HPLC Method for Simultaneous Determination of Dextromethorphan Hydrobromide, Chlorpheniramine Maleate and Potassium Sorbate in Cough Syrup

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Abstract. The development and validation of an analytical method for the simultaneous determination of Dextromethorphan Hydrobromide, Chlorpheniramine Maleate, and Potassium Sorbate in cough syrup using the High-Performance Liquid Chromatography (HPLC) method have been conducted. The determination was carried out on the simulation sample consisting of three substances and prepared five different concentrations, with three replications for each concentration. The optimum HPLC conditions were obtained using the C18 column, with UV detection at 230 nm. The mobile phase of acetonitrile & phosphate buffer pH 2.5 (containing sodium-heptane sulfonate as ion-pair), with a gradient elution system, at a flow rate of 1 mL/minute, while the flow rate column temperature is set at 35°C. The results showed that the method has met the parameters set in the validation test with the recovery ranged from 98% - 102%, the precision test results (RSD) < 2%, and the linearity (r) ≥ 0.98. The method obtained is quite selective by confirmed identity with the maximum wavelength scanning of each analyte peak by PDA detector. The limit of detection (LoD) for Dextromethorphan-HBr, Chlorpheniramine maleate and Potassium sorbate were 3.13 µg/mL, 0.94 µg/mL and 0.12 µg/mL respectively and the limit of quantitation (LoQ) were 9.49 µg/mL, 2.85 µg/mL and 0.38 µg/mL respectively.

1. Introduction
Dextromethorphan-HBr (DTM) is chemically (+)-3-methoxy-17-methylmorphinan hydrobromide. The molecular formula is C_{18}H_{25}NO.HBr and molecular weight is 370.3 g/mol. DTM is an antitussive cough medicine used to suppress coughs caused by irritation of the throat and bronchial airways. Chlorpheniramine maleate (CTM) is chemically 3-(4-Chlorophenyl)-N,N-dimethyl-3-pyridine-2-ylpropan-1-aminium (2Z)-3-carboxyprop-2-enolate. The molecular formula is C_{16}H_{19}ClN_{2}.C_{4}H_{4}O_{4}, and the molecular weight is 390.87 g/mol. CTM is an antihistamine that functions to treat hypersensitivity reactions. Potassium sorbate (PS) is chemically Potassium (2E,4E)-Hexa-2,4-dienoate. The molecular formula is C_{6}H_{7}KO_{2}, and the molecular weight is 150.22 g/mol [1]. PS acts as a medicinal preservative, namely as an anti-bacterial and antifungal. The use of this preservative...
must be regulated and controlled so that it does not cause harm such as poisoning and long-term use is a carcinogen [2]

Many methods have been described in the literature to determine DTM, CTM, and PS individually and in combination [3-7]. However, no HPLC method was reported for the simultaneous determination of these drugs in combined dosage forms. Fixed-dose combination containing DTM (5.0 mg), CTM (2.0 mg), and PS (10.0 mg) in the cough syrup and the HPLC method was validated following ICH Guidelines [8-9]. This study aimed to analyze the three substances simultaneously under the same conditions to simplify and shorten analysis time.

2. Methodology

2.1. Material and Reagents

Pure DTM from USP reference standard, pure CTM from Suriya Life Science, and PS from Celanese, Germany [10]. The cough syrup sample simulation consists DTM (5.0 mg), CTM (2.0 mg) & PS (10.0 mg) dissolved in sample matrix cough syrup. The reagents used were HPLC grade, KH2PO4, a reagent for ion pair chromatography (sodium 1-heptane sulfonate) from TCI, High purity aqua DM was prepared by using Millipore. Mobile phase and working solutions were filtered through 0.45 µm and degassed using a sonicator before use.

2.2. Apparatus

An HPLC system (Shimadzu LC-20AD), with LC solutions data handling system (Shimadzu LC Solution) and an autosampler (SIL-20AC), was used for the analysis. The detector consists of a photodiode array (SPD-M20A)). The column used was C-18, Inertsil ODS3, (15 cm x 4.6 mm i.d. x 5 µm) with UV detection at 230 nm.

