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QUANTITATION OF ACETAMINOPHEN, CHLORPHENIRAMINE MALEATE, PHENYLTOLOXAMINE CITRATE AND PSEUDOEPHEDRINE HYDROCHLORIDE IN COMBINATIONS IN CAPSULES AND TABLETS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high-performance liquid chromatography method has been developed for the quantitation of acetaminophen, chlorpheniramine and pseudoephedrine in combination in capsules and tablets using a non-polar (μ/C18) column and 3 different mobile phases. A very simple preliminary extraction procedure is required before injecting onto the chromatograph. The method is accurate and precise with percent relative standard deviations based on 6 injections of 0.9, 1.9 and 1.1 for acetaminophen, chlorpheniramine and pseudoephedrine, respectively. Phenyltoloxamine citrate which is often mixed with acetaminophen has also been quantified using the developed method. The percent relative standard deviation based on 6 injections of phenyltoloxamine has been determined to be 1.0.

BACKGROUND

There are a number of pharmaceutical dosage forms available for the temporary relief of sinus headache and nasal congestion. One combination which is very popular contains acetaminophen (an analgesic), chlorpheniramine maleate (an antihistamine) and pseudoephedrine hydrochloride (a decongestant). The most popular dosage forms contain (per tablet/capsule) 325-500 mg of acetaminophen, 2 mg of chlorpheniramine maleate and 30 mg of pseudoephedrine hydrochloride. Phenyltoloxamine citrate (30 mg) is often mixed with acetaminophen (325 mg) in solid dosage forms by a number of manufacturers. A number of excipients, colors, lubricants, disintegrating agents, binders and fillers are usually added to dosage forms which can interfere with the quantitative analysis of the active ingredients.

The problem of analysis is further complicated by the presence of a high concentration of acetaminophen (an excellent absorbant in the useful UV range) versus very small quantities of chlorpheniramine maleate and pseudoephedrine hydrochloride. Moreover, pseudoephedrine is a poor absorbant as compared with acetaminophen. The high performance liquid chromatography (HPLC) method for the quantitation of acetaminophen and chlorpheniramine maleate were reviewed by Gupta and Heble[1]. A paired ion chromatography method for the quantitation of chlorpheniramine maleate[2] has been reported recently. The quantitation of pseudoephedrine[3] by ion-paired HPLC has also been reported. It is well known that ion-paired chromatography shortens the life of the column and is prefered only as the last alternative. None of the reported methods is applicable to ingredients when present in the above combination.

This paper reports the quantitation of acetaminophen (I), chlorpheniramine maleate (II), and pseudophedrine hydrochloride (III) in combination using reversed phase HPLC without ion-pairing. The developed method can also be used for the quantitation of phenyltoloxamine in combination with acetaminophen.

MATERIALS AND METHODS

Materials: All the chemicals and reagents were USP-NF or ACS grade and used without further purification.

Apparatus and Column: A high performance liquid chromatograph (Waters ALC 202) attached to a multiple wavelength detector (Schoeffel's 770) and a recorder (Omniscribe 5312-12) was used. Two micro/C18 columns (Waters, 30 cm x 3.9 mm i.d. with 10 micron size particles and 15 cm x 3.9 mm i.d. with 5 micron size particles) were purchased and used as received.

Mobile Phases: For acetaminophen, 5.5% methanol (V/V), 1% glacial acetic acid (V/V) and 0.02 M ammonium acetate in water. The pH was 3.8 (± 0.05). For chlorpheniramine maleate and phenyltoloxamine citrate, 42% methanol (V/V), 1% glacial acetic acid (V/V) and 1% (V/V) of solution of ammonium formate prepared according to directions in the literature[4] in water. The pH was 4.3 (± 0.05). For pseudoephedrine, same as for acetaminophen except that methanol concentration was 4% (with 30 cm column) and 8% (with 15 cm column).

Chromatographic Conditions: The flow rate was 2.0 ml/min and the temperature was ambient. The sensitivity for acetaminophen was 0.04 AUFS (at 257 nm) and 0.02 AUFS (at 257 nm for pseudoephedrine, 261.5 nm for chlorpheniramine and 269 nm for phenyltoloxamine) for others. The chart speed was 30.5 cm/hr.

Preparation of Stock Solutions: A 50.0 mg quantity of acetaminophen was dissolved in enough methanol to make 100.0 ml of solution. A 250.0 mg quantity of salicylamide (the internal standard) was dissolved in methanol to make 50.0 ml of the solution. One hundred (100.0 mg) of chlorpheniramine maleate was dissolved in water to make 100.0 ml of the solution. A 200.0 mg of phenyltoloxamine citrate was mixed with enough water to make 100.0 ml of a clear solution.

