VALIDATION OF HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PSEUDOEPHEDRINE HCl, GUAIIFENESIN, CHLORPHENIRAMINE MALEATE AND DEXTROMETHORPHAN HBr

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ABSTRACT
Objectives: Pseudoephedrine HCl, Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan HBr combination is a common combination cough syrup. Many validated methods are available for the determination of each compound alone and in combination with other drugs. The local pharmaceutical industry used to analyze such combination in individual assessment which is efforts and time consuming. The objective of this study is to validate a method for simultaneous determinations of the four compounds in one single injection.

Methods: HPLC method had been develop using detector at 210 nm, column C18 4.6 mm × 250 mm, 3µm and mobile phase of Potassium dihydrogen orthophosphate, acetonitrile, orthophosphoric acid, triethanolamine and water. The column oven temperature is 40°C, flow rate 0.8 ml/min and 60 minutes run time. The method had been validated according to the ICH guidelines with respect to method specificity, linearity and range, precision, accuracy and robustness. Limit of detection, quantitation limit and solution stability had been assessed.

Results: The average retention times the 4 compounds are 5.5, 1, 15.85, 50.44 minutes. The RSD% is less than 1%, the correlation coefficient r2, M, and coefficient ration parameters. The method is qualified for two analysts in two different apparatus in two days above 20. The Method showed an appropriate linearity having correlation coefficient r2 0.9996 – 0.9998. The RSD% of results for two analysts in two different apparatus in two days was less than 2. The test solution is stable for 48 hours.

Conclusion: The method is simple and fulfilled all acceptable criteria for all validation parameters. The method is qualified enough to be used for routine analysis of products containing the four components.

Key words: Chemical method validation, chlorpheniramine, chromatographic system validation, dextromethorphan, guaifenesin, pseudoephedrine.

INTRODUCTION
Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical application. As per the ICH guidelines, the validation process of the method includes the specificity, linearity and range, precision, accuracy, solution stability, assay of pharmaceutical product and robustness.

Compounds structural formula: Pseudoephedrine is a systemic decongestant, Guaifenesin is used as an expectorant and to liquefy the bronchial secretion, Chlorpheniramine is used for symptomatic relief of allergy, and Dextromethorphan is a cough suppressant. The USP HPLC method for its individual assay uses water/ methanol/glacial acetic acid as mobile phase, 4.6 mm×250 mm column packed with Li1 10µm, 276 nm detector and 2ml/min rate flow. The retention time is 7 min. The USP method for assay of solution three or more of Acetaminophen, Chlorpheniramine Maleate, Dextromethorphan HBr and Pseudoephedrine HCl uses menthol/ water, monobasic potassium phosphate, triethylamine, sodium lauryl sulphate and phosphoric acid as mobile phase. Column 4.6 mm×150 mm, L11, 214 nm detector and...
2m/min flow rate. Many studies to assay Guaifenesin alone and in combination of other drugs had been done using Spectrophotometric methods HPLC methods and volumetric methods. The separation and determination of pseudoephedrine, dextromethorphan, diphenhydramine and chlorpheniramine in cold medicines had been done using Non-aqueous Capillary electrophoresis. A HPLC method for simultaneous determination of the four compound plus pyrilamine and pheniramine had been performs using Kromasil C18 column, mobile phase of methanol and dihydrogen phosphate at pH 3 and wavelength 220 nm, run time of 13 minutes had been achieved. The objective is to validate a method for quantitative determination of Pseudoephedrine HCL, Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan HBr simultaneously in one single HPLC injection.

\[\text{Figure 1: The structural formulas of the compounds.}\]

\[\text{Figure 2: Chromatogram for system suitability.}\]

**MATERIALS AND METHODS**

Purified water, Blue Nile Research Centre, Sudan.

Potassium Dihydrogen Orthophosphate and Acetonitrile HPLC grade, Sharlau Chemie, Spain.

Triethanolamine 99.8% AR, Chem lab NV; Belgium.

Orthophosphoric acid 88% Luba Chemie.

Chlorpheniramine Maleate, Guaifenesin, Dextromethorphan hydrobromide and Pseudoephedrine working standards and test samples.

High Performance Liquid Chromatography, Prominee – LC 2030, Shimadzu, Japan.

Software Lab solution, Shimadzu, Japan.

Column: insert Sustain C18; 4.6 mmx 250 mm; 3 µm. Electronic Balance AY 220, Schimadzu. pH meter Mi 150; Hanna instruments, Romania. Rocking Shaker SK-330-pro, USA.

