was injected simultaneously and also intraperitoneally (eight animals per dose). Salivation was observed 30 minutes after injection by wiping the mouth of the animal on a blotting paper; the ED_{50} was determined by the same procedure employed in the experiment on mice and rats.

Action on Pupillary Width.—The pupillary width in unanesthetized mice was measured with a binocular lens with a magnification of 15 times 30 minutes after subcutaneous administration of the drugs (Pulewka, P., *Arch. Exptl. Pathol. Pharmakol.*, **168**, 307(1932)). The ED_{300} (dose which increases the pupil size to 300% of the control value) was determined.

RESULTS

Pharmacologic results of methixene hydrochloride and atropine are shown in Table II.

In three species tested, the relative potency of methixene hydrochloride to atropine reveals that with respect to inhibition of gastrointestinal motility, atropine is 2.2 to 16.7 times as potent, but with reference to inhibition of salivation, atropine is 32 to 87 times as potent. In the mouse, atropine is 20 times as potent as methixene hydrochloride with respect to mydriatic effect.

ACUTE TOXICITY

The values of the LD_{50} and their confidence limits have been calculated according to the Litchfield-Wilcoxon method (7). The intravenous LD_{50} for methixene hydrochloride is 18.0 (15.8 to 20.5) mg./Kg. in mice and 24.0 (21.0 to 27.4) mg./Kg. in rats. Orally, the LD_{50} is 430 (350–530) mg./Kg. in mice and over 1,500 mg./Kg. in rats.

CHRONIC TOXICITY

Initial studies on the tolerance of methixene hydrochloride during long term administration were carried out in rats. Three groups of ten animals each received 1, 4, and 15 mg./Kg. of the drug with the food for 9 months. An untreated group served as the controls. Weight gain in the treated animals and controls, hematological findings, and macroscopic and histologic examination of the organs gave no indication of toxic action of methixene hydrochloride in this dosage (2). Studies in rats for 18 months and in dogs for 12 months and suitable progeny studies are in progress and will be reported when completed.

CONCLUSION

It is evident that methixene hydrochloride is somewhat less active than atropine in the intestinal passage test and also with regard to inhibition of the peristaltic reflex. However, if the characteristic secondary actions of parasympatholytic agents, inhibition of salivation, and mydriatic action are considered, far higher doses of methixene hydrochloride are required to produce these effects than those of atropine.

In the three animal species studied, the therapeutic ratio of methixene hydrochloride (range between gastrointestinal effect and undesired side effects) is from four to 21 times higher than that of atropine.

Studies in experimental animals justify the therapeutic evaluation of methixene hydrochloride in the symptomatic management of conditions associated with gastrointestinal hypermotility or spasm.

Long term toxicity studies and progeny studies should be completed before methixene hydrochloride is released for general use.

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Analysis of Dosage Forms Containing Ephedrine and Barbiturate Combinations

By MARTIN I. BLAKE and DANIEL A. NONA

Procedures are presented for the determination of ephedrine salts in combination with barbiturates in tablets and capsules. An aliquot prepared from the dosage form is passed through a strong anion exchange resin. The ephedrine, contained in the eluate, is determined by titration with standard hydrochloric acid. The barbiturate is eluted from the column with acetic acid in ethanol and determined by nonaqueous titration. A modification of this procedure is proposed for formulations containing the sodium salt of the barbiturate. The methods are simple, accurate, and less time consuming than the official assay.

THE OFFICIAL ASSAY (1) for ephedrine sulfate and phenobarbital capsules involves ether extraction of the phenobarbital and a Kjeldahl distillation procedure for the estimation of the ephedrine content. The assay procedure has remained essentially unchanged since N.F. VIII when this dosage form first became official. The distillation technique was originally proposed by Hilty (2). The ephedrine sulfate solution is refluxed with hydrochloric acid and distilled in the presence of zinc dust and sodium hydroxide into a solution of standard acid. The ephedrine is determined by residual titration of the distillate. The method is tedious and time consuming.

Hilty and Wilson (3) analyzed tablets containing a combination of ephedrine sulfate and a barbiturate

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by ether extraction of the ephedrine and chloroform extraction of the barbiturate. Mattson (4) developed an ultraviolet spectrophotometric procedure for phenobarbital when in the presence of ephedrine sulfate. Comer and Bourne (5) analyzed capsules containing a combination of aminophylline, amobarbital, and ephedrine hydrochloride. The barbiturate and ephedrine were separated by solvent extraction and determined by infrared spectrophotometry.

Methods of analysis of ephedrine salts and barbiturates as individual components in various dosage forms were reviewed in earlier papers (6–8), and procedures based on ion exchange and nonaqueous titration were proposed for their determination.

In this paper procedures are presented for the analysis of capsules and tablets which contain combinations of ephedrine salts with barbiturates or barbiturate salts. The components are separated by passage through ion exchange resins and estimated by an aqueous or nonaqueous titration procedure.

