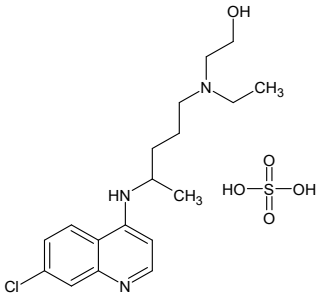
# Robust Separation of Hydroxychloroquine and Chloroquine in Hydroxychloroquine Sulfate Tablets Using the Kinetex® 5 µm C18

**Zeshan Aqeel, J Preston, Ryan Splitstone, and Phil Koerner**
**Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA**




Monograph: Hydroxychloroquine Sulfate
p$K_a$: 9.67
LogP: 3.87

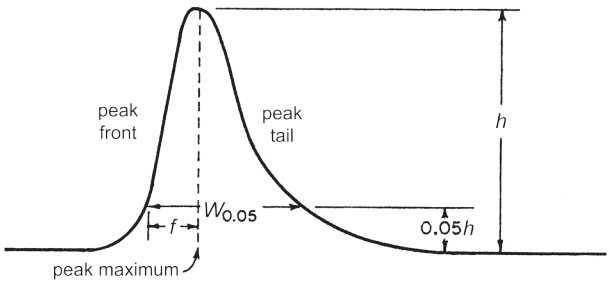
## Overview

In this application is the comparison of two particle morphologies of similar L1 (C18) phase in the separation of Hydroxychloroquine and related impurity Chloroquine under the USP conditions for assay of hydroxychloroquine sulfate tablets. The application demonstrates the potential method improvements that can be achieved per the allowable adjustments outlined in USP General Chapter <621> relative to the original column and conditions referenced in the monograph.

| USP Monograph: Hydroxychloroquine Sulfate Tablet Assay | |
|---|---|
| **Standard Stock Solution** | Dissolve 1.0 mg/mL of USP Hydroxychloroquine Sulfate RS in *Diluent* |
| **Standard Solution** | 0.05 mg/mL of USP Hydroxychloroquine Sulfate RS from *Standard Stock solution* in *Mobile Phase* |
| **System Suitability Stock Solution** | Dissolve 1.0 mg/mL of Chloroquine Phosphate in Methanol |
| **System Suitability Solution** | Transfer 5.0 mL of the *System Suitability Stock solution* into 100-mL volumetric flask, add 5.0 mL of *Standard Stock solution*, and dilute to volume with *Mobile Phase* |
| **Diluent** | Mixture of Methanol:Water (1:1) |
| **Column** | |
| **Size** | Method 1: 250 x 4.6 mm, Method 2: 250 x 4.6 mm |
| **Stationary Phase** | Method 1: Fully Porous 5 µm C18, Method 2: Kinetex 5 µm C18 |
| **Temperature** | 26 °C |
| **Mobile Phase (premixed)** | A = Methanol, Acetonitrile, water, and phosphoric acid (100:100:800:2). Mix and add 96 mg of sodium 1-pentanesulfonate in the resulting solution, and filter Mobile Phase |
| **Isocratic** | Isocratic: (100:100:800:2, Methanol: Acetonitrile: Water: Phosphoric Acid)<br>Total Run Time: 20 min |
| **Flow Rate** | 1.0 mL/min |
| **Detector** | UV @ 254 nm |
| **Injection Volume** | 10 µL of System Suitability solution and Standard solution |
| **System Suitability – System Suitability solution and Standard solution** | |
| Sample: Standard solution and System Suitability solution: | |

- Resolution (Rs): NLT 1.8 between Chloroquine and Hydroxychloroquine for System Suitability solution
- Relative Standard Deviation: NMT 1.5 % for Standard solution (5 replicate injections)

USP Tailing Factor, <621> Chromatography**




Symmetry factor ($A_S$): Also known as the "tailing factor", of a peak is calculated by:

