Abstract:

A novel, simple, sensitive and specific spectrophotometric method has been developed for the simultaneous estimation of Codeine Phosphate and Chlorpheniramine Maleate in combined liquid dosage form (i.e., syrup). This method involves solving simultaneous equation method. Codeine phosphate has absorbance maxima at 284nm and Chlorpheniramine maleate has absorbance maxima at 264nm in 0.5N H₂SO₄.

The proposed method was validated in terms of Linearity and Range, Accuracy, Precision, Limit of Detection and Limit of Quantitation. Beer’s Law was obeyed in the concentration range of 25 - 175µg/ml and 5 - 35 µg/ml for codeine phosphate and chlorpheniramine maleate respectively with regression coefficient r = 0.999 for both.

Key words: Codeine Phosphate, Chlorpheniramine Maleate, Simultaneous Equation, Multicomponent Analysis.

2. Introduction:

Codeine Phosphate (CP) is 7,8-didehydro-4,5-epoxy-3- methoxy-17-methylmorphinan-6-ol dihydrogen phosphate hemihydrate, an alkaloid occurring in *Papaver somniferum* or obtained from morphine by methylation. The phosphate salt of codeine, a naturally occurring phenanthrene alkaloid and opioid agonist with analgesic, antidiarrheal and antitussive activities. It mimics the actions of endogenous opioids by binding to the opioid
receptors at many sites within the central nervous system (CNS). Stimulation of mu-subtype opioid receptors results in a decrease in the release of nociceptive neurotransmitters such as substance P, GABA, dopamine, acetylcholine and noradrenaline; in addition, the codeine metabolite morphine induces opening of G-protein-coupled inwardly rectifying potassium (GIRK) channels and blocks the opening of N-type voltage-gated calcium channels, resulting in hyperpolarization and reduced neuronal excitability. Stimulation of gut mu-subtype opioid receptors results in a reduction in intestinal motility and delayed intestinal transit times. Antitussive activity is mediated through codeine’s action on the cough center in the medulla.

![Fig. 1: Structure of Codeine Phosphate](image)

Chlorpheniramine Maleate is (RS)-3-(4-chlorophenyl)-3-(pyridyl)propyldimethylamine hydrogen maleate. Chlorpheniramine Maleate is a first generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria. It antagonize all actions of histamine (on GIT, blood vessels, respiratory tract) except for those mediated solely by H₂ receptors. The action of all of the H₁ receptor blockers is qualitatively similar. However, most of these blockers have additional effects unrelated to their blocking of H₁ receptors; these effects probably reflect binding of the H₁ antagonists to cholinergic, adrenergic, or serotonin receptors. It is useful in treating allergies caused by antigens acting on IgE-antibody sensitized mast cells.

![Fig. 2: Structure of Chlorpheniramine Maleate](image)
3. Experimental
3.1. Chemicals and Reagent:
The CP and CPM were obtained as a gift sample from Biological E Ltd., Hyderabad, Andhra Pradesh. 0.5N H$_2$SO$_4$ was used as solvent. The marketed formulation of this combination (Label claim: 5ml of the syrup CP 10mg and CPM 4mg), Coscodin were obtained from Biological E Ltd., Hyderabad.

3.2. Instrumentation:
UV-Visible spectrophotometer – Hitachi V-2000 with 10mm matched quartz cell.

4. Experimental Part
4.1. Preparation of Stock Solutions:
10mg of CP and 10mg of CPM were weighed accurately and transferred to two separate 100 ml volumetric flasks. Both the drugs were dissolved in 50ml of 0.5N H$_2$SO$_4$ with shaking and then volume is made upto the mark with 0.5N H$_2$SO$_4$ to get standard stock solution of each drug. These stock solutions are filtered through 0.2µm Nylon 66 (N66) and 7 mm membrane filter paper to give concentration of 1000µg/ml of both CP and CPM.

4.2. Calibration Curve:
From each stock, appropriate aliquots were pipette out from each standard stock solution in to a series of 100ml of volumetric flasks. The volume was made upto the mark with sovent (0.5N H$_2$SO$_4$) to obtain concentration of 50, 75, 100, 125, 150 µg/ml of CP and 10, 15, 20, 25, 30 µg/ml of CPM.

100µg/ml of CP and 20µg/ml of CPM were scanned in UV-range (i.e., 400 – 220nm) and their absorbance maxima ($\lambda_{\text{max}}$) were recorded as 284nm and 264nm respectively.

