ANTIDEPRESSANT DRUGS: HIGHLY SENSITIVE AND VALIDATED SPECTROPHOTOMETRIC TECHNIQUE

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ABSTRACT

A simple, rapid, selective and highly sensitive spectrophotometric method is described for the quantitative determination of the tricyclic antidepressant drugs, desipramine hydrochloride (DPH), clomipramine hydrochloride (CPH) and imipramine hydrochloride (IMH) in pure and in pharmaceutical preparations. The proposed method is based on the bromination of above drugs with known excess of bromine. The unreacted bromine is determined based on its ability to bleach the dye Eiochrome blue black R quantitatively at 530 nm for all the three drugs obeying Beer's law in the range, 0.0 – 8, 0.0 – 10 and 0.0 – 9.0 µg ml^{-1} for DPH, CPH and IMH, respectively. The molar absorptivity values were found to be 1.61×10^4, 1.62×10^4 and 1.57×10^4 l $mol^{-1}cm^{-1}$, respectively with the corresponding Sandell's sensitivity values 0.0187, 0.0216 and 0.0202 **µg cm**$^{-2}$. The limits of detection and (LOD) and quantification (LOQ) are also reported for the developed method. Intra- and inter-day precision and accuracy of the method were established according to the current ICH guidelines. Applications of the procedure to the analysis of various pharmaceutical preparations gave reproducible and accurate results. Further, the validity of the procedure was confirmed by applying the standard addition technique and the results were evaluated in terms of Student's *t*-test and variance ratio *F*-test to find out the significance of proposed method over the reference method.

Keywords: Eriochrome blue black R, antidepressant drugs, desipramine hydrochloride, clomipramine hydrochloride, imipramine hydrochloride, unreacted bromine.

INTRODUCTION

DPH, CPH and IMH are tricyclic antidepressants. Desipramine hydrochloride (DPH), is chemically known as [3-(10,11-Dihydro-dibenzo[b,f] azepin-5-yl)-propyl]-methyl-amine hydrochloride (Fig 1. a). It inhibits the reuptake of norepinephrine and to a lesser extent serotonin. It is used to treat depression, but not considered a first line treatment since the introduction of Selective serotonin re-uptake inhibitors (SSRI) antidepressants. It is the active *in vivo* metabolite of imipramine and as such, shares many of imipramine's pharmacologic effects. Chemically, clomipramine hydrochloride (CPH) is 3-(3-chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-aminehydrochloride (Fig. 1. b). Clomipramine is used for the treatment and relief of obsessive and compulsive disorders as well as in depression and other emotional disturbances. In spite of new atypical drugs such as those of the SSRI group (fluoxetine, fluvoxamine etc), clomipramine is still the reference compound in the treatment of these psychiatric disorders [1-3]. Chemically, imipramine hydrochloride (IMH) is (10,11-Dihydro-N,N-dimethyl)-5H-dibenz[b,f] azepine-5-propanamine hydrochloride (Fig. 1. c). It is a potent inhibitor of noradrenaline reuptake at noradrenergic nerve endings. All the three drugs DPH, CPH and IMH are official in European Pharmacopoeia [4, 5, 6]. The report describes a potentiometric titration of DPH, CPH and IMH using 0.1 M sodium hydroxide as titrant in acidic medium.

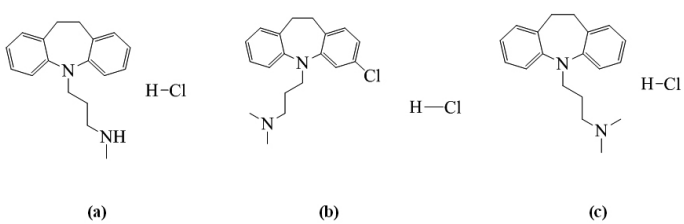

Fig. 1-Structure of (a) DPH, (b) CPH and (c) IMH.