Chromatographic System

Column: insert Sustain C18; 4.6 mmx 250 mm; 3 µm. Flow rate: 0.8 ml/min. Wavelength 210 nm. Detector: PDA. Oven temperature: 40C. Injection volume: 20 µL. Run time: 60 min.

Preparation of 0.2 M Potassium dihydrogen orthophosphate: dissolve 27.218 gram in 700 ml water and complete to 1000 ml.

Preparation of mobile phase: to 550 ml of 0.2 M Potassium dihydrogen Orthophosphate in a 1 litre volumetric flask add 200 ml of Acetonitrile, 30 ml of 10% Orthophosphoric acid and 1 ml Triethanolamine 99.8%. Dilute to volume by water and adjust the pH to 3 with orthophosphoric acid or Sodium hydroxide.

Preparation of diluent: use the mobile phase as a diluent. Preparation of the Storage: 100 mg Guaifenesin, 30 mg Pseudoephedrine HCL, 10 mg Dextromethorphan and 2 mg Chlorpheniramine maleate working standards into 100 ml volumetric flask, add 60 diluent, shake and sonicate for 5 minutes, cool and make up to volume with diluent. Mix well, transfer to 10 ml to 50 ml volumetric flask make up to volume with the diluent, mix and filter using 0.45 µL nylon syringe filter. Preparation of the Sample: Transfer 2 ml of the sample of specific gravity 1.2779 g/cm³= 2.5558 grams to 100 ml volumetric flask, add 60 ml diluent, shake well for 10 minutes, make up to volume with diluent, filter using0.45 µL nylon syringe filter.

Procedure

Equilibrate the column with mobile phase for sufficient time until stable baseline is obtained. Separately inject equal volumes 20µL of the standard preparation and the assay preparation into the chromatographic system, record the chromatogram and measure the areas of the major peaks. Inject the blank once, the standard solution for 6 replicates and the sample preparation in triplicates. The tailing factor for each peak should not be more than 2 and the RSD should not be more than 2.
Table 1: Results of the method Precision.

| 6 replicates | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|--------------|-----------------|-------------|------------------|------------------|
| Average RT   | 5.5 mins        | 12.63 mins  | 15.85            | 50.44            |
| RSD%         | 0.07            | 0.05        | 0.08             | 0.07             |
| Average Area | 2850535.33      | 11585256.33 | 201544.17        | 936327           |
| RSD%         | 0.04            | 0.04        | 0.19             | 0.05             |
| Plates       | 46780           | 72286.83    | 79354            | 81109.17         |
| Tailing factor | 1.38        | 1.27        | 1.28             | 1.23             |
| Peaks resolution | -          | 20.47      | 5.6              | 28.65            |

Figure 3: The peak purity without interference of Placebo and excipients.

Calculate the quantity in percentage by the formula:

\[
\frac{R_u/R_s \times C \times (100/W_u) \times D \times P/100 \times 1/L \times 100}{L}
\]

Where, \( D \) is the density in mg/ml, \( W_u \) is the weight in mg of the sample taken, \( R_u \) and \( R_s \) are the peak areas responses from the assay preparation and the standard preparation respectively, \( P \) is the potency of tested API in % and \( L \) is the labeled quantity.

**Steps on Method Validation**

1. Develop a validation protocol or operating procedure for the validation.
2. Define the application, purpose, and scope of the method.
3. Define the performance parameters and acceptance criteria.
4. Define validation experiments.
5. Verify relevant performance characteristics of equipment.
6. Qualify materials (e.g., standards and reagents).
7. Perform pre-validation experiments.
8. Adjust method parameters or/and acceptance criteria if necessary.
9. Perform full internal (and external) validation experiments.
10. Develop SOPs for executing the method in the routine.
11. Define criteria for revalidation.
12. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine.
13. Document validation experiments and results in the validation report.

Table 2: Levels of concentration of Standard µg/ml.

| Conc. Level | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|-------------|-----------------|-------------|------------------|------------------|
| 1-5%        | 3 µg/ml         | 10 µg/ml    | 0.2 µg/ml        | 1 µg/ml          |
| 2-10%       | 6 µg/ml         | 20 µg/ml    | 0.4 µg/ml        | 2 µg/ml          |
| 3-25%       | 15 µg/ml        | 50 µg/ml    | 1 µg/ml          | 5 µg/ml          |
| 4-50%       | 30 µg/ml        | 100 µg/ml   | 2 µg/ml          | 10 µg/ml         |
| 5-75%       | 45 µg/ml        | 150 µg/ml   | 3 µg/ml          | 15 µg/ml         |
| 6-100%      | 60 µg/ml        | 200 µg/ml   | 4 µg/ml          | 20 µg/ml         |
| 7-125%      | 75 µg/ml        | 250 µg/ml   | 5 µg/ml          | 25 µg/ml         |
| 8-150%      | 90 µg/ml        | 300 µg/ml   | 6 µg/ml          | 30 µg/ml         |
| 9-175%      | 105 µg/ml       | 350 µg/ml   | 7 µg/ml          | 35 µg/ml         |
| 10-200%     | 120 µg/ml       | 400 µg/ml   | 8 µg/ml          | 40 µg/ml         |

