which formed was recrystallized from a solution of hot chloroform; yield, 48.0 Gm., 84%; m. p. 110–111°.

Anal.—Calcd. for $C_{10}H_{12}NO\text{-}COOH$: neut. equiv., 205.2. Found: 201.0.

Ten grams (0.048 mole) of the allyl phthalamide was dissolved in 50 ml. of methyl alcohol. A solution of mercuric acetate was prepared by dissolving 15.35 Gm. (0.048 mole) of the acetate in 300 ml. of hot methyl alcohol containing 0.5 ml. of nitric acid. The hot acetate solution was filtered into the hot amide solution with the formation of a white precipitate. The product was recrystallized from dilute methyl alcohol; yield, 18.0 Gm., 85%; m. p., 152–154°.

Anal.—Calcd. for $C_{12}H_{13}HgNO_4$: C, 33.08; H, 2.98; Hg, 46.05. Found: C, 33.11; H, 3.39; Hg, 45.05.

SUMMARY

1. Synthesis of the required intermediates for ultimate mercuration, such as monoallyl succinate, monoallyl phthalate, N-allylsuccinamic acid, and N-allylphthalamic acid is described.

2. Mercuration of the above compounds to form anhydro zwitterion structures is described.

3. The structures of these anhydro derivatives is established by elemental analysis and infrared data.

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Ion Exchange Separation and Colorimetric Determination of Phenylephrine in Pharmaceutical Products

By CLARK A. KELLY and MORRIS E. AUERBACH

Since the introduction of phenylephrine, it has been used in combination with many other drugs. With some formulations it has been found useful to isolate the drug via a cationic exchange resin before determining the intact phenylephrine by the usual colorimetric procedures (coupling with diazotized *p*-nitroaniline, or reacting with Millon's reagent when catecholamines are present).

IN THE DECADE since Auerbach (1) described the azo colorimetric determination of phenylephrine hydrochloride U. S. P. (*l*-1-(*m*-hydroxyphenyl)-2-methylaminoethanol hydrochloride), the number of formulations in which this sympathomimetic is used have increased enormously. In most cases the presence of other active constituents has complicated the analysis for phenylephrine, particularly in stability testing. Methods involving paper chromatography, spectrophotofluorometry, ultraviolet spectrophotometry, bromination, and a number of colorimetric procedures (2–14) have been used, but are limited in most cases to simple phenylephrine preparations. The important step is then the quantitative separation of the phenylephrine from substances which interfere with its colorimetric determination. By the use of a cationic exchange resin (nuclear sulfonic type) separation from acidic and neutral substances is accomplished, and at the same time the selectivity of the intact phenylephrine assay is greatly increased. If the side chain were lost, or its basicity significantly lessened, the remaining molecule would not be held by the resin and so would be discarded with the water washes. The intact phenylephrine held by the resin is eluted with acid along with any basic material that may be eluted at the stated acid strength. Almost the only compounds which cannot be separated from phenylephrine by ion exchange,

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and which cause serious interference, are the catecholamines. In such cases, Millon's reagent, which is selective for mono-phenols, is used.

Cationic exchange resins have been used in this laboratory with good success in the separation of phenylephrine from many other drugs. Another laboratory (15) has applied a similar technique but elutes the phenylephrine from the resin with 0.1 *M* sodium hydroxide instead of acid. In special cases this may be useful, but it is well to remember that an alkaline medium favors oxidative destruction of the phenolic group, and might thus be responsible for erratic assays.

Vincent, *et al.* (16), have used a variety of ion exchange resins in the separation of sympathomimetic amines. They reported, and this laboratory confirms, that Amberlite IRA-400 (a strongly basic anion exchanger) holds the phenylephrine so firmly that it is not eluted. With Amberlite IRC-50 (a weakly acidic cation exchanger) phenylephrine is not held quantitatively. Amberlite IR-45 (a weakly basic anion exchanger) was used successfully in their work. The phenylephrine is then determined through titration of its basic nitrogen. In this procedure, both the separation and assay depend upon the basic function, thus no information results as to the qualitative status of the phenolic group. The questions raised by Schriftman (6) in regard to determining intact phenylephrine in complex formulations during stability testing are met by the dual nature of the recommended procedure: (*a*) separation depending on the intact basic side chain (*b*) subsequent assay depending upon intact hydroxyphenyl function.