Several analytical methods are available in the literature for the determination of DPH, CPH and IMH in biological fluids and/or pharmaceutical formulations. These methods include chromatographic techniques like HPLC-DAD method using 7,7,8,8-tetracyano-quinodimethane (TCNQ) as a derivatisation agent [7], HPLC with liquid-liquid micro extraction technique [8], RP-HPLC and HPTLC [9] GC [10], GC-MS [11-13,], electrogenerated chemiluminescence [14,], voltammetry [15], spectrofluorimetry [16] and spectrophotometry [17- 27].

The above reported analytical methods suffer from one or the other disadvantages. The reported chromatographic techniques [7-13] require expensive experimental set-up, requires sophisticated instrumentation [15] and involvement of scrupulous experimental conditions which are not affordable in every laboratory for routine analysis; Whereas the reported spectrophotometric methods were associated with some major drawbacks such as, lesser sensitivity [16-17, 23-26], tedious extraction procedures [16-17, 26], maintenance of strict experimental conditions [16, 17, 21] and time consuming [18, 20, 27]. For these reasons, the development of a new, simple, rapid, selective, sensitive and inexpensive spectrophotometric method that overcomes the drawbacks of the existing methodologies was very essential.

Comparison between the reported spectrophotometric methods for determination of DPH, CPH and IMH with the proposed method is shown in Table 1.

The method suggested is devoted to study a highly sensitive, selective, reproducible and economically viable method that could be used to determine DPH, CPH and IMH in bulk drug and in dosage forms. The proposed method employs the use of bromate-bromide solution and Eriochrome blue black R as reagents and is based on the bromination of above drugs with known excess of bromine followed by reaction with Eriochrome blue black R to produce violet colored dye. The linearity, accuracy, precision, ruggedness and recovery of the assay were validated according to ICH guidelines. The method is suitable for routine analytical use, as it is simple and does not involve any complicated extraction procedures and was found to be simple, selective and cost-effective compared too many reported methods.

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Table 1-Comparison of the performance characteristics of the proposed method with the existing visible spectrophotometric/chromatographic methods.

Sl. No.	Reagent's used	Methodology	Linear range, µg ml^{-1}	Remarks	Ref
1	Alizarin red S	The ion-pair complex showed maximum fluorescence intensity at 561 nm with excitation at 490 nm	1 – 20	Requires extraction procedure and maintenance of strict pH control	16
2	Molybdenum(V) thiocyanate and hexa kis iron (III) solution	Ion-pair formation between molybdenum (V) thiocyanate and hexa kis iron (III) solution	10 – 200	Less sensitive and requires extraction procedure	17
3	Neocuproine bathocuproine	Formation of copper(I)-drug complex and subsequently reacting with neocuproine or bathocuproine complex which shows maximum absorption at 460 and 480 nm	0.2 – 2.8	Sensitive, but time consuming and requires heating for 30 min	18
4	*p*-phenylenediamine dihydrochloride	Formation of diazotized *p*-phenylenediamine dihydrochloride, shows maximum absorption at 565 nm	1.1 –3.6	Sensitive but requires high acidic conditions and cooling to 0 to 5 ºC	19
5	Picryl chloride	Based on reaction with picryl chloride in chloroform medium and measurement at 395 nm	0.16 – 1.6 0.4 – 2.4	Sensitive but time consuming and require the use of organic solvent	20
6	Ammonium meta vanadate	Oxidation of the drugs by ammonium metavanadate with maximum absorption at 618 nm	0.7 – 35	Requires maintenance of high acidic conditions and less sensitive	21
7	$NaClO_4$–buffer–ethanol	derivative spectrophotometry	0.63 – 10.04	Sensitive but of lack of selectivity	22
8	Methyl orange	Formation of ion-pair with methyl orange and the absorbance was measured at 425 nm	0.79 – 25.3	Less sensitive	23
9	eriochrome cyanine R	Reacts in neutral medium with imipramine forming reddish compound	10 – 80	Less sensitive	24
10	Iminodibenzyl p-chloranilic acid	First derivative of ratio spectra Formation of charge transfer complex	5 – 30 20 – 200	Less sensitive	25
11	azocarmine G (ACG) naphthalene blue (NB) woolfast blue BL (WFB BL)	Formed ion-associates exhibit absorption maxima at 550 nm(ACG), 620 nm (NB) and 590 nm (WFB BL)	2.0 – 12.0 4.0-16.0 1.0-12.0	Less sensitive and requires extraction procedures	26
12	Picryl chloride	Based on reaction with picryl chloride in chloroform medium and measurement at 395 nm	0.16 – 1.6 0.4 – 2.4	Time consuming and require the use of organic solvent	27
12	Eriochrome blue balck R – Bromate bromide mixture	Bromination of the drugs with known excess of bromine followed by reaction with Eriochrome blue balck R and exhibits absorption maxima (λ_{max}) at 530 nm	0.0 – 8 0.0 – 10 0.0 – 9	Highly sensitive, less time consuming, no heating is required, no use of organic solvents (eco-friendly) and inexpensive	Developed method