RESULTS AND DISCUSSION

**Precision**

The Table 1 presents the average of 6 injection of the standard. The RSD% for the retention times and he peaks areas of all substances is less than 1%, the theoretical plates is more than 2000, the tailing factors are not more than 2 and the resolution between the peaks is more than 2. Thus complying, the precision acceptance criteria.

**Specificity**

Using placebo suspension in the same weight and way of the sample test, following the same procedure, no
interference from the placebo was observed at the retention time of the drugs peaks (Figure 3). Peak purity demonstrates that the observed chromatographic peak is attributed to a single component that the excipients were not interfering with the component peaks at the specific retention time. The acceptance criteria for the peak purity are to be attributed to 90-100% purity.

### Table 3: Peak area and RSD% for linearity.

| Level | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|-------|-----------------|-------------|------------------|------------------|
|       | Area            | RSD%        | Area             | RSD%             |
| 1     | 164023.3        | 0.08        | 709131           | 0.24             | 15970.67 | 0.4  | 52153.33 | 0.89 |
| 2     | 302652.3        | 0.08        | 1305768          | 0.06             | 22415.67 | 0.54 | 88761.67 | 0.38 |
| 3     | 720954.7        | 0.04        | 3096022          | 0.03             | 54007    | 0.42 | 228668.7 | 0.25 |
| 4     | 1488262         | 0.15        | 6191429          | 0.10             | 106605   | 0.38 | 480969.3 | 0.77 |
| 5     | 2153761         | 0.19        | 8860252          | 0.31             | 162422   | 0.46 | 704803.7 | 0.53 |
| 6     | 2853314         | 0.57        | 11555520         | 0.64             | 218128   | 0.92 | 938008   | 0.82 |
| 7     | 3512556         | 0.03        | 14304351         | 0.02             | 267495.3 | 0.33 | 1159430  | 0.2  |
| 8     | 4250768         | 0.88        | 17240602         | 0.35             | 324816   | 1.0  | 1402909  | 0.83 |
| 9     | 4882828         | 0.04        | 19679804         | 0.04             | 371301   | 0.16 | 1613694.7| 0.13 |
| 10    | 5535872         | 0.24        | 22624204         | 0.26             | 427313   | 0.33 | 1888020  | 0.6  |

### Table 4: Linearity Results.

| Parameter                  | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|----------------------------|-----------------|-------------|------------------|------------------|
| Correlation Coefficient r² | 0.9998          | 0.9996      | 0.9997           | 0.9997           |
| Slope                      | 46098.9590      | 55897.1449  | 53117.76         | 46687.1513       |
| y- intercept               | 56476.2818      | 337530.7956| 2636.4341        | 1366.97          |
| Regression line equation   | Y = 46098.959x  | Y = 55897.1449x | Y = 53117.76x + | Y = 46687.15x + |
|                           | +56476.2818     | +337530.7956| 2636.4341        | 1366.97          |

### Table 5: Results for Accuracy.

| Conc | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|------|-----------------|-------------|------------------|------------------|
|      | % Mean recovery | RSD%        | % Mean recovery  | RSD%             |
| 50%  | 100.85%         | 0.11%       | 100.94           | 0.01             | 100.74 | 0.07   | 99.71  | 0.56 |
| 100% | 100.85%         | 0.11%       | 99.43            | 0.16             | 100.41 | 0.16   | 100.21 | 0.18 |
| 150% | 100.83%         | 0.06%       | 99.39            | 0.06             | 100.73 | 0.48   | 100.12 | 0.19 |

Figure 4: Peak Purity of Pseudoephedrine.  
Figure 5: Peak Purity of Guaifenesin.  
Figure 6: Peak Purity of Chlorpheniramine.  
Figure 7: Peak Purity of Dextromethorphan.
The Peak for Pseudoephedrine is detected at 5.154 min, for Guaifenesin 12.615 min, for Chlorpheniramine 15.83 min and for Dextromethorphan 50.362 min giving rise to peak purity 99.16%, 92.2%, 94.95% and 96.28% as shown in figures 4,5,6,7 respectively.

