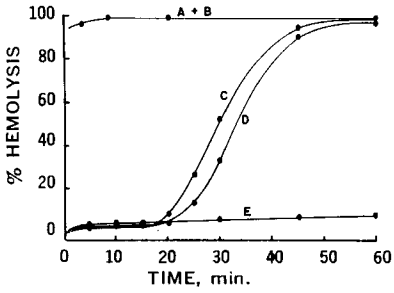

Fig. 5—Kinetic study of the hemolytic activity of various test solutions. Key: A, water; B, 15% DMSO; C, 110 mcg./ml. chlorhexidine diacetate in 0.6% NaCl; D, 110 mcg./ml. chlorhexidine diacetate, 0.6% NaCl, and 1% DMSO; E, 110 mcg./ml. chlorhexidine diacetate, 0.6% NaCl, and 15% DMSO.

ing concentrations of DMSO exceeding 35% caused the denaturation of blood, as noted in an earlier report (14).

As can be seen in Fig. 5, both distilled water and 15% DMSO induced total hemolysis within the first 5 min. of the study. Hemolysis occurring in the 15% DMSO test solution can be largely attributed to osmotic hemolysis resulting from the penetration of this hygroscopic material through the erythrocytic membrane (14). The addition of 0.6% sodium chloride to DMSO solutions of less than 35% concentration prevents osmotic hemolysis (14); hemolysis occurs in solutions containing greater amounts of DMSO due to the cytotoxic action of the more concentrated solutions. The addition of 1% DMSO to a solution containing 0.6% sodium chloride and 110 mcg./mg. of chlorhexidine diacetate slowed the rate of hemolysis induced by the latter and the addition of 15% DMSO all but prevented the chlorhexidine diacetate-induced hemolysis. The suspicion that DMSO exerts an independent cellular effect such as to prevent the preservative from exerting its own cellular activity has been bolstered by recent data obtained in this laboratory (21) showing a similar influence of DMSO on the hemolytic activities of such chemically diverse preservatives as phenylethyl alcohol, benzalkonium chloride, *p*-chlorophenol, benzyl alcohol, phenylmercuric acetate, and *m*-cresol. In each instance, the concentration of DMSO required to all but eliminate the hemolysis normally induced by the hemolytic concentrations of these preservatives was between 7 and 15%. Spectrophotometric analysis failed to reveal complexation between the DMSO and the chlorhexidine diacetate, further adding support to interference by DMSO by a cellular mechanism.

REFERENCES

(1) Davies, G. E., Francis, J., Martin, A. R., Rose, F. L., and Swain, G., *Brit. J. Pharmacol.*, **9**, 192(1954).
(2) Hugo, W. B., and Longworth, A. R., *J. Pharm. Pharmacol.*, **16**, 655(1964).
(3) *Ibid.*, **16**, 751(1964).
(4) *Ibid.*, **17**, 28(1965).
(5) Hugo, W. B., and Foster, J. H. S., *ibid.*, **16**, Suppl. 124T(1964).
(6) Brown, M. R. W., and Richards, R. M. E., *ibid.*, **16**, Suppl. 41T(1964).
(7) *Ibid.*, **16**, Suppl. 51T(1964).
(8) Wiseman, D., *ibid.*, **16**, Suppl. 56T(1964).
(9) Rye, R. M., and Wiseman, D., *ibid.*, **17**, 295(1965).
(10) Brown, M. R. W., and Norton, D. A., *J. Soc. Cosmetic Chemists*, **16**, 369(1965).
(11) Ansel, H. C., and Cadwallader, D. E., *J. Pharm. Sci.*, **53**, 169(1964).
(12) Ansel, H. C., *ibid.*, **54**, 1159(1965).
(13) Cadwallader, D. E., and Ansel, H. C., *ibid.*, **54**, 1010 (1965).
(14) Ansel, H. C., and Leake, W. F., *ibid.*, **55**, 685(1966).
(15) Grosicki, T. S., and Husa, W. J., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 632(1954).
(16) Herschler, R. J., unpublished data; through Jacob, S. W., Bischel, M., and Herschler, R. J., *Current Therap. Res.*, **6**, 193(1964).
(17) Jacob, S. W., Bischel, M., and Herschler, R. J., *Current Therap. Res.*, **6**, 193(1964).
(18) *Ibid.*, **6**, 134(1964).
(19) Horita, A., and Weber, L. J., *Life Sci.*, **3**, 1389(1964).
(20) Ben, M., *et al.*, *Pharmacologist*, **6**, 189(1964).
(21) Ansel, H. C., and Cabre, G. E., unpublished data.

