Simultaneous Assay of Dexchlorpheniramine Maleate, Betamethasone, and Sodium Benzoate in Syrup by a Reliable and Robust HPLC Method

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The simultaneous determination of betamethasone, dexchlorpheniramine maleate, and sodium benzoate in pharmaceutical syrup was done by using a simple validated HPLC method. The chromatographic separation of the three analytes was done in a C18 column maintained at 25°C, using a mixture of acetonitrile and 0.02 M phosphate buffer solution pH 2.70 (35:65, v:v) as mobile phase. The isocratic elution was chosen with total flow rate of mobile phase maintained at 1.0 mL per minute. The analytes were detected by a UV-Vis detector set at 254 nm. Injection volume was set at 50 μL. The method was fully validated in terms of specificity, linearity, precision, accuracy, and robustness according to requirements of current guidelines and was proven to be suitable for the intended application.

1. Introduction

Betamethasone is a synthetic glucocorticoid possessing anti-inflammatory [1] and antiallergic properties [2]. It works by affecting the synthesis of prostaglandin [3] and leukotriene [4]. Dexchlorpheniramine, usually used in form of maleate salt, is the pharmacologically active dextrorotatory enantiomer of chlorpheniramine [5], an antihistamine working on H1 receptors to reduce the allergic reactions [6]. Because betamethasone and dexchlorpheniramine produce their pharmacologic effects through different mechanisms, they can be used in combination in different dosage forms like tablets, oral solutions, or syrup to enhance the resulting therapeutic efficacy.

Besides the active principles, many pharmaceutical dosage forms also contain excipients for many purposes, including enhancing the efficacy of active principles (by ameliorating their solubility, by slowing down their deterioration, etc.) and assuring the efficiency and safety of the dosage form by prohibiting the development of micro-organisms. These agents must be present in the pharmaceutical dosage forms at a proper level during the shelf life of these products to ensure their effectiveness. Therefore, the assay of solubility enhancers or antimicrobial preservatives, particularly the latter, is usually necessary in specification of pharmaceutical preparations, besides the assay of active principles.

Sodium benzoate can be used for both the above-mentioned purposes: it can increase the solubility of active principle [7], and it can also be used as antimicrobial preservative [8, 9] to inhibit the development of micro-organism. So, the assay of sodium benzoate is required in these cases for quality control purpose.

Betamethasone and dexchlorpheniramine maleate were determined separately in bulk active compound and pharmaceutical dosage forms by HPLC in C18 column [10–13]. Betamethasone and dexchlorpheniramine maleate were simultaneously determined by UV-Vis spectrometry [14] and high-performance thin-layer chromatography [15]. The assay of sodium benzoate can be done by volumetric titration [7]. However, for the simultaneous assay of sodium benzoate and other analytes, HPLC in C18 column was the most common choice of analytical technique [8, 9, 16]. Up to now, no method has yet been published for simultaneous
determination of betamethasone, dexchlorpheniramine maleate, and sodium benzoate in pharmaceutical dosage forms.

To assure the reliability of analytical results, any method intended for the assay of active principle(s) and other components, such as preservative(s), in pharmaceutical dosage forms must be able to satisfy suitable performance levels, such as those required by AOAC International for quantitative method [17] and must be able to provide obligated validation data to authorities according to guidelines on analytical method validation, such as those issued by ICH [18] or FDA [19].

In the current guideline “Validation of Analytical Procedures: Text and Methodology Q2 (R1)” of ICH, assay tests for drug substances and drug product must be validated in terms of specificity, precision, accuracy, linearity, and range [18]. The validation of an analytical procedure ensures the reliability and reproducibility of results obtained from the applied analytical technique and the particular analytical conditions of the method. The validation report of all analytical methods must be submitted to the regulation agency as an integral part of the technical document for the registration of pharmaceuticals for human use.

In this study, an HPLC method using C18 column was developed and validated for simultaneous assay of betamethasone, dexchlorpheniramine maleate, and sodium benzoate in syrup.

2. Materials and Methods

2.1. Instrumentation. The method was developed and validated on a Shimadzu LC-20AT HPLC system (Shimadzu, Kyoto, Japan) consisting of a pump (model LC-20AD), a degasser (model DGU-20A5), a PDA detector (model SPD-M20A), an autosampler (model SIL-20AChT), and a control module (model CBM-20A1ite). This system used LC solution software version 1.25 SP4 for data processing and evaluation. Analytical column was a Luna C18 column (250 × 4.6 mm, 5 μm) of Phenomenex (Torrance, CA, USA).

