Assay Procedure for Spectrophotometric Determination of Dextroamphetamine Sulfate and Amobarbital Formulated into Timed-Release Capsules

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Abstract □ An analytical method is presented for the quantitative spectrophotometric determination of a combination of dextroamphetamine sulfate and amobarbital blended in long-acting pellets. The powdered mixture is dissolved in 50% ethanol, and the solution is poured onto an alginic acid column which holds dextroamphetamine. Amobarbital, which passes through the column with the alcoholic washings, is collected in a 100-ml. volumetric flask. The assay technique offers a rapid and accurate determination of the two therapeutically active ingredients. Synthetic mixtures were analyzed by the proposed method, and recovery was 98–101%.

Keyphrases □ Amobarbital–dextroamphetamine sulfate timed-release formulations—GLC separation, UV spectrophotometric analysis □ Dextroamphetamine sulfate–amobarbital timed-release formulations—GLC separation, UV spectrophotometric analysis □ Timed-release formulations, amobarbital–dextroamphetamine sulfate—separation and analysis □ GLC—separation, amobarbital–dextroamphetamine □ UV spectrophotometry—analysis, amobarbital and dextroamphetamine in combination

Pharmaceutical preparations containing dextroamphetamine and amobarbital have been on the market for a number of years. However, no standard analytical procedure exists for the determination of these two ingredients in combination. Several methods are available in the literature for the analysis of dextroamphetamine and amobarbital as individual pure substances and in combination with other drugs. Worrel and Ebert (1) obtained quantitative recovery of amphetamine in combination with aspirin and phenacetin following a procedure involving the precipitation of amphetamine using tetraphenylboron, with the subsequent volumetric determination. Colorimetric methods for dextroamphetamine and amobarbital have been developed (2, 3). Analytical methods involving UV spectrophotometry also were reported (4, 5). Dextroamphetamine may be analyzed by a distillation method (6), which is the analytical procedure presently official in the British Pharmacopoeia.

Wells (7) described a GLC method for the determination of the optical isomers of amphetamine which, according to the author, could be extended to the dosage of dextroamphetamine in the pharmaceutical preparation. Although full consideration was given to this method in early studies of the problem, the separation of dextroamphetamine sulfate and amobarbital using GLC did not permit their analysis in combination. Even the current widely accepted method (8) for the analysis of mixtures of these two active ingredients, based on extraction procedure, is not specific and is time consuming. When the procedure was applied as the method of analysis to the determination of dextroamphetamine and amobarbital in a timed-release preparation, the extraction was often complicated by formation of emulsions.

Table I—Analyses of Synthetic Mixtures

Analysis Number	Dextroamphetamine Sulfate Recovered, mg.	Amobarbital Recovered, mg.
1	10.09	65.00
2	10.10	64.87
3	9.21	65.30
4	9.97	63.91
5	10.14	64.88
6	10.15	64.91
7	9.98	65.10
8	10.10	65.20
$\bar{X}$	9.97	64.89
SD, mg.	±0.31	±0.41

Since this popular pharmaceutical combination is assayed extensively, a more efficient procedure was needed; therefore, a fast and accurate spectrophotometric method for the determination of the combination of dextroamphetamine sulfate and amobarbital has been devised. In the proposed method, the two active ingredients are separated on a chromatographic column containing alginic acid; this cation-exchanger (9) is now widely employed in pharmaceutical analysis. The separated compounds are determined by UV spectrophotometry. Dextroamphetamine is eluted from the column with 0.5 *N* sulfuric acid and its absorbance is determined at 257 nm.; amobarbital, which is washed out of the column, is collected into a 100-ml. volumetric flask and its absorbance is evaluated at 240 nm.

EXPERIMENTAL

Apparatus—A spectrophotometer (Beckman DB) with 1- and 4-cm. square fused silica cells was used. The glass column, 30 × 1.8 cm. with a 5-cm. stem, was fitted with a buret key.

Reagents—The following were used: cation-exchange resin alginic acid[1], 40–100 mesh; 0.5 *N* sulfuric acid in water; 50% ethanol in water; and 2 *N* hydrochloric acid in water.

Buffer Solutions—The following were used: (*a*) pH 6—250 ml. of 0.2 *M* KH_2PO_4 and 28 ml. of 0.2 *N* NaOH diluted to 1000 ml. with distilled water; and (*b*) pH 10—250 ml. 0.2 *M* H_3BO_3, 250 ml. of 0.2 *M* KCl, and 220 ml. of 0.2 *M* NaOH diluted to 1000 ml. with distilled water. In all cases, reagent grade materials were used.

