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Ion-pair Complexometric Determination of Cyproheptadine Hydrochloride Using Bromophenol Blue

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ABSTRACT: Two simple, rapid and inexpensive methods based on spectrophotometry and turbidimetry have been developed for the determination of cyproheptadine hydrochloride (CPH) in pure form and in dosage forms with Bromophenol Blue (BPB) as the reagent. The spectrophotometric method is based on the formation of an ion-pair complex between the drug and BPB in an acidic buffer. The ion-pair complex formed, which has an absorption maximum at 420 nm, is quantitatively extracted into chloroform. The turbidimetric procedure is also based on the formation of the ion-pair complex which will be present in the form of a turbid suspension at controlled pH and ionic strength conditions. The absorbance of the turbid suspension is measured at 650 nm and is correlated to drug concentration. All experimental variables were studied and optimised for both the methods. The calibration graph is linear in the range of 2-12 μg ml^{-1} for spectrophotometry, and 10-70 μg ml^{-1} for turbidimetry. Statistical treatment of the experimental results indicated that the methods are precise and accurate. Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedures. The procedures were successfully applied to the determination of the bulk drug and its pharmaceutical formulations.

KEYWORDS: Cyproheptadine hydrochloride, determination, spectrophotometry, turbidimetry, ion-pair complex, Bromophenol Blue, pharmaceuticals.

INTRODUCTION

Cyproheptadine hydrochloride (CPH) is a serotanin and a histamine antagonist with anticholinergic and sedative effects. It also blocks voltage sensitive calcium channels in pancreatic islet cells and smooth muscle. CPH is also used as an appetite stimulant to assist weight gain. Chromatographic techniques such as gas chromatography (GC)[1,2] and high performance liquid chromatography (HPLC)[3-6] have been applied for the determination of CPH in biological fluids[3] including serum,[4] plasma,[1,4,5] and urine,[1,5,6] and also in tissue.[2] Kashyap *et al*.[7] have described a derivative UV-spectroscopic method for the determination of CPH in blood and tissue extracts with recoveries ranging from 70 to 82 and 73 to 113%, respectively. Separation of the drug by TLC on Silica gel G containing 1% zinc acetate with benzene - DMF - acetic acid (30:10:7) as the mobile phase and detection by spraying with Drangendorff reagent has been reported by Bhushan et al[8]. There is also a report on thermal[9] studies of the drug for its identification.

A limited number of methods have been reported for the quantification of the drug in pharmaceutical dosage forms. Two GC[10,11] and three HPLC[12-14] methods for the assay of CPH in dosage forms are found in the literature. Xhou[15] reported a UV- spectrophotometric assay of CPH in compound tablets containing pemoline. The tablets were dissolved in dilute HCl and the absorbance measured at 286 nm with a recovery of 100.8%. Adanski[16] was, perhaps, the first to report a colorimetric method for the assay of CPH in tablets. The method involved the extraction of the drug with chloroform, extraction of the chloroform extract with bromocresol green in phosphate buffer, pH 5.4, reextraction of the aqueous layer with chloroform, and finally adding 0.1 N sodium hydroxide followed by absorbance measurement at 615 nm. In another extractive colorimetric method,[17] the drug was precipitated with reineckate and the ion-pair complex was filtered, dissolved in acetone and absorbance measured at 525 nm. Sane *et al*.[18] reported a similar ion-pair extraction photometric method using three dyes Solochrome Black T, Solochrome Dark Blue and Fast Sulphon Black FF, the absorbance of the complex being measured at 520 nm. The drug has also been determined spectrophotometrically based on ion-pair complex formation with Benzyl Orange[19] at pH 4.7-4.9, followed

by extraction into dichloromethane and measurement at 404 nm. Shingbal and Naik[20] have described a spectrophotometric method based on the complexation reaction. Based on ion-pair formation reactions, two cyproheptadinium-responsive electrodes were prepared with tetraphenylborate[21] and dinonylnaphthalene sulphonic acid[22] as ion-pair reagents and applied for the determination of CPH in pharmaceuticals with recoveries >98%.

