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Simultaneous Determination of Acetaminophen, Guaifenesin, Pseudoephedrine, Pholcodine, and Paraben Preservatives in Cough Mixture by High-Performance Liquid Chromatography

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Abstract □ The separation and simultaneous determination, by high-performance liquid chromatography, of acetaminophen (I), guaifenesin (II), pseudoephedrine hydrochloride (III), and pholcodine (IV), together with a series of parabens (methyl to butyl, V–VIII) in a cough mixture, has been demonstrated using a chemically bonded octadecylsilane stationary phase with a mobile phase of methanol–water–acetic acid (45:55:2) containing the ion-pairing agent octanesulfonic acid. Retention volumes for the active ingredients were 3.8 ml, 5.4 ml, 9.4 ml, and 15.6 ml for compounds I–IV, respectively. Corrected retention volumes for the parabens [5.4 ml for methyl (V), 9.6 ml for ethyl (VI), 18.5 ml for propyl (VII), and 37.9 ml for butyl (VIII)] showed an exponential relationship with chain length of the esterifying alcohols. Excipients did not interfere with the estimation of any of the compounds, hence pretreatment of the sample was unnecessary. Average recoveries of the active ingredients and of the parabens from laboratory prepared samples were essentially 100% of theoretical with standard deviations of 1.7, 0.3, 1.5, 0.3, 0.3, 3.3, 0.7, and 2.7% for I–VIII, respectively.

Keyphrases □ Acetaminophen—simultaneous determination of guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture by high-performance liquid chromatography □ Pseudoephedrine—simultaneous determination of acetaminophen, guaifenesin, pholcodine, and paraben in preservatives in cough mixture by high-performance liquid chromatography □ Paraben—simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, and pholcodine in preservatives in cough mixture by high-performance liquid chromatography □ High-performance liquid chromatography—simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture

High-performance liquid chromatography (HPLC) has become a powerful tool for the analysis of pharmaceutical products. Mixtures used for the treatment of coughs and colds may be complexes containing several active ingredients including a decongestant, an antihistamine, frequently an analgesic, preservatives, dyes, and flavors. The active materials cover a range of structures with widely varying polarities and include both acidic and basic compounds.

A number of conventional methods have been applied to the present series. Pholcodine has been estimated by UV spectrophotometry following separation by TLC and colorimetry (1, 2). Pseudoephedrine and acetaminophen have been determined spectrophotometrically (3, 4) and by GLC (5, 6). Guaifenesin has been determined in pharmaceutical preparations by GLC (7, 8). The parabens may be assayed by GLC (9) or by UV spectroscopy following sample clean-up by column chromatography (10).

Spectrophotometric, GLC, or methods requiring TLC separation when applied to samples such as cough mixtures can be lengthy and/or subject to interferences by the matrix of the sample, and they are generally not suitable for simultaneous assay. The simultaneous assay of the drugs and preservatives described in this report cannot be achieved by any of the techniques mentioned here.

The application of HPLC procedures to various combinations of drugs and parabens has been reported (11–19), and some attention has been given to the effect of carbon chain length of the alkylsulfonic acid ion-pairing agents on retention times of several drugs (20, 21). The effect of chain length on resolution of mixtures of materials with varying polarity was tested, and a procedure was developed by which eight components, four active materials and four preservatives, may be determined with one injection.

EXPERIMENTAL

Materials—All active ingredients and paraben preservatives were of BP quality except guaifenesin which conformed to BPC standard. All were used without further purification.

Mobile Phase—The mobile phase, methanol[1]–water–glacial acetic acid (45:55:2) containing 0.005 *M* octanesulfonic acid[2], was filtered through a 0.45-μm filter[3]. The flow rate was 2.5 ml/min.

Instrumentation—The liquid chromatograph consisted of a constant flow pump[4], a low-pressure injector[5], a dual channel absorbance detector[6] set at 254 and 280 nm, and a 30-cm × 4-mm i.d. octadecylsilane column[7]. Outputs from the 280-[8] and 254-nm[9] channels were quantitated. Separate monitoring at 254 nm was required for pseudoephedrine which is not detected on the 280-nm channel.

