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Short communication

Simultaneous HPLC analysis of pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride in liquid dosage forms

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ABSTRACT

An HPLC method using UV detection is proposed for the simultaneous determination of pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride in liquid formulation. C18 column (250 mm × 4.0 mm) is used as the stationary phase with a mixture of methanol:acetate buffer:acetonitrile (85:5:10, v/v) as the mobile phase. The factors affecting column separation of the analytes were studied. The calibration graphs exhibited a linear concentration range of 0.06–1.0 mg/ml for pseudophedrine hydrochloride, 0.02–1.0 mg/ml for codeine phosphate, and 0.0025–1.0 mg/ml for triprolidine hydrochloride for a sample size of 5 μl with correlation coefficients of better than 0.999 for all active ingredients studied. The results demonstrate that this method is reliable, reproducible and suitable for routine use with analysis time of less than 4 min.

1. Introduction

The combination of pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride is used in pharmaceutical preparations as cough–cold syrup. This combination, however, is not present in USP or BP. In this respect, a method for the analysis of this combination is needed. In the scientific literature, analysis of pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride has been reported as individual ingredients and in combination products. Analytical methods have included gas–liquid chromatography (GLC) [1], UV [2–6], thin layer chromatography (TLC) [7], and HPLC [8–26]. Codeine phosphate in combination with other compounds has been determined in different pharmaceutical preparations by GLC [1], TLC [7], UV [2–3], and HPLC [8–10,14,16–18,20–26]. Pseudophedrine hydrochloride in combination with other compounds has been determined in different pharmaceutical preparations by HPLC [11,15,18]. Pseudophedrine hydrochloride and triprolidine hydrochloride was simultaneously determined in different pharmaceutical dosage forms by HPLC [12–13]. Pseudophedrine hydrochloride and codeine phosphate was also determined simultaneously by HPLC [19]. All of the above mentioned methods, however, have not been employed for the simultaneous determination of pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride in any pharmaceutical formulation. In this work, we introduce an HPLC method for simultaneous determination of this combination in liquid formulation. Validation of the current method will be performed according to the requirements of USP for assay determination which include accuracy, precision, selectivity, linearity and range.

2. Experimental

2.1. Equipments and setting

The system used was a Merck Hitachi HPLC (Hitachi, Ltd. Tokyo, Japan) equipped with manual loop injector and connected to a variable diode array wavelength detector and recorder. A C18 reversed-phase column (250 mm × 4.0 mm i.d) bonded onto 5 μm silica gel manufactured by Merck was used for the analysis. The flow rate was 1.5 mL min^{-1}, wavelength was 254 nm; and the injection volume was 5 μL.

2.2. Reagents

All chemicals were of analytical grade and used without further purification. Water was distilled and deionised by passing through water purification system. Acetonitrile and methanol HPLC grade are from J.T. Baker (NJ, USA). Ammonium acetate is from Merck (Darmstadt, Germany). The active ingredients pseudophedrine HCl, codeine phosphate, triprolidine HCl, and the excipients used in manufacturing the liquid syrup

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2.3. Standards and sample preparation

The mobile phase used after optimisation was a mixture of methanol:acetate buffer:acetonitrile with a ratio of (85:5:10, v/v), respectively. A buffer solution (pH = 6.9) was prepared by dissolving 20.0 g ammonium acetate in 200.0 mL distilled water. The mobile phase was filtered using 0.45 μm microporous polyamide filters and was degassed with the aid of a sonnicator (Fisher Scientific, FS220).

Standard solution of the three active ingredients of the drug was prepared in the following manner: 150 mg of pseudophedrin hydrochloride and 50 mg of codeine phosphate were dissolved in 50 mL methanol (solution 1). 12.5 mg of triprolidine hydrochloride was dissolved in 50 mL methanol (solution 2) 10 mL of the first solution, and 5 mL of the second solution were diluted to 50 mL with methanol.

Sample solution of the drug was prepared by diluting 5.0 mL of the syrup to 50.0 mL with methanol, to get a concentration equal to the concentration of the analytes in the standard solution, i.e. 0.6 mg mL^{-1} of pseudophedrine hydrochloride, 0.2 mg mL^{-1} of codeine phosphate, and 0.025 mg mL^{-1} of triprolidine hydrochloride. The solutions were filtered through a 0.45-μm membrane before use.

