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SENSITIVE AND SELECTIVE METHODS FOR THE DETERMINATION OF CYPROHEPTADINE IN TABLETS USING *N*-BROMOSUCCINIMIDE AND TWO DYES

One titrimetric and two spectrophotometric methods are described for the determination of cyproheptadine hydrochloride (CPH) in bulk drug and tablets employing N-bromosuccinimide (NBS) as a brominating agent and two dyes, erioglaucine (EG) and meta-cresol purple (MCP) as auxiliary reagents. In titrimetry, a measured excess of NBS is added to an acidified solution of CPH and the unreacted NBS is determined iodometrically. Spectrophotometry involves the addition of a known excess of NBS to CPH in acid medium followed by estimation of residual NBS by reacting with a fixed amount of either erioglaucin and measuring the absorbance at 630 nm (method A) or meta-cresol purple and measuring the absorbance at 540 nm (method B). The titrimetric procedure is applicable over the range of 1.5-15 mg of CPH, and the reaction stoichiometry is found to be 1:2 (CPH: NBS). The spectrophotometric methods are applicable over the ranges of 0.1-2.0 μg mL^{-1} (method A) and 0.4-12 μg mL^{-1} (method B). The molar absorptivities are calculated to be 1.4×10^5 and 2.2×10^4 L mol^{-1} cm^{-1} for methods A and B, respectively, and the corresponding Sandell sensitivity values are 0.0023 and 0.0141 μg cm^{-2}. The limits of detection are calculated and found to be 0.03 and 0.24 μg mL^{-1} for methods A and B, respectively with corresponding limits of quantification 0.09 and 0.71. The methods were applied to the assay of CPH in tablets, and the results were compared statistically with those of a reference method.

Keywords: cyproheptadine; titrimetry; spectrophotometry; N-bromosuccinimide; pharmaceuticals.

Cyproheptadine hydrochloride (CPH, Figure 1), chemically known as 4-(5*H*-dibenzo[*a*,*d*]-cyclohepten--5-ylidene)-1-methylpiperidine hydrochloride, is a sedating antihistamine with antimuscarinic, serotonin-antagonist, and calcium-channel blocking action in pancreatic islet cells and smooth muscle [1]. It is used to treat some hormonal disorders and may also be used for treating side effects of taking antidepressants [2]. CPH is also used in clinical and veterinary medicine [3] as an antiserotonergic and antihistaminic agent with sedative and anticolinergic effects, as well as to stimulate appetite and weight gain in human and veterinary medicine [4]. Special attention has recently been paid to CPH in the literature because significant structural similarities between CPH and tryciclic antidepressants (TCAs) have induced false positive results in TACs analysis [5].

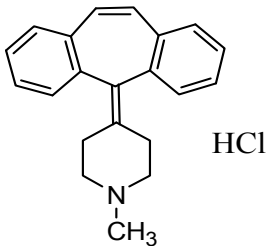

Figure 1. Structure of cyproheptadine hydrochloride.

The drug is official in the Indian Pharmacopeia [6] which describes a UV-spectrophotometric method

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for its assay in tablet in which the absorbance in 0.1 M HCl is measured at 286 nm. The United Sates Pharmacoepia [7] describes non-aqueous titration with perchloric acid as titrant where the end point was located visually using crystal violet as indicator. Literature survey revealed the availability of few methods for the assay of CPH in pharmaceutical formulations. Liquid chromatography mass spectrometry (LC-MS) [8], gas liquid chromatography [9,10] and high performance liquid chromatography (HPLC) [11-17] have been used to assay CPH. Recently, HPLC has been used for the assay of CPH in feed stuff [18]. Ion-selective based potentiometry is another technique that has found application in the analysis of CPH-containing tablets. The drug has been assayed by potentiometry using CPH-tetraphenylborate [19], CPH-dinonylnaphthalenesulphonicacid [20], CPH-tetrakis(4--chlorophenyl)borate [21] as electroactive compounds. Application of derivative UV-spectrophotometry for the assay of CPH in two- component system has also been reported [22].

Liu and Lu [23] developed a chemiluminescence method for the determination of CPH, where riboflavin was used as a chemiluminescence reagent. Suling and Limin [24] developed a method for the assay of CPH in serum, urine and in pharmaceuticals based on the measurement of enhancement of resonance light scattering at 364 nm after formation of ion-association complex with ammonium molybdate.

There is a report on the titrimetric assay of CPH [25], in which the drug is treated with known excess of bromate-bromide mixture in HCl medium followed by the determination of unreacted bromine iodometrically. In the same article, a kinetic assay of CPH is also described.

The reported visible spectrophotometric methods [26-32] currently available suffer from disadvantages such as critical dependence on pH, poor sensitivity, labor-intensive, and tedious and time-consuming liquid-liquid extraction step, use of large amount of organic solvents etc. (Table 1). The reported chromatographic techniques, although sensitive, require expensive instrumental set-up. The present investigation aims to develop simple, sensitive, and cost-effective methods for the determination of CPH in pure form and in dosage forms using titrimetric and spectrophotometric techniques. The methods utilize NBS, erioglaucine and meta-cresol purple as reagents. The developed methods offer the advantages of simplicity, speed, accuracy, and precision without the need for costly equipment/chemicals.

