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Short communication

A chromatographic method for rapid and simultaneous analysis of codeine phosphate, ephedrine HCl and chlorpheniramine maleate in cough–cold syrup formulation

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Abstract

The present paper describes a simple, accurate and precise reversed phase HPLC method for rapid and simultaneous quantification of codeine phosphate, ephedrine HCl and chlorpheniramine maleate in a cough–cold syrup formulation. Separations were carried out on a Zorbax® XDB C8 column (150 × 4.6 mm ID), 5 μm particle size. A gradient elution system was developed using varying percentages of two mobile phases: methanol–glacial acetic acid–triethylamine (980:15:6 v/v) and water–glacial acetic acid–triethylamine (980:15:6 v/v). The elution of the analytes was achieved in less than 7 min with a flow rate of 1.5 ml/min. Detection was by UV absorbance at a wavelength of 254 nm. Quantification of the components in actual syrup formulations was calculated against the responses of freshly prepared external standard solutions. The method was validated and met all analysis requirements of quality assurance and quality control recommended by FDA of the USA.
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Keywords: Simultaneous quantification; Codeine phosphate; Ephedrine HCl; Chlorpheniramine maleate; Cough–cold syrup; HPLC

1. Introduction

Codeine phosphate (CP) is chemically 7,8–didehydro-4,5-α-epoxy–3–methoxy -17-methyl-morphinan-6-α-ol phosphate salt. Its structure is shown in Fig. 1. In pharmaceutical preparations, the compound is used as a sedative, an analgesic and an antitussive agent [1–4]. Ephedrine HCl (EP) is chemically benzenemethanol, α-[1-(methylamino)ethyl]-, [*R*-*R**,*S**]- chloride salt. Its structure is shown in Fig. 2. EP is a decongestant and was once used as a broncodilator in the treatment of asthma [1–4]. Chlorpheniramine maleate (CLP) is chemically 2-pyridinepropanamine, γ(4-chlorophenyl) -*N*,*N*-dimethyl, (*Z*)-2-butenedioate. Its structure is shown in Fig. 3. It is an antihistamine and is widely used as an ingredient in antitussive

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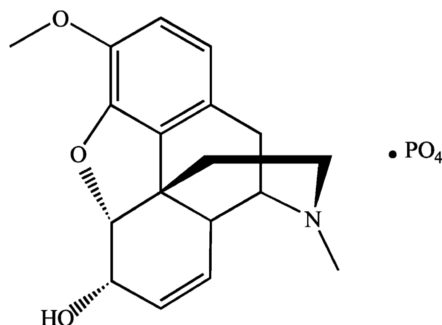

Fig. 1. Structure of codeine phosphate.

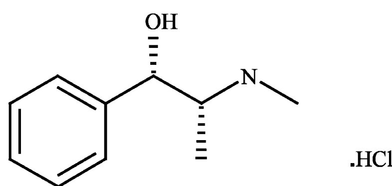

Fig. 2. Structure of ephedrine HCl.

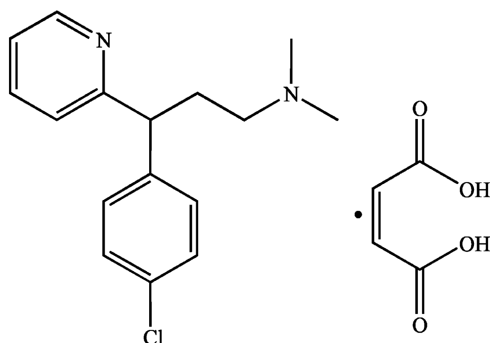

Fig. 3. Structure of chlorpheniramine maleate.

formulations [1]. These three compounds are the pharmacologically active constituents found in most cough–cold syrups [3].

There have been numerous publications describing various methods for the quantification of these three compounds individually and in combination with other drugs. Recently, CLP and EP have been successfully quantified in cough cold formulations by micellar liquid chromatography using sodium dodecyl sulfate [5]. CLP and pseudoephedrine (PEP), an isomer of ephedrine, have also been separated by more conventional reversed phase

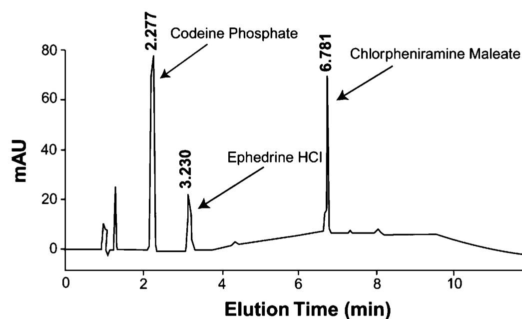

Fig. 4. Typical chromatogram of mixed standard solution.

