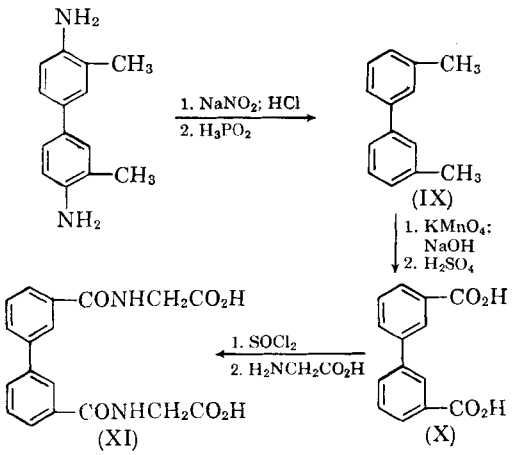

Gm. (0.01 mole) of glycine, and 1.0 Gm. (0.025 mole) of sodium hydroxide in 50 ml. of water were refluxed for 30 minutes. The mixture was cooled and the product precipitated by the slow addition of a 5% solution of hydrochloric acid and recrystallized from ethanol, yielding 1.1 Gm. of product (78.6%); m.p. 204° (decompn.).

Anal.—Calcd. for $C_{18}H_{16}N_2O_6$: N, 7.86. Found: N, 7.88, 7.89.

SUMMARY

Five new derivatives of diphenic acid have been prepared for pharmacological testing as possible antibacterial, antihyperglycemic, antispasmodic, and/or local anesthetic activity: *o,o'* - bis(2-nitro - 1,3 - dihydroxypropyl)biphenyl disodium salt; *o,o'* - bis(3 - benzoxy - 2 - benzamido - 1-hydroxypropyl)biphenyl; *o,o'* - bis(β - dichloroacetamidoethyl)diphenate; N,N' - bis - (benzenesulfonyl) - *o,o* - diphenoylurea, and *m,m'*-bis(N-carboxymethyl)diphenamide.

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Analysis of Phenobarbital Elixir by an Ion Exchange and Nonaqueous Titration Procedure

By MARTIN I. BLAKE and FREDERICK P. SIEGEL

Phenobarbital elixir is analyzed by passing an aliquot through a strongly basic anion exchange resin. The phenobarbital is eluted from the column with acetic acid in ethanol. After evaporation of the solvent, the residue is dissolved in dimethylformamide and titrated with sodium methoxide in benzene-methanol. Other barbiturate elixirs are assayed similarly. Nonionic components, coloring agents, bases, most salts, and synthetic sweetening agents do not interfere.

THE ANALYSIS of barbiturate salts in a variety of dosage forms by ion exchange and nonaqueous titration was recently reported (1). Application to the assay of elixirs proved unsuccessful. Ion exchange resins have been employed for the isolation of barbiturates (2) and a variety of nonaqueous techniques have been described for determining barbiturates in dosage forms. These have been reviewed in an earlier paper (1). The usual procedure in applying a nonaqueous titration procedure to the analysis of elixirs involves extraction of the barbiturate with an organic solvent prior to titration in a nonaqueous medium.

Potentiometric titration of phenobarbital elixir with silver nitrate was reported by Mattocks and Voshall (3) and later by Bodin (4). Cohen and Lordi (5) developed an amperometric and a potentiometric titration procedure with mercuric ion for analyzing the elixir. A general review of the literature dealing with the analysis of the barbituric acids is presented by Connors (6).

The official assay for phenobarbital elixir is a gravimetric one in which the phenobarbital is extracted with chloroform. High results are usually obtained because of extraction of other chloroform soluble components. This has been noted by several workers (3, 7). In addition, the official assay is tedious and time consuming.

This report describes a procedure in which the phenobarbital is removed from the elixir by

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passage through an anion exchange resin. The phenobarbital is eluted from the column with acetic acid in ethanol followed by titration in dimethylformamide with sodium methoxide in benzene-methanol. The assay is applied to other barbiturate elixirs.