Standard Solutions—The stock solution of analytes in methanol contained 25.00, 5.00, 5.00, 0.75, 0.61, 0.20, 0.33, and 0.20 mg/ml of I–VIII, respectively. Aliquots (2, 3, 4, 5, 6, and 7 ml) of this solution were diluted to 100 ml with water and filtered through a 0.45-μm filter[10]. A calibration

[1] Methanol BDH, redistilled in glass.
[2] PIC B8, Waters Associates, Milford, Mass.
[3] Millipore Type FH organic.
[4] Model 6000A, Waters Associates, Milford, Mass.
[5] Model U6K, Waters Associates, Milford, Mass.
[6] Model 440, Waters Associates, Milford, Mass.
[7] μ-Bondapak C-18, Waters Associates, Milford, Mass.
[8] Data Module, Waters Associates, Milford, Mass.
[9] Varian CDS III integrator.
[10] Millipore Type HA aqueous.

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Table I—Percent Recovery of Compounds I–IV from Prepared Mixtures

Amount added as Percent of Theoretical	Acetaminophen (I)	Guaifenesin (II)	Pseudoephedrine Hydrochloride (III)	Pholcodine (IV)
80	103.8	98.5	96.4	100.0
100	99.7	98.5	98.7	99.3
120	100.9	99.1	99.9	99.9
Mean	101.5	98.7	98.3	99.7
SD	2.1	0.3	1.8	0.4

curve for each compound was prepared from triplicate, 20-μl injections of these solutions.

Quantitation of the Actives and Preservatives in Synthetic and Commercial Cough Mixtures—For the determination of recoveries, small laboratory batches of a cough mixture were prepared containing 80, 100, and 120% of the theoretical amount of each of the active ingredients and the four parabens: 500.0, 100.0, 30.0, 15.0, 13.4, 4.0, 6.6, and 4.0 mg of I–VIII/20 ml, respectively.

A control sample containing all ingredients except the actives and parabens was also prepared. Sample analysis was carried out by diluting 5–100 ml with water and filtering through a 0.45-μm filter.

Duplicate, 20-μl injections were made of each filtered sample solution and the results were calculated by reference to a standard containing the theoretical sample concentration. The response of the standard was determined by making four replicate injections, two before and two after the samples were run.

Commercial mixtures were assayed by diluting 5–100 ml with water, filtering with a 0.45-μm filter, and injecting 20 μl. The results were calculated by reference to a single standard containing the theoretical sample level of actives and preservatives.

RESULTS

A typical chromatogram from a cough syrup prepared with the four actives and the four preservatives is shown in Fig. 1. Detector response was linear for all eight compounds between 40 and 140% of the theoretical sample level. Deviations of points from linearity for compounds I–VIII were ≤3.9%.

Preparation of the control sample (*i.e.*, without I–VIII) permitted checking on the possibility of the presence of interfering peaks. The results of the recoveries, shown in Tables I and II, indicate satisfactory

