The authors thank Professor Stanley J. Brumleve and Professor Roland G. Severson for their advice and encouragement. Grateful acknowledgment is made to the Indian National Science Academy, New Delhi, India, and to the State Council of Scientific and Industrial Research, Uttar Pradesh, Lucknow, India, for providing Research Fellowships to A. Chaudhari and S. Kumar, respectively.

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Spectrophotometric Determination of Diphenhydramine Hydrochloride Using Dipicrylamine

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Abstract □ A spectrophotometric procedure for the determination of diphenhydramine hydrochloride based on the reaction with dipicrylamine was developed. A yellow complex forms and is easily extractable by chloroform at pH 5. The mole ratio of diphenhydramine hydrochloride to dipicrylamine in the complex is 1:3. The absorbance of the complex obeys Beer's law over the concentration range of 3–10 μg of diphenhydramine hydrochloride per ml of chloroform. This procedure can be carried out in the presence of other compounds without interference.

Keyphrases □ Diphenhydramine hydrochloride—spectrophotometric analysis, color complex with dipicrylamine, pharmaceutical formulations □ Dipicrylamine—color complex formation for spectrophotometric analysis of diphenhydramine hydrochloride, pharmaceutical formulations □ Spectrophotometry—analysis, diphenhydramine hydrochloride, pharmaceutical formulations □ Antihistaminic agents—diphenhydramine hydrochloride, spectrophotometric analysis, pharmaceutical formulations

5-(Hydroxymethyl)-2-furaldehyde is a well-known product of the Maillard reaction (1–3). It is frequently present in pharmaceutical preparations, particularly in syrups, and can interfere in the chemical analysis for active components.

USP XVIII (4) described a UV assay for diphenhydramine hydrochloride (I) elixir, in which 5-(hydroxymethyl)-2-furaldehyde interferes with the chemical analysis, the nonaqueous titrimetry for the powder, and also the UV assays for the capsule and injection preparations. The separation of the base on a cation exchanger, followed by quantitative spectrophotometric determination, was reported (5, 6).

A UV method was developed to measure the steam-distillable compound after hydrolysis and oxidation of diphenhydramine (7). An addition compound formation between diphenhydramine and tetrabromophenolphthalein ethyl ether in ethylene-dichloride was used (8), as was ion-pair formation between the amine and an indicator dye (9). Nonaqueous titrimetry and salt partition were used in analysis procedures for organic bases (10, 11).

Quantitative TLC also was described (12). A modification of the USP XVIII method was described in which the effect of 5- (hydroxymethyl)-2-furaldehyde was eliminated (13). A column chromatography procedure was reported for the separation of I from a capsule formulation with determination by UV (14). However, these methods lack the simplicity and sensitivity to determine microamounts of I.

The present study describes a direct, simple, and sensitive procedure for the determination of I spectrophotometrically. This method is applicable for powder, syrup, capsule, injection, and elixir dosage forms without interferences. The procedure depends on the formation of a complex between I and dipicrylamine (II) which is extractable by chloroform at pH 5. This method can be carried out successfully in the presence of 5-(hydroxymethyl)-2-furaldehyde and other compounds.

EXPERIMENTAL[1]

Reagents and Chemicals—A 0.001 *M* diphenhydramine hydrochloride (I) aqueous solution (USP reference standard) and a 0.001 *M* dipicrylamine (II) in 0.4% sodium carbonate solution were prepared. All buffers used were of the BP standard.

General Procedure—To a 50-ml separator were added 1-5 ml of 0.001 *M* I, 1 ml of 0.001 *M* II, and 10 ml of pH 5 buffer solution. The mixture was shaken, and the complex formed was extracted with 5, 3, and 2 ml of chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and then diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 420 nm *versus* a similarly prepared blank. Any other pharmaceutical preparations should be diluted to contain less than 100 μg/ml of I and then analyzed as described.

RESULTS AND DISCUSSION

A yellow-colored complex with a maximum absorption at 420 nm developed (Fig. 1) when I reacted with II. It was completely extractable by chloroform at pH 5. A calibration curve was plotted for various concentrations of I. Beer's law was followed over the concentration range of 30–100 μg of I/10 ml of chloroform. The molar absorptivity was 1.45×10^4.

The effects of temperature, pH, and the presence of many compounds were studied. A pH of 5 gave optimum results and different temperatures had no effect on complex formation and extraction.