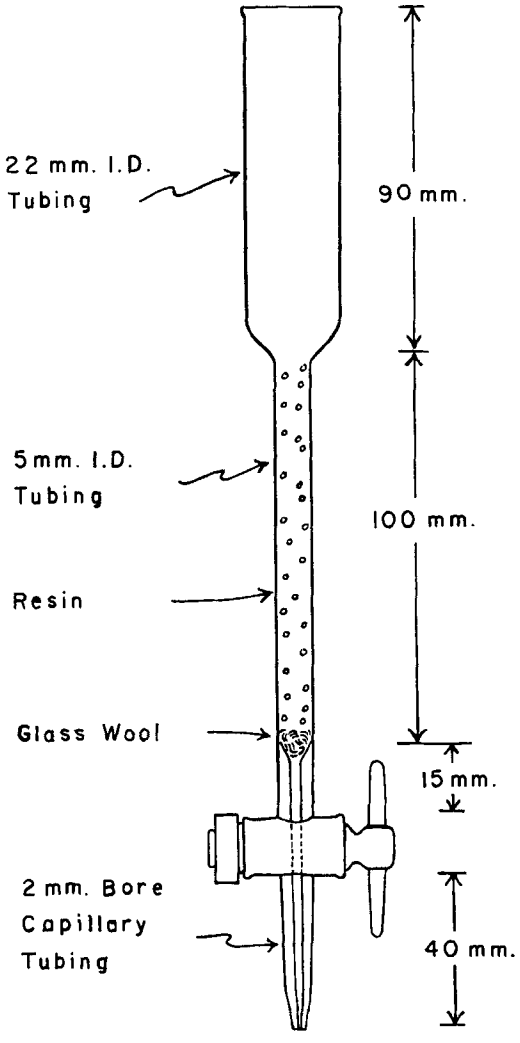

Fig. 1.—Ion exchange column for isolation of phenylephrine.

EXPERIMENTAL

Apparatus.—Glass column, see Fig. 1. The stopcock can be of glass or teflon; less "freezing" occurs with the latter.

Water bath, a 600-ml. beaker with a stainless steel screen support resting on the bottom.

Any suitable photocolorimeter, capable of selecting light bands at about 420 mμ and at about 500 mμ.

Reagents.—Cationic exchange resin in the hydrogen form, Dowex 50-X-1, 50–100 mesh or its equivalent. To be assured that the resin is free of any foreign matter which might interfere in the colorimetric procedures it is washed as follows: mix with a magnetic stirrer 100 Gm. resin in a 2 L. beaker with 500 ml. 3 *M* hydrochloric acid for fifteen minutes. Repeat washes twice more, discarding each acid wash. Wash the resin free of acid (pH tape color remains neutral). Store resin in distilled water until needed. Enough resin, about 2 Gm., is added in the form of a slurry to the glass column which has a small loose glass wool plug resting on the constriction above the stopcock. The resin height is 100 mm. which fills the column just to the reservoir portion of the column. The reservoir capacity is about 25 ml.

Hydrochloric acid, 0.5 *M*.

Nitric acid, 1 *M*.

Sodium hydroxide, 1 *M*.

Sodium hydroxide solution, 10%.

Borax (sodium tetraborate decahydrate) solution, 5%.

Diazo solution. Dissolve 30 mg. recrystallized *p*-nitroaniline[1] in 2 ml. dilute (6 *M*) hydrochloric acid. Chill in ice bath, add 0.5 ml. of 7% sodium nitrite solution. After two minutes add 100 ml. ice cold water and 1 ml. 4% sulfamic acid solution. Mix, and keep in the ice bath until needed. Even when cold, this reagent should not be used if more than three hours old.

Millon's reagent. Add 2 ml. triple distilled mercury to a 125-ml. conical flask. With swirling add

[1] Dissolve 10 Gm. of the *p*-nitroaniline in as little of hot 95% ethanol as possible, filter, let cool slowly, and then keep in an ice bath for at least an hour. Filter again. Dry the yellow crystals at 60° overnight. The melting range of the purified compound is 147°–149° corrected.

20 ml. concentrated (16 *M*) nitric acid and allow to stand for ten minutes in a well ventilated hood. Add 35 ml. water and mix. Add by pipet 10% sodium hydroxide solution until a permanent turbidity results (about 3 ml.). Add 5 ml. dilute (3 *M*) nitric acid. Keep this clear solution stoppered. Do not use after the first day of preparation. Millon's reagent reacts readily with protein matter (skin in particular) so great care should be used in handling it at all times.

Standard Solution.—Weigh exactly 100 mg. phenylephrine hydrochloride (U. S. P. grade) and transfer to a 100-ml. volumetric flask. Dissolve and make to volume with water (solution A). By appropriate dilution with water prepare the desired working standard.

For the Azo method.—Pipet 10 ml. of solution A to a 100-ml. volumetric flask and dilute to volume with water (solution B). Pipet 10 ml. of solution B to a 100-ml. volumetric flask and dilute to volume with water. This last dilution contains 10 mcg. phenylephrine hydrochloride per ml.