Table 1. Comparison of the performance characteristics of the present methods with published methods

Reagent/s used	Methodology	λ_{max} nm	Linear range, μg mL^{-1} (ε = L mol^{-1} cm^{-1})	Remarks	Ref.
Bromocresol Green	Chloroform extractable ion-pair complex measured	615	1-10	Requires close pH control and involved extraction steps	26
a)Solochrome Black T b)Solochrome Dark Blue Fast c) Sulphon Black FF	Extractable ion-pair Complex measured	520	4-18	Narrow linear range, require extraction	28
Benzyl Orange	Dichloromethane extractable ion-pair complex measured	404	10-60	Required close pH control and involved extraction steps	29
Reineckate	Ion-pair complex measured after filtering Precipitate formed	525	100-600	Less sensitive, involves precipitation, filtration and dissolution steps	27
Bromophenol Blue	Yellow chloroform extractable 1:1ion-pair complex was measured. Chloroform extractable turbid suspension was measured	420 650	2-12 (10-70)	Requires close pH control and involves extraction steps	30
Bromate-Bromide	Residual bromine treated with Methyl Orange and measured	520	0.5-4 5.25×10^4	Requires slightly higher acid concentration	25
Chloranilic acid	Charge-transfer complex was measured	520	25-125 1.48×10^3	Less sensitive, requires organic solvent	31
a)NBS-Erioglaucin	Unbleached dye colour measured	630	0.1-2.0 1.4×10^5	Highly sensitive and selective, no heating or extraction step, no pH-adjustment, employ mild acid conditions, inexpensive instrumental setup.	Present methods
b)NBS-Metacresol purple	-do-	540	0.4-12.0 (2.2×10^4)		

EXPERIMENTAL

Apparatus and reagents

A Systronics model 106 digital spectrophotometer with 1 cm matched quartz cells was used for all absorbance measurements.

All the reagents used were of analytical reagent grade and distilled water was used throughout the investigation.

N-Bromosuccinimide (NBS)

An approximately 0.01 M solution was prepared by dissolving about 1.8 g of NBS (SRL Research Chemicals, Mumbai, India) in water with the aid of heat and diluted to one litre with water. The solution was standardized iodometrically [33] and kept in an amber coloured bottle stored in a refrigerator, and used as such in titrimetry. It was diluted appropriately to get 65 and 120 μg mL^{-1} NBS for use in spectrophotometric methods A and B, respectively.

Solutions of 5 and 2 M HCl, 10% KI and 1% starch were prepared in water in the usual manner.

Erioglaucine solution, EG (300 μg mL^{-1})

The solution was prepared by dissolving 30 mg of dye (Loba Chemie, Mumbai, India) in water and diluting to the mark with water in a 100 mL calibrated flask and used in spectrophotometric method A.

Meta-cresol purple solution, MCP (80 μg mL^{-1})

A 200 μg mL^{-1} stock solution was first prepared by dissolving 20 mg of dye (Loba Chemie, Mumbai, India) in 2 mL of 0.1 M NaOH, and diluted to volume with water in a 100 mL calibrated flask. The solution (200 μg mL^{-1}) was diluted with water to get the working concentration of 80 μg mL^{-1} MCP for use in spectrophotometric method B.

Preparation of stock solution

Pharmaceutical grade cyproheptadine hydrochloride (certified to be 99.85%) was obtained from Cipla India Ltd., Bangalore, India, and used as received. A stock standard solution equivalent to 1.5 mg mL^{-1} of CPH was prepared by dissolving accurately weighed 150 mg of pure drug in water, and diluted to the mark in a 100 mL calibrated flask and used in titrimetric work. Another stock solution equivalent to 200 μg mL^{-1} of CPH was prepared by dissolving accurately weighed 20 mg of pure drug in water and diluting to the mark in a 100 mL calibrated flask. This was diluted appropriately with water to get working concentrations of 5 and 20 μg mL^{-1} of CPH for use in spectrophotometric methods A and B, respectively. The standard solutions were kept in an amber coloured bottle and stored in a refrigerator when not in use.

Pharmaceutical preparations

Two brands of tablets containing CPH, Practin-4 mg (Wockhardt Ltd., India) and Ciplactin-4mg (Cipla India Ltd., India) used in the investigation were purchased from local commercial sources.

Procedures

Titrimetry

A 10 mL aliquot of pure drug solution containing 1.5-15 mg of CPH was accurately measured and transferred into a 100 mL titration flask. The solution was acidified by adding 5 mL of 2 M HCl followed by the addition of 10 ml of 0.01 M NBS by means of pipette. The content was mixed well and the flask was kept aside for 10 min with occasional swirling. Then, 5 mL of 10% potassium iodide was added to the flask and the liberated iodine was titrated with 0.02 M sodium thiosulphate to a starch end point. A blank titration was run under the same conditions. The drug content in the aliquot was calculated as:

$$\text{Amount}(\text{mg}) = \frac{V \times Mol.wt \times R}{n}$$

where V = volume of NBS solution reacted with the drug, mL; $Mol.wt$ = relative molecular mass of drug; R = strength of NBS, mol mL^{-1} and n = number of moles of NBS reacting with each mole of drug.

Spectrophotometry using erioglaucine (method A)

Different aliquots (0.2, 0.5, 1.0,…, 4.0 mL) of a standard 5 μg mL^{-1} CPH solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 4 mL by adding adequate quantity of water. To each flask were added 1 mL each of 2 M HCl and 1.0 mL of 65 μg mL^{-1} NBS solution (accurately measured), the last being measured accurately. The flasks were stoppered, content mixed and left to stand for 10 min with occasional shaking. Finally, 1.0 mL of 300 μg mL^{-1} EG solution (accurately measured) was added and the volume was made up to 10 mL and mixed. The absorbance of each solution was measured at 630 nm against a reagent blank after 5 min.