2.2. Chemicals and Reagents. Reference substances of betamethasone (purity 100.4%) and sodium benzoate (purity 98.6%) were established at National Institute of Drug Quality Control (Hanoi, Vietnam); reference substance of dexchlorpheniramine maleate (purity 99.9%) was purchased from Institute of Drug Quality Control of Ho Chi Minh City (Ho Chi Minh City, Vietnam). Xinfadro syrup (containing 3.0 mg of betamethasone, 24.0 mg of dexchlorpheniramine maleate, and 120.0 mg of sodium benzoate per 60 mL of syrup) was purchased from market. A placebo mixture consisting of citric acid, sodium citrate, vanillin, sorbitol, ethanol, refined sugar, and water was prepared from the information provided in the label of syrup bottle to be used in method validation steps. Acetonitrile HPLC grade, methanol HPLC grade, orthophosphoric acid PA grade, potassium dihydrogen phosphate PA grade, and triethylamine PA grade were purchased from Merck Vietnam (Ho Chi Minh City, Vietnam).

2.3. Chromatographic Conditions. Mobile phase was a mixture of acetonitrile and 0.02 M phosphate buffer solution pH 2.7 (35:65, v:v). The 0.02 M phosphate buffer solution pH 2.7 was prepared by dissolving 2.72 g of potassium dihydrogen phosphate and 3 mL of triethylamine in 900 mL of water, adjusting the pH to 2.7 ± 0.1 by orthophosphoric acid, adding water to make 1000 mL, mixing the solution well, filtering it through 0.45 μm membrane filter, and degassing it by sonication for 15 minutes before using it. The flow rate of mobile phase was maintained at 1.0 mL/min. The analysis was carried out on an Shimadzu LC-20AT series HPLC system equipped with a PDA detector set at 254 nm for recording chromatograms. The chromatographic separation was conducted on a Luna C18 column (250 × 4.6 mm, 5 μm) maintained at 25°C. The injection volume was 50 μL.

2.4. Preparation of Standard Solution. Stock standard solutions of betamethasone (1.0 mg/mL), dexchlorpheniramine maleate (1.0 mg/mL), and sodium benzoate (5.0 mg/mL) were prepared by dissolving an accurately weighed quantity of corresponding reference standards using mobile phase as diluents. Working mixed standard solutions were prepared by accurately diluting stock standard solutions to the intended concentration with the same diluents. Standard solutions were filtered through 0.45 μm membrane filter before being used for chromatographic analysis.

2.5. Preparation of Sample Solution and Placebo Solution. To prepare sample solution, an amount of syrup equivalent to about 0.25 mg of betamethasone was accurately weighed into a 25 mL volumetric flask and was diluted to volume with mobile phase as diluent. This solution was filtered through 0.45 μm membrane filter before being used for chromatographic analysis.

For method validation, placebo solution was prepared by weighing accurately a quantity of placebo mixture (as described in 2.2) equivalent to the amount of syrup used to prepare sample solution and diluted afterward as with the sample solution.

2.6. Method Validation. To assure the suitability of the method for simultaneous assay of dexchlorpheniramine, betamethasone, and sodium benzoate in syrup, it was validated in accordance with the current guideline of ICH [18] in the following criteria.

2.6.1. Specificity. In the case of HPLC method, the specificity is assured by the complete separation of analytes of interest from other components in the sample matrix [16]. To evaluate the capacity of the developed method to yield well-separated peaks corresponding to dexchlorpheniramine, sodium benzoate, and betamethasone, mixed standard solution of these analytes, sample, placebo, and blank solution were injected separately at the same volume (50 μL) into the chromatographic system.
2.6.2. Linearity and Range. According to the guideline of ICH [18], an assay method must maintain linear relation between the concentration of analyte(s) and the intensity of response (i.e., peak area for HPLC method) within a certain range around the target concentration (at least from 80% to 120% of target concentration). In this study, the target concentration was about 10.0 μg/mL for betamethasone, 80.0 μg/mL for dexchlorpheniramine, and 400.0 μg/mL for sodium benzoate. Accordingly, mixed standard solutions containing exact concentrations of betamethasone, dexchlorpheniramine maleate, and sodium benzoate at different levels of betamethasone (6.0, 8.0, 10.0, 12.0, 13.9, and 15.9 μg/mL), dexchlorpheniramine maleate (48.2, 64.3, 80.4, 96.5, 112.6, and 128.6 μg/mL), and sodium benzoate (240.2, 320.3, 400.4, 480.5, 560.6, and 640.6 μg/mL) were prepared, corresponding to 60%, 80%, 100%, 120%, 140%, and 160% of target concentration, respectively. Three injections of mixed standard solution at each concentration were executed and calibration curve for each analyte was established between the standard concentration and average peak area. The significance of the linearity of each calibration curve was assessed by one-way ANOVA (the linearity is significant if P<0.05 in expression of SPSS 16.0 software) [16].

