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Short Communication

Simultaneous determination of guaiphenesin and codeine phosphate in tablets by high-performance liquid chromatography

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Abstract

A reversed-phase high-performance liquid chromatographic method has been developed for the simultaneous determination of guaiphenesin and codeine phosphate in an antitussive tablet formulation. A 10-μm C_{18} column is used with a 1:1 methanol–water mixture (pH 3.0) as the mobile phase and spectrophotometric detection is carried out at 212 nm. The total elution time is shorter than 4.4 min. The method showed good linearity, precision and reproducibility. The proposed method was successfully applied to the determination of guaiphenesin and codeine phosphate in tablets.

Keywords: Guaiphenesin; Codeine phosphate; High-performance liquid chromatography; Simultaneous determination; Tablet

1. Introduction

Guaiphenesin [3-(2-methoxyphenoxy)-1,2-propanediol, guaiacol glyceryl ether, glyceryl guaiacolate] has expectorant properties and is widely used in cough remedy formulations [1,2]. Other uses of this drug are as an agent for reducing platelet adhesiveness, as a hypocholesteremic reagent, as a muscle relaxant, and as a general anaesthetic for veterinary use [3]. Codeine phosphate is an antitussive and analgesic drug, which is available worldwide. Coughing is one of the most common symptoms of respiratory infections for which patients seek relief. The pharmaceutical combination of guaiphenesin and codeine phosphate increases the volume of secretions in the respiratory tract, assists the patient in coughing up viscid mucus and is thus used in the treatment of coughs.

Guaiphenesin and codeine phosphate together with their dosage forms and in combination with other drugs have been listed in various pharmacopeias [4–6]. Several methods (liquid and gas chromatographic, spectrophotometric and titrimetric methods) are described in these pharmacopeias for guaiphenesin in the raw material and dosage forms. Numerous analytical methods such as gas chromatography (GC), liquid chromatography and non-aqueous titrimetry have been reported for the determination of codeine phosphate in bulk drug and dosage forms. In the presence of other drugs, guaiphenesin has been quantitated by derivative UV spectrophotometry [7], high-performance liquid chromatography (HPLC) [8–11] and GC [12]. Codeine phosphate in combination with other drugs has been determined using spectrophotometry [13,14], non-aqueous titrimetry [15] and HPLC [16,17].

Although there is an assay method for guaiphenesin and codeine phosphate described for a syrup formulation in the USP, this method is time consuming because of the separate determination of the two drugs. Thus, it was considered desirable to develop a simpler and faster assay that would serve as an alternative to the current official method.

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In this work, our objective was to develop and validate a specific, precise and reproducible method for the simultaneous quantitation of guaiphenesin and codeine phosphate. Analytical data is presented to illustrate the usefulness of the method for the determination of the two drugs together in tablet formulation using HPLC.

2. Experimental

2.1. Chromatographic conditions

The chromatograph consisted of a solvent delivery pump (Waters 510 HPLC Isocratic Pump), an automatic sample injection system (Waters 717 Plus Autosampler), a 300 mm × 3.9 mm (C_{18}) stainless steel reverse phase column packed with 10 μm dimethyloctadecylsilyl-bonded amorphous silica (Bondapak Waters Associates, Milford, MA), a photodiode array detector (Waters 996 Photodiode Array Detector). The mobile phase was 50:50 methanol–water (pH 3.0 adjusted with 10% orthophosphoric acid) at a flow rate of 1.7 ml/min, and elution was monitored at 212 nm. The mobile phase mixture was filtered through a 0.45 μm pore nylon membrane filter (Millipore, Bedford, MA) and degassed by sonication.

2.2. Chemicals

Codeine phosphate was received from United Pharmaceutical Works, Holland. Phenacetine, used as internal standard, and guaiphenesin were obtained from Sigma Laboratories, USA. Orthophosphoric acid and HPLC grade methanol were received from E. Merck Corporation. Water used in analysis was demineralized and bidistilled using Aqua Nova distillation apparatus (Degerfors, Sweden).

2.3. Stock and standard solutions

Guaiphenesin (10 mg), codeine phosphate (10 mg) and phenacetin (internal standard, 30 mg), were accurately weighed and dissolved in water to a volume of 100 ml in a volumetric flask. Serial dilutions of these stock solutions were made with the mobile phase to obtain a standard solution containing 3 μg/ml of codeine phosphate, 30 μg/ml of guaiphenesin and 30 μg/ml of the internal standard.

2.4. Calibration graphs

Mixed standard solutions containing codeine phosphate (1–5 μg/ml), guaiphenesin (10–50 μg/ml) with a fixed concentration of phenacetin (internal standard, 30 μg/ml) were prepared in the mobile phase. These solutions were filtered through a 0.45 μm membrane before use. A total of 50 μl of each solution was repeatedly injected into the column. The five concentrations of each compound were subjected to regression analysis and the slope and intercept were calculated.