EXPERIMENTAL

Preparation of Columns.—Two ion-exchange resins were employed in this study. The strongly basic anionic exchange resin Dowex 2-X8, 50–100 mesh, was prepared for use by adding about 10 Gm. of the resin in the form of an aqueous suspension to a chromatographic column, 1 × 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 strongly acidic cation exchange resin Dowex 50-X8, 200–400 mesh, was prepared by adding about 10 Gm. of resin to a column, 1 × 30 cm. When the resin settled, the column was washed with 200 ml. of distilled water, 100 ml. of 2 *N* hydrochloric acid, and finally with distilled water until the eluate was neutral. A solvent layer was always maintained above the resin column.

Table I.—Analysis of Ephedrine Salts and Phenobarbital Mixtures

Mixture	Weighed Amount, mg.	Recovery, %
Ephedrine sulfate	100.0	99.50 ± 1.14[a]
Phenobarbital	100.0	99.78 ± 0.26
Ephedrine HCl	100.0	99.48 ± 0.98
Phenobarbital	100.0	100.90 ± 0.09

[a] Average of at least six determinations ± standard deviation.

Analysis of Ephedrine and Phenobarbital Mixtures.—A sample containing 100 mg. of ephedrine sulfate or ephedrine hydrochloride and 100 mg. of phenobarbital, accurately weighed, was dissolved in 25 ml. of 50% ethanol. The solution was added to the column containing the strongly basic anion exchange resin. Additional 50% ethanol was passed through the column until 75 ml. of eluate was collected. The ephedrine, as free base, passed through the column and appeared in the eluate. The ephedrine was determined by visual titration with 0.1 *N* hydrochloric acid, using 2 drops of methyl red T.S. as the indicator.

The phenobarbital, retained by the column, was eluted with 50% acetic acid in 95% ethanol at a flow rate of 1 ml. per minute. Sixty milliliters of eluate was collected, and the solution was evaporated to dryness on a steam plate. Five milliliters of water was added, and the solution was again evaporated to dryness. This procedure was repeated once. The residue was dissolved in 20 ml. of dimethyl formamide, and the solution, magnetically stirred, was titrated with 0.1 *N* sodium methoxide in benzene-methanol, prepared and standardized as described previously (9). Titration was effected potentiometrically with a Fisher titrimeter equipped with a calomel and glass electrode system. The end point was determined from the break in the curve obtained by plotting millivolts *versus* volume of titrant.

The analysis of ephedrine and phenobarbital in synthetic mixtures is reported in Table I.

Analysis of Dosage Forms.—The proposed procedure was applied to solid dosage forms containing ephedrine salts combined with barbiturates.

Ephedrine sulfate and phenobarbital capsules N.F. XI and commercially available capsules of ephedrine sulfate and amobarbital were analyzed by transferring the contents of 20 capsules to a beaker. Forty milliliters of 50% ethanol was added, and the solution was mixed thoroughly. The solution was filtered into a 100-ml. volumetric flask, using washings of 50% ethanol to effect the transfer. Sufficient diluted alcohol was passed through the filter to fill

Table II.—Analysis of Dosage Forms Containing Ephedrine Salt and Barbiturate Mixtures

Dosage Form	Components	Labeled Amount Per Dosage Unit, mg.	Recovery, %
Ephedrine sulfate and phenobarbital capsules N.F.	Ephedrine sulfate	50.0	98.90 ± 0.46[a]
	Phenobarbital	50.0	98.85 ± 0.65
Ephedrine and Amytal pulvules	Ephedrine sulfate	25.0	99.50 ± 1.01
	Amobarbital	50.0	99.59 ± 0.34
Ephedrine and Amytal tablets	Ephedrine sulfate	8.0	101.50 ± 1.68
	Amobarbital	16.0	98.88 ± 1.02
Ephedrine and phenobarbital sodium capsules	Ephedrine	25.0	98.74 ± 0.98 99.51 ± 0.91[b]
	Sodium phenobarbital	50.0	99.42 ± 0.62
Ephedrine and Seconal sodium capsules	Ephedrine sulfate	25.0	98.94 ± 1.46 99.11 ± 0.32[b]
	Sodium secobarbital	50.0	99.22 ± 1.96
Ephedrine and Nembutal capsules	Ephedrine HCl	25.0	99.73 ± 1.37 99.42 ± 0.86[b]
	Sodium pentobarbital	25.0	99.46 ± 0.41

[a] Average of at least six determinations ± standard deviation. [b] By nonaqueous titration method.

the flask to the mark. An aliquot of this solution, containing 50 to 100 mg. of both ephedrine sulfate and barbiturate, was transferred by pipet to the column containing the strongly basic anion exchanger. The column was then eluted with 50% ethanol and 50% acetic acid in ethanol as described for ephedrine and phenobarbital mixtures. The data for these analyses are reported in Table II.