Spectrophotometry using meta-cresol purple (method B)

Varying aliquots (0.2, 0.5, 1.0,…, 6.0 mL) of a standard 20 μg mL^{-1} CPH solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 6 mL by adding water. To each flask were added 1 mL of 2 M hydrochloric acid and 1.0 mL of NBS solution (120 μg mL^{-1}) by means of a micro burette. The content was mixed well and the flasks were kept aside for

10 min with intermittent shaking. Finally, 1.0 mL of 80 μg mL^{-1} MCP solution was added to each flask, the volume was made up to 10 mL, mixed well and absorbance measured against a reagent blank at 540 nm after 5 min.

In either spectrophotometric method, a standard graph was prepared by plotting the absorbance *versus* the concentration of CPH. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beer's law data.

Assay procedure for tablets

Forty tablets (practin and ciplactin), each containing 4 mg of CPH, were weighed accurately and ground into a fine powder. An amount of the powder equivalent to 150 mg of CPH was accurately weighed into a 100 mL volumetric flask, 60 mL water was added and content shaken thoroughly for about 20 min. The volume was diluted to the mark with water, mixed well and filtered using Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was rejected and a convenient aliquot of filtrate (containing 1.5 mg mL^{-1} CPH) was taken for assay by titrimetric procedure. The tablet extract was diluted stepwise to get 5 and 20 μg mL^{-1} CPH concentrations for use in spectrophotometric methods A and B, respectively. A suitable aliquot was then subjected to analysis following the procedures described earlier.

Placebo blank analysis

A placebo blank of the composition: talc (35 mg), starch (25 mg), acacia (25 mg), methyl cellulose (30 mg), sodium citrate (25mg), magnesium stearate (25 mg) and sodium alginate (20 mg) was made and its solution was prepared as described under the procedure for tablets, and then analysed using the procedures described above.

Synthetic mixture analysis

A solution of 1.5 mg mL^{-1} CPH was prepared and used for titrimetric assay and diluted stepwise diluted stepwise with water to obtain working concentrations of 20 μg mL^{-1} and 5 CPH for spectrophotometric methods B and A, respectively.

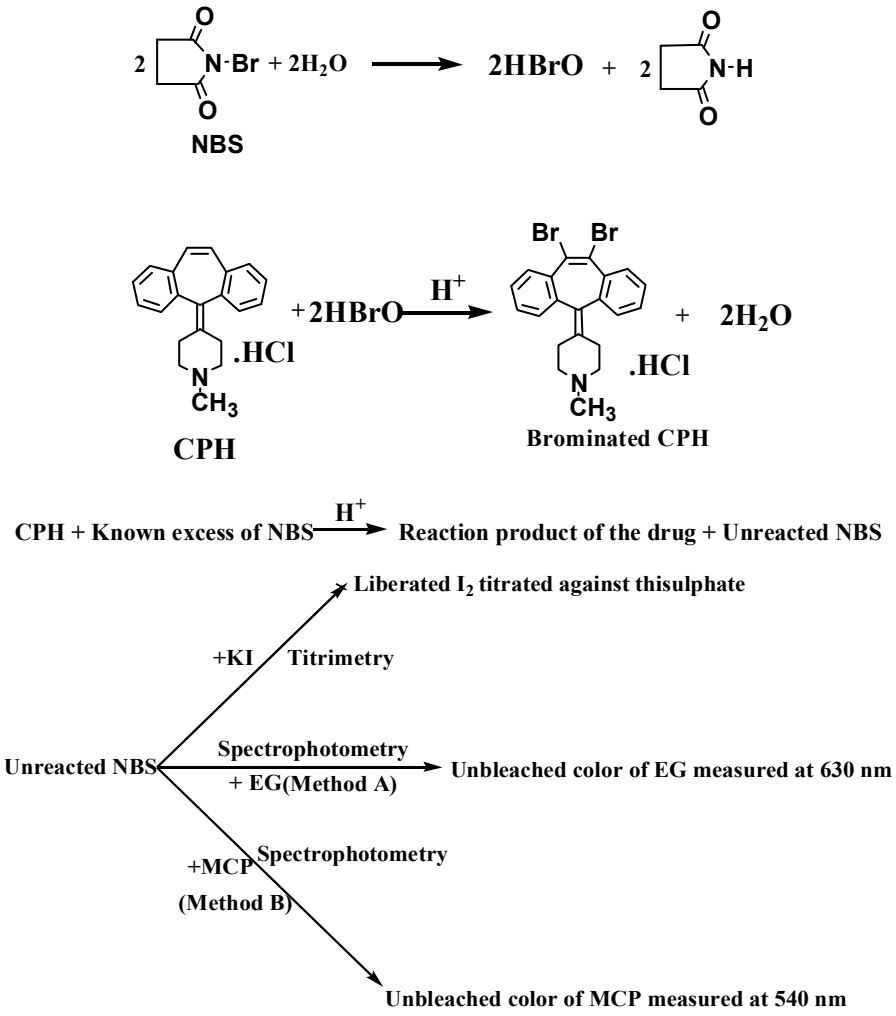
RESULTS AND DISCUSSION

The analytical applications of NBS are broadly based on bromination and oxidation reactions. These reactions have found extensive applications in the determination of a variety of organic compounds including those of pharmaceutical interest [34-37]. Ziegler *et al*. [38] made detailed studies of NBS applications for allylic bromination. In the reactions where NBS was used as brominating agent, the monovalent positive bromine of the NBS (the bond between bromine and nitrogen is polarized by the two neighboring carbonyl groups) was responsible for bromination. A close examination of the literature search presented in the introduction revealed that NBS has not yet been used for the determination of CPH.

