RESEARCH PAPER

Accepted 5 April 2005
Revised 6 October 2004
Received 12 March 2003
Indian J. Pharm. Sci., 2005, 67(2):175-181

Neocuproine and Bathocuproine as New Reagents for Sensitive Spectrophotometric Determination of Certain Dibenzazepine Drugs

N. G. KESHAVACHAR, AYESHA SYEDA, M. A. PASHA[1] AND A. A. SYED*
Department of Studies in Chemistry, University of Mysore, Mysore-570 006
[1]Department of Studies in Chemistry, Central College, Bangalore-560 001

Two new reagents, neocuproine and bathocuproine are proposed for simple, sensitive and selective spectrophotometric methods for the determination of dibenzazepine class of drugs. In the proposed method copper(II) reacts with imipramine hydrochloride, desipramine hydrochloride, clomipramine hydrochloride, trimipramine maleate and opipramol and subsequently with neocuproine or bathocuproine in an acetic acid medium to yield yellow or orange-red colour, with maximum absorption at 460 or 480 nm, respectively. A reaction mechanism is proposed with drug – metal and reagent ratio of 1:2.

Imipramine hydrochloride (IPH), desipramine hydrochloride (DPH), clomipramine hydrochloride (CPH), trimipramine maleate (TPM) and opipramol (OPP) are dibenzazepine class of antidepressant drugs with a seven membered central ring[1]. Dibenzazepine class of antidepressant drugs have been broadly classified as derivatives of iminodibenzyl and iminostilbenes. IPH, DPH, CPH and TPM belong to the category of iminodibenzyl compounds, while opipramol (OPP) is a derivative of iminostilbene. These drugs are believed to block the uptake of biogenic amines into neurons thereby increasing the concentration of neurotransmitters. They are prescribed over a course of weeks to months; depending upon the elevation of patient's symptoms. To ensure proper results from medication, purity of drugs is of paramount importance. Besides, the determination of purity of tricyclic antidepressants drugs has received greater attention in recent years by stringent quality control measures adopted in pharmaceutical industries.

The various methods proposed for the determination of dibenzazepine derivatives include: titrimetry[2-4], chromatography[5-7], thermoanalytical[8], electroanalytical[9,10], radioanalytical[11,12] and optical methods[13-15]. Among the optical techniques, simple methods based on UV/Vis spectrophotometry have become an accepted analytical tool for the assay and evaluation of drugs.

Visible spectrophotometric methods are convenient, simple, sensitive and can be relatively inexpensive. These methods employ different routes in the determination of chromogen produced such as dye[16], coupled product[17,18], ionpair[19], molecular complex[20,21], charge transfer complex[22] and radical cation[23,24]. However, the above methods have never utilized a co-ordinated complex as a chromogen for the determination of dibenzazepine drugs.

Therefore an attempt was made to meet the ever increasing demand for the stringent quality control in the pharmaceutical industries by developing a simple, sensitive and selective spectrophotometric procedure for the determination of IPH, DPH, CPH, TPM and OPP in both preformulations and dosage forms. The proposed procedure involves the reduction of copper(II) to copper(I) and complexing with neocuproine or bathocuproine in acetic acid medium to produce yellow or orange-red colour.

MATERIALS AND METHODS

UV/Vis spectrophotometer, Uvidec-610 type with 1.0 cm matched cell (Jasco, Tokyo, Japan) was employed for

*For correspondence
E-mail: syedakheelahmed@hotmail.com

measuring the absorbance values. Imipramine hydrochloride was obtained from SG Pharmaceuticals, while, desipramine hydrochloride, clomipramine hydrochloride, trimipramine maleate and opipramol were procured from Ciba-Geigy, Switzerland. Cupric sulphate (BDH), 2,9-dimethyl-1,10-phenonthroline (neocuproine) and 2,9-dimethyl-4,7-diphenyl-1,10-phenonthroline (bathocuproine) (SRL) were obtained. All other chemicals and solvents used were of analytical grade. Double distilled water was used throughout. Samples of the drugs (100 mg) were dissolved in 100 ml of distilled water; a few drops of 0.1N HCl were added to improve the solubility of the drugs. Distilled water was added to obtain 1 mg ml^{-1} of the solution. An aqueous solution of 5.0×10^{-4}M copper(II) sulphate, 0.10% (*w/v*) and 0.05% (*w/v*) of neocuproine and bathocuproine solutions were prepared in double distilled water and alcohol, respectively.

