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Use of bromophenol blue in the spectrophotometric and turbidimetric determination of mebrophenhydramine in tablets

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Simple, sensitive, rapid and cost-effective methods are described for the determination of mebrophenhydramine hydrochloride (MPH) in pure form and in tablets using Bromophenol Blue (BPB) as a reagent. The spectrophotometric method is based on the formation of an ion-association complex of the drug with BPB in phthalate buffer of pH 3.0, its extraction into chloroform and absorbance measurement at 420 nm. In turbidimetry BPB was used to produce stable turbidity arising from the formation of drug-dye complex of very low solubility at an altered pH condition and reagent-concentration and in the presence of potassium chloride. The absorbance of the turbid suspension was measured at 650 nm. Various parameters affecting the analytical procedures were investigated and optimised. In spectrophotometry, Beer's law is obeyed over the concentration range of 2-12 μg mL^{-1} with an apparent molar absorptivity of 1.98×10^4 L moL^{-1} cm^{-1} and Sandell sensitivity of 19.39 ng cm^{-2}. Turbidimetry is applicable in the concentration range of 10-70 μg mL^{-1}. The limits of detection are 0.10 and 0.44 μg mL^{-1} for spectrophotometry and turbidimetry, respectively. The methods were used to determine MPH in standard solution with a coefficient of variation of less than 2%, and were further applied to the determination of MPH in tablets. The results obtained by the proposed methods agree with the label claim. The reliability of the methods was further established by recovery studies applying standard-addition technique.

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Mebrophenhydramine hydrochloride (MPH) is one of the most potent antihistaminic drugs. Literature on the methods for the determination of MPH is scarce. Sastry *et al.*[1] have described an extractive spectrophotometric method using cobaltthiocyanate as a reagent. But, the method suffers from the disadvantage of poor sensitivity with the linear range being 50-500 μg mL^{-1}. Ultra-violet spectrophotometry[2,3], potentiometry[4] and capillary isotachophoresis[5] are some of the methods reported for the determination of MPH.

This work describes the spectrophotometric and turbidimetric determination of the drug based on ion-pair complex formation with BPB as the ion-pair complexing agent. The complex formed at pH 3.0 was extracted into chloroform and the absorbance measured at 420 nm in spectrophotometry while in turbidimetry the drug-dye ion-pair complex was produced as an insoluble suspension at pH 2.40 ± 0.10 and ionic strength 0.21 M, and the absorbance of the turbid suspension was measured at 650 nm. The methods are potentially very useful for the estimation of MPH in bulk drug and in tablets.

Experimental Procedure

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements. Pharmaceutical grade MPH with an assigned purity of 99.8% was gifted by Smithkline Beecham India Ltd., and was used as received. Mebryl tablets were obtained from local commercial sources. All chemicals used were of analytical grade and double distilled water was used throughout the investigation. Stock standard solution of MPH was prepared by dissolving 100 mg of pure drug in water and diluting to 100 mL in a volumetric flask. Working standards of 20 and 200 μg mL^{-1} were prepared for spectrophotometry and turbidimetry, respectively, by appropriate dilution of stock standard solution. Phthalate buffer of pH 3.0, hydrochloric acid

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(0.1 M), potassium chloride (1 M) were prepared in the usual way. A 0.5% aqueous solution of BPB was prepared and filtered for use in turbidimetry. The same was diluted to 0.1% solution for spectrophotometric work. Spectroscopic grade chloroform was used for extraction.

Preparation of tablet solution

Twenty tablets were weighed and ground into a fine powder. Portion of the powdered tablets equivalent to 100 mg of the active component was transferred into a 100 mL standard flask, 60 mL of water were added and shaken thoroughly for 20 min. The volume was made upto the mark, mixed well and filtered using a quantitative filter paper. Finally, the filtrate was diluted stepwise to get 20 and 200 μg mL^{-1} solutions for spectrophotometric and turbidimetric work, respectively.

