



Method Optimization of Hydroxychloroquine in Reversed Phase Mode

- Rapid Analysis Using Volatile Mobile Phase -

YMC CO., LTD.

(F140925JE)

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.

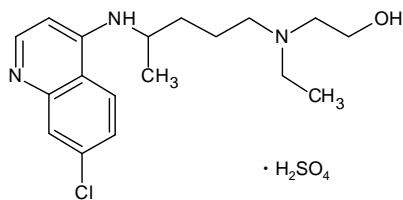
In this technical data sheet, we will introduce method optimization for rapid analysis of Hydroxychloroquine and Chloroquine mixture using organic/inorganic hybrid silica column, Triart C18, and volatile mobile phase that is compatible with LC-MS(/MS).

Mobile Phase Screening with Triart C18

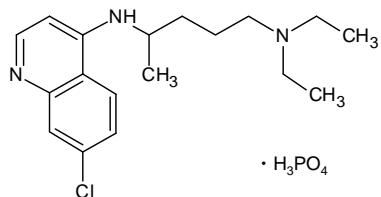
HPLC Conditions



Column	: YMC-Triart C18 1.9 μm (50 X 2.0 mmI.D)
Eluent	: A) 20 mM formate buffer B) acetonitrile or methanol 5-95%B (0-10 min), 5%B (10-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 25
Detection	: UV at 254 nm
Injection	: 4 μL (50 $\mu\text{g/mL}$)
Sample	: 1. Hydroxychloroquine sulfate 2. Chloroquine phosphate



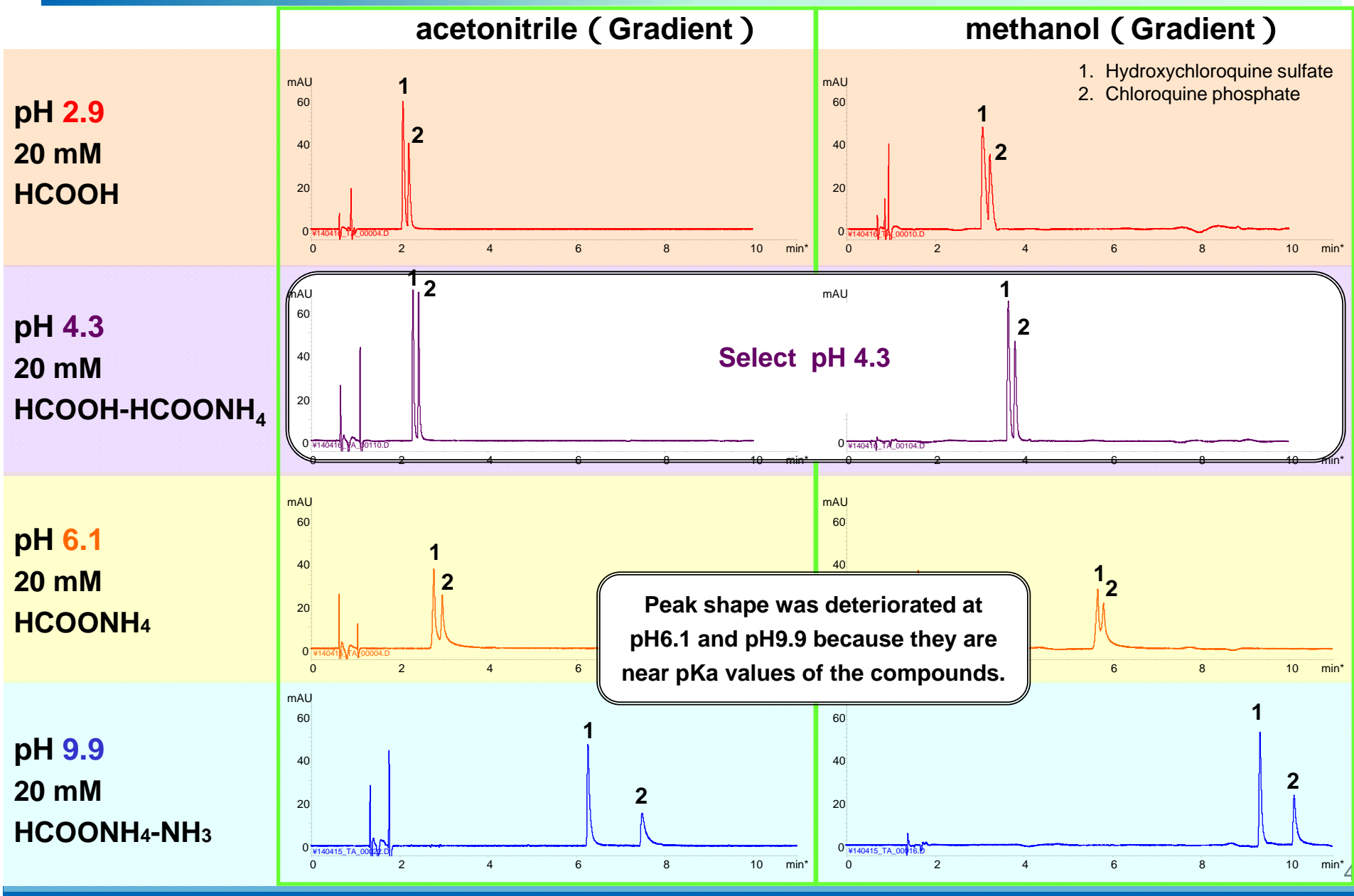
Hydroxychloroquine sulfate
(pKa1 = 7.28)
(pKa2 = 9.76)