The present work involves the bromination of CPH by NBS followed by determination of surplus NBS after allowing the bromination reaction to complete. In titrimetry, the unreacted NBS is determined iodometrically. Under the optimized reaction condition, there was found to be a definite reaction stoichiometry of 1:2 between CPH and NBS within the range of 1.5-15 mg CPH and all the calculations are based on this fact. The piperidine ring in CPH, because of its steric hindrance bromination, is vulnerable and hence, bromination preferably takes place in the cycloheptene ring (Scheme 1). In spectrophotometric methods, it is determined by reacting with a fixed amount of either EG and measured at 630 nm (Figure 2a) or MCP and measured at 540 nm (Figure 2b). The spectrophotometric methods make use of the bleaching action of NBS on either of the two dyes, where the discolouration is caused by oxidative destruction of the dye. The tentative reaction scheme of titrimetric and spectrophotometric methods is shown in Scheme 1.

Titrimetry

Direct titration of CPH with NBS in acid medium was not successful. However, the back titrimetric assay was found possible when the reactants were allowed to stand for some time in acid medium. In this procedure, a known excess of NBS was allowed to react with CPH in acid medium and the unreacted NBS was subsequently determined iodometrically. The reaction stoichiometry was found to be 1:2 (CPH:NBS). Reproducible and stoichiometric results were obtained when HCl medium (0.2 to 0.6 M) was employed. At optimum acid concentration of 0.4 M (5 mL of 2 M HCl in a total volume of 25 mL), the reaction goes to completion in 10 min and contact time up to 45 min had no effect on the stoichiometry or the results. At lower acid concentration (less than 5 mL of 2 M HCl) the reaction stoichiometry was slightly less than 2 and higher acid concentrations exceeding 0.6 M HCl overall resulted in slightly higher *n* values. Under the optimized reaction condition, there was found to be a definite reaction stoichiometry of 1:2 between CPH and NBS was found within the range of 1.5-15 mg CPH. A 10 mL volume of 0.01 M NBS was found adequate for quantitative reaction CPH in the range investigated and contact time of 5 min was sufficient for the complete liberation of iodine from the unreacted NBS.



Scheme 1. Tentative reaction scheme for titrimetry and spectrophotometric methods A and B.

Spectrophotometry

Many dyes are irreversibly destroyed to colourless species by oxidizing agents in acid medium [39] and this observation has been exploited for the indirect spectrophotometric determination of some bioactive compounds [40-43]. In the proposed spectrophotometric methods, the ability of NBS to effect bromination of CPH and irreversibly destroy EG and MCP to colourless products in acid medium has been capitalized. Both methods are based on the bromination of the drug by measured excess of NBS and subsequent determination of the latter by reacting with EG or MCP, and measuring the absorbance at 540 or 630 nm. In either method, the absorbance increased linearly with increasing concentration of drug. CPH when added in increasing concentrations to a fixed concentration of NBS consumes the latter and there will be a concomitant decrease in the concentration of NBS. When a fixed concentration of either dye is added to decreasing concentration of NBS, a concomitant increase in the concentration of dye is obtained. This is observed as a proportional increase in the absorbance at the respective wavelengths of maximum absorption with increasing concentration of CPH (Figures 2a and 2b).

Method development: optimization of experimental variables

Absorption spectra

The proposed methods are based on the determination of unreacted bromine after the reaction between the drug and NBS is judged to be complete. The greenish colour of the unreacted EG in acid medium absorbed maximally at 630 nm (method A) and the reddish-pink colour of unreacted MCP in acid medium peaked at 540 nm (method B). The absorption spectra of both the methods are presented in Figure 2.

Effect of reagent concentration

Preliminary experiments were performed to fix the upper limits of the EG and MCP that could pro-

