High-Pressure Liquid Chromatographic Determination of Methscopolamine Nitrate, Phenylpropanolamine Hydrochloride, Pyrilamine Maleate, and Pheniramine Maleate in Tablets

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Received August 8, 1980, from the *Analytical Research Department, Dorsey Laboratories (Division of Sandoz, Inc.), Lincoln, NE 68501.* Accepted for publication December 18, 1980.

Abstract □ Methscopolamine nitrate, phenylpropanolamine hydrochloride, pyrilamine maleate, and pheniramine maleate were determined in a multilayer film-sealed tablet with an enteric-coated core by reversed-phase high-pressure liquid chromatography without interference from active components and/or matrix decomposition.

Keyphrases □ Methscopolamine nitrate—high-pressure liquid chromatographic analysis, tablets □ Phenylpropanolamine hydrochloride—high-pressure liquid chromatographic analysis, tablets □ Pyrilamine maleate—high-pressure liquid chromatographic analysis, tablets □ Pheniramine maleate—high-pressure liquid chromatographic analysis, tablets □ High-pressure liquid chromatography—simultaneous determination of methscopolamine nitrate, phenylpropanolamine hydrochloride, pyrilamine maleate, and pheniramine maleate in tablets

Phenylpropanolamine, pyrilamine, pheniramine, and tropane alkaloids are active components employed in cold preparations. Simultaneous analysis of these compounds is complicated by the low concentration of tropane alkaloids in relationship to the other three amines and by the weak UV absorbance of the tropane alkaloid molecule.

BACKGROUND

Various spectrophotometric methods have been employed for the analysis of phenylpropanolamine, pheniramine, and pyrilamine; however, extensive sample cleanup and separation are involved prior to the UV finishes (1, 2). Colorimetric methods and other indirect UV methods employed for the determination of methscopolamine and other tropane alkaloids are tedious and nonspecific (3–5).

Various stability-indicating high-pressure liquid chromatographic (HPLC) and GLC methods were reported for the determination of products containing one or more tropane alkaloids (6–12). The problem of simultaneously determining scopolamine and atropine with a preponderant concentration of phenylpropanolamine and chlorpheniramine was addressed by Santoro *et al.* (13) using GLC. However, this method also involved tedious sample preparation prior to injection.

The procedure described in the present study for the simultaneous determination of methscopolamine nitrate, phenylpropanolamine hydrochloride, pyrilamine maleate, and pheniramine maleate is rapid and stability indicating.

EXPERIMENTAL

Reagents—Phenylpropanolamine hydrochloride[1], pyrilamine maleate[2], pheniramine maleate[3], methscopolamine nitrate[4], tropic acid[5], and acetonitrile[6] were used as obtained. Ethylenediamine sulfate was prepared by precipitating the salt from an aqueous solution of ethylenediamine[5] and sulfuric acid with methanol. All other chemicals were reagent grade and were used without further purification.

Apparatus—A constant-flow high-pressure liquid chromatograph[7] was used in conjunction with a variable-wavelength detector[8]. Effluents were monitored at 216.5 nm. Peak areas were determined using an electronic digital integrator[9]. The stainless steel column (25 cm × 4.6 mm i.d.) was obtained prepacked with 10-μm reversed-phase material[10]. An automatic injection system was used to introduce the sample onto the column inlet[11].

The mobile phase was 2.85×10^{-3} *M* ethylenediamine sulfate buffer adjusted to pH 7.44 ± 0.02 with 1 *M* ammonium hydroxide–acetonitrile (1:1 v/v). The mobile phase was prepared fresh daily, and the flow rate was 3.8 ml/min.

Sample—The formulation investigated was a film-sealed tablet with an outer layer and an enteric-coated core[12]. The samples were assayed initially and after extended storage at elevated temperatures.

Ten tablets were crushed between paper and transferred to a 500-ml volumetric flask. Then 250 ml of 0.05 *N* hydrochloric acid in 50% alcohol was added. The preparation was heated for 15 min on a steam bath and shaken automatically for 2 hr. The sample was diluted to volume with water and filtered through glass microfiber[13]. The standard was prepared