Chloroquine phosphate
(pKa1 = 7.29)
(pKa2 = 10.32)

Mobile Phase Screening

Triart C18 X pH X Organic Solvent



Mobile Phase Optimization

Selection of Organic Solvent and Adjustment of Composition

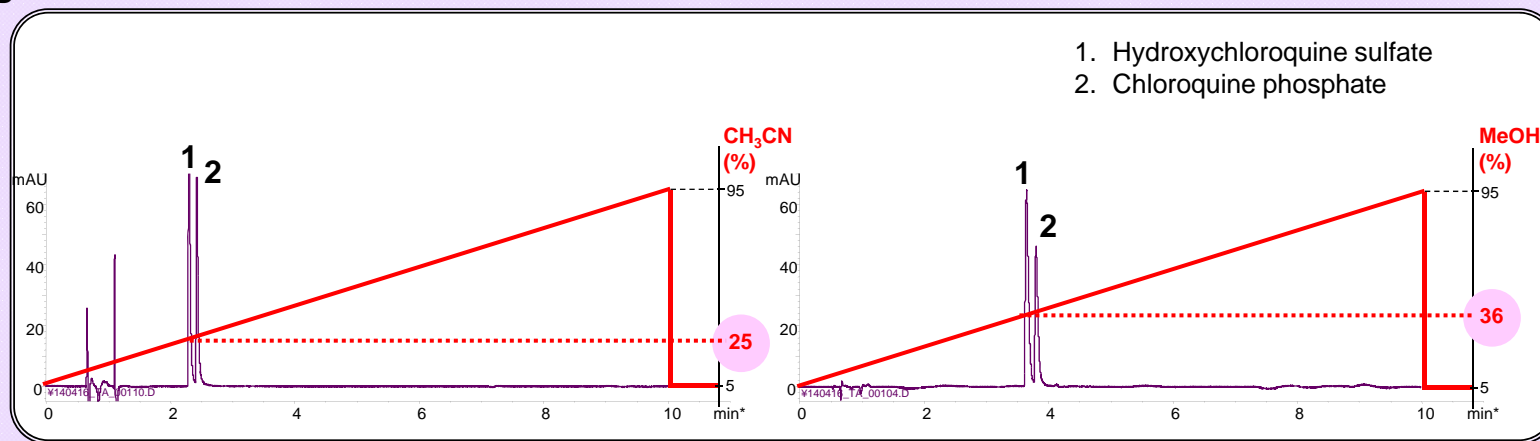


Result of screening

acetonitrile (Gradient)

methanol (Gradient)

pH 4.3
20 mM
HCOOH-HCOONH₄



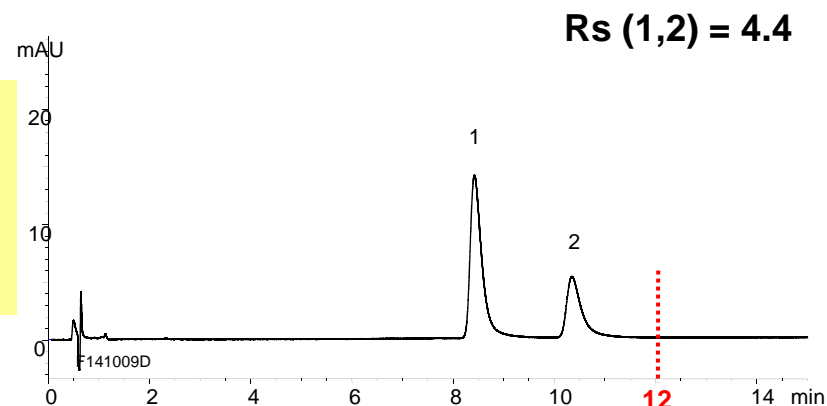
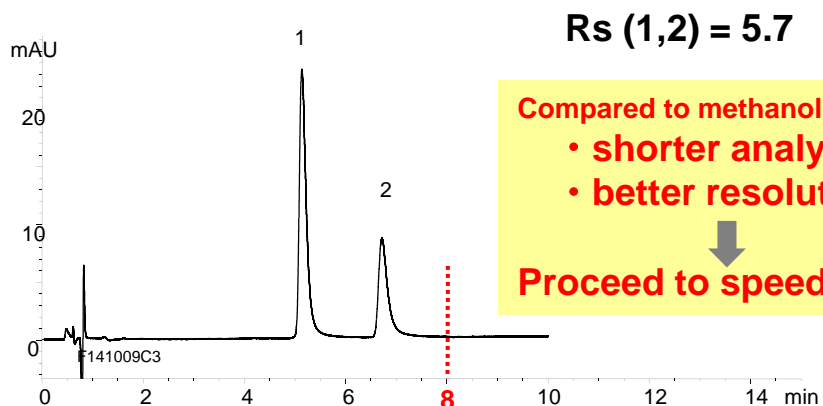
Elution method change from Gradient to Isocratic