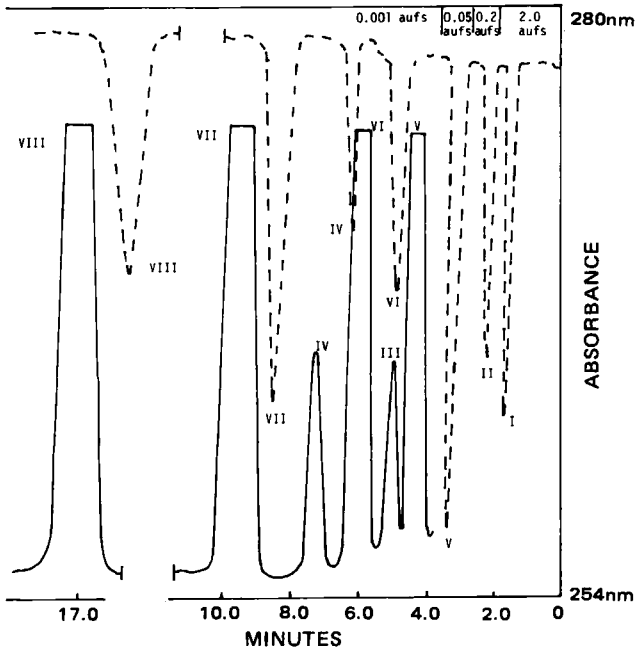

Figure 1—*High-performance liquid chromatogram of a cough preparation. Peaks I–VIII are acetaminophen, guaifenesin, pseudoephedrine hydrochloride, pholcodine, methyl–butyl paraben, respectively.*

Table II—Percent Recovery of Compounds V–VIII from Prepared Mixtures

Amount added as Percent of Theoretical	Methyl Paraben (V)	Ethyl Paraben (VI)	Propyl Paraben (VII)	Butyl Paraben (VIII)
80	99.3	103.1	99.4	105.0
100	99.3	98.0	100.2	100.0
120	99.9	104.2	98.5	104.2
Mean	99.5	101.8	99.4	103.1
SD	0.3	3.3	0.9	2.7

method precision and accuracy, the highest standard deviation for the recovery of any one compound being 3.3% and the average recoveries for all eight compounds lying within the range of 98.3–103.1%.

A result emerging coincidentally from this work was the determination of the structure–retention volume relationship for the parabens. A plot of corrected retention volume *versus* chain length of the esterifying alcohol shows the exponential type relationship which reduces to a semilog linear plot (Fig. 2).

The overall accuracy was evaluated by making a single injection of each of nine commercial preparations of known composition and calculating the results by comparison with nine replicate injections of a single standard solution (Table III).

DISCUSSION

The simultaneous analysis of this group of compounds presented several problems. The difference in polarity between I and VIII rendered separation of I–VI difficult, if VII and VIII were to be eluted within a reasonable time. The large differences in detector response and concentration between acetaminophen and the other components restricted the amount of sample that could be injected without overloading the detector on both channels. Even at the concentration described, maximum absorbance of acetaminophen on the 280-nm channel approaches 2.0. However, this concentration was necessary in order to obtain quantifiable peaks for pseudoephedrine and pholcodine. Difficulties were encountered because there were three classes of compounds [acidic (I), basic (III, IV) and nonpolar (II, V–VIII)] undergoing analysis simultaneously. Ideally, two types of chromatography would be required to achieve the desired separation: reverse-phase with ion-pairing reagents for acidic and basic compounds and ordinary reverse-phase for nonpolar compounds. An alkylsulfonic acid was added to the mobile phase to obtain retention and separation of the two basic compounds (III and IV) present in the mixture. The acetaminophen elutes close to the solvent front but does not suffer from interference by coeluting impurities from the sample matrix.