2.6.3. Sensitivity. For HPLC methods, generally, the sensitivity is assessed by measuring the signal-to-noise ratio between the peak height of analyte and the variation of the neighboring baseline in chromatograms. The concentrations of analyte giving a signal-to-noise ratio about 3:1 and about 10:1, respectively, are considered as the lowest detectable level or limit of detection (LOD) and the lowest quantifiable level or limit of quantitation (LOQ) [16, 20]. The LOD and LOQ of betamethasone, dexchlorpheniramine maleate, and sodium benzoate were determined by analyzing solutions containing these substances at different concentrations and measuring the signal-to-noise ratio for each analyte.

2.6.4. Accuracy. According to the current guideline [18] and the previously published work [16], the accuracy for an assay method must be validated by recovery studies at least three concentrations of each analyte within the range from 80% to 120% of target concentration. Therefore, to evaluate the accuracy in quantitative determination of each analyte, exact quantities of reference substances of betamethasone, dexchlorpheniramine maleate, and sodium benzoate were mixed with placebo matrix in such a way that the spiked samples, after preparation process, yielded solutions containing each analyte at three concentration levels, corresponding to 80%, 100%, and 120% of target concentration, i.e., about 0.008, 0.010, and 0.012 mg/mL with betamethasone; 0.064, 0.080, and 0.096 mg/mL with dexchlorpheniramine maleate; and about 0.320, 0.400, and 0.480 mg/mL with sodium benzoate. At each concentration level, three samples were prepared and analyzed to obtain the percentage recovery of each analyte and the RSD for variation of recovery rate at each concentration level.

2.6.5. Precision. The precision of chromatographic system, or system suitability, was validated by estimating the variation of peak performance of each analyte after six repetitive injections of mixed standard solution of dexchlorpheniramine, betamethasone, and sodium benzoate at 100% of target concentrations [16, 18, 20].

The method's precision, including repeatability (intraday precision) and intermediate precision (interday precision), was determined by calculating the variation of quantitative result obtained from six independent analyses of sample solutions containing dexchlorpheniramine, betamethasone, and sodium benzoate at approximately 100% of target concentration on the same day and on two different days, respectively.

2.6.6. Range. Range of concentrations of each analyte where the accuracy and precision of quantitative analysis are assured must be at least from 80% to 120% of the target concentration of each analyte [18, 19]. This requirement was validated simultaneously with the accuracy of the method as mentioned above.

2.6.7. Robustness. The current ICH guideline [18] does not obligate the robustness on validation of assay method but welcomes any attempt to confirm the robustness of an analytical method, particularly a quantitative one. In this study, following small and deliberate changes on HPLC conditions was applied to assess the impact on analytical results:

(i) Flow rate: ±0.2 mL/min

(ii) Percentage of 0.02 M phosphate buffer solution in mobile phase: ±1%

(iii) pH of the 0.02 M phosphate buffer solution: ±0.5 pH units

At each condition, a mixed standard solution of dexchlorpheniramine maleate, sodium benzoate, and betamethasone at 100% of target concentration and three sample solutions at approximately 100% target concentration were prepared and injected into chromatography system. The robustness of the method was verified by investigating the variation in peak area of each analyte in repetitive analysis of standard solution and the variation in the content of each analyte found in sample solutions [16, 20, 21].

2.6.8. Stability of Analytical Solution. Although the standard and sample solutions were analyzed immediately after preparation, there is always a delay time during which these solutions waited to be analyzed in the autosampler tray. Therefore, their stability was investigated by analyzing the standard and sample preparations at 0 h and after one day of cool storage (at 10°C in refrigerator) and at 25°C. For each solution, three injections were executed at each time, and the stability of analytical solutions was evaluated from the variation of average peak area and RSD value of peak area among repeated injections.
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2.7. Data Processing. IBM SPSS software (version 16.0) (IBM, Armonk, NY, USA) was used for statistical analysis of analytical results.

3. Results and Discussion

3.1. Method Development and Optimization. The objective of this method is to provide a chromatographic solution that permits simultaneous assay of the two active principles dexchlorpheniramine maleate and betamethasone and the preservative sodium benzoate in syrup. From the information gathered after our bibliographic research, an analytical column with end-capped octadecylsilyl stationary phase, the Luna C18 column, the stationary phase was selected for method development.

To obtain chromatographic conditions suitable for the intended application of the method, preliminary trials were carried out. The results obtained from these trials were summarized in Table 1. They pointed out that the use of acetonitrile as the organic component in mobile phase would give better peak shape for dexchlorpheniramine maleate and sodium benzoate and give shorter analysis time than methanol when used at the same percentage in mobile phase. Preliminary test also found that phosphate buffer gives better peak shapes for analytes and better resolution between analytes and other matrix components. From these results, Luna C18 column with acetonitrile and 0.02 M phosphate buffer solution pH 2.7 (35:65, v:v) in isocratic elution mode was selected for the final method.