Ion-pair extraction spectrophotometry[23] has received considerable attention for quantitative estimation of pharmaceutical compounds. BPB being an anionic dye has been reported to form ion-pair complexes thus offering simple and rapid spectrophotometric determination of a number of organic pharmaceuticals.[24-34] Although a few visible spectrophotometric methods[16-20] for CPH are found in the literature no method using BPB has been reported.

This paper describes two newly developed procedures for the determination of CPH. The first method is based on the complexation of the drug with BPB at pH 3.0 to form an ion-pair, which is extracted into chloroform and the absorbance measured at 420 nm. The other method is based on the fact that CPH reacts with BPB under slightly altered conditions of pH, ionic strength and reagent concentration to form an insoluble ion-pair complex. The resulting suspension is measured at 650 nm. The methods are more simple and sensitive than many reported earlier (Table 1).

Materials and Methods

Apparatus

Absorbance measurements were carried out using a Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, India) with 1-cm quartz cells.

Materials and Reagents

Pharmaceutical grade CPH (Cipla India, Ltd., Mumbai, India) was used as working standard. All chemicals used were of analytical reagent grade and double distilled water was used throughout the investigation. A stock standard solution containing 1000 μg ml^{-1} of CPH was prepared in water. Working standards of 20 μg ml^{-1} (for spectrophotometry) and 200 μg ml^{-1} (for turbidimetry) were prepared by appropriate dilution of the stock solution. Phthalate buffer, pH 3.0, was prepared by dissolving 2.04 g of potassium hydrogen phthalate in 100 ml of water and the pH was adjusted by using 0.1 M hydrochloric acid. A 0.5% solution of Bromophenol Blue was prepared by dissolving 0.5 g of the reagent (RANBAXY Fine Chemicals Ltd., New Delhi, India) in 100 ml of water, and filtering it to remove the insoluble residue. This was used for turbidimetry. For use in spectrophotometry, the 0.5% solution was diluted to 0.1%. Potassium chloride reagent was prepared by dissolving 7.4 g of the salt (S. d. Fine Chem. Ltd., Mumbai, India) in water and diluting to 100 ml. Hydrochloric acid (0.1 M) was prepared by diluting 8.5 ml of concentrated acid to 1 litre. Spectroscopic grade chloroform was used for extraction.

Procedure for Pharmaceutical Preparations

Ciplactin and Practin tablets each containing 4 mg of CPH were used in the investigation. Forty tablets were weighed and powdered. An accurately weighed quantity of finely ground powder equivalent to 100 mg of CPH was transferred into a 100 ml volumetric flask and extracted with 60 ml of water by vigorous shaking for 20 min. Then, the volume was diluted to the mark, mixed well and filtered using Whatman No.41 filter paper. The first 10 ml portion of the filtrate was discarded

Table 1. Comparision of the reported methods with the proposed methods.

Method	Linear range of response	Remarks	Ref
GC	100-500 μg ml^{-1}	Less sensitive	10
GC	30-585 μg ml^{-1}	Less sensitive	11
HPLC	15-672 μg ml^{-1}	Less sensitive	12
HPLC	27-80 ng ml^{-1}	Narrow linear range of response	13
Vis-spectrophotometry	100-600 μg ml^{-1}	Procedure is tedious; involves precipitation, filtration and dissolution steps	17
Vis-spectrophotometry	4-18 μg ml^{-1}		18
Vis-spectrophotometry	10-60 μg ml^{-1}	Critical pH dependence	19
Vis-spectrophotometry		Involves multiple extraction steps lasting more than 2 h	16
Potentiometry	0.026 - 3.2 mg ml^{-1}	Least sensitive	21
Potentiometry	0.032 - 3.2 mg ml^{-1}	Least sensitive	22
Vis-spectrophotometry	2-12 μg ml^{-1}	More sensitive	**Proposed method**
Turbidimetry	10-60 μg ml^{-1}	No extraction involved Uses a simple instrument	**Proposed method**

and the filtrate was diluted to 20 and 200 μg ml^{-1} for spectrophotometric and turbidimetric work, respectively.

