Analysis of Phenylephrine and Phenylpropanolamine Hydrochlorides in Combination

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Abstract □ An accurate and precise method for determination of phenylephrine hydrochloride and phenylpropanolamine hydrochloride is described. This method is based on measuring the aryl aldehydes, which are selectively extracted after periodate oxidation of the two phenylethanolamines. It is sufficiently sensitive for individual dosage unit measurements. No assay interferences were apparent with a stressed mixture where phenylephrine was acetylated by aspirin.

Keyphrases □ Phenylephrine–phenylpropanolamine combination—assay in aspirin mixture □ Phenylpropanolamine–phenylephrine combination—assay in aspirin mixture □ Aspirin mixture—determination of phenylephrine and phenylpropanolamine □ UV spectrophotometry—determination, phenylethanolamines in aspirin mixture □ Periodate oxidation—assay, separation of phenylethanolamines

Periodate oxidation of vicinal glycols, α-hydroxyketones, and α-primary or secondary aminoalcohols is a well-known reaction. Heimlich *et al.* (1) utilized this reaction for the measurement of phenylpropanolamine in urine by spectrophotometric analysis of the oxidation product, benzaldehyde. Alkaline periodate oxidation also was used by Wallace (2) to determine phenylpropanolamine and related compounds in biologic specimens. The assay of phenylephrine hydrochloride and phenylpropanolamine hydrochloride in separate formulations using periodate oxidation was described by Chafetz (3). A modified version of this oxidative technique is reported here for the assay of these two drugs in combination in a mixture with aspirin. By careful adjustment of pH, selective separation of the two oxidation compounds, benzaldehyde and *m*-hydroxybenzaldehyde, is quantitative.

EXPERIMENTAL

Apparatus—A Beckman DBG with a 25.4-cm. (10-in.) recorder was used for UV scans and absorbance measurements.

Reagents—Analytical grade reagents were used.

0.3 N Sodium Metaperiodate—Dissolve 3.2 g. of sodium metaperiodate in 50 ml. of water.

pH 7.5 Buffer (1.0 M)—Dissolve 69.0 g. of monobasic sodium phosphate in 250 ml. water. Adjust the pH to approximately 7.5 using 10% NaOH. The final adjustment can be made with 0.1 *N* NaOH. Then add water to make 500 ml. total volume.

1.0 N Sodium Hydroxide—Dissolve 40.0 g. of sodium hydroxide in 1.0 l. of water.

1.0 N Hydrochloric Acid—Add 83 ml. of concentrated hydrochloric acid to enough water to make 1.0 l.

Standard Solutions—Prepare three solutions containing both phenylephrine hydrochloride[1] and phenylpropanolamine hydrochloride[2] in the following amounts, respectively: 25 and 60 mcg./ml., 50 and 120 mcg./ml., and 75 and 180 mcg./ml.