Ephedrine sulfate and amobarbital tablets were assayed by triturating 20 tablets in a mortar with approximately 25 ml. of 50% ethanol. The mixture was filtered into a 100-ml. volumetric flask with the aid of diluted alcohol rinsings. Sufficient diluted alcohol was passed through the filter to bring the volume to the mark. An aliquot containing 60 to 100 mg. of both ephedrine sulfate and amobarbital was treated as described in the previous paragraph for the capsule dosage forms. Results are reported in Table II.

A modified procedure was used for the analysis of ephedrine sulfate and phenobarbital sodium, ephedrine sulfate and secobarbital sodium, and ephedrine hydrochloride and pentobarbital sodium. A solution was prepared as described for the capsules, and an aliquot containing between 50 and 100 mg. of each active component was added to the column of the strongly acidic cation exchange resin. The column was eluted with 50% ethanol until 75 ml. of eluate was collected. This solution containing both the barbituric acid derivative and hydrochloric or sulfuric acid was passed through the column of strongly basic anionic exchange resin. The column was washed with water, and the barbiturate was eluted from the column with 50% acetic acid in ethanol. Sixty milliliters of eluate was collected, and the solution was evaporated to dryness on a steam plate and assayed nonaqueously as described earlier.

The strongly acidic exchange column which retained the ephedrine and sodium ion was eluted with a solution of 5% ammonia in 95% ethanol. A total of 60 ml. of eluate was collected in a 100-ml. beaker. The solution was gently aerated by passing a stream of air over the beaker until the ammonia was completely expelled. At least 3 hours was required for this step. During the aeration the liquid level in the beaker was maintained constant by frequent addition of alcohol. The solution was titrated visually with 0.1 *N* hydrochloric acid using 2 drops of methyl red T.S. as indicator. This solution containing ephedrine hydrochloride was evaporated to dryness on a steam plate, and the residue was analyzed for ephedrine content by nonaqueous titration. Ten milliliters of glacial acetic acid, 20 ml. dioxane, and 10 ml. of 6% mercuric acetate in glacial acetic acid were added to the beaker. The solution, magnetically stirred, was titrated potentiometrically using a Fisher titrimeter equipped with a calomel and glass electrode system. The titrant was 0.1 *N* perchloric acid in dioxane. Titration was also effected visually by adding 2 drops of methyl violet T.S. to the solution. The indicator color change was noted by using indicator solution in conjunction with a potentiometric titration. The color change corresponding to the graphic end point was from violet to an intense blue coloration.

DISCUSSION

Simple and accurate procedures are proposed for the analysis of combinations of ephedrine salts and barbiturates. They are less cumbersome and less time consuming than the official assay. Four or five assays may be conveniently conducted simultaneously.

When a solution of ephedrine sulfate or ephedrine hydrochloride and a barbiturate is passed through a strongly basic anion exchange resin, quaternary base type, the resin quantitatively splits the ephedrine salt; the free base appears in the eluate, and the acid component remains on the column. The barbiturate is also retained by the column. The ephedrine content of the eluate is readily determined by titration with standard acid. As reported in an earlier paper (7), 50% acetic acid in ethanol effectively removes the barbiturate from the column, but does not displace the hydrochloride or sulfate. The barbiturate content is determined by nonaqueous titration. Data are reported in Table I for the analysis of synthetic mixtures of ephedrine salts and phenobarbital. Application of the proposed procedure to the analysis of the official capsules and several commercially available capsules and tablets is reported in Table II.

A modification of the proposed method is required where the ephedrine salt is combined with a sodium barbiturate. When such a mixture is passed through a strong anionic exchange resin, sodium and ephedrine appear in the eluate. Potentiometric titration does not differentiate these bases. In the modified procedure the solution is first passed through the strong cation exchanger Dowex 50-X8. The ephedrine and sodium ion are retained by the column, and the barbiturate and hydrochloric acid or sulfuric acid appear in the eluate which is then passed through the strong anion exchanger Dowex 2-X8. By displacement elution with acetic acid, the barbiturate is removed from the column while the hydrochloride or sulfate remains on the column. The barbiturate is determined by nonaqueous titration. The strong cation exchanger which has retained the ephedrine and sodium is eluted with 5% ammonium hydroxide in alcohol. The ammonia displaces the weaker base ephedrine from the column; the sodium remains on the column. The ammonia is expelled from the eluate by gentle aeration, and the ephedrine content is determined by titration with standard hydrochloric acid and then by nonaqueous titration with perchloric acid in dioxane. This modification has been applied to three capsule dosage forms containing combinations of ephedrine an barbiturate salts. The data are recorded in Table II.

The modified procedure is applicable where sodium chloride and similar salts are present. Insoluble fillers, lubricants and diluents, and nonionic components in general, will not interfere with the assays. However, acids comparable in strength to the barbiturates and bases similar to ephedrine and their salts will interfere.

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