Analytical Procedures

Spectrophotometry

Aliquots of the standard solution containing 2 to 12 μg ml^{-1} CPH were transferred into a 125 ml separating funnel. Then, 5.0 ml of phthalate buffer of pH 3.0 and 2.0 ml of 0.1% BPB dye solution were added to the separating funnel. The total volume was adjusted to 15 ml by the addition of water and the contents were mixed well. Then, 5.0 ml of chloroform were added and the contents were shaken vigorously for 1 min. The two layers were allowed to separate, the chloroform layer was passed through anhydrous sodium sulphate and the absorbance was measured at 420 nm against a reagent blank.

Turbidimetry

In each of a series of 10 ml calibrated flasks were placed 0.50 to 3.50 ml of 200 μg ml^{-1} CPH solution. To each flask, 1 ml each of 0.1 M hydrochloric acid and 1 M KCl were added and the total volume was adjusted to about 7 ml by adding water, and mixed well. Finally, 2.0 ml of BPB reagent was added and the volume was made up to 10 ml. The flasks were shaken for 1 min by inverting them once every second and they were allowed to stand for 10 min. The absorbance of the resulting turbid suspension was measured with a spectrophotometer. All measurements were made at 650 nm at room temperature (30±2^{0}C).

In both the methods, a calibration graph was prepared by plotting the measured absorbance as a function of concentration or a regression equation was calculated. The concentration of the unknown was read from the calibration graph or deduced from the regression equation.

Results and Discussion

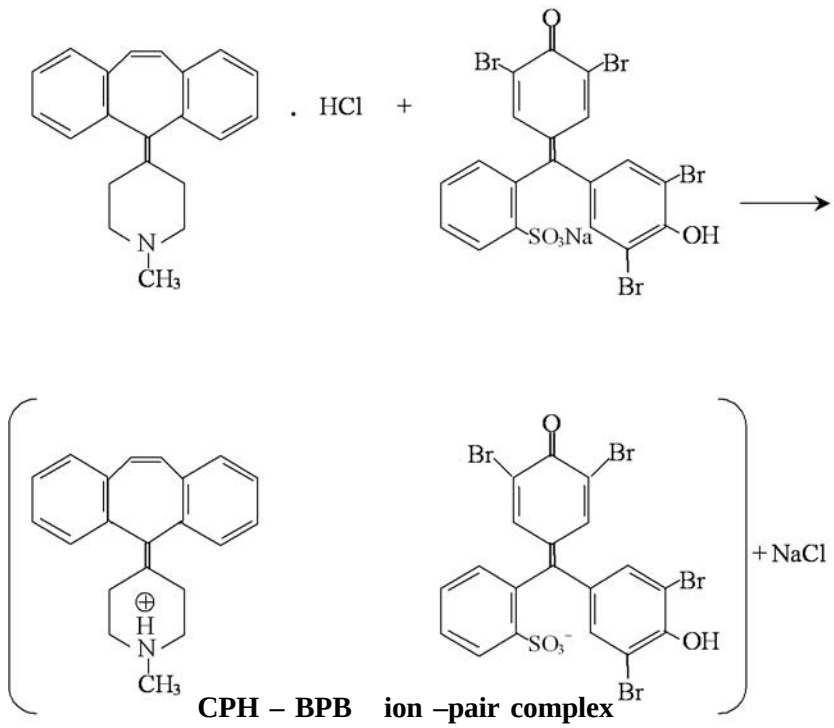
The proposed procedures are based on the reaction between CPH and BPB resulting in the formation of an ion-pair complex which could be either extracted into chloroform and measured spectrophotometrically or obtained as a stable suspension and measured turbidimetrically. The experimental conditions were optimised and the methods validated.

Spectrophotometry

CPH reacted with BPB in aqueous solution in acidic medium to form an yellow ion-pair complex which was extracted into chloroform. The formation of the complex is shown in the reaction scheme given below.