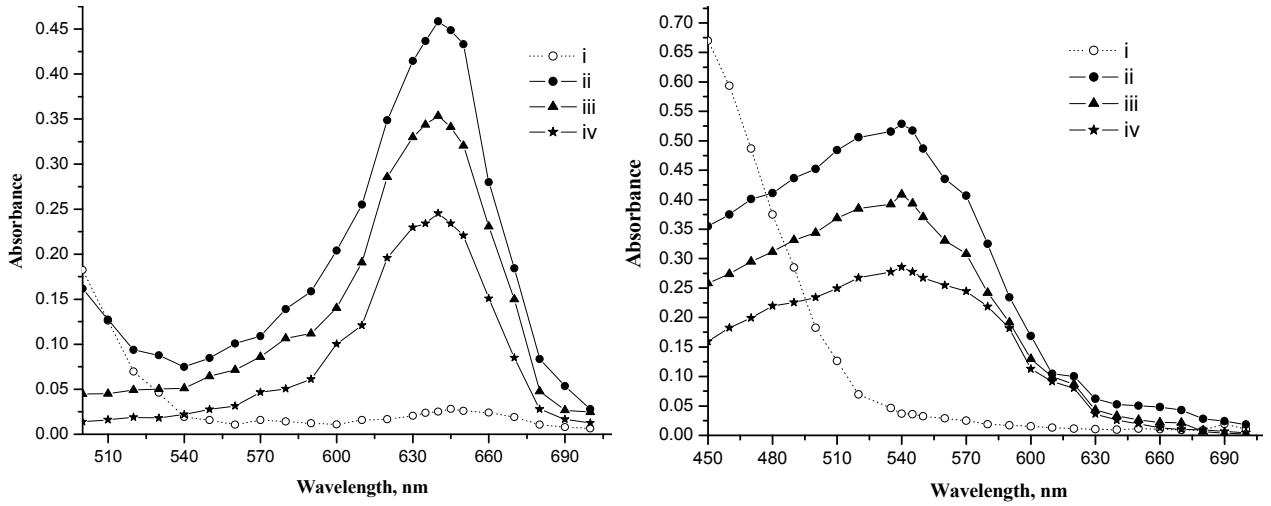


Figure 2. Absorption spectra: a) method A, I - without CPH; ii - 1 μg mL^{-1} CPH; iii - 0.75 μg mL^{-1} CPH; iv - 0.5 μg mL^{-1} CPH; b) method B. I - without CPH; ii - 8 μg mL^{-1} CPH; iii - 6 μg mL^{-1} CPH; iv - 4 μg mL^{-1} CPH.

duce a reasonably high absorbance, and these were found to be 300 μg mL^{-1} EG in method A and 80 μg mL^{-1} MCP in method B. To fix the optimum concentration of NBS, different concentrations of NBS were reacted with a fixed concentration of EG or MCP (300 and 80 μg mL^{-1}) in HCl medium and the absorbance was measured at 630 and 540 nm, respectively. A constant and minimum absorbance resulted with 65 and 120 μg mL^{-1} NBS for methods A and B, respectively. Different concentrations of CPH were reacted with 1mL NBS of 65 μg mL^{-1} in method A and 120 μg mL^{-1} in method B in HCl medium before determining the residual NBS *via* the reaction scheme illustrated earlier. This facilitated the optimization of the linear dynamic range over which each procedure could be applied for the assay of CPH.

Effect of reaction medium

The reaction between CPH and NBS was performed in different acid media, *viz.* sulphuric acid, hydrochloric acid and perchloric acid. Hydrochloric acid was found to be the ideal medium for the bromination of CPH by NBS as well as the latter's determination employing either dye. The effect of acid concentration on the reaction between CPH and NBS was studied by varying the concentration of HCl keeping the concentrations of NBS and drug fixed. Higher the acid concentrations showed lower sensitivity, hence 1 mL of 2 M HCl in a total volume of 10 mL was found optimal to achieve maximum absorbance of sample and minimum absorbance of blank in both methods.

Study of reaction time and stability

Under the described experimental conditions, for a quantitative reaction between CPH and NBS, contact time of 10 min was found necessary in both methods at room temperature. After addition of dye, the reaction between NBS and dye was instantaneous and absorbance of the unreacted dye was stable at least 45 min in method A and 60 min in method B.

Validation of the proposed methods

The proposed methods have been validated for linearity, sensitivity, selectivity, precision, accuracy, and recovery.

Linearity

A linear relation is found between absorbance and concentration of CPH within the Beer's law range given in Table 2. The calibration graphs are described by the equation obtained by the method of least squares:

$$Y = a + bX$$

where Y = absorbance, a = intercept, b = slope and X = concentration in μg mL^{-1}. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values and the limits of detection and quantification are calculated as per the current ICH guidelines [44] which are compiled in Table 2 that speaks of the excellent sensitivity of the proposed method. Limits of detection (*LOD*) and quantification (*LOQ*) were calculated from the following equations:

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

Table 2. Analytical and regression parameters of spectrophotometric methods

Parameter	Method A	Method B
λ_{max} / nm	630	540
Beer's law limits, μg mL^{-1}	0.1 - 2.0	0.4 - 12
Molar absorptivity, L mol^{-1} cm^{-1}	1.4×10^5	2.2×10^4
Sandell sensitivity[a], μg cm^{-2}	0.0023	0.0141
Limit of detection, μg mL^{-1}	0.03	0.24
Limit of quantification, μg mL^{-1}	0.09	0.71
Regression equation, Y[b]		
Intercept, a	0.0051	0.0095
Slope, b	0.4389	0.0658
Correlation coefficient, r	0.9992	0.9997
Standard deviation of intercept, S_a	0.4765	0.0605
Standard deviation of slope, S_b	0.3552	0.0166

[a]Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of A = 0.001measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. [b] $Y = a + bX$, where Y is the absorbance and X concentration in μg mL^{-1}

where σ is the standard deviation of n reagent blank determinations and S is the slope of the calibration curve.

Accuracy and precision

In order to study the precision and accuracy of the proposed methods, three amounts/concentrations of pure CPH within the linearity range were analyzed, each determination being repeated seven times (intra-day precision) on the same day and one time each for five days (inter-day precision). The percentage relative standard deviation (*%RSD*) was ≤ 2.71% (intra-day) and ≤3.14% (inter-day). In addition, the accuracy of the proposed method was measured by calculating the percentage relative error (*%RE*), which varied between 0.46 and 3.04%. The results of this study compiled in Table 3 indicate the high accuracy and precision of the proposed methods.

