GLC Analysis of Theophylline, Hydroxyethyltheophylline, and Diphenylpyraline Hydrochloride Syrup

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Abstract □ A GLC method for determining theophylline, hydroxyethyltheophylline, and diphenylpyraline hydrochloride is presented. The method permits the separation and quantitative determination of the therapeutically active ingredients with a single injection in an overall time of approximately 2.5 hr.

Keyphrases □ Theophylline—GLC analysis, in syrup □ Hydroxyethyltheophylline—GLC analysis, in syrup □ Diphenylpyraline hydrochloride—GLC analysis, in syrup □ GLC—analyses, theophylline, hydroxyethyltheophylline, diphenylpyraline hydrochloride

Analysis of theophylline (I), hydroxyethyltheophylline (II), and diphenylpyraline hydrochloride (III) by spectrophotometry and a titrimetric procedure (1) or a combination of ion-exchange chromatography and spectrophotometry was not applicable because of large amounts of ammonium chloride (24 mg/ml) and sodium citrate (10 mg/ml) contained as ingredients in the syrup formulation. This paper presents a uniform and simple GLC method.

Compounds I–III have been determined by GLC singly and in combination (2, 3), but their simultaneous quantitative analysis has never been recorded. The polar nature of certain xanthines causes absorption and tailing peaks, which complicate quantitative determination. In the present study, this problem was resolved by methylating the xanthine bases in the flash heater of a gas chromatograph with a methanolic solution of their trimethylanilinium salts (4).

EXPERIMENTAL

Apparatus—A gas chromatograph[1] with a flame-ionization detector was fitted with a 1.8-m × 2-mm i.d. glass column packed with 10% OV-101 on 80–100-mesh Chromosorb W.

Reagents[2]—A commercially prepared 0.2 *M* trimethylanilinium hydroxide solution[3] was used for methylating the xanthine bases. The pH 3.8 buffer was prepared as follows. Monobasic potassium phosphate, 6.805 g, was dissolved in 900 ml of water, and the pH was adjusted to 3.8 with 1% (v/v) phosphoric acid in water. Then the solution was diluted to 1000 ml with water. The mixed solvent system was chloroform–isopropanol (3:1).

Standard Solutions—To prepare the theophylline standard solution, 100 mg of anhydrous theophylline USP reference standard was weighed and dissolved in 50 ml of pH 3.8 buffer (2 mg/ml). Hydroxyethyltheophylline (Austria P. grade), 100 mg, was dissolved in 50 ml of pH 3.8 buffer (2 mg/ml). Diphenylpyraline hydrochloride, 80 mg, was dissolved in 50 ml of pH 3.8 buffer (1.6 mg/ml). The internal standard, pyrilamine maleate, 100 mg, was dissolved in 100 ml of pH 3.8 buffer (1 mg/ml).

Chromatography—The oven was programmed at 215–230° at 2°/min, the injection port was set at 275°, and the detector was set at 280°. The nitrogen carrier flow rate was 15 ml/min. Hydrogen and air flow rates were adjusted to give maximum response. An attenuation of 10 was reduced to 7 after 4.8 min.

Standard Preparation—Standard solutions of I (10 ml), II (1 ml), III (1 ml), and pyrilamine maleate (1 ml) were pipetted into a 150-ml separator and extracted with two 25-ml and two 15-ml portions of mixed solvent. Each organic extract was filtered through anhydrous sodium sulfate into a 250-ml conical flask. The aqueous phase was made alkaline with 10% NaOH, and the extraction was continued with two 20-ml and two 10-ml portions of chloroform.

Organic phases were pooled, and the sodium sulfate was rinsed with two 10-ml aliquots of chloroform. Then the combined extracts were evaporated in a rotating evaporator at 45°. The residue was redissolved in 5 ml of mixed solvent and then pipetted in 20-μl aliquots into vials for evaporation at room temperature. The residue in each vial was redissolved in 30 μl of reagent and then injected[4] in 1-μl samples into the chromatograph.

Sample Preparation—The weight per milliliter was determined, and an amount of syrup[5] equivalent to about 6 ml was weighed in a 50-ml beaker. The pH was adjusted to 3.8 after addition of 10 ml of pH 3.8 buffer. Then the solution was transferred into a 150-ml separator, and the beaker was rinsed with small portions of pH 3.8 buffer. Internal standard solution, 1 ml, was pipetted into a 150-ml separator and extracted according to the *Standard Preparation* procedure previously described.

RESULTS AND DISCUSSION

Well-resolved chromatographic peaks were obtained for the three active ingredients and the internal standard (Fig. 1). The retention times of I–III and pyrilamine maleate were 3.52, 5.64, 7.56, and 9.97, respectively, based on freshly prepared solutions. The solution was analyzed six times (Table I).