For the Millon method.—Pipet 5 ml. of solution A to a 100-ml. volumetric flask and dilute to volume with 1 *M* nitric acid. This last dilution contains 50 mcg. phenylephrine hydrochloride per ml.

Sample solutions.—Solutions or aqueous extracts of phenylephrine should be of such concentrations that not more than a 5-ml. aliquot containing a minimum of 250 mcg. phenylephrine hydrochloride for the azo method, and not more than a 25 ml. aliquot containing a minimum of 1.25 mg. for the Millon method is added quantitatively to the resin column. If larger aliquots are required, the washing step will involve more water than described for the isolation step.

An example for a tablet containing 5 mg. phenylephrine hydrochloride is as follows: determine the average weight of 20 tablets. Grind to a fine powder. Transfer a weight of the powder equivalent to one average tablet to a 250-ml. glass-stoppered conical flask. Add exactly 100 ml. water and shake mechanically for ten minutes. Filter through Whatman No. 1 paper or its equivalent, discarding the first 25 ml. Take a 5-ml. aliquot of the clear filtrate by pipet and add to the resin column reservoir.

Azo Method (No Catecholamines Present).—An example is given below, in a formulation containing N - acetyl - *p* - aminophenol, phenylephrine hydrochloride, thenyldiamine hydrochloride, and caffeine. Pass an aqueous solution containing at least 250 mcg. phenylephrine hydrochloride in a volume not greater than 5 ml. through the resin column at a flow rate of 1 to 2 ml. per minute. Wash the column with 5-ml. portions of water at the same flow rate, draining each wash to the top of the resin column, until 25 ml. have been collected in a graduated cylinder. Discard these washes. Place a 25-ml. volumetric flask under the column and elute the phenylephrine with five 5-ml. portions of 0.5 *M* hydrochloric acid collecting exactly 25 ml. of eluate. Mix, and transfer by pipet 2 ml. to a test tube graduated accurately at the 10 ml. volume. To an ordinary test tube add a 2-ml. aliquot and one drop of phenolphthalein test solution U. S. P. From a 5 or 10 ml. semimicro buret graduated in 0.05 ml. titrate this 2-ml. aliquot with 1 *M* sodium hydroxide to the phenolphthalein end point (first permanent red). To the aliquot in the graduated test tube add the same amount of 1 *M* sodium hydroxide less 0.05 ml. Great care must be taken at this step to assure valid results.

To this nearly neutral solution and to a series of similar tubes containing standard phenylephrine hydrochloride (0, 10, 20, 30 mcg.) add water so that each tube contains 5–6 ml. solution. Add 2 ml. of the 5% borax solution and mix. Add 0.5 ml. of the fresh cold diazo solution, mix, and let stand for ten minutes. Add 1 ml. 10% sodium hydroxide. Dilute to the 10 ml. mark with water and mix. Read in a photocolorimeter at 500 mμ, setting the instrument with the reagent blank. Calculate the amount of phenylephrine hydrochloride present from the standard values obtained at the same time the sample is analyzed.

Millon Method (Catecholamines Present).—An example is a formulation containing 0.125% phenylephrine hydrochloride and 0.5% N-isopropyl-ethyl norepinephrine hydrochloride. Pass an aqueous solution containing at least 1.25 mg. phenylephrine hydrochloride in a volume not greater than 25 ml. through the resin column at a flow rate of 1 to 2 ml. per minute. Wash the column with 5-ml. portions of water, draining each wash to the top of the resin column, until 100 ml. has been collected in a graduated cylinder. Discard these washes. Place a 25-ml. volumetric flask under the column and elute the phenylephrine with five 5-ml. portions of 1 *M* nitric acid, collecting exactly 25 ml. of the eluate. Mix, and transfer by pipet 3 ml. to a test tube graduated accurately at the 10 ml. volume. Prepare a series of similar tubes containing standard phenylephrine hydrochloride (0, 100, 150, 200 mcg.). Adjust the volume in each tube to 5 ml. with 1 *M* nitric acid. Add 4 ml. Millon's reagent to each tube. Mix, and keep in a boiling water bath for fifteen minutes. Allow the tubes to cool for at least thirty minutes at room temperature. Adjust the volume in each tube to 10 ml. with 1 *M* nitric acid. Mix, and read the clear solutions in a photocolorimeter at 420 mμ, setting the instrument with the reagent blank. Calculate the amount of phenylephrine hydrochloride present from the standard values obtained at the same time the sample is analyzed.

