Anal.—Calc. for $C_{14}H_{13}Cl_2NO_4$: C, 50.93; H, 3.98; N, 4.24. Found: C, 51.10; H, 4.02; N, 4.27.

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Rapid, Stability-Indicating, High-Pressure Liquid Chromatographic Determination of Theophylline, Guaifenesin, and Benzoic Acid in Liquid and Solid Pharmaceutical Dosage Forms

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Abstract □ Theophylline, guaifenesin, and benzoic acid were determined by reversed-phase high-pressure liquid chromatography without interference from active and/or vehicle decomposition. A degradation product of sucrose, 5-hydroxymethylfurfural, can be identified and quantified in liquid samples simultaneously.

Keyphrases □ Theophylline—analysis, high-pressure liquid chromatography, in liquid and solid pharmaceutical dosage forms, stability □ Guaifenesin—analysis, high-pressure liquid chromatography, in liquid and solid pharmaceutical dosage forms, stability □ Benzoic acid—analysis, high-pressure liquid chromatography, in liquid and solid pharmaceutical dosage forms, stability □ High-pressure liquid chromatography—analysis of theophylline, guaifenesin, benzoic acid in liquid and solid pharmaceutical dosage forms

Determination of active components and preservatives in pharmaceutical products subjected to aging and stress requires a highly specific method. The actives and preservatives must be determined in the presence of vehicle degradation as well as their degradation products.

Vehicle degradation is particularly critical in syrups, where the decomposition of hexose sugars results in a series of complex reaction products (1–3). Resulting UV-absorbing species, such as 5-hydroxymethylfurfural, can interfere with some assays (4).

Theophylline and guaifenesin, widely used in asthmatic preparations, have been assayed by various methods. Spectrophotometric determinations are rapid but nonspecific unless the actives are separated from interfering species adequately (5–7).

While GLC methods demonstrate greater specificity, derivatization is generally required to avoid excessive tailing due to the polar nature of theophylline and guaifenesin (8–15). In addition, theophylline sodium glycinate, a commonly used water-soluble form of theophylline, is not readily soluble in organic solvents required by GLC methods.

Determination of theophylline and guaifenesin by high-pressure liquid chromatography (HPLC) in biological

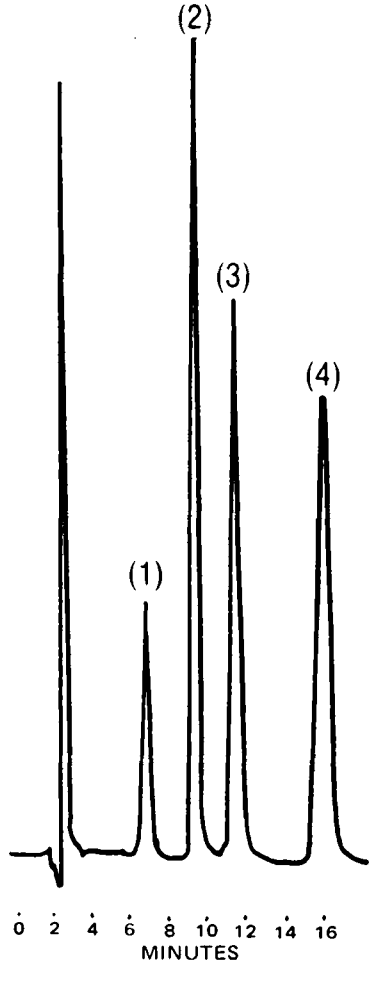

Figure 1—*Representative chromatogram of an elixir. The concentrations of components in the sample were: (1), benzoic acid, 0.01 mg/ml; (2), theophylline sodium glycinate, 0.2 mg/ml; (3), guaifenesin, 0.067 mg/ml; and (4), methylparaben internal standard, 0.1 mg/ml.*

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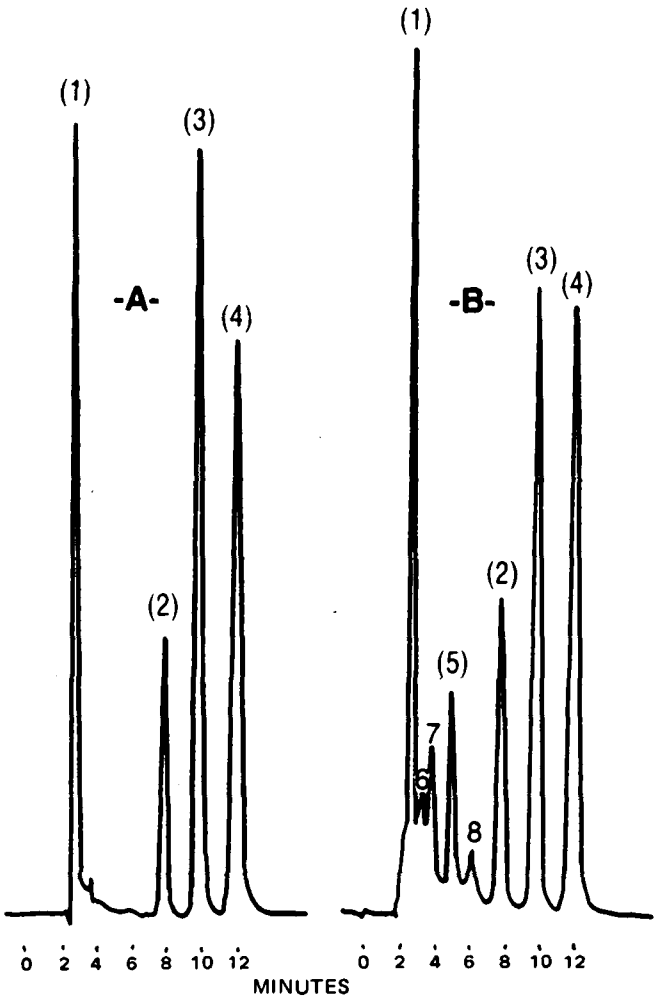

