Method Optimization of Hydroxychloroquine in Reversed Phase Mode

- Rapid Analysis Using Volatile Mobile Phase -
Introduction

Hydroxychloroquine was first approved in the United States in 1955. And nowadays, it has been approved in more than 70 countries all over the world as a remedy of malaria, systemic lupus erythematosus, cutaneous lupus erythematosus and a variety of disease.

Official HPLC analytical method of Hydroxychloroquine is stipulated in the USP (The United States Pharmacopeia), section of “Hydroxychloroquine Sulfate Tablets, part of Assay”. However, the USP method cannot be used with LC-MS(/MS) because a non-volatile ion-pairing reagent is used.

In this technical data sheet, we will introduce method optimization for rapid analysis of Hydroxychloroquine and Chloroquine mixture using organic/inorganic hybrid silica column, Triart C18, and volatile mobile phase that is compatible with LC-MS(/MS).
Mobile Phase Screening with Triart C18
HPLC Conditions

<table>
<thead>
<tr>
<th>Column</th>
<th>YMC-Triart C18 1.9 µm (50 X 2.0 mmI.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent</td>
<td>A) 20 mM formate buffer</td>
</tr>
<tr>
<td></td>
<td>B) acetonitrile or methanol</td>
</tr>
<tr>
<td></td>
<td>5-95%B (0-10 min), 5%B (10-20 min)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>25 °</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 254 nm</td>
</tr>
<tr>
<td>Injection</td>
<td>4 µL (50 µg/mL)</td>
</tr>
<tr>
<td>Sample</td>
<td>1. Hydroxychloroquine sulfate</td>
</tr>
<tr>
<td></td>
<td>2. Chloroquine phosphate</td>
</tr>
</tbody>
</table>

Hydroxychloroquine sulfate
(pKa1 = 7.28)
(pKa2 = 9.76)

Chloroquine phosphate
(pKa1 = 7.29)
(pKa2 = 10.32)
### Mobile Phase Screening

**Triart C18 X pH X Organic Solvent**

<table>
<thead>
<tr>
<th>pH</th>
<th>Solvent</th>
<th>Peaks</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9</td>
<td>Acetonitrile</td>
<td>1, 2</td>
<td>1. Hydroxychloroquine sulfate, 2. Chloroquine phosphate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Select pH 4.3</strong></td>
</tr>
<tr>
<td>4.3</td>
<td>Methanol</td>
<td>1, 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCOOH-HCOONH₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.1</td>
<td>Acetonitrile</td>
<td>1, 2</td>
<td>Peak shape was deteriorated at pH 6.1 and pH 9.9 because they are near pKa values of the compounds.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.9</td>
<td>Methanol</td>
<td>1, 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCOONH₄-NH₃</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mobile Phase Optimization
Selection of Organic Solvent and Adjustment of Composition

Result of screening

<table>
<thead>
<tr>
<th>pH 4.3</th>
<th>20 mM HCOOH-HCOONH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>acetonitrile (Gradient)</td>
</tr>
<tr>
<td></td>
<td><img src="image1.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

1. Hydroxychloroquine sulfate
2. Chloroquine phosphate

Elution method change from Gradient to Isocratic

It is effective to choose 15 to 20% lower organic composition than where a sample is eluted on gradient elution.
e.g. 25% on gradient >>> 25-15=10% on Isocratic

Tips

20 mM HCOOH-HCOONH₄ (pH 4.3)/acetonitrile (90/10)

Rs (1,2) = 5.7

Compared to methanol
- shorter analysis time
- better resolution

Proceed to speeding up

20 mM HCOOH-HCOONH₄ (pH 4.3)/methanol (85/15)

Rs (1,2) = 4.4
Speeding Up of Analysis

Column : Triart C18 1.9 µm, 50 X 2.0 mm I.D.
Eluent : 20 mM HCOOH-HCOONH₄ (pH 4.3)/acetonitrile (90/10)

0.2 mL/min
- 16.6 MPa (2,410 psi)
- 8.0 min

0.4 mL/min
- 31.0 MPa (4,500 psi)
- 4.0 min

0.6 mL/min
- 44.3 MPa (6,430 psi)
- 2.6 min

0.8 mL/min
- 57.2 MPa (8,300 psi)
- 2.0 min

1.0 mL/min
- 69.0 MPa (10,000 psi)
- 1.6 min

Temperature : 25 °C
Detection : UV at 254 nm
Injection : 2 µL (10 µg/mL)

1. Hydroxychloroquine sulfate
2. Chloroquine phosphate
Comparison with USP Method

YMC method
: volatile mobile phase, UHPLC column
Triart C18 1.9 µm, 50 X 2.0 mmI.D.

Analysis time: 1/10 of USP method
LC-MS(MS) compatible

USP method
: non-volatile mobile phase, conventional column
Triart C18 5 µm, 250 X 4.6 mmI.D.

Eluent : acetonitrile/methanol/water/phosphoric acid
(100/100/800/2)
containing 96 mg of sodium 1-pentanesulfonate
Flow rate : 1.0 mL/min
Temperature : 25 °C
Detection : UV at 254 nm
Injection : 20 µL (50 µg/mL)
Pressure : 9.5 MPa (1,380 psi)

Eluent : 20 mM HCOOH-HCOONH₄ (pH 4.3)/acetonitrile
(90/10)
Flow rate : 1.0 mL/min
Temperature : 25 °C
Detection : UV at 254 nm
Injection : 2 µL (10 µg/mL)
Pressure : 69.0 MPa (10,000 psi)