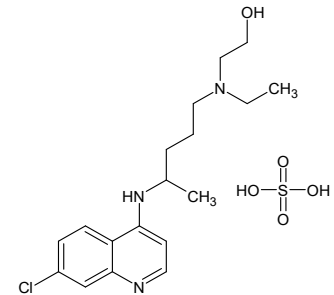


# APPLICATIONS

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

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Monograph: Hydroxychloroquine Sulfate  
pK<sub>a</sub>: 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

<b>Standard Stock Solution</b>	Dissolve 1.0 mg/mL of USP Hydroxychloroquine Sulfate RS in <i>Diluent</i>
<b>Standard Solution</b>	0.05 mg/mL of USP Hydroxychloroquine Sulfate RS from <i>Standard Stock solution</i> in <i>Mobile Phase</i>
<b>System Suitability Stock Solution</b>	Dissolve 1.0 mg/mL of Chloroquine Phosphate in Methanol
<b>System Suitability Solution</b>	Transfer 5.0 mL of the <i>System Suitability Stock solution</i> into 100-mL volumetric flask, add 5.0 mL of <i>Standard Stock solution</i> , and dilute to volume with <i>Mobile Phase</i>
<b>Diluent</b>	Mixture of Methanol:Water (1:1)

### Column

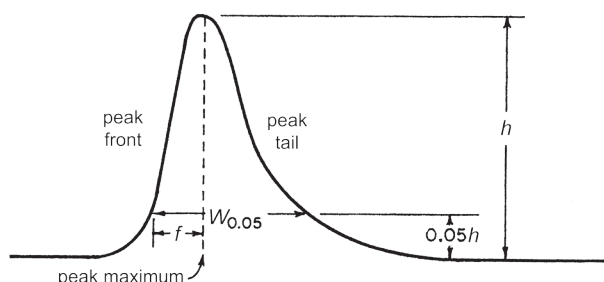
<b>Size</b>	Method 1: 250 x 4.6 mm, Method 2: 250 x 4.6 mm
<b>Stationary Phase</b>	Method 1: Fully Porous 5 µm C18, Method 2: Kinetex 5 µm C18
<b>Temperature</b>	26 °C
<b>Mobile Phase (premixed)</b>	A = Methanol, Acetonitrile, water, and phosphoric acid (100:100:800:2). Mix and add 96 mg of sodium 1-pentanesulfonate in the resulting solution, and filter Mobile Phase
<b>Isocratic</b>	Isocratic: (100:100:800:2, Methanol: Acetonitrile: Water: Phosphoric Acid) Total Run Time: 20 min
<b>Flow Rate</b>	1.0 mL/min
<b>Detector</b>	UV @ 254 nm
<b>Injection Volume</b>	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<sub>S</sub>): Also known as the “tailing factor”, of a peak is calculated by:

$$A_S = W_{0.05}/2f$$

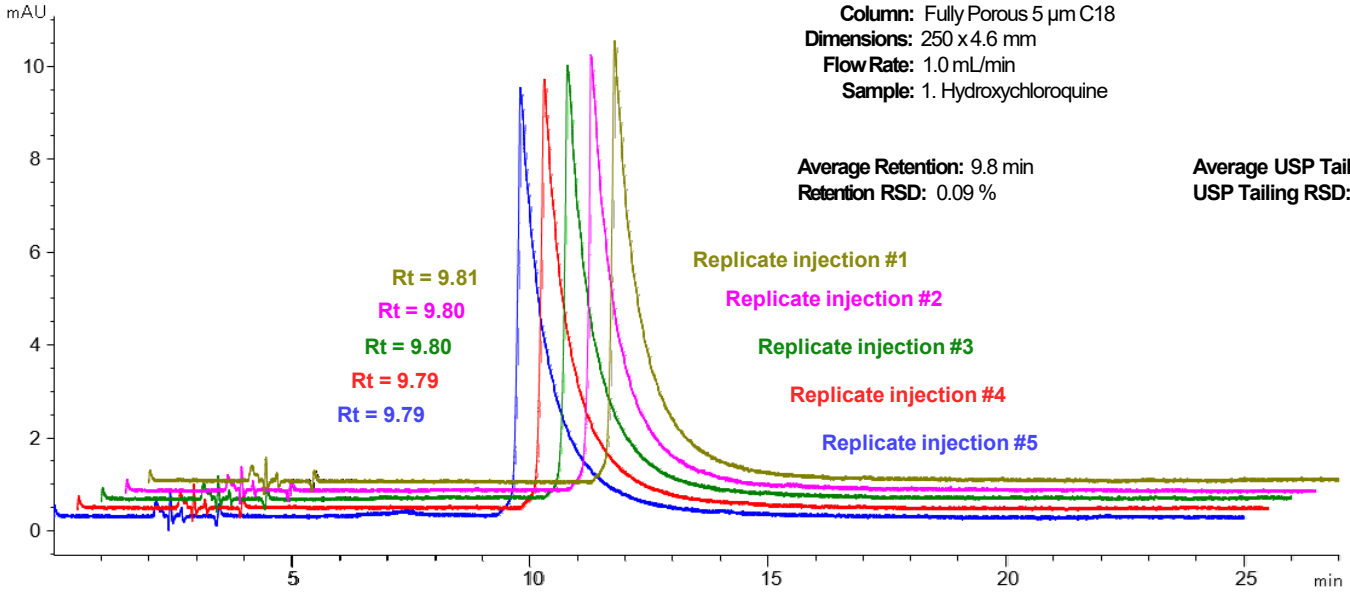
where W<sub>0.05</sub> is the width of the peak at 5% height and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

\*\* 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.