2.4. Solutions for validation study

2.4.1. Linearity and range

Stock standard of pseudophedrine hydrochloride with a concentration of 1.0 mg mL^{-1} was prepared by dissolving 100 mg of pseudophedrine hydrochloride in 100 mL of methanol. Nine different concentrations of pseudophedrine hydrochloride was prepared in the following manner: 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 4.0, 6.0, and 8.0 mL of the stock standard was diluted to 10 mL with methanol to get the following concentrations: 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.4, 0.6, and 0.8 mg mL^{-1} of pseudophedrine hydrochloride. The same procedure was repeated for codeine phosphate and triprolidine hydrochloride.

2.4.2. Accuracy (recovery)

For recovery study, 100 mL of simulated syrup was prepared by dissolving 600 mg pseudophedrine hydrochloride, 200 mg codeine phosphate, and 25 mg of triprolidine hydrochloride in the required excipients of the drug formulation. A tenfold dilution was performed, and 10 μL were injected into the column. The peak areas resulting were compared with that of the standard.

The standard of the three active ingredients (prepared in Section 2.3) was used for precision study, while the sample of the syrup (also prepared in Section 2.3) was used for intermediate-precision and selectivity studies.

3. Results and discussion

3.1. Method development

Reversed-phase LC-method was employed in the current work for the separation of these three analytes. To this end, reversed-phase C8 and C18 columns using a mixture of organic solvents (acetonitrile, and methanol) and aqueous buffer as a mobile phase were tested. While C8 column does not show enough resolution between these analytes, C18 column shows adequate resolution (resolution factor is 2.0 between pseudophedrine hydrochloride and codeine phosphate, and 2.5 between codeine phosphate and

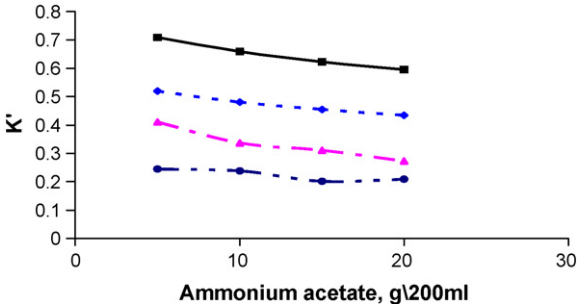

Fig. 1. Plot of the capacity factor, k', versus concentration of ammonium acetate (g/200 mL water) in the mobile phase. (■) Triprolidine hydrochloride, (♦) codeine phosphate, (▲) pseudophedrine hydrochloride, (●) solvent.

triprolidine hydrochloride). In order to optimise the chromatographic parameters, the effect of changing the composition of mobile phase on the capacity factor (k') was studied. Results have shown that the capacity factors of the three analytes were decreased with increasing percentage of methanol and acetonitrile in the mobile phase (data not shown), which is expected for reversed-phase LC mode. The capacity factors were also decreased with increasing the concentration of ammonium acetate in the buffer (Fig. 1) as the peaks of the three analytes become sharper with lower retention times and consequently lower capacity factors. Therefore, the selection of the concentration of ammonium acetate in buffer and the composition of mobile phase was based on providing good baseline, adequate separation, and sharp peaks in reasonable time. A typical chromatogram of standard solution containing each of the components listed above is shown in Fig. 2.

3.2. Method validation

After method development, validation of the current method was performed in accordance with USP requirements for assay determination (Category-I: analytical methods for quantitation of active ingredients in finished pharmaceutical products) which include accuracy, precision, selectivity, linearity and range.

