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Spectrophotometric Determination of Diphenhydramine in Pharmaceutical Preparations

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A spectrophotometric method is proposed for the determination of diphenhydramine in pharmaceutical preparations. The method is based on an addition-compound formation between diphenhydramine and the tetrabromophenolphthalein ethyl ester in 1,2-dichloroethane. Beer's law is obeyed over the range of 2.0×10^{-6}—1.0×10^{-5}M of diphenhydramine at 594 mμ. Optimum conditions are described. The effect of diverse substances and the composition of the colored species were also investigated.

The titrimetric method[1] has been used for the determination of diphenhydramine, which is widely used as antihistaminate. Cobalt nitrate - potassium thiocyanate - sulfuric acid[2] and nitric acid -sulfuric acid[3] have been described as spectrophotometric reagents for diphenhydramine. A fluorometric method[4] based on the oxidation of diphenhydramine has also been described.

Recently, plasmocorinth B[5] was proposed for the spectrophotometric determination of diphenhydramine by solvent extraction. This method can not, however, be used in the analysis of injections because it suffers from the interference of halide and calcium ions.

This paper will report the results of a study designed to develop a new spectrophotometric method for diphenhydramine by means of solvent extraction. The tetrabromophenolphthalein ethyl ester forms an addition compound with colorless diphenhydramine in 1,2-dichloroethane. In the absence of diphenhydramine, a yellow color was observed, while in its presence a red-violet color was developed.

Experimental

Apparatus. The spectrophotometric measurements were made with a Shimadzu QR-50 spectrophotometer, equipped with 10-mm cuvettes. An Iwaki Model KM shaker with a time switch was used for the extraction. The pH measurements were carried out with a Toa Denpa Model HM-5 pH meter.

Reagents. *Tetrabromophenolphthalein Ethyl Ester (TEE)*

1) J. P. VII-1, Part II, Nankodo (1965), p. 594.
2) G. Sekules and G. Guadagnini, *Boll. Chim. Farm.*, **103**, 432 (1964); *Chem. Abstr.*, 11853 (1964).
3) L. Molle, *J. Pharm. Belg.*, **5**, 339 (1950).
4) R. E. Jensen and R. T. Pflaum, *J. Pharm. Sci.*, **53**, 835 (1964).
5) H. Koike, K. Ohashi, M. Matsuo and A. Kawasaki, *Bunseki Kagaku*, **17**, 603 (1968).

Solution. Weighed amounts of tetrabromophenolphthalein ethyl ester potassium salt were dissolved in ethyl alcohol.

Standard Diphenhydramine Solution. A stock solution was prepared by dissolving 2.919 g of diphenhydramine hydrochloride (dried at 105°C) and diluting it to 1 *l* with water to make the solution 1.0×10^{-2}M. The stock solution was used to prepare the standard solution in the desired concentration.

Buffer Solution. The pH 8 buffer was prepared by mixing a 0.4M potassium dihydrogen phosphate solution and a 0.4M disodium hydrogen phosphate solution.

All the chemicals were of a reagent grade, and the water used was passed through an ion-exchange resin.

Procedure. *Standard Procedure.* Pipette 5 m*l* of a diphenhydramine solution (less than 5×10^{-5}M), 5 m*l* of a TEE solution (10^{-3}M), and 5 m*l* of the buffer solution into a 100-m*l* separatory funnel. Dilute the mixture to 25 m*l* with water and shake the solution for 2 min with 10 m*l* of 1,2-dichloroethane. After the separation of the two layers, run off the extract into a glass tube through filter paper to remove any droplets of water. Measure the absorbance of the extract at 594 mμ, using a reagent blank as a reference.

Injection. Dilute a sample with water and treat the solution in the same manner as with the standard procedure.

Powder and Tablet. Dissolve a sample containing about 5 mg of diphenhydramine in 50 m*l* of 0.01N sulfuric acid. Filter the solution with a glass filter and dilute the filtrate to 500 m*l* with water. Treat the solution in the same manner as with the standard procedure.

Ointment. Place a sample containing about 5 mg of diphenhydramine in a separatory funnel, and add 15 m*l* of ether. Extract the diphenhydramine three times with 30-m*l* portions of 0.03N sulfuric acid. Put all the extracts together into a 500-m*l* volumetric flask and make up to the mark with water. Treat the solution in the same manner as with the standard procedure.

Results and Discussion

Absorption Spectra. Figure 1 shows the visible absorption spectra of extracts treated as with the standard procedure. It can be seen that the presence of diphenhydramine leads to a considerable increase in the absorbance. The absorbance maximum of the extracts is at 594 mμ. This red-violet color in the organic layer may be attributed to the effect of the association between the TEE and the diphenhydramine.

Effect of pH. The absorbance of the extract is constant over the 7—9 pH range. In a more acidic or a more alkaline solution, the absorbance decreases, presumable because the TEE is not extracted into an organic solvent from a strong alkaline solution and because the diphenhydramine is not extractable from an acidic solution.

Effect of Reagents. Excess amounts of the phosphate buffer had no influence on the absorbance of the extract. When the buffer concentration was less than 0.05M in the aqueous layer, a good separation of the two layers was not observed.

The absorbance of the extract was constant if the TEE concentration was more than the 1.2×10^{-4}M initially present in the aqueous layer when the concentration of diphenhydramine was 1.0×10^{-5}M. With smaller amounts of TEE, low absorbances were obtained.

