gy and Therapeutics, King George's Medical College, Lucknow University, Lucknow 226003, India, and the ‡Department of Pharmacology and Center in Toxicology, Department of Biochemistry, School of Medicine, Vanderbilt University, Nashville, TN 37232

Accepted for publication December 4, 1974.

Supported by U.S. Public Health Service Grants ES00267, ES00782, and DA00141 from the National Institutes of Health and by the Indian Council of Medical Research, New Delhi, India.

The authors thank Professor K. P. Bhargava for his advice and encouragement and Dr. M. L. Dhar and Dr. Nitya Nand of the Central Drug Research Institute, Lucknow, India, for providing microanalysis facilities.

* To whom inquiries should be directed. Present address: Department of Physiology and Pharmacology, School of Medicine, University of North Dakota, Grand Forks, ND 58201

PHARMACEUTICAL ANALYSIS

GLC Trace Analysis of Dextromethorphan and Bromhexine Salts in Pharmaceutical Preparations

J. L. FABREGAS and A. MARGALET *

Abstract □ GLC proved to be particularly well suited for trace analysis of dextromethorphan and bromhexine salts in different pharmaceutical preparations, using diphenhydramine as the internal standard.

Keyphrases □ Dextromethorphan salts—GLC analysis in pharmaceutical preparations □ Bromhexine salts—GLC analysis in pharmaceutical preparations □ GLC—analysis, dextromethorphan and bromhexine salts in pharmaceutical preparations

Due to its bronchial mucolytic action, bromhexine has been incorporated in pharmaceutical preparations, especially since the preparation of *N*-(2-amino-3,5-dibromobenzyl)-*N*-cyclohexylmethylamine hydrochloride (1). Other studies reported its pharmacology (2), antitussive (3) and bronchosecretolytic activities (4), and metabolism (5, 6).

However, analytical procedures have been inadequate to determine bromhexine and dextromethorphan quantitatively in a pharmaceutical mixture, because of their similar solubilities, analogous UV absorptions (7, 8), and similar colorimetric reactions (9). Methods utilizing GLC analysis of dextromethorphan have been reported (10). None of these methods has been used for the quantitative determination of bromhexine. Under the conditions described here, GLC was particularly well suited for the analysis of dextromethorphan and bromhexine in pharmaceutical formulations containing antibiotics and essential oils.

Table I—Determination of Bromhexine and Dextromethorphan in Pharmaceutical Formulations

Formulation	Label Claim, mg	Range Found, mg	*SD*, mg	*CV*, %
Syrup				
Bromhexine	1.80	1.770–1.801	0.033	1.83
Dextromethorphan	2.90	2.904–2.940	0.037	1.28
Granulation				
Bromhexine	1.80	1.770–1.800	0.033	1.83
Dextromethorphan	2.90	2.860–2.901	0.042	1.45

EXPERIMENTAL

Chromatographic Conditions—A gas chromatograph[1] equipped with a −0.2–1.0-mv recorder[2] was used. A 1.5-m × 0.3-cm (5-ft × 0.125-in.) stainless steel column packed with 3% SE-30 (silicone rubber) on 100–120-mesh Varaport 30 was used. The column temperature was 180°, and the detector and injection port temperatures were 255 and 200°, respectively. The nitrogen carrier gas flow rate was 30 ml/min at 37 psi. A flame-ionization detector was used with a hydrogen flow rate of 30 ml/min and an air flow rate of 300 ml/min. The optimal working attenuation was 8×10^{-10} amp/mv.

Reagents—Carbon tetrachloride and chloroform[3], spectroscopic grade, were used.

Internal Standard—After a number of trials with other materials (codeine, ephedrine, chlorpromazine, and papaverine), diphenhydramine was chosen as the internal standard because of its commercial availability as a pure reagent[4] and its adequate separation from the other compounds, resulting in a symmetrical peak of low retention time (≃2.5 min).

For the internal standard solution, 30 mg of diphenhydramine hydrochloride was extracted with carbon tetrachloride, in alkaline media, following the procedure described for the sample preparation. The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness, and the residue was dissolved and diluted to 100 ml with carbon tetrachloride.

Preparation of Samples—An accurately weighed sample, containing dextromethorphan and bromhexine salts equivalent to

[1] Varian Aerograph 1740, Walnut Creek, Calif.
[2] Electronik 15, Honeywell, Inc., Philadelphia, Pa.
[3] Merck.
[4] Benadryl hydrochloride, Parke-Davis & Co., Detroit, Mich.