Methods

Spectrophotometry

Accurately measured volumes of 20 μg mL^{-1} drug solution containing 2-12 μg mL^{-1} of MPH were transferred into separate 125 mL separating funnels. Five mL of buffer (pH 3.0) and 2.0 mL of 0.1% BPB reagent were added to each separating funnel. The total volume was brought to 15 mL by adding requisite volume of water and the contents were mixed well. Chloroform (5 mL) was added and the contents were shaken vigorously for 1 min. The two phases were allowed to separate and the chloroform layer was collected and passed over anhydrous sodium sulphate, and its absorbance was measured at 420 nm against a reagent blank.

Turbidimetry

Suitable aliquots (0.5-3.5 mL) of the drug solution (200 μg mL^{-1}) were transferred into a series of 10 mL volumetric flasks. One mL each of 0.1 M hydrochloric acid and 1 M potassium chloride were added to each flask. The total volume was adjusted to 7.0 mL by adding requisite volume of water, and mixed well. Finally, 2.0 mL of 0.5% BPB reagent were added. The volume was made upto the mark with water. The flasks were shaken for 1 min by inverting the flask once every second. The absorbance of the resulting turbid solution was measured with a spectrophotometer at 650 nm against a reagent blank.

The calibration graphs were drawn for both the methods by plotting the absorbance values versus concentration. Suitable aliquots of tablet solution were treated in the same manner as described under each procedure and absorbance measured.

Results and Discussion

The proposed procedures are based on the formation of an ion-pair complex between the drug and BPB, and the complex was either extracted into chloroform and measured spectrophotometrically at 420 nm or obtained as a stable suspension and measured turbidimetrically at 650 nm.

Spectrophotometry

BPB being an anionic dye, in the acidic pH reacts with MPH and forms a yellow ion-pair complex (λ_{max} 420 nm) which is soluble in chloroform. The absorption spectra of the complex and the reagent blank are shown in Fig. 1. The experimental conditions were established by varying one parameter at a time and observing its effect on the absorbance of the coloured species.

In order to establish the effective buffer and optimum pH range, the drug was allowed to react with BPB in the presence of various buffers of different acidic pH values. The phthalate buffer was found to be most suitable. The effect of pH on extraction is shown in Fig. 2. Nearly constant absorbances were obtained in the pH range of 2.6-3.0, hence, a pH of 3.0 was selected. Complexation was found to be unaffected when 1.0 to 7.0 mL of buffer of selected pH was used. Five mL of buffer were used in a total volume of 15 mL. The optimum volume of the dye solution was also studied. Figure 3 reveals an

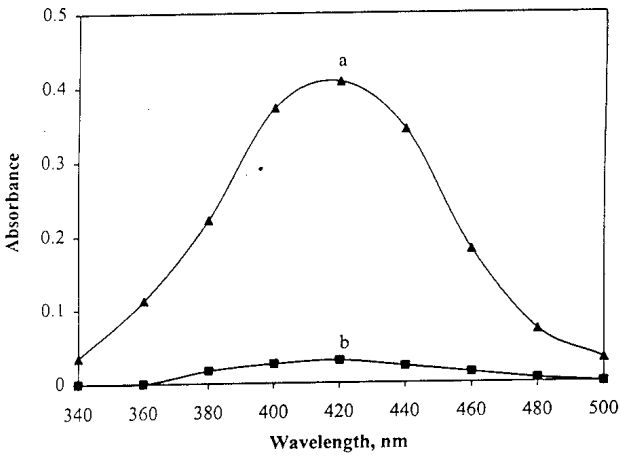

Fig. 1 — Absorption spectra of: (a) MPH-BPB complex formed in aqueous phthalate buffer of pH 3.0 containing 8 μg/mL MPH and extracted with chloroform; (b) Blank

