# Improved Method of Assay of Pyrvinium Pamoate and Its Official Preparations

By H. D. BECKSTEAD and S. J. SMITH

**A simple, rapid, and reliable assay procedure for pyrvinium pamoate and its official preparations using 80 per cent acetic acid solutions and absorption measurement at 508 mμ is presented. Results with this method are found to be more precise and can be obtained with greater facility than with the U.S.P. method. In addition a thin-layer chromatography system using Silica Gel G and dioxane–water (3:1), for the identification of pyrvinium base is described.**

THE ASSAY procedure described in the "United States Pharmacopeia" XVII (1) for pyrvinium pamoate {6-dimethylamino-2-[2-(2,5-dimethyl-1-phenyl-3-pyrrolyl)vinyl]-1-methylquinolinium 4,4′-methylenebis[3-hydroxy-2-naphthoate]} specifies the use of low actinic glassware and minimal exposure to light of the sample being analyzed. In the revised method of assay of the bulk drug (2) the use of sodium hydroxide in methoxyethanol has been deleted since this was found to depress the absorbance of solutions.

Analysis of oral suspensions and tablets containing pyrvinium pamoate requires (in addition to protecting the sample from unnecessary exposure to bright light) the use of a rather involved partition chromatography system [both phases of chloroform–hexane–water–methoxyethanol–acetic acid (30:20:10:10:1) on acid washed siliceous earth].

In this laboratory, results from analyses of official preparations of pyrvinium pamoate were found to be closely coupled to the rate of column elution. A maximum elution rate of 4 ml./min., as specified in the U.S.P., gave results with very low values and broad analytical ranges (Table I). When the elution rate was held at a maximum of 3 ml./min. (elution time: 30 to 35 min.) reasonable results were obtained. However, when duplicate samples of U.S.P. reference standard pyrvinium pamoate were eluted through columns on each of 3 consecutive days, a broad analytical range was obtained while standard deviations between days varied from 1.53 to 2.45.

This apparent sensitivity of the official method to variation in elution rates (and perhaps variation in column density), together with the resulting imprecision and inaccuracy, led to the search for a more satisfactory assay procedure.

The only other method of assay of pyrvinium pamoate apparent in the literature is that described by Alves, who used absorption measurement in dimethylformamide (DMF) and acetic acid solutions (3). In this paper a simple, rapid, and reliable spectrophotometric assay procedure for pyrvinium pamoate and its preparations employing 80% acetic acid solutions is reported.

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## EXPERIMENTAL

Light absorption measurements in stability studies were made using a Beckman model DK2 and in assays a Beckman model DU spectrophotometer. Both were calibrated previously by means of a mercury source.

**Solvents**—Reagent grade DMF, acetic acid, dimethylsulfoxide, and methoxyethanol were used as supplied. Technical grade formamide was distilled (b.p. 97–99°/14 mm. Hg) before use.

**Stability Studies**—Ten micrograms per milliliter solutions of pyrvinium pamoate (U.S.P. reference standard) in the various solvents were prepared and their absorbances recorded at suitable time intervals.

**Assay Procedure**—*U.S.P.*—Pyrvinium pamoate: assay according to the official revised procedure (2). Oral suspension and tablets: assay as described in the U.S.P. (1) observing either the maximum elution rate of 4 ml./min. or 3 ml./min.

**Proposed Method**—Standard solution: accurately weigh 10 mg. of U.S.P. reference standard pyrvinium pamoate and transfer to a 10-ml. volumetric flask. Add approximately 5 ml. of 80% acetic acid, shake mechanically for 5 min., make up to volume, and mix. Dilute 1.0 ml. of this solution to 100 ml. in a volumetric flask with 80% acetic acid.

Pyrvinium pamoate: prepare as described for the standard solution.

Oral suspension: into a 100-ml. volumetric flask weigh by difference 0.7 Gm. oral suspension. Add 50 ml. 80% acetic acid, shake mechanically for 5 min., make up to volume, and mix. Dilute 10 ml. of this solution to 100 ml. with 80% acetic acid in a volumetric flask.

Tablets: determine the average weight of 20 tablets. Powder 5 tablets and transfer an aliquot equivalent to about 50 mg. pyrvinium base to a 100-ml. volumetric flask. Add 50 ml. 80% acetic acid, shake mechanically for 5 min., make up to volume, mix, and filter through Whatman No. 1 paper, or equivalent. Pipet 1 ml. of the filtrate into a 100-ml. volumetric flask and dilute to volume with 80% acetic acid.