Solvent for Extraction. The behavior of various solvents in the extraction was studied. The solvents tested were benzene, butyl acetate, carbon tetrachloride, chloroform, cyclohexane, 1,2-dichloroethane, ether, ethyl acetate, *n*-hexane, isoamyl alcohol, methyl isobutyl ketone, monochlorobenzene, and toluene. Of these, dichloroethane was chosen for the present work because it gave the deepest color.

The Other Variables. Full color development took about 1 min of shaking. Continued shaking up to 5 min product no further change in the absorbance. The color intensity of the dichloroethane extracts remained constant for 1 hr. Normal room temperature fluctuations (14—26°C)

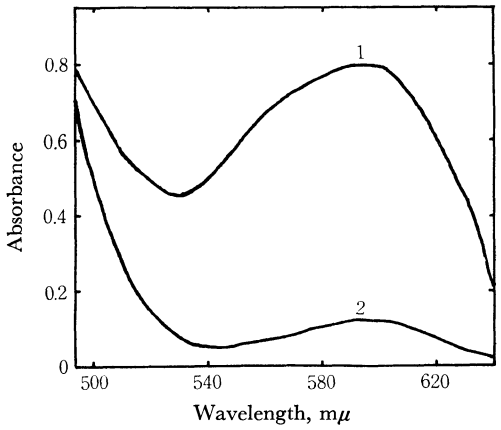

Fig. 1. Absorption spectra.
1: 1.0×10^{-5}M of diphenhydramine
2: Reagent blank
Reference: water

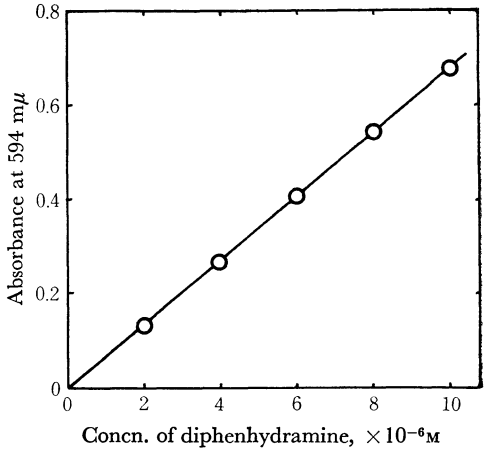

Fig. 2. Calibration curve.
Reference: Reagent blank

TABLE 1. EFFECT OF FOREIGN SUBSTANCES

Substance	Mole Ratio	Recovery (%)
Ammonium sulfate	1000	104.3
Calcium chloride	1000	99.0
Sodium carbonate	1000	102.1
Sodium chloride	1000	100.0
Sodium nitrate	1000	98.8
Potassium bromide	1000	100.0
Aniline	1000	99.7
Aminopyrine	5	125.1
Antipyrine	20	115.5
Benzyl alcohol	1000	99.6
Caffeine	10	117.7
Ethoxybenzamide	50	119.2
Glucose	1000	100.0
Lactose	1000	100.1
Noscapine	1	112.3
Phenacetine	5	116.5
Phenol	1000	93.8
Sodium acetate	1000	100.0
Sodium cirate	1000	100.2
Sodium salicylate	1000	99.8
Sodium tannate	1000	100.2
Vitamine C	1000	99.4
Starch	0.4 %	100.0

Diphenhydramine taken : 1.0×10^{-5}M

were without measurable effect on the absorbance.

Calibration and Precision. Figure 2 shows the calibration curve obtained according to the standard procedure.

The reproducibility of the proposed method was setimated from the results for ten sample solutions, each with a final diphenhydramine concentration of 1.0×10^{-5} M. The mean absorbance was 0.675, with a standard deviation of 0.006 absorbance unit.

Composition of the Colored Species. A sample (25 m*l*) containing 1.0×10^{-5}M of diphenhydramine was extracted with 10 m*l* of dichloroethane at pH 8. Then the amounts of diphenhydramine in the aqueous layer and organic layer were determined according to the proposed method. The extraction rate was about 94% in the absence of TEE, and 95% in its presence. These results indicate that diphenhydramine is extracted independently of TEE and is then associated with TEE in the organic solvent.

TABLE 2. ANALYSIS OF DIPHENHYDRAMINE IN COMMERCIAL SAMPLES

Sample	Diphenhydramine content	
	Titrimetric method	Proposed method
Injection*	9.98 mg/m*l*	9.97 mg/m*l*
Tablet*	91.0 mg/g	90.6 mg/g
Powder	99.7 mg/g	99.4 mg/g
Ointment	9.84 mg/g	9.87 mg/g

* Contained as diphenhydramine hydrochloride
All samples were obtained from a drugstore.

Continuous-variation plots were employed in order to investigate the composition of the colored species. The total concentration of TEE and diphenhydramine was kept constant at 1.6×10^{-5}M. The resulting curves at 594 and 560 mμ, in which the reagent blank has been subtracted, have a maximum at 0.5 mol fraction of diphenhydramine. It may, therefore, be suggested that a 1 : 1 addition compound is formed in the dichloroethane phase between TEE and diphenhydramine.

Effect of Foreign Substances. Table 1 shows the effect of diverse substances on the determination of diphenhydramine. Sodium chloride, tannate, benzyl alcohol, and starch, all of which are apt to be present in a pharmaceutical preparation with diphenhydramine, do not interfere.

Analysis of Practical Samples. Commercial samples used as antihistaminates were analyzed according to the proposed method and the titrimetric method.[1] The results obtained are summarized in Table 2.

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