in mechanism studies, of the possibilities of conductional as well as junctional sites of action for psychotropic agents.

The need to determine site and mechanism of the conduction rate alteration is apparent. The drug concentrations used in this study were near the concentration of chlorpromazine found by Nathan and Friedman (8) to alter the permeability of resting cells of *Tetrahymena pyriformis*. They concluded that this alteration had a lipid site of action. Tasaki (7), in his experiments with saponin, has demonstrated that pharmacological alteration of lipid can change impulse conduction rate. He reported that this agent, by an effect on lipid of the myelin sheath, increased the time required for impulse transmission across the internodal segment. He found, however, that urethan altered conduction rate by a nodal rather than an internodal effect. A study of conduction parameters to provide information as to the site of the rate alteration by these psychotropic agents is planned.

REFERENCES

(1) Bircher, R., "Psychotropic Agents," lecture to Columbia University, College of Physicians and Surgeons, New York, N. Y., 1962.
(2) Randall, L. O., *Diseases Nervous System*, **21**, 7(1960).
(3) Zbinden, G., *et al.*, *Toxicol. Appl. Pharmacol.*, **3**, 619 (1961).
(4) Taussig, H. B., *Sci. Am.*, **207**, 29(1962).
(5) Kopera, J., and Armitage, A. K., *Brit. J. Pharmacol.*, **9**, 392(1954).
(6) Krivoy, W. A., *J. Pharmacol. Exptl. Therap.*, **129**, 186 (1960).
(7) Tasaki, I., "Nervous Transmission," Charles C Thomas, Springfield, Ill., 1953.
(8) Nathan, H. A., and Friedman, W., *Science*, **135**, 793 (1962).
(9) Stacy, R. W., "Biological and Medical Electronics," McGraw-Hill Book Co., New York, N. Y., 1960.

# Ion-Exchange Separation and Ultraviolet Spectrophotometric Determination of Dextromethorphan in Pharmaceutical Products

By K. O. MONTGOMERY* and M. H. WEINSWIG

**A method for the isolation and determination of dextromethorphan is presented. The dextromethorphan is extracted with strong cation exchange resin, AG 50W-X4, and is subsequently eluted with 1 *N* hydrochloric acid in 60 per cent methanol in water. The dextromethorphan is determined in the eluate by ultraviolet spectrophotometry. The assay is used successfully on several commercial products.**

THE POPULAR acceptance of dextromethorphan as an antitussive agent has brought numerous pharmaceutical products to the consumer with this as the main ingredient. There has been very little information reported on the analytical procedures for the determination of dextromethorphan concentrations in liquid dosage forms.

Saques (1) was able to obtain satisfactory results using ultraviolet spectrophotometry and nonaqueous titration on the pure compound but control blanks had to be utilized to overcome interferences from excipients. Lee (2) demonstrated the use of the classical reineckate precipitation and colorimetric determination on the pure compound. Horioka (3) tested a dye complexation and extraction. The official method (4) for the assay of the pure compound and for the official tablets utilizes nonaqueous titration. Ultraviolet spectrophotometry and paper chromatography (5, 6) have also been studied.

The official assay procedure for the syrup requires an involved immiscible solvent extraction and reextraction followed by the determination using ultraviolet spectrophotometry.

The present paper utilizes the ability of strongly acidic cation exchange resins to separate an amine such as dextromethorphan from common dosage form ingredients prior to determination on a spectrophotometer. A weakly acidic solution is used prior to the use of the strongly acidic solution to remove traces of aromatic amines from flavors or coloring agents that may be present in pharmaceutical products. This type of separation using ion-exchange resins has been employed in the determination for phenylephrine by Kelly and Auerbach (7) and by Blake and Nona (8) in the determination for ephedrine salts.

Received May 9, 1966, from the College of Pharmacy, Butler University, Indianapolis, Ind. 46207.
Accepted for publication June 24, 1966.
Abstracted from a thesis submitted by K. O. Montgomery to the Graduate Division, Butler University, Indianapolis, Ind., in partial fulfillment of Master of Science degree requirements.
* Present address: Pitman-Moore Division, The Dow Chemical Co., Indianapolis, Ind.

## EXPERIMENTAL

**Apparatus.**—Glass column 20 cm. × 1 cm. with stopcock made of Teflon and containing built in needle valve for control of flow rate. The column is also fitted with a reservoir with a capacity of 250 ml.

A suitable recording ultraviolet spectrophotometer such as the Beckman DK-2A or Spectronic 505 which records in absorbance units.

**Reagents.**—Cationic exchange resin AG 50W-X4 100–200 mesh in hydrogen form available from Bio-Rad Laboratories, Richmond, Calif. Enough resin, about 3 Gm., is added in the form of a slurry to the glass column and rinsed with water. Hydrochloric acid, 0.05 *N* in 60% methanol in water. Hydrochloric acid, 1.0 *N* in 60% methanol in water.

**Standard Solution.**—Weigh exactly 150 mg. of dextromethorphan hydrobromide N.F. reference standard and transfer to a 100-ml. volumetric flask. Dissolve and adjust the volume with distilled water to prepare the desired stock standard.

Pipet exactly 10 ml. of the stock standard into a 200-ml. volumetric flask and adjust the volume with 1.0 *N* hydrochloric acid in 60% methanol in water.