Measure the absorbance of all solutions at 508 mμ on a suitable instrument using 80% acetic acid as a blank.

TABLE I.—ANALYSIS OF PYRVINIUM PAMOATE

| Method → | U.S.P. XVII Method, 4 ml./min. Max. Elution | | | U.S.P. XVII Method, 3 ml./min. Max. Elution | | | 80% Acetic Acid | | | | 80% Acetic Acid | | | | N,N-Dimethylformamide | | | | N,N-Dimethylformamide | | | | Dimethylsulfoxide[e] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glassware → | Low Actinic | | | Low Actinic | | | Low Actinic | | | | Normal | | | | Low Actinic | | | | Normal | | | | Low Actinic | |
| Preparation | Av.,[a] % | Range, % | S.D. | Av.,[a] % | Range, % | S.D. | Av.,[a] % | Range, % | S.D. | | Av.,[a] % | Range, % | S.D. | | Av.,[a] % | Range, % | S.D. | | Av.,[a] % | Range, % | S.D. | | Av.,[b] % | Range, % | S.D. |
| Powder | 95.72 | 5.05 | 1.82[d] | 98.08 | 3.15 | 1.15[e] | 99.49 | 0.64 | 0.25 | | 105.51 | 7.55 | 2.80 | | 99.50 | 0.32 | 0.13 | | 97.08 | 3.42 | 1.25 | | 106.19 | 20.97 | 3.29 |
| Oral suspension No. 1 | 85.64 | 12.42 | 4.30 | 94.01 | 6.29 | 2.20 | 92.73 | 0.60 | 0.26 | | 93.19 | 2.93 | 1.06 | | 92.53 | 0.60 | 0.26 | | 88.81 | 3.42 | 1.19 | | 102.39 | 1.07 | 0.57 |
| Oral suspension No. 2 | 89.30 | 5.75 | 2.00 | ... | ... | ... | 91.93 | 0.82 | 0.28 | | 92.45 | 2.05 | 0.72 | | 92.03 | 0.70 | 0.30 | | 90.69 | 3.54 | 1.36 | | 102.81 | 0.84 | 0.47 |
| Oral suspension No. 3 | 80.63 | 8.43 | 3.41 | 97.02 | 5.13 | 2.00 | 103.88 | 0.59 | 0.23 | | 101.08 | 0.62 | 0.24 | | 104.48 | 0.58 | 0.20 | | 101.15 | 1.97 | 0.82 | | 101.95 | 0.49 | 2.58 |
| Oral suspension No. 4 | 79.93 | 15.63 | 5.73 | 92.73 | 4.66 | 1.27 | 91.63 | 1.10 | 0.43 | | 90.87 | 1.01 | 0.38 | | 91.80 | 0.60 | 0.23 | | 88.96 | 0.77 | 0.30 | | 105.05 | 5.06 | 0.29 |
| Tablets No. 1 | 87.39 | 13.37 | 5.10 | 89.99 | 4.20 | 1.64 | 97.30 | 0.60 | 0.22 | | 99.21 | 1.52 | 0.64 | | 98.68 | 0.90 | 0.37 | | 95.97 | 2.75 | 1.05 | | 99.39 | 19.94 | 11.10 |
| Tablets No. 2 | 82.81 | 9.54 | 3.69 | 84.27 | 3.44 | 1.18 | 93.65 | 1.10 | 0.72 | | 94.06 | 7.14 | 2.29 | | 94.41 | 0.84 | 0.30 | | 93.21 | 3.18 | 1.06 | | 110.41 | 17.36 | 9.40 |
| Simulated suspension[f] | ... | ... | ... | ... | ... | ... | 99.51 | 0.97 | 0.35 | | ... | ... | ... | | ... | ... | ... | | ... | ... | ... | | ... | ... | ... |
| Simulated tablet[f] | ... | ... | ... | ... | ... | ... | 100.14 | 0.70 | 0.29 | | ... | ... | ... | | ... | ... | ... | | ... | ... | ... | | ... | ... | ... |

[a] Average of six assays. [b] Average of three assays. [c] Formamide was also tried; results for powder were 108.12%; 106.56%; 102.64%. Mean: 106.56%; standard deviation: 3.28. [d] Superceded procedure for powder. [e] Revised procedure for powder. [f] Corresponding in composition to a commercial preparation.

