can be obtained by their use than from purely crystalline modification. This is because the system utilizes, in effect, the free energy of dilution of the complexing agent to raise the solubility of the drug. Since molecular complexes of this type are readily produced, particularly by relatively insoluble drugs, this approach may often provide the answer for those products which are poorly available because of slow rates of dissolution.

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Drug Standards

Determination of Amphetamine in Dosage Forms by Partition Chromatography

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A procedure is presented for the assay of preparations of amphetamine and several other sympathomimetic amines. The amine is isolated by extraction from the stationary aqueous phase of a partition column with a chloroform solution of triethylamine. The chloroform solution is then extracted with aqueous acid and the amine determined spectrophotometrically. Data obtained on several commercial samples of dextro-amphetamine sulfate tablets by three different assay procedures are reported, and the inapplicability of the U.S.P. XVI assay procedure to some of the commercial products is demonstrated. An analysis by the proposed column-ultraviolet procedure can be completed in about 1 hour.

MANY METHODS of analysis of sympathomimetic amines have been reported (1). They include direct titration of free bases, residual titration of free bases, extraction of bases combined in salts, distillation and titration of volatile bases, nonaqueous titration, gravimetric methods, ultraviolet absorption, and various chromatographic techniques. The widespread use of these preparations in medical practice and their extensive distribution in illegal channels creates a necessity for a simple and rapid method of assay.

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This assay procedure was submitted to the Committee on Revision of the "United States Pharmacopeia" for consideration as a substitute for the currently official method. Subsequent to the preparation of this manuscript, the committee expressed a preference for the modification, using ammonium hydroxide rather than triethylamine as reagent. We have concurred that this modification is preferable for an official assay method.

The proposed procedure is designed to permit the assay of small samples, thus making it applicable to the analysis of a single dosage unit. In this procedure, the extraction of the amphetamine from the dosage form is achieved by elution from an immobile aqueous phase of a column with a solution of triethylamine in chloroform. The eluate is collected in a separator containing an accurately measured quantity of sulfuric acid solution (2 in 100) into which the amphetamine is then extracted. It is quantitatively determined by ultraviolet absorption of the acid solution.

In the development of the method, several other elution procedures were investigated. In a published report of an extraction procedure for alkaloids (2), *p*-toluenesulfonic acid and the alkaloid are incorporated in an aqueous solution as the stationary phase on a partition column. Ether is used to elute the acidic and neutral

substances without removal of the alkaloid. The *p*-toluenesulfonic acid salt of the alkaloid is then eluted with chloroform, and the eluate is passed over an alkaline column which removes the *p*-toluenesulfonic acid moiety. Several variations of this procedure were tried for dextro-amphetamine sulfate, but quantitative recoveries were not achieved.

Another of the procedures investigated employed the addition of a layer of infusorial earth with concentrated ammonium hydroxide solution to an acidic column containing the amphetamine sample. When chloroform was passed through the column, it carried through a sufficient amount of the ammonia to neutralize the acid and allow the amphetamine to be eluted as the free base. The excess ammonia was then removed by warming on a steam bath under a stream of air. The chloroform was transferred to a separator containing sulfuric acid (2 in 100) and extracted as in the method given. Though this procedure gave quantitative results, it required two more steps than the method employing triethylamine. These are the mixing and packing of the ammoniacal infusorial earth onto the column and the removal of the large excess of ammonia which is needed to permit the elution of the amphetamine from the column.

PROCEDURE

Reagents

Triethylamine.—Prepare a 1 in 200 solution of triethylamine in water-saturated chloroform. Extract 20 ml. of this solution with 10 ml. of sulfuric acid solution (2 in 100). Determine the absorbance of the acid solution at 257 mμ, using the sulfuric acid solution as the blank. If the absorbance is 0.010 or greater, purify the amine as follows.