EXPERIMENTAL SECTION

Apparatus

All the absorbance measurements were performed using a Systronics Model 166 digital spectrophotometer provided with 1-cm matched quartz cells.

Reagents and Standards

Analytical reagent grade chemicals were used, and double distilled water was used throughout the experiment to prepare all solutions.

i. Standard DPH, CPH and IMH solutions. Pure samples (99.95 %) of DPH, CPH and IMH were used and received as gift from La Pharma, Ahmedabad, Gujarat, India, and Aventis Pharma. Ltd., Mumbai, India. A stock standard solutions equivalent to 100 µg ml^{-1} of the cited drugs was prepared separately by dissolving 10 mg of the pure drug in 100 ml distilled water. Working solutions were prepared as required by dilution in water.

Pharmaceutical formulations of DPH such as Norpramin and Pertofrane (Aventis Pharmaceuticals), CPH such as Ocifril (La Pharma) and Clonil (Intas) and Impramine (Sun) and Depranil (La Pharma) tablets were purchased from local commercial sources.

ii. Bromate-Bromide solution (10 µg ml^{-1}). A stock standard solution of 100 µg ml^{-1} bromate solution for bromine generation was prepared by dissolving accurately weighed 0.05 g of potassium bromate (Sarabhai M. Chemicals, India) and 0.5 g of potassium bromide (S. D. Fine Chem., India) in water and diluted to the mark in a 500 ml calibrated flask. To get 20 µg ml^{-1} of $KBrO_3$ stock solution, 20 ml of bromate-bromide mixture was diluted to 100 ml in distilled water. Then, 50 ml of this stock solution was transferred in to a 100 ml calibrated flask containing 40 ml of 4.25 M H_2SO_4 and diluted to 100 ml with water to get the working concentration of 10 µg ml^{-1} of $KBrO_3$. This bromate solution (10 µg ml^{-1}) was freshly prepared on the day of use.

iii. Erio chrome blue black R (EBBR) (0.1 %, w/v). It was prepared by dissolving 0.1 g of Erio chrome blue black R (BDH chemicals, Poole, England) in 100 ml water to get 0.1 % solution.

General procedures

Calibration curve

Appropriate aliquots of aqueous working solution containing 0.0, 0.25, 0.5, 1.0, ……4.0 ml of DPH (20 µg ml^{-1}), 0.0, 0.25, 0.5, 1.0, …5.0 ml (20 µg ml^{-1}) of CPH and 0.0, 0.05, 0.125, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.25 ml of IMH (40 µg ml^{-1}) were transferred into a series of separate 10 ml calibrated flasks using a micro burette. To each flask were added 6 ml of bromate-bromide mixture (10 µg ml^{-1} w.r.t $KBrO_3$), and the flasks were stoppered. Finally, 0.75 ml of 0.1 % EBBR was added to each flask and mixed well before being diluted to 10 ml with distilled water and the absorbance of the bright pink color solution (violet) was measured at 530 nm against the reagent blank. The reagent blank was prepared similarly, but without drug content.