Assay procedure:

Assay with copper(II) and neocuproine/bathocuproine. Aliquots of standard solution (5-70 μg) of IPH, DPH, CPH, TPM or OPP were transferred into calibrated flasks (25-ml). To each flask 2 ml of cupric sulphate, 3 ml of neocuproine and 1 ml of acetic acid solution were added and the flasks were kept in a boiling water bath for 30 min and cooled to room temperature. The solutions were made up to the volume with distilled water.

The procedure was also repeated with bathocuproine and the contents after mixing thoroughly were kept in a boiling water bath for 20 min. It was cooled to room temperature and diluted to the mark with alcohol. The absorbance was measured at 460 and 480 nm with neocuproine and bathocuproine, respectively against the corresponding reagent blank and calibration graphs were constructed.

Twenty tablets of tricyclic antidepressant drugs were finely powdered in a small dish. The powdered drug (50 mg) was dissolved in ~20 ml of 1N HCl and filtered through a Whatman No. 2 filter paper. The filtrate was made up to 100 ml with water in a volumetric flask. The filtrate was diluted with distilled water to obtain a sample concentration of 10 μg ml^{-1}. An aliquot of this solution was used for the determination of tricyclic drugs as per the procedure described earlier.

RESULTS AND DISCUSSION

The method involves the reaction of tricyclic antidepressant class of drugs with copper(II) salts, in the presence of neocuproine and bathocuproine, under mild acidic condition (1M acetic acid) to produce yellow and orange-red colour with maximum absorption at 460 and 480 nm, respectively. The reaction involves the formation of initial copper(II) – IPH, DPH, CPH, TPM or OPP complex in mild acetic acid medium which undergoes reduction to copper(I)-drug complex and subsequently reacting with neocuproine or bathocuproine to form yellow or orange-red colour complex. Copper(I) and copper(II) have the characteristic features that both weak and strong field ligands can bind to the metal ions.

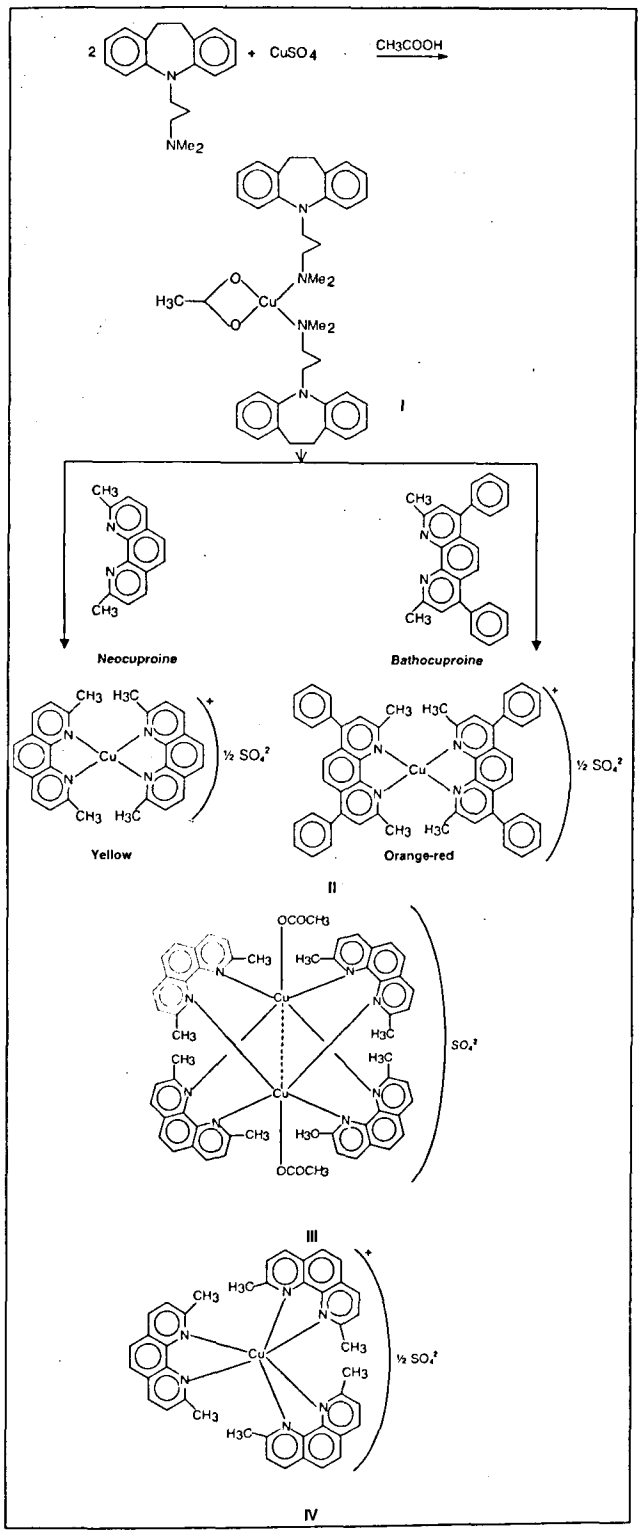
Copper(II) sulphate on treatment with 2 moles of imipramine in acetic acid medium may give the initial complex-I. As CH_3COO^- and $R^{\frown}N(CH_3)_2$ ligands are weak field ligands, on treatment with neocuproine/bathocuproine (a strong field ligand), get replaced readily to give a bis-chelated complex-II as shown in Scheme 1.