3.2. Method Validation

3.2.1. Specificity. To evaluate the specificity of the method, blank solution, placebo solution, standard solution, and sample solution (containing betamethasone, dexchlorpheniramine maleate, and sodium benzoate at target concentrations, i.e., 0.010 mg/mL, 0.080, and 0.400 mg/mL, respectively) were injected separately into HPLC system, and the chromatogram results are shown in Figures 1(a)–1(c). Betamethasone, dexchlorpheniramine maleate, and sodium benzoate were eluted into 3 well-separated peaks, and purity analysis (Figures 1(d)–1(f)) confirmed that there was no coeluted element at the retention times of any analyte. Therefore, the chromatographic separation was capable of isolating each of the analytes of interest and permitting their specific analysis without interference from other components of the sample matrix.

3.2.2. Linearity. The mean peak area of each analyte obtained from the chromatogram of mixed standard solution was plotted against corresponding concentration to establish the calibration line. The summarized graphs (Figure 2) revealed linearity over the concentration range of 6.0–15.9 μg/mL for betamethasone, of 48.2–128.6 μg/mL for dexchlorpheniramine maleate, and of 240.2–640.6 μg/mL for sodium benzoate. From the regression analysis, the linear equation was obtained: \( y = 88252x - 6294 \) for betamethasone, \( y = 36578x - 8537 \) for dexchlorpheniramine maleate, and \( y = 15438x + 28569 \) for sodium benzoate, and the coefficient of determination \( R^2 \) was 0.999 for all the three analytes. ANOVA analysis for all analytes (Tables 2–4) confirmed the statistical significance of the linear regression model in predicting the outcome variable \( (P < 0.05) \).

3.2.3. Limit of Detection (LOD) and Limit of Quantification (LOQ). For betamethasone, the concentration of injected solution at LOD and LOQ was 2.0 μg/mL and 6.0 μg/mL, equivalent to injected quantity of betamethasone of 0.10 μg and 0.30 μg, respectively. For dexchlorpheniramine maleate, the concentration of injected solution at LOD and LOQ was 16.0 μg/mL and 48.0 μg/mL, equivalent to injected quantity of potassium guaiacolsulfonate of 0.80 μg and 2.40 μg, respectively. For sodium benzoate, the concentration of injected solution at LOD and LOQ was 80.0 μg/mL and 240.0 μg/mL, equivalent to injected quantity of sodium benzoate of 4.00 μg and 12.00 μg, respectively.

3.2.4. Accuracy. The ICH guideline [18] requires that recovery rate for assay method must fall between 98.0% to 102.0% of the true concentration and variation of recovery rate at one concentration level in terms of RSD must not exceed 2.0%. The recovery studies with dexchlorpheniramine, betamethasone, and sodium benzoate, summarized in Table 5, showed recovery rate from 99.8% to 102.0% at all three levels for all analytes and at RSD values at each level for each analyte varying from 0.1 to 0.6%, within the limits recommended by ICH. So, the method was of acceptable accuracy for simultaneous assay of dexchlorpheniramine, betamethasone, and sodium benzoate in syrup.

3.2.5. Precision. The system precision, or system suitability, of the method was revealed by the peak performance for each analyte. For dexchlorpheniramine, betamethasone, and sodium benzoate, the variations of peak properties (repeatability) and interday (intermediate precision), were carried out. Results obtained from these trials in Table 5, showed recovery rate from 99.8% to 102.0% at all three levels for all analytes and at RSD values at each level for each analyte varying from 0.1 to 0.6%, within the limits recommended by ICH. So, the method was of acceptable accuracy for simultaneous assay of dexchlorpheniramine, betamethasone, and sodium benzoate in syrup.

3.2.6. Range. As discussed in Section 3.2.4 and summarized in Table 5, the range from 80% to 120% of target concentration for dexchlorpheniramine, betamethasone, and
Table 1: Results of preliminary optimization.

