In the case of dyes, enhanced adsorption is desirable for uniform coloring of solid dosage forms. However, in the case of drugs, adsorption to a seemingly inert excipient such as starch may affect their release and availability. Problems due to drug adsorption by excipients may also arise during *in vitro* evaluation of the solid dosage forms and during quantitative analysis for the active ingredient. This would be particularly true for potent drugs that have low effective doses. Adsorption studies during the preformulation stages in the development of a drug product can provide clues to such problems.

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DRUG STANDARDS

Spectrophotometric Determination of Diphenhydramine Hydrochloride in an Antiallergic Cream

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Abstract □ A specific method for the quantitative determination of diphenhydramine hydrochloride in a cream formulation has been developed. The method entails the extraction of diphenhydramine by chloroform, further purification of the extracted base by column chromatography using alginic acid, and its spectrophotometric determination in the eluate at 258 mμ.

Keyphrases □ Diphenhydramine cream—analysis □ Column chromatography—separation □ UV spectrophotometry—analysis

The isolation of a pharmacologically active ingredient from a pharmaceutical formulation containing surfactants is often difficult. Various authors have followed different procedures for the removal of unwanted ingredients. Jones (1) described a method for the determination of diethylstilbestrol in a water-dispersible suppository using column chromatography followed by TLC. Gottlieb (2) used refluxing with an organic solvent to break down the emulsion and subsequently recovered the active drug (which was also diethylstilbestrol) using an aluminum column. More recently, Forman (3) developed an assay for dienestrol in a cream[1] using urea-inclusion chemistry to remove the excess of monostearin. The information obtained from any of these studies is valuable and may indicate a general approach to analysis employing the two basic steps of extraction and cleanup. However, each of them is a specific case which depends upon the physical and chemical properties of the active component.

Therefore, it is reasonable to assume that different methods of separation may be required for differing formulations containing the same active ingredients and for differing active ingredients contained in similar formulations. Diphenhydramine hydrochloride is found in various combinations in commercially available pharmaceutical preparations, and various assays for its determination have been reviewed (4–6). No procedure, however, has been reported involving the quantification of diphenhydramine hydrochloride in an

[1] The analysis was applied to a product containing 2% of diphenhydramine hydrochloride and marketed as "Allergin Cream."

Table I—Results of Determination of Diphenhydramine Hydrochloride in Eight Synthetic Mixtures[a]

Mixture Containing	Taken, g.	Found for Eight Determinations, g.[b]
Diphenhydramine hydrochloride	2.00	1.97, 2.01, 2.10, 1.98, 2.04, 1.96, 2.01, 2.10

[a] The preparation also contains emulsifying wax, liquid paraffin, parabens, glycerin, water, color, and menthol. [b] Mean percentage recovery of diphenhydramine hydrochloride = 100.5%.

emulsified cream base. The procedure described in this paper is a modification of an analysis method for the determination of diphenhydramine hydrochloride in a cough mixture (6). The method entails the recovery of the diphenhydramine by chloroform extraction and further purification through an alginic acid column, followed by quantitative spectrophotometry at 258 mμ.

EXPERIMENTAL

Apparatus—A Beckman DB spectrophotometer and 4-cm. square, fused silica cells were used. A glass column, 30 × 1.8 cm., with a stem, 5 cm., was fitted with a buret key.

Reagents—The following were used: cation-exchange resin, alginic acid, 40–100 mesh (available from British Drug Houses); 2 *N* hydrochloric acid in water; 0.1 *N* hydrochloric acid in water; 5% hydrochloric acid in water; 95% ethanol in water; and 80% ethanol in water. Except where otherwise specified, all reagents were of British Drug Houses' Analar quality.

Standard Solutions—The following solutions were prepared with suitable reference standards: (*a*) diphenhydramine hydrochloride, 2 g./100 ml. in 5% hydrochloric acid; and (*b*) diphenhydramine hydrochloride, 3.2 mg./100 ml. in 0.1 *N* hydrochloric acid.

Column Preparation—Alginic acid, about 4 g., was slurried in water and allowed to soak for 4 hr. The slurry was poured into a glass column which had been fitted with a cotton wool plug and allowed to settle. The column was washed with 2 *N* hydrochloric acid, until the absorbance of the eluate (pathlength 4 cm.) was less than 0.005 at 258 mμ, and then with distilled water until the eluate was neutral to litmus. Finally, 25 ml. of 80% ethanol was passed through the column.

Sample Treatment—An amount corresponding to approximately 2 g. of cream (about 40 mg. diphenhydramine hydrochloride) was accurately weighed into a 150-ml. separator. The cream was suspended in 6 ml. of 5% hydrochloric acid. The suspension was extracted with four successive portions of 15 ml. each of chloroform; each extraction was filtered through a pledget of cotton wool into a 100-ml. volumetric flask, and the solution was brought to volume. An aliquot of 20 ml. was pipeted into a 25-ml. volumetric flask. The chloroform was evaporated to dryness on a water bath with the aid of a current of air. The residue was dissolved in 95% ethanol, and the solution was brought to volume. An aliquot, 10 ml., was pipeted onto the prepared column, and the solution was allowed to pass through the column at a rate of 1 ml./min. The column was then washed with 50 ml. of 80% ethanol divided into two portions, also at a rate of 1 ml./min., and finally with 200 ml. of water at a rate of 4 ml./min. Diphenhydramine was subsequently eluted with 0.1 *N* hydrochloric acid at a rate of 1 ml./min. The first 5 ml. of eluate was discarded and the balance collected into a 100-ml. volumetric flask until 100 ml. was collected. The absorbance of the solution was then determined at 258 mμ, using 4-cm. cells and 0.1 *N* hydrochloric acid as blank.

Table II—Analysis of Diphenhydramine Hydrochloride in Eight Commercial Formulations

Preparation	Claim for Diphenhydramine Hydrochloride, g. %	Found, % of Claim
A	2	105.30
B	2	98.73
C	2	100.23
D	2	99.45
E	2	102.70
F	2	100.68
G	2	98.10
H	2	99.79

RESULTS AND DISCUSSION

The cream examined was of the oil–water type. The oil phase contained menthol as a coolant. A nonionic emulsifying wax was used as the emulgent, as well as providing a cream of the required consistency. The aqueous phase contained diphenhydramine hydrochloride, parabens, red color, and glycerin as a humectant. In a preliminary study, attention was focused on the possibility of finding a combination of solvents which would help liquify the cream and at the same time facilitate the diphenhydramine extraction. The most suitable system found was chloroform–water. By initially acidifying the cream, the difficulty, which is normally encountered in alkaloidal extractions using chloroform–water systems, of extraction of the active ingredient favoring the aqueous phase instead of the chloroform phase is overcome. In addition to diphenhydramine, the chloroform extract contains menthol, liquid paraffin, parabens, and the emulsifying wax. For this reason, the residue after the evaporation of chloroform was dissolved in 95% ethanol instead of water which would have given a turbid solution. Although the alcoholic solution used does not dissolve the oily particles in the residue, these are easily eliminated by filtration of the solution through a pledget of cotton wool. When the procedure was followed to analyze eight synthetic mixtures of the cream (prepared in a manner similar to commercial formulation), the results in Table I were obtained.

When the method was applied to eight commercial products, the results in Table II were obtained.

The reasonable results obtained with commercial and empirical products establish the validity of this procedure.

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