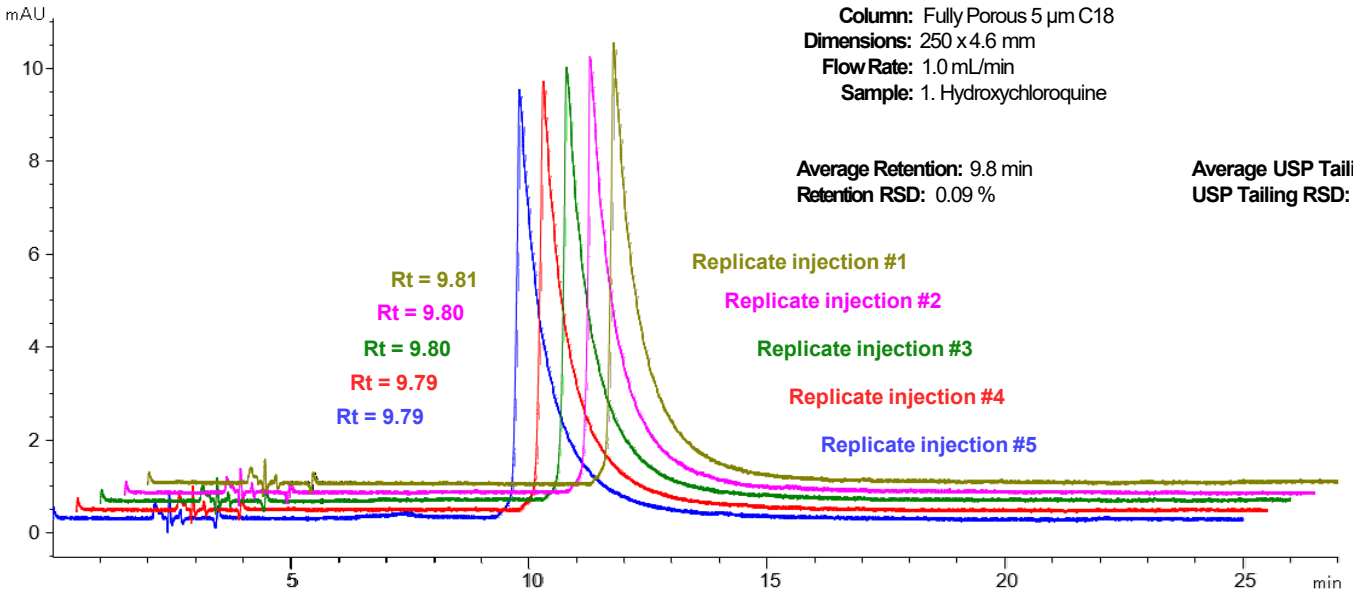
$A_S = W_{0.05}/2f$

where $W_{0.05}$ is the width of the peak at 5 % height and *f* is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5 % of the peak height from the baseline.

** General Chapter <621> "Chromatography" in United States Pharmacopeia 40 National Formulary 35 (USP 40-NF 35, United States Pharmacopeial Convention, Rockville, Maryland, 2017), p. 6.