Usual Standard Solutions: Acetaminophen, a 3.2 ml quantity of the stock solution was mixed with 8.0 ml of the stock solution of salicylamide and brought to volume (100.0 ml) with water. Chlorpheniramine maleate, a 4.0 ml of quantity of the stock solution was mixed with 10.0 ml quantity of the stock solution of phenyltolaxamine citrate (the internal standard) and brought to volume (100.0 ml) with water. Phenyltolaximine citrate, a 10.0 ml quantity of the stock solution was mixed with 10.0 ml quantity of the stock solution of chlorpheniramine maleate (the internal standard) and brougnt to volume (100.0 ml) with water. Pseudoephedrine hydrochloride, a 60.0 mg quantity of the powder was dissolved in 60 ml of water, 3.0 ml of the stock solution of salicylamide (the internal standard) added and brought to volume (100.0 ml) with water.

Extraction Procedures (Sample Preparation) from Capsules and Tablets: Ten tablets or contents of ten capsules (single tablet or capsule for content uniformity) were ground to a fine powder using a pestle mortar. This powder was used for extraction procedure. Acetaminophen, a portion of the powder representing 50.0 mg of acetaminophen was weighed accurately and mixed with 80 ml of methanol in a 150 ml

beaker. The mixture was stirred for 2-3 minutes and brought to volume (100.0 ml) with methanol. The suspension was mixed, filtered (Whatman #1), first 15 ml of the filtrate was rejected and then a portion of clear filtrate was collected for further dilution. A 3.2 ml quantity was mixed with 8.0 ml quantity of the stock solution of salicylamide and brought to volume (100.0 ml) with water.

Chlorpheniramine Maleate: A portion of the fine powder representing 2.0 mg of chlorpheniramine maleate was mixed with one ml of dilute sulfuric acid, 5.0 ml quantity of the stock solution of phenyltoloxamine citrate, and 35 ml of water. The mixture was stirred for about 5 minutes, brought to volume (50.0 ml) with water and filtered. First 10 ml of the filtrate was rejected and then a portion of the clear filtrate was collected for analysis.

Phenyltoloxamine Citrate: The extraction procedure was same as for chlorpheniramine maleate except that powder representing 20.0 mg of phenyltoloxamine citrate was treated, to which 10.0 ml quantity of the stock solution of chlorpheniramine maleate and 80 ml of water were added. After 5 minutes stirring, volume was brought to 100.0 ml with water.

Pseudoephedrine Hydrochloride: A portion of powder representing 30.0 mg of pseudoephedrine hydrochloride was mixed with one ml of dilute sulfuric acid, 35 ml of water and 3.0 ml of the stock solution of salicylamide. The mixture was stirred for about 3 minutes, brought to volume (50.0 ml) and filtered. First 10 ml of the filtrate was rejected and then a portion collected for assay.

TABLE 1
ASSAY RESULTS

Dosage Form	Assay Results - Percent of the Label Claim			
	Acetaminophen	Chlorpheniramine	Pseudoephedrine	Phenyltoloxamine
Tablets[a] (pink)	99.0	100.5	100.3	-[b]
Tablets[c] (pink)	100.1	100.0	100.5	-[b]
Tablets[c] (pink) (different lot)	99.6	100.3	100.5	-b
Capsules[c] (white powder)	100.2	-[b]	100.0	-[b]
Tablets[d] (white)	-[b]	-[b]	101.2	-[b]
Tablets[a] (yellow)	100.2	-[b]	-[b]	98.6
Tablets[e] (orange)	100.2	-[b]	-[b]	99.2
Syntnetic Mixture[f] 1	99.8	100.2	100.0	-[b]
Synthetic Mixture[g] 2	100.2	-[b]	100.6	99.2

Content Uniformity Results

(For Pseudoephedrine and Chlorpheniramine Maleate in Tablets[a])

Tablet 1	-	102.5	100.6	-
Tablet 2	-	101.4	100.8	-
Tablet 3	-	97.6	97.7	-
Tablet 4	-	99.0	100.6	-
Tablet 5	-	101.8	99.2	-
Tablet 6	-	98.6	100.2	-
Tablet 7	-	103.2	98.4	-
Tablet 8	-	98.1	100.2	-
Tablet 9	-	96.9	97.6	-
Tablet 10	-	97.9	102.1	-

[a]Duramed Pharmaceuticals, Inc., Westbury, NY.
[b]Did not contain this ingredient.
[c]Warner-Lambert Co., Morris Plains, NJ.
[d]Phillips-Roxane Laboratories, Columbus, OH.
[e]Rugby Laboratories, Rockville Center, NY.
[f]Contained 325 mg of acetaminophen, 30 mg of pseudoephedrine hydrochloride, 2 mg of chlorpheniramine maleate and 150 mg of lactose.
[g]Contained 450 mg of acetaminophen, 30 mg of pseudoephedrine hydrochloride, 30 mg of phenyltoloxamine citrate and 150 mg of lactose.