Robustness and ruggedness

To evaluate the robustness of the methods, two important experimental variables, *viz*. the amount of acid and reaction time were slightly varied, and the capacity of the methods was found to remain unaffected by small deliberate variations. The results of this study are presented in Table 4 and indicate that the proposed methods are robust. Method ruggedness is expressed as *%RSD* of the same procedure applied by three analysts and using three different spectrophotometers by the same analyst. The inter-analysts' and inter-instruments' *RSD* values were ≤3.4% indicating ruggedness of the proposed methods. The results of this study are presented in Table 4.

Selectivity

In the present methods, interference by the excipients often used in pharmaceutical formulations or as possible co-active substances was studied. Selectivity was evaluated by both placebo blank and synthetic mixture analyses. The placebo blank, consisting the composition as mentioned under "Analysis of Placebo blank" was prepared and analyzed as described under the recommended procedures. The resulting

Table 3. Intra-day and inter-day precision and accuracy studies; mg in titrimetry and μg mL^{-1} in spectrophotometry (%RE - relative error and %RSD - relative standard deviation)

Method	CPH taken mg μg^{-1} mL^{-1}	Intra-day accuracy and precision (n = 5)			Inter-day accuracy and precision (n = 5)		
		CPH found, μg mL^{-1}	*%RE*	*%RSD*	CPH found, μg mL^{-1}	*%RE*	*%RSD*
Titrimetric method	3.0	2.92	2.2	2.0	2.91	2.8	3.1
	6.0	6.10	2.3	1.4	5.89	1.8	2.6
	9.0	9.15	2.5	2.5	8.77	2.5	1.9
Spectrophotometric method A	0.5	0.49	1.8	2.7	0.48	2.9	3.1
	1.0	1.02	2.2	2.2	0.98	1.2	2.4
	1.5	1.47	1.8	1.6	1.46	2.2	2.9
Spectrophotometric method B	4.0	3.92	2.0	1.8	3.87	3.0	2.0
	6.0	6.08	1.4	2.0	5.83	2.7	2.7
	8.0	8.13	1.7	1.6	7.78	2.7	3.0

Table 4. Results of method robustness and ruggedness (all values in % RSD) studies

Method	Nominal amount concentration[a]	Reaction times (*n* = 3)	Different analysts (*n* =3)	Different instruments[b] (*n* = 3)
Titrimetric	3.0	1.58	0.76	0.62
	6.0	1.26	0.85	0.72
	9.0	1.34	1.04	0.58
Spectrophotometric method A	0.5	2.65	1.04	2.42
	1.0	3.14	1.28	2.16
	1.5	3.38	0.92	1.85
Spectrophotometric method B	4.0	1.85	0.73	2.16
	6.0	2.16	1.12	2.72
	8.0	2.08	1.08	3.04

[a]mg in titrimetry and μg mL^{-1} in spectrophotometry; [b]burettes in titrimetry and spectrophotometers in methods A and B

absorbance readings for the methods were same as the reagent blank, inferring no interference from the placebo. The selectivity of the methods was further confirmed by carrying out recovery study from synthetic mixture. The percent recoveries of CPH were 102.1±1.35 for titrimetry and 98.7±1.18 and 101.4±1.63 for methods A and B, respectively. This confirms the selectivity of the proposed methods in the presence of the commonly employed tablet excipients.

Application to analysis of tablets

The proposed methods were successfully applied to the determination of CPH in two different brands of tablets, namely, practin and ciplactin. The results presented in Table 5 showed that there was a close agreement between the results obtained by the proposed methods and the label claim. The results were also compared with those of the reference method [7] statistically by a Student's *t*- test for accuracy and variance ratio *F*-test for precision at 95% confidence level. The reference method describes non-aqueous titration with perchloric acid as titrant where the end point was located visually using crystal violet as indicator. The calculated *t*- and *F*-values indicate that there is no significant difference between the proposed methods and the reference method with respect to accuracy and precision.

Recovery studies

To further ascertain the accuracy of the proposed methods, a standard addition technique was followed. A fixed amount of drug from pre-analyzed tablet powder was taken and pure drug at three different levels (50, 100 and 150% of that in tablet powder) was added. The total was found by the proposed methods. The determination at each level was repeated three times and the percent recovery of the added standard was calculated. Results of this study presented in Table 6 reveal that the accuracy of methods was unaffected by the various excipients present in the formulations.

CONCLUSIONS

The methods for the determination of CPH described in this paper are simple, selective, accurate and precise. The chromatographic techniques [8-17], although sensitive, require judicious control of pH of the mobile phase besides requiring expensive and sophisticated instruments. A large volume of solvents is required for these techniques, which are expensive, hazardous to health, and harmful to the environment. The reported visible spectrophotometric [26-31] methods require liquid-liquid extraction step, strict pH

Table 5. Results of assay of tablets by the proposed methods and statistical evaluation

Tablet brand name	Label claim[b]	Found[a] (Percent of label claim ± *SD*)			
		Reference method	Proposed methods		
			Titrimetric	Spectrophotometric method A	Spectrophotometric method B
Ciplactin[c]	4	99.36±1.65	97.98±0.86	98.78±1.42	98.62±1.38
			t = 2.61	*t* = 2.80	*t* = 2.90
			F = 3.68	*F* = 1.35	*F* = 1.42
Practin[d]	4	100.4±1.86	100.8±1.27	100.65 ±1.58	99.16±1.22
			t = 2.09	*t* = 2.79	*t* = 2.94
			F = 2.14	*F* = 1.38	*F* = 2.32

