (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 540 nm (M_1), 620 nm (M_2), 580 nm (M_3) against the reagent blank. The amount of CLP was calculated from the calibration plot.

RESULTS AND DISCUSSION

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

Optimum conditions fixation

Conditions under which the reaction of CLP with each dye fulfills the essential analytical requirements were investigated. All the experimental conditions studied were optimized at room temperature (25 ± 1°C) and were established by varying one parameter at a time[11] and observing its effect on the absorbance of the coloured species.

In the preliminary experiments, in view of developing methods of analysis suitable for assaying small quantities of CLP, seven acidic dyes (Table-1) such

as tropaeolin-00, alizarin red-S, bromo cresol green, azocarmine-G, naphthalene blue 12 BR, naphthalene blue black and erichrome black T were tested at various pH ranges as the colour 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 CLP 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 (Cl No. 50085), NB 12 BR (Cl No. 20500), NBB (Cl No. 20470) 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 coloured complex with respect to maximum stability.

TABLE-1
λ_{max} AND ε_{max} VALUES OF CLP-DYE COMPLEXES

S. No.	Dye	Category	λ_{max} (nm)	ε_{max} ($L\ mol^{-1}\ cm^{-1}$)
1.	NBB*	Azo dye	580	2.43×10^{4}
2.	NB 12 BR*	Azo dye	620	$\times 10^{4}$
3.	TPOO	Azo dye	430	1.10×10^{4}
4.	EBT	Azo dye	520	6.21×10^{3}
5.	ACG*	Phenazine dye	540	$\times 10^{4}$
6.	BCG	Triphenyl methane dye	425	2.72×10^{4}
7.	ARS	Anhraquinone dye	430	5.87×10^{3}

*Chosen for further CLP investigation.

The absorption spectra of the ion-association complexes of CLP with the three dyes, extracted into chloroform and of the reagent blank, were obtained as described in the procedure. The spectra are very similar in shape to the absorption spectrum of an aqueous solution of the respective dye, indicating that these ion-association complex spectra show the characteristics λ_{max} (540 nm, method M_1; 620 nm, method M_2 and 580 nm method M_3) values of the respective dye itself.

In order to establish the optimum pH range (for M_1–M_3), the CLP 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 measurement. The results show that a quantitative extraction was produced between pH 1.1–1.5 (for M_1–M_3). All subsequent studied were carried out at pH 1.5 (for M_1–M_3). 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 M_1, M_2 and M_3, respectively. A 6.0 mL portion of buffer was

found to be optimal in methods M_1, M_2 and M_3. 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 coloured 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 colour products were stable up to 30 min. The stoichiometric ratio of the CLP to dye was found as 1 : 1 with ACG or 2 : 1 with NB 12 BR or NBB through slope analysis method.

Chemistry of the coloured species

CLP being basic in nature forms an ion-association complex with the acidic dye which is extractable into chloroform. The stoichiometric ratio of the CLP to dye was found as 1 : 1 with ACG or 2 : 1 with NB 12 BR or NBB through slope ratio method[12]. The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be interpretable in terms of electronic, steric and other effects of complexing behaviour. The possible structure of the ion-association complex in each instance was established based on the analogy reports for similar types of molecules with acidic dyes and was further confirmed by slope-ratio studies. The protonated nitrogen (positive charge) of the drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction as given below.

Interference studies

The interference studies in the determination of CLP 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 of 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 CLP in formulations for methods A, B and C, respectively.

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 CLOMIPRAMINE

Parameters	Method M_1	Method M_2	Method M_3
	ACG	NB 12 BR	NBB
λ_{max}	540	620	580
Beer's Law limits (μg/mL)	2.0–6.0	2.0–10.0	2–10
Molar absorptivity (L mol^{-1} cm^{-1})	3.529×10^4	2.509×10^4	2.439×10^4
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.0110	0.0140	0.0160
Regression equation (y = a + bc):			
Slope (b)	0.1011	0.0714	0.0610
Intercept (a)	–0.0026	–0.0011	0.0009
Correlation coefficient (r)	0.9999	0.9999	0.9998
Relative standard deviation*	0.2690	0.2460	0.3560
% error in bulk sample (95% confidence limit)†	0.2250	0.2060	0.2320

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

TABLE-3
ASSAY OF CLP IN PHARMACEUTICAL FORMULATIONS

Pharmaceutical sample (Labelled amount)	% Recovery (mg)			
	Proposed method			Ref. method[8]
	M_1	M_2	M_3	
T_1 (50 mg)	99.76 ± 0.43	99.80 ± 0.85	99.20 ± 0.88	99.51 ± 0.70
	t = 1.08	t = 1.44	t = 0.62	
	F = 2.65	F = 1.47	F = 1.58	
T_2 (50 mg)	99.17 ± 0.67	99.21 ± 0.51	99.28 ± 0.54	98.93 ± 0.77
	t = 1.21	t = 1.22	t = 1.31	
	F = 1.32	F = 2.27	F = 2.03	
T_3 (50 mg)	99.58 ± 0.71	99.81 ± 0.69	99.25 ± 0.65	99.48 ± 0.88
	t = 1.21	t = 0.96	t = 0.75	
	F = 1.53	F = 1.62	F = 1.83	
T_4 (50 mg)	99.91 ± 0.22	99.82 ± 0.56	99.56 ± 0.69	98.90 ± 0.35
	t = 1.23	t = 0.89	t = 0.15	
	F = 2.53	F = 2.56	F = 3.88	

Average (± 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 extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multi-component 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, CLP 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 CLP in bulk samples and formulations with reasonable precision and accuracy.

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