**Calculations**—For pyrvinium pamoate:

$$\frac{A \times \text{wt. of ref.}}{B \times \text{wt. of sample taken}} \times 100 = \% \text{ (w/w) pyrvinium pamoate} \quad \text{(Eq. 1)}$$

For the oral suspension:

$$\frac{A \times SG \times 0.6644}{B \times \text{wt. of sample taken}} \times 100 = \% \text{ (w/v) pyrvinium base} \quad \text{(Eq. 2)}$$

For tablets:

$$\frac{A \times 100 \times \text{av. wt./tablet (Gm.)} \times 0.6644}{B \times \text{wt. of sample taken (Gm.)} \times \text{labeled wt. (mg.)}} \times 100 = \% \text{ (w/w) pyrvinium base/tablet} \quad \text{(Eq. 3)}$$

where

$A$ = absorbance of sample solution,
$B$ = absorbance of standard solution,
SG = specific gravity of the suspension,
0.6644 = ratio of the formula weight or pyrvinium base to one-half of the molecular weight of pyrvinium pamoate (the pamoate moiety being dibasic).

### Thin-Layer Chromatography

**Plates**—Prepare plates with standard equipment for thin-layer chromatography (*cf. Reference 4*). To obtain five plates 20 × 20 cm., mix 25 Gm. of Silica Gel G (Merck) with 50 ml. of 0.5 *N* NaOH. Apply the slurry to plates (layer thickness about 250 $\mu$), air dry, and activate within 1 hr. of use by heating at 110° for 20 min.

**Solvent System**—1,4-Dioxane–water (3:1 v/v).

**Spray Reagents**—(*a*) Mix 20 ml. of 10% w/v aqueous $FeCl_3$ and 10 ml. 5% w/v aqueous $K_3Fe(CN)_6$ with 70 ml. water. (*b*) Prepare 2 *N* HCl.

**Preparation of Samples**—Dissolve sufficient bulk drug, suspension, or powdered tablet in methoxyethanol to give a concentration of about 1 mg./ml. pyrvinium pamoate. If the solution is turbid, filter or centrifuge it before spotting.

**Chromatographic Procedure**—Equilibrate a suitable jar with the chromatographic solvent. Spot 5 $\mu$l. of solutions being examined next to a similar amount of U.S.P. reference pyrvinium pamoate. Insert the plate in the chromatographic jar and allow the solvent to rise to a height of 15 cm. (about 2 hr.). Air dry plates and view under shortwave U.V. light or treat with spray (*a*) followed by spray (*b*).

## RESULTS AND DISCUSSION

The revised assay of pyrvinium pamoate has deleted the use of sodium hydroxide in the methoxyethanol. Figures 1 and 2 clearly indicate the deleterious effects of this base. Furthermore, the figures indicate that the degradative effects of the base are greatly in excess of breakdown due to actinic activity, since the sample curves obtained for solutions stored in both low actinic and normal glassware are superimposable (Figs. 1 and 2). The deleterious activity of sodium hydroxide is further demonstrated when the lower analytical results from the now-superseded procedure for pyrvinium pamoate powder (1) are compared with data obtained from the revised method (2) (Table I).

The use of heat recommended to aid solution of

pyrvinium pamoate may reduce the accuracy of the official method of assay due to possible breakdown of the drug. Furthermore, analyses of oral suspensions and tablets require that aliquots of material subjected to assay be adsorbed on chromatographic support prior to transfer to the column. Each of these factors increases the exposure of the sample to light and introduces the possibility of error. A major factor causing variability in assay results employing the chromatographic column was found to be variation in the rate of column elution. Elution of the column at a maximum rate of 4 ml./min. as specified in the U.S.P. gave results which were, in all cases, below U.S.P. limits and exhibited broad analytical ranges (Table I). In all analyses pink material was noted to remain on the column. However, when the elution rate was kept at a maximum of only 3 ml./min. (elution time: 30–35 min.), results and standard deviations were found to be reasonable (Table I).