The amount of the each drug present in the respective samples was computed from the concurrent calibration curve or the regression equation. All measurements were made at room temperature (27 ± 3 °C).

Procedure for commercial samples

The applicability of the proposed method for the determination of the above cited drugs was tested using two brands of each tablet in pharmaceutical formulation. For this purpose, twenty tablets of DPH (25 mg and 50 mg), CPH (10 and 25 mg) and IMH (20 mg) were weighed accurately and ground into fine powder. An amount of the powder equivalent to 10 mg of each drug was weighed accurately into a three separate 100 ml calibrated flasks and 50 ml distilled water was added. The content was shaken for about 30 min; the volume was diluted to the mark with water and mixed well and filtered using a Whatman No.41 filter paper. The filtrate containing the cited drugs were at a concentration 100 µg ml^{-1} was subjected to analysis by the procedure described above after suitable dilution step.

Analysis of placebo blank

A placebo blank of the composition: talc (15 mg), acacia (10 mg), starch (15 mg), methyl cellulose (20 mg), sodium citrate (25 mg), magnesium stearate (20 mg) and sodium alginate (15 mg) was made and its solution was prepared in 50 ml calibration flask as described under '*Procedure for commercial samples',* and then subjected to analysis using the procedure described under '*Procedure for Calibration curve'.*

Analysis of Synthetic mixture

To the placebo blank of the composition described above, 10 mg each of DPH and CPH was added into a separate 100 ml calibrated flasks and homogenized, and the solution was prepared as described under '*Procedure for commercial samples',* and then subjected to analysis by the procedure described under '*Procedure for Calibration curve'.* The analysis was used to study the interferences of excipients such as talc, acacia, starch, methyl cellulose, sodium citrate, sodium alginate and magnesium stearate.

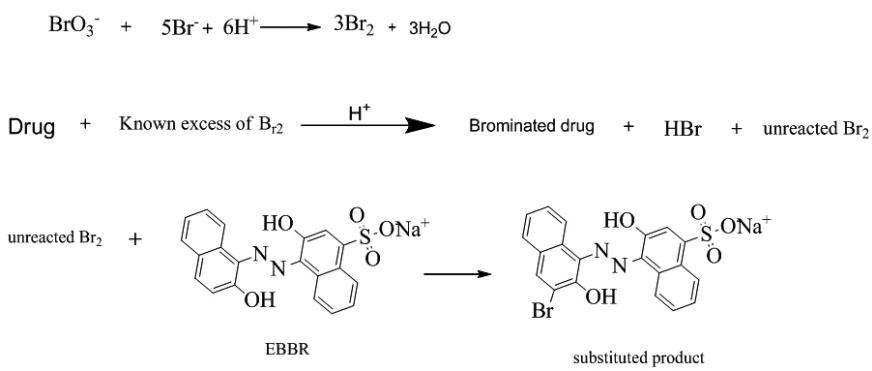
RESULTS AND DISCUSSION

Chemistry of the method

Bromate in acid medium acts as an oxidizing agent [28]. Preliminary experiments revealed that DPH, CPH and IMH drugs are prone to bromination reaction by bromine generated *in situ* by the action of acid on bromate-bromide mixture.

In the present study, a known excess of bromine is used to brominate the studied drugs (DPH, CPH and IMH) in an acidic condition. The unreacted bromine bleaches the color of the azo-dye, EBBR, thereby a decrease in bromine concentration. This reaction formed the basis of DPH, CPH and IMH determination. When EBBR is bleached (brominated) completely with bromine, the absorbance at 530 nm decreases and reaches minimum value. In the presence of drug concentration (DPH, CPH and IMH), bromine is reduced to bromide and the unreacted bromine decolorized the EBBR. Thus, with increasing concentration of drug, higher amount of bromine is reduced and this is observed by a linear increase in the absorbance due to the unbleached EBBR at 530 nm.