## Method 1

Standard solution: 0.05 mg/mL Hydroxychloroquine Sulfate

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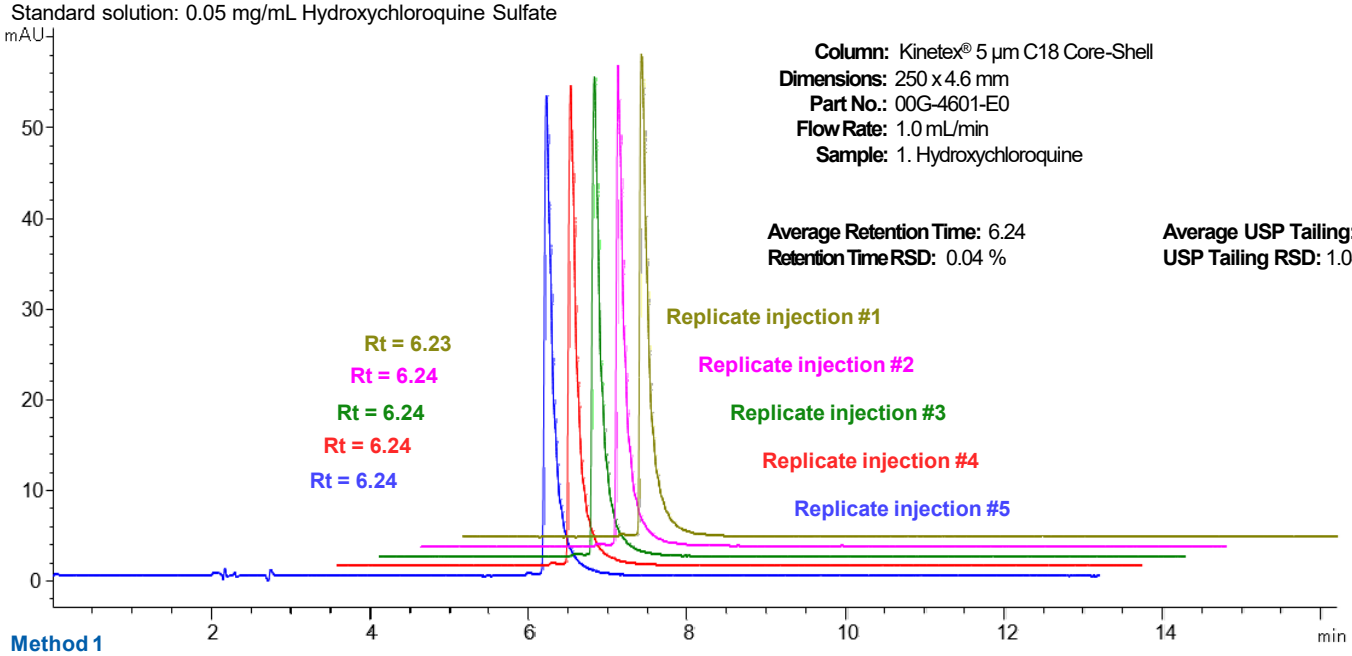
## Method 2

Standard solution: 0.05 mg/mL Hydroxychloroquine Sulfate

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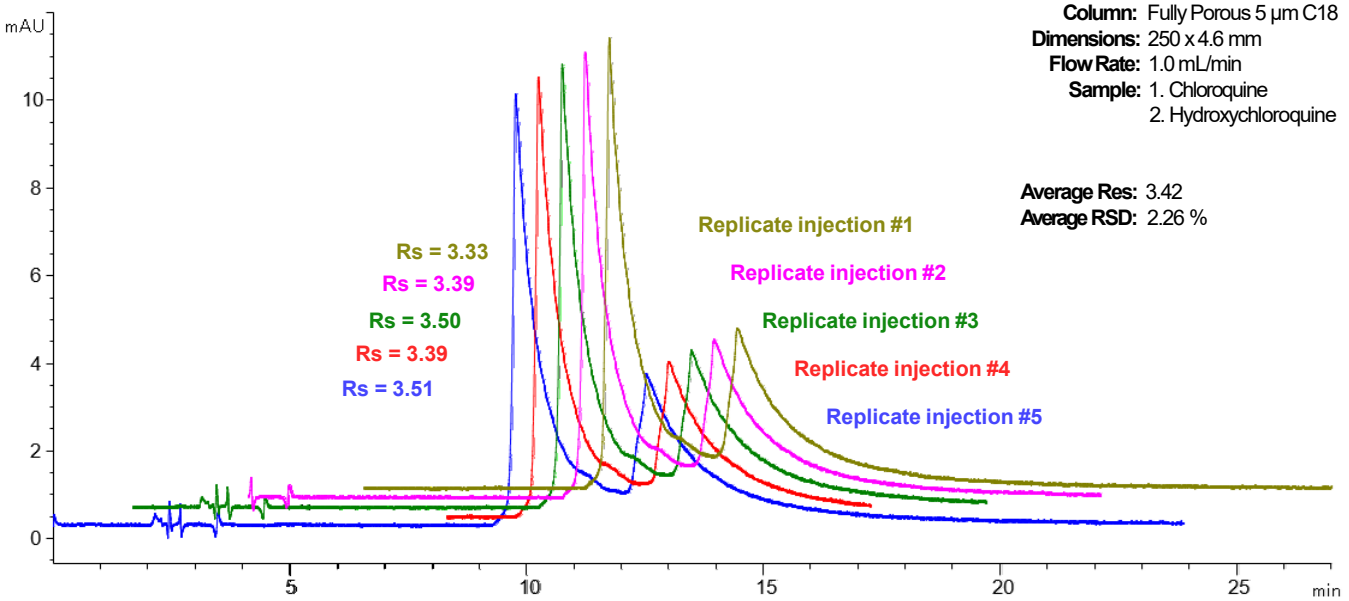
## Method 1