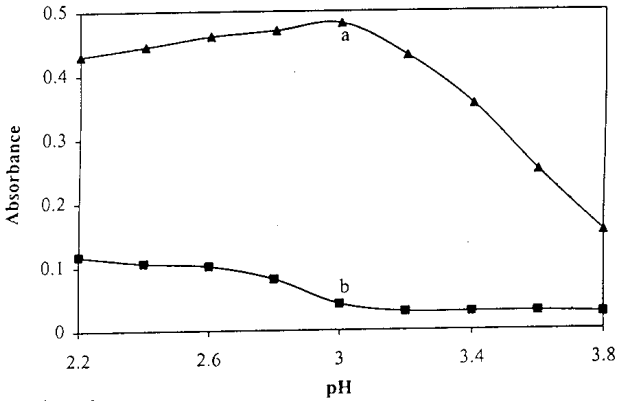

Fig. 2 — Effect of pH on complex formation: (a) MPH-BPB complex obtained with 10 mg/mL MPH, 0.006% BPB; (b) Blank

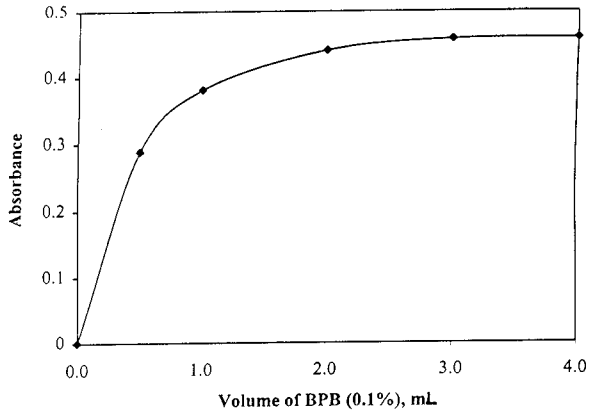

Fig. 3 — Effect of BPB concentration on complex formatio with 8 μg/mL MPH, pH = ± 0.10

increase in the absorbance readings with the increase of volume of BPB used upto 1.5 mL. However, a small increase in the absorbance value of the blank was observed at volumes larger than 2.5 mL. Two mL of 0.1% (w/v) BPB were found to be quite enough.

In the preliminary experiments, methylene chloride, toulene, benzene, carbon tetrachloride and isoamyl alcohol were tried as solvents for extraction. None of these systems showed better results than the chloroform. Shaking time of 0.5-5 min produced constant absorbance, and a shaking time of 1 min was chosen for use. A ratio of 3:1 of aqueous to organic phases was required for efficient extraction of the coloured species. Under optimum conditions, the drug-dye complex in the aqueous phase was extracted with three 5 mL portions of chloroform and the absorbance was measured each time. Only one extraction was found to be adequate to achieve a quantitative recovery of the complex. The yellow coloured complex was stable for about 3 days. Applying Job's method of continuous variations, the reaction stoichiometry of this ion-pair complex was proved to be 1:1.

Turbidimetry

A qualitative study of the formation of the ion-pair complex was carried out by mixing 3 mL of 200 μg mL^{-1} MPH solution and 2 mL of BPB solution of various (from 0.1 to 0.5%) concentrations. The pH was adjusted prior to the addition of the reagent by the dropwise addition of 0.1 M HCl or NaOH solution. Precipitation was observed in acidic media.

The influence of pH on the precipitation indicated the formation of solid only over the pH range of 1.00-3.90 with the maximum precipitation in the pH range of 2.40-2.90. At lower pH values the co-precipitation of the dye was observed. The addition of NaOH to an acidic suspension resulted in the dissolution of the solid at pH 4.80. Although the absorbance of the suspension was more or less same in the pH range 1.60±0.10 to 3.00 ± 0.10 (Fig. 4) a working pH of 2.40±0.10 was selected. Above pH 3.0 the blank was found to show a slight absorbance. This effective pH was achieved by adding 1 mL of 0.1 M HCl in a total volume of 10 mL. The influence of ionic strength was studied by adjusting the total ionic strength from 0.035 to 0.41 M with potassium chloride after adjusting the pH to 2.40 ± 0.10. It was observed that the values above 0.21 M resulted in the co-precipitation of the reagent. An ionic strength of 0.11 M was considered the optimum, which was achieved by the addition of 1 mL of 1 M KCl in