Drug Standards

Determination of Phenylephrine in Combinations with Other Drugs

By JOSEPH LEVINE and THOMAS D. DOYLE

Phenylephrine combines with di-(2-ethylhexyl) phosphoric acid to form an ion-pair which can be extracted with immiscible solvents. Application of this means of extraction in conjunction with partition chromatography provides a method for the analysis of phenylephrine in its various combinations with other drugs.

THE ISOLATION and determination of phenylephrine by the usual methods for the analysis of alkoids[1] is not feasible because of the highly unfavorable extraction characteristics of the compound. Most of the published procedures for its analysis are not applicable in the presence of the drugs with which it is compounded in many formulations. Pratt (2), applying the acetylation procedure of Welsh (3), converted phenylephrine in aqueous solution to the diacetyl derivative, which is readily extracted with im-

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[1] Because alkaloids and many pharmaceutically important synthetic organic bases present identical analytical problems, and since the word "alkaloid" is reserved for the natural product, it has been proposed (1) that the word "alkoid" be used to encompass the entire group.

miscible organic solvents. Clark and Rosenberg (4) and Hyatt (5) modified the acetylation procedure to permit its application to combinations containing various antihistamines. In these procedures, an alkaline solution of the sample is used as the immobile phase in a chromatographic column. A preliminary chloroform elution removes the antihistamines only. A chloroform solution of acetic anhydride passed over the column acetylates the phenylephrine *in situ*, and the resultant diacetyl compound is readily eluted. This procedure is inapplicable to samples containing acetaminophen, with which phenylephrine is frequently combined. Smith (6) separated phenylephrine from a number of other drugs by eluting them with chloroform from a partition chromatographic column in which the ratio of diatomaceous earth[2] to immobile phase is much higher than that usually employed; he then washed off the phenylephrine with ethanol. He reported the method to be unsuccessful for mixtures containing several accompanying drugs, including sodium benzoate and potassium guaiacolsulfonate. Hiskey and Levin (7) described a colorimetric procedure applicable directly to solutions without prior isolation of the phenylephrine. Reaction with 4-aminoantipyrine and potassium ferricyanide in sodium bicarbonate solution produces a red color, which can be measured directly. This method must be applied with great care, since the intensity of the color developed varies significantly with the concentration of each of the reagents, and both the intensity of the color and the wavelength of maximum absorbance change rapidly with time. Acetaminophen also produces a color with the reagent, while phenolphthalein is converted to its red salt; therefore, this method is inapplicable in the presence of these drug materials.

Phenylephrine may be extracted readily by converting it to an ion-pair with di-(2-ethylhexyl) phosphoric acid (DEHP).[3] Use of this reagent is based upon a report by Temple and Gillespie (8) that chloroform solutions of this "liquid ion-exchanger" will extract physiologically active amines from aqueous solutions at pH 4–8; in turn, the amines will be re-extracted by aqueous solutions at low pH levels. However, the procedure as described by Temple and Gillespie is not directly applicable to the determination of phenylephrine in pharmaceutical combinations, since other alkoids will be extracted together with that substance. Also, the unfavorable partition coefficient of ion-pairs formed with phenolic amines would require multiple extractions for complete recovery of phenylephrine.

Quantitative extraction of the phenylephrine–DEHP complex is readily achieved through the application of partition chromatography. A solution of the sample itself adjusted to a pH of 5–6 constitutes the immobile phase of the system. An ether solution of DEHP passed over the column elutes the phenylephrine; this is quantitatively extracted from the eluate with dilute sulfuric acid and determined spectrophotometrically. Because alkaline solutions of phenylephrine absorb more strongly and with a maximum at a longer wavelength than acid solutions, the extract is made alkaline prior to measurement.

This partition system is effective in the analysis of phenylephrine in combination with other drugs. A chloroform or ether wash of the column prior to the elution of phenylephrine with the DEHP solution will remove a variety of substances, including acetaminophen, sulfonamides, aspirin, phenolphthalein, glyceryl guaiacolate, dextromethorphan, dihydrocodeinone, and the antihistamines, as well as flavoring matter present in syrups.