(RP)-HPLC for analysis of cold relief chewing gum formulations [6]. All three components, along with a number of other common active ingredients, were resolved by RP-HPLC with the use of indirect conductometric detection for quantification purposes [7]. Gas–liquid chromatographic methodologies have also been developed for the determination of such ingredients in cold syrups, including the three of interest here [8]. Different combinations of related analgesics have also been quantified using post-column photochemical derivatization following RP-HPLC separation [9]. Ion-pairing agents have also been utilized successfully in the separation of the active agents found in cough cold pharmaceutical preparations [10,11].

Whilst all of the above listed procedures have been successfully validated and applied in routine analysis, none of them addresses simultaneous quantification of all three combined components in one step. There are limited reagents available for development and no choice as to the mode of detection to be used, i.e. UV, as is the case in many laboratories. The present paper describes the development of a RP-HPLC method using gradient mobile phase that offers certain advantages in its simplicity and time saving. Development of the method was undertaken with a prerequisite to be as uncomplicated as possible, in deference to the training of staff in the use of advanced analytical equipment such as HPLC for routine analysis. It also describes the development of validation work as per guidelines recommended by the Food and Drug Administration (FDA) of the United States [12].

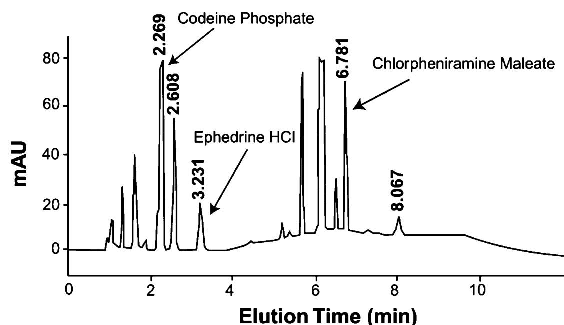

Fig. 5. Typical chromatogram of cough–cold syrup solution.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade triethylamine and AR grade orthophosphoric acid were purchased from Riedel-de Haen (Seelze, Germany). Analytical grade glacial acetic acid and HPLC grade methanol were purchased from Merck (Darmstadt, Germany). Distilled and deionised water was obtained from an in house Millipore Milli-Q 50 ultra pure water system (Millipore, Molsheim, France). Reference standards of codeine phosphate (CP), ephedrine HCl (EP) and chlorpheniramine maleate (CLP) were provided by a pharmaceutical company (Vida Laboratories Ltd, HK). They were all of BP or USP quality and were used without further purification. A placebo syrup mix to be used in recovery analysis was prepared by the company also.

2.2. Preparation of solutions

A 10 mM solution of orthophosphoric acid was prepared by adding 10 ml of 85% orthophosphoric acid to 1000 ml of deionised water. This was the diluent solution used in solution preparations.

A mixed standard solution was prepared containing 400 μg/ml of CP, 200 μg/ml of EP and 40 μg/ml of CLP in diluent. Sample solution was prepared by adding five ml of syrup formulation to a 25 ml volumetric flask along with around 15 ml of diluent. This was unltrasonicated for a few minutes, allowed to cool and made up to the mark with diluent. The sample solution was filtered through 0.45 μm acrodisc filters before being placed in vials for HPLC analysis. Theoretical concentrations of analyte compounds in the sample solution, according to stated label strengths, should have been 400 μg/ml of CP, 200 μg/ml of EP and 40 μg/ml of CLP.

2.3. Chromatographic conditions

A Hewlett–Packard 1100 HPLC (Palo Alto, CA, USA) system was used, coupled with an UV–diode array detector. Separations were carried out on a Zorbax® XDB C8 column (150 × 4.6 mm I.D) packed with 5 μm particle size of silica as the stationary phase.

The basic nature of the three compounds for quantification usually leads to peak tailing, broad bands and small plate numbers during RP-HPLC. This is related to 'silanol effects'- interaction of basic drugs with free silanol groups on the column. As a consequence, triethylamine was employed as an additive to eliminate silanophilic retention of the basic solutes. The addition of amines is a common solution to the problem of peak tailing in RP-HPLC [13,14]. The amount of triethylamine necessary was investigated in a stepwise fashion in order to find a balance between sufficient resolution of the compounds, satisfactory peak shape and minimal run time in order to conserve solvent. Eventually the following mobile phase compositions were employed. Mobile phase A consisted of methanol –glacial acetic acid–triethylamine (980:15:6 v/v). Mobile phase B was water–glacial acetic acid–triethylamine (980:15:6 v/v). Both mobile phases were ultrasonicated for 15 min in order to degas them. The flow rate was 1.5 ml/min, injection volume 20 μl and a column temperature

Table 1
Mobile phase timetable

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	18	82
2	18	82
5	60	40
8	60	40
10	18	82
12	18	82

Table 2
Linearity study results

Analyte	pK_a	Equation of calibration curve	Correlation coefficient squared (r^2)
CP	8.2	$y = 10.840x + 16.355$	1.0000
EP	9.6	$y = 1.180x - 1.499$	0.9999
CLP	4.0	$y = 3.331x - 3.532$	1.0000

of 30 °C. Method development led to the mobile phase timetable shown in Table 1 to be both satisfactory in resolution of the compounds and rapid enough to enable multiple sample runs in a single day of analysis.