[a]mg/tablet in tablets; [b]mean value of five determinations; [c]Cipla India Ltd., India; [d]Wockhardt Ltd., India. The value of *t* and *F* (tabulated) at 95 % confidence level and for four degrees of freedom are 2.77 and 6.39, respectively

Table 6. Results of recovery experiments by standard addition method

Method	Tablet studied	CPH in tablet mg/μg mL^{-1}	Pure CPH added mg/μg mL^{-1}	Total found μg mL^{-1}	Pure CPH recovered[a] Percent ± *SD*
Titrimetry	Ciplactin	4.43	3.0	7.53	103.33±1.45
		4.43	4.5	9.04	102.42±1.02
		4.43	6.0	10.58	102.5±0.98
		4.52	3.0	7.55	101.0±0.74
	Practin	4.52	4.5	9.12	102.22±1.24
		4.52	6.0	10.61	101.5±1.16
		0.48	0.25	0.73	97.85 ± 1.19
Spectrophotometric method A	Ciplactin	0.48	0.50	1.09	101.50 ± 0.79
		0.48	0.75	1.27	102.50 ± 1.76
		0.53	0.25	0.74	98.56 ± 1.19
	Practin	0.53	0.50	1.02	102.00 ± 0.79
		0.53	0.75	1.28	103.02 ± 1.27
		3.96	2.0	5.97	98.22 ± 1.32
Spectrophotometric method B	Ciplactin	3.96	4.0	7.96	99.10 ± 1.02
		3.96	6.0	10.16	102.78 ± 1.26
		3.98	2.0	5.96	98.48 ± 1.62
	Practin	3.98	4.0	8.07	101.79 ± 1.02
		3.98	6.0	10.19	103.14 ± 1.82

[a]Mean value of three determinations. mg in titrimetry and μg mL^{-1} in spectrophotometry

control, poor sensitivity, and large amounts of high purity solvents, which are often hazardous and resulting the production of toxic lab waste. In contrast to the published methods, the present methods are simple, and use eco-friendly chemicals, and free from such unwelcome steps as heating or extraction and also from critical pH conditions. The proposed methods are more sensitive compared to other reported visible spectrophotometric methods as indicated by the molar absorptivity values of 1.4×10^5 and 2.2×10^4 L mol^{-1} cm^{-1}. The relative cheapness of the apparatus and reagents demonstrate their advantageous characteristics. The methods are also useful due to high tolerance limit for common excipients found in drug formulations. These merits, coupled with the use of a simple and inexpensive instrument, make the proposed methods acceptable in quality control laboratories for routine use.

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REFERENCES

[1] S.C. Sweetman, Martindale - The Complete Drug Reference, 33rd ed., Pharmaceutical Press, London, 2002, p. 414

[2] Maria Gorczyca, Alfred Zejc, Chemia Lekow, Wydawnictwo Lekarskie PZWL, Warszawa, 1998 (in Polish)

[3] X. Feás, L. Ye, P. Regal, C. A. Fente, A. Cepeda, J. Sep. Sci. **32** (2009) 1740-1747

[4] X. Feás, J.A. Seijas, M.P. Vázquez-Tato, P. Regal, A. Cepeda, Anal. Chim. Acta **631** (2009) 237-244

[5] X. Feás, C.A. Fente, S.V. Hosseini, J.A. Seijas, B.I. Vázquez, C. M. Franco, A. Cepeda, Mater. Sci. Eng., C **29** (2009) 398-404

[6] The Indian Pharmacopoeia, Vol. I (The controller of Publications, Govt. of India, Ministry of Health and Family Welfare, New Delhi), 1996, p. 218

[7] The United States Pharmacopoeia, XXIV Revision, the National Formulary XIX Rockville, USP Convention, 2000

[8] X. Feás, Y. Lei, S.V. Hosseini, C.A. Fente, J. Pharm. Biomed. Anal. **50** (2009) 1044-1049

[9] C. Yang, Q. Men, Yaowu Fenxi Zazhi **11** (1991) 113-118 (in Chinese)

[10] R.T. Sane, P.P. Karkhanis, P.G. Anaokar, Ind. J. Pharm. Sci. **43** (1981) 111-112

[11] A.D. Mao, B.F. Wang, Yaowu Fenxi Zazhi **21** (2001) 60--61 (in Chinese)

[12] P.A. Williams, Pharmacopeial Forum **14** (1988) 3463--3472

[13] G.R. Rao, S. Raghuveer, Indian Drugs **22** (1985) 377-380

[14] G.W. Burrows, C.L. Alliger, J. Pharm. Sci. **72** (1983) 1212-1213

[15] K. Basavaiah, V.S. Charan, U. chandrashekar, P. Nagegowda, B.C. Somashekar, Bulg. Chem. Comm. **36** (2004) 112-116

[16] Z. Lingya, China Pharmacis 09, 2009

[17] A. El-Gindy, F. El-Yazby, A. Mostafa, M.M. Maher, J. Pharm. Biomed. Anal. **35** (2004) 703-713

[18] C. Ying, J. Yin, Z. Wengang, China Feed **2**, 2011

[19] Y.M. Issa, M.S. Rizk, S.S. Mohammed, Anal. Lett. **25** (1992) 1617-1619

[20] A.A. Bunaciu, M.S. Ioneseu, I. Enacheseu, G.E. Baiuleseu, V. V. Cosofret, Analusis **16** (1988) 131-134

