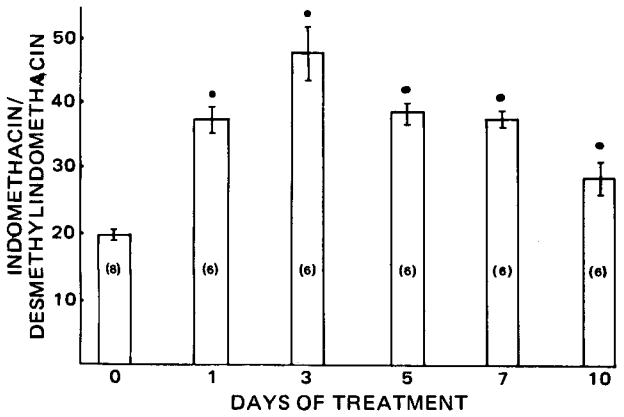

Figure 5—*Ratio of indomethacin divided by desmethylindomethacin. Key: ●, significant differences from control, p < 0.05, as determined by the Student t test.*

glucose levels (Fig. 2) were significantly elevated after 1 day and remained so. Although their blood levels were higher than those of the controls, none of the experimental animals had glucose in their urine on the day of sacrifice.

Figures 3 and 4 represent the serum levels of I and II, respectively. There were no significant differences in the substrate during the experiment. However, there were significant differences in the product level from Day 1 on. The maximum inhibition occurred at Day 3, followed by a tendency toward control levels as time progressed.

Elevated blood glucose levels obtained by oral administration of the 20% glucose solution rapidly led to inhibition of the hepatic microsomal mixed-function oxidase, which catalyzed *O*-dealkylation within 1 day. Although serum insulin levels were not measured, it can be projected from the urine levels at sacrifice and from the data presented by Hartshorn *et al.* (2) that the results were not due to hypoinsulinemia. Inversion of the inhibition after the maximum level at Day 3 confirmed earlier findings (7) that inhibition is short lived and returns to normal even though glucose administration is prolonged.

Since there were no significant differences in the blood substrate levels, the biotransformation inhibition could only be accounted for by the glucose affecting enzymatic activity. Kinetic studies (3) indicated that glucose caused mixed inhibition (competitive and noncompetitive) and altered the enzyme at the active site and at an allosteric site. The inversion of inhibition may have been caused by induction of new enzyme as well as displacement of old enzyme, freeing more active sites. Figure 5 shows the ratio of substrate to product, representing the biotransformation of I to II under the influence of glucose and time.

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Simultaneous Determination of Hydroxyzine Hydrochloride and Benzyl Alcohol in Injection Solutions by High-Performance Liquid Chromatography

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Abstract □ A stability-indicating, high-performance liquid chromatographic method was developed for the simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol in injection solutions. Separation was achieved using a μBondapak C_{18} column and the eluent [60% water, 25% acetonitrile, and 15% methanol containing 0.06% (v/v) sulfuric acid, 0.5% (w/v) sodium sulfate, and 0.02% (w/v) heptanesulfonic acid sodium salt] at a flow rate of 2 ml/min. Isobutyrophenone and *p*-nitroacetophenone were used as internal standards. The UV detector response at 257 nm was linear for hydroxyzine hydrochloride in the 3–10-mg/ml range and for benzyl alcohol in the 0.54–1.8-mg/ml range under analysis conditions. The method is accurate, simple, and precise.

Keyphrases □ High-performance liquid chromatography—simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol, injection solutions □ Analysis, simultaneous—high-performance liquid chromatographic method for hydroxyzine hydrochloride–benzyl alcohol injection solutions □ Injection solutions—high-performance liquid chromatographic method for simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol

Hydroxyzine hydrochloride is usually formulated with benzyl alcohol as a preservative (bacteriostatic) in injection solutions. Depending on conditions such as pH, light exposure, and temperature, photolysis occurs in solution, resulting in the formation of *p*-chlorobenzophenone, *p*-chlorobenzaldehyde, *p*-chlorobenzoic acid, and 1-[2-(2-hydroxyethyl)ethyl]piperazine dihydrochloride (1, 2). The compendial assay for hydroxyzine hydrochloride injection, based on titration of the free base with perchloric acid, is not indicative of drug stability in solution (3). From an analytical standpoint, a simple, rapid, and precise method is desirable for the simultaneous determination of both the drug substance and the preservative in the formulation.