The acceptance criteria for the correlation Coefficient $r^2$ should be $\geq 0.999$ for the range of concentration 75 – 125% of the target concentration. Thus, the method comply the requirement for linearity.

### Range

The data obtained from the accuracy studies may be used to assess the range of the method. 50% to 150% of the target concentration is utilized.

| Variable | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|----------|-----------------|-------------|-------------------|------------------|
| 35°C     | 5.27            | 2899525     | 50442             | 1.32             |
| RSD%     | 0.01            | 0.08        | 0.2               | 0.4              |
| 40°C     | 5.19            | 2910973     | 50395             | 1.36             |
| RSD%     | 0.25            | 0.39        | 0.55              | 0.19             |
| 45°C     | 5.1             | 2897807     | 49702             | 1.42             |
| RSD%     | 0.00            | 0.08        | 0.2               | 0.4              |

| Variable | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|----------|-----------------|-------------|-------------------|------------------|
| 35°C     | 17              | 1838365     | 88614             | 1.25             |
| RSD%     | 0.04            | 0.62        | 0.13              | 0.23             |
| 40°C     | 17.51           | 190058      | 88982             | 1.25             |
| RSD%     | 0.4             | 1.02        | 0.42              | 0.14             |
| 45°C     | 17.88           | 189603      | 88894             | 1.24             |
| RSD%     | 0.14            | 0.49        | 0.43              | 0.12             |

| Variable | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|----------|-----------------|-------------|-------------------|------------------|
| 35°C     | 5.27            | 3165558     | 51109             | 1.35             |
| RSD%     | 0.07            | 0.3         | 0.55              | 0.0              |
| 40°C     | 5.19            | 2818701     | 50945             | 1.35             |
| RSD%     | 0.07            | 0.29        | 0.55              | 0.04             |
| 45°C     | 5.2             | 2614346     | 50723             | 1.35             |
| RSD%     | 0.07            | 0.27        | 0.56              | 0.04             |

| Variable | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|----------|-----------------|-------------|-------------------|------------------|
| 208 nm   | 17.66           | 208296      | 88796             | 1.25             |
| RSD%     | 0.13            | 0.53        | 0.19              | 0.09             |
| 210 nm   | 17.66           | 189909      | 88911             | 1.25             |
| RSD%     | 0.13            | 0.29        | 0.2               | 0.09             |
| 112 nm   | 17.66           | 175248      | 89032             | 1.25             |
| RSD%     | 0.13            | 0.4         | 0.19              | 0.05             |

| Column temp | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|-------------|-----------------|-------------|-------------------|------------------|
| 208 nm      | 5.2             | 12.8        | 21                | 17.7             |
| 210 nm      | 5.22            | 12.7        | 21.3              | 17.6             |
| 212 nm      | 5.2             | 12.8        | 21.5              | 17.7             |

The Peak for Pseudoephedrine is detected at 5.154 min, for Guaifenesin 12.615 min, for Chlorpheniramine 15.83 min and for Dextromethorphan 50.362 min giving rise to peak purity 99.16%, 92.2%, 94.95% and 96.28% as shown in figures 4,5,6,7 respectively.

The acceptance criteria for the correlation Coefficient $r^2$ should be $\geq 0.999$ for the range of concentration 75 – 125% of the target concentration. Thus, the method comply the requirement for linearity.
Table 11: Results of robustness on change of flow rate.

| Variable | Pseudoephedrine | Guaifenesin |
|----------|-----------------|-------------|
|          | Mean RT min    | Mean area   | Theoretical plates | Tailing factor | Mean RT min | Mean area   | Theoretical plates | Tailing factor |
| 0.7 ml/min | 5.89 | 3294494 | 55188 | 1.32 | 14.43 | 13380124 | 84056 | 1.24 |
| 0.8 ml/min | 5.23 | 2908409 | 51315 | 1.35 | 12.8 | 11810239 | 7808 | 1.25 |
| 0.9 ml/min | 4.66 | 2582571 | 45811 | 1.42 | 11.42 | 10478534 | 73876 | 1.26 |

| Variable | Chlorpheniramine | Dextromethorphan |
|----------|-----------------|-----------------|
|          | Mean RT min    | Mean area   | Theoretical plates | Tailing factor | Mean RT min | Mean area   | Theoretical plates | Tailing factor |
| 0.7 ml/min | 19.86 | 213070 | 93639 | 1.24 | 57.9 | 1067617 | 90142 | 1.2 |
| 0.8 ml/min | 17.71 | 189127 | 89044 | 1.25 | 51.6 | 939955 | 86973 | 1.2 |
| 0.9 ml/min | 15.75 | 167279 | 85353 | 1.24 | 4.2 | 832535 | 86286 | 1.22 |