[21] J. Drozd, H. Hopkala, Desalination **163** (2004) 119-125

[22] X. Zhou, Zhongguo Yaoke Daxue Xuebao **20** (1989) 307--308 (in Chinese)

[23] R.X. Liu, J.R. Lu, Fenxi Huaxue **37** (2009) 267-270 (in Chinese)

[24] S. Feng, L. Guo, Chemical Papers **62** (2008) 350-357

[25] K. Basavaiah, Ind. J. Chem. Tech. **13** (2006) 360-366

[26] S. Adamski, Acta Pol. Pharm. **22** (1965) 311-314

[27] J. Emmanuel, T.V. Yegnanarayan, Indian Drugs **19** (1982) 505-507

[28] R.T. Sane, U.M. Vaidya, V.G. Nayak, A.Y. Dhamankar, S.K. Joshi, V.J. Doshi, S.V. Sawant, V.B. Malkar, U.R. Pandit, AT. Sathe, S. Jukar, A.D. Nadakarni, Indian Drugs **19** (1982) 398-403

[29] C.N. Carducci, G. Barcic, A. Mascaro, Application to drugs SAFYBI **19** (1979) 1358-1361

[30] K. Basavaiah, V.S. Charan, Science Asia **30** (2004) 163--170

[31] K. Basavaiah, V.S. Charan, Turk. J. Chem. **26** (2002) 653-661

[32] D.M. Shingbal, S.D. Naik, Indian Drugs **18** (1981) 444--446

[33] A. Berka, J. Vulterin, J. Zyka Newer Redox Titrants, 1st ed., Pergamon Press, London, 1965, p. 38

[34] N.K. Mathur, C.K. Narang The determination of organic compounds with *N*-bromosuccinimide and allied reagents, Academic Press, New York, 1975, p.13

[35] K.B. Vinay, H.D. Revanasiddappa, O.Z. Devi, P.J. Ramesh, K. Basavaiah, Braz. J . Pharm. Sci. **47** (2011) 1-10

[36] O. Zenita, K. Basavaiah, Int. J. Anal. Chem. **2011** (2011), Article ID 581372, doi:10.1155/2011/581372

[37] N. Rajendra Prasad, K Basavaiah, K.B. Vinay, **2011** (2011), Article ID 138628, doi:10.1155/2011/138628

[38] K. Ziegler, A. Spath, E. Schaaf, W. Schumann, E. Winkelmann Ann. Chem. **551**(1942) 80-119

[39] I.M. Kolthoff, R. Belcher, V.A. Stenger, G. Matsuyama Volumetric Analysis, III, 1st ed., Interscience Publishers, Inc., New York, 1957, p. 504

[40] C.S.N. Sharma, C. Kamala Sastry, C.S.P. Sastry Acta Cienc. Indica **28** (2002) 221-225

[41] C.S.P. Sastry, V.A.N. Sarma, U.V. Prasad, C.S.R. Lakshmi, Ind. J. Pharm. Sci. **59** (1997) 161-164

[42] K. Basavaiah, H.C. Prameela Anal. Sci. (Japan) **19** (2003) 779-784

[43] K. Basavaiah, U.R.A. Kumar, K. Tharpa, Chem. Ind. Chem. Eng. Q. **14** (2008) 185−190

[44] International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R 1), Complementary Guideline on Methodology dated 06 November 1996, London.

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NAUČNI RAD

OSETLJIVE I SELEKTIVNE METODE ZA ODREĐIVANJE KUPROHEPTADINA U TABLETAMA POMOĆU *N*-BROMOSUKCINIMIDA I DVE BOJE

U radu su razvijene jedna titrimetrijska i dve spektrofotometrijske metode za određivanje kuproheptadin hidrohlorida (CPH) u rasutom stanju i u tabletama primenom N-bromosukcinimida (NBS) kao reagensa za bromovanje i dve boje: erioglaucina (EG) i meta-krezol purpurnog (MCP). Kod titrimetrije se tačno poznata koncentracija NBS dodaje u zakišeljeni rastvor CPH, a onda se višak neizreagovanog NBS određuje jodometrijski. Spektrofotometrijsko određivanje se zasniva na dodatku poznate količine NBS u CPH u kiseloj sredini, zatim NBS reaguje sa određenom količinom erioglaucina (metoda A-meri se apsorbanca na 540 nm) ili meta-krezola purpurnog (metoda B: meri se apsorbanca na 630 nm). Nađeno je da je odnos CPH:NBS u reakcionoj spektrofotometriji 1:2. Titrimetrijska procedura je primenljiva za određivanje CPH u opsegu koncentracija 1,5-15 mg, dok se spektrofotometrija može primenjivati za određivanje CPH u opsegu koncentracija 0,1-2,0 μg mL^{-1} po metodi A i 0,4-12 μg mL^{-1} po metodi B. Limiti detekcije za spektrofotometrijske metode iznose 0,0023 μg cm^{-2} za metodu A i 0,0141 μg cm^{-2} za metodu B. Limiti kvantifikacije za spektrofotometrijske metode iznose 0,09 μg cm^{-2} za metodu A i 0,71 μg cm^{-2} za metodu B. Metode su primenjene za određivanje CPH u tabletama, a rezultati su statistički poređeni sa referentnom metodom.

Ključne reči: kuproheptadin; titrimetrija; spektrofotometrija; N-bromosukcinimid, lekovi.