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Research Article

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# DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE DETERMINATION OF GUAIPHENESIN AND CHLORPHENIRAMINE MALEATE IN SYNTHETIC MIXTURE

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## ABSTRACT

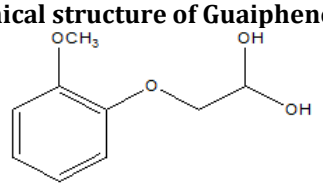
A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of guaiphenesin and chlorpheniramine maleate in synthetic mixture. A reversed-phase C-18 column (250 mm × 4.6 mm i.d., particle size 5 µm) column with mobile phase consisting of acetonitrile and phosphate buffer 20:80 (v/v) (pH 3 ±0.02, adjusted with orthophosphoric acid) was used. The flow rate was 1.0 ml/ min and effluents were monitored at 267 nm. The retention times of guaiphenesin and chlorpheniramine maleate were found to be 7.801 min and 4.047 min, respectively. The method was validated in terms of linearity, range, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The linearity for both the drugs was found in the range of 2-32 µg/ml. The % recoveries of guaiphenesin and chlorpheniramine maleate were found to be between 100.13 ±0.09 % and 100.42 ±0.40 %. The proposed method was successfully applied to the estimation of guaiphenesin and chlorpheniramine maleate in synthetic mixture.

**Keywords:** Chlorpheniramine maleate; Guaiphenesin; Simultaneous estimation; RP-HPLC.

## INTRODUCTION

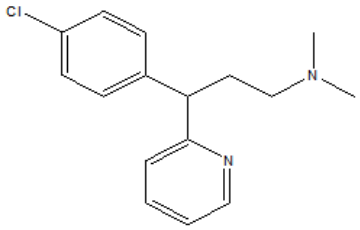
Guaiphenesin (GPN) is chemically 3-(2-methoxyphenoxy)propane-1,2-diol (Figure **1**) is a well know expectorant drug[1]. It is official in Indian Pharmacopoeia[2], British Pharmacopoeia[3], United State Pharmacopoeia[4], European Pharmacopoeia[5] and Japanese Pharmacopoeia[6].

**Figure 1. Chemical structure of Guaiphenesin (GPN)**




Literature survey reveals HPLC[7] and UV spectrophotometry[8] methods for estimation of GPN in single dosage form. Literature survey also reveals UV spectrophotometry[9] and HPLC[10] methods for determination of GPN with other drugs in combination. Chlorpheniramine Maleate (CPM) is chemically [3-(4-chlorophenyl)-3-(pyridin-2yl) propyl]dimethylamine (Figure **2**) is a well know antitussive class of drug[11]. Chlorpheniramine Maleate (CPM) is official in Indian Pharmacopoeia[12], British Pharmacopoeia[13], United State Pharmacopoeia[14], European Pharmacopoeia[15] and Japanese Pharmacopoeia[16]. Literature survey reveals UV spectrophotometry[17] methods for determination of CPM in single dosage form. Literature survey also reveals HPLC[18-19], UV spectrophotometry[20] method for the determination of CPM with other drugs in combination. Therefore, the present work was aimed to develop and validate a new RP- HPLC method for simultaneous estimation of GPN and CPM in synthetic mixture.

**Figure 2. Chemical structure of Chlorpheniramine Maleate (CPM)**




## MATERIALS AND METHODS

### Chemicals and Reagents

Reference standards of GPN and CPM were procured as gift samples from Camper Healthcare, Ganpat Vidhyanagar, Kherva, Mehsana, Gujarat. HPLC grade acetonitrile and water were obtained from Finar Chemicals Ltd., Mumbai, India. Potassium dihydrogen orthophosphate AR grade, orthophosphoric acid AR grade and triethylamine AR grade were procured from Central Drug House (P) Limited, New Delhi, India.

### Instrumentation

HPLC instrument equipped with a UV-visible detector and a photodiode array detector, (Shimadzu, LC-2010 $C_{HT,}$ Japan), auto sampler, Thermic Hypersil $C_{18}$ column (250 mm x 4.6 mm, 5 µm particle size) and LC-solution software were used.

### Chromatographic conditions

The mobile phase consist acetonitrile and phosphate

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buffer 20:80 (v/v) (pH 3 ±0.02, adjusted with orthophosphoric acid). The mobile phase was sonicated for 10-15 min and filtered through a Whatman filter paper no. 41. Flow rate of mobile phase was 1.0 ml/min. The variable wavelength UV–visible detector was set at 267nm. All analyses were performed at ambient temperature.

**Preparation of standard stock solutions**

An accurately weighed standard GPN (10mg) and CPM (10mg) powder was transferred to separate 100ml volumetric flasks, dissolved and diluted up to the mark with distilled water to obtain standard stock solutions having concentration 100 µg/ml of both the drugs.