Further, as Cu belongs to d^9 system, the complex-II may suffer from tetragonal distortion, hence, an alternating octahedral field complex-III may also form under the above said conditions. Also copper belongs to group XI elements their penultimate shell contains 10 d electrons having the electronic configuration[25] $[Ar]3d^{10}4s^1$. The molar ratio 1:2 of (copper(II) – IPH and neocuproine or bathocuproine) is interesting as it is different (1:3) from iron(III)–1,10-phenonthroline, even though both metal and the ligand have striking similarities with copper and neocuproine or bathocuproine. However, trischelated complex of the type IV is highly susceptible due to John-Teller distortion[26], hence, formation of IV is ruled out under the conditions studied.

The factors affecting the colour development like reproducibility, sensitivity and adherence to Beer's Law were investigated with imipramine hydrochloride as the model representative compound, since the reaction of other tricyclic antidepressants will not be different.

A yellow and orange-red coloured product with maximum absorbance at 460 and 480 nm was formed when IPH was allowed to react with copper(II) sulphate, in the presence of neocuproine and bathocuproine, respectively in acetic acid medium. The absorbance spectra of the yellow and orange-red coloured products along with the reagent blank are shown in fig. 1.

Maximum and constant absorbance values were obtained when the standard flasks were kept in a boiling water bath for 30 min (for neocuproine) and 20 min (for



Scheme 1: Proposed mechanism for the reaction of dibenzazepines with neocuproine/bathocuproine

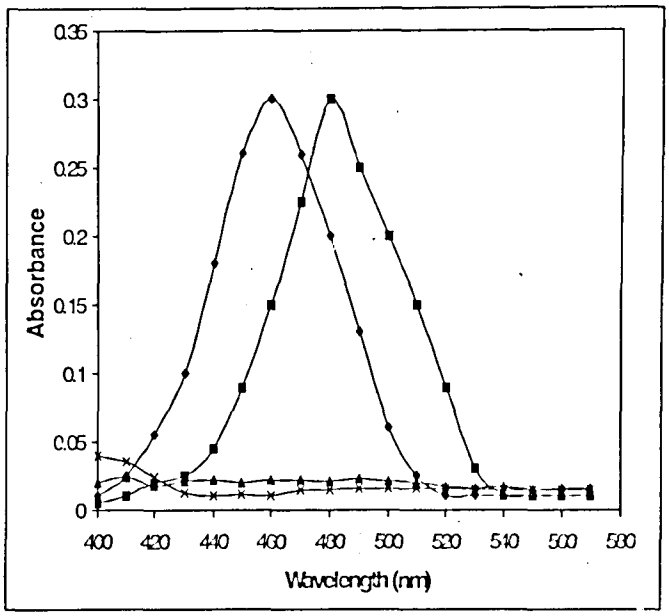


Fig. 1: Absorption spectra of coloured products

1 and 3 are IPH + Copper(II) + neocuproine complex (IPH=1000 ng ml^{-1}) and IPH + Copper(II) + bathocuproine complex (IPH=1000 ng ml^{-1}), respectively 2 and 4 are their corresponding reagent blanks.

bathocuproine) after adding the reagents to the drug solutions in 1-5 ml of 1 M acetic acid solution and they remained stable for 4-5 h and 5-6 h with neocuproine and bathocuproine, respectively. Less than 1 ml and above 5 ml of 1 M acid concentration, the colour development was slow and the absorbance gave erratic values. The development of yellow and orange-red colour with other acids like hydrochloric acid, sulphuric acid, nitric acid, phosphoric acid and formic acid was slow. Acetic acid was selected as the appropriate reaction medium, because of the colour stability and less interference from other excipients present in drug solutions.