Absorption Spectra

Fig 1 shows the absorption spectra of the CPH-BPB ion-pair complex and of the reagent blank in chloroform. The absorption maximum of the ion-pair



CPH – BPB ion –pair complex

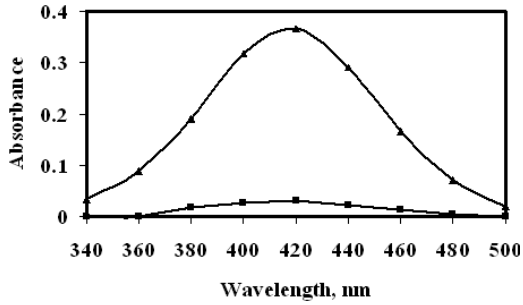


Fig 1. Absorption spectra of: (a) CPH-BPB complex formed in aqueous phthalate buffer of pH 3.0 containing 8 μg mL^{-1} CPH and extracted with chloroform (b) Blank

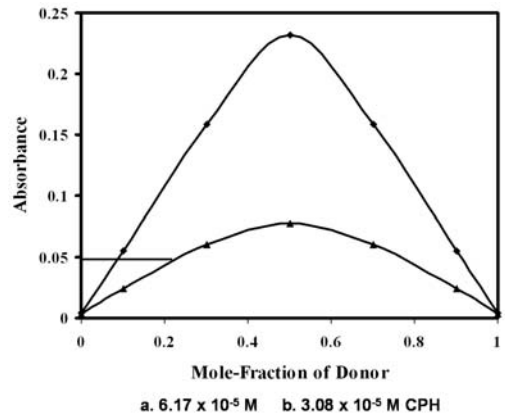


Fig 2. Job's Continuous Variations plots for CPH

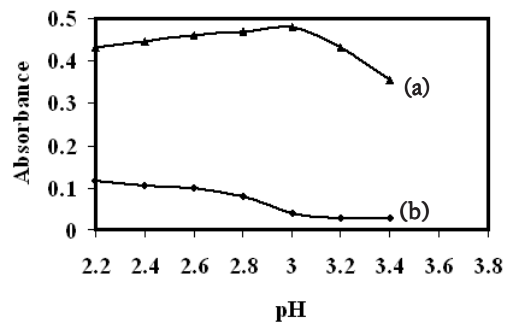


Fig 3. Effect of pH on complex formation: (a) CPH-BPB complex obtained with 10 μg mL^{-1} CPH, 0.02% BPB (b) Blank.

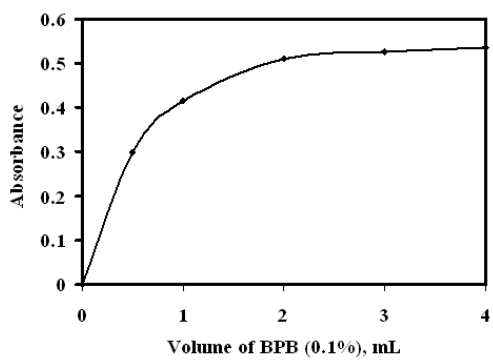


Fig 4. Effect of BPB concentration on complex formation with CPH = 10 μg mL^{-1}

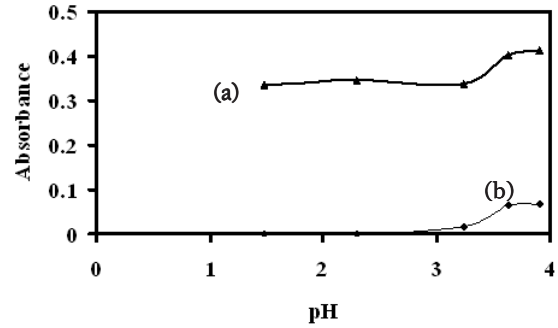


Fig 5. Effect of pH on suspension formation: (a) CPH-BPB complex obtained on suspension with 50 μg mL^{-1} CPH, 0.1% BPB at ionic strength 0.21 M (b) Blank.

