

Simultaneous High-Performance Liquid Chromatographic Determination of Paracetamol, Phenylephrine HCl, and Chlorpheniramine Maleate in Pharmaceutical Dosage Forms

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Abstract

A rapid, precise, and specific high-performance liquid chromatographic method is described for the simultaneous determination of paracetamol, phenylephrine HCl, and chlorpheniramine maleate in combined pharmaceutical dosage forms. The method involves the use of a μ Bondapak CN RP analytical column (125 Å, 10 μ m, 3.9 \times 150 mm) at 22°C as the stationary phase with the mixture of acetonitrile and phosphate buffer (pH 6.22, 78:22) as the mobile phase. Derivatization of the drugs is not required. The method is applied to commercial pediatric cough-cold syrups, tablets, and capsules marketed in Turkey. The relative standard deviation for 10 replicate measurements of each drug in the medicaments is always less than 2%.

Introduction

Combinations of decongestant, antihistaminic, and analgesic preparations are widely used for cough and cold treatment.

Several methods have been described for the quantitative determination of these drugs. High-performance liquid chromatography (HPLC) methods have been investigated by many workers. Most of them were based on ion-pair formation, and the detection methods were typically based on measuring the UV absorbance of the analytes (1–9).

The methods given in the literature were applied to these active ingredients, but the methods could only determine two components simultaneously. In the given methods, paracetamol and phenylephrine HCl give peaks with the same retention times. Other analytical techniques such as derivative spectrophotometry (2), high-performance thin-layer chromatography (10), liquid chromatography (LC)–mass spectrometry (11), gas–liquid chro-

matography (12), and spectrofluorometric (13) methods have also been reported, but none of the methods are applicable for the simultaneous determination of three components with a large excess of paracetamol content.

In USP 24, the determination of these components has also been performed with HPLC, but all of them were determined separately and the method does not involve simultaneous determination (14).

In this study, we propose to employ UV detection to determine active ingredients in cough–cold syrups, tablets, and capsules after HPLC separation.

The advantages of the proposed method are that the method works well for all cold drugs with UV absorptivity, the detector response for all drugs are similar, they are easily applicable in large excess amounts of paracetamol drugs without any fitting into one another, and they have a very short analysis time of approximately 4 min.

Experimental

Apparatus

The system consisted of a Hewlett Packard (Waldborn, Germany) Series 1100 LC including an HP UV–vis detector, vacuum degasser, gradient pump module, auto injector with a variable injection valve, and column compartment oven. A μ Bondapak CN RP analytical column from Waters (Milford, MA) (125 Å, 10 μ m, 3.9 \times 150 mm) was used. Instrumental settings were a flow rate of 1.5 mL/min, a column temperature at 22°C, and a detector wavelength of 265 nm.

Materials and reagents

All the drugs were of USP quality. Methanol and acetonitrile were obtained from J.T. Baker (Griesheim, Germany) in HPLC gradient grade. Orthophosphoric acid and triethylamine were obtained from Merck Inc. (Darmstadt, Germany). The water used

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was distilled and deionized by using a Millipore (Vienna, Austria) Milli-Q ultrapure system. Other chemicals were of analytical or HPLC grade.

Mobile phase

The mobile phase consisted of an aqueous solution of phosphate buffer (pH 6.22) and acetonitrile (22:78, v/v). The phosphate buffer was prepared by dissolving 1.36 mL orthophosphoric acid in 1 L water. Triethylamine was added to the phosphate buffer solution in order to adjust the pH to 6.22. Acetonitrile and water were previously filtered under vacuum through 0.45- μ m nylon filters before injection into the HPLC apparatus.

Standard stock solutions

Standard solutions were prepared by dissolving the drugs in methanol and diluting them to the desired concentrations.

Paracetamol

A 320-mg sample of paracetamol was accurately weighed and dissolved with methanol up to volume in a 10-mL volumetric flask. A 31.2- μ L volume of this solution was again diluted with methanol to volume in a 10-mL volumetric flask.

Phenylephrine HCl

A 10-mg sample of phenylephrine HCl was accurately weighed and dissolved with methanol up to volume in a 10-mL volumetric flask. A 1000- μ L volume of this solution was again diluted with methanol to volume in a 10-mL volumetric flask.

Chlorpheniramine maleate

A 10-mg sample of chlorpheniramine maleate was accurately weighed and dissolved with methanol up to volume in a 50-mL volumetric flask.

