High-Performance Liquid Chromatographic Determination of Cyproheptadine Hydrochloride in Tablet Formulations

GREGORY W. BURROWS ˣ **and CHERYL L. ALLIGER**

Received November 6, 1981, from the *Analytical Chemistry Department, Food and Drug Research Laboratories, Inc., Waverly, NY 14892.* Accepted for publication August 26, 1982

Abstract □ A high-performance liquid chromatographic method is described which determines cyproheptadine hydrochloride in tablet formulations. Tablets were dissolved in water–acetonitrile (50:50) and analyzed using an octadecylsilane column with a mobile phase of 85% acetonitrile and 15% of an aqueous solution of 0.01 *M* 1-octanesulfonic acid, 0.5% triethylamine, and 1% acetic acid using UV absorbance detection at 280 nm.

Keyphrases □ Cyproheptadine hydrochloride–high-performance liquid chromatography, tablet formulations □ High-performance liquid chromatography—cyproheptadine hydrochloride in tablet formulations

Cyproheptadine hydrochloride, an oral antihistamic agent used in the treatment of perennial and seasonal rhinitis and other allergic reactions (1), is formulated in various ways. In this analysis, cyproheptadine was separated from vitamins, plant extracts, and fillers. The standard spectrophotometric analysis described in USP XX (2) proved unsatisfactory with these formulations. Excipients coeluting from the silica gel column caused an elevated absorbance and erroneous assay.

Various authors have described separations of antihistamines and cyproheptadine hydrochloride by GLC (3, 4) and reverse-phase high-performance liquid chromatography (HPLC) (5, 6). A buffered, normal phase, ion-paired separation has also been reported (7). The purpose of this study was to develop a chromatographic analysis to separate a complex mixture of ingredients in a tablet formulation.

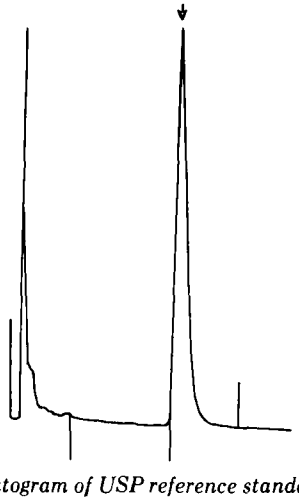

Figure 1—*Chromatogram of USP reference standard cyproheptadine hydrochloride.*

EXPERIMENTAL

The high-performance liquid chromatograph[1] with an octadecylsilane column[2] was used for all separations. The detector was equipped with a 280-nm filter and set at 0.05 AUFS. Mobile phase at ambient temperature was pumped at 2 ml/min. (500–1000 psi) until the baseline was stable.

Chemicals and Reagents—Acetonitrile[3] was HPLC grade, 1-octanesulfonic acid[4] and glacial acetic acid[5] were reagent grade, triethylamine[5] was reagent grade and redistilled in glass, and cyproheptadine HCl[6] was USP reference standard.

Mobile Phase—The mobile phase was delivered using two pumps and a solvent programmer. The programmer was set to pump a mixture of 85% acetonitrile and 15% aqueous phase. The aqueous phase consisted of a solution of 0.01 *M* 1-octanesulfonic acid, 0.5% triethylamine, and 1% acetic acid.

Standard Curve—Standard solutions were prepared to concentrations ranging from 26.68 to 80.04 μg/ml representing results of 40 to 120% of theoretical. A plot of concentration *versus* area was linear (correlation coefficient = 0.99997). The line equation is $y = mx + b$, where m (slope) = 1.06×10^7 and b (intercept) = -0.005×10^7. Run on 3 successive days, the average slope had an *RSD* of 0.43%.

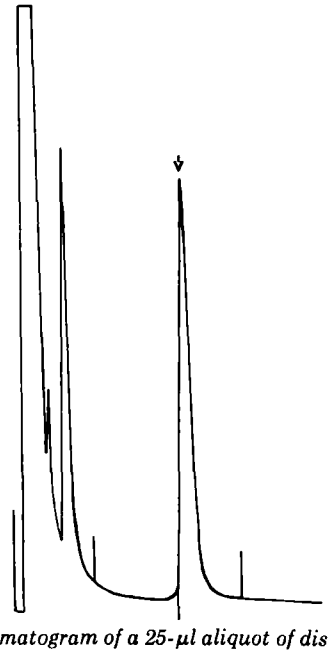

Figure 2—*Chromatogram of a 25-μl aliquot of dissolved tablets.*

[1] Model 204 Chromatograph with two M6000A pumps, Model 660 Solvent programmer, U6K Universal Injector, M730 data module, M440 UV detector, Waters Associates, Milford, Mass.
[2] 5 mm (10 μm) Radial Pak A, Waters Associates, Milford, Mass.
[3] J. T. Baker Chemical Co., Phillipsburg, NJ 08865
[4] Eastman Kodak Co., Rochester, NY 14650
[5] Fisher Scientific Co., Fair Lawn, NJ 07410
[6] U.S. Pharmacopeial Convention, Inc., Rockville, MD 20852

0022-3549/83/1000-1212$01.00/0

Table I—Dissolution of Cyproheptadine Hydrochloride

Dissolution Medium	Sample Identification	Peak[a] Area ($\times 10^6$)	Cyproheptadine Hydrochloride Available, %
Distilled water	USP reference standard	6.859	100
Distilled water	Cyproheptadine hydrochloride (used in tablet formulation)	6.410	93.4
Distilled water	Blank powder	None detected	
Simulated gastric fluid	USP reference standard	2.973	43.3
Simulated gastric fluid	Tablets	2.932	42.7
Simulated intestinal fluid	USP reference standard	3.369	49.1
Simulated intestinal fluid	Tablets	0.784	11.4

[a] One determination each.