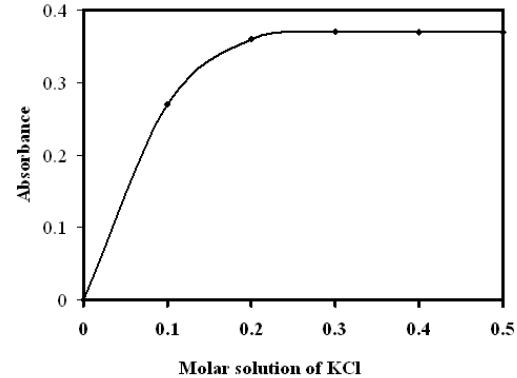


Fig 6. Effect of ionic strength.

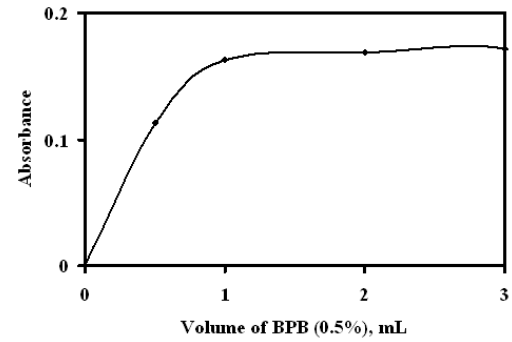


Fig 7. Effect of BPB concentration on complex formation: CPH=30 μg mL^{-1}, pH=2.40±0.10, ionic strength = 0.21 M

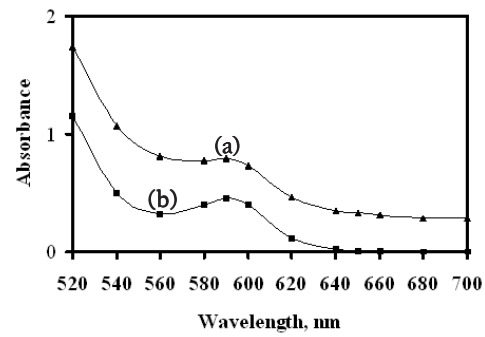


Fig 8. Absorption spectra of: (a) Suspension: 40 μg mL^{-1} CPH, 0.1% BPB, ionic strength 0.21 M, pH = 2.40±0.10 (b) Blank.

in chloroform is at 420 nm where the absorbance of the reagent blank is insignificant (0.03). Therefore, a wavelength of 420 nm was used for the examination of the conditions for the determination of CPH.

Applying Job's method of continuous variations,[35] the reaction stoichiometry of the ion-pair complex was found to be 1:1 (Fig 2). The Turner-Anderson method[36] was used to calculate the formation constant, which was found to be 6.08×10^4.

Effect of pH

The effect of pH on extraction is shown in Fig 3. The solution was adjusted to a pH between 2.2 and 3.8 with hydrochloric acid-phthalate buffer solution. Maximum and constant absorbances were obtained in the pH range 2.6 to 3.0. The absorbance decreased at pH values below 2.6 and above 3.0. In this work, the extraction of the CPH-BPB complex was, therefore, carried out at pH 3.0 (HCl-phthalate buffer solution). Also, the effect on the absorbance of varying volumes of buffer solution from 0.5 to 7.0 ml was examined. As a result, 5.0 ml of buffer solution were employed because the absorbance was constant irrespective of the volume of buffer solution added.

Effect of Amount of BPB

BPB solution (0.1%) was added to a solution containing 2.5 ml of 20 μg ml^{-1} CPH and the effect of the amount of BPB was examined by the proposed procedure. The absorbance increased in the range of 0.25 to 1.5 ml of 0.1% BPB solution and remained constant in the concentration range of 2.0 - 4.0 ml of 0.1% BPB solution (Fig 4). Hence, 2.0 ml of 0.1% solution were used as the optimum volume in a total volume of 15 ml.

