nant mare's urine. Since the quality of the diethylene glycol succinate liquid phase and packed column is critical to the success of the method, details are provided relating to their use and conditioning.

Preliminary collaborative testing is underway to evaluate the use of this procedure as a routine control method. This method has also been used for the analysis of free estrogens, which might be present in formulations containing conjugated estrogens, by simply omitting the hydrolysis step and carrying out the balance of the procedure.

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# Analysis of Pholcodine in Cough Preparations


**JACOB S. SHOHET**



**Abstract** □ A rapid and simple method for the assay of pholcodine in various syrup and linctus formulations, based on color reaction with *p*-dimethylaminobenzaldehyde, is suggested. At specified conditions, the results obtained show good reproducibility.



**Keyphrases** □ Pholcodine—analysis in cough preparations □ Antitussives—analysis of pholcodine in cough preparations □ *p*-Dimethylaminobenzaldehyde—reagent, analysis of pholcodine in cough preparations


Pholcodine resembles codeine in its action as a cough suppressant for the relief of unproductive cough. It has some pharmacological advantages over codeine including the absence of side effects such as constipation and other withdrawal symptoms (1).

Although pholcodine, like codeine, is a common component in syrups and linctus formulations, little has been written concerning the assay methods of pholcodine in pharmaceutical preparations. It is often the sole active component in a preparation[1] but may be combined with ephedrine hydrochloride, papaverine hydrochloride, promethazine, *etc.*

[1] Examples for such preparations are Copholco (Wade Co.), Falcodyl (Norton), Sancos (Sandoz), *etc.*

## DISCUSSION

Pholcodine linctus formulations have appeared in the BPC (2). The official assay procedure involves extraction with chloroform from an alkaline solution followed by nonaqueous titration. The accuracy of the method depends on the purity of inactive components, such as sucrose, sorbitol, glycerol, citric acid, and chloroform spirit, used in the formulation. Since these components constitute the majority of the linctus weight, any impurity may introduce error. Theoretically, this difficulty would be overcome by running a blank linctus containing all components except pholcodine. However, since there are so many formulations and variations, running a blank cannot be considered a practical solution. This method also suffers from the use of a relatively dilute titrant (0.02 *N* $HClO_4$) which affects the sharpness of the end-point.

No method or modification has been proposed (2) for the assay of pholcodine combined with other drugs. The nonaqueous titration cannot be applied, since the free bases of most drugs commonly formulated with pholcodine are miscible in chloroform and would be titrated simultaneously with the pholcodine. This is the case with ephedrine, pseudoephedrine, oxomemazine, promethazine, codeine, promazine, papaverine, chlorpheniramine, trimeprazine, *etc.* Other methods of analysis of pholcodine in syrups are the reaction with hexamethylenetetramine–lactose reagent (3) (ephedrine interferes) and TLC (4, 5).

The method proposed in this paper utilizes the reaction of pholcodine with *p*-dimethylaminobenzaldehyde in strong acidic medium. Under the specified conditions, reproducible results are obtained in the assay of pholcodine when formulated alone or in combination with other drugs. The present method also has the

**Table I**—Assay Results of Pholcodine in Commercial Pharmaceuticals

| Preparation | Labeled Amount, mg/ml | Found, mg/ml | Mean | *SD* | Coefficient of Variation, % |
|---|---|---|---|---|---|
| 1. Pholcodine | 1.00 | 0.960, 0.980, 0.970 | 0.970 | 0.0100 | 1.031 |
| Ephedrine hydrochloride | 1.00 | | | | |
| Ammonium chloride | 15.00 | | | | |
| 2. Pholcodine | 1.00 | 0.990, 0.970, 0.975 | 0.978 | 0.0104 | 1.063 |
| Ephedrine hydrochloride | 1.00 | | | | |
| Pyrilamine maleate | 2.50 | | | | |
| Ammonium chloride | 15.00 | | | | |
| 3. Pholcodine | 0.80 | 0.790, 0.795, 0.795 | 0.792 | 0.0032 | 0.400 |
| Ephedrine hydrochloride | 0.80 | | | | |
| 4. Pholcodine | 1.60 | 1.590, 1.600, 1.630 | 1.607 | 0.0208 | 1.294 |
| Bromoform | 0.03 | | | | |
| 5. Pholcodine | 1.00 | 0.965, 0.980, 0.995 | 0.980 | 0.0150 | 1.531 |
| Ephedrine hydrochloride | 1.60 | | | | |
| Chlorpheniramine maleate | 0.40 | | | | |
| 6. Pholcodine | 1.00 | 0.960, 0.975, 1.010 | 0.982 | 0.0256 | 0.607 |
| Papaverine hydrochloride | 0.20 | | | | |

advantage of determining pholcodine without preliminary separation from other combined drugs.