It was found that a 5.0×10^{-4}M copper(II) sulphate solution in the range of 1-5 ml and 0.1% (*w/v*) of neocuproine in the range of 2-5 ml and 0.05% (*w/v*) of bathocuproine in the range of 2-5 ml gave maximum colour intensity and stability. Hence, 2 ml of copper(II) sulphate, 3 ml of neocuproine and 3 ml of bathocuproine solutions were found optimum. The sequence of addition of copper(II) sulphate, neocuproine/bathocuproine, drug solution and acetic acid was studied *via* the formation of the yellow/orange-red complex. There was no appreciable change in the absorbance or colour of the product when the sequence of these reac-

tants was altered. Table 1 and 2 shows the linear calibration ranges and equation parameters for these procedures. Separate determinations at different concentrations of each drug gave a coefficient of variation not exceeding 2%.

Sandell's sensitivity (S)[27] represents the number of micrograms of the determinand per ml of a solution having an absorbance (A) of 0.001 for a path length (l) of 1 cm. Thus, $S=10^{-3}/a=\mu g\ cm^{-2}$, where a is the specific absorptivity, and its value (ml/g.cm) corresponds to determine and the absorbance of a 1μg/ml solution of the determinand in a cuvette with an optical path length of 1 cm.

Job's method[28] of continuous variation was employed

TABLE 1: OPTICAL CHARACTERISTICS OF THE CHROMOGEN FOR THE DETERMINATION OF TRICYCLIC ANTI-DEPRESSANTS WITH NEOCUPROINE

Drugs Parameters	IPH	DPH	CPH	TPM	OPP
Colour	yellow	yellow	yellow	yellow	yellow
l_{max} (nm)	460	460	460	460	460
Stability (h)	5	5	5	5	4
Beer's law (ng/ml)	200-2800	200-2800	200-2400	200-2800	200-2800
Recommended drug concentration (ng/ml)	1000	1000	1000	1000	1000
Molar absorptivity (l/mol.cm)	5.45×10^4	5.41×10^4	5.64×10^4	6.42×10^4	5.7×10^4
Sensitivity	5.81×10^{-3}	5.5×10^{-3}	6.22×10^{-3}	6.39×10^{-3}	6.3×10^{-3}
Regression equation*					
Slope	0.157	0.180	0.195	0.181	0.181
Intercept	0.023	0.003	-0.038	-0.026	-0.024
Correlation coefficient	0.9949	0.9601	0.9899	0.9950	0.9989

*y=ax+b, where x is the concentration of IPH (imipramine HCl), DPH (desipramine HCl), CPH (clomipramine HCl), TPM (trimipramine HCL) or OPP (opipramol) in ng/ml, n=6.

TABLE 2: OPTICAL CHARACTERISTICS OF THE CHROMOGEN FOR THE DETERMINATION OF TRICYCLIC ANTI-DEPRESSANTS WITH BATHOCUPROINE

Parameters	IPH	DPH	CPH	TPM	OPP
Colour	orange-red	orange-red	orange-red	orange-red	orange-red
lmax (nm)	480	480	480	480	480
Stability (h)	6	5	5	6	6
Beer's law (ng/ml)	200-2800	200-2800	200-2800	200-2800	200-2800
Recommended drug concentration (ng/ml)	1000	1000	1000	1000	1000
Molar absorptivity (l/mol.cm)	7.59x104	7.97x104	7.8x104	8.50x104	7.22x104
Sensitivity	4.17x10-3	3.8x10-3	4.5x10-3	4.83x10-3	5.03x10-3
Regression equation*					
Slope	0.135	0.140	0.154	0.144	0.132
Intercept	-0.106	0.117	-0.071	0.077	0.010
Correlation coefficient	0.9802	0.9969	0.9901	0.9603	0.9786

* y=ax+b where x is the concentration of IPH (imipramine HCl), DPH (desipramine HCl), CPH (clomipramine HCl), TPM (trimipramine HCL) or OPP (opipramol) in ng/ml. n=6

for the determination of the molar ratio of IPH-copper(II) reaction with neocuproine and bathocuproine as an example. Aqueous solutions of IPH (100 ppm) and copper(II) [5×10^{-4}M] and a concentration of [5×10^{-4}M] neocuproine or bathocuproine were used. A series of standard solutions of IPH and neocuproine or bathocuproine copper(II) in different complimentary proportions were prepared and then absorbance was measured at 460 or 480 nm against blanks prepared under the same conditions.

For the complexation reaction, the metal was reduced by the drug and subsequent complexation with the reagents. Investigations of the continuous molar variation of IPH-copper(II) and neocuproine or bathocuproine showed that the copper(I) interacts with neocuproine and bathocuproine in 1:2 ratio. Similar results have been observed with the mole-ratio method. A reaction mechanism based on the above observations is presented in Scheme 1.