Analytical Procedure—Five tablets (each containing approximately 0.7 mg cyproheptadine hydrochloride) were placed in a 50-ml volumetric flask. Twenty-five ml 50:50 (v/v) acetonitrile–water was added and swirled until the tablets were completely dissolved and then brought to volume with acetonitrile–water. The amount of cyproheptadine in the tablets was calculated from the linear regression of the standard curve.

A solution of powdered excipients without cyproheptadine hydrochloride was prepared at a concentration equal to five tablets in a 50-ml solution. No interferences from other ingredients or contaminants in the analysis were detected in the blank solution.

Three samples of the powdered excipient spiked with cyproheptadine gave recoveries of 100.4, 100.0, and 99.8% (mean = 100.1 ± 0.3%).

RESULTS AND DISCUSSION

Cyproheptadine (a weak aliphatic base) pairs with octanesulfonate in a weakly acidic mobile phase. A mobile phase of acetonitrile–aqueous solution (85:15) (0.01 *M* octanesulfonic acid, 1% acetic acid, 0.5% triethylamine) gave the best separation (Fig. 1) with cyproheptadine resolved from other ingredients (Fig. 2) with no interferences from contaminants or excipients.

Table II—Assays of Three Production Lots

No. Lot	Theoretical Concentration, mg/tablet	Obtained Concentration, mg/tablet Replicate Analyses 1	2	3	Mean	*SD*
1	0.667	0.639	0.635	0.637	0.637	0.002
2	0.667	0.649	0.628	0.667	0.648	0.020
3	0.667	0.616	0.653	0.627	0.632	0.019

A comparison of analyses of cyproheptadine dissolved in distilled, deionized water, simulated gastric fluid [USP XX (2)], and simulated intestinal fluid [USP XX (2)] showed irregular results (Table I). Preliminary results indicate that dissolution is slow in the simulated fluids with total release times of several hours.

Since water appeared to be the best dissolution solvent, a mixture of acetonitrile–water was used, which made the resultant solution more compatible with the mobile phase. The assay of three production lots of tablets was measured to be 95.5, 97.2, and 94.8% of theoretical content (Table II). The method precision by triplicate assays was 0.3, 3.1, and 3.0%, respectively.

REFERENCES

(1) "Physicians Desk Reference," 35th ed. Medical Economics Co., Oradell, N.J.

(2) "The United States Pharmacopeia XX/The National Formulary XV," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 193–194.

(3) A. W. Missen, Rep.—*N.Z. Dep. Sci. Ind. Res., Chem Div. Rep.*, ISS C. D. 2282, 36 (1979).

(4) E. C. G. Clarke, "Isolation and Identification of Drugs," Vol. I, Pharmaceutical Press, London, 1974, p. 278.

(5) B. B. Wheals, *J. Chromatogr.*, **187**, 65 (1980).

(6) D. L. Massaret and M. R. Detavernier, *J. Chromatogr.*, **187**, 139 (1980).

(7) D. L. Massaret and G. Hoogewijis, *Anal. Lett.*, **13**, 389 (1980).

ACKNOWLEDGMENTS

Acknowledgment is given to the sponsor for funding of this work and giving permission to publish. Details of the formulation have been omitted to protect client confidentiality.

Synthesis and Anticonvulsant Testing of 4-Phenylsemicarbazides

MILTON J. KORNET ˣ and JOHN YEOU-RUOH CHU

Abstract □ A series of compounds based on the semicarbazide structure have been synthesized. Anticonvulsant activity was found in a majority of the compounds using both the maximal electroshock seizure and the subcutaneous pentylenetetrazol seizure threshold tests. Activity of the compounds was weaker than the 1,1,2-trisubstituted semicarbazides previously reported.

Keyphrases □ 4-Phenylsemicarbazides—synthesis and anticonvulsant activity □ Anticonvulsants—synthesis of 4-phenylsemicarbazides

Earlier work (1–5) on the synthesis and anticonvulsant activity of 4-phenylsemicarbazides was concerned primarily with compounds in which N-1 and N-2 are fully substituted by alkyl or aryl residues. To obtain additional information regarding structure–activity relationships for these types of compounds, it was desirable to prepare the series of compounds represented by III. This series differs from all of the previous series in that the compounds contain a hydrogen atom at N-2. This series includes 2-methyl, 2,6-dimethyl, and 2-chloro-6-methyl substituents in the aromatic ring since such substitution generally proved to be optimal in the previous series of 4-phenylsemicarbazides studied (1, 3).

RESULTS AND DISCUSSION

1,1-Disubstituted hydrazines (II) readily added to aryl isocyanates (I) and afforded III in good yields (Scheme I, Table I). Several different 1,1-disubstituted hydrazines were used (Table I) including the cage-like compound, 3-amino-3-azabicyclo[3.2.2]nonane. The latter compound

0022-3549/83/1000-1213$01.00/0