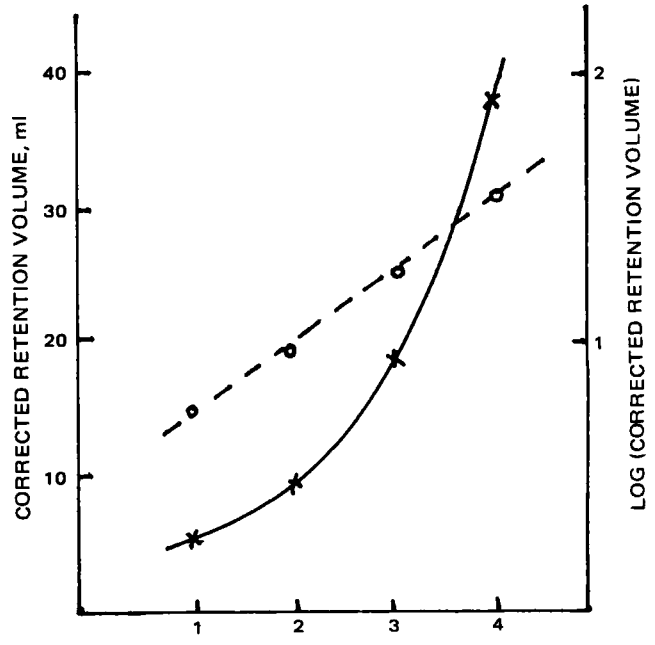

Figure 2—*Carbon chain length of esterifying alcohol of parabens.*

Table III—Typical Assay Results of Commercial Preparations

Sample[a]	Acetaminophen (I)	Guaifenesin (II)	Pseudoephedrine Hydrochloride (III)	Pholcodine (IV)	Methyl Paraben (VI)	Propyl Paraben (VII)
1	100.0	100.1	100.3	100.0	101.5	101.2
2	102.4	98.8	99.2	98.3	100.0	101.8
3	98.9	98.5	101.0	99.5	100.5	97.3
4	100.0	97.0	98.2	101.1	97.7	98.5
Mean Recovery[b]	100.6	98.1	100.0	100.6	99.2	99.9
SD[c]	1.5	1.6	1.5	2.3	1.8	2.1

[a] Recoveries expressed as percent of theoretical. [b] Calculated from nine replicates. [c] Standard deviations of a single determination calculated from nine replicates.

Initially hexanesulfonic acid[11] was chosen with a water–methanol ratio of 70:30, but this resulted not only in a long retention time for VIII, as its retention is not dependent on the ion-pairing agent, but also gave a poor separation of III and V. By using octanesulfonic acid as the ion-pairing agent and changing the water–methanol–acetic acid ratio of the mobile phase to 55:45:2, a satisfactory separation was achieved. Addition of acetic acid reduced tailing of III and peak broadening of IV.

The analytical results demonstrate the ability of ion-pair reverse-phase HPLC to simultaneously assay four actives and four paraben preservatives. A particular advantage of the method is the minimum time required for sample preparation and analysis of the complete separation requiring only 18 min. The method has been successfully used on a routine basis for over 6 months. Special column clean-up procedures have not been required during this time and no significant loss of column performance has been observed.

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Synthesis and Pharmacological Activity of Benzo[*b*]thiophene-3-carboxylic Acid Derivatives

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Received December 7, 1981, from the **Department of Chemistry, College of Pharmacy* and the ‡*Department of Animal Physiology and Pharmacology, School of Veterinary Medicine, Tehran University, Tehran, Iran.* Accepted for publication March 31, 1982.

Abstract □ Several dialkylaminoethyl benzo[*b*]thiophene-3-carboxylates, *N*-(2-dialkylaminoethyl)benzo[*b*]thiophene-3-carboxamides, 2-dialkylaminoethyl benzo[*b*]thiophene-3-carbamates, and substituted ureas with benzo[*b*]thiophene moiety, were prepared and tested for local anesthetic, anticholinergic, and antihistaminic activities. Several of the compounds showed significant activity

Keyphrases □ Benzo[*b*]thiophene-3-carboxylic acid derivatives—synthesis and pharmacological activity, local anesthetic, anticholinergic, and antihistaminic activity □ Anesthetics, local—synthesis and pharmacological activity of benzo[*b*]thiophene-3-carboxylic acid derivatives □ Anticholinergics—synthesis and pharmacological activity of benzo[*b*]thiophene-3-carboxylic acid derivatives □ Antihistamines—synthesis and pharmacological activity of benzo[*b*]thiophene-3-carboxylic acid derivatives

Many of the clinically active local anesthetics are dialkylaminoalkyl esters and dialkylaminoalkylamides of carboxylic acids (1). Some dialkylaminoalkylesters of 2- or 3-benzo[*b*]thiophenecarboxylic acid have been reported to have hypotensive, antiviral, and antifungal activities (2), as have some benzo[*b*]thiophene-2-carboxamides (3). It

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