A new HPLC method development and validation for simultaneous determination of acetaminophen and codeine phosphate in bulk and dosage forms

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ABSTRACT
In current research an easy, accurate, reliable, specific and economical HPLC approach is established to analyze acetaminophen and codeine phosphate. Quantification and separation of acetaminophen and codeine phosphate is done with C18 water's column using mobile phase -0.1M, pH 3.5 NaH2PO4: acetonitrile (50:50). Linearity for acetaminophen was 150µg/ml -450µg/ml and for codeine phosphate was 30µg/ml -90µg/ml. LOD was 2.770µg/ml for acetaminophen and 0.842 µg/ml for codeine phosphate. Precision on was lesser than 2.0% and accuracy was near to 100%. The objective of this research deals with optimization of method conditions to estimate acetaminophen and codeine phosphate also includes method validation, specificity, linearity, precision, accuracy and robustness. This developed procedure can therefore employed in pharmaceutical formulations for intent of quality control.

Keywords: Acetaminophen, Codeine Phosphate, RP-HPLC.

INTRODUCTION
Pain is an uncomfortable sensory experience, like prick, stinging, burning, or ache. It can be sluggish and come and go, or might be continuous. Pain is indeed a notification that anything might be incorrect in the nervous system. Pain can be divided as acute pain and chronic pain. Acute pain normally occurs and exists for a limited duration. Chronic pain continues longer than acute pain and is usually resistant to medical therapy. It is generally linked to a long term disease.

Treatment of slight and moderate pain involves the mixture of acetaminophen (non-salicylate) and codeine (opiate antagonist).

Acetaminophen
Acetaminophen is divided as analgesic, anti pyretic, non steroidal anti inflammatory, cyclooxygenase inhibitor. Technical name is N-(4- hydroxyphenyl) acetamide. Half life of the drug is estimated as 2.5 hr and discharged out through urine (90% of the given drug).
It raises the pain limit by blocking synthesis of two cyclooxygenase isoforms (COX 1 & COX 2). These enzymes produce prostaglandins which are accountable for the intense feeling of pain.

It is an antagonist for opioid, analgesic drug, antidiarrheal drug, antitussive drug. Technical name is (4R,4aR,7S,7aR,12bS)-9-methoxy-3-methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinolin-7-ol:phosphoric acid. Half life of the drug is estimated as 2.5 hr. It is discharged through urine (90%). It acts by binding to µ-opioid receptors and block their function. µ opioid receptors transmit pain all through the body and the central nervous system. It is used in treating pain of mild to moderate type and cough relief. It is also used in treating irritable bowel syndrome.

**MATERIALS AND METHOD**

**Apparatus**

- Waters HPLC system, photodiode detector with empower software version
- Water column C18:5 µm, 4.5 mm and 250 mm dimensions.
- Ultrasonicator
- Weighing balance
- soreson pH meter

**Chemicals**

- Sodium dihydrogen phosphate
- Acetonitrile
- Phosphoric acid

**Conditions to assay drugs:**

- Flow velocity - 1.0 ml / min
- Temperature -25 * c
- Vol. injected - 10µl
- Detection - 228

**Mobile phase**

- 0.1 M NaH₂PO₄ buffer, pH 3.5 and acetonitrile were mixed in 50:50 volume ratios. Same solvent mix was used as diluent for making solutions of drug stock and standard solution.

**Codeine phosphate & acetaminophen stock solution**

- Weighed about 300 mg acetaminophen and 60 mg codeine phosphate was transferred to 100 ml
volumetric flask. 50 ml diluent was added, mixed well and make up volume to mark by diluent. Concentration of acetaminophen and codeine phosphate stock solution was 3000 µg/ml acetaminophen and 600 µg/ml codeine phosphate.

### Solutions for study of calibration graph
Solution with five concentrations of acetaminophen and codeine phosphate were prepared from stock solution by diluent.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Acetaminophen in µg/ml conc.</th>
<th>Codeine phosphate in µg/ml conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>225</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>375</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>450</td>
<td>90</td>
</tr>
</tbody>
</table>

### Tablet solution
Tylenol with codeine no.4 (strength: acetaminophen 300 mg and codeine 60 mg) tablets are powdered and weighed. Acetaminophen 300 mg and codeine 60 mg weight equivalent powder was deported to 100 ml flask. 50 ml diluent was added, 30 min sonicated, filtered through membrane finally make up to 100 ml by diluent. Concentration of stock tablet solution is 3000 µg/ml acetaminophen and 600 µg/ml codeine phosphate.

### Analysis of codeine phosphate and acetaminophen in tablet
10 microliters of tablet sample for analysis is prepared and is infused to HPLC system. Chromatograms and peak response of acetaminophen and codeine phosphate were noted. Content of codeine phosphate and acetaminophen was determined by using the peak response data.