System Suitability solution: 0.05 mg/mL Hydroxychloroquine Sulfate & 0.05 mg/mL Chloroquine

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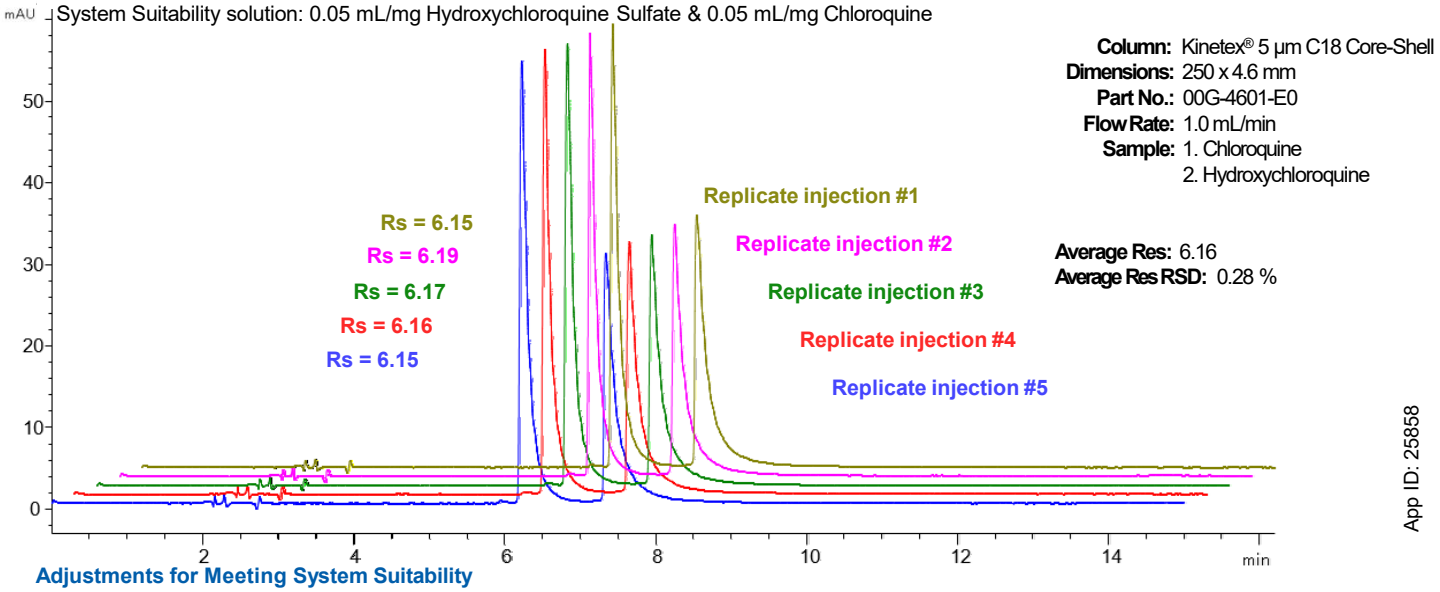
## Method 2

System Suitability solution: 0.05 mL/mg Hydroxychloroquine Sulfate & 0.05 mL/mg Chloroquine