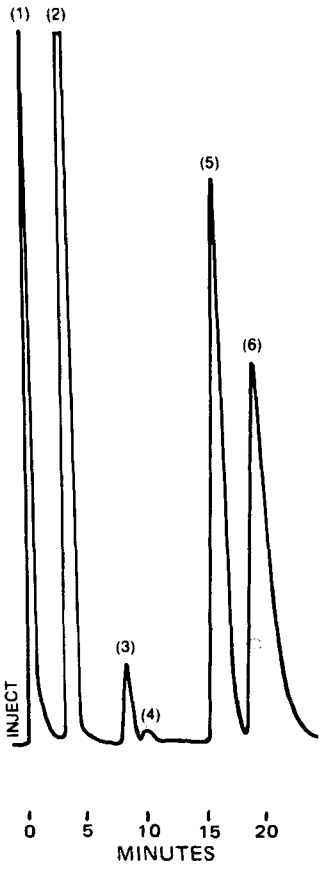

Figure 1—*Representative chromatogram of a tablet. Key: 1, excipients; 2, phenylpropanolamine; 3, methscopolamine; 4, excipient; 5, pyrilamine; and 6, pheniramine.*

[1] R. W. Greeff & Co., Dolton, IL 60419.
[2] Sandoz Ltd., Basel, Switzerland.
[3] Delamar, Elk Grove Village, IL 60007.
[4] Henley & Co., Lake Bluff, IL 60044.
[5] Aldrich Chemical Co., Milwaukee, WI 53233.
[6] HPLC grade, Fisher Scientific Co., St. Louis, MO 63132.
[7] Model 6000A, Waters Associates, Milford, MA 01757.
[8] Model 450, Waters Associates, Milford, MA 01757.
[9] Model 730, Waters Associates, Milford, MA 01757.
[10] Partisil-10-ODS, Whatman, Clifton, NJ 07014.
[11] Model 710A, Waters Associates, Milford, MA 01757.
[12] Dorsey Laboratories, Lincoln, NE 68501.
[13] GF/A, Whatman, Clifton, NJ 07014.

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Table I—Recovery of Active Ingredients

Active Ingredient	Label, mg/tablet	Assay, mg/tablet	CV^a
Phenylpropanolamine hydrochloride	50	49.9	0.57
Methscopolamine nitrate	4	3.95	1.35
Pyrilamine maleate	25	24.8	1.14
Pheniramine maleate	25	25.4	0.63

[a] Standard deviation from the mean of 10 determinations divided by the mean expressed as a percent.

simultaneously in the same manner. The resulting theoretical concentrations of active components in the preparation were 1.0 mg of phenylpropanolamine hydrochloride, 0.5 mg of pyrilamine maleate, 0.5 mg of pheniramine maleate, and 0.08 mg of methscopolamine nitrate.

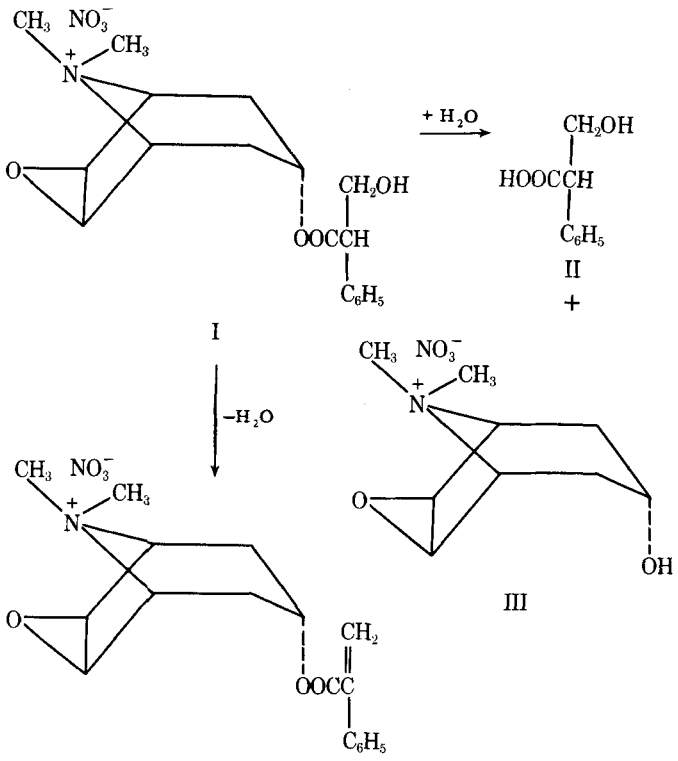
Assay—Thirty microliters of standard and sample preparations was injected into the chromatograph, and the peak areas were determined. The quantity of active components was determined by comparing the peak area of the sample to the respective peak area of the standard of known concentration.