2.3. Preparation & Standard stock solution

A combined standard stock solution containing DMP, CTM & PS were prepared by 50.0 mg, 20.0 mg, and 10 mg, respectively. All of them are transferred to a 50.0 ml volumetric flask and add the mixed solution of phosphate buffer pH 2.5: methanol (90:10 v/v) and sonicated in a bath sonicator for 15 minutes. After sonication, the volume was made up to the mark 50 mL with the same mixed solution. A solution was made five times dilutions for the working standard to obtain the final concentrations of DTM, CTM, and PS 200, 80, and 40 µg/mL, respectively.

2.4. Preparation sample solution

First, we weigh 200.0 mg DTM and 80.0 mg CTM in a 50 mL volumetric flask, then dissolved in a phosphate buffer: methanol mixture (90:10 v/v) up to the limit marked (solution A) and weighed separately 400 mg KS in a 50 mL volumetric flask then dissolved in the same mixed solution to the limit marked (solution B). We weighed 10 x density cough syrup matrix into 5 of 50mL volumetric flasks to make a simulation sample solution. Subsequently added 7, 9, 10, 11 and 13 mL solution A and 4, 9, 10, 11, 13 mL solution B, respectively. Then diluted with a mixed solution of phosphate buffer pH 2.5: methanol (90:10 v/v) and after sonicated for 15 minutes, volume was made up to 50 mL by the same mixed solution. For PS determination, it is necessary to make a 10x dilution of each simulated solution.
3. Results and Discussion

3.1 Method Development

Firstly, we tested several mobile phase compositions for effective separation of dextromethorphan hydrobromide, chlorpheniramine maleate, and potassium sorbate. The mobile phase containing acetonitrile and phosphate buffer pH 2.5 (containing sodium-heptane sulfonate as ion-pair) (5:95, v/v) with gradient elution system up to 30% acetonitrile in 15 minute. The flow rate of 1 mL/min, the injection volume of 20 μL, the column temperature 35°C, and a detection wavelength of 230 nm afforded the best separation of these analytes. (see Figure 1 and Table 1)

3.2. Validation Method

3.2.1. Linearity, Limit of Detection, and Limit of Quantification

To evaluate the linearity of the method, different concentrations of the three analytes in the range of 140.0–260.0 μg/mL for DTM, 56.0–104.0 μg/mL for CTM, 16.0–52.0 μg/mL for PS were analyzed, and the linearity between the peak area and the concentration was examined for each analyte. The results obtained show that the current method is linear for the three analytes in the range specified above with a correlation coefficient of better than 0.99, see Table 2.

![Figure 1. Chromatogram of Dextromethorphan HBr (DTM), Chlorpheniramine maleate (CTM) and Potassium Sorbate (PS) in cough syrup formulation](image)

**Table 1.** Chromatographic parameters of the separated analytes

| Analyte                  | Retention time (minute) | Resolution | Asymmetry |
|--------------------------|-------------------------|------------|-----------|
| Dextromethorphan HBr     | 25.622                  | 9.76       | 1.20      |
| Chlorpheniramine maleate | 22.557                  | 5.37       | 1.09      |
| Potassium Sorbate        | 20.982                  | 31.12      | 1.06      |
3.2.2 Accuracy (% Recovery)

The method’s accuracy was studied by preparing a sample matrix of the syrup cough formulation according to the formulation procedure. The required quantity of placebo, a known amount of the three active ingredients (DTM, CTM & PS) with the same proportion as in the cough syrup formulation, was added to get simulated drug formulation. Results have shown that the assay’s mean recovery is (100.64 ± 0.63)% for three ingredients, and the RSD is lower than 1.0%, see Table 3.

| Analyte                  | Equation                  | Coef. Correl (r) | LoD (µg/mL) | LoQ (µg/mL) |
|--------------------------|---------------------------|------------------|-------------|-------------|
| Dextromethorphan HBr     | Y = 19091X + 105144       | 0.9998           | 3.13        | 9.49        |
| Chlorpheniramine maleate | Y = 24693X + 17506        | 0.9999           | 0.94        | 2.85        |
| Potassium Sorbate        | Y = 42941X + 17782        | 1.0000           | 0.12        | 0.38        |

3.2.3 Precision

This method’s precision was evaluated by calculating the RSD of five simulation samples with different concentration levels and three replicates for each concentration. The result show %RSD ranged is (0.05 – 0.16)%, (0.02 – 0.26)% and (0.01 – 0.20)% for DTM, CTM and PS respectively. It was found to be less than RSD repeatability by Horwitz. See Table 3. These results show that the current method is repeatable.