# APPLICATIONS

## Method 1

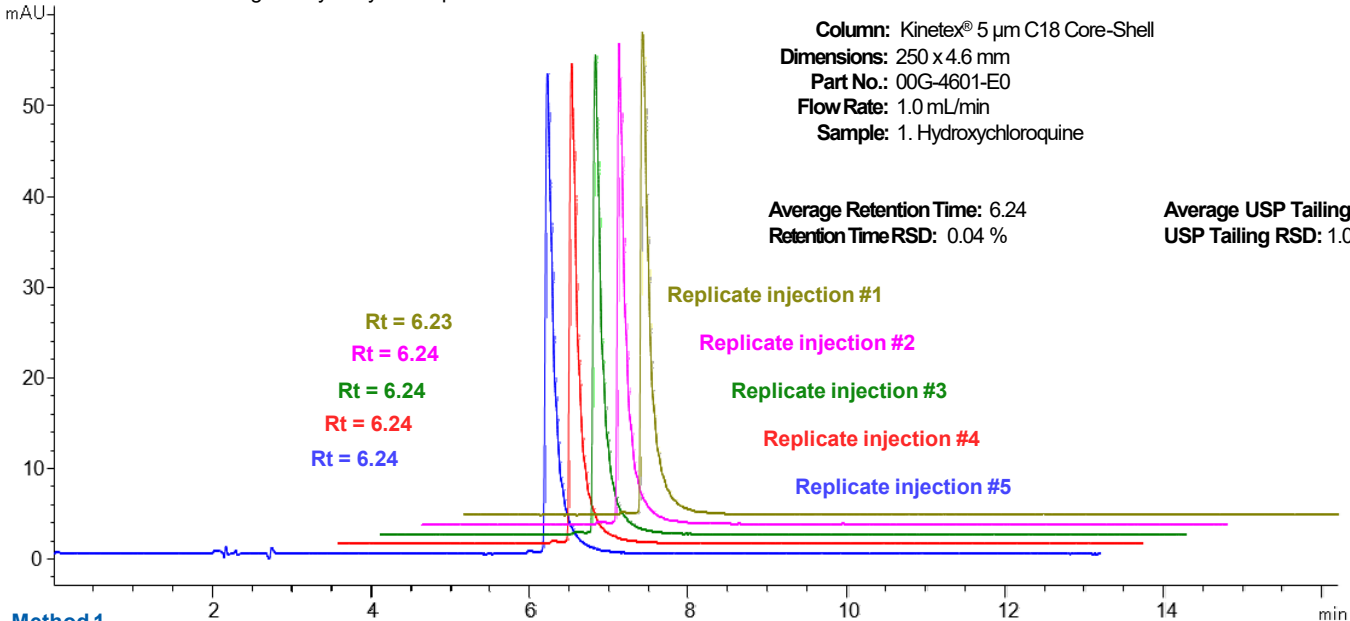
Standard solution: 0.05 mg/mL Hydroxychloroquine Sulfate



App ID: 25859

## Method 2

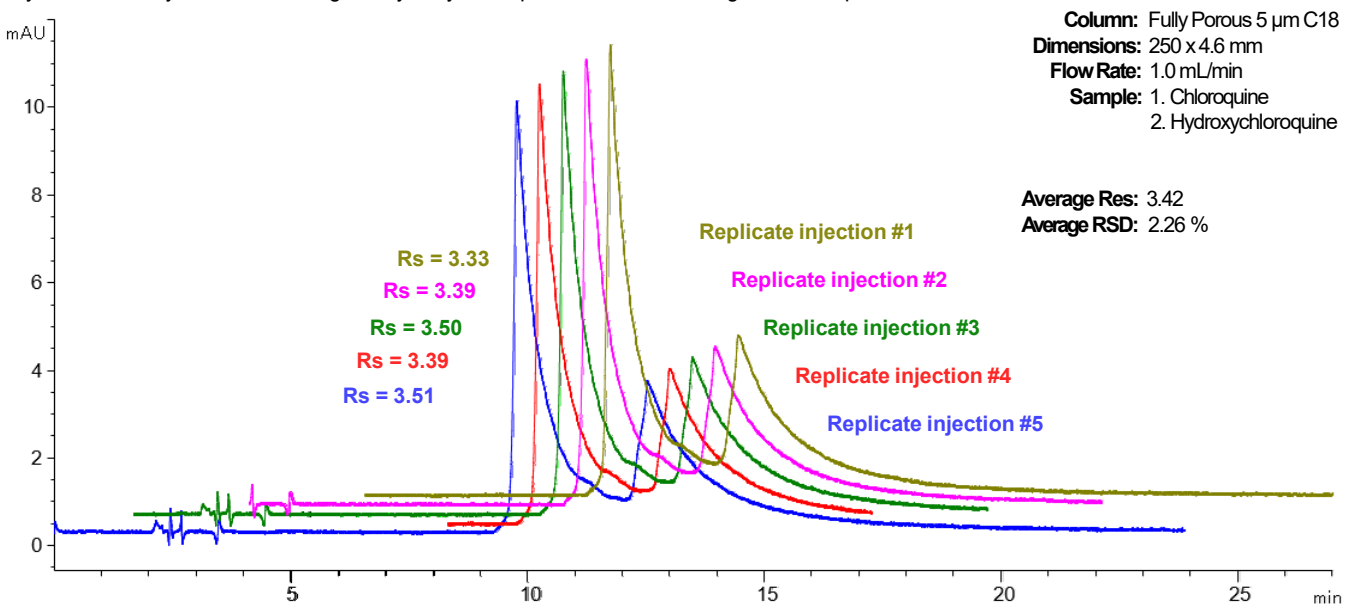
Standard solution: 0.05 mg/mL Hydroxychloroquine Sulfate