Phenylpropanolamine is not completely removed by this treatment. This compound is separated from phenylephrine by passing the DEHP complex of both compounds through a sodium hydroxide column. This will trap only the phenylephrine, which will subsequently be released when the column is neutralized.

EXPERIMENTAL

Chromatographic Columns—Attach a 50-mm. length of 6 or 8-mm. tubing to a 25 × 250-mm. test tube. Use a tamping rod consisting of a disk of stainless steel, aluminum, or glass, of a diameter 1 mm. less than that of the column, attached to a rod 12 to 18 in. long. Pack fine glass wool[4] in the base of the column as support.

Buffers—*pH 5.8 Buffer*—Mix 1 vol. of 1 *M* dibasic potassium phosphate with 9 vol. of 1 *M* monobasic potassium phosphate. Adjust to pH 5.80 ± 0.05, using a pH meter.

pH 5.1 Buffer—Mix 2 vol. of 1 *M* dibasic potassium phosphate with 1 vol. of 1 *M* citric acid. Adjust to pH 5.10 ± 0.05.

Preparation of Samples—*Syrups*—For samples containing the usual concentration of 5 mg. of phenylephrine HCl per 5 ml., measure 10.0 ml. of pH 5.8 buffer into a 25-ml. volumetric flask. Add syrup to volume, being careful to avoid wetting the flask above the graduation mark.

Tablets—Weigh a ground sample containing about 2 mg. of phenylephrine into a 50-ml. beaker. If components of tablet are water-soluble, add 2 ml. of water, warm slightly to dissolve, and add 1 ml. of pH 5.8 buffer. If some components are not water-soluble (*e.g.*, acetaminophen), add 1 ml. of dimethyl-

[2] Marketed as Celite 545 by the Johns-Manville Corp., New York, N. Y.

[3] Listed in some catalogs as bis-2(ethylhexyl) phosphate.

[4] Pyrex Filtering Fibre, Corning Glass catalog No. 3950.

sulfoxide, warm to dissolve, and then add 2 ml. of pH 5.8 buffer. For tablets containing antacids such as magnesium and aluminum hydroxides, heat the powdered sample with 5 ml. of ethanol and 1 ml. of concentrated HCl to dissolve the alkaline material; add 10 ml. of *n*-butanol and evaporate to dryness.[5] Dissolve residue in 1 ml. of dimethylsulfoxide and add 2 ml. of pH 5.8 buffer.

Procedure

Use water-saturated solvents throughout.

A—*Samples Without Phenylpropanolamine*—Transfer mixture of 1 Gm. of acid-washed diatomaceous earth with 0.8 ml. of pH 5.1 buffer to column containing a pledget of glass wool, and tamp to a uniform mass. Mix 4 Gm. of diatomaceous earth with 3 ml. of syrup sample or with tablet preparation and transfer to column directly above pH 5.1 layer. Dry-wash beaker with 1 Gm. of diatomaceous earth, add to column, and tamp. Cover with wad of glass wool.

Pass 75 ml. of $CHCl_3$ over column, followed by 100 ml. of ether. Evaporate final 10 ml. of eluate separately; if residue remains, pass additional ether over column.

With a rubber bulb, draw DEHP[6] into a graduated 1-ml. pipet to a level estimated to provide a total of about 1.2 ml.[7] Transfer DEHP into 50 ml. of ether; then flush the pipet several times with the ether. Avoid contact with the reagent.

Place a separator containing about 20 ml. of approximately 0.1 *N* H_2SO_4, as a receiver under the column. Elute the column with the DEHP–ether solution, then follow with 25 ml. of ether. Shake the separator, and transfer aqueous layer to 50-ml. volumetric flask containing 4 ml. of 1 *N* NaOH. Re-extract ether with 15 ml. of 0.1 *N* H_2SO_4, transfer extract to the flask, and adjust to volume with water. (If sample contains magnesium hydroxide, gel will separate from this solution; remove by filtration or centrifugation.) Determine absorbance with a spectrophotometer at maximum at about 291 mμ, and compare with that of a standard solution containing about 2 mg. of phenylephrine HCl in 50 ml. of approximately 0.01 *N* NaOH.