Reflux 100 ml. of triethylamine with 20 ml. of water and 2 Gm. of sodium hydrosulfite for about 6 hours. Wash the amine with water and dry it by refluxing through a Dean-Stark trap. Finally, distill, collecting the first 75 ml. of distillate, and store it over anhydrous sodium carbonate. Test the purified amine occasionally to determine if the absorbance is below the specified maximum.

Chloroform.—Saturate reagent grade chloroform with water.

Hydrochloric Acid.—Use 1 in 100 solution.

Sulfuric Acid.—Use a 2 in 100 chloroform saturated solution.

Infusorial Earth.—Use acid washed Celite.[1]

Preparation of the Sample

Weigh and finely powder a sufficient number of tablets to provide a representative sample. Weigh accurately a portion of the powder, equivalent to about 5 mg. of dextro-amphetamine sulfate, in a 100-ml. beaker. Add 2 ml. of hydrochloric acid solution and swirl gently to wet the powder thoroughly. Warm on a steam bath for about 1 minute with occasional gentle swirling and cool. Add 3 Gm. of infusorial earth to the beaker, and mix until a fluffy mixture is obtained.

Chromatographic Column

Pack a pledget of fine glass wool with the aid of a tamping rod in the base of a chromatographic tube made from approximately 5 cm. of 6–8 mm. tubing fused to a 25 × 300-mm. test tube. The tamping rod consists of a disk of stainless steel, aluminum, or glass, with a diameter about 1 mm. less than that of the column, attached to a rod approximately 18 in. long. To 2 Gm. of infusorial earth in a 100-ml. beaker, add 1 ml. of hydrochloric acid solution and mix thoroughly with a spatula. Transfer the mixture to the column and tamp lightly to compress the material into a uniform mass. Transfer the sample preparation to the column, "dry rinse" the beaker with about 1 Gm. of infusorial earth, and transfer it to the column. Add a pledget of fine glass wool and tamp as above. Wash the column with 100 ml. of water-saturated chloroform and discard the washings.

Assay

Place a 125-ml. separator containing 10.0 ml. of chloroform-saturated sulfuric acid solution under the column to receive the eluate. Pass 35 ml. of a 1 in 200 solution of triethylamine in water-saturated chloroform through the column. Complete the elution with 70 ml. of water-saturated chloroform. Shake the separator vigorously for 1 minute, allow the layers to separate, and discard the chloroform. Scan the spectra of the acid solution of the sample and of a standard solution, containing about 0.5 mg. of dextro-amphetamine sulfate per ml., from 350 to 250 mμ in 1-cm. cells with a suitable recording spectrophotometer using chloroform-saturated sulfuric acid as the blank. Determine the baseline-corrected absorbance of the

TABLE I.—RESULTS OBTAINED IN THE ASSAY OF 5-MG. DEXTRO-AMPHETAMINE SULFATE TABLETS

Commercial Product	Per cent of Claim U.S.P. Assay	Distillation Assay	Proposed Assay
A	95.4	97.4	97.6
	95.0	97.4	97.0
B	91.2	97.6	97.8
	91.8	96.8	98.4
C	91.2	94.0	95.6
	90.2	95.6	95.6
D	96.0	97.4	97.6
	96.0	97.4	97.4
E	94.0	97.4	99.6
	94.4	97.8	98.8
F	92.0	98.0	98.4
	95.2	98.0	99.0
G	93.0	98.8	100.4
	91.6	99.0	100.0
H	82.6	94.2	93.8
	79.6	94.2	93.4
I	90.6	95.6	95.6
	91.2	95.2	96.8
J	88.8	94.4	95.0
	89.0	94.8	95.4
K	95.0	100.6	103.9
	95.0	101.0	102.8

[1] Marketed by Johns-Manville Corp., Manville, N. J.