| Column | Mobile phase | Elution mode | Flow rate | Observation | Result |
|--------|--------------|--------------|-----------|-------------|--------|
| Phenomenex C18 | Methanol—phosphate buffer 0.02 M pH 2.7 (40:60, v:v) | Isocratic | 1.0 mL/min | Poor peak shapes for dexchlorpheniramine maleate and sodium benzoate, retention too long for betamethasone (more than 20 minutes), betamethasone still partially coeluted with a matrix peak | Rejected |
| Phenomenex C18 | Acetonitril—phosphate buffer 0.02 M pH 2.7 (40:60, v:v) | Isocratic | 1.0 mL/min | Better peak shape for dexchlorpheniramine maleate and sodium benzoate, shorter analysis time, but betamethasone still partially coeluted with a matrix peak | Rejected |
| Phenomenex C18 | Acetonitril—acetate buffer 0.025 M pH 3.0 (40:60, v:v) | Isocratic | 1.0 mL/min | Bethamethasone was resolved from matrix peak but peak shape was poor and peak response unstable | Rejected |
| Phenomenex C18 | Acetonitril—phosphate buffer 0.02 M pH 2.7 (35:65, v:v) | Isocratic | 1.0 mL/min | Good peak shape for all three analytes and all three analytes were completely separated from matrix components | Accepted |

Figure 1: Continued.
Figure 1: Chromatogram of mix standard solution (a), Xifapro sample solution (b), placebo (c) and peak purity of analytes (peak of dexchlorpheniramine (d), peak of sodium benzoate (e) and peak of betamethasone (f)). 1, peak of dexchlorpheniramine, 2, peak of sodium benzoate, 3, peak of betamethasone.

Figure 2: Calibration curves of dexchlorpheniramine maleate (a), sodium benzoate (b), and betamethasone (c).

Table 2: Results of ANOVA analysis for calibration curve of dexchlorpheniramine maleate.

| Model     | Sum of squares | df | Mean square | F       | Sig. |
|-----------|----------------|----|-------------|---------|------|
| Regression| 6.054E12       | 1  | 6.054E12    | 4.306E4 | 000a |
| Residual  | 5.624E8        | 4  | 1.406E8     |         |      |
| Total     | 6.055E12       | 5  |             |         |      |

*aPredictors (constant), dexchlorpheniramine_concentration. bDependent variable, dexchlorpheniramine_peak_area.
Table 3: Results of ANOVA analysis for calibration curve of sodium benzoate.

| Model       | Sum of squares | df | Mean square | F       | Sig. |
|-------------|----------------|----|-------------|---------|------|
| Regression  | 2.675E13       | 1  | 2.675E13    | 3.571E4 | 000a |
| Residual    | 2.996E9        | 4  | 7.490E8     |         |      |
| Total       | 2.675E13       | 5  |             |         |      |

*Predictors (constant), sodium_benzoate_concentration. bDependent variable, sodium_benzoate_peak_area.

Table 4: Results of ANOVA analysis for calibration curve of betamethasone.

| Model       | Sum of squares | df | Mean square | F       | Sig. |
|-------------|----------------|----|-------------|---------|------|
| Regression  | 5.408E11       | 1  | 5.408E11    | 1.014E4 | 000a |
| Residual    | 2.134E8        | 4  | 5.335E7     |         |      |
| Total       | 5.411E11       | 5  |             |         |      |

*Predictors (constant), betamethasone_concentration. bDependent variable, betamethasone_peak_area.

Table 5: Results of accuracy.

| Spiked level (%) | Replicate number | Spiked amount of standard (mg) | Peak area (mAU·s) | Recovery (%) | Spiked amount of standard (mg) | Peak area (mAU·s) | Recovery (%) | Spiked amount of standard (mg) | Peak area (mAU·s) | Recovery (%) |
|------------------|-------------------|-------------------------------|-------------------|--------------|-------------------------------|-------------------|--------------|-------------------------------|-------------------|--------------|
| 80%              | 1                 | 1.606                         | 2326791           | 99.7         | 7.895                         | 5013454           | 101.6        | 0.199                         | 695365           | 100.5        |
|                  | 2                 | 1.606                         | 2334539           | 100.0        | 7.895                         | 4991733           | 101.2        | 0.199                         | 703190           | 101.6        |
|                  | 3                 | 1.606                         | 2327665           | 99.7         | 7.895                         | 5017325           | 101.7        | 0.199                         | 696949           | 100.7        |
| Mean             | RSD (%)           |                               |                  |              |                               |                   |              |                               |                   |              |
| 80%              |                   |                               | 99.8             | 0.2          |                               | 3.0               | 0.6          |                               |                   |              |
| 100%             | 1                 | 2.008                         | 2890627           | 99.1         | 9.869                         | 6225259           | 101.0        | 0.249                         | 867571           | 100.3        |
|                  | 2                 | 2.008                         | 2899677           | 99.4         | 9.869                         | 6238642           | 101.2        | 0.249                         | 871281           | 100.8        |
|                  | 3                 | 2.008                         | 2885840           | 98.9         | 9.869                         | 6216278           | 100.8        | 0.249                         | 863282           | 99.8         |
| Mean             | RSD (%)           |                               | 99.1             | 0.2          |                               | 1.0               | 0.6          |                               |                   | 100.3        |
| 120%             | 1                 | 2.409                         | 3480064           | 99.4         | 11.843                        | 7478069           | 101.1        | 0.298                         | 1048690          | 101.1        |
|                  | 2                 | 2.409                         | 3469911           | 99.1         | 11.843                        | 7470749           | 101.0        | 0.298                         | 1047360          | 100.9        |
|                  | 3                 | 2.409                         | 3476467           | 99.3         | 11.843                        | 7477084           | 101.1        | 0.298                         | 1047448          | 100.9        |
| Mean             | RSD (%)           |                               | 99.3             | 0.1          |                               | 1.0               | 0.1          |                               |                   | 1.0          |