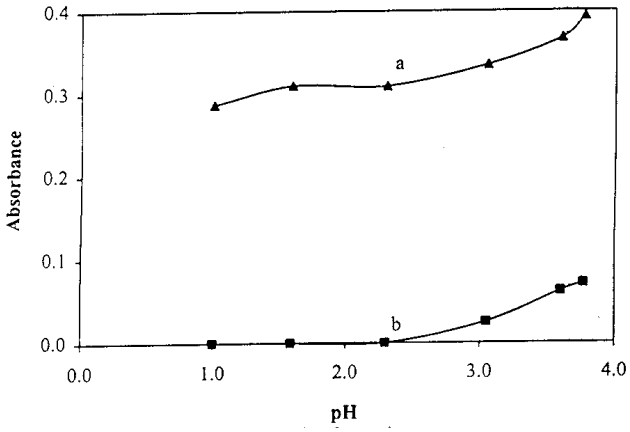

Fig. 4 — Effrect of pH on suspension formation: (a) MPH-BPB complex obtained on suspension with 50 μg/mL MPH, 0.05% BPB at ionic strength 0.21 M; (b) blank

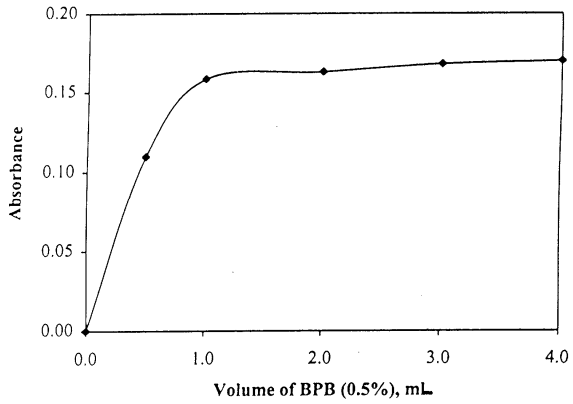

Fig. 5 — Effect of BPB concentration on complex formation with 20 μg/mL MPH, ionic strength 0.21 M, pH = 2.40 ± 0.10

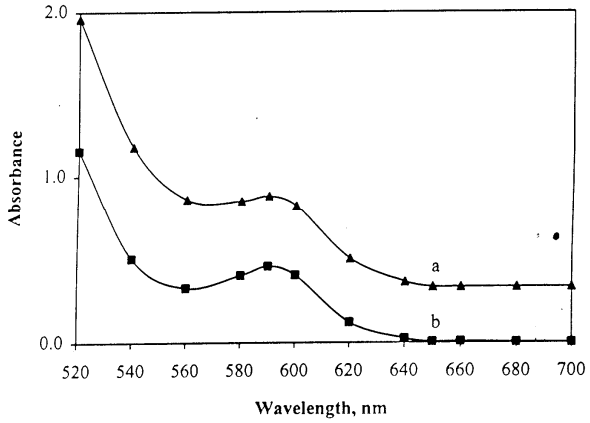

Fig. 6 — Absorption spectra of: (a) Suspension: 40 μg/mL MPH, 0.1% BPB, ionic strength 0.21 M, pH = 2.4 ± 0.10; (b) Blank

addition to HCl added to produce the required pH. The formation of the turbid suspension was found to be affected by the concentration of BPB. To assess the effect of its concentration, different volumes of 0.5% BPB were added to a solution containing 1.0 mL of 200 μg mL^{-1} of MPH. A steady rise in the absorbance occurs upto 1.0 mL, beyond which a plateau was observed (Fig. 5). On the other hand, the absorbance was found to decrease at higher concentration, (> 4 mL). Hence 2.0 mL of 0.5% solution were used throughout the study.

