Table IV—Liquid Water Permeation of Coated Lactose Tablets

Plasticizer	Percent Weight Gain after 1 hr.	4 hr.
None	1.9	5.9
Diethyl phthalate, 25%	0.73	1.3
Triacetin, 20%	1.9	6.1
Fully acetylated monoglycerides, 20%	0.95	2.4
Butyl phthalyl butyl glycolate		
10%	1.0	2.4
20%	1.1	2.6
30%	0.47	1.0
Tributyl citrate, 20%	0.81	2.9

late, however, suggests that this parameter normally need not be a prime criterion in selecting a plasticizer.

Enteric Coating Performance—Table III summarizes the comparison of tablet coating performance among the plasticizers. The uncorrected columns report the minimum amount of total coating weight required to prevent tablet disintegration for 1 hr. in simulated gastric fluid. The corrected columns report the same data calculated in terms of cellulose acetate phthalate only.

Overall, considerably more coating was required to protect the lactose tablets. While this may be due in part to a difference in tablet porosity, the large differences for most plasticizers suggest that the type of tablet base is indeed of significance.

Greater amounts of coating generally were required when plasticizers were added to cellulose acetate phthalate. Among the six plasticizers tested on lactose, only fully acetylated monoglycerides and butyl phthalyl butyl glycolate performed better than unplasticized cellulose acetate phthalate. On dicalcium phosphate, only partially acetylated monoglycerides performed better.

Furthermore, the plasticizers that promoted good performance on one type of tablet base performed poorly on another base. Partially acetylated monoglycerides, for example, performed excellently on dicalcium phosphate but poorly on lactose. Similar (though opposite) results were found for fully acetylated monoglycerides.

These data show that a plasticizer may be carefully chosen to promote good performance on a given tablet substrate and yet may not perform well on a different substrate.

Tablet-Disintegration Time—Table III also summarizes the disintegration times in simulated intestinal fluid and shows that the plasticizers had little effect.

Liquid Water Permeation—Most enteric coated tablets are finished with sugar coatings. During this operation, the cellulose acetate phthalate film is most likely in contact with water. Transport through the film is therefore by means of liquid water in addition to water vapor permeation. Table IV illustrates the weight increases of lactose tablets after immersion in water (the values again reflect data on tablets coated with the minimum amounts of coating to resist disintegration in simulated gastric fluid for 1 hr.).[6] Varying amounts of water permeation were observed; diethyl phthalate and 30% butyl phthalyl butyl glycolate contributed to the most effective barriers.

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[6] The results would, of course, be inaccurate if some lactose was lost by diffusion through the coating. It was determined by reweighing tablets, dried for 16 hr. at 37° after exposure to water (25°) for 6 hr., that this did not occur.

Facile Isolation of Phenylephrine from Syrups

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Abstract □ Although phenylephrine HCl is easily isolated from most of its dosage forms, the high water solubility of both the base and salt forms often makes its isolation from syrups and elixirs difficult. The partition coefficient for extraction of phenylephrine from water to *n*-butyl alcohol is unfavorable, but the drug can be salted into the organic solvent with an excess of sodium chloride and recovered from butanol by extraction with aqueous alkali. Interference to spectrophotometric determination caused by certain coal tar dyes can be circumvented by preliminary extraction of them as ion pairs with quaternary salts. Recovery of at least 98.0% and excellent precision were obtained in trials of the procedure with syrup USP and an elixir placebo as the test vehicles.

Keyphrases □ Phenylephrine—isolation from syrups □ Syrups—separation, isolation of phenylephrine □ UV spectrophotometry—analysis

Phenylephrine HCl is extensively formulated with other drugs in a variety of liquid and solid dosage forms. Because both the free base and salt forms of the drug are very water soluble, its isolation from excipients and many coformulated drugs often can be accomplished by simple solvent extraction of interferences from a dilute acid or base extract of the product. With syrups and elixirs, however, separation of phenylephrine to provide an unaltered UV spectrum is often difficult.

Several chromatographic methods have been described for isolation of phenylephrine from its dosage forms. Schriftman separated it by paper chromatography (1) and electrophoresis (2). Kelly and Auerbach (3) and Montgomery *et al.* (4, 5) used ion-exchange chromatography. Clark and Rosenberg (6) eluted phenylephrine as its diacetyl derivative, formed on a partition chromatography column, and then determined it spectrophotometrically after hydrolysis of the acetyl functions. Levine and Doyle (7) described the partition chromatographic separation of phenylephrine as its ion pair with di-(2-ethyl)hexylphosphoric acid. Ponder (8) exploited the observation that phenyl-

Table I—Partition of Phenylephrine HCl between Butanol and Aqueous Systems

Aqueous phase:	Water[a]	Water	pH 8.4	pH 10.0	pH 11.0	Syrup USP
% in butanol:	7	70	78	84	78	38

[a] All but this sample were saturated with excess sodium chloride.

ephrine base can be extracted with ether from saturated sodium chloride solution, and he described a partition chromatography method which requires elution of 1 mg. of drug with 300 ml. of ether. All of these methods are time consuming and tedious.