Table 6: Results of system precision for betamethasone and dexchlorpheniramine maleate.

| No. of injection | Retention time (minutes) | Peak area (mAU·s) | Asymmetry of peak | Number of theoretical plates | Resolution |
|------------------|--------------------------|-------------------|-------------------|-------------------------------|------------|
| **Dexchlorpheniramine maleate** |                         |                   |                   |                               |            |
| 1                | 4.721                    | 2919244           | 1.3               | 3962                          | 5.2        |
| 2                | 4.708                    | 2920606           | 1.3               | 3940                          | 5.2        |
| 3                | 4.703                    | 2922790           | 1.3               | 3932                          | 5.2        |
| 4                | 4.689                    | 2915735           | 1.3               | 3909                          | 5.2        |
| 5                | 4.689                    | 2913275           | 1.3               | 3909                          | 5.2        |
| 6                | 4.689                    | 2910823           | 1.3               | 3909                          | 5.2        |
| Average          | 4.709                    | 2917079           | 1.3               | 3927                          | 5.2        |
| RSD (%)          | 0.3                      | 0.2               | 0.1               | 0.6                           | 0.3        |
| **Sodium benzoate** |                         |                   |                   |                               |            |
| 1                | 9.410                    | 6172807           | 1.3               | 2891                          | 9.4        |
| 2                | 9.413                    | 6173718           | 1.3               | 2893                          | 9.4        |
| 3                | 9.415                    | 6171398           | 1.3               | 2894                          | 9.4        |
| 4                | 9.397                    | 6162984           | 1.3               | 2883                          | 9.4        |
| 5                | 9.386                    | 6161866           | 1.3               | 2877                          | 9.4        |
| 6                | 9.377                    | 6150875           | 1.3               | 2871                          | 9.4        |
| Average          | 9.400                    | 6165608           | 1.3               | 2885                          | 9.4        |
| RSD (%)          | 0.2                      | 0.1               | 0.1               | 0.3                           | 0.2        |
| No. of injection | Retention time (minutes) | Peak area (mAu·s) | Asymmetry of peak | Number of theoretical plates | Resolution |
|------------------|--------------------------|-------------------|-------------------|----------------------------|------------|
| 1                | 17.917                   | 863391            | 1.1               | 8025                       | 11.3       |
| 2                | 17.895                   | 868096            | 1.1               | 8006                       | 11.3       |
| 3                | 17.869                   | 868453            | 1.1               | 7983                       | 11.3       |
| 4                | 17.818                   | 860990            | 1.1               | 7937                       | 11.2       |
| 5                | 17.787                   | 864537            | 1.1               | 7909                       | 11.2       |
| 6                | 17.757                   | 863288            | 1.1               | 7883                       | 11.2       |
| Average          | 17.841                   | 864793            | 1.1               | 7957                       | 11.3       |
| RSD (%)          |                          | 0.4               | 0.3               | 0.7                        | 0.6        |

Table 7: Results of repeatability and intermediate precision.