Standard Solutions—The following were used: (*c*) dextroamphetamine sulfate, 0.4 mg./ml. in 50% ethanol; and (*d*) amobarbital, 2.4 mg./ml. in 50% ethanol.

Column Preparation—Alginic acid, about 4 g., is slurried in water and allowed to soak 4 hr. The slurry is poured into a glass column fitted with glass wool and allowed to settle. The column is washed with 2 *N* hydrochloric acid until the absorbance of the eluate (path-

[1] British Drug Houses.

Table II—Analyses of Commercial Product

Product	Dextroamphetamine Sulfate Found, mg.	Amobarbital Found, mg.
A	10.06	65.19
B	10.12	64.60
C	9.20	65.00
D	10.10	65.68
E	9.91	65.30
F	10.10	63.93
G	10.00	65.90
H	10.09	65.00

length 4 cm.) is less than 0.005 at 257 nm., and it is then washed with distilled water until the eluate is neutral to litmus solution. Finally, 25 ml. of 50% ethanol is passed through the column.

Sample Treatment—Grind as completely as possible, to pass through a 100-mesh sieve, the contents of not less than 20 capsules[2]. Transfer an accurately weighed portion of powder, equivalent to about two capsules, to a 50-ml. volumetric flask. Add 50% ethanol to volume and mix. Allow the mixture to stand for 30 min., shaking occasionally; filter it through a tight pledget of cotton wool and then discard the first 10 ml. of filtrate. Pipet 10 ml. of clear solution onto the prepared alginic acid column; place a 100-ml. volumetric flask under the column and start collecting the eluate at a rate of 1 ml./min. Then pass more 50% ethanol through the column to the 100-ml. mark. This eluate contains amobarbital. Wash the column with 100 ml. of distilled water at as fast a rate as possible, and allow the level of the liquid in the column to drop to just above the resin. Elute the column with 0.5 *N* sulfuric acid at a rate of 1 ml./min. Discard the first 10 ml. of eluate, and collect the rest in a 100-ml. volumetric flask to volume. This solution contains dextroamphetamine.

Determination—By following the baseline technique, compare the absorbances of the standard solution passed through the column with those of the samples. Quantitatively adjust the claimed concentrations of the sample eluates as closely as possible to the corresponding standard solutions. Calculate the amount of amobarbital in the sample from the value of the absorption of the corresponding standard at 240 nm. in a pH 10 buffer, using the same aliquot in a pH 6 buffer as the blank. Similarly calculate the amount of dextroamphetamine sulfate at 257 nm., using 4-cm. cells and 0.5 *N* sulfuric acid as the blank.

RESULTS AND DISCUSSION

The accuracy of the proposed method is based upon results of eight synthetic mixtures containing a proportion of 10–65 mg. of dextroamphetamine sulfate and amobarbital, respectively. Recoveries for each drug are presented in Table I.

Eight different batches of a commercial product were assayed by the proposed method. The results of these determinations are given in Table II.

Table III—Recovery of Amobarbital Added to Commercial Preparation of Dextroamphetamine Sulfate

Mixture	Amobarbital		
	Milligrams Added	Milligrams Recovered	Percent Recovery
a	35.00	34.90	99.7
b	40.00	39.70	99.3
c	65.00	65.40	100.6
d	95.00	93.90	98.9

To eliminate interferences from flavoring, sweetening, and coating agents, amobarbital was determined utilizing the pH chromophoric procedure. Since the absorption maximum of amobarbital can be readily shifted, this technique was employed as the means of quantitative assay. To prove that the absorption was unaffected by the presence of the excipients, standard amobarbital was added to powdered samples of various commercial timed-release granules containing only dextroamphetamine sulfate. A twofold excess of granules over the amount usually found in amobarbital and dextroamphetamine sulfate preparations was added; the mixtures were assayed by the proposed method. Recoveries of the added amobarbital are listed in Table III.

When the capsule contents were not ground finer than 100 mesh, the assay indicated less dextroamphetamine sulfate and amobarbital than the amount declared. This method is simple, rapid, and designed to determine specifically dextroamphetamine sulfate and amobarbital in combination and in the presence of numerous other organic compounds found in timed-release pharmaceuticals.

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[2] The analysis was applied to capsules containing 10 mg. of dextroamphetamine sulfate and 65 mg. of amobarbital; these capsules are marketed as Dexabarb L.A.