2.5. Pharmaceutical formulation

Ekspektoran tablet (Military Pharmaceutical Industry, Turkey) labeled to contain 15 mg codeine phosphate and 150 mg guaiphenesin per tablet.

2.6. Assay

The average weight per tablet was calculated from the weight of 20 tablets. Quantities of the finely powdered tablets equivalent to 15 mg codeine phosphate and 150 mg of guaiphenesin were accurately weighed into a 100-ml volumetric flask, and dissolved in water. The solution was sonicated for a few minutes and brought to volume with water. A total of 0.2 ml of the completely dissolved solution was directly transferred to a 10-ml volumetric flask, 1 ml of the internal standard solution was added and the contents were diluted to volume with the mobile phase. The solution was filtered through a 0.45 μm membrane before use and 50 μl was chromatographed as previously described. The contents of codeine phosphate and guaiphenesin were calculated from linear regression equations of the calibration graph.

3. Results and discussion

In order to effect simultaneous analysis of codeine phosphate and guaiphenesin peaks under isocratic conditions, the mobile phase composition was optimized. A satisfactory separation was obtained with a mobile phase consisting of 1:1 methanol–water. The optimum resolution of the components was obtained at pH 3.0. Large changes in the pH (± 0.5) of the mobile phase affected the resolution of peaks. Using reversed-phase column (C_{18}) and at a flow rate of 1.7 ml/min, the retention times for codeine phosphate, guaiphenesin, and phenacetine (internal standard) were observed to be 2.3, 2.8, and 3.5 min, respectively. Total time of analysis was less than 4.4 min. Capacity factors (k') were found to be 0.92, 1.33, and 1.92 for codeine phosphate, guaiphenesin, and phenacetin, respectively (t_o value was 1.2 min). The maximum absorption of codeine phosphate was found to be at 212 nm and this wavelength was chosen for the analysis. Fig. 1 shows a typical chromatogram of the standard solution of analytes. The limit of detection was calculated to be 0.12 and 1.75 μg/ml for codeine phosphate and guaiphenesin, respectively. The limit of quantitation was found to be 0.4 and 5.83 μg/ml for codeine phosphate and guaiphenesin, respectively.

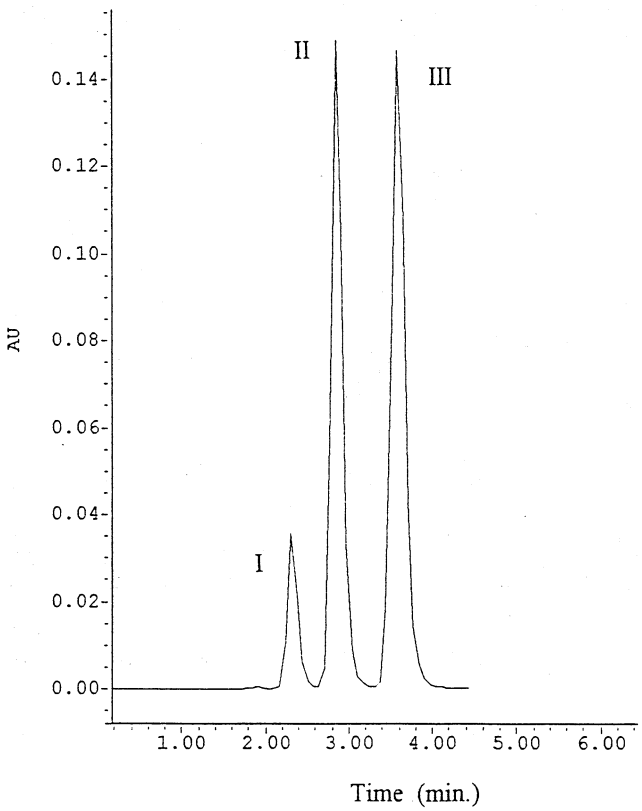

Fig. 1. HPLC chromatogram of a working standard solution containing codeine phosphate (I), guaiphenesin (II) and phenacetin (III). Eluting solvent, 1:1 methanol–water (pH 3.0), flow rate 1.7 ml/min; ambient temperature; $\lambda = 212$ nm.