Fig. 3: Spectra of Codeine Phosphate of 100 µg/ml
Fig. 4: Spectra of Chlorpheniramine Maleate of 20 µg/ml

4.3. Analysis of Syrup:

1ml of Coscodin syrup (Label claim: Each 5ml of syrup contains CP 10mg and CPM 4mg) was measured and taken into a 100ml volumetric flask. It is then dissolved in 0.5N H₂SO₄ and is made up to the mark. The solution is then filtered through 0.2µm Nylon 66 (N66) and 7 mm membrane filter paper. Now the solution is scanned in UV-range (i.e., 400 – 220nm).

4.4. Simultaneous Equation Method or Vierodt’s Method:

As per Simultaneous Equation Method (Vierodt Method), if a sample contains two absorbing drugs each of which absorbs at λₘₐₓ of the other (λ₁ and λ₂), it is possible to determine both drugs.
---------- CP

----- CPM

λ₁ (λ_max of CP): 284nm
λ₂ (λ_max of CPM): 264nm

Absorptivities of CP at λ₁ (α₁):
Absorbance at 284 = 0.399

\[ A = abc \] (where a = absorptivity, b = path length (1 cm), c = concentration)
\[ a_1 = \frac{A}{c} \]
where c = 100µg/ml
\[ a_1 = \frac{0.399}{100} = 0.00399 \]

Absorptivities of CP at λ₂ (α₂):
Absorbance at 264 = 0.157

\[ A = abc \] (where a = absorptivity, b = path length (1 cm), c = concentration)
\[ a_2 = \frac{A}{c} \]
where c = 100µg/ml
\[ a_2 = \frac{0.157}{100} = 0.00157 \]

Absorptivities of CPM at λ₁ (α₁):
Absorbance at 284 = 0.098

\[ A = abc \] (where a = absorptivity, b = path length (1 cm), c = concentration)
\[ a_1 = \frac{A}{c} \]
where c = 20µg/ml
\[ a_1 = \frac{0.098}{20} = 0.0049 \]

Absorptivities of CPM at λ₂ (α₂):
Absorbance at 264 = 0.447

\[ A = abc \] (where a = absorptivity, b = path length (1 cm), c = concentration)
\[ a_1 = \frac{A}{c} \]
where c = 20µg/ml
\[ a_1 = \frac{0.447}{20} = 0.02235 \]

Absorbance of sample at 284 (A₁) = 0.275
Absorbance of sample at 264 (A₂) = 0.95

Let the concentrations of CP and CPM are Cₓ and Cᵧ
5. Calculations:

5.1. Concentration of CP (C_x):

As per Simultaneous Equation Method,

\[ C_x = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \]

\[ = \frac{(0.95 \times 0.0049) - (0.275 \times 0.02235)}{(0.00157 \times 0.0049) - (0.00399 \times 0.02235)} \]

\[ C_x = 18.3012 \]

5.2. Concentration of CPM (C_y):

As per Simultaneous Equation Method,

\[ C_y = \frac{A_1 a_{X2} - A_2 a_{x1}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \]

\[ = \frac{(0.275 \times 0.00157) - (0.95 \times 0.0039)}{(0.00157 \times 0.0049) - (0.00399 \times 0.02235)} \]

\[ C_y = 40.17 \]

**Amount of the drug present in sample** = Concentration x Dilution Factor

**Amount of CP present (for 1ml)** = \( C_x \times D.F \)

Dilution Factor for CP = 100

Amount of CP = 18.3012 x 100

= 1830.12

= 1.83mg

**Amount of CPM present (for 1ml)** = \( C_y \times D.F \)

Dilution Factor for CPM = 20
Amount of CP = 40.17 x 20
= 803.4µg
= 0.803mg

6. Method Validation:

The developed analytical method was subjected to validation as per ICH guidelines.

6.1. Linearity and Range:

The standard curve was obtained in concentration range of 25 - 150µg/ml for CP and 5 – 30µg/ml for CPM. The linearity and range of this method was evaluated by Linear regression analysis, using Least squares method.

6.2. Precision:

Intra-day Precision: In intra-day precision, the sample mixture containing--- µg/ml of CP and --- µg/ml of CPM were analyzed six times (n=3) at different time interval on same day.