This paper describes the development of a high-performance liquid chromatographic (HPLC) method for hydroxyzine hydrochloride and benzyl alcohol in an injectable formulation. The method is stability indicating of both ingredients in solution.

EXPERIMENTAL

Instrumentation—The chromatographic system was equipped with a dual-piston reciprocating pump[1], a universal injector[2], and a vari-

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model U6K, Waters Associates, Milford, Mass.

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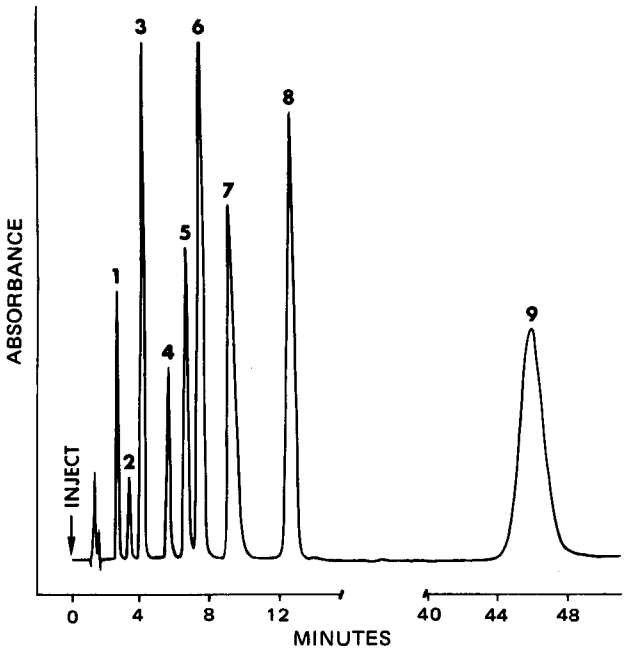

Figure 1—*Synthetic mixture of formulation ingredients, potential degradation products, and internal standards for quantitation. Key: 1, benzyl alcohol (0.81 mg/ml); 2, benzoic acid (0.093 mg/ml); 3, benzaldehyde (0.041 mg/ml); 4,* p-*nitroacetophenone (0.024 mg/ml); 5,* p-*chlorobenzoic acid (0.19 mg/ml); 6,* p-*chlorobenzaldehyde (0.073 mg/ml); 7, hydroxyzine hydrochloride (5.0 mg/ml); 8, isobutyrophenone (0.269 mg/ml); and 9,* p-*chlorobenzophenone (0.18 mg/ml).*

able-wavelength UV detector[3]. The separation was performed on a 30-cm × 4-mm i.d. column containing microparticulate (10-μm) bonded octadecylsilane material[4]. The chromatographic peaks were electronically integrated and recorded[5].

Eluent—The eluent was 60% water, 25% acetonitrile[6], and 15% methanol[6] containing 0.06% (v/v) sulfuric acid, 0.5% (w/v) sodium sulfate, and 0.02% (w/v) heptanesulfonic acid sodium salt[7]. The eluent pH was 2.6.

Internal Standard Solution—A methanolic solution of 0.2 mg of *p*-nitroacetophenone[8]/ml and 2.5 mg of isobutyrophenone[8]/ml was used as the internal standard solution.

Standard Preparation—The standard solution for analysis was prepared by dissolving 250 mg of hydroxyzine hydrochloride NF reference standard in 5 ml of water. Five milliliters of a 9-mg/ml methanolic solution of benzyl alcohol NF reference standard and 5.0 ml of the internal standard solution were pipetted into the hydroxyzine hydrochloride reference standard solution; this mixture was diluted to 50 ml with methanol.

Sample Preparation—The method was developed utilizing a formulation of 50 mg of hydroxyzine hydrochloride/ml containing 9 mg of benzyl alcohol/ml. The analysis sample was prepared by mixing 5.0 ml of formulation and 5.0 ml of internal standard solution and diluting the mixture to 50 ml with methanol.

Analysis—The following chromatographic conditions were used: flow rate, 2 ml/min; detector wavelength, 257 nm; absorbance, 0.2 aufs; and injection volume, 10 μl. Duplicate analyses were performed for both the standard and sample preparations.