Assay Procedure: A 20.0 µl quantity of the sample was injected into the chromatograph using the described conditions. For comparison purpose, an identical volume of the standard solution was injected after the assay sample eluted.

Calculations: Since preliminary investigations indicated that ratio of peak heights (drug/internal standard) were directly related (range tested ± 50% of the standard solutions), the results were calculated using

$$\frac{(Ph)a}{(Ph)s} \times 100 = \text{Percent of the label claim found}$$

where (Ph)a is the peak heights ratio (drug/internal standard) of the assay solution and (Ph)s that of the standard solution of an identical concentration based on the label claim. The results are presented in Table 1 and Figures 1-4.

RESULTS AND DISCUSSION

Acetaminophen - The results indicate (Table 1) that the developed method can be used to quantify acetaminophen in capsules and tablets without interferrence from other ingredients (Figure 1). Actually to develop a method for acetaminophen was the easiest of all since it formed bulk of the capsule/tablet. In a previous study[1] for the quantitation of acetaminophen, chlorpheniramine maleate, dextromethorphen hydrobromide and phenylpropranolamine hydrochloride in combination, a microphenyl column and four different mobile phases were used to quantify all the four ingredients. In the present study, the authors were unable to separate pseudoephedrine from acetaminophen using microphenyl column. Therefore, a different column (micro/Clo)

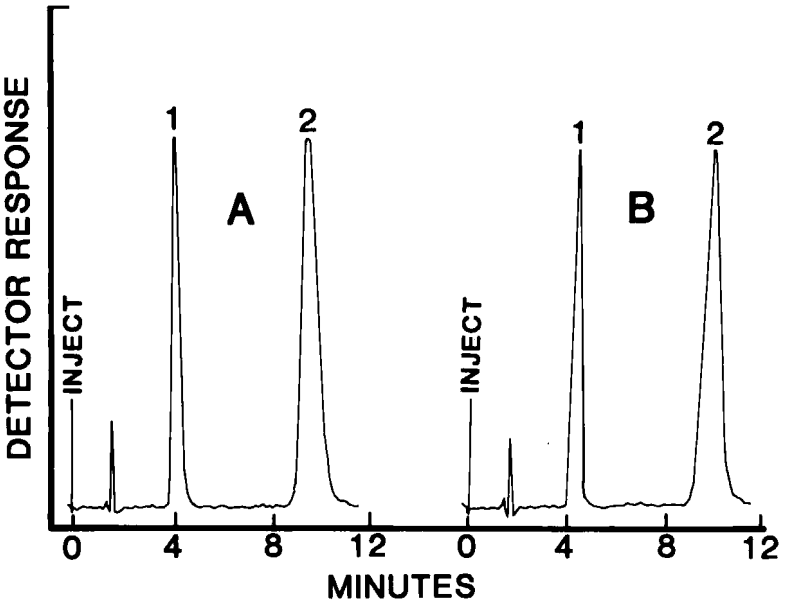

FIGURE 1

Sample chromatograms. Peaks 1-2 are from acetaminophen (0.32 µg) and salicylamide (AUFS 0.04), respectively. Chromatogram A is from a standard solution and B from tablets. For other chromatographic conditions, see text.

was used to separate pseudoephedrine hydrochloride from acetaminophen. In the previous method[1], no internal standard was developed. Since it is easier to quantify all the ingredients of a dosage form using a single column, therefore, acetaminophen assay method using micro/C18 was developed. The method is accurate and precise with a percent relative standard deviation based on 6 injections (0.32 µg each) of 0.9.