App ID: 25857

## Method 1

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

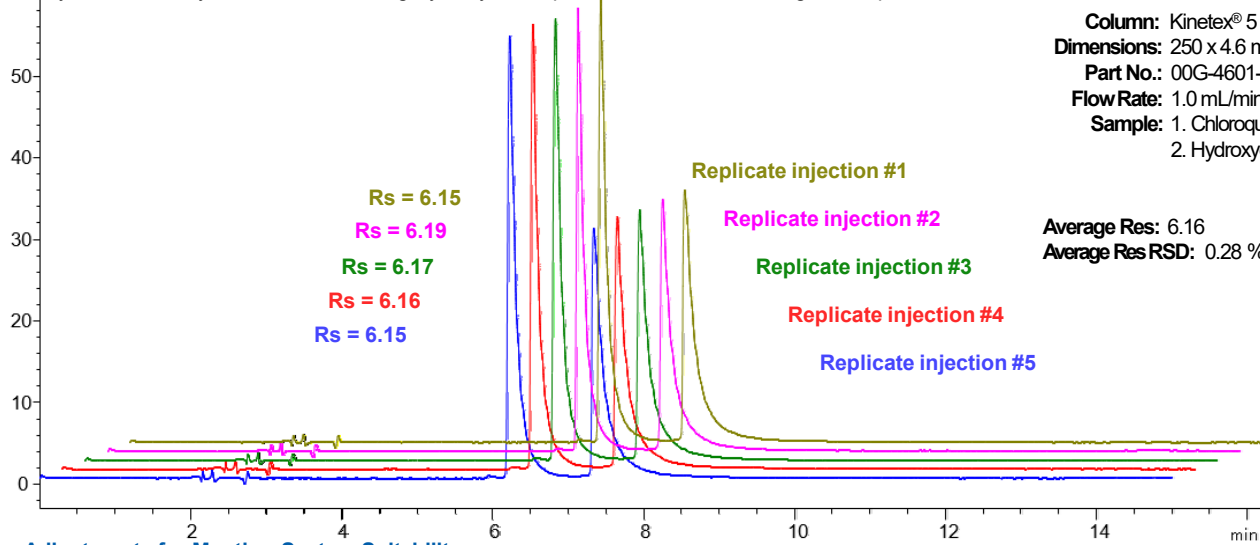


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# APPLICATIONS

## Method 2

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



**Column:** Kinetex<sup>®</sup> 5  $\mu$ m C18 Core-Shell  
**Dimensions:** 250 x 4.6 mm  
**Part No.:** 00G-4601-E0  
**Flow Rate:** 1.0 mL/min  
**Sample:** 1. Chloroquine  
2. Hydroxychloroquine

**Average Res:** 6.16  
**Average Res RSD:** 0.28 %

### Adjustments for Meeting System Suitability

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1	Method 2
Mobile Phase pH	$\pm 0.2$ units	As specified	As specified
Concentration of Salts in Buffer	$\pm 10\%$	As specified	As specified
Composition of the Mobile Phase	$\pm 30\%$ Relative; cannot exceed $\pm 10\%$ 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 $\mu$ L (as specified)	10 $\mu$ L (Allowed)
Column Temperature	$\pm 10^\circ\text{C}$	Ambient (as specified)	26 $^\circ\text{C}$ (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 $\mu$ m (as specified)	5 $\mu$ m (Allowed)
Flow Rate	$\pm 50\%$ (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 ( $\mu$ m)	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
<b>Column</b>	Fully Porous 5 $\mu$ m C18	Kinetex 5 $\mu$ m C18
<b>Hydroxychloroquine Average Rt</b>	9.8 min	6.2 min
<b>Hydroxychloroquine Average Rs</b>	3.42	6.16
<b>Standard Solution Hydroxychloroquine Peak Area</b>	391.6	412.8
<b>Standard Solution Hydroxychloroquine Peak Area RSD (n=5)</b>	2.26 %	0.28 %
<b>Backpressure (Bar)</b>	176	190

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