## EXPERIMENTAL

**Reagents and Chemicals**—The following were used: dilute sulfuric acid, 10% (w/v); dilute ammonium hydroxide, 10% (w/v); chloroform (analytical reagent) and ether (analytical reagent); and pholcodine standard BP.

*p*-Dimethylaminobenzaldehyde reagent was prepared just prior to use by dissolving 1 g of *p*-dimethylaminobenzaldehyde (analytical reagent) in 100 ml of sulfuric acid, 70% (w/v).

**Standard Solution**—A solution of pholcodine standard, 0.4 mg/ml, was prepared by dissolving the solid in dilute sulfuric acid.

**Sample Solution**—An accurately weighed portion of syrup or linctus, equivalent to about 20 mg of pholcodine, was transferred into a 125-ml separator. The sample was made alkaline by addition of dilute ammonium hydroxide solution. The mixture was extracted successively with four 30-ml portions of chloroform. The chloroform extracts were combined, washed with 10 ml of distilled water, and evaporated to dryness. The residue obtained was dissolved in dilute sulfuric acid and diluted to a volume of 100 ml.

**Procedure**—Volumes of 0.5 ml of sample and standard solutions were separately transferred, in triplicate, into a series of test tubes. Dilute sulfuric acid (0.5 ml) was pipetted into another test tube to serve as a blank. *p*-Dimethylaminobenzaldehyde reagent (10 ml) was added; after vigorous shaking, the tubes were transferred into a water bath maintained at 60°. After 10 min, the tubes were removed and quickly cooled to room temperature. At 0.5 hr after removal from the bath and cooling to room temperature, the absorbance was read at a wavelength of 450 nm against blank reagents.

If the syrup contains orange extract, two preliminary extractions are necessary using a mixture of 15 ml of ether and 1 ml of dilute sulfuric acid. Then the procedure is followed beginning with "The sample was made alkaline . . ." under *Sample Solution*.

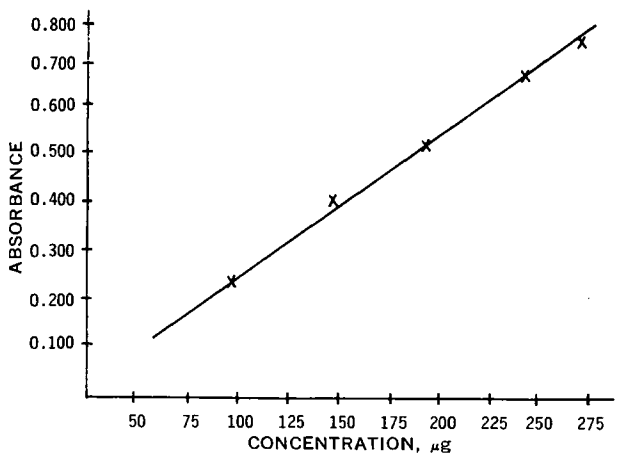


**Figure 1**—*Standard curve for pholcodine.*

## RESULTS

When this method was tested on pholcodine samples containing various amounts of the drug, strict obedience to the Beer–Lambert law was achieved over the range of 50–250 μg of pholcodine (Fig. 1).

Both the accuracy and precision of the proposed method were checked on drug mixtures simulating existing commercial pharmaceuticals. Table I represents the composition of the pharmaceuticals, the labeled amounts of the active components, the amounts of pholcodine found by triplicate analysis, and the related interpretation of data. As shown, high accuracy and a small acceptable coefficient of variation were obtained. Table I also shows that the reaction of pholcodine with *p*-dimethylaminobenzaldehyde is specific as far as combination with common drugs is concerned.

The dependence of the absorbance on time, measured on samples just cooled to room temperature, is summarized in Table II. According to Table II, the color of the reaction product is sufficiently stable. The color reaction of pholcodine with *p*-dimethylaminobenzaldehyde is as sensitive as that of codeine with the same reagent (6, 7). The reaction mixture of codeine is not stable in light, but the reaction of pholcodine is not affected by such limitations.