**Preparation of sample solution**

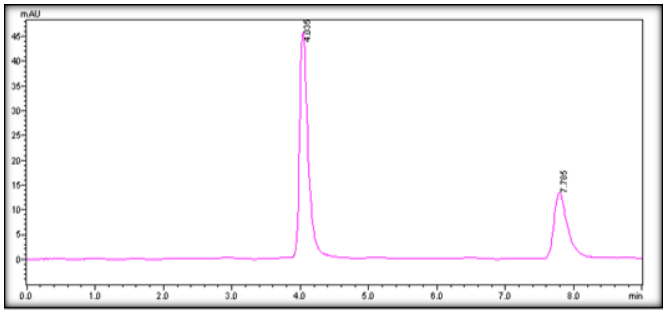
Synthetic mixture containing 50mg GPN and 4mg CPM. From this synthetic mixture weight a quantity of powder which is equivalent to 25mg of GPN and 2mg of CPM and transferred to 100 ml volumetric flask and mark up to 100ml by distilled water. The solution was ultrasonicated for 10-15 min and filtered through Whatman filter paper no. 41. Take 1ml from above solution in 10ml volumetric flask and mark up to 10ml by distilled water to obtain solution containing 25 µg/ml of GPN and 2 µg/ml of CPM.

## RESULTS AND DISCUSSION

**Method Development**

Several mobile phase compositions were tried to resolve the peaks of GPN and CPM. The optimum mobile phase containing Acetonitrile and phosphate buffer 20:80 (v/v) (pH 3 ± 0.02, adjusted with orthophosphoric acid) was selected because it could resolve the peaks of GPN (RT = 7.801 ±0.03 min) and CPM (RT = 4.047±0.05 min) with a resolution 12.05. Quantification was achieved with UV detection at 267 nm on the basis of peak area at 1.0 ml/min flow rate. A typical HPLC chromatogram obtained during simultaneous determination of GPN and CPM is given in (Figure **3**).

**Figure 3. HPLC chromatogram obtained during simultaneous determination of GPN and CPM**




**Method Validation**

***Linearity and Range:*** Six different concentrations (2, 8, 14, 20, 26 and 32 µg/ml) of the mixture of two drugs were prepared for linearity studies. The calibration curves obtained by plotting peak area against concentration showed linear relationship over a concentration range of 2-32 µg/ml for both the drugs. The linear regression equations for GPN and CPM were found to be y = 4159.3x + 51596 and y = 9700.7x + 220078 respectively. The regression coefficient values ($r^2$) were found to be 0.9994 and 0.9995 respectively indicating a high degree of linearity. Calibration curves of GPN and CPM are shown in (Figure **4**) and (Figure **5**) and (Table **1**).

***Precision:*** The intra-day an inter-day precisions (n=3) were assessed by analyzing standard solutions. The % RSD was found to be between 0.51-0.96 % and 0.41-0.88 % and 0.72-1.28 % and 1.17-1.59 % for both the drugs (Table **1**). The lower values of % RSD indicate that the method is precise.