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

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

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



Compared to methanol
• shorter analysis time
• better resolution
↓
Proceed to speeding up

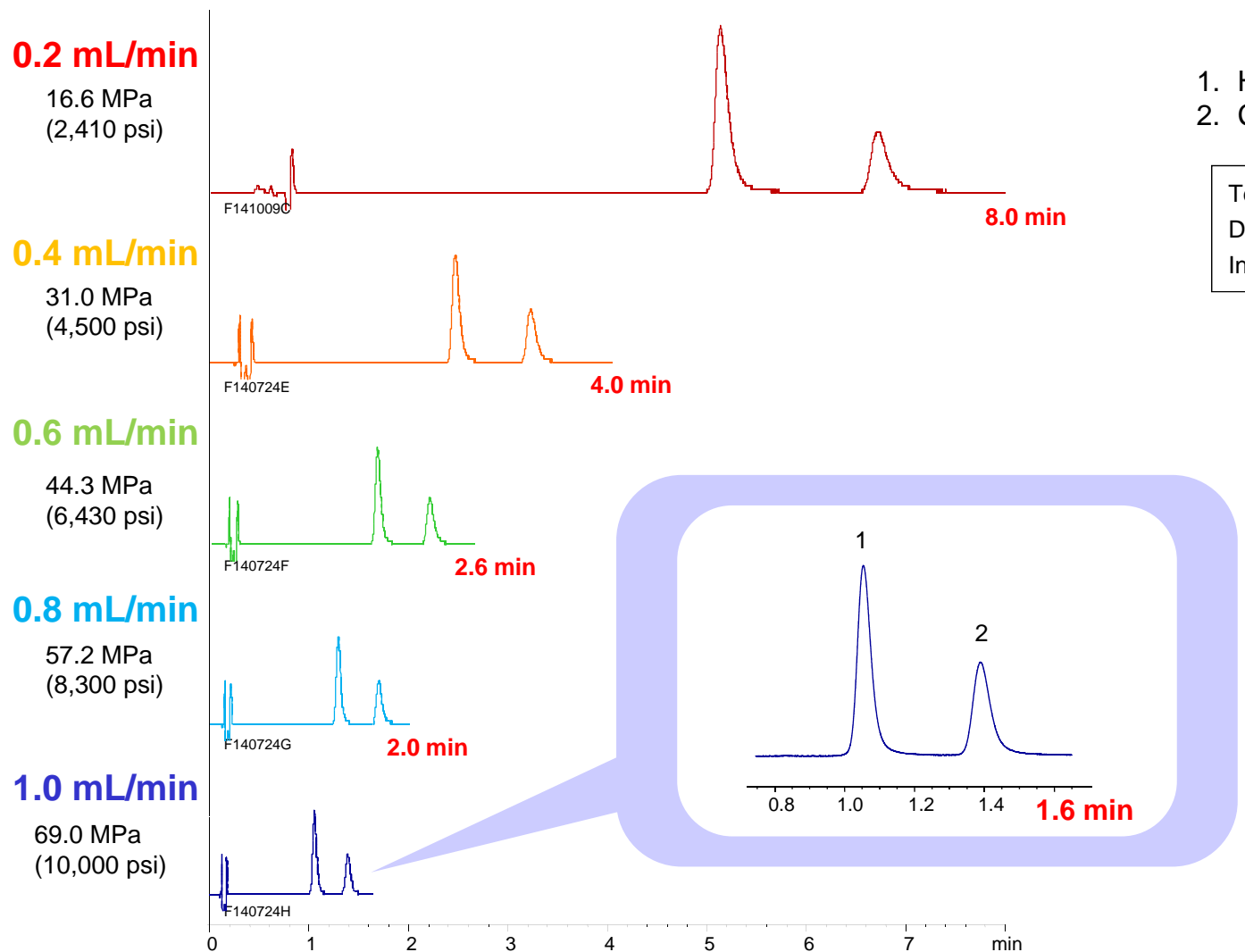
Speeding Up of Analysis

Column : Triart C18 1.9 μm , 50 X 2.0 mm I.D.