Effect of Other Compounds—To determine the effect of other compounds, a standard solution containing 90 μg of I and the compound in question were placed in a separator and analyzed by the described method. The following compounds did not interfere when added in the indicated amounts: ammonium chloride[2] (10 mg), menthol[2] (100 mg), sucrose[2] (100 mg), sodium citrate[2] (10 mg), saccharin sodium[2] (5 mg), acetaminophen[3] (10 mg), phenylephrine hydrochloride[3] (100 mg), ascorbic acid[3] (10 mg), 5-(hydroxymethyl)-2-furaldehyde[4] (20 mg), orange oil[5] (0.1 ml), cinna-

[1] A Coleman Junior II model 6/20 spectrophotometer with 1-cm glass cells and a Beckman 10 recorder were used. A Beckman H_3-type pH meter was used for pH measurements.
[2] Present with I in syrups.
[3] Components of a diphenhydramine capsule marketed as Flustop.
[4] Frequently present in syrups.
[5] Constituents of I elixir.

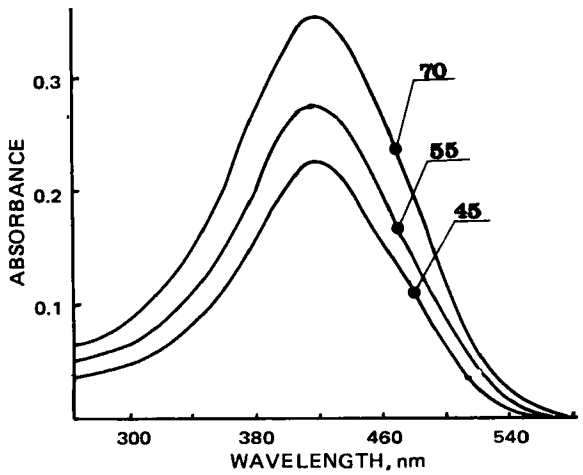

Figure 1—*Absorption spectra of the I–II complex at various I concentrations. (Numbers on curves indicate concentration of I in micrograms per 10 ml of chloroform.)*

mon oil[5] (0.003 ml), clove oil[5] (0.1 ml), coriander oil[5] (0.05 ml), anethol (0.2 ml), amaranth[5] (5 mg), and alcohol[5] (0.5 ml). The percent recovery of I from different synthetic pharmaceutical preparations is shown in Table I.

Stability of Complex—The I–II complex was very stable in chloroform and began to fade slowly only after 10 days. Before the extraction, the mixture was put in a boiling water bath for 30 min; the absorbance did not change after extraction with chloroform.

Nature of Complex—The mole ratio method (15) was employed to determine the composition of the I–II complex as follows.

The concentration of I was kept constant at 0.0002 *M* while the concentration of II was varied between 0.0001 and 0.002 *M*. Spectrophotometric measurements were made as already described.

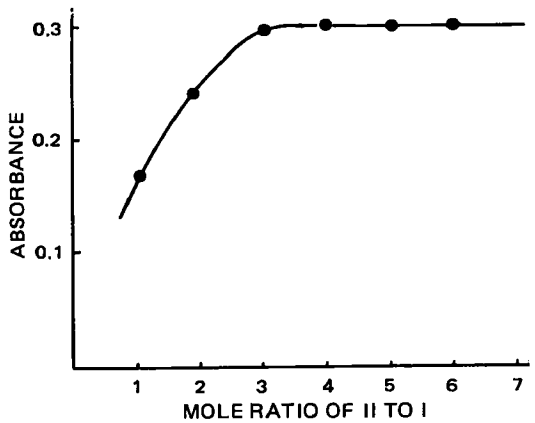

Figure 2—*Variation of the absorbance* versus *the mole ratio of II to I at 420 nm when the concentration of I was kept constant at 2* $\times 10^{-4}$ M.

Table I—Percent Recovery of Diphenhydramine Hydrochloride

Synthetic Preparation	I Added, μg	Absorbance Values: Before Addition	Absorbance Values: After Addition	Recovery, %	Deviation, %
Syrup	40	0.250	0.452	101.0	1
Capsule	40	0.250	0.450	100	0.0
Elixir	40	0.250	0.447	98.5	1.5
Injection	40	0.200	0.400	100	0.0
Capsules[a]	40	0.200	0.400	100	0.0

[a] The analysis was applied to Flustop capsules containing 400 mg of acetaminophen, 5 mg of phenylephrine hydrochloride, 10 mg of diphenhydramine hydrochloride, and 50 mg of ascorbic acid.

Figure 2 shows that the absorbance of the complex increased as the concentration of II was increased up to a I–II ratio of 1:3. The absorbance of the complex did not change with further increases, indicating a 1:3 ratio of I to II in the complex.

Precision and Accuracy—A set of 10 identical samples, each with a final I concentration of 90 μg, was treated according to the recommended procedure and the absorbances were measured. The percent accuracy and the confidence limits were calculated to be 1% and 89.1 ± 0.85 μg, respectively.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 8, 1975, from the *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Tehran University, Tehran, Iran.*

Accepted for publication July 21, 1975.

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