RESULTS AND DISCUSSION

The recovery and coefficient of variation for each active component are shown in Table I. The standard demonstrated linearity for the four active ingredients over a region of ±20% of the theoretical product content. The addition of a sample blank to the standard resulted in less than a ±1% effect for all active components. Figure 1 represents a typical chromatogram.

Tropane alkaloids are subject to degradation by both hydrolysis and dehydration. The degradation pathway of methscopolamine is shown in Scheme I (6–8). Tropic acid, methscopine, and aposcopolamine were resolved from all peaks. Tropic acid eluted at the void volume, and aposcopolamine eluted after methscopolamine (Fig. 2). Methscopine has little UV absorbance at this wavelength and concentration and was not detected. Figure 3 represents a sample spiked with tropic acid and aposcopolamine bromide.

The degradation pathway of phenylpropanolamine is not established. However, stability-indicating spectrophotometric methods such as



Scheme I—Degradation pathway of methscopolamine nitrate (I) to tropic acid (II), methscopine nitrate (III), and aposcopolamine methylnitrate (IV).

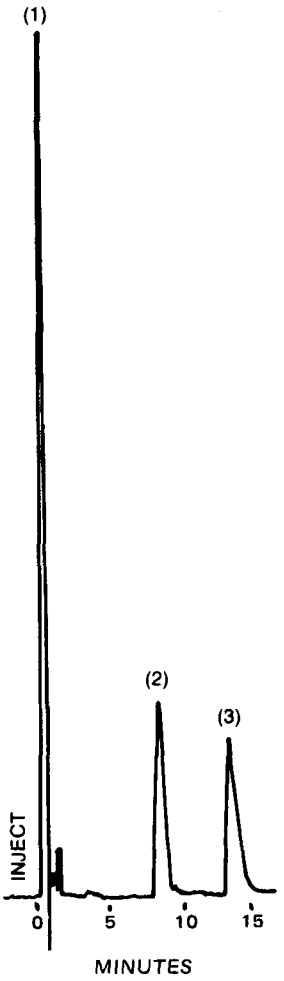


Figure 2—*Chromatogram of methscopolamine and its UV-active degradation products. Key: 1, tropic acid; 2, methscopolamine; and 3, aposcopolamine.*

periodate oxidation of phenylpropanolamine to benzaldehyde followed by extraction into hexane (1) indicate that the molecule is subject to decomposition in pharmaceutical preparations with time at normal and

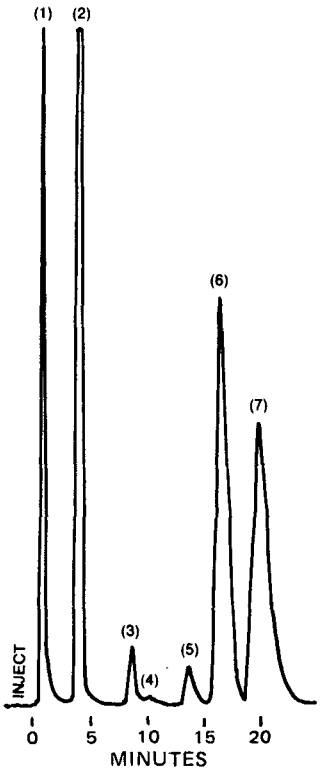


Figure 3—*Representative chromatogram of sample spiked with tropic acid and aposcopolamine bromide. Key: 1, tropic acid and excipients; 2, phenylpropanolamine; 3, methscopolamine; 4, excipient; 5, aposcopolamine; 6, pyrilamine; and 7, pheniramine.*

elevated temperatures. While the degradation products of phenylpropanolamine cannot be quantified by this method, a significant decrease in phenylpropanolamine was observed in stressed samples. Assay results for fresh and stressed samples by periodate oxidation correlated well with this assay, thus supporting the stability-indicating properties of this assay for phenylpropanolamine.

The recovery of the antihistamines, pyrilamine maleate[5] and pheniramine maleate, from a tablet matrix containing resinous material became increasingly difficult as the tablet was subjected to stress. Consequently, the acidic–alcoholic sample solution was exposed to heat to free the remaining antihistamines from the matrix.

In general, the chromatograms of stressed samples showed a decrease in phenylpropanolamine and methscopolamine but no loss in the pheniramine or pyrilamine content. There was no indication that excipients or degradation products interfered with the four peaks.

Careful control of buffer pH was necessary to obtain adequate separation of the pyrilamine and pheniramine peaks. The monitoring wavelength of 216.5 nm was selected as the optimum response *versus* baseline noise for the four species. At this wavelength, trace amounts of α-aminopropiophenone, a precursor and possible trace contaminant in phenylpropanolamine, do not absorb to a significant amount.