RESULTS AND DISCUSSION

A chromatographic method that is stability indicating of hydroxyzine hydrochloride was outlined previously (4); the chromatography was performed on a Bondapak phenyl/corasil column using a 5:3 mixture of 0.25% $(NH_4)_2CO_3$–acetonitrile as the eluent. A similar system using a 45:55 mixture of 0.01 *M* phosphate buffer (pH 7.8)–acetonitrile as the eluent and a μBondapak C_{18} column also was used successfully to monitor hydroxyzine hydrochloride stability[9]. In both methods, a high proportion of acetonitrile is needed to elute the drug substance as the free base. Benzyl alcohol, another component in the formulation, could not be analyzed with hydroxyzine hydrochloride in either chromatographic system; it could not be retained on either column due to the high acetonitrile concentration in the eluent.

The ion-pair reversed-phase chromatography used in this method allowed benzyl alcohol and hydroxyzine hydrochloride to be determined simultaneously. The acidity of the eluent was adjusted to a pH at which hydroxyzine hydrochloride and heptanesulfonic acid sodium salt are ionized, resulting in ion-pair formation. The ionic strength of the eluent was increased by the addition of sodium sulfate, which generally decreases the retention of compounds being chromatographed *via* the ion-pairing mechanism (5). Although the proportion of organic solvent in the eluent is high, the polarity of organic solvent was increased by using a mixture of methanol and acetonitrile. Therefore, benzyl alcohol was retained on the C_{18} column; it was unaffected by the addition of heptanesulfonic acid sodium salt and sodium sulfate to the eluent, as it did not form an ion-pair.

This analysis is stability indicating of both hydroxyzine hydrochloride and benzyl alcohol (Fig. 1). A synthetic mixture of benzyl alcohol, hydroxyzine hydrochloride, several potential degradation products (benzoic acid[8], benzaldehyde[8], *p*-chlorobenzoic acid[8], *p*-chlorobenzaldehyde[8], and *p*-chlorobenzophenone[8]), and the internal standards used for quantitation, *p*-nitroacetophenone and isobutyrophenone, were separated under analytical conditions.

The detector wavelength of 257 nm was selected for maximum response for both hydroxyzine hydrochloride and benzyl alcohol. The linearity of the detector response under analytical conditions was established for hydroxyzine hydrochloride in the 3–10-mg/ml range (*y* intercept, −0.001; *r*, 0.9999) and for benzyl alcohol in the 0.54–1.8-mg/ml range (*y* intercept, 0.003; *r*, 0.9999). Since the resulting standard curves for both hydroxyzine hydrochloride and benzyl alcohol were linear and passed through the origin, sample analysis could be performed *versus* a single standard preparation.

Experiments were performed to demonstrate the accuracy of the method. Six solution mixtures were prepared ranging from 60 to 200% of the theoretical concentrations of the two components. Recoveries of benzyl alcohol varied from 99.4 to 103.0% with an average recovery of 101.0 ± 1.35%; recoveries of hydroxyzine hydrochloride varied from 99.8 to 100.7% with an average recovery of 100.2 ± 0.34%.

The reproducibility of the method was demonstrated by replicate analyses of hydroxyzine hydrochloride and benzyl alcohol in a commercial product which had been stored at 25° for 2 years. The statistical data are presented in Table I.

Table I—Statistical Data Resulting from Simultaneous Analyses of Hydroxyzine Hydrochloride and Benzyl Alcohol in Hydroxyzine Hydrochloride Injection [a]

Parameter	Hydroxyzine Hydrochloride	Benzyl Alcohol
Number of analyses	7	7
Milligrams per milliliter (mean)	48.7	8.47
SD, mg/ml	±0.44	±0.09
RSD, %	±0.9	±1.0

[a] Abbott Laboratories, North Chicago, Ill.; stored at 25° for 2 years.

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[3] Model LC-55, Perkin-Elmer, Norwalk, Conn., or model SF 770 Spectroflow, Schoeffel, Westwood, N.J.

[4] μBondapak C_{18}, Waters Associates, Milford, Mass.

[5] Autolab System I (Spectra-Physics, Santa Clara, Calif.) with model 023 strip-chart recorder (Perkin-Elmer, Norwalk, Conn.) or model 3385A Automation System (Hewlett-Packard, Avondale, Pa.).

[6] Distilled in glass, Burdick & Jackson, Muskegon, Mich.

[7] Eastman Organic Chemicals, Rochester, N.Y.

[8] Aldrich, Milwaukee, Wis.

[9] L. Marchant, Abbott Laboratories, North Chicago, Ill., unpublished data.