The overall run time for each injection was therefore 12 min. Prior to analysis, the column was flushed with 24% mobile phase A and 76% mobile phase B at a flow rate of 2.0 ml/min until a flat baseline was observed. The optimum UV wavelength for detection was determined to be 254 nm from inspection of the individual spectra of the analyte peaks as provided by the diode array detector. All data analysis and chromatogram processing was performed by HP CHEMSTATION software.

2.4. Chromatographic analysis

A typical (post method validation) chromatographic run involved the preparation of duplicate standard solutions (A and B) and duplicate sample solutions (A and B). The injection sequence was as follows: blank (diluent solution), standard solution A × 5, standard solution B × 2, blank, sample solution A × 2, sample solution B × 2, blank and so on with any other samples to be analyzed. Such a sequence was necessary for system suitability to be established on the day of analysis.

From the peak areas obtained the percentage label strengths (%LS) of each compound were calculated using the following formula:

$$\%\text{LS} = \frac{\text{StdA concentration (mg/ml)}}{\text{Average StdA peak area}} \times \frac{\text{Average Sample peak area}}{\text{Label strength (mg/ml)}} \times 500$$

On a day of analysis, the average peak area of analytes in the five standard A injections was used for single point calibration and a check recovery value for each was calculated using the standard B area response.

2.5. Validation of the method

Specificity of the method was investigated by analysis of the UV spectra obtained for standard and sample analyte peaks from the diode array detector. The chemstation software calculated resolution values of the peaks from each other.

Accuracy of the method was studied by recovery investigation. This also provided the working range for the method. Placebo syrup solution containing all components apart from codeine phosphate, ephedrine HCl and chlorpheniramine maleate was used. Known amounts of each of these three were then 'spiked' into separate 25 ml aliquots of placebo to give pseudo sample solutions of approximately 80, 100 and 120% of stated label strength values. These samples were then analyzed according to procedure and percentage recoveries calculated.

Linearity of the method was evaluated by preparing a standard solution containing 600 μg/ml of codeine phosphate, 300 μg/ml of ephedrine

Table 3
Precision results

	Concentration determined (μg/ml)		
	CP	EP	CLP
Within-run precicion			
Mean	403.7	196.8	41.8
%R.S.D. ($n = 6$)	0.20	0.45	0.33
Between-run precision			
Mean	401.9	195.7	42.7
%R.S.D. ($n = 12$)	0.57	0.53	1.13

HCl and 60 μg/ml chlorpheniramine maleate (150% of targeted level of the assay concentration). Sequential dilutions of this solution were performed to give solutions at 120, 100, 80 and 50% of the target assay concentration. These were injected in triplicate and the peak areas used to plot calibration curves.

Precision (method repeatability) was investigated using one batch of freshly manufactured cough cold syrup formulation. From this six separate sample solutions (A–F) were prepared as per procedure. Each was injected twice and the peak areas obtained used to calculate mean and percentage R.S.D. values. Injecting a freshly prepared standard solution ten times and calculating mean and percentage R.S.D. values evaluated injection (system) repeatability.

Ruggedness of the method was studied by using different sources of solvents and reagents, a different HPLC system and evaluation of the stability of standard and sample solutions over a 3-day period.

3. Results and discussion

3.1. Separation performances

Figs. 4 and 5 show examples of the standard and sample solution chromatograms obtained using the aforementioned chromatographic conditions. The retention times of CP, EP and CLP were found to be 2.27, 3.23 and 6.78 min, respectively. Elution of the maleate counter-ion occurred just after the solvent front at around 1.35 min. This peak did not interfere with any of the three main analytes. These retention times did not vary to any considerable degree during and in between analyses (%R.S.D. less than 2% for the retention time of each peak). Resolution of the EP from CP was 7.16, whilst resolution of the CLP from the EP was 30.83. Both these values meet the acceptance criteria for resolution of greater than or equal to two. The number of theoretical chromatographic plates for CP, EP and CLP were approximately 6500, 7000 and 13 000, respectively. No significant column degradation was noted in over a month of consistent usage. During this month over 400 injections of sample and standard solutions were run through the column, adding up to total run time of over 80 h with more than 10 l of solvent having been passed through the system.