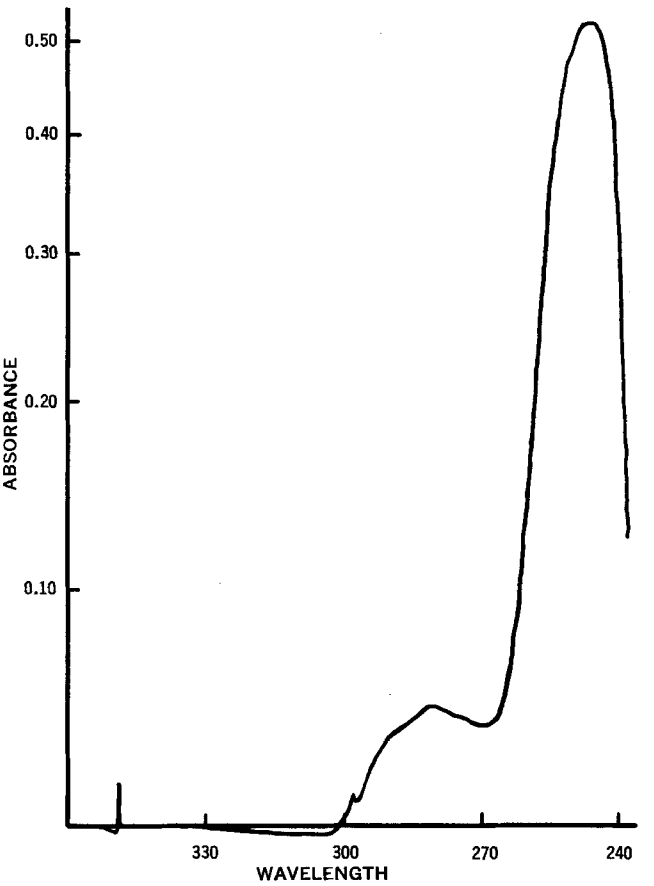

Figure 1—*Absorption spectrum of benzaldehyde in chloroform (8.0 mcg./ml.).*

Periodate Oxidation Method—A 10-ml. aliquot of a filtered solution containing not more than 0.75 mg. of phenylephrine hydrochloride and 1.8 mg. of phenylpropanolamine hydrochloride is pipeted into a 60-ml. separator. To the sample aliquot and to a 10-ml. aliquot of distilled water (reagent blank), add 1.5 ml. 1.0 *N* sodium hydroxide and mix. Then add 1.5 ml. of 0.3 *N* sodium metaperiodate, mix, and let stand 10–30 min. (The oxidation is complete at 10 min. and remains stable for at least 30 min.). Extract three times with 20 ml. of chloroform. Filter the combined chloroform extract through chloroform-washed cotton into a 200-ml. volumetric flask. Wash the cotton with a small amount of chloroform and then bring the total volume up to 200 ml. with chloroform. Measure the absorbance of this solution at 247.5 nm. *versus* the reagent blank and use it for the calculation of the concentration of phenylpropanolamine hydrochloride. The UV spectrum obtained by this method is characteristic of benzaldehyde (Fig. 1).

[1] Winthrop Laboratories.

[2] Penick.

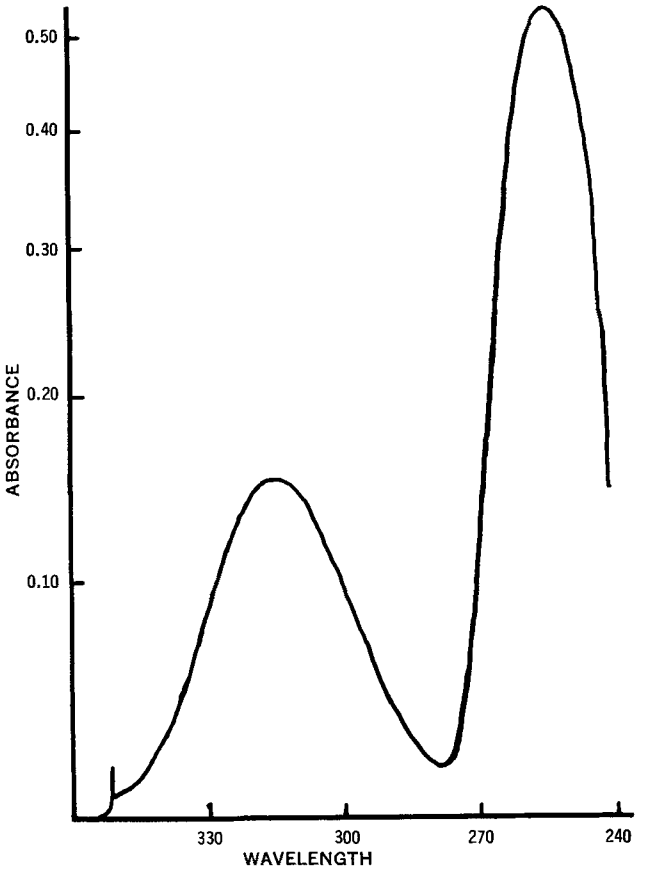

Figure 2—*Absorption spectrum of* m-*hydroxybenzaldehyde in chloroform (12.5 mcg./ml.).*

Adjust the pH of the aqueous phase of the extraction to 7.5 ± 0.5 by adding 5 ml. of the pH 7.5 phosphate buffer (1 *M*) and 1.0 ml. of 1.0 *N* hydrochloric acid. Extract three times with 15 ml. of chloroform. Filter the combined chloroform extract through chloroform-washed cotton into a 50-ml. volumetric flask. Wash the cotton with less than 5 ml. of chloroform and then bring the total volume up to 50 ml. with chloroform. Measure the absorbance of this solution at 252.5 nm. *versus* the reagent blank and use it for the calculation of the concentration of phenylephrine hydrochloride. The UV spectrum of this chloroform solution is characteristic of *m*-hydroxybenzaldehyde (Fig. 2).

For calculations, standard curves are obtained by assaying 10.0-ml. aliquots of the three standard solutions. From these curves, the measured absorbances can be converted to micrograms per milliliter; then, by multiplying by the appropriate dilution factor, the assay values of the phenylephrine hydrochloride and phenylpropanolamine hydrochloride are determined.