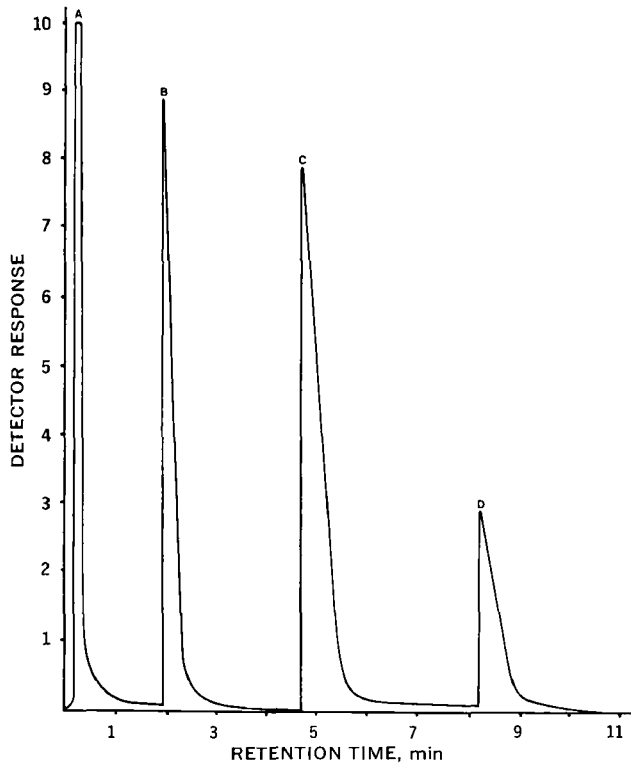

Figure 1—*Typical gas chromatogram. Key: A, chloroform; B, diphenhydramine; C, dextromethorphan; and D, bromhexine.*

2.3–3.2 and 1.4–2.5 mg of the respective bases, was extracted with five 35-ml portions of carbon tetrachloride in alkaline media. To the combined extracts, dried over anhydrous sodium sulfate, was added 5.0 ml of the internal standard solution. After evaporating to dryness, the residue was dissolved in 1.0 ml of chloroform (Solution A).

Preparation of Standards—For the working standard solution, a mixture was prepared of the same dextromethorphan and bromhexine salts[5] that were subjected to analysis and of known content as free bases. Four portions, equivalent to 110, 100, 90, and 80% of the formulation label claim, were accurately weighed and extracted following the procedure described for the sample preparation. To the extracts, dried over anhydrous sodium sulfate, 5.0 ml of the internal standard solution was also added. The resulting solutions were evaporated to dryness, and the four residues were dissolved in 1.0 ml of chloroform.

Assay Procedure—One microliter of the sample (Solution A) and standard solutions was injected into the gas chromatograph. The chromatograph was operated isothermally for the first 2 min, temperature-programmed at 2°/min, and switched to 12°/min just when the dextromethorphan peak reaches its maximum height. Under these conditions, the retention times relative to the internal standard were approximately 2.3 and 3.7 for dextromethorphan and bromhexine, respectively.

[5] The salts used in this study were prepared by the Research Division of Laboratorios Almirall, S.A., Barcelona, Spain.

Calculations—The peak areas were measured by multiplying their peak height times their width at half-height. Two standard curves were then established from the four standard tests by plotting the ratio of peak areas of dextromethorphan base to the internal standard against the weight of dextromethorphan base and the ratio of peak areas of bromhexine base to the internal standard against the weight of bromhexine base.

The concentrations of dextromethorphan and bromhexine bases in the sample were determined by computing the dextromethorphan–internal standard and bromhexine–internal standard peak area ratios, respectively, from the chromatogram and obtaining the corresponding weights from the standard curves. The values so obtained were then converted to percents of these two active ingredients in the original pharmaceutical formulation sample.

RESULTS AND DISCUSSION

To determine the recovery of bromhexine, samples were prepared containing eight different levels of known amounts of bromhexine base (1.55–2.55 mg); the precision of the chromatographic analysis was satisfactory. It was found that a linear relationship existed between the ratios of the observed peak areas of bromhexine base to the internal standard and the actual weight of bromhexine.

The results of this study led to the quantitative analysis of standard mixtures of bromhexine and dextromethorphan salts, with good agreement in the working ranges of 1.40–2.00 and 2.30–3.20 mg of the respective bases for 15 samples.

The results obtained under the experimental conditions and the good performance of the column during several months of continued use clearly indicate the column suitability for the proposed analytical system for conducting quality control assays of dextromethorphan and bromhexine in pharmaceutical preparations. The results are shown in Table I. A typical gas chromatogram is shown in Fig. 1.

REFERENCES

(1) J. Keck, *Justus Liebigs Ann. Chem.*, **662**, 171(1963).

(2) R. Engelhorn and S. Pueschmann, *Arch. Exp. Pathol. Pharmakol.*, **246**, 54(1963).

(3) M. Nakama, G. Hayashi, Y. Kowa, Y. Ishida, and S. Nakagami, *Nippon Yakurigaku Zasshi*, **65**, 446(1969).

(4) H. Eigelsreiter and M. Mair, *Arzneim.-Forsch.*, **17**, 353(1967).

(5) O. Leder, Z. Kopitar, and G. Beisenherz, *Nucl. Med., Suppl. 1968*, **8**, 199(1970).

(6) Z. Kopitar, O. Leder, and V. Heinz, *Arzneim.-Forsch.*, **21**, 914(1971).

(7) E. G. C. Clarke, "Isolation and Identification of Drugs," Pharmaceutical Press, London, England, 1969, p. 599.

(8) *Ibid.*, p. 289.

(9) O. W. A. Weber and J. E. Heveran, *J. Pharm. Sci.*, **62**, 1174(1973).

(10) K. H. Goebbeler, *Deut. Apoth. Ztg.*, **111**, 1291(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 17, 1974, from the *Research and Development Laboratory, Quality Control Division, Laboratorios Almirall, S.A., Cardoner, 72-74, Barcelona, Spain.*

Accepted for publication November 8, 1974.

The authors thank Dr. R. G. W. Spickett for helpful discussions.

[x] To whom inquiries should be directed.