Inter-day Precision: In inter-day precision, a set of three sample mixtures containing 100 µg/ml of CP and 25 µg/ml of CPM were prepared and analyzed on different day.

The variation of the results on same day and on different days were analyzed and statistically validated.

6.3. Accuracy:

Recovery studies were carried out by applying the method to drug sample present in liquid dosage form (syrup) to which known amount of CP and CPM corresponding 80%, 100% and 120% of label claim was added (standard addition method). After the addition of the standards, the contents were analyzed by the same procedure used for the syrup.

6.4. Limit of Detection and Limit of Quantification:

LOD and LOQ values are obtained from the slope and standard deviation of calibration curve.

LOD = 3 x Standard Deviation/slope

LOQ = 10 x Standard Deviation/slope
7. Results and Discussion:

7.1. Assay:

Table 1: Assay results of CP and CPM in combined liquid dosage form.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim (for 5ml)</th>
<th>% Drug found ± SD*</th>
<th>RSD(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine Phosphate</td>
<td>10 mg</td>
<td>9.59 ± 0.1035</td>
<td>1.0824</td>
</tr>
<tr>
<td>Chlorpheniramine Maleate</td>
<td>4 mg</td>
<td>4.05 ± 0.04384</td>
<td>1.0824</td>
</tr>
</tbody>
</table>

* n=3, SD : Standard Deviation, RSD : Relative Standard Deviation

As per ICH guidelines RSD(%) should not be more than 2%, which shows that the proposed method is satisfactory for simultaneous determination of Codeine Phosphate and Chlorpheniramine Maleate.

7.2. Linearity and Range:

Linearity was established by least square regression analysis of the calibration curve. The linearity range for CP and CPM were found to be 50 - 150µg/ml and 5 - 30 µg/ml respectively. The regression coefficients were found to be 0.9999 for both CP and CPM.

Fig. 6: Linearity curve for Codeine Phosphate.
Fig. 7: Linearity curve for Chlorpheniramine Maleate.

7.3. Precision:

For intra-day studies, 3 concentrations were analyzed on the same day and for inter-day studies, 3 concentrations were analyzed. The data showed that RSD was found to be less than 2% for both; intra-day and inter-day studies, which shows that the method is precise. Results are reported in Table 2.

Table 2: Precision

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/ml)</th>
<th>Measured concentration (µg/ml) ± SD</th>
<th>%CV</th>
<th>Measured concentration (µg/ml) ± SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine Phosphate</td>
<td>100</td>
<td>99.33 ± 0.4509</td>
<td>0.4539</td>
<td>99 ± 0.4582</td>
<td>0.4628</td>
</tr>
<tr>
<td>Chlorpheniramine Maleate</td>
<td>20</td>
<td>19.46 ± 0.1258</td>
<td>0.6464</td>
<td>19.58 ± 0.104</td>
<td>0.5314</td>
</tr>
</tbody>
</table>
7.4. Accuracy:

Recovery studies were performed to determine the accuracy of the method. Recovery experiments were performed at three levels, in which the preanalyzed sample at 80%, 100% and 120% of the label claim. The replicate samples of each concentration levels were prepared and the % recovery at each level was determined. Results are reported in table 3.

Table 3: Accuracy.

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>%</th>
<th>Amount present (mg/5ml)</th>
<th>Amount of standard drug added (in mg)</th>
<th>Amount recovered (mg Mean ± SD) (n=3)</th>
<th>Mean ± SD of % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>CPM</td>
<td>CP</td>
<td>CPM</td>
</tr>
<tr>
<td>80%</td>
<td></td>
<td>10</td>
<td>4</td>
<td>8</td>
<td>3.2</td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>120%</td>
<td></td>
<td>10</td>
<td>4</td>
<td>12</td>
<td>4.8</td>
</tr>
</tbody>
</table>

7.5. Limit of Detection (LOD) and Limit of Detection (LOQ):

LOD and LOQ were determined based on standard deviation of response and slope of calibration curve.

LOD and LOQ were found to be 0.8577 and 2.8591 for CP and 0.1395 and 0.4652 for CPM respectively.

8. Conclusion:

A newly developed spectrometric method can be used for routine analysis as a method for the simultaneous estimation of CP and CPM in pharmaceutical liquid dosage form. The developed method was validated and found to be simple accurate and precise. Statistical analysis of the developed method has been carried out, which shows good accuracy and precision.
9. References:


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