TLC—This procedure is a modified form of the method used by Troup and Mitchner (4). The absorbent used is silica gel F-254[3]. The solvent system is the chloroform layer from a mixture of chloroform–glacial acetic acid–methanol–water (85:20:8:20) which has been equilibrated for 16 hr. The spotting solution is a 4:1 mixture of ethanol–acetone, of which enough is spotted to leave 10 mcg. of phenylephrine hydrochloride and 25 mcg. of phenylpropanolamine hydrochloride on the plate. Detection is made by spraying the plate with a diazo reagent (25 ml. of 0.3% *p*-nitroaniline plus 1.5 ml. of 5% sodium nitrite). The plate is then heated at 70° for 15 min., after which the plate is sprayed with 20% sodium carbonate. Acetylated phenylpropanolamine and the mono-, di-, and triacetyl derivatives of the phenylephrine can be detected in this manner (Fig. 3). For reference standards, three acetylated forms of phenylephrine hydrochloride were synthesized according to the methods reported by Troup and Mitchner (4). Phenylpropanolamine hydrochloride was readily acetylated by heating it on a steam table in the presence of acetic anhydride.

[3] Merck.

Aspirin Assay—The aspirin was assayed by a nonaqueous titration method, using dimethylformamide as the solvent and lithium methoxide (0.1 *N*) as the titrant. Since both phenylephrine hydrochloride and phenylpropanolamine hydrochloride are also titrated by the lithium methoxide, a correction factor was used in the calculations.

Free Salicylic Acid Assay[4]—A weighed sample, equivalent to 400 mg. of aspirin, is transferred to a 125-ml. separator. To this, add 50.0 ml. of solvent (1:1 *n*-pentane–ether)[5] and shake for 1 min. Draw off the solids and wash the solvent two times with 10 ml. of distilled water, shaking each for 15 sec. Discard the water washes. Evaporate a 20-ml. aliquot of the pentane–ether extract in a stream of air or nitrogen. Dissolve the residue in 2.0 ml. of 95% ethanol. To this, add 20 ml. of distilled water, mix, and immediately add 0.4 ml. of iron reagent (to 8.0 ml. of 10% ferric ammonium sulfate, add 7.0 ml. of 1 *N* hydrochloric acid and dilute to 100 ml. with distilled water). Mix and immediately determine the absorbance *versus* a reagent blank in a suitable spectrophotometer at 526 nm. Compare with a standard curve prepared by assaying 200, 300, and 400 mcg. of salicylic acid in 2.0 ml. of 95% ethanol with water and iron reagent as described previously.

RESULTS AND DISCUSSION

Both phenylephrine hydrochloride and phenylpropanolamine hydrochloride, together in the presence of aspirin, can be quantitatively measured by this periodate assay. The standard curves obtained are linear and reproducible over the ranges indicated. Absorbances representative of the solutions used for the standard curves are as follows: phenylephrine hydrochloride—5 mcg./ml.

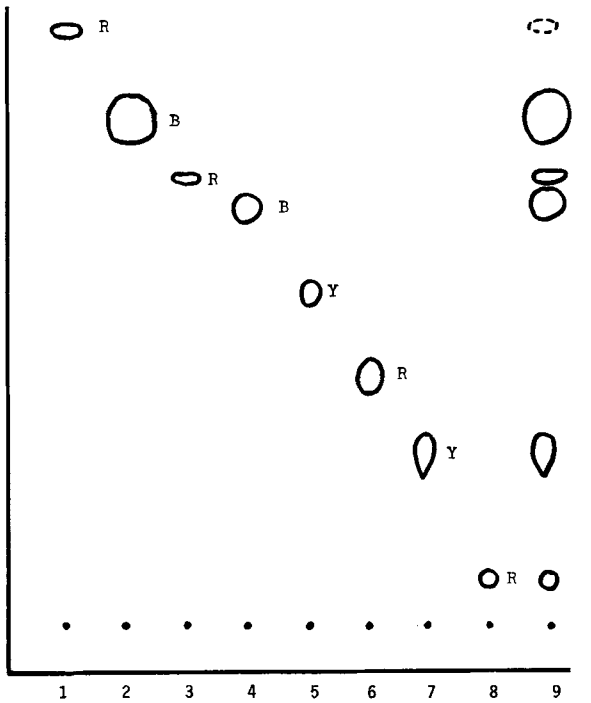

Figure 3—*TLC of components of stability sample with their respective degradation products and a typical stressed stability sample. Key: 1, triacetylphenylephrine* [N-(m,β-*diacetoxyphenethyl*)-N-*methylacetamide*]; *2, aspirin; 3, diacetylphenylephrine* [N-(m-*acetoxy*-β-*hydroxyphenethyl*)-N-*methylacetamide*]; *4, salicylic acid; 5, diacetylated phenylpropanolamine; 6,* N-*monoacetylphenylephrine* [N-(m,β-*dihydroxyphenethyl*)-N-*methylacetamide*]; *7, phenylpropanolamine hydrochloride; 8, phenylephrine hydrochloride; and 9, sample stressed at 70° for 42 days. Colors: Y = yellow, R = red, and B = brown.*