| Analyte                  | % Recovery (n = 5) | % RSD (n = 5) |
|--------------------------|-------------------|---------------|
| Dextromethorphan HBr     | 101.3 ± 0.7       | 0.10          |
| Chlorpheniramine maleate | 100.1 ± 0.3       | 0.13          |
| Potassium Sorbate        | 100.5 ± 0.9       | 0.11          |

3.2.4 Selectivity

Analytical selectivity relates to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior. On the other hand, an analytical approach is selective if only one component can be determined independently from all the different elements that give no analytical signal (9-10). The current method’s selectivity was demonstrated by the excellent separation of the three analytes. (See Figure 1 and confirmed by scanning the wavelength for each analyte as shown in the Figure. 2, 3, and 4). The method’s selectivity is strengthened in the absence of other compounds’ coelution, which is indicated by the value of the purity angle less than the purity threshold and the resolution value greater than 1.5 as shown in Table 4.
Table 4. Comparing purity Angle vs. Purity Threshold

| Analyte          | Resolution | Purity Angle | Purity Threshold |
|------------------|------------|--------------|------------------|
| DTM standard     | 11.11      | 0.205        | 0.232            |
| DTM in sample    | 4.39       | 1.261        | 1.522            |
| CTM standard     | 5.50       | 0.211        | 0.226            |
| CTM in sample    | 5.78       | 0.190        | 0.566            |
| PS standard      | 39.64      | 0.065        | 0.250            |
| PS in sample     | 11.43      | 0.110        | 0.249            |

Figure 2. Dextromethorphan HBr spectrum

Figure 3. Chlorpheniramine maleate spectrum
3.2.5 Robustness

The robustness test of the analytical method measures its capacity to remain unaffected by small but deliberate method parameters and indicates its reliability during normal usage. Robustness of the current approach was investigated by analyzing samples of the cough syrup using the same chromatographic conditions specified in method development but with a small change in the following chromatographic parameters: (a) pH of phosphate buffer: 2.0 and 3.0 instead of 2.5, (b) detection wavelength: 228 nm and 232 nm instead of 230 nm, (c) column temperature: 30°C and 40°C instead of 35°C. All variation parameters three-time repeatedly. RSD of dextromethorphan HBr, chlorpheniramine maleate, and potassium sorbate assay under these conditions is calculated to be less than 1%, except phosphate buffer pH 2, which is obtained > 2% (see Table 5).

**Table 5. Robustness testing**

| Parameter                  | Assay                  |                 |                 |
|----------------------------|------------------------|-----------------|-----------------|
|                            | Dextromethorphan HBr   | Chlorpheniramine maleate | Potassium sorbate |
| pH phosphate buffer        | 2.0                    | 100.91 ± 0.07   | 88.44 ± 4.58    | 99.91 ± 0.92   |
|                            | 2.5                    | 100.73 ± 0.06   | 98.92 ± 0.17    | 99.83 ± 0.11   |
|                            | 3.0                    | 100.56 ± 0.05   | 98.28 ± 0.07    | 99.63 ± 0.13   |
| Wavelength (nm)            | 228                    | 100.01 ± 0.16   | 98.94 ± 0.10    | 100.05 ± 0.10  |
|                            | 230                    | 100.91 ± 0.06   | 99.03 ± 0.05    | 100.18 ± 0.02  |
|                            | 232                    | 100.32 ± 0.12   | 99.00 ± 0.05    | 100.35 ± 0.25  |
| Column temperature (°C)    | 30                     | 99.08 ± 0.22    | 98.72 ± 0.18    | 99.95 ± 0.14   |
|                            | 35                     | 100.73 ± 0.18   | 98.92 ± 0.02    | 100.26 ± 0.14  |
|                            | 40                     | 99.45 ± 0.15    | 98.37 ± 0.16    | 100.27 ± 0.15  |
4. Conclusion

A simple, accurate, precise HPLC method is developed and validated for simultaneous determination of dextromethorphan-HBr, chlorpheniramine maleate, and potassium sorbate simultaneously in cough syrup formulations as long as the pH more than 2.

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