EXPERIMENTAL

Preparation of Column.—The strongly basic anionic exchange resin Dowex 2-X8, 50-100 mesh, was used in this study. A suspension in water of about 10 Gm. of the resin was added to a chromatographic column, 1 cm. × 30 cm. The resin column was washed with 100 ml. of distilled water, 100 ml. of 5% sodium hydroxide, and 200 ml. of distilled water. The column was ready for use.

Assay Procedure for Pure Phenobarbital.—Approximately 100 mg. of pure phenobarbital, accurately weighed, was dissolved in 50 ml. of 50% ethanol. The solution was passed through the resin column. This was followed by the addition of 50 ml. of distilled water. The phenobarbital was eluted from the column with a solution of 50% acetic acid in 95% ethanol at a flow rate of 1 ml. per minute. The first 10 ml. of eluate contained no phenobarbital and was discarded. The next 50 ml. of eluate was collected and evaporated to dryness on a steam bath. Five milliliters of distilled water was added and the solution was again evaporated to dryness. This was repeated once. The residue was dissolved in 20 ml. of dimethylformamide and the solution was titrated with 0.1 *N* sodium methoxide in benzene-methanol prepared and standardized as described earlier (8). Titration was effected potentiometrically with a Fisher titrimeter equipped with a calomel and glass electrode system. The end point was determined by plotting millivolts *vs.* volume of titrant. The point of inflection was readily determined from the curve. A blank run was conducted with each series of three determinations. Recovery of phenobarbital was quantitative as indicated by data reported in Table I.

TABLE I.—ANALYSIS OF PHENOBARBITAL ELIXIR

Sample	Phenobarbital Theoretic or Labeled Amount, mg.	Proposed Method, % Recovery[b]	Official Method, % Recovery[c]
Pure phenobarbital	100.0	99.76 ± 0.09[d]	...
Elixir A	100.0[a]	99.12 ± 1.19	100.7
Elixir B	100.0	97.29 ± 0.77	99.5
Elixir C	100.0	96.15 ± 0.78	99.0
Elixir D	100.0	100.96 ± 0.29	104.7

[a] Mg. per 25 ml. [b] Average of at least 5 runs. [c] Average of duplicate runs. [d] Standard deviation.

Phenobarbital Elixir.—Exactly 25 ml. of phenobarbital elixir was transferred by pipet to a 150-ml. beaker. About 25 ml. of distilled water or 50% ethanol was added. This reduced the viscosity of the elixir and permitted a more rapid flow rate through the column. The solution was added to the column. When the last of the solution disappeared below the surface of the resin, distilled water was added to the column. The column was washed with 100-150 ml. of water. The phenobarbital was eluted from the column with 50% acetic acid in 95% ethanol. The eluate was treated as described previously for the pure phenobarbital sample. The data for the analysis of phenobarbital elixir are reported in Table I. Elixirs *A* and *B* represent different batches of phenobarbital elixir prepared in the manufacturing pharmacy of our pharmacy department. Elixirs *C* and *D* were commercial samples.

Other Barbiturate Elixirs.—The proposed assay procedure was applied to the analysis of elixirs containing secobarbital, pentobarbital, and amobarbital. Commonly used commercial varieties of the elixirs were employed in this study. These were treated in the same manner as described for phenobarbital elixir. The data are reported in Table II.

TABLE II.—ANALYSIS OF OTHER BARBITURATE ELIXIRS

Sample	Barbiturate Concn. Labeled Amount, mg.	% Recovery
Secobarbital elixir	110.0[a]	101.86 ± 0.49[c]
Amobarbital elixir	110.0	99.74 ± 0.69
Pentobarbital sodium elixir	91.3[b]	103.83 ± 1.07

[a] Mg. per 25 ml. [b] As pentobarbital. [c] Standard deviation.

DISCUSSION

The shortcomings of the official assay procedure for phenobarbital elixir have been noted here and have been indicated previously by other workers. Although a variety of methods and modifications have been proposed which appear to be superior to the official assay procedure the latter has remained essentially unchanged over the years.