Shore and Olin (9) found that epinephrine and norepinephrine can be extracted to the extent of about 65% with 10 vol. of *n*-butyl alcohol from 1 vol. of sodium chloride-saturated aqueous phase, although these catecholamines are poorly extracted into butanol from aqueous solution in the absence of salt. Phenylephrine, the monophenolic analog of epinephrine, has a much better partition coefficient between butanol and aqueous salt solution, 70% of it being extracted by an equal volume. A study of the partition behavior of phenylephrine in this system was undertaken with the objective of providing a simple solvent extraction method for separation of the drug from syrups and elixirs. Such a procedure is reported here.

EXPERIMENTAL

Reagents and Supplies—All of the reagents used were official grades or equivalent in quality. Ammonia buffer was prepared by adjusting the pH of 1 *N* ammonium hydroxide solution to 10.0 ± 0.1 with concentrated hydrochloric acid. Salt-saturated butanol was prepared by equilibrating *n*-butyl alcohol with saturated aqueous sodium chloride solution. Cetylpyridinium chloride was used to remove dye interferences. The method was tested by adding phenylephrine HCl to each of two vehicles to obtain concentrations of about 1 mg./ml. The test vehicles were syrup USP (85% aqueous sucrose) and an elixir vehicle which had been available as a clinical test placebo for an experimental formulation. The latter contained sucrose, sorbitol, glycerin, alcohol, citrate, artificial sweeteners, flavors, FD&C Red No. 2, FD&C Yellow No. 5, and D&C Green No. 5. Spectra were determined in 1-cm. cells in a Cary model 14 recording spectrophotometer.

Procedure—Transfer exactly 2.0 ml. of syrup or elixir to a 125-ml. separator, and add 2 ml. of water. If the preparation is too viscous to afford convenient pipeting, dilute it quantitatively with an equal volume of water and then transfer exactly 4.0 ml. of the dilution to a separator. Add about 2 g. of sodium chloride and 1 ml. of ammonia buffer, and extract the mixture with two 50-ml. portions of salt-saturated butanol. Combine the butanol extracts in a 250-ml. separator, and extract them with successive 35-, 30-, and 30-ml. portions of 1 *N* sodium hydroxide, collecting the alkali extracts in a 100-ml. volumetric flask. Dilute the solution to volume with alkali.

Concomitantly scan the UV absorption spectra of the solution and of a standard preparation containing about 2 mg. of phenylephrine hydrochloride reference standard USP, accurately known, per 100 ml. of 1 *N* sodium hydroxide solution in 1-cm. cells in a suitable spectrophotometer from 350 to 250 nm., using 1 *N* alkali as the blank. If the spectrum of the solution from the assay preparation matches that from the standard preparation, calculate the content of $C_9H_{13}NO_2 \cdot HCl$, in milligrams per milliliter, from the relation $50C(A_U/A_S)$, where C is the concentration in milligrams per milliliter of phenylephrine HCl in the standard preparation, and A_U and A_S are the absorbances of the solution from the assay preparation and the standard preparation, respectively, determined at the absorption maximum at about 292.5 nm.

If the spectrum of the assay preparation shows the presence of interfering colorants, the following expedient has been shown to be effective with most dyes permitted for use in U. S. drug preparations. Add about 10 mg. of cetylpyridinium chloride to a mixture of 2.0 ml. of the elixir or syrup and 2 ml. of water in a 125-ml. separator, and extract the mixture with successive small portions of chloroform until the aqueous layer is colorless. Discard the extracts and follow the method already described beginning with: "Add about 2 g. of sodium chloride. . .."

Table II—Recovery of 1.00 mg./ml. Phenylephrine HCl Added to Two Vehicles

Trial	Sample Found, mg. per ml.	
	Syrup USP	Elixir Placebo
1	0.980	0.985
2	0.980	0.980
3	0.9825	0.9975
4	0.9775	0.9875
5	0.980	0.9875
Mean recovery, %	98.0	98.75
Relative standard deviation, %	0.2	0.6

RESULTS AND DISCUSSION

Partition Behavior of Phenylephrine—The data in Table I were obtained by equilibrating equal volumes of mutually saturated aqueous solutions and butanol containing known amounts of phenylephrine HCl and an excess of sodium chloride. The amount of phenylephrine extracted was determined spectrophotometrically. The phenomenon appears to be simple "salting out," because sodium sulfate and other salts may be substituted for sodium chloride with equivalent results. The relatively low extractability of the drug from syrup USP may be attributed to lower salt solubility in the concentrated sugar solution; however, this effect is minimized in practice since the syrup is diluted 2 in 5 in the procedure.