### Results

#### Method development conditions
Mobile phase: NaH$_2$PO$_4$: ACETONITRILE (50:50)
Column: WATERS, C18, 250×4.6mm, 5 µm
Flow rate: 1.0 ml/min
Temperature: 25°C
Volume: 10 µl
Run time: 10 min
Detector: 228
pH: 3.5

#### Validation

#### Linearity
Linearity for acetaminophen and codeine phosphate was examined between the range from 150 µg/ml to 450 µg/ml and 30 µg/ml to 90 µg/ml. The made five dissimilar concentration solution solutions were infused to HPLC column.
### Acetaminophen area response

<table>
<thead>
<tr>
<th>Acetaminophen in µg/ml conc.</th>
<th>Codeine phosphate area response</th>
</tr>
</thead>
<tbody>
<tr>
<td>2426888</td>
<td>557912</td>
</tr>
<tr>
<td>3638921</td>
<td>835834</td>
</tr>
<tr>
<td>4857571</td>
<td>1115288</td>
</tr>
<tr>
<td>6064280</td>
<td>1396662</td>
</tr>
<tr>
<td>7277269</td>
<td>1671158</td>
</tr>
</tbody>
</table>

Regression equation:
For acetaminophen:
\[
y = 48504x + 2537
\]
\[R^2 = 0.9998\]

For codeine phosphate:
\[
y = 11149x + 442.8
\]
\[R^2 = 0.9997\]

### CODEINE PHOSPHATE LINEARITY

Limit of detection and limit of quantitation

To establish detection and quantitation limits, the signal to noise technique was utilized. The concentration of acetaminophen and codeine phosphate giving signal to noise ratio of 3 value could be identified as their LOD and 10 value could be identified as their LOQ.

Acetaminophen
- LOD - 0.831 µg / ml
- LOQ - 2.770 µg / ml

Codeine phosphate
- LOD - 0.252 µg / ml
- LOQ - 0.842 µg / ml
Accuracy

Three samples were prepared at 50%, 100%, and 150% concentration of target test solution by spiking acetaminophen and codeine phosphate standard to tablet solution. The solutions are injected to HPLC column. Calculated the recovered percentage of acetaminophen and codeine phosphate at 50%, 100% and 150% concentration levels.

<table>
<thead>
<tr>
<th>Area response</th>
<th>µg/ml Conc. spiked</th>
<th>µg/ml Conc. determined</th>
<th>Percent Recover</th>
</tr>
</thead>
<tbody>
<tr>
<td>2422031</td>
<td>150</td>
<td>149.43</td>
<td>99.62</td>
</tr>
<tr>
<td>2427451</td>
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<td>149.77</td>
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<tr>
<td>2421126</td>
<td>150</td>
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<td>99.58</td>
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<td>4856543</td>
<td>300</td>
<td>299.63</td>
<td>99.88</td>
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<td>4851560</td>
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<tr>
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<td>99.80</td>
</tr>
<tr>
<td>7270596</td>
<td>450</td>
<td>448.57</td>
<td>99.68</td>
</tr>
</tbody>
</table>

Acetaminophen accuracy results

<table>
<thead>
<tr>
<th>Area response</th>
<th>µg/ml Conc. spiked</th>
<th>µg/ml Conc. determined</th>
<th>Percent Recover</th>
</tr>
</thead>
<tbody>
<tr>
<td>557647</td>
<td>30</td>
<td>29.90</td>
<td>99.68</td>
</tr>
<tr>
<td>557577</td>
<td>30</td>
<td>29.90</td>
<td>99.66</td>
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<tr>
<td>557254</td>
<td>30</td>
<td>29.88</td>
<td>99.61</td>
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<td>60</td>
<td>59.57</td>
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<td>60</td>
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<tr>
<td>1114393</td>
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<tr>
<td>1677273</td>
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<td>1677619</td>
<td>90</td>
<td>89.96</td>
<td>99.96</td>
</tr>
<tr>
<td>1678429</td>
<td>90</td>
<td>90.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Codeine phosphate accuracy

Results of Precision

Six standard solutions were made and injected to the HPLC column. Calculated the RSD percentage for peak response of acetaminophen and codeine phosphate. RSD percent was less than 2.0%.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Acetaminophen peak response</th>
<th>Codeine phosphate peak response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4851947</td>
<td>1111980</td>
</tr>
<tr>
<td>2</td>
<td>4852730</td>
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<td>3</td>
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<td>1114734</td>
</tr>
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<td>4</td>
<td>4858078</td>
<td>1113377</td>
</tr>
<tr>
<td>5</td>
<td>4859673</td>
<td>1119315</td>
</tr>
<tr>
<td>6</td>
<td>4853345</td>
<td>1117554</td>
</tr>
<tr>
<td>Average</td>
<td>4855952</td>
<td>1115205</td>
</tr>
<tr>
<td>SD</td>
<td>3673.898</td>
<td>2729.539</td>
</tr>
<tr>
<td>RSD</td>
<td>0.076</td>
<td>0.245</td>
</tr>
</tbody>
</table>
CONCLUSION

HPLC approach established in current research is easy, accurate, reliable, specific and economical to analyze acetaminophen and codeine phosphate. The validated strategy exhibits adequate results for all the parameters studied. This developed procedure can therefore be employed in pharmaceutical formulations for intent of quality control.

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