3.2. Calibration graphs and linearity study

Linearity of the method was evaluated by preparing a standard solution containing 600 μg/ml of CP, 300 μg/ml of EP and 60 μg/ml CLP (150% of targeted level of the assay concentration). Sequential dilutions of this solution were performed to give solutions at 120, 100, 80 and 50% of the target assay concentration. These were injected in triplicate and the peak areas used to plot calibration curves. Results were inputted into a Microsoft excel spreadsheet program so calibration curves could be plotted. The square of the correlation coefficients and equations for the curves are shown in Table 2. All three r^2 values are greater than 0.999, therefore acceptable. From these results it is acceptable to use a single point calibration in analysis of actual samples. Limits of detection and quantification were not evaluated as this method is only applicable to active agent assays, not impurities etc. In any case, if an active ingredient falls below 90% label strength, a value well within the linear range of the method, it fails the assay.

3.3. Precision (repeatability and reproducibility)

Method precision was investigated by the analysis of six separately prepared samples of the same batch of syrup. The repeatability (within-run precision) was evaluated by one operator within

Table 4
Accuracy (recovery) study results

Percentage of target concentration*	CP% recovery	EP% recovery	CLP% recovery
80	100.96 (0.25)	101.92 (0.62)	101.95 (0.65)
100	100.82 (0.34)	101.93 (0.28)	99.99 (0.21)
120	99.87 (0.44)	101.26 (0.55)	100.58 (0.79)

*, Hundred percent of target concentration is equivalent to 400 μg/ml of codeine phosphate, 200 μg/ml of ephedrine HCl and 40 μg/ml of chlorpheniramine maleate. The figures in brackets represent %R.S.D. values for three replicates.

a day, whilst reproducibility (between-run precision) was evaluated by different operators on 2 separate days. The results obtained are shown in Table 3. In all instances the accepted criteria of %R.S.D. of less than 2% was met.

Precision of the system (injector repeatability) was evaluated by injecting a freshly prepared standard solution ten times. The %R.S.D. results obtained were 0.08, 0.16 and 0.41 for CP, EP and CLP, respectively, all well below the accepted maximum of 1%.

3.4. Accuracy

Accuracy of the method was studied by recovery investigation as described in Section 2.5. The results of this investigation are shown in Table 4. For all three analytes at the different concentration levels evaluated the recovery values meet the acceptance criteria of 100 ± 2%. In addition, these results provide the working range for the method. The method can accurately determine CP levels between 320 and 480 μg/ml, EP levels between 160 and 240 μg/ml, and CLP levels between 32 and 40 μg/ml.

3.5. Selectivity

Selectivity of the method was demonstrated by the fact that the UV spectra obtained for each analyte peak in the sample solutions were matching with the corresponding standard analyte peaks. There appeared to be no interference from formulation excipients or other impurities present.

3.6. Robustness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different solvent sources were used in conjunction with a different HPLC system. Stability studies of standard and sample solutions found them to be stable for at least 2 days if stored in a refrigerator at about 5 °C.

4. Conclusion

The present paper describes a highly precise, convenient, rapid and accurate method for the simultaneous quantification of CP, EP and CLP by RP-HPLC with UV detection. Certainly this method is not the first to describe such multi-component analysis, but it is believed to be advantageous in its ease of usage due to minimal sample and standard preparation, as well as economical due to the small amount of reagents required. With regards to the methodologies referenced in the introduction of this paper, a number of comparisons can be made. One such method described the use of indirect conductometric detection for the determination of active ingredients in cough cold syrups [7]. Whilst this does describe the simultaneous assay of CP, EP and CLP, the CLP did not elute until around 22 min and resolution of EP from CP appears quite poor in the published chromatograms. Another paper details the quantification of CLP and pseudoephedrine by micellar liquid chromatography [5]. Although this method does provide a similarly short run time with CLP eluted in around 6 min, quantification of CP is not described and

more obscure reagents are required (pentanol, sodium dodecyl sulfate). The presently described method provides successful resolution of all three analytes in less than 7 min, utilizing common laboratory reagents in a simple, easy to follow procedure. Further subsequent improvements to the methodology may still be worthy of investigation, with application to the quantification of other ingredients possibly present in cough–cold syrup; e.g. pseudoephedrine, promethazine, bromhexine and paracetamol.

Following on from development, the method has been successfully applied to the routine analysis of syrup formulation manufactured by a local pharmaceutical company, with consistent percentage label strength values for all three compounds in the 90–110% range as per US Pharmacopoeia requirements.

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