Interferences—The effect of possible phenylephrine degradation products was not evaluated in this study. (Stability considerations in phenylephrine assay will be the subject of a future communication from these laboratories.) Colorants interfered in the determination of phenylephrine added to the elixir placebo used; this interference was circumvented by extracting the sulfonate dyes as ion pairs with cetylpyridinium chloride.[1] Experiments with the certified dyes permitted in U. S. drug preparations showed that all except FD&C Blue No. 1, FD&C Blue No. 2, FD&C Red No. 4, and D&C Red No. 33 could be removed by this method. Interference by coformulated drugs and excipients in many cases can be circumvented by solvent extraction from acid or basic solution. An unidentified component of a proprietary flavor mixture interfered in application of the method described here to a syrup placebo. In such cases, the highly selective periodate oxidation method described by Chafetz (10) can be used. Although periodate oxidation is inapplicable to many syrup formulations because the sugar reduces the oxidant, such products can be assayed by this method when the drug is isolated by the procedure described in this article.

Precision and Recovery—Application of the method to syrup USP and the elixir placebo resulted in the data shown in Table II. Concomitant extraction of a standard improves the recovery; however, a recovery of not less than 98.0% and not more than 102.0% is this laboratory's acceptance criterion.

SUMMARY

Phenylephrine can be isolated from many elixir or syrup preparations by saturating a diluted sample buffered to pH 10.0 with sodium chloride and extracting with salt-saturated butanol. The drug can be recovered from the butanol phase by extraction with *N* sodium hydroxide for subsequent analysis by spectrophotometry or other means. Certain colorants which interfere in the extraction scheme can be removed by ion-pair formation with a quaternary ammonium salt such as cetylpyridinium chloride.

[1] This technique was demonstrated to Lester Chafetz in 1958 by M. E. Auerbach, who used benzalkonium chloride as the quaternary amine component.

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Antibacterial Activity of Certain Mannich Bases against *Escherichia coli* and *Staphylococcus aureus*

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Abstract □ A group of 28 Mannich bases comprising four different amino functional groups was tested for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The zones of inhibition were measured, and the results of the tests were tabulated. Postulations are offered to explain the superior activity of one compound when compared with another, and these explanations are combined with suggested modes of action.

Keyphrases □ Mannich bases—antimicrobial activity testing, *Staphylococcus aureus*, *Escherichia coli*, structure–activity relationships, mode of action proposed □ Antimicrobial activity—Mannich bases tested, *Staphylococcus aureus*, *Escherichia coli*

Some of the 5-chloro-2-thienyl-3-dialkylaminoethyl ketones reported by Britton and Nobles (1) possessed *in vitro* activity against certain bacteria. In addition, Taylor and Nobles (2) stated that some of their ketonic Mannich bases demonstrated *in vitro* antitubercular and amoebicidal action. Schlingman *et al.* (3) reported the synthesis of vinylogs of β-aminopropiophenones. These substances were Mannich bases and were shown to possess antibacterial activity *in vitro*. The Mannich bases of rifomycin SV were effective against both Gram-positive and Gram-negative bacteria, possessed low toxicity, and could be maintained at high blood levels (4). Blanton and Nobles (5) prepared Mannich bases using 3-azabicyclo[3.2.2]nonane as an amine moiety, and the resulting compounds had a broad spectrum of antimicrobial activity. In addition, Mannich bases derived from tetracycline retained a high activity (6).

These observations prompted the present survey of the antibacterial activity of a series of Mannich bases, including those reported by Khullar and Chatten (7).

EXPERIMENTAL

Materials—All Mannich bases employed in this study were synthesized by Method A, as reported earlier (7), and are listed in

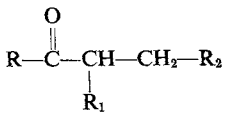

Table I—Mannich Bases Employed in This Study

Compound Number	R	R_1	Melting Ranges, R_2 = 2,6-dimethylmorpholino	R_2 = morpholino	R_2 = piperidino	R_2 = 3-methylpiperidino
1	Me—	H	156–157° (7)[a]	—	175°	—
2	C_6H_5—	H	195–196.5° (7)	186.5° (11)	191–192° (11)	172–174° (11)
3	*p*-Me—C_6H_4—	H	207–208° (7)	224–225° (12)	176.5° (11)	177–178°
4	*p*-MeO—C_6H_4—	H	203–205° (7)	209–210° (11)	209–211° (14)	—
5	*p*-Cl—C_6H_4—	H	203.5–204° (7)	201–202°	187°	186–187.5°
6	*p*-O_2N—C_6H_4—	H	203–204° (7)	206° (13)	193–195° (13)	—
7	*m*-O_2N—C_6H_4—	H	196–197° (7)	186–187° (11)	178–179.5° (15)	—
8	*o*-O_2N—C_6H_4	H	—	192–193°	—	—
9	C_6H_5CH=CH—	H	196–197° (11)	—	—	—
10	C_6H_5—	Me	187–188.5° (7)	—	—	—
11		H	—	200–201.5°	186.5–187.5°	—

[a] Number in parentheses is reference to previously reported melting point.