Table 12: Resolution of peaks in changing the rate flow.

| Flow rate | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|-----------|-----------------|-------------|-----------------|-----------------|
|           | RT Resolution  | RT Resolution | RT Resolution  | RT Resolution  |
| 0.7 ml/min | 5.9  | -   | 14.4 | 22.1 | 19.9 | 9.2  | 57.9 | 28.6 |
| 0.8 ml/min | 5.2  | -   | 12.8 | 21.4 | 17.7 | 9    | 51.6 | 28  |
| 0.9 ml/min | 5.2  | -   | 12.8 | 20.5 | 17.7 | 8.8  | 51.5 | 27.9 |

Table 13: The average and RSD% of peaks for solution stability.

| Parameter          | Pseudoephedrine | Guaifenesin | Chlorpheniramine | Dextromethorphan |
|--------------------|-----------------|-------------|-----------------|-----------------|
| Mean peaks areas   | 2879033         | 11675642    | 187949          | 98897           |
| RSD%               | 0.12            | 0.19        | 0.15            | 0.48            |

Limit of detection DL and limit of quantitation QL

\[
DL = 3.3 \times MRSE \\
QL = 10x MRSE
\]

MRSE = Mean Root Square Error, DL µg/ml: 2.67, 10, 0.15, 0.86 for Pseudoephedrine, Guaifenesin, Chlorpheniramine, Dextromethorphan respectively. QL µg/ml: 8.08, 31.14, 0.47, 2.6

Accuracy

According to the ICH guide lines Q2 the accuracy is assessed using three replicates of each of the concentrations 50%, 100% and 150% were analyzed for theoretical values, RSD and percent recovery. Since the acceptance criteria is that the measured recovery should be 95%–105%, so the method comply the requirement for accuracy

Precision

Repeatability

10 replicates of the sample were used and the mean, stand deviation and relative standard deviation were obtained. The FDA and ICH stated that the RSD should be ±1% for the drug substance and ±2% for the drug product. Thus, the method fulfilled the repeatability criterion.
Intermediate Precision
Intermediate precision within laboratory variations had been demonstrated by two analysts, using two HPLC systems on different days and evaluating the relative percent purity data across the two HPLC systems at three concentration levels; 50%, 100% and 150%. The following results were obtained: S1A and S1B is the RSD% of concentration 50% for analysts A and B. S2A and S2B is the RDS% of concentration 100% for analysts A and B. S3A and S3B is the RSD% of concentration 150% for analysts A and B. Two different systems at two different days technique were used. S2a + S2b are 0.52, 0.27, 0.09, and 0.17 for the four compounds respectively. S3a +S3b are 0.97, 1.0, 0.34, and 0.21 for the four compounds respectively.

Since the acceptance criterion for intermediate precision is that the results obtained by two analysts using two instruments at different days should have statistical RSD≤2%, thus the method comply the acceptable criteria20.

Robustness
Effect of change in column temperature
Acceptance Criteria for Robustness
1. The number of the theoretical plates should be less than 2000.
2. The tailing factor for compounds should not be more than 2.0.
3. The RSD% of the peaks areas of the replicates of either the standard solution or the compounds should not be more than 2.0%.
4. The resolution between the peaks of the compounds should be ≥ 2.0.

The method fulfilled the acceptance criteria as the number of the theoretical plates in all variables is more than 2000, the RSD% of the retention time and peaks area are less than 2.0%, the tailing factor for all peaks of the different variables are less than 2.0 and the resolution between the peaks is more than 2.0.

Thus, the method satisfied the requirements for robustness on changing the column temperature, on changing the detective wavelength and on changing the flow rate.

Solution Stability
The test had been carried out by initial testing then after preservation of the test solution for 6 hours, 12 hours, 18 hours, 24 hours and 48 hours. The RSD% for the peaks areas of all compounds is less than 2%, therefore, the standard preparation is stable for 48 hours at room temperature.

CONCLUSION
The analytical method used for determination of Pseudoephedrine HCL, Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan HBr in syrup as four-in-one was found to be consistent and precise and in conformance with the acceptable criteria of validation parameters of specificity, system suitability, linearity and range, precision, accuracy, reproducibility and robustness. The method is fully validated and can be used in routine testing for simultaneous determination of such combination products.

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AUTHOR’S CONTRIBUTION
The manuscript was carried out, written, and approved in collaboration with all authors.

CONFLICT OF INTEREST
No conflict of interest associated with this work.

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