B—*Samples Containing Phenylpropanolamine*—Prepare sample column, and wash with $CHCl_3$ and ether as under *A*. Mount a second column, prepared with 3 Gm. of diatomaceous earth and 2 ml. of 0.5 *N* NaOH, directly below sample column. Elute phenylpropanolamine with a solution of 0.1 ml. of DEHP in 50 ml. of ether, followed by 25 ml. of ether. Finally, elute the phenylephrine from the column train with 1.2 ml. of DEHP in ether exactly as described above.

RESULTS AND DISCUSSION

Standard quantities of phenylephrine, both alone and combined with other drugs, were analyzed by the procedure as described. Recoveries are presented in Table I. The analysis of phenylephrine in commercial samples of various combinations is presented in Table II. In several cases, analyses of selected accompanying materials were made as outlined below. The ultraviolet spectra of these were identical with those of the pure material, and the recoveries were in accord with the label claims. Since no standard recovery data were collected for these compounds, the results of the analyses are not included.

In accord with their distribution coefficients and dissociation constants, the majority of pharmaceutically important bases can be completely eluted from chromatographic columns at pH levels ranging down to about pH 3, using moderate volumes of ether or chloroform; the rate of elution increases rapidly with increase of pH up to the pKa value of the base. At pH 5.8 alkoids such as most of the antihistamines are readily removed; phenylpropanolamine is eluted slowly with chloroform and phenylephrine very slowly with ether at this pH level. The latter two compounds are retained on the pH 5.1 trap while the others pass through.

Phenylpropanolamine is separated from phenylephrine by passing the ether solution of their DEHP complexes over a diatomaceous earth–sodium hydroxide column. Phenylephrine is retained as its phenol salt while the phenylpropanolamine complex is eluted. For this separation, the quantity of DEHP used initially must be sufficient to remove the phenylpropanolamine completely while being insufficient to neutralize the entire NaOH column. This column must, in turn, be completely neutralized by the DEHP used to elute the phenylephrine. The proper balance is achieved with the concentrations specified under *Experimental*. It can be noted that phenylephrine can be quantitatively determined with procedure *A* even though the phenylephrine fraction is not free of phenylpropanolamine, since solutions of the latter

Table I—Standard Recovery of Phenylephrine Hydrochloride

Other Drugs Present	Phenylephrine HCl Taken, mg.	Recovery Found, mg.	Recovery %
...	4.38	4.43	101.0
...	2.37	2.39	100.6
Phenylpropanolamine HCl, 24.8 mg.	5.31	5.34	100.4
Phenylpropanolamine HCl, 25.8 mg.	5.83	5.74	98.4
Phenylpropanolamine HCl, 19.3 mg.	5.22	5.14	98.3
Phenylpropanolamine HCl, 9.8 mg.	2.05	2.07	101.0
Dextromethorphan HBr, 9.9 mg. Glyceryl guaiacolate, 37.3 mg. Acetaminophen, 65.8 mg.	2.39	2.40	100.4
Chlorpheniramine maleate, 12.5 mg. Phenyltoloxamine citrate, 150 mg.	2.14	2.15	100.4
Aspirin, 132.6 mg.	2.06	2.08	101.0
Aspirin, 82.8 mg. Phenolphthalein, 24.5 mg. Magnesium hydroxide, 220.5 mg.	2.39	2.38	99.6

[5] Butanol is used to effect azeotropic evaporation of water, thereby insuring complete removal of HCl.

[6] No differences were observed from using DEHP from two sources. One, with no grade indicated, was colorless; the other, labeled "Practical Grade," was quite yellow.

[7] One milliliter of DEHP was found to be a marginal amount in several instances.