The difference in concentration of unbleached EBBR and the reacted bromine in the reaction mixture give the exact concentration of the drug. The absorption spectra [Fig. 2] show that, a linear increase in absorbance with increasing drug concentration. The possible reaction pathway is given in Scheme 1.



Scheme 1-Proposed reaction scheme.

Absorption spectra

EBBR has a maximum absorbance at 530 nm (Curve A). On treatment with bromine, the color of the EBBR bleaches due to bromination. When EBBR is bleached completely with bromine, the absorbance at 530 nm decreases and reaches minimum value (Curve B). With an increase in drug concentration (DPH/CPH/IMH), there is a corresponding decrease in bromine concentration, and as a result less brominated/bleached EBBR is obtained (Curves C, D and E). Here, DPH is used as a model compound, since CPH/IMH is also behaved similarly to it. The concentration of bromine, EBBR and the DPH are given in Fig. 2

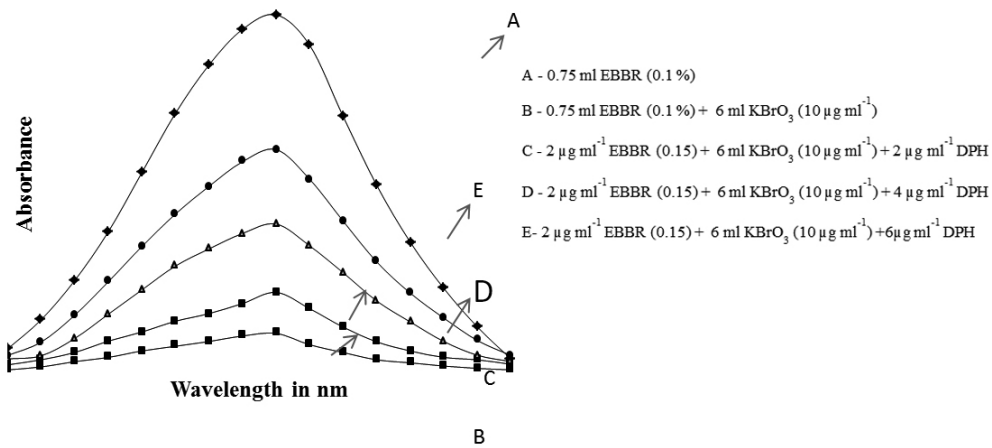


Fig. 2-Absorption spectra for DPH.

Optimization of variables and method development

The factors affecting reaction conditions (effect of dye, bromine concentration and standing time) were studied separately by measuring the absorbance of the brominated products at 530 nm for both the drugs such as DPH, CPH and IMH, respectively by varying one parameter at a time and keeping the others constant.

Effect of dye and reagent concentration

In spectrophotometric analytical methods where maximum sensitivity is desired, the reagent concentration in solution is an important parameter to be studied. In order to achieve this objective, preliminary experiment was performed to fix the upper and lower limits of the dye that could be determined spectrophotometrically at 530 nm. The upper limit was of the absorbance (0.728) adjusted was obtained by the addition of 0.75 ml of 0.1 % EBBR in 10 ml of the reaction mixture. The lower limits of the absorbance was reached by the addition of 6 ml of bromate-bromide mixture (10 µg ml^{-1} w r. t $KBrO_3$) in the same volume. The absorbances of these are given in Fig.2.

Reaction Time and Color Stability

Experiment was carried out to optimize the reaction time and stability of the substituted product formed. The effect of reaction time between the drugs (DPH, CPH and IMH) and the bromine generated *in situ* was completed in 3 min (Fig. 2, Curves C, D and E). After completion of the reaction between the drug and the bromine, a 5 min standing time was necessary for the complete bleaching of the dye color by the residual bromine and this bromination process was instantaneous and found to be complete within 8 min. The absorbance of the measured species was stable up to 30 min.