**Table II**—Dependence of Absorbance on Time

| Minutes | Absorbance | Change in Absorbance, % |
|---|---|---|
| 0 | 0.485 | — |
| 5 | 0.485 | — |
| 10 | 0.488 | 0.62 |
| 20 | 0.492 | 1.44 |
| 30 | 0.496 | 2.27 |
| 60 | 0.505 | 4.13 |

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# Determination of Iodochlorhydroxyquin and Corticosteroids in Pharmaceutical Formulations


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**Abstract** □ Iodochlorhydroxyquin was separated from various corticosteroids using an acetonitrile–diatomaceous earth column. Iodochlorhydroxyquin was eluted with cyclohexane, and the corticosteroid was eluted with chloroform. Iodochlorhydroxyquin was determined by both a UV absorbance method and a new complexiметric method using the nickel chelate of iodochlorhydroxyquin. The corticosteroid was determined by the blue tetrazolium and isoniazid procedures. The average percent recovery for these four methods was 100.8, 99.4, 100.7, and 99.9, respectively, for 10 known mixtures. The standard deviation for the absorbance for 10 determinations of the nickel complex was 0.002 absorbance unit (0.31%). Various characteristics of the nickel and other complexes were evaluated, including the sensitivity, solubility, and wavelength of maximum absorbance in 14 different solvents. The analyses of 23 typical products are reported, for which the standard deviation, expressed as a percentage of the amount declared, was 1.31% for the UV, 1.34% for the complexiметric, 1.49% for the blue tetrazolium, and 1.22% for the isoniazid procedures. Methods of determination in the presence of interferences are discussed.



**Keyphrases** □ Iodochlorhydroxyquin and corticosteroid formulations—partition chromatographic separation, complexiметric analysis of iodochlorhydroxyquin, blue tetrazolium analysis of corticosteroid □ Corticosteroid and iodochlorhydroxyquin formulations—partition chromatographic separation, complexiметric analysis of iodochlorhydroxyquin, blue tetrazolium analysis of corticosteroid □ Partition chromatography—separation, iodochlorhydroxyquin and corticosteroid formulations □ Nickel complex formation—analysis, iodochlorhydroxyquin after separation from iodochlorhydroxyquin and corticosteroid formulations


The "American Drug Index 1973" (1) lists 36 manufacturers of pharmaceutical formulations that contain iodochlorhydroxyquin (5-chloro-7-iodo-8-quinolinol) (I). Five of these manufacturers prepare products containing only I, while 33 manufacture products containing I plus a corticosteroid such as hydrocortisone, hydrocortisone acetate, or prednisolone.

USP XVIII (2) specifies an IR procedure that is specific for I in creams, ointments, and suppositories. NF XIII (3) requires the same IR technique to determine I in creams, lotions, and ointments containing I plus hydrocortisone and uses the blue tetrazolium method for the corticosteroid. IR methods specific for I in pharmaceutical products were described (4, 5), but these procedures are difficult to use due to the volatility and odor of the carbon disulfide and the small volumes required in the extraction step.

Other reported methods include UV absorption (3), gravimetry (3), combustion in an oxygen flask followed by titration (6), polarography (7), fluorescence (8), GLC (9), and spectrophotometry (10–12). Spectrophotometric methods depend upon the formation of a colored metallic complex with I. The iron (III) complex (10, 11) and the copper (II) complex (12) have been utilized for the determination of I in certain pharmaceutical products.

This paper reports a simple, rapid, quantitative method for the separation of I from corticosteroids by partition chromatography using the diatomaceous earth–acetonitrile column described previously (13, 14). After separation, I is determined by conversion to the nickel complex and measurement of the absorption at 406 nm while the corticosteroid is analyzed by the blue tetrazolium procedure of USP XVIII (15). Twenty-one different pharmaceutical formulations of creams, lotions, ointments, and suspensions were analyzed by the proposed method. When necessary, the method may be supplemented by the use of IR (3), GLC (9), or TLC (16) to detect the presence of impurities such as 8-hydroxyquinoline, 5-chloro-8-hydroxyquinoline, 5-iodo-8-hydroxyquinoline, 5,7-diiodo-8-hydroxyquinoline, and 5,7-dichloro-8-hydroxyquinoline.

## EXPERIMENTAL

**Equipment**—The following were used: a UV–visible recording