Calibration curves were generated from the chromatograms of standard solutions. The calibration graphs showed good linearity between peak-area ratios and concentrations of 1–5 μg/ml with $r^2 = 0.9960$ and 10–50 μg/ml with $r^2 = 0.9988$ for codeine phosphate and guaiphenesin, respectively (Table 1). A mixture containing known amounts of the codeine phosphate and guaiphenesin was used for the determination of the recovery of the compounds. The quantitation was performed using the slope and intercept data of regression analysis for each compound. The average percentage recoveries were found to be 100.00 ± 0.69 and 99.11 ± 0.74 for codeine phosphate and guaiphenesin at the concentrations of 3.5 and 35 μg/ml, respectively (Table 2).

The precision (within-day variations of replicate determinations) and the reproducibility (day to day variation of the determinations) of the proposed method were shown by chromatographing the solutions (five times) of compounds at various concentrations in the presence of the internal standard. The concentration of each compound was calculated from its peak height or peak-area ratio using the appropriate regression equation (Table 1). The results obtained for the precision and reproducibility are given in Table 3. The relative standard deviations (RSD) for the intra-day variations were 0.34 and 0.46% at the concentrations of 4 and 5 μg/ml for codeine phosphate, and 0.13 and 0.39% for guaiphenesin at the concentrations of 40 and 50 μg/ml, respectively. The RSD values for inter-day precision

Table 1
Results of regression analyses from standard solutions

Compound	Concentration (μg/ml)	$A_D/A_{I.S.}$[a] ± SD[b]	Slope	Intercept	$r \pm$ SD[b]
Codeine phosphate	1.0	0.0566 ± 0.002	0.0577	0.0029	0.9980 ± 0.0015
	2.0	0.1120 ± 0.003			
	3.0	0.1842 ± 0.005			
	4.0	0.2298 ± 0.002			
	5.0	0.2904 ± 0.002			
Guaiphenesin	10.0	0.2821 ± 0.003	0.0268	0.0230	0.9994 ± 0.0001
	20.0	0.5651 ± 0.005			
	30.0	0.8501 ± 0.004			
	40.0	1.0832 ± 0.004			
	50.0	1.3672 ± 0.006			

[a] Data represents ten replicate injections of standard solutions. $A_D/A_{I.S.}$ is the ratio of the integrated area of the drug peak at a given concentration divided by the integrated area of internal standard (phenacetine) peak at a concentration of 30 μg/ml.
[b] SD, standard deviation.

Table 2
Recovery of guaiphenesin and codeine phosphate from laboratory-made mixtures ($n = 5$) [a]

Compound	Concentration (μg/ml)	Amount found (μg/ml)	% Recovery ± SD
Codeine phosphate	3.5	3.5	100.00 ± 0.69
Guaiphenesin	35	34.69	99.11 ± 0.74

[a] Other ingredients of the formulation are amidon, gelatin, talc, and magnesium stearate.

Table 3
Summary of intra-day and inter-day precision data for HPLC assay of guaiphenesin and codeine phosphate ($n = 5$)

Compound	Concentration (μg/ml)	Intra-day	Inter-day			
		Mean ± RSD% [a]	Amount found ± RSD% [a]			Mean ± RSD% [a]
			Day 1	Day 2	Day 3	
Codeine phosphate	4	99.60 ± 0.34	99.60 ± 0.34	99.47 ± 0.37	99.80 ± 0.22	99.62 ± 0.17
	5	99.54 ± 0.46	99.54 ± 0.46	100.32 ± 0.91	99.61 ± 0.50	99.82 ± 0.43
Guaiphenesin	40	99.39 ± 0.13	99.86 ± 0.14	99.19 ± 0.22	99.39 ± 0.13	99.48 ± 0.34
	50	100.27 ± 0.39	100.28 ± 0.47	100.27 ± 0.39	100.19 ± 0.34	100.25 ± 0.05

[a] RSD, relative standard deviation.

Table 4
Results from quantitation analysis of guaiphenesin and codeine phosphate in tablet formulation ($n = 5$)

Compound	Label (mg/tablet)	Amount found (%) ± RSD
Codeine phosphate	15	101.33 ± 0.24
Guaiphenesin	150	99.68 ± 0.09

were found to be 0.17 and 0.43 for codeine phosphate and 0.05–0.34 for guaiphenesin at the same concentrations.

The proposed HPLC method was applied to the quantitation of compounds in tablets. Table 4 summarizes the analytical results from the commercial dosage form.

The aim of the study was to develop a method for the simultaneous quantitation of codeine phosphate and guaiphenesin in tablet dosage form. The developed method allows the quantitation of both compounds in tablets using the same dilution and the same injection volume and with reasonable responses for the two well-resolved peaks. This is an advantage over the current USP procedure, which involves separate quantitation of codeine phosphate and guaiphenesin using GC.

In conclusion, our results indicate that the developed method could be used for the simultaneous quantitation of codeine phosphate and guaiphenesin in pharmaceutical formulations.

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