Standard mixture solution

A standard mixture solution was prepared from these stock solutions by mixing 2000 μ L of a paracetamol standard solution, 152 μ L of a phenylephrine HCl standard solution, 14.8 μ L of a chlorpheniramine maleate standard solution, and 1232 μ L methanol.

Sample preparations

Syrup samples

The syrup solution was homogenized by shaking and diluted with methanol to give a final concentration of 40 to 120 μ g for paracetamol, 1 to 4 μ g for phenylephrine HCl, and 0.3 to 1 μ g for chlorpheniramine maleate in 1 mL.

Tablet and capsules

Twenty tablets or capsule contents were weighed, their mean weight determined, and they were finely powdered. An equivalent weight of the tablet or capsule content was transferred into a 10-mL volumetric flask containing 6 mL methanol, ultrasonicated for 20 min, and diluted to 10 mL with methanol. The solution was filtered through a 0.45- μ m nylon filter.

This solution was again diluted with methanol to give a final concentration mentioned in the syrup samples.

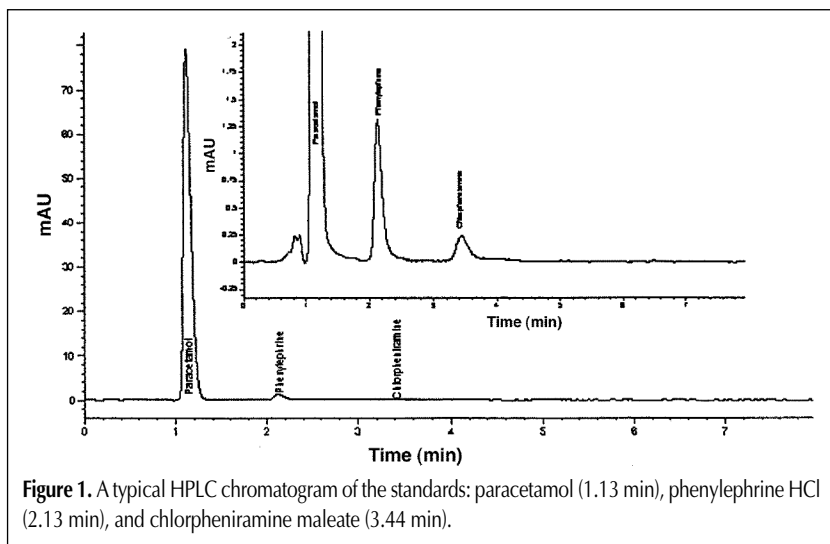


Figure 1. A typical HPLC chromatogram of the standards: paracetamol (1.13 min), phenylephrine HCl (2.13 min), and chlorpheniramine maleate (3.44 min).

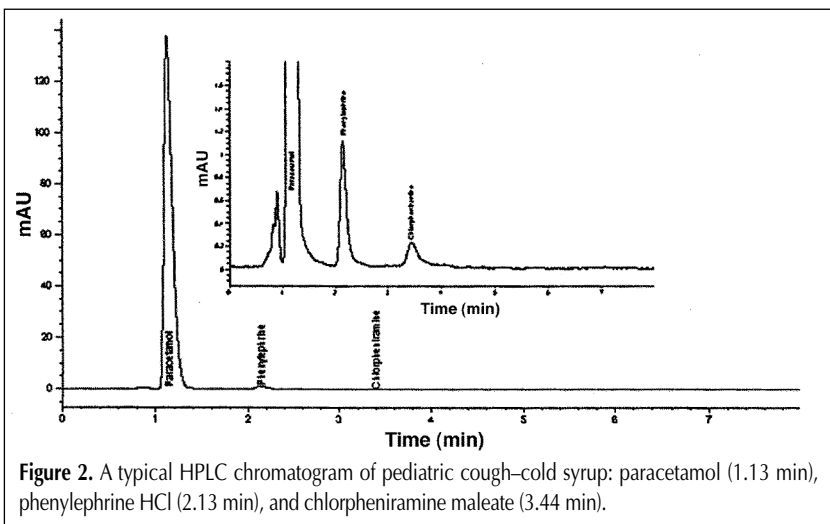


Figure 2. A typical HPLC chromatogram of pediatric cough-cold syrup: paracetamol (1.13 min), phenylephrine HCl (2.13 min), and chlorpheniramine maleate (3.44 min).