Extraction Solvent and Shaking Time

Several organic solvents were examined for their ability to extract drug - BPB ion-pair complex. Among those organic solvents, chloroform was found to be the most suitable for quantitative extraction. A ratio of 3:1 (v:v) of aqueous to organic phases was required for efficient extraction of the coloured species. Shaking times of 0.5 to 5 min produced constant absorbance, hence a shaking time of 1 min was chosen for use. The drug-dye complex in the aqueous phase was extracted with 5 ml portions of chloroform three times. The absorbance was measured each time under the optimum conditions and only one extraction was found to be adequate to achieve a quantitative recovery of the complex.

Turbidimetry

BPB forms a suspension with CPH, which can be used for the latter's determination. The ion-pair formation was carried out by mixing 2 ml of 200 μg ml^{-1} CPH and 2 ml of 0.1% - 0.5% dye. Prior to adding the reagent, the pH of solution was adjusted with 0.1 M HCl. Precipitate was observed in the acid medium. The influence of pH on the precipitation was also investigated; the formation of solid was observed only over the pH range of 2.00 - 3.90, with the greatest precipitation in the pH range 2.40 - 2.90. Lower pH values resulted in the coprecipitation of the dye. The addition of NaOH to an acidic suspension resulted in the dissolution of the solid at pH 4.80. In other words, the absorbance was uniform in the pH range of 1.40-3.20. A working pH of 2.40 was selected, since above pH 3.0 the blank was found to show slightly increased absorbance (Fig 5). This pH was achieved by the addition of 1 ml of 0.1 M HCl. The influence of ionic strength was also studied by adjusting the total ionic strength from 0.035 to 0.41 M by adding different volumes of 1 M KCl in the presence of 1 ml of 0.1 M HCl. It was observed that ionic strength values above 0.21 M resulted in the coprecipitation of the reagent. An ionic strength of 0.21 M KCl was considered the optimum. The effect of ionic strength is shown in Fig 6.

Effect of BPB Concentration

The formation of turbid suspension was found to be affected by the concentration of BPB. To examine this, different volumes of 0.5% BPB solution were added to a solution containing 1 ml of 300 μg ml^{-1} of CPH. A gradual increase in the absorbance was observed up to 1.0 ml, beyond which a plateau was obtained. A decrease in absorbance was found at higher concentrations (Fig 7). Hence, 2 ml of 0.5% BPB solution in 10 ml was maintained.

Absorption Spectra

Fig 8 shows the absorption spectra of the turbid suspension and of the blank. The spectra exhibit more or less similar features in the region 520-640 nm. However, between 640 and 700 nm, the turbid suspension has a significant absorbance, whereas the blank has negligible absorbance in this region. Therefore, all absorbance measurements were made at 650 nm.

Stability of Suspension

The suspension was obtained by the formation of the CPH-BPB ion-pair complex under the conditions described in the procedure. A continuous increase in the absorbance occurred in the first 10 min. After that time, the absorbance remained constant up to 2 hours. Hence for quantification purposes, it is advisable to take absorbance readings 15 min after the addition of the dye.

Table 2. Accuracy and precision of the procedures.

Concentration of CPH taken, μg ml^{-1}	Concentration of CPH found*, Range, μg ml^{-1}		Relative error, %	RSD,%
Spectrophotometry				
4.00	4.11	0.10	2.75	0.93
6.00	5.92	0.16	1.33	1.28
10.00	9.92	0.21	0.80	0.77
Turbidimetry				
20.00	20.32	1.31	1.60	2.39
40.00	40.18	0.53	0.45	0.60
60.00	59.78	1.69	0.37	1.02

Table 3. Results of interference study.

Excipient added	Tolerance limit (μg ml^{-1}) Spectrophotometry[a]	Turbidimetry[b]
Talc powder	110	470
Starch	90	540
Lactose	120	1000
Magnesium stearate	150	1350
Sodium alginate	160	1560
Sodium acetate	80	400
Calcium gluconate	130	480
Calcium dihydrogen orthophosphate	150	1280

Table 4. Results of analysis of pharmaceutical preparations containing CPH.

Preparation*	Nominal amount, mg/tablet	Found+ (% recovery±SD) Spectrophotometry	Turbidimetry
Ciplactin tablet[a]	4.00	101.7±2.0	103.9±1.6
Practin tablet[b]	4.00	98.0±2.1	99.4±1.3

+Average of five replicate analyses.
* Marketed by : a) Cipla and b) Merind

Table 5. Results of recovery study by the standard addition method.