TABLE 3: RECOVERY OF IMIPRAMINE HYDROCHLORIDE (IPH) IN THE PRESENCE OF EXCIPIENTS AND OTHER SUBSTANCES

Material	Amount (mg)	% Recovery of IPH* ±SD	
		Neocuproine	bathocuproine
Glucose	50	99.8±0.60	100.6±1.12
Lactose	50	99.2±0.57	100.8±0.89
Dextrose	50	98.9±0.61	101.9±1.27
Starch	50	97.1±1.72	99.2±0.69
Sodium alginate	50	98.8±1.21	98.8±0.93
Sodium lauyrl sulphate	50	99.1±0.85	101.2±1.14
Vitamin C	10	>50<60	>50<60

SD: standard deviation, *1000 ng/ml of IPH (imipramine HCl) taken. n=5.

TABLE 4: DETERMINATION OF CERTAIN DIBENZAZEPINES IN COMMERCIAL AND LABORATORY PREPARED TABLETS BY THE PROPOSED METHODS AND OFFICIAL METHODS WITH NEOCUPROINE

Formulation (tablets)	Label claim (mg/tablet)	Recovery* %	Additional Analyte added (mg)	Recovery* %	Official method[29-31] (found %)
Depranil (imipramine HCl)	75	100.3±1.2	75	99.8±0.85	101.1±0.51
Laboratory prepared (desipramine HCl)	50	99.8±0.50	50	100.1±0.6	99.4±0.92
Clofranil (clomipramine HCl)	25	99.6±0.91	25	100.4±0.83	99.6±0.53
Surmontil (trimipramine maleate)	10	100.3±1.11	10	100.5±0.91	99.8±0.71
Laboratory prepared (opipramol)	50	99.7±0.41	50	99.3±0.82	99.8±0.52

*Proposed method; average±standard deviation of 5 determinations.

TABLE 5: DETERMINATION OF CERTAIN DIBENZAZEPINES IN COMMERCIAL AND LABORATORY-PREPARED TABLETS BY THE PROPOSED METHODS AND OFFICIAL METHODS WITH BATHOCUPROINE

Formulation (tablets)	Label claim	%Recovery* (mg/tablet)	Additional Analyte added (mg)	%Recovery*	Official method (found %)
Depranil (imipramine HCl)	75	99.8±0.95	75	100.6±0.9	101.1±0.51
Laboratory prepared (desipramine HCl)	50	100.4±0.62	50	99.3±0.72	99.4±0.92
Clofranil (clomipramine HCl)	25	100.1±0.73	25	100.2±0.92	99.6±0.53
Surmontil (trimipramine maleate)	10	99.6±0.64	10	99.5±0.80	99.8±0.71
Laboratory prepared (opipramol)	50	100.1±0.52	50	99.8±0.63	99.8±0.52

*Proposed method; average±standard deviation of 5 determinations, *Ref. 29-31

The development of the coloured product was slow at room temperature. The absorbance values were maximum and remained constant above temperatures 80°. After cooling to room temperature the products with neocuproine and bathocuproine were stable for 4-5 h and 5-6 h, respectively.

The interference by various substances that often accompany tricyclic antidepressant drugs in pharmaceutical preparations were studied by taking imipramine hydrochloride as a representative drug. It was found that commonly encountered pharmaceutical additives and excipients such as glucose, lactose, dextrose, starch, sodium alginate and sodium lauryl sulphate did not interfere, while vitamin C was found to interfere seriously (Table 3).

The applicability of the method to assay pharmaceutical preparations was examined. Commercial tablets containing imipramine hydrochloride, clomipramine hydrochloride, trimipramine hydrochloride and laboratory prepared tablets of desipramine hydrochloride and opipramol were analyzed by the proposed methods. The results obtained (Table 4 and 5) compared favourably with those reported by the USP 24, 2000 method[29-31]. The USP 24 method suggests four time extraction with ether and measurement of absorbance in the UV region at 250 nm at 25 $\mu g\ ml^{-1}$ concentration of IPH. Our proposed methods have the advantage that the active drug component can be determined directly without any preconcentration step.

The ever-increasing consumer awareness, consequently, stringent quality control procedures adopted in pharmaceutical industries demands the development of simple and sensitive methods for the routine assay and evaluation of drugs in preformulations and dosage forms. The use of common reagents and simplicity of the method is a step forward to assure high standard in quality control. Of the two new reagents studied by us bathocuproine is recommend for routine analysis of IPH, DPH, CPH, TPM and OPP in preformulation and dosage forms.

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