Method validation

The spectrophotometric method validation characteristics were tested in accordance with ICH [29] guidelines. The proposed method was evaluated under the optimum conditions with respect to linearity, accuracy, precision, molar absorptivity, Sandell's sensitivity and Student's *t*- and *F*-test.

Linearity

A linear calibration graph was constructed using the standard solutions of DPH, CPH and IMH. Under established experimental conditions, a linear correlation was found between the absorbance at 530 nm and concentrations of DPH, CPH and IMH in the ranges are given in Table 2. Regression analysis of the calibration curves are described by the equation:

$$y = a + bx$$

where y = absorbance, a = intercept, b = slope and x = concentration and the values are presented in Table 2. The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity and Sandell's sensitivity values [30] are also given in Table 2. The calibration curves are shown in Figures 3. a, b and c, respectively.

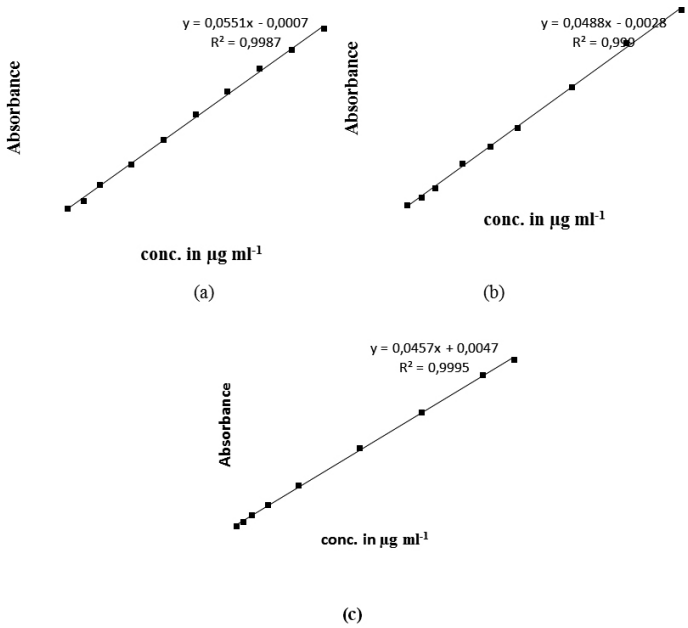


Fig. 3-Calibration curves for (a) DPH, (b) CPH and (c) CPH.

Sensitivity

The detection limits (LOD) and limits of quantitation (LOQ), for the proposed method was also evaluated as per ICH guidelines using the formula:

where σ is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte, and s is the slope of the calibration graph. The high values of molar absorptivity (ε) and low values of Sandell's sensitivity and LOD indicate the high sensitivity of the proposed method (Table 2).

$$\text{LOD} = \frac{3.3\times\sigma}{s} \quad \text{and} \quad \text{LOQ} = \frac{10\times\sigma}{s}$$

Table 2-Analytical and regression parameters of the proposed method.

Parameter	DPH	CPH	IMH
λ_{max} nm	530	530	530
Beer's law range (µg ml^{-1})	0.0 - 8	0.0 – 10	0.0 – 9.0
Linear range (µg ml^{-1})	0.3 - 8	0.5 – 10	0.3 – 9.0
Molar absorptivity (ε), (l $mol^{-1}cm^{-1}$)	1.624×10^4	1.615×10^4	1.572×10^4
Sandell's sensitivity (µg cm^{-2})	0.0187	0.0217	0.0202
Regression equation, Y* Intercept (a) Slope (b)	-0.0023 0.0559	-0.0028 0.0488	0.0047 0.0457
Correlation coefficient (r)	0.999	0.999	0.9996
S_a	0.0375	0.0009	0.0060
S_b	0.0061	0.0199	0.0006
LOQ (µg ml^{-1})	0.2125	0.1018	0.2151
LOD (µg ml^{-1})	0.0701	0.0336	0.0709

*y=a+bx, where x is the concentration of DPH, CPH and IMH in µg ml^{-1} and y is the absorbance at the respective λ_{max}., S_a is the standard deviation of the intercept, S_b is the standard deviation of the slope.