In the technique proposed in the present study, a strongly basic anion exchange resin, quaternary base type, is used to remove the barbiturate from the other components of the elixir. This obviates the tedious and time consuming extraction process used in the official assay and in most of the proposed methods based on nonaqueous titration. The eluent, 50% acetic acid in ethanol, effectively removes the barbiturate from the column. Acetic acid, a stronger acid than the barbiturates, displaces the latter from the resin. Although the barbiturate may be quantitatively recovered from only 30 ml. of eluate, 50 ml. is collected in the assay to ensure complete extraction. Quantitative recoveries are reported for phenobarbital elixir in Table I and for other barbiturate elixirs in Table II. Comparison was made with the official assay for phenobarbital elixir. The data are shown in Table I. It should be noted that somewhat higher results were consistently obtained with the official procedure. This is undoubtedly due to the extraction of chloroform soluble components in addition to the phenobarbital. This was reported earlier by others (3, 7).

The interference by other components in the elixirs was studied. Nonionic ingredients such as sucrose, glycerol, and propylene glycol were readily washed

from the column with water. They appeared in the eluate. The amaranth coloring agent used in phenobarbital elixir did not pass through the column, but concentrated in the upper 0.5 cm. of the resin column. When the resin was to be regenerated, the upper centimeter of the column was removed and discarded. The column was then washed with distilled water, sodium hydroxide solution, and distilled water until the eluate showed pH 5 to 6. The column was ready for a second run. Caramel, the coloring agent used in certain barbiturate elixirs, appeared to be washed completely from the column with water. Barbiturate salts, as pentobarbital sodium in pentobarbital elixir, do not complicate the assay since only the anion is retained by the resin; the cation appears in the eluate. Synthetic sweetening agents as saccharin sodium and cyclamate calcium may be used in commercial barbiturate elixirs. A mixture containing 100 mg. of saccharin and 100 mg. of phenobarbital was titrated in dimethylformamide with sodium methoxide. Differentiation of the two acids was noted. This is shown in Fig. 1, curve *A*; the first inflection is due to saccharin and the second is attributed to phenobarbital. Recovery was quantitative for both components. A mixture of 100 mg. of saccharin sodium and 100 mg. of phenobarbital was dissolved in 50% ethanol and passed through the resin column. The column was eluted with acetic acid in ethanol as described for the barbiturate elixirs. Titration in dimethylformamide with sodium methoxide showed quantitative recovery of the barbiturate. See Fig. 1, curve *B*. Elution of the column with 1 *M* hydrochloric acid in 50% ethanol, evaporation of the solvent, and subsequent nonaqueous titration indicated quantitative recovery of the saccharin. Since acetic acid is a stronger acid than the barbiturates used in this study, but weaker than saccharin, the former and not the latter is displaced from the resin column. Similar results were obtained with cyclamate calcium. Hydrochloric acid, found in pentobarbital elixir, does not interfere since acetic acid will not displace it from the column. Salts, in general, will not interfere unless the anion represents an acid which is weaker than acetic

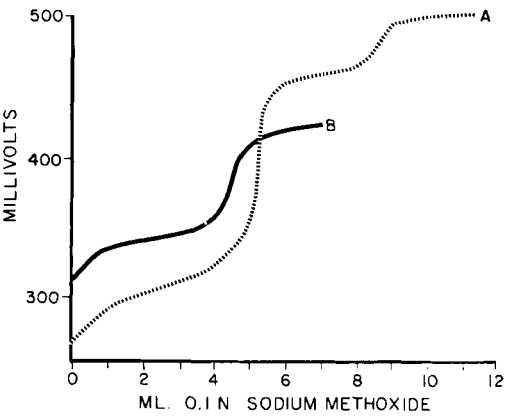

Fig. 1.—Curve *A*, titration of saccharin and phenobarbital mixture; curve *B*, titration of phenobarbital after passage of mixture through resin column.

acid. Even then, it may be possible to differentiate between it and the barbiturate by nonaqueous titration if the two differ significantly in acid strength. Methenamine and bases do not interfere.

The proposed assay procedure is simple, accurate, and less time consuming than the official procedure. It should have wide application. Although it has been used only for elixirs in this study, it should be applicable to other dosage forms and to certain combinations of barbiturates with other agents.

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