Relatively minor variations in elution rates, and perhaps also in the density of the column packing, may have given rise to relatively broad ranges in the U.S.P. assay results. This can be inferred from the data obtained when duplicate samples of U.S.P. reference material were chromatographed in a uniform manner on 3 consecutive days. Standard deviations between pairs of results were 1.82, 1.53, and 2.45. The six results examined *in toto* had a standard deviation of 3.49. The variation in absorbance of the eluates was the equivalent of an analytical range of 9.29%. Thus in addition to variation within the sample itself inconsistencies are even obtained with the reference standard using the official method.

To find a suitable medium for the assay of pyrvinium pamoate, the stability of the drug in 80% acetic acid, DMF, dimethylsulfoxide, and methoxyethanol in low actinic and normal glassware was determined (Figs. 1 and 2). Solutions kept in low actinic glassware were found to be quite stable, in all cases exhibiting at least 97% of the original absorption at 508 mμ after 60 min. Solutions stored in normal glassware were somewhat less stable (Fig. 2), showing a rapid initial decrease in absorption and thereafter their absorbances remaining fairly constant. No satisfactory absorption curve could be obtained with formamide solutions of the drug, although the trend of readings appeared to indicate that the order of stability was very similar to that in solvents listed above.

Concomitant with these studies, assays of pyrvinium pamoate and its preparations were carried out in both low actinic and normal glassware employing the five solvent systems listed in Table I. Results obtained are shown therein. As anticipated, low actinic glassware always afforded more reliable results than normal glassware. With formamide and also with dimethylsulfoxide solutions, satisfactory results could not be obtained. Assays were calculated as shown under *Experimental*, and for consistency all figures in Table I have been quoted as per cent of labeled claim. To conform with the U.S.P. procedure, values quoted for the suspension (Eq. 2; Table I) should be divided by 100 to read grams per 100 ml.

The data in Table I indicate that results from analyses carried out in dimethylsulfoxide and formamide (powder only) gave the highest values.

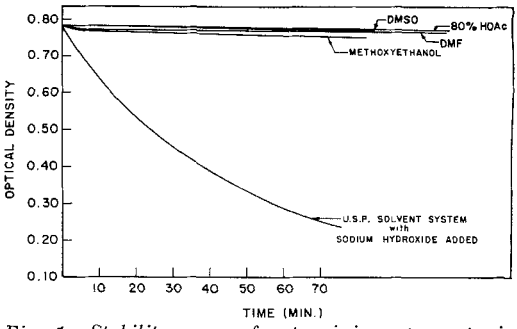


*Fig. 1—Stability curves for pyrvinium pamoate in low actinic glassware.*

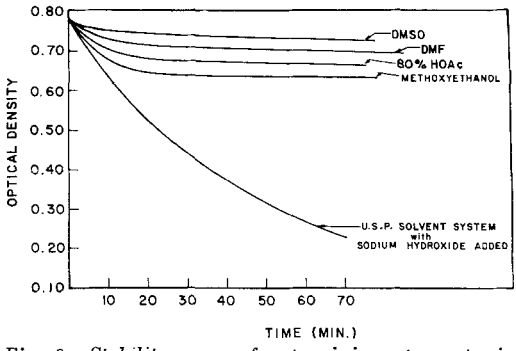


*Fig. 2—Stability curves for pyrvinium pamoate in normal glassware.*

In addition, results from both of these methods exhibited, in some instances, large standard deviations. Analyses based on the use of both 80% acetic acid and DMF in low actinic glassware showed consistently smaller experimental ranges and smaller standard deviations (probability $<0.05$) than analyses based on the other systems. These ranges were also smaller than those obtained from assays using 80% acetic acid and DMF in normal glassware. There is little difference in the variability between 80% acetic acid and DMF solvent systems when low actinic glassware (aluminum foil wrapped around the volumetric flask suffices) is employed. However, the greater stability of pyrvinium pamoate in acetic acid solution compared to DMF solution (Fig. 1), coupled with the more general availability, lower toxicity, and lower cost of acetic acid, leads to the proposal of the acetic acid solvent system as the medium of choice for the assay of pyrvinium pamoate and its preparations.

Most of the preparations of pyrvinium pamoate examined did not completely dissolve in methoxyethanol. In addition, when some brands were brought into contact with the solvent, a copious precipitate was formed which appeared to adsorb pyrvinium pamoate, thus making it difficult to obtain homogeneous aliquots for subsequent chromatography. This source of error was eliminated by the use of 80% acetic acid in which all excipients were found to be completely soluble.