Results and Discussion

Calibration and linearity

An external standard method was used for quantitative determinations. Triplicate 1-, 3-, 6-, 8-, 9-, 10-, and 15- μ L injections were made for the standard mixture solution. The retention times of the standards were 1.13 min for paracetamol, 2.13 min for phenylephrine HCl, and 3.44 min for chlorpheniramine maleate. A typical HPLC chromatogram of the standard mixture is shown in Figure 1. The calibration graphs were obtained by plotting the peak area against the concentration of the drugs. In the simultaneous determination, the calibration graphs were found to be linear in the mentioned concentrations (the correlation coefficients are shown in Table I).

Precision (reproducibility)

The precision of the method was studied by

determining the concentrations of each drug in a syrup, capsule, and tablet ten times. The results of the precision study (shown in Table I) indicate that the method is reliable (relative standard deviation percentage < 2).

Ingredients	Precision (%RSD [†])	Coefficient of correlations	Linearity (µg/mL)
Paracetamol	0.13	0.9999	25–120
Phenylephrine HCl	1.95	0.9999	0.3–10
Chlorpheniramine maleate	1.36	0.9999	0.2–3

* $n = 10$.
† %RSD, relative standard deviation percentage.

Matrix	Ingredient	Amount added	%Recovery [†]
Syrup (mg/mL)	Paracetamol	30.0	99.73 (0.18)
	Phenylephrine HCl	1.0	98.40 (1.55)
	Chlorpheniramine maleate	0.2	98.00 (4.55)
Tablet (mg)	Paracetamol	325.0	99.99 (0.04)
	Phenylephrine HCl	5.0	100.56 (1.56)
	Chlorpheniramine maleate	2.0	99.60 (0.65)

* $n = 5$.
† Relative standard deviation shown in parentheses.

Samples	Ingredient	Label value (mg)	Found % (mg)	Label claim
Syrup (mg/mL)	Paracetamol	32	32.100	100.30
	Phenylephrine HCl	1	1.000	100.00
	Chlorpheniramine maleate	0.2	0.200	100.00
Dristan	Paracetamol	325	325.010	100.00
	Phenylephrine HCl	5	5.037	100.74
	Chlorpheniramine maleate	2	2.019	100.99
Deflu	Paracetamol	300	300.051	100.02
	Phenylephrine HCl	5	5.019	100.38
	Chlorpheniramine maleate	2	2.010	100.50
Coldeks	Paracetamol	325	325.015	100.01
	Phenylephrine HCl	5	5.012	100.24
	Chlorpheniramine maleate	1	1.006	100.60

* Average of 10 analyses.

Recovery tests

Recovery tests were performed by adding a known amount of each drug to a cough–cold syrup and tablet where it was known to be absent.

The mean results of five analyses ranged from 97.10 to 99.53 (Table II), and these can be considered to be good recoveries.

Determination of the limit of detection and quantitation

The limit of detection (LOD) was defined as the concentration of phenylephrine HCl and chlorpheniramine maleate (calculated as 0.0325 µg/mL and 0.0279 µg/mL, respectively) that produce analytical signals equal to thrice the deviation of the background signals. The limit of quantitation (LOQ) was the lowest levels of phenylephrine HCl and chlorpheniramine maleate (determined to be 0.251 µg/mL and 0.184 µg/mL, respectively) in the simultaneous quantitative assay. The relative standard deviation percentage results of the LOQ studies were 1.29 for phenylephrine HCl and 2.51 for chlorpheniramine maleate ($n = 10$).

Selectivity

Selectivity was assessed by a quality control of the chromatograms obtained from samples and placebo. Possible interferences resulting from substances present in the medicaments were not observed.

Determination of active ingredients in pharmaceutical dosage forms

The contents of three drugs in ten different pediatric cough–cold syrups, capsules (Coldeks), and tablets for each brand (Dristan and Deflu) were determined by the proposed method, and the results are presented in Table III.

The chromatogram of a pediatric cough–cold syrup is shown in Figure 2.

Conclusion

The concentration of phenylephrine HCl, chlorpheniramine maleate, and a large excess of paracetamol in pharmaceutical samples can be satisfactorily determined using HPLC with UV detector. This study has shown that UV detection is a sensitive, reliable, reproducible, and accurate method for the determination of the active ingredients in pediatric cough–cold syrups, capsules, and tablets.

The method is straightforward and simpler than the commonly used HPLC methods involving ion pairing or derivatization. As can be seen in the figures, 3.5 min is enough for all of the active ingredients to be released.

This method has been found suitable for the routine analysis of the pharmaceutical dosage forms in quality control and R&D laboratories for products of similar type and composition.

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Manuscript accepted December 7, 2001.