TABLE II.—COLLABORATIVE RESULTS ON PRODUCT K OF TABLE I BY THE COLUMN-UV PROCEDURE

Collaborator	Per cent of Claim
A	102.0
	103.0
B	102.8
	102.0
C	102.0
	104.2
D	103.9
	102.8
E	103.2
	103.6

TABLE III.—RESULTS OF THE ASSAY OF OTHER SYMPATHOMIMETIC AMINES

Product	Dosage Form	Label Claim	Per cent of Claim Found
Mephentermine sulfate	Tablets	25 mg./tab.	99.0
			98.6
Ephedrine sulfate	Capsules	25 mg./cap.	100.4
			100.8
Phenylpropanol-amine HCl	Elixir	4 mg./ml.	97.5
			96.8
Methamphet-amine HCl	Tablets	5 mg./tab.	100.8
			101.2

sample and standard solutions at the maximum at about 257 mμ by drawing the baseline as a continuation of the curve between 350 and 300 mμ.

Calculate the milligrams of dextro-amphetamine sulfate per tablet by the formula $10\ C\ (A_u/A_s)(W_t/W_u)$, in which C is the concentration of the standard solution in milligrams per milliliter, A_u is the absorbance of the sample solution, A_s is the absorbance of the standard solution, W_t is the average tablet weight, and W_u is the weight of the sample taken.

RESULTS AND DISCUSSION

A simulated dextro-amphetamine sulfate tablet mixture was prepared for use in standard recovery tests to check the accuracy of the method. Duplicate assays of the mixture gave recovery values of 100.0 and 100.2%.

Eleven samples of commercial 5-mg. dextro-amphetamine sulfate tablets, were assayed by three different methods: (*a*) the U.S.P. XVI procedure, (*b*) a distillation-ultraviolet absorption procedure, and (*c*) the method described herein. A sufficient number of tablets for all three procedures was weighed, finely powdered, and the appropriate aliquot taken for each method. All of the analyses were run in duplicate, the data from which are shown in Table I.

The distillation procedure was run on the 11 samples as a check on the accuracy of the proposed column procedure. A weighed sample, equivalent to about 15 mg. of dextro-amphetamine sulfate, was distilled from a strongly alkaline solution and collected in a solution of mineral acid. The acid solution was made alkaline and extracted with chloroform. The chloroform solution was then extracted and the amphetamine measured as it is in the column method.

Product K, one of the eleven commercial samples of compressed tablets, was distributed to other chemists of this laboratory for collaborative study. The resulting data are shown in Table II.

To investigate further the scope of the method, a timed release preparation of dextro-amphetamine sulfate, consisting of encapsulated pellets, was analyzed by the column procedure. The per cent of label claim found was 103.1, 102.0, and 102.8%. The method can also be used to analyze other sympathomimetic amines with similar physical and chemical properties. The results of the analyses of four such compounds are shown in Table III.

Amphetamine has a relatively low absorptivity. Using the Beckman spectrophotometer, a concentration of the order of 0.5 mg. per ml. is required to give an optimum absorbance value for quantitative determination. Since for single-dose-unit analysis a small sample is specified, the column eluate is concentrated by extraction into an acid solution. The small quantity of triethylamine employed is sufficient to neutralize the acid and release the amphetamine from the column. When quantities of triethylamine approaching 1 ml. were used, the assay results were consistently about 2% low. The excess triethylamine reacted with the sulfuric acid to form the water-soluble amine sulfate which increased the acid solution volume and caused the low results. Levine (3), in the determination of codeine in mixtures with antihistamines, used more than 1 ml. of triethylamine in the elution. The eluate was collected in a volumetric flask and diluted to volume with chloroform from which the absorbance was determined; therefore, no interference with the concentration was encountered.

The official method of assay of dextro-amphetamine sulfate tablets U.S.P. XVI (4) was shown to have several undesirable aspects. The data in Table I show that the results from the official assays are both low and are the least precise for most of the 11 samples. The results of the distillation-ultraviolet absorption and the column-ultraviolet absorption methods are practically the same. The longer time required to complete the official assay procedure and the low assay results make its use as a general method unsatisfactory. For the varied purposes of a compendium assay, a method suitable to all available products is necessary. The proposed method meets this criterion and at the same time provides a simpler and more rapid method of assay than does the official method.

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