Precision was also examined by analyzing a production lot of the syrup. Each of three replicate extractions was chromatographed five times (Table II). Precision and accuracy data indicate good reproducibility. The precision with which diphenylpyraline hydrochloride was determined was less than expected when compared to that of theophylline and

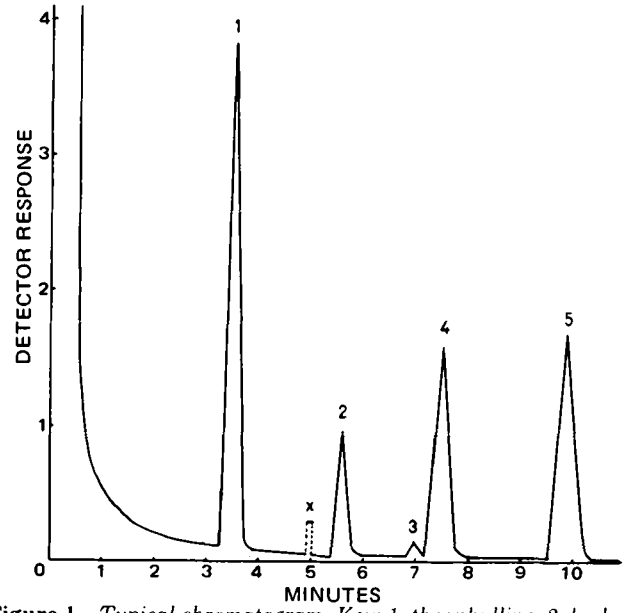

Figure 1—*Typical chromatogram. Key: 1, theophylline; 2, hydroxyethyltheophylline; 3, precursor peak; 4, diphenylpyraline hydrochloride; and 5, pyrilamine maleate.*

[1] Hewlett-Packard 5840-A.
[2] All reagents were Analar grade by BDH.
[3] Pierce Chemical Co., Rockford, Ill.
[4] Microliter syringe, 10 μl, Hamilton Co., Reno, Nev.
[5] The commercial syrup contained (per 30 ml): theophylline, 100 mg; hydroxyethyltheophylline, 10 mg; and diphenylpyraline hydrochloride, 8 mg.

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Table I—Standard Solution

Trial	Theophylline		Hydroxyethyltheophylline		Diphenylpyraline Hydrochloride	
	Sample, mg	Recovery, %	Sample, mg	Recovery, %	Sample, mg	Recovery, %
1	100.7	97.9	9.3	98.9	8.6	104.7
2	99.3	99.1	10.7	98.1	7.9	101.3
3	101.5	100.8	11.1	97.3	8.1	97.5
4	98.9	101.2	9.8	98.0	8.3	97.6
5	99.2	102.1	10.5	100.0	7.9	102.5
6	99.1	99.4	10.5	98.1	8.4	97.2
Mean relative error, %	1.26		1.43		2.63	
$\bar{X}$	100.1		98.4		100.1	
SD, %	±1.55		±0.93		±3.4	
RSD, %	1.55		0.95		3.4	

Table II—Production Lot of Syrup

	Theophylline	Hydroxyethyltheophylline	Diphenylpyraline Hydrochloride
Theoretical concentration, mg/30 ml	100.0	10.0	8.0
$\bar{X}$ of 15 samples	99.3	10.5	8.3
RSD, %	1.87	1.20	3.50

hydroxyethyltheophylline. Diphenylpyraline hydrochloride appeared not to undergo any chemical reaction in methanol and reaction mixtures as shown by the retention time. A preliminary 1-μl injection of reagent alone showed a small precursor peak just before the diphenylpyraline hydrochloride peak. This peak may insignificantly affect measurement of diphenylpyraline hydrochloride.

Linearity was also determined. Linear curves passing through the origin were obtained for solutions containing 40–100% of the quantities of drug ingredients being sought. These curves related the peak area and drug concentration.

The initial pH 3.8 of the sample and standard solutions was necessary to ensure the extraction of theophylline and hydroxyethyltheophylline.

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Stereochemical Preferences for Curarimimetic Neuromuscular Junction Blockade IV: Monoquaternary Ammonium Probes Possessing Carbon and Nitrogen Asymmetry

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Abstract □ Two enantiomeric pairs of neuromuscular junction blocking agents were prepared in which an asymmetric carbon atom is adjacent to an asymmetric quaternized nitrogen moiety. The blocking agents were obtained from the enantiomers of laudanosine by stereoselective quaternization with benzyl and ethyl iodides. Curarimimetic potencies were measured with an *in vivo* cat hypoglossal nerve–tongue muscle preparation. The studies suggest that the asymmetry present in these structures does not lead to significant differences in blocking potency between enantiomers.

Keyphrases □ Enantiomers—benzyl- and ethyllaudanosinium iodides synthesized, curarimimetic potencies compared □ Neuromuscular junction blocking agents—enantiomers of benzyl- and ethyllaudanosinium iodides synthesized, curarimimetic potencies compared □ Curarimimetic potency—compared in enantiomers of benzyl- and ethyllaudanosinium iodides □ Quaternary ammonium probes—enantiomers of benzyl- and ethyllaudanosinium iodides synthesized, curarimimetic potencies compared □ Structure–activity relationships—enantiomers of benzyl- and ethyllaudanosinium iodides, curarimimetic potencies compared

Previous reports (1–4) described stereochemical preferences exhibited by the neuromuscular junction toward nondepolarizing blocking agents having carbon asymmetry alpha to quaternary ammonium functionality. Initial studies (1) employed monoquaternary structures (hemicurares) as probes and indicated a modest blocking preference for the (*S*)-enantiomers; subsequent investigations (2, 3) employed bisquaternary probes and indicated that the order of blocking preference is (*R*) > (*S*) for these structures. Since the quaternary ammonium function is the most distinguishing pharmacophore of this pharmacological class, structures having nitrogen asymmetry could possess larger enantiomeric potency differences that might also be independent of other, more remote structural modification.

An early example of variation in curarimimetic potency due to nitrogen asymmetry is the report (5) that the diastereomers derived from asymmetric quaternization of coniine show small potency differences. Larger potency differences were reported for the stereoisomers resulting from quaternization of canadine (6, 7). Similarly, certain optically active inorganic onium ions also showed small

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