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ACKNOWLEDGMENTS

The author thanks Dorsey Laboratories and Dr. Eugene Brockemeyer, Director of Pharmaceutical Research and Quality Assurance, for support, and Murial V. Moffitt for technical assistance.

Antiparasitic Structure–Activity Relationships of Congocidine Derivatives

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Received October 23, 1980, from the **Department of Pharmacy, School of Pharmacy, the* [‡]*Department of Parasitology, Hadassah Medical School, and the* [§]*Department of Natural Products, School of Pharmacy, Hebrew University of Jerusalem, Jerusalem, Israel.* Accepted for publication December 17, 1980.

Abstract □ Several congocidine analogs were synthesized and tested for *in vivo* activity against *Trypanosoma congolense* and *in vitro* activity against amastigotes of *Leishmania tropica*. The tripyrrole derivative, β-{[*N*-methyl-4-[*N*-methyl-4-(guanidinoacetamido)pyrrole -2- carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido}butyroamidine dihydrochloride, was less toxic and more active than congocidine. The guanidinoacetyl moiety appears to be a structural requirement for antiparasitic activity in the congocidine series.

Keyphrases □ Congocidine derivatives—tri- and monopyrrole analogs, synthesized and evaluated for antiparasitic activity in mice, *in vivo* and *in vitro* studies □ Structure–activity relationships—tripyrrole derivatives of congocidine synthesized and evaluated for antiparasitic activity in mice, *in vivo* and *in vitro* studies □ Antiparasitic activity—tripyrrole derivatives of congocidine synthesized and evaluated for activity in mice, *in vitro* and *in vivo* studies

Congocidine (I) and distamycin A (II) are basic oligopeptide antiviral antibiotics isolated from the medium of *Streptomyces* sp. (1–3). The structure of congocidine was established by a series of degradations and a total synthesis (4, 5) and was shown to be identical to that of netropsin, which was isolated from a medium of *Streptomyces netropsis* (6, 7).

BACKGROUND

Congocidine (I) and distamycin A (II) are the major components of a group of pyrrole amidine antiviral antibiotics. Unlike distamycin A and in addition to its antiviral and antibacterial activities (8, 9), congocidine also shows antiparasitic activity against *Trypanosoma congolense* (the source of its name) and other parasites (1, 2, 7). Little information is available on the structural requirements for the antiparasitic activity of congocidine. The only reported study (10) demonstrated that replacement of the *N*-methylpyrrole ring in congocidine by thiophene, pyridine, or benzene led to the loss of its trypanocidal activity.

Several studies on the distamycin series showed that when distamycin A (a tripeptide) was converted to its tetra- or pentapeptide analogs, the antiviral and antibacterial activities against some viruses and bacteria increased (11–14). At the same time, cytotoxicity was somewhat reduced, indicating that the biological and cytotoxic activities were separable.

A similar observation was made in the congocidine series. Several tripyrrole derivatives of congocidine were less cytotoxic and more active than the parent drug in three tests against Herpes simplex virus (15). The present study evaluated the antiparasitic activity of different tri- and monopyrrole derivatives of congocidine to establish some structural requirements in the congocidine molecule. Congocidine and its derivatives were tested against *Leishmania tropica*[1] *in vitro* in mouse peritoneal exudate cells and against *T. congolense*[2] *in vivo*.

EXPERIMENTAL

Chemistry—The syntheses of the compounds were described previously (15, 16).

Parasitology[3]—*Animals*—Male mice of the inbred strains C_3H and

[1] The *L. tropica* LRC-L137 strain was obtained from the strain collection of the World Health Organization's International Reference Center for Leishmaniasis (WHO-LRC) at the Department of Protozoology, Hadassah Medical School, Jerusalem, Israel.

[2] The *T. congolense* TREU 1183 strain was kindly supplied by Professor Curtis L. Patton, Department of Epidemiology and Public Health, Yale University, New Haven, Conn.

[3] Some of the parasitological work was presented at the annual meeting of the Israeli Society of Protozoologists (April 1980) and will appear as the following abstracts: J. El-On, C. L. Greenblat, D. T. Spira, R. Mechoulam, and M. Bialer, "The Effect of Congocidine Derivatives on *Leishmania tropica* and *Trypanosoma congolense*," in *J. Protozoology Supplement* (1981).

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