Method	Ciplactin tablet				Practin tablet			
	CPH in the formulation solution, μg	Pure CPH added, μg	Total found, μg	Recovery of pure CPH, % *	CPH in the formulation solution, μg	Pure CPH added, μg	Total found, μg	Recovery of pure CPH, % *
Spectrophotometry	20.3	40.0	62.0	104.2	19.6	40.0	60.4	102.1
	20.3	60.0	78.9	97.6	19.6	60.0	81.5	103.2
	20.3	80.0	97.9	97.0	19.6	80.0	100.4	101.0
Turbidimetry	207.7	200.0	414.4	103.3	198.7	200.0	399.4	100.3
	207.7	300.0	519.8	104.0	198.7	300.0	496.8	99.4
	207.7	400.0	615.3	101.9	198.7	400.0	599.7	100.3

* Average of three determinations

Analytical Data

Spectrophotometry

Beer's law was obeyed in the range of 2-12 μg ml^{-1}. The linear regression equation was Y_{420} = - 0.0177 + 0.0515 X, where Y is absorbance and X is concentration in μg ml^{-1}. The regression co-efficient was found to be 0.9989. The molar absorptivity and Sandell sensitivity were 1.65 x 10^4 l mol^{-1} cm^{-1} and 19.61 ng cm^{-2}, respectively. The detection limit, calculated from D_L=3.3σ/*S* (where *S* is the standard deviation of seven blank determinations and S slope of the calibration curve) was found to be 0.14 μg ml^{-1}, and the quantification limit given by Q_L=10σ/*S* was calculated to be 0.48 μg ml^{-1}. The complex extracted into chloroform was stable up to 72 hours.

Turbidimetry

The absorbance - concentration graph was linear over the range of 10-70 μg ml^{-1} of CPH and could be described by the equation, Y_{650} = -0.0264+0.0084 X with a correlation coefficient of 0.9987. The detection and quantification limits were 0.63 and 1.91 μg ml^{-1}, respectively.

Accuracy and Precision

Accuracy and precision were found by analysis of seven replicate samples containing three different amounts (within the linear ranges). The range, percent error and relative standard deviation obtained are given in Table 2, and reveal that the methods are reasonably accurate and precise.

Interference

Talc powder, starch, lactose, magnesium stearate, sodium alginate, sodium acetate, calcium gluconate and calcium dihydrogenorthophosphate are normally added to tablets as excipients. These compounds were added in amounts usually present in tablets which resulted in solutions containing 8 μg ml^{-1} of CPH (spectrophotometry) and 50 μg ml^{-1} (turbidimetry) and analysed. The results are presented in Table 3. The tolerance limits of the excipients were established at those concentrations which do not cause more than ± 2% error in the recovery of CPH at the concentrations used (8 or 50 μg ml^{-1}).

Application

The proposed methods were applied to the determination of CPH in the tablets containing CPH. Table 4 shows the assay results from the average of five determinations. The recoveries range from 97.98 to 103.85% indicating the efficiency and reproducibility of the methods. The accuracy and reliability of the methods were further ascertained by performing recovery studies by applying standard-addition technique. To a fixed amount of the drug in the tablet powder (pre analysed), pure drug was added at three different levels and the total was found by the proposed methods. Determination at each level of pure drug added was repeated three times. The results presented in Table 5 indicate the absence of interference from commonly encountered excipients in amounts normally present in tablets.

Conclusions

Cyproheptadine has been assayed in tablets using two different techniques. The methods are simple, accurate and precise. The sensitivity of the spectrophotometric method is significantly higher than that of all the spectrophotometric, potentiometric, GC and some HPLC methods proposed earlier. The methods are also easier and cheaper to carryout than an HPLC separation, and do not require expensive or toxic chemicals, and are free from matrix interference. The proposed methods should therefore be generally applicable to the determination of the drug.

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