TABLE II—ANALYSIS OF COMMERCIAL SAMPLES

Sample	Drugs Present		Phenylephrine HCl Found, mg.	Phenylephrine HCl % of Labeled Claim
Syrup	Phenylephrine HCl, 25 mg.		24.12	96.4
	Glyceryl guaiacolate, 250 mg.		24.05	96.2
	Pyrilamine maleate, 30 mg.			
	Methapyrilene fumarate, 30 mg./fl. oz.			
Syrup	Phenylephrine HCl, 5 mg.	Sample A	4.96	99.2
	Dextromethorphan HBr, 15 mg.			
	Chlorpheniramine maleate, 1 mg./5 ml.	Sample B	4.72	94.6
	Alcohol, 10%		4.78	95.6
Syrup	Phenylephrine HCl, 5 mg.		5.08	101.6
	Phenylpropanolamine HCl, 20 mg.		5.12	102.4
	Phenyltoloxamine citrate, 7.5 mg.			
	Chlorpheniramine maleate, 2.5 mg./5 ml.			
	Sodium benzoate, 0.1%			
Nasal soln.	Phenylephrine HCl, 2.5 mg.		2.60	104.0
	Sulfisoxazole, 40 mg./ml. (as diethanolamine salt)		2.58	103.2
Expectorant	Phenylephrine HCl, 5.0 mg.		4.86	97.2
	Brompheniramine maleate, 2.0 mg.		4.94	98.8
	Phenylpropanolamine HCl, 5.0 mg.			
	Glyceryl guaiacolate, 100 mg./5 ml.			
Tablets	Phenylephrine HCl, 10 mg.		10.45	104.5
	Dihydrocodeinone bitartrate, 5 mg.		10.62	106.2
	Chlorpheniramine maleate, 2 mg.			
	Acetaminophen, 250 mg.			
	Caffeine, 30 mg.			
	Homatropine methylbromide, 1.5 mg./tab.			
Tablets	Phenylephrine HCl, 5 mg./tab.		4.24	84.8
	Aspirin	Sample A	4.21	84.2
	Yellow phenolphthalein		4.24	84.8
	Magnesium hydroxide	Sample B	4.80	96.0
			4.86	97.2
Tablets	Phenylephrine HCl, 5 mg.		4.84	96.8
	Phenindamine tartrate, 10 mg./tab.		4.82	96.4
	Aspirin		4.88	97.6
	Caffeine			
	Aluminum–magnesium hydroxide gel			

do not absorb at 291 mμ, the wavelength at which phenylephrine is measured.

With minor modification, the procedure as described can be adapted to the concurrent determination of many of the drugs which accompany phenylephrine in commercial preparations. Phenylpropanolamine, for example, can be extracted from the initial DEHP eluate with sulfuric acid in the same manner as phenylephrine. To compensate for the low absorbance of phenylpropanolamine, smaller volumes of acid may be used for the extraction. To determine acetaminophen, the initial chloroform and ether eluates from the sample column are passed over a diatomaceous earth–1 *N* NaOH column, which will retain the acetaminophen while alkoids, together with flavoring material, pass through. The acetaminophen is eluted after acidification of the column *in situ* with acetic acid, as previously described (9). For syrups containing dextromethorphan, a diatomaceous earth–2 *N* HCl column is placed in series with the sample column, and 100 ml. of ether is passed over the columns. This will elute the greater part of the dextromethorphan, together with flavoring matter, from the sample column; the dextromethorphan alone will be retained on the HCl column. Then 75 ml. of chloroform is passed over both columns to elute the total quantity of dextromethorphan. The eluate can be received directly in a volumetric flask containing a small amount of acidified methanol (1) and its absorbance measured directly. Chlorpheniramine, if present in this same combination, will be retained on the HCl column, and it can be eluted with ammoniacal chloroform by the procedure described for other alkoids (10). Finally, the phenylephrine is eluted with DEHP as above.

REFERENCES

(1) Levine, J., *J. Pharm. Sci.*, **54**, 485(1965).
(2) Pratt, E. L., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 505 (1957).
(3) Welsh, L. H., *ibid.*, **44**, 507(1955).
(4) Clark, W. R., and Rosenberg, L. A., *J. Assoc. Offic. Agr. Chemists*, **48**, 579(1965).
(5) Hyatt, R., *ibid.*, **48**, 594(1965).
(6) Smith, D. J., *J. Assoc. Offic. Anal. Chemists*, **49**, 536 (1966).
(7) Hiskey, C. F., and Levin, N., *J. Pharm. Sci.*, **50**, 393(1961).
(8) Temple, D. M., and Gillespie, R., *Nature*, **209**, 714 (1966).
(9) Levine, J., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 687(1957).
(10) Levine, J., and Roe, J. E., *J. Assoc. Offic. Agr. Chemists*, **42**, 693(1959).