Intra-day and Inter-day precision and accuracy

In order to determine the accuracy and precision (intra- and inter-day) of the proposed method, solutions containing three different concentrations (low, medium and high) (Table 3) within the working limits of drugs were prepared and analyzed in five replicates in the same day (intra-day precision) and in after 3 days (inter-day precision). The relative error, RE (%) and relative standard deviation, RSD (%) values of both intra and inter-day studies were satisfactory and showed that the best appraisal of the procedure in daily use.

Interferences

The selectivity of the proposed method to pharmaceutical samples was tested by a systematic study under the optimum experimental conditions which were made for the effect of the additives and excipients. The recommended procedure was applied to the analysis of both placebo blank and synthetic mixtures prepared in the laboratory as described under *Analysis of placebo blank and Synthetic mixture.* The usual diluents and excipients such as starch sodium alginate, talc, gelatin, dextrose, methyl cellulose and acacia were found not to interfere with the analysis by the proposed method and the results were obtained in the range 98.5 % to 101 % for DPH, CPH and IMH, respectively. These results further showed clearly the accuracy and precision of the developed method.

Application to analysis of pharmaceutical samples

To check the validity of the proposed method, the drugs under investigation (DPH, CPH and IMH) was determined in some commercial formulations and the results are presented in Table 4. The results of an assay of the cited drugs were statistically compared with the reference method [19, 24] by applying the Student's *t*- test for accuracy and *F*-test for precision. The results in the Table

4 showed that there is no significant difference between the proposed and reference methods [19, 24] at the 95 % confidence level with respect to accuracy and precision. The calculated *t*- and *F*- values (Table 4) did not exceed the tabulated values (t = 2.77 and F = 6.39), they could therefore be used easily for the routine analysis of pure DPH, CPH and IMH in its dosage forms.

The results obtained are presented in Table 3 and show that the accuracy and precision of the proposed method have good repeatability and reproducibility.

Table 3-Evaluation of intra-day and inter-day accuracy and precision.

Drug	Drug added, µg ml^{-1}	Intra-day accuracy and precision (n=5)			Inter-day accuracy and precision (n=5)		
		Drug found, µg ml^{-1}	% RE	% RSD	Drug found, µg ml^{-1}	% RE	% RSD
CPH	0.4	0.393	1.79	1.32	0.408	1.89	1.29
	0.8	0.786	1.78	1.54	0.813	1.67	1.28
	1.2	1.204	0.36	1.89	1.210	0.80	2.04
DPH	0.4	0.395	1.28	1.05	0.41	2.28	1.79
	0.6	0.592	1.41	1.17	0.62	2.97	2.04
	0.8	0.782	2.31	1.58	0.82	2.97	2.42
IMH	2	1.96	1.19	0.77	2.03	-1.64	2.79
	4	3.97	1.23	1.42	4.04	-0.88	2.08
	6	5.92	1.30	1.59	5.97	0.55	2.25

% RE: percent Relative error; % RSD: percent Relative standard deviation.

Table 4-Results of determination of DPH and CPH in dosage form and statistical comparison with the reference method.