Figure 2—*Chromatograms of a liquid product. Key: A, fresh sample; B, after 50° storage for 9 months; (1), excipients; (2), benzoic acid; (3), theophylline; (4), guaifenesin; (5), 5-hydroxymethylfurfural; and (6, 7, and 8), unidentified decomposition products.*

fluids is highly specific for both actives in the presence of dietary metabolites and various other drugs (16–23). In this study using stability-indicating HPLC methods, theophylline, guaifenesin, and benzoic acid were determined in the presence of both active and vehicle degradation in liquid and solid pharmaceutical dosage forms. 5-Hydroxymethylfurfural, resulting from the degradation of sucrose, can also be measured by this method.

EXPERIMENTAL

Reagents—Acetonitrile[1], theophylline sodium glycinate[2], guaifenesin[3], methylparaben[4], benzoic acid[5], 5-hydroxymethylfurfural[6], guaiacol[6], and 1,3-dimethyluric acid[7] were used as obtained. All other chemicals were reagent grade and were used without further purification.

Apparatus—A constant-flow, high-pressure liquid chromatograph[8] was used in conjunction with a variable wavelength detector[9]. Effluents were monitored at 230 nm. Peak areas were determined using an electronic digital integrator[10]. The stainless steel column, 4.6 mm i.d. × 25 cm, was obtained prepacked with 10-μm reversed-phase material[11]. A sample injection valve was used to introduce the samples into the column inlet[12]. The mobile phase was 10^{-3} *M* sodium citrate–citric acid buffer adjusted to pH 4.15 and acetonitrile in the ratio of 9:1 (v/v). The flow rate was 2 ml/min.

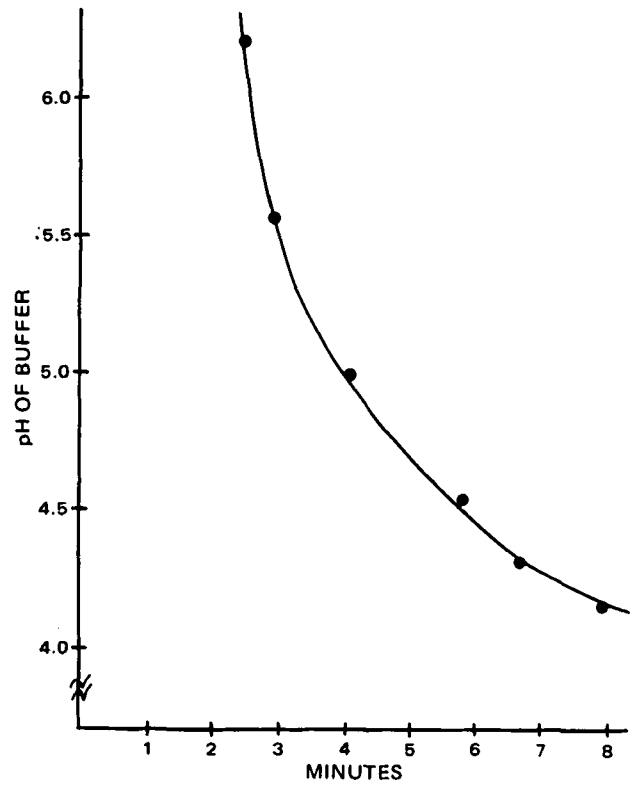

Figure 3—*Relationship of retention time of benzoic acid to the pH of the buffer in the mobile phase.*

Samples—The following formulations were investigated: tablet, elixir, and syrup containing theophylline sodium glycinate and tablet and elixir containing theophylline sodium glycinate and guaifenesin[13]. The preservative in the liquid products was benzoic acid. Samples were assayed initially and after storage at 30, 40, 50, and 65° for varying periods.

Liquid preparations were diluted with water and an internal standard. Solid dosage forms were ground, wetted with alcohol, diluted with water, and stirred automatically for 1 hr. A filtered portion of the solution was diluted with water and an internal standard. Methylparaben was the internal standard for products containing both theophylline and guaifenesin. The internal standard for single-entity theophylline products was guaifenesin.