Chlorpheniramine Maleate and Phenyltoloxamine Citrate - The results indicate (Table 1) that the developed method can be used to quantify chlorpheniramine maleate and phenyltoloxamine citrate in capsules and tablets. As reported under acetaminophen, chlorpheniramine maleate

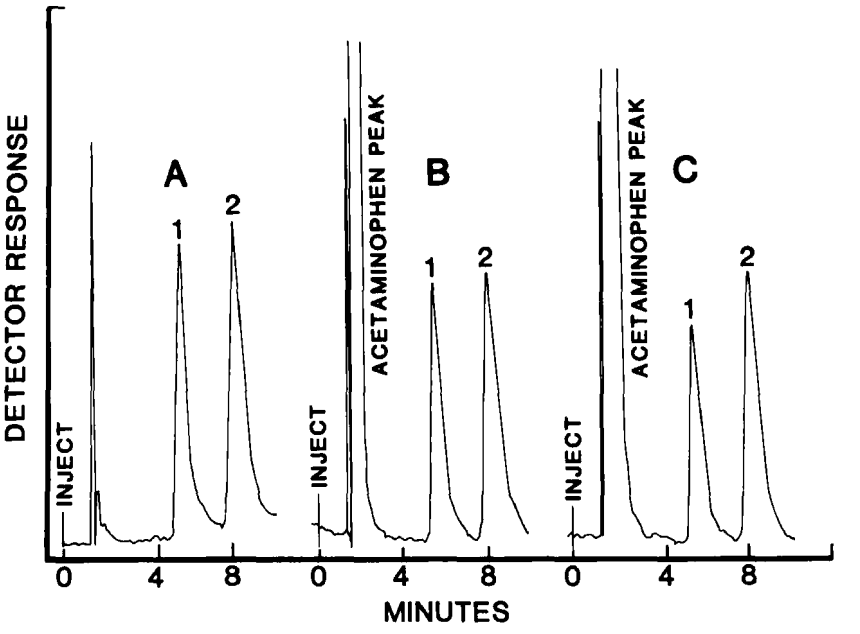

FIGURE 2

Sample chromatograms. Peaks 1-2 are from chlorpheniramine (0.8 µg) and phenyltoloxamine (AUFS 0.02), respectively. Chromatogram A is from a standard solution (at 269 nm) containing 100 µg/ml of chlorpheniramine maleate and 200 µg/ml of phenyltoloxamine citrate. Chromatogram B is from tablets (at 269 nm) containing acetaminophen and phenyltoloxamine citrate and C from tablets (at 261.5 nm) containing acetaminophen, chlorpheniramine maleate and pseudoephedrine hydrochloride. For other chromatographic conditions, see text.

was also quantified previously[1] using a microphenyl column without an internal standard. The present method was developed to keep the same column and an internal standard (phenyltoloxamine citrate) was also used successfully (Figure 2). The method is accurate and precise with a percent relative standard deviation based on 6 injections (0.8 µg each) of 1.9. Since phenyltoloxamine citrate is often mixed with acetaminophen in many dosage forms, the authors tried successfully to quantify it using the method developed. In this case, the

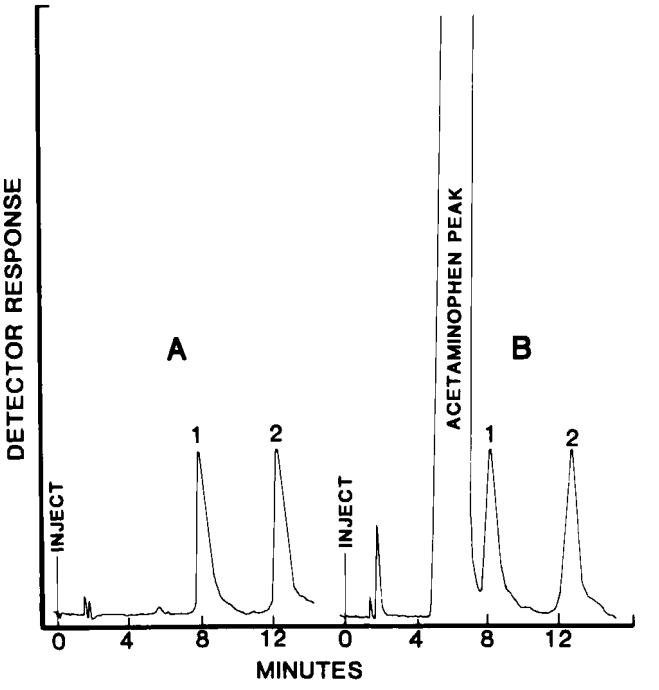

FIGURE 3

Sample chromatograms using column with 10 micron size particles and mobile phase containing 4% methanol. Peaks 1-2 are from pseudoephedrine (12 µg) and salicylamide (AUFS 0.02), respectively. Chromatogram A is from a standard solution and B from tablets. For other chromatographic conditions, see text.

only differences were the wavelength of detector (269 nm i.e. wavelength of maximum absorption for phenyltoloxamine) and the concentration of the internal standard (chlorpheniramine maleate) in the usual standard solution. Due to poor absorption of chlorpheniramine at 269 nm, its concentration as an internal standard was increased from 40 µg/ml (at 261.5 nm) to 100 µg/ml. Using this method, the percent relative standard deviation based on 6 injections (4 µg each) was 1.0 for phenyltolaxamine citrate.