| No. of sample solution | Sample weight (g) | Density: 1.095 g/mL | Content of dexchlorpheniramine maleate in syrup (%, comparing to labeled amount) | Content of sodium benzoate in syrup (%, comparing to labeled amount) | Content of betamethasone in syrup (%, comparing to labeled amount) |
|------------------------|-------------------|----------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------------------|
| Day 1, analyst 1       |                   |                      |                                                                                  |                                                                 |                                                                |
| 1                      | 5.4324            | 101.0                | 101.2                                                                           | 101.5                                                          | 101.3                                                           |
| 2                      | 5.4630            | 100.4                | 100.5                                                                           | 101.6                                                          | 101.6                                                           |
| 3                      | 5.3981            | 100.7                | 101.1                                                                           | 99.2                                                           | 99.8                                                            |
| 4                      | 5.5087            | 99.0                 | 99.2                                                                             | 99.2                                                           | 99.8                                                            |
| 5                      | 5.4672            | 100.0                | 100.2                                                                           | 99.5                                                           | 100.2                                                           |
| 6                      | 5.5064            | 98.6                 | 98.8                                                                             | 99.5                                                           | 99.5                                                            |
| Average (1–6)          |                   | 99.9                 | 100.2                                                                           | 99.5                                                           | 100.7                                                           |
| RSD (%) (1–6)          |                   |                      | 1.0                                                                              | 0.9                                                            |                                                                  |
| Day 2, analyst 2       |                   |                      |                                                                                  |                                                                 |                                                                |
| 7                      | 5.5623            | 99.6                 | 101.2                                                                           | 101.5                                                          | 101.3                                                           |
| 8                      | 5.4786            | 101.4                | 100.5                                                                           | 101.6                                                          | 101.6                                                           |
| 9                      | 5.5368            | 100.2                | 101.1                                                                           | 99.8                                                           | 99.8                                                            |
| 10                     | 5.4361            | 101.7                | 99.2                                                                             | 99.8                                                           |                                                                  |
| 11                     | 5.5698            | 100.1                | 100.2                                                                           | 99.5                                                           | 100.2                                                           |
| 12                     | 5.5345            | 100.1                | 98.8                                                                             | 99.5                                                           |                                                                  |
| Average (1–12)         |                   | 100.2                | 99.5                                                                             | 101.2                                                          |                                                                  |
| RSD (%) (1–12)         |                   |                      | 0.9                                                                              | 1.1                                                            | 1.0                                                             |

Results obtained in day 1 by analyst 1 (sample no. 1–6) were used for evaluating repeatability and those obtained in day 1 and day 2 (sample no. 1–12) were used together for evaluating intermediate precision.

Table 8: Results of robustness.

| Variation                        | Specific condition | Dexchlorpheniramine maleate | Sodium benzoate | Betamethasone |
|----------------------------------|--------------------|------------------------------|-----------------|---------------|
|                                  |                    | RSD (%) for peak area        | RSD (%) for content in syrup | RSD (%) for peak area | RSD (%) for content in syrup | RSD (%) for peak area | RSD (%) for content in syrup |
| Flow rate (mL/min)               | 0.8                | 0.1                          | 0.4             | 0.1           | 0.4             | 0.3             | 0.4             |
|                                  | 1.0 (normal)       | 0.2                          | 0.6             | 0.2           | 0.6             | 0.4             | 0.5             |
|                                  | 1.2                | 0.4                          | 0.4             | 0.4           | 0.4             | 0.3             | 0.8             |
| pH of 0.02 M phosphate buffer solution | 2.65               | 0.2                          | 0.6             | 0.2           | 0.6             | 0.2             | 0.6             |
|                                  | 2.70 (normal)      | 0.1                          | 0.5             | 0.3           | 0.9             | 0.4             | 0.3             |
|                                  | 2.75               | 0.3                          | 0.3             | 0.5           | 0.9             | 0.4             | 0.5             |
| Percentage of 0.02 M phosphate buffer in mobile phase | 64                 | 0.1                          | 0.5             | 0.2           | 0.9             | 0.2             | 0.5             |
|                                  | 65 (normal)        | 0.1                          | 0.6             | 0.3           | 0.5             | 0.3             | 0.7             |
|                                  | 66                 | 0.1                          | 0.4             | 0.2           | 1.1             | 0.5             | 0.4             |
| Wavelength of detector          | 252 nm             | 0.5                          | 0.4             | 0.2           | 0.5             | 0.2             | 0.5             |
|                                  | 254 nm (normal)    | 0.1                          | 0.3             | 0.3           | 0.5             | 0.3             | 0.4             |
|                                  | 256 nm             | 1.0                          | 0.4             | 0.4           | 0.9             | 0.2             | 0.5             |
sodium benzoate assured the accuracy and precision of assay results for these analytes.

3.2.7. Robustness. After implementing deliberate minor changes as mentioned in Section 2.6.7, the peak area and assay results for each analyte obtained at each modified condition were presented in Table 8. For all applied changes, variation of peak area for all analytes was small (RSD less than 2.0%) and good separation was achieved for each analyte. The contents of betamethasone, dexchlorpheniramine maleate, and sodium benzoate found in sample were not varied significantly when any change described in Section 2.6.7 was implemented, as one-way ANOVA analysis found $F < F_{\text{crit}}$ for both analytes (as shown in Table 9).