**Figure 4. Calibration curves of GPN**

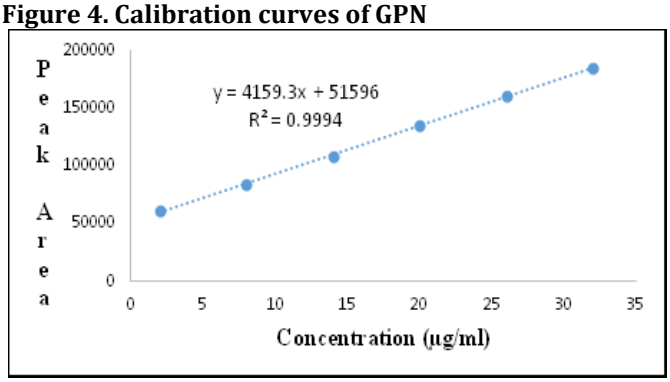



**Figure 5. Calibration curves of CPM**

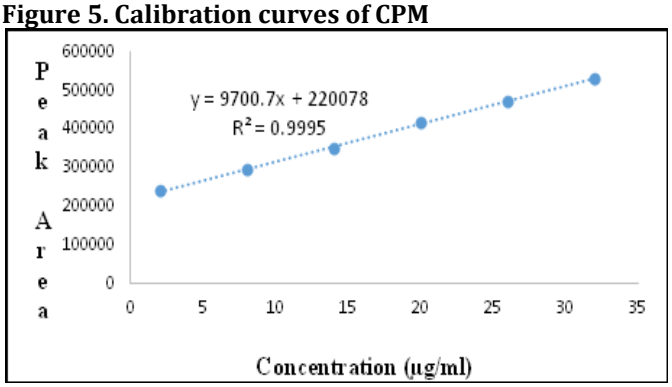



**Table 1. Regression analysis data and summary of validation parameters for the proposed method**

| Parameters | | GPN | CPM |
|---|---|---|---|
| Concentration limit (µg/ml) | | 2-32 | 2-32 |
| Regression equation<br>y = mx + C<br>Slope (m)<br>Intercept (C) | | y = 4159.3x + 51596<br>4159.3<br>51596 | y = 9700.7x + 220078<br>9700.7<br>220078 |
| Correlation coefficient ($R^2$) | | 0.9994 | 0.9995 |
| LOD (µg/ml) | | 0.53 | 0.12 |
| LOQ (µg /ml) | | 1.63 | 0.36 |
| Repeatability (% RSD, n=6) | | 0.35 | 0.87 |
| Precision (% RSD, n=3) | Intraday | 0.51 – 0.96 | 0.41 – 0.88 |
| | Interday | 0.72 – 1.28 | 1.17 – 1.59 |
| Accuracy ± S.D. (% Recovery, n=3) | | 100.13 ± 0.09 | 100.42 ± 0.40 |
| %Assay ± S.D. (n=6) | | 100.15 ±0.24 | 100.89 ± 0.67 |

***Repeatability:*** The values of % RSD for GPN and CPM were found to be 0.35 % and 0.87 %, respectively (Table **1**). The values of %RSD were found to be <1 %, which indicates that the proposed method is repeatable for estimation of GPN and CPM.

***Limit of detection (LOD) and limit of quantification (LOQ):*** The values of LOD for GPN and CPM were found to be 0.53 µg/ml and 0.12 µg/ml, respectively and the values of LOQ for GPN and CPM were found to be 1.63 µg/ml and 0.36 µg/ml, respectively. These data show (Table **1**) that the proposed method is sensitive for estimation of GPN and CPM.

***Accuracy:*** Recovery studies were carried out by applying the standard addition method. Known amounts of standard of GPN and CPM corresponding to 50%, 100%, and 150% of the label claim were added separately. The average % recoveries for GPN and CPM in synthetic mixture were found to be between 100.13 ± 0.09 % and 100.42 ± 0.40 %. The results revealed that there was no interference of excipients. The results of accuracy are shown in (Table **2**).

**Analysis of Synthetic Mixture**

Analysis of synthetic mixture was carried out using

optimized mobile phase and HPLC conditions. The % drug content obtained by the proposed method for GPN and CPM was found to be 100.15 ±0.24 % and 100.89 ± 0.67 % respectively. The results are given in Table **3**.

**Table 2. Recovery data for the proposed method**

| Drug | Level | Amount present (µg/ml) | Amount added (%) | Mean% Recovery ± S.D. |
|---|---|---|---|---|
| GPN | I | 25 | 50 | 100.24 ± 0.12 |
| | II | 25 | 100 | 100.10 ± 0.28 |
| | III | 25 | 150 | 100.05 ± 0.11 |
| CPM | I | 2 | 50 | 99.98 ± 0.11 |
| | II | 2 | 100 | 100.77 ± 0.56 |
| | III | 2 | 150 | 100.51 ± 0.41 |

**Table 3. Estimation of drugs in synthetic mixture (n=6)**

| Sample No. | Label claim | | Amount found | | %Label claim | |
|---|---|---|---|---|---|---|
| | GPN (mg/ml) | CPM (mg/ml) | GPN (mg/ml) | CPM (mg/ml) | GPN % | CPM % |
| 1 | 50 | 4 | 50.18 | 4.01 | 100.28 | 100.28 |
| S.D. | | | 0.12 | 0.03 | 0.24 | 0.67 |

**Table 4. System suitability parameters**

| Parameters | GPN ± %RSD (n = 6) | CPM ±% RSD (n = 6) |
|---|---|---|
| Retention time (min) | 7.801 ± 0.19 | 4.047 ± 0.15 |
| Tailing factor | 1.673 ± 1.37 | 1.452 ± 0.57 |
| Theoretical plates | 7603.49 ± 1.26 | 4104.42 ± 0.75 |
| Resolution | 12.504 ± 0.69 | - |

**System Suitability Parameters**

For system suitability parameters such as the resolution, tailing factor, theoretical plate and retention time of the peaks were calculated. The results are shown in Table **4**.

## CONCLUSION

A novel RP- HPLC method has been developed for the simultaneous estimation of GPN and CPM in synthetic mixture. The method gave good resolution for both the drugs with a short analysis time below 8 minutes. The developed method was validated. It was found to be novel, simple, precise, accurate, and sensitive. The good % recovery in synthetic mixture suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 1 % showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of GPN and CPM in synthetic mixture.

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