Assay—Standard and sample preparations, 20 μl, were injected into the chromatograph, and the peak areas were determined. The quantity of actives and preservative present was determined by comparing the peak area ratio of the sample to the respective peak area ratio of the standard of known concentration, where the ratio was component peak area divided by internal standard peak area.

All standards demonstrated linearity over a region ±20% of theoretical product content. The addition of sample blank to the standard did not alter the peak areas of interest.

Figure 1 represents a typical chromatogram for elixir containing the active components theophylline sodium glycinate and guaifenesin and the preservative benzoic acid.

RESULTS AND DISCUSSION

Jensen (24) reported the hydrolysis of guaifenesin to guaiacol and glycerol by boiling guaifenesin with hydrochloric acid and the oxidation

[1] Burdick & Jackson Laboratories, Muskegon, MI 49440.
[2] Chattem Chemicals, c/o Austin Chemical Co., Chicago, IL 60631.
[3] Ganes Chemical, New York, NY 10036.
[4] Tenneco Chemicals, Chicago, IL 60630.
[5] Monsanto Co., St. Louis, MO 63166.
[6] Aldrich Chemical Co., Milwaukee, WI 53233.
[7] Adams Chemical Co., Round Lake, IL 60073.
[8] Model 830 with model 833, Du Pont, Wilmington, DE 19898.
[9] Model 837, Du Pont, Wilmington, DE 19898.
[10] Autolab System IV, Spectra-Physics Corp., Mountain View, CA 94040.
[11] Partisil-10-ODS, Whatman, Inc., Clifton, NJ 07014.
[12] Model 7105, Rheodyne, Berkeley, CA 94710.
[13] Dorsey Laboratories, Lincoln, NE 68501.

of guaifenesin with periodic acid to o-methoxyphenoxy acetaldehyde and formaldehyde. The extreme conditions required to degrade guaifenesin indicate that its decomposition is highly unlikely in pharmaceutical products subjected to several years of aging at normal conditions or several months at 50–70°. The present study supports this assumption in that no significant loss in guaifenesin was observed for the products assayed.

Cohen (25) stated that theophylline solutions subjected to strongly alkaline pH showed decomposition and apparent ring opening after several weeks and that theophylline was also susceptible to oxidation, resulting in the formation of 1,3-dimethyluric acid.

Significant loss of theophylline was observed in liquid products subjected to 50–70° for several months. A comparison of the chromatograms from fresh liquid products and high stress samples showed a loss in theophylline as well as increases in the 5-hydroxymethylfurfural peak and several additional unidentified peaks (Fig. 2). Sample blanks subjected to similar stress contained corresponding unidentified peaks. If 1,3-dimethyluric acid is present, it is well removed from the peaks of interest.

The degradation of sucrose solutions results in a highly complex series of decomposition products, including 5-hydroxymethylfurfural. Because of the quasistable state of this strongly UV-absorbing species in solution, it is frequently regarded as an indication of vehicle decomposition (1).

The amount of 5-hydroxymethylfurfural present in the sample can readily be quantified by the addition of this component to the standard. A study conducted on a liquid theophylline product indicated that decreasing the pH over the 6–5 range decreased theophylline stability and increased the amounts of 5-hydroxymethylfurfural and a second unidentified vehicle decomposition product.

The retention time of benzoic acid was highly sensitive to the mobile phase pH (Fig. 3). When the pH of the buffer in the mobile phase was adjusted to 4.15, the benzoic acid eluted between the last vehicle decomposition product peak and the theophylline peak.

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COMMUNICATIONS

Precaution in Use of High-Pressure Liquid Chromatographic Simultaneous Plasma Procainamide and *N*-Acetylprocainamide Determination

Keyphrases □ Procainamide—analysis, plasma, high-pressure liquid chromatography deproteinization *versus* extraction methods □ *N*-Acetylprocainamide—analysis, plasma, high-pressure liquid chromatography, deproteinization *versus* extraction methods □ High-pressure liquid chromatography—analysis, procainamide and *N*-acetylprocainamide in plasma, deproteinization *versus* extraction methods

To the Editor:

A high-pressure liquid chromatographic (HPLC) method for the simultaneous plasma procainamide and *N*-acetylprocainamide determination was reported recently from this laboratory (1). The method involved microvolume acetonitrile plasma protein precipitation and injection of an aliquot of the resultant supernatant solution onto a cation-exchange column. Detection was by UV absorption at 274 nm. *N*-Acetylprocainamide and procainamide eluted from the system with retention times of 4 and 5 min, respectively, and appeared to be symmetrical peaks that were satisfactorily resolved from each other and other plasma components. Total analysis time per sample was ~7 min. The method required extremely simple sample preparation and short analysis time. The purpose of this communication is to report some precautions in the use of this method.

Due to an irreversible loss of column performance, a new cation-exchange column obtained from the same manufacturer[1] was used as previously reported (1). This new column demonstrated that the peak in patient samples

[1] Partisil PXS 10/25 SCX, Whatman, Clifton, N.J.

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