3.2.1. Linearity and range

To evaluate linearity of the method, ten different concentrations of the three analytes in the range of 0.06–1.00 mg mL^{-1}

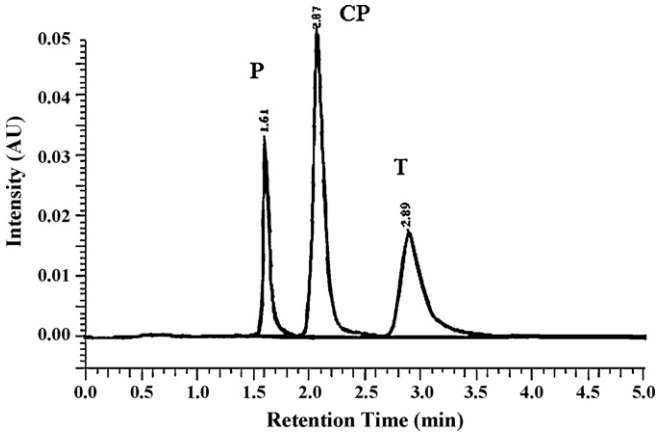

Fig. 2. A typical chromatogram of pharmaceutical combination contains 0.3 mg mL^{-1} pseudophedrine hydrochloride, 0.1 mg mL^{-1} codeine phosphate, and 0.0125 mg mL^{-1} triprolidine hydrochloride. Column: C18 (25 cm × 4.0 mm). Mobile phase: methanol:acetate buffer pH 6.9:acetonitrile (85:5:10, v/v). Flow rate: 1.5 mL min^{-1}, wavelength: 254 nm. P: pseudophedrine hydrochloride, CP: codeine phosphate, T: triprolidine hydrochloride.

Table 1
Intermediate-precision of the method (% of the three active ingredients during 6 days).

Day	Pseudophedrine hydrochloride (%[a])	Codeine phosphate (%[a])	Triprolidine hydrochloride (%[a])
1	103 ± 0.4	97.2 ± 0.6	96.3 ± 2.0
2	101 ± 1.3	97.5 ± 1.5	96.5 ± 1.9
3	102 ± 1.1	98.5 ± 0.9	97.5 ± 1.7
4	101 ± 0.9	97.1 ± 1.2	96.8 ± 1.8
5	101 ± 1.5	96.8 ± 1.9	97.7 ± 1.2
6	102 ± 0.9	97.5 ± 1.3	97.2 ± 1.5

[a] Mean ± R.S.D. for five samples.

for pseudophedrine hydrochloride, 0.02–1.0 mg mL^{-1} for codeine phosphate and 0.0025–1.0 mg mL^{-1} of triprolidine hydrochloride were analysed and the linearity between the peak area and the concentration was examined for each analyte. The results obtained show that the linearity range is 0.06–1.0 mg mL^{-1} for pseudophedrine hydrochloride with a correlation coefficient of 0.9996, 0.02–1.0 mg mL^{-1} for codeine phosphate with a correlation coefficient of 0.9997, and 0.0025–1.0 mg mL^{-1} for triprolidine hydrochloride with a correlation coefficient of 0.9993.

3.2.2. Accuracy (recovery)

Percentage recovery of the three active ingredients using this method was determined using the simulated syrup sample which prepared in experimental part. Results have shown that the mean recovery is 99.8%, 99.4%, and 98.6% for pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride, respectively, and the R.S.D. for 6 samples is lower than 1.0%.

3.2.3. Precision

Precision of this method was determined by injecting the standard solution of the three analytes six times. The R.S.D. of peak area of six replicates was found to be less than 1.0%.

Intermediate-precision of the method was also evaluated by analyzing five samples of the three analytes at different days (6 days). Results which are represented in Table 1 show good intermediate-precision of the method (average percentage of pseudophedrine hydrochloride for the 6 days is 102% with a R.S.D. of 0.7%, while it is 97.5% for codeine phosphate with a R.S.D. of 0.6%, and 97.0% for triprolidine hydrochloride with a R.S.D. of 0.6%).

3.2.4. Selectivity

Selectivity of the current method was demonstrated by good separation of the three active ingredients (pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride). Furthermore, matrix components, e.g. excipients, do not interfere with the three analytes as they have no absorbance.

4. Conclusion

This method represents a fast analytical procedure for the simultaneous quantitation of pseudoephedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride. The sample preparation is simple, the analysis time is short and the elution is isocratic. The method is amenable to the analysis of large numbers of samples with excellent precision and accuracy.

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