App ID: 25858

### Adjustments for Meeting System Suitability

| Method Parameter | Allowed Adjustments (isocratic elution) | Method 1 | Method 2 |
|---|---|---|---|
| **Mobile Phase pH** | ± 0.2 units | As specified | As specified |
| **Concentration of Salts in Buffer** | ± 10% | As specified | As specified |
| **Composition of the Mobile Phase** | ± 30% Relative; cannot exceed ± 10% Absolute adjustment; cannot be reduced to zero | As specified | As specified |
| **Wavelength of Detector** | No deviations permitted | 254 nm (as specified) | As specified |
| **Injection Volume** | Can be adjusted as much as needed; must be consistent with linearity, precision, and detection requirements | 20 µL (as specified) | 10 µL (Allowed) |
| **Column Temperature** | ± 10°C | Ambient (as specified) | 26 °C (Allowed) |
| **Stationary Phase** | No change of the identity of the substituent permitted (e.g. no replacement of C18 by C8) | L1 (as specified) | As specified |
| **Column Length** | Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%* | 250 mm (as specified) | 250 mm (Allowed) |
| **Column Internal Diameter** | Can be adjusted so long as linear velocity is maintained | 4.6 mm (as specified) | 4.6 mm (Allowed) |
| **Particle Size** | Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%* | 5 µm (as specified) | 5 µm (Allowed) |
| **Flow Rate** | ± 50 % (at given ID) | 1.0 mL/min (as specified) | 1.0 mL/min** |

*Alternatively (as for the application of particle size adjustment to superficially porous particles), other L/dp combinations can be used provided that the number of theoretical plates (N) is within -25% to +50%.

**Maintained volumetric flow rate at the given column ID.

### Allowable Column Adjustments: L/dp Ratio -25 % to 50 %

| Column | Length (mm) | ID (mm) | dp (µm) | L/dp | Allowable Range |
|---|---|---|---|---|---|
| **Fully Porous** | 250 | 4.6 | 5 | 50,000 | 37,500 - 75,000 |
| **Core-Shell** | 250 | 4.6 | 5 | 50,000 | ALLOWED |

### Method Summary and Comparison

| | Method 1 | Method 2 |
|---|---|---|
| **Column** | Fully Porous 5 µm C18 | Kinetex 5 µm C18 |
| **Hydroxychloroquine Average Rt** | 9.8 min | 6.2 min |
| **Hydroxychloroquine Average Rs** | 3.42 | 6.16 |
| **Standard Solution Hydroxychloroquine Peak Area** | 391.6 | 412.8 |
| **Standard Solution Hydroxychloroquine Peak Area RSD (n=5)** | 2.26 % | 0.28 % |
| **Backpressure (Bar)** | 176 | 190 |

## Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/ChatNow.


**Australia**
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

**Austria**
t: +43 (0)1-319-1301
anfrage@phenomenex.com

**Belgium**
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

**Canada**
t: +1 (800) 543-3681
info@phenomenex.com

**China**
t: +86 400-606-8099
cninfo@phenomenex.com

**Denmark**
t: +45 4824 8048
nordicinfo@phenomenex.com

**Finland**
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

**France**
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

**Germany**
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

**India**
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

**Ireland**
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

**Italy**
t: +39 051 6327511
italiainfo@phenomenex.com

**Luxembourg**
t: +31 (0)30-2418700
nlinfo@phenomenex.com

**Mexico**
t: 01-800-844-5226
tecnicomx@phenomenex.com

**The Netherlands**
t: +31 (0)30-2418700
nlinfo@phenomenex.com

**New Zealand**
t: +64 (0)9-4780951
nzinfo@phenomenex.com

**Norway**
t: +47 810 02 005
nordicinfo@phenomenex.com

**Poland**
t: +48 (12) 881 0121
pl-info@phenomenex.com

**Portugal**
t: +351 221 450 488
ptinfo@phenomenex.com

**Singapore**
t: +65 800-852-3944
sginfo@phenomenex.com

**Spain**
t: +34 91-413-8613
espinfo@phenomenex.com

**Sweden**
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

**Switzerland**
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

**United Kingdom**
t: +44 (0)1625-501367
ukinfo@phenomenex.com

**USA**
t: +1 (310) 212-0555
info@phenomenex.com

**All other countries/regions**
**Corporate Office USA**
t: +1 (310) 212-0555
info@phenomenex.com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