TABLE I.—ANALYSIS OF PRODUCTS CONTAINING DEXTROMETHORPHAN HYDROBROMIDE

| Dextromethorphan Hydrobromide Product No. | Amt. Claimed | Amt. Found | % Label Claim |
|---|---|---|---|
| 1[a] | 0.9 Gr./fl. oz. | 0.906 Gr./fl. oz. | 100.7 |
| 2[b] | 15 mg./5 ml. | 14.93 mg./5 ml. | 99.5 |
| 3[c] | 5 mg./5 ml. | 5.18 mg./5 ml. | 103.6 |
| 4[d] | 7.5 mg./5 ml. | 7.76 mg./5 ml. | 103.5 |

[a] Marketed as Cheracol D by The Upjohn Co., Kalamazoo Mich. [b] Marketed as Robitussin D M by A. H. Robins, Richmond, Va. [c] Marketed as Thorexin by Isodine Pharmacal Corp., New York, N. Y. [d] Marketed as Actin by Chesebrough-Pond's, Inc., New York, N. Y.

**Sample Treatment.**—Pipet a sample equivalent to 15 mg. of dextromethorphan hydrobromide into the reservoir, rinse the pipet with distilled water, and add to the reservoir.

Add distilled water to the sample to make the volume approximately 100 ml. and mix well.

Allow the sample solution to flow through the resin bed at the rate of 2–3 ml./min. Wash the column by adding 100 ml. of distilled water and allow it to flow through the resin at the rate of 5 ml./min.

Traces of aromatic amines from flavors or coloring agents are removed by allowing 50 ml. of 0.05 *N* hydrochloric acid in 60% methanol in water to flow through the column at 5 ml./min.

Position a 200-ml. volumetric flask under the column and add 190 ml. of 1.0 *N* hydrochloric acid in 60% methanol in water. Allow this to flow through the column at the rate 3 ml./min. The volume is adjusted with 1.0 *N* hydrochloric acid in 60% methanol in water.

**Determination.**—The ultraviolet spectrum of the sample effluent and of the working standard is recorded with a suitable spectrophotometer. Absorbance units should be used.

Using the baseline technique determine the absorbance at the maximum at about 278 mμ. Calculate the amount of dextromethorphan hydrobromide present from the standard values obtained at the same time the sample is analyzed

## DISCUSSION AND RESULTS

Standard solutions, when subjected to the above procedure, yield an average recovery or accuracy of 99.04% with a standard deviation of ±0.52% based on ten determinations. The data for the analysis of marketed products are shown in Table I.

The analytical grade resin used was found to be satisfactory without pretreatment except for washing with a simple water rinse.

A slightly raised baseline was noted with some products where a small portion of the coloring agent was held and eluted with the sample. This did not cause any problem since the baseline technique was used in the calculations.

Other ingredients present in the various formulations included potassium guaiacolsulfonate, ammonium chloride, tartar emetic, sodium citrate, glyceryl guaiacolate, white pine and wild cherry bark extractives, and various common ingredients in syrups and elixirs.

## SUMMARY

A method for the isolation and determination of dextromethorphan has been presented. The procedure has been used successfully on commonly available liquid dosage forms and the results are accurate and reproducible.

## REFERENCES

(1) Saques, C. P., *Anales Fac. Quim Farm., Univ. Chile*, **11**, 171(1959).
(2) Lee, K., *J. Pharm. Pharmacol.*, **12**, 666(1960).
(3) Horioka, M., *Yakugaku Zasshi.*, **77**, 200(1957); through *Chem. Abstr.*, **51**, 8367*b*(1957).
(4) "National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965.
(5) Oestreicher, P. M., Formilo, C. G., and Levi, L., *Bull. Narcotics U. N. Dept. Social Affairs*, **6**, 42(1954); through *Chem. Abstr.*, **49**, 14272*gh*(1955).
(6) Schmall, M., Wollish, E., and Shafer, E. G. E., *Anal. Chem.*, **28**, 1373(1956).
(7) Kelly, C. A., and Auerbach, M. E., *J. Pharm. Sci.*, **50** 490(1961).
(8) Blake, M. I., and Nona, D. A., *ibid.*, **52**, 945(1963).

# Effect of Red Cedar Chip Bedding on Hexobarbital and Pentobarbital Sleep Time

By HUGH C. FERGUSON*

**Male albino mice, in a red cedar chip environment, show a decreased sleep time to sodium hexobarbital and sodium pentobarbital. The effect appears to be reversible.**

THROUGHOUT the years various papers have been published citing controllable factors that modify drug action and toxicity in experimental animals. Examples of these are the age of the animal reported by Petty and Karler (1), cage design by Winter and Flataker (2), exercise and limitation of movement by Hardinge and Peterson (3), strain difference by Weaver and Kerley (4), type of anesthetic and pain response by Gutman and Charmovitz (5), grouping and amphetamine toxicity by Chance (6), and volume of fluid administered by Ferguson (7). Thus, by taking advantage of such knowledge, variations in results might be minimized within this laboratory and from laboratory to laboratory.

In the course of a screening program, control results changed abruptly in one of the author's standard tests. Investigation of all possible varia-

Received April 18, 1966, from the School of Pharmacy, University of Georgia, Athens. 30601.
Accepted for publication June 13, 1966.
* Present address: Mead Johnson Research Center, Evansville, Ind. 47721.