Table 9: Results of ANOVA analysis for content of betamethasone, dexchlorpheniramine maleate and sodium benzoate.

|                      | Sum of squares | df  | Mean square | $F$  | Sig  |
|----------------------|----------------|-----|-------------|------|------|
| Dexchlorpheniramine content |                |     |             |      |      |
| Between groups       | 976            | 11  | 089         | 460  | 910  |
| Within groups        | 4,633          | 24  | 193         |      |      |
| Total                | 5,610          | 35  |             |      |      |
| Sodium benzoate content |              |     |             |      |      |
| Between groups       | 2,014          | 11  | 183         | 317  | 974  |
| Within groups        | 13,853         | 24  | 577         |      |      |
| Total                | 15,868         | 35  |             |      |      |
| Betamethasone content |                |     |             |      |      |
| Between groups       | 1,123          | 11  | 102         | 403  | 941  |
| Within groups        | 6,073          | 24  | 253         |      |      |
| Total                | 7,196          | 35  |             |      |      |

Table 10: Results of stability studies.

| Studies               | Average retention time (minutes) | Average peak area (mAu.s) | Average asymmetry of peak | Average number of theoretical plate | RSD (%) of peak area | Recovery (%) |
|-----------------------|----------------------------------|---------------------------|---------------------------|-------------------------------------|----------------------|--------------|
| Dexchlorphenamine maleate |                                 |                           |                           |                                     |                      |              |
| Standard solution     |                                 |                           |                           |                                     |                      |              |
| 0 h                   | 4.698                            | 2921785                   | 1.3                       | 3924                                | 0.1                  | —            |
| 24 h at refrigerator  | 4.702                            | 2917643                   | 1.3                       | 3930                                | 0.1                  | 99.9         |
| 24 h at 25°C          | 4.691                            | 2914786                   | 1.3                       | 3912                                | 0.2                  | 99.8         |
| Sample solution       |                                 |                           |                           |                                     |                      |              |
| 0 h                   | 4.705                            | 2917613                   | 1.3                       | 3935                                | 0.2                  | —            |
| 24 h at refrigerator  | 4.695                            | 2914534                   | 1.3                       | 3919                                | 0.1                  | 99.9         |
| 24 h at 25°C          | 4.701                            | 2912677                   | 1.3                       | 3929                                | 0.4                  | 99.8         |
| Sodium benzoate       |                                 |                           |                           |                                     |                      |              |
| Standard solution     |                                 |                           |                           |                                     |                      |              |
| 0 h                   | 9.403                            | 6169304                   | 1.3                       | 2887                                | 0.1                  | —            |
| 24 h at refrigerator  | 9.391                            | 6167025                   | 1.3                       | 2880                                | 0.1                  | 100.0        |
| 24 h at 25°C          | 9.415                            | 6149723                   | 1.3                       | 2894                                | 0.1                  | 99.7         |
| Sample solution       |                                 |                           |                           |                                     |                      |              |
| 0 h                   | 9.411                            | 6170542                   | 1.3                       | 2892                                | 0.2                  | —            |
| 24 h at refrigerator  | 9.396                            | 6165339                   | 1.3                       | 2883                                | 0.1                  | 99.9         |
| 24 h at 25°C          | 9.409                            | 6148732                   | 1.3                       | 2891                                | 0.2                  | 99.6         |
| Betamethasone         |                                 |                           |                           |                                     |                      |              |
| Standard solution     |                                 |                           |                           |                                     |                      |              |
| 0 h                   | 17.807                           | 862754                    | 1.1                       | 7927                                | 0.1                  | —            |
| 24 h at refrigerator  | 17.789                           | 861859                    | 1.1                       | 7911                                | 0.1                  | 99.9         |
| 24 h at 25°C          | 17.814                           | 860934                    | 1.1                       | 7933                                | 0.3                  | 99.8         |
| Sample solution       |                                 |                           |                           |                                     |                      |              |
| 0 h                   | 17.792                           | 861055                    | 1.1                       | 7914                                | 0.1                  | —            |
| 24 h at refrigerator  | 17.801                           | 859823                    | 1.1                       | 7922                                | 0.2                  | 99.9         |
| 24 h at 25°C          | 17.797                           | 858506                    | 1.1                       | 7918                                | 0.2                  | 99.7         |
3.2.8. Solution Stability. The percentage of recovery was within the range of 98.0% to 102.0% and RSD was not more than 2.0%, indicating a good stability of the sample and standard solutions for 24 hr at both conditions, as shown in Table 10. These results proved that both analytes were stable in sample and standard solutions prepared as described in 2.4 and 2.5, and the preparation procedure for sample and standard solution was suitable for intended application of the method.

4. Conclusion

In this paper, the development and validation of an HPLC method for simultaneous assay of dexchlorpheniramine maleate, sodium benzoate, and betamethasone in syrup have been discussed. The method has been proven to be able to quantify these three analytes specifically, without interference from sample matrix. The reliability and robustness of the method were also assured by validation results, demonstrating its suitability for intended application.

Data Availability

The data used to support the findings of this study are available from corresponding author (hoalethiuong@gmail.com) upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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