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

1. Hydroxychloroquine sulfate
2. Chloroquine phosphate

Temperature	: 25
Detection	: UV at 254 nm
Injection	: 2 μL (10 $\mu\text{g}/\text{mL}$)

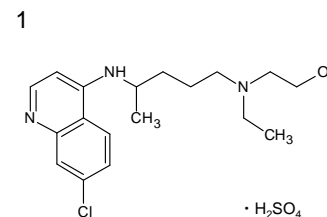
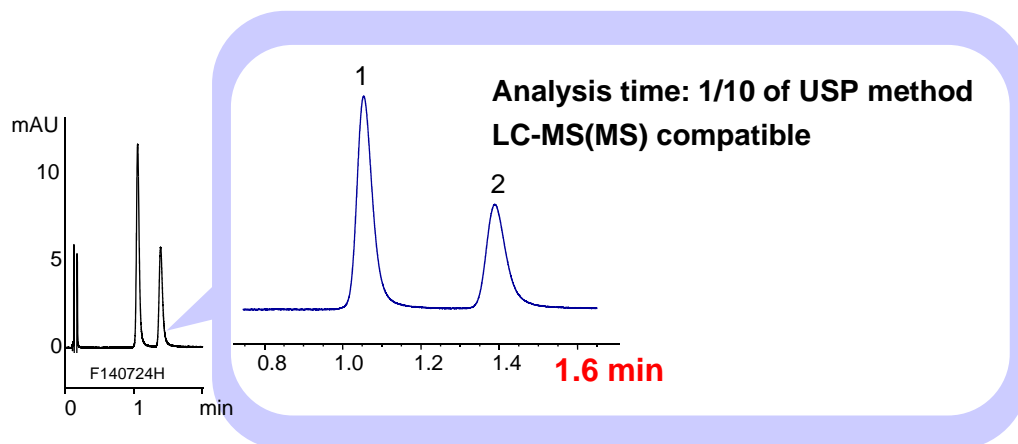


Comparison with USP Method

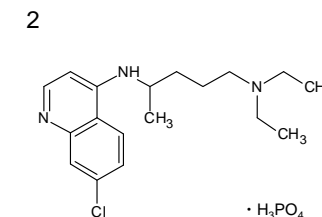
YMC method

: volatile mobile phase, UHPLC column

Triart C18 1.9 μm , 50 X 2.0 mmI.D.



Hydroxychloroquine sulfate



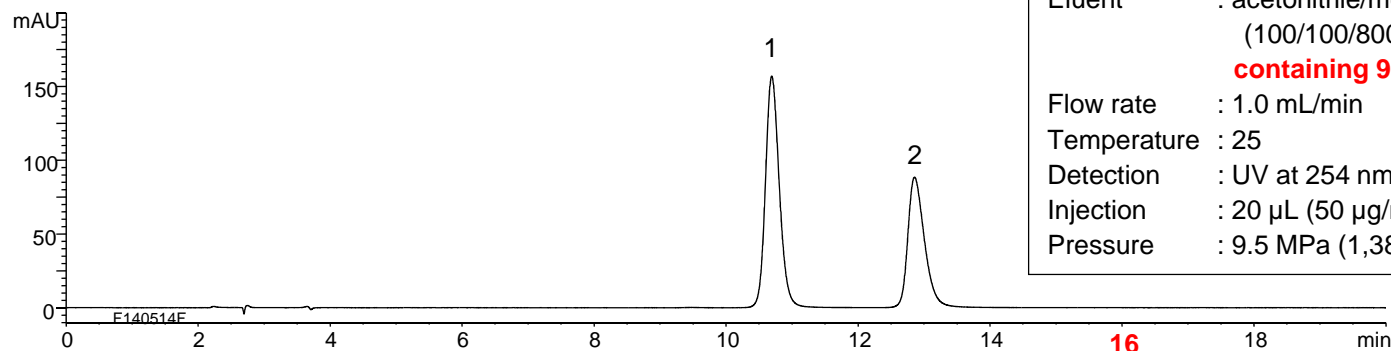
Chloroquine phosphate

Eluent : **20 mM HCOOH-HCOONH₄ (pH 4.3)/acetonitrile (90/10)**
 Flow rate : 1.0 mL/min
 Temperature : 25
 Detection : UV at 254 nm
 Injection : 2 μL (10 $\mu\text{g}/\text{mL}$)
 Pressure : 69.0 MPa (10,000 psi)

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
 Detection : UV at 254 nm
 Injection : 20 μL (50 $\mu\text{g}/\text{mL}$)
 Pressure : 9.5 MPa (1,380 psi)