[4] This method was developed by the Analytical Chemistry Department, Sterling-Winthrop Research Institute.

[5] The *n*-pentane should be passed through a column of silica gel before it is used.

Table I—Results of Stressing Mixture of Phenylephrine Hydrochloride, Phenylpropanolamine Hydrochloride, and Aspirin at 70°

Assay	Original	21 Days	42 Days
Phenylephrine hydrochloride	5.0 mg.	3.7 mg.	2.3 mg.
Phenylpropanolamine hydrochloride	12.6 mg.	12.5 mg.	11.4 mg.
Aspirin	325.8 mg.	—	—
Free salicylic acid	0.03%	0.63%	2.74%
Free salicylic acid (theoretical)[a]	—	0.88%	2.67%
Moisture (Karl Fischer titration)	0.25%	—	—

TLC

21 Days

1. Trace amount of mono- and triacetylated phenylephrine
2. Moderate amount of diacetylated phenylephrine
3. No detectable amount of acetylated phenylpropanolamine

42 Days

1. No detectable amount of monoacetylated phenylephrine
2. Large amount of diacetylated phenylephrine
3. Trace amount of triacetylated phenylephrine
4. No detectable amount of acetylated phenylpropanolamine

[a] Calculated by using the assayed amount of phenylephrine hydrochloride and by assuming that salicylic acid is formed only during the acetylation of phenylephrine by aspirin and that this acetylation is the only cause of phenylephrine decrease in potency.

(0.209), 10 mcg./ml. (0.424), and 15 mcg./ml. (0.634); phenylpropanolamine hydrochloride—3 mcg./ml. (0.209), 6 mcg./ml. (0.411), and 9 mcg./ml. (0.612). The assays and their standard deviations, based on six replicate values for the low and high quantities of phenylephrine hydrochloride, are 0.249 ± 0.011 mg. and 0.745 ± 0.006 mg. Likewise, for phenylpropanolamine hydrochloride, they are 0.567 ± 0.011 mg. and 1.81 ± 0.01 mg.

To obtain an indication of the validity of the assay, the following mixture was stressed at 70°: phenylephrine hydrochloride, 5.0 mg., phenylpropanolamine hydrochloride, 12.5 mg.; and aspirin, 325 mg. The mixture was assayed at 0, 21, and 42 days for phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and free salicylic acid. Aspirin was assayed in only the unstressed mixture. TLC was used to detect any acetylated forms of phenylephrine or phenylpropanolamine.

The results of the stressing test (Table I) indicate that the periodate assay is capable of accurately measuring the decomposition of phenylephrine hydrochloride and phenylpropanolamine hydrochloride. In this case, the phenylephrine hydrochloride is being acetylated, as indicated by TLC (Fig. 3). Also, the increase in free salicylic acid corresponds to the theoretical amount that would be formed by the acetylated amount of phenylephrine hydrochloride, as indicated by the assay results, assuming that acetylation of phenylephrine is the only reaction. The phenylpropanolamine hydrochloride proves to be quite stable under these conditions as compared to phenylephrine hydrochloride. The acetylated phenylephrine and phenylpropanolamine compounds were tested for possible interferences in the assay procedure. As expected, none of the acetylated compounds was oxidizable with the periodate, and no absorbance was obtained in the range of interest (360–240 nm.) under the conditions of assay. Also, there were no interferences due to the presence of the excipients: starch, sugar, and talc.

SUMMARY

A periodate oxidation assay method for phenylephrine hydrochloride and phenylpropanolamine hydrochloride in combination with aspirin was shown to be accurate, sensitive, and precise. This assay is based on the oxidation of the two phenylethanolamines to their respective aryl aldehydes, which are selectively extracted by pH manipulation. The assay was also proven to be stability indicating by following the decomposition of a stressed mixture of phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and aspirin. For the usual dosages used in capsule and tablet forms of these two drugs, this assay procedure is sufficiently sensitive for individual dosage unit measurements.

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