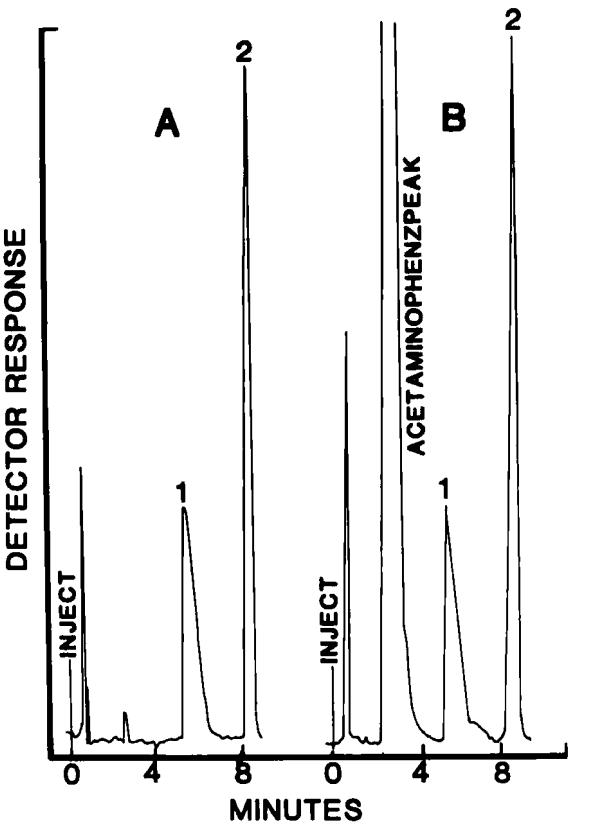

FIGURE 4

Sample chromatograms using column with 5 micron size particles and mobile phase containing 8% methanol. Peaks 1-2 and chromatograms A-B are same as given above (Figure 3). For other chromatographic conditions, see text.

Pseudoephedrine Hydrochloride - The developed method can be used to quantify pseudoephedrine hydrochloride (Table 1) in capsules and tablets. It was very difficult to separate pseudoephedrine from acetaminophen because of its low concentration and poor absorption versus acetaminophen whose concentration was almost 11 times and absorption almost 40 times at 257 nm (the wavelength of maximum absorption of pseudeoephedrine). Initially, the authors tried many different mobile

phases with different pH values and microphenyl column (as discussed above under acetaminophen) without success to separate pseudoephedrine from acetaminophen. On using a more non-polar column (10 micron particle size μ/C18), an incomplete separation (Figure 3) was possible. Later on in these studies, a 5 micron particle size μ/C18 column was used with higher percentage of methanol (8% versus 4% for 10 micron particle size column) to separate pseudoephedrine from acetaminophen completely (Figure 4). The developed method is accurate and precise with percent relative standard deviation based on 6 injections (12 μg each) of 1.1. The small particle size column can be used for the quantitation of other ingredients also i.e. acetaminophen, chlorpheniramine maleate and phenyltoloxamine citrate. For chlorpheniramine and phenyltoloxamine there was no need to increase the percent of methanol in the mobile phase. It should be pointed out that it was very difficult to extract pseudoephedrine hydrochloride from "Sinutab" (Warner-Lambert) tablets, the results were consistantly lower (86%) even on 2 different lots. The authors first tried to use 1.5 ml of dilute hydrochloric acid per tablet to facilitate the extraction. The results were not uniform. Also, both with water and water containing hydrochloric acid, the mixture was like an emulsion and hard to filter probably due to the addition of some filler not identified on the label. With the use of one ml of dilute sulfuric acid, the results were consistant and the filtration was very easy since formation of emulsion did not occur.

Briefly, the developed method can be used to quantify all the ingredients using a single column and 3 different mobile phases.

Furthermore, the method is sensitive for the determination of content uniformity of capsules and tablets (Table 1). The method was also tried on synthetic mixtures with excellent results (Table 1). There was no need of adding a counterion (for ion-pairing) in the mobile phase which shortens the life of column.

ACKNOWLEDGEMENT

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