The absorption spectra of the turbid colloid suspension in the visible region is illustrated in Fig. 6. The sloping curve exhibits no wavelength of maximum absorbance. The turbid suspension and the blank exhibit more or less similar spectral features in the region, 520-640 nm. The blank has no absorbance beyond 640 nm whereas MPH-BPB complex has significant absorbance between 640 and 700 nm, which can be attributed to the suspension (drug-dye complex). All absorbance measurements were made at 650 nm.

Analytical data

Spectrophotometry

Beer's law was obeyed in the concentration range of 2-12 μg mL^{-1}. The linear regression equation is Y_{420} = 0.0112+0.0483 *X*, where *Y* is the absorbance of concentration *X* in μg mL^{-1}. The regression co-efficient was found to be 0.9998. The molar absorptivity and Sandell sensitivity were 1.98×10^4 L moL^{-1} cm^{-1} and 19.39 ng cm^{-2}, respectively. The limit of detection was 0.10 μg mL^{-1} and the limit of quantification was 0.51 μg mL^{-1}.

Turbidimetry

The calibration graph was linear over the concentration range of 10-70 μg mL^{-1} of MPH. The linear regression equation is Y_{650}=−0.0046+0.0083 *X*, with a correlation coefficient of 0.9991. The limit of detection and quantification were calculated to be 0.44 and 1.46 μg mL^{-1}, respectively. The drug-dye complex obtained as a turbid suspension was found to be stable for more than 2 h. However, a standing time of 10 min after mixing the reactants was found necessary.

Accuracy and precision

In order to determine the accuracy and precision of the recommended procedures, seven replicate determinations at three different concentrations of drug were carried out. The range, percent error and relative standard deviation obtained are given in Table 1 and indicate that the proposed methods are highly accurate and reproducible.

Application

The recommended procedures were applied satisfactorily to the determination of MPH in tablets. Table 2 shows the assay results of five determinations. The recoveries agree well enough with the label claim (25 mg per tablet) and the precision is quite satisfactory. The accuracy and reliability of the proposed methods were further established by performing recovery studies. To a fixed amount of the drug in tablet solution (pre-analysed) pure drug was added at three different levels, and the total amount was determined by the proposed methods. Each determination was repeated

Table 2 — Results of analysis of pharmaceutical preparations containing MPH

Preparation [b]	Label claim	Found [a] (% recovery ± SD)	
		Spectrophotometry	Turbidimetry
Mebryl tablet [c]	25 mg per tablet	97.25 ± 0.06	102.65 ± 0.32

[a] Average value of five replicate analyses, [b] Marketed by, [c] Smithkline Beecham India Ltd.

Table 3 — Recovery studies by standard-addition technique

	Mebryl tablet			
Method	MPH in the tablet solution, μg	Pure MPH added, μg	Total found, μg	Recovery of pure MPH, % [a]
Spectrophotometry	19.5	60.0	82.8	105.6
	19.5	80.0	102.0	103.1
	19.5	100.0	121.9	102.5
Turbidimetry	205.3	300.0	504.8	99.8
	205.3	400.0	606.2	100.2
	205.3	500.0	705.2	99.9

[a] Average value of three determinations

three times. The percent recoveries of the pure drug added (Table 3) indicate that the tablet excipients like talc, starch, lactose, alginate, stearate and gum acacia in amounts present in tablet preparation do not interfere in the determination.

Conclusions

The methods presented here are direct, simple and rapid for the analysis of MPH in tablets which is the pharmaceutical form most frequently prescribed. The results obtained demonstrate good precision and accuracy and compare well with the label content. The spectrophotometric method described is more simple and sensitive than the oxidative UV-spectrophotometric[2,3] and the cobaltthiocyanate[1] methods described earlier. The turbidimetric method though less sensitive than the spectrophotometric method offers the advantage of further simplicity since no liquid-liquid extraction is required. The other advantages of the turbidimetric method include the use of water as reaction medium and measurement in the visible region using an ordinary spectrophotometer.

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