To confirm the accuracy of the proposed method a simulated tablet as well as a suspension was prepared and assayed according to the new method.

The values shown in Table I are per cent recovery. These results appear to confirm that the proposed method is both accurate and precise. In addition to its greater precision, it is also much faster than the official method; about 8 min. compared with near 40 min. in the latter.

Thin-layer chromatographic examination of official dosage forms of pyrvinium pamoate and a U.S.P. reference standard indicated that the drug consists of a number of components. Pyrvinium pamoate contained in official preparations separated into two major red spots at $R_f$ 0.33 and 0.44 and a minor red spot at $R_f$ 0.50. Upon spraying, these spots stained intense blue, while pamoic acid, previously visible only by fluorescence at $R_f$ 0.50, stained light blue. U.S.P. reference standard pyrvinium pamoate was found to contain all of the above components as well as another minor red constituent, staining intense blue, at $R_f$ 0.15. Pamoic acid could be separated from the red material also appearing at $R_f$ 0.50 by using 0.1 *N* NaOH instead of 0.5 *N* NaOH in preparing the chromatographic plates (new $R_f$ of pamoic acid: 0.69).

The occurrence of a greater number of red spots than expected, on the basis of two isomeric configurations of the compound, should not be attributed to the degradative effect of sodium hydroxide in the aqueous system since, for example, chromatography with chloroform–ethanol (7:3) on neutral Silica Gel G (Merck), although giving very poor separation, clearly indicated three red constituents in commercial and four such components in U.S.P. reference standard pyrvinium pamoate. These compositional characteristics of the drug will be examined further.

## REFERENCES

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# Analysis of Combinations Containing Phenylephrine in Liquid Dosage Forms

By K. O. MONTGOMERY, P. V. JENNINGS, and M. H. WEINSWIG

**A method is presented for the isolation, separation, and determination of phenylephrine, an antitussive, and an antihistamine in commercial products containing the combination. All three compounds are extracted with strong cation exchange resin AG 50W-X4. The phenylephrine is eluted from the resin bed with 8.0 *N* phosphoric acid. The antitussive is then eluted with 1.0 *N* hydrochloric acid in 60 per cent methanol in water. Finally the antihistamine is eluted with 3.5 *N* hydrochloric acid in 40 per cent methanol in water. The compounds are determined by subjecting the eluates to ultraviolet spectrophotometry. The method is used successfully on several commercial products.**

THE ASSAY of complex formulations containing several active ingredients is usually a difficult and time-consuming problem. This is especially true if the active ingredients are similar in their chemical and physical properties. Ion-exchange chromatography was selected as the method of choice for this assay because of its ability to isolate completely the cationic active ingredients from the inactive ingredients, and separate these active ingredients by selective elution. Each ingredient was then quantitatively eluted in a form suitable for determination by ultraviolet spectrophotometry.

Ion-exchange chromatography has been used extensively to accomplish difficult separations of inorganic ions. Hofer (1) used an ion-exchange resin[1] to absorb calcium and magnesium from tissue extracts. Magnesium was then eluted with 2 *N* hydrochloric acid and calcium was eluted with 3 *N* hydrochloric acid. Grasselly (2) used anion-exchange resins to separate iron, aluminum, manganese, calcium, and magnesium. Pollard (3) separated magnesium, strontium, barium, and calcium on the ion-exchange resin[1] utilizing a gradient elution technique. Pitstick and associates (4) separated magnesium, cobalt, zinc, copper, and iron with anion-exchange resins.

Most of the references to organic separations utilize a simple adsorption–elution technique to separate anions or cations from neutral components. Wang and Hunter (5) assayed 11 different alkaloids separately by adsorbing them on an ion-exchange resin,[2] eluting with glacial acetic acid, and titrating the eluate with perchloric acid.

Morphine has been separated from the nonphenolic alkaloids of opium utilizing ion exchange (6). Blake and associates (7, 8) applied tech-

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[1] Marketed as Dowex 50-X8 by the Dow Chemical Co., Midland, Mich.
[2] Marketed as Amberlite IRC-50 by Rohm & Haas, Philadelphia, Pa.