Drug studied	Tablet brand Name*	Nominal amount mg per tablet	Found** (% of nominal amount ± SD)	
			Reference Method [17]	Proposed method
DPH	Norpramin[a]	25 mg	99.9 ± 1.14	100.20 ± 0.112 t = 1.26, F = 5.36
	Pertofrane[b]	50 mg	100.2 ± 0.15	100.54 ± 0.11 t = 1.98, F = 1.58
CPH	Ocifril[c]	25 mg	99.8 ± 0.78	100.41 ± 0.120 t = 2.43, F = 5.48
	Clonil[d]	10 mg	100.5 ± 0.21	100.57 ± 0.126 t = 0.41, F = 2.79
IMH	Impramine[e]	25 mg	99.68 ± 0.20	99.87 ± 0.29 t = 0.61, F = 0.46
	Depranil[f]	25 mg	99.55 ± 0.25	100.89 ± 0.17 t = 1.49, F = 2.21

*Marketed by: a and b. (Aventis Pharmaceuticals), c. (La Pharma), d. (Intas), e. (Sun), and f. (La Pharma)
**Mean value of five determinations
Tabulated *t*- and *F*-values at 95 % confidence level are 2.77 and 6.39, respectively.

Evaluation of accuracy by recovery study (standard addition technique)

To further assess the accuracy of the proposed method, recovery experiment was performed by applying the standard addition technique. The recovery test was done by adding the drugs (DPH, CPH and IMH) to the previously analyzed tablets. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of pure drugs. The recovery of the pure drug added was quantitative and the recovery percentage values ranged between 99.18 – 101.59 % for both the drugs and are close to 100 % indicating that the recovery was good, and that the co-formulated substance did not interfere in the determination. The results of recovery study are summarized in Table 5.

Table 5-Results of Recovery Experiments *via* the Standard Addition Technique.

Tablet studied*	DPH				CPH				IMH			
	DPH tablet µg ml^{-1}	Pure DPH added, µg ml^{-1}	Total found, µg ml^{-1}	Pure DPH recovered** % ± SD	CPH tablet added, µg ml^{-1}	Pure CPH added, µg ml^{-1}	Total found, µg ml^{-1}	Pure CPH recovered** % ± SD	IMH tablet added µg ml^{-1}	Pure IMH added, µg ml^{-1}	Total found µg ml^{-1}	Pure IMH recovered** % ± SD
Tablet A	2	2	4.01	100.35 ± 0.06	2	2	4.02	101.07 ± 0.09	2	2	3.98	99.40 ± 0.16
	2	4	6.04	100.92 ± 0.26	2	4	5.96	99.18 ± 0.15	2	4	5.97	99.23 ± 0.11
	2	6	7.95	99.28 ± 0.19	2	6	8.02	100.36 ± 0.19	2	6	8.06	100.98 ± 0.62
Tablet B	2	2	0.399	101.25 ± 0.04	2	2	4.03	101.59 ± 0.08	2	2	4.01	100.60 ± 0.09
	2	4	0.603	99.02 ± 0.17	2	4	6.03	100.72 ± 0.07	2	4	6.03	100.74 ± 0.16
	2	6	0.805	99.73 ± 0.15	2	6	8.04	100.63 ± 0.16	2	6	8.08	101.34 ± 0.25

* Tablet A- [a] Norpramin, [b] Ocifril and [c] Impramine
Tablet B- [a] Pertofrane, [b] Clonil and [c] Depranil
**Mean value of three determinations

CONCLUSIONS

In the present investigation a simple, highly sensitive, accurate and precise spectrophotometric method for the routine estimation of DPH, CPH and IMH in pure form and in dosage forms is described. The method developed does not involve multi steps and do not take more operator time and expensive experimental set up like chromatographic methods. Moreover, the proposed method is free from the usual analytical complications like extraction steps [16-17, 26] or heating [18] or cooling to 0 to 5 ºC [19], and free from interference by common additives and excipients. Further, the proposed method using EBBR-bromate bromide mixture as reagent can be applied at ambient temperature and color development is instantaneous. Another advantage of the developed method is more sensitive than the reported spectrofluorometric [16] and spectrophotometric methods [16-17, 23-26]. In addition, organic solvents [16-17, 20, 22, 25-27] are not used in the determination. These advantages give the proposed method a great value and make it applicable for the analysis of DPH and CPH in routine quality control pharmaceutical laboratories.

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