DISCUSSION AND RESULTS

The ion exchange resins used successfully under these conditions are Dowex 50-X-1, 2, 8, 12, and 16, Dowex 50-W-X-1, and Amberlite IR-120 analytical grade. With Dowex 50-X-1 in the sodium form, phenylephrine is also recovered quantitatively.

As much as 100 mg. phenylephrine hydrochloride can be held by the resin column and subsequently eluted and determined quantitatively (100 ± 2% recovery). In using these columns one must consider the presence of all other basic substances as well as salts which are present, as the resin may become so saturated that the phenylephrine is not retained during the water washes. It should be kept in mind that neutral salts such as sodium chloride are split by the resin so that an equivalent amount of hydrochloric acid is released. If the wash water contained over 0.1 *M* acid, the phenylephrine would be eluted. With the various formulations analyzed no such interference has been noted.

Phenylephrine is taken and held by the resin from aqueous solutions containing as much as 0.1 *M* hydrochloric acid so the original sample or extract can be this acidic without affecting the recovery.

Once the phenylephrine is held by the resin any amount of water may be passed through the column. The amount of washing depends on the amount and kinds of interfering substances removed by this step.

Quantitative acid elution requires a minimum of 0.2 *M* hydrochloric acid. The maximum permissible molarity depends upon the elution characteristics of other basic substances which may be held by the column. Acid above 1 *M* elutes thenyldiamine and interferes with the azo dye method. Experience has shown that different lots of nominally the same resin require somewhat different acid strength to get recoveries of 100 ± 2%. For most purposes, 0.5 *M* acid represents a good compromise, but it is advisable to check this point on each new lot of resin purchased.

Dowex resins show some shrinkage during the acid elution. Thus the volume of water freed from a given resin during the elution is variable. It follows that the acid molarity is variable, and therefore it is important to neutralize each eluate carefully before proceeding with color development.

The colorimetric methods are well known and have been modified only slightly, to fit the needs of the procedure presented. Only one point merits particular emphasis, namely that the azo coupling reaction must be run at about pH 9 (borax) to get good color production.

In the technique described for the Millon method, the nitric acid resin eluate is colorless as it comes off the column. If a catecholamine is also in solution, the eluate soon turns yellow. This color does not interfere with the assay. If a precipitate occurs during colorimetry, the presence of chloride ion should be suspected. However, if glassware is scrupulously clean and the directions are carefully followed, the solutions will remain clear and the color stable for at least three days.

The recommended procedure was developed to handle the analysis of a tablet of the following composition:

	Per Tablet, mg.
N-Acetyl-*p*-aminophenol (APAP).....	150
Phenylephrine hydrochloride.....	5
Thenyldiamine hydrochloride.....	7.5
Caffeine.....	15

Both thenyldiamine and APAP interfere with the phenylephrine determination, thus preliminary physical separation is required. All the intact APAP and over 99% of the caffeine pass through the column during the washing step while the phenylephrine and thenyldiamine remain fixed to the column. On eluting with 0.5 *M* hydrochloric acid only the phenylephrine is removed. Of course, new interferences may appear as the result of decomposition during stability studies. Thus APAP might conceivably hydrolyze to *p*-aminophenol (PAP), a serious interference. For a purely hypothetical case, if 1% of the APAP were hydrolyzed, the final colorimetric solution would contain 4.3 mcg. PAP which would read as 0.9 mcg. phenylephrine hydrochloride, and would thus result in a falsely high value for phenylephrine.

As a matter of fact, during stability studies at 40° for a year, little change was noted in phenylephrine values for the formulation quoted.

A large number of other complex formulations, including syrups and elixirs, as well as tablets, have been assayed successfully by the recommended procedure. Other substances in these formulations included acetophenetidin, dextromethorphan hydrobromide, pyrilamine maleate, aspirin, phenolphthalein, various common pharmaceutical dyes, ascorbic acid, codeine phosphate, chlormezanone, chlorpheniramine maleate, meperidine, potassium guaiacolsulfonate, potassium iodide, ammonium chloride, benzocaine, bismuth subgallate, tannic acid, boric acid, various suppository bases, and catecholamines. As already indicated, only when catecholamines are present is it useful to abandon the azo colorimetric procedure and resort to Millon's reagent.

SUMMARY

Working details have been presented for the isolation (via a cationic exchange resin) and colorimetric determination of intact phenylephrine in various pharmaceutical preparations. The combination ion exchange-colorimetric procedures measure intact phenylephrine only: the ion exchange involves the basic nitrogen side chain while the colorimetric methods (coupling with diazotized *p*-nitroaniline or reaction with Millon's reagent) depend upon the presence of the unchanged phenolic group.

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