

# APPLICATIONS

## USP Assay and Organic Impurities (LC-UV) for Chloroquine Phosphate on Luna® 5 µm C18(2) and Kinetex® 5 µm C18 150 x 4.6 mm HPLC Columns

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### Overview

Chloroquine phosphate is a 4-aminoquinoline compound used to prevent and treat malaria, and is currently being studied for its anti-viral effects as a potential treatment for COVID-19.

In this application for the LC-UV assay of chloroquine phosphate, the mobile phase conditions outlined by the USP for Chloroquine Phosphate were used. Two C18 (USP L1) columns containing different particle morphologies, but the same 150 x 4.6 mm dimension and 5 µm particle size, were compared. The standard solution (0.3 mg/mL of Chloroquine Phosphate in mobile phase) and system suitability solution (2.0 µg/mL each of USP Chloroquine Phosphate RS, USP Phenol RS, USP Hydroxychloroquine Sulfate RS, USP Chloroquine Related Compound A RS, USP Chloroquine Related Compound D RS, USP Chloroquine Related Compound E RS, and USP Chloroquine Related Compound G RS in Mobile phase) were prepared in accordance with the USP monograph. The resulting chromatograms and system suitability results are summarized below.

The [Kinetex 5 µm C18](#) column based on core-shell particle technology gave higher efficiency and resolution versus the fully porous [Luna 5 µm C18\(2\)](#) column of the same dimension and particle size. However, a minor selectivity difference was also observed with the Kinetex C18 column that resulted in a reversal of the elution order of Hydroxychloroquine sulfate and Chloroquine Related Compound D. This elution order was confirmed by injection of individual standards for all sample compounds. The expected elution order per the USP monograph for all compounds were (relative retention times): Phenol (0.2), Chloroquine Related Compound G (0.27), Chloroquine Related Compound D (0.42), Hydroxychloroquine Sulfate (0.49), Chloroquine Related Compound A (0.73), Chloroquine Phosphate (1.0), and Chloroquine Related Compound E (1.5). This reversal in elution order for Hydroxychloroquine Sulfate and Chloroquine Related Compound D did result in improved separation of these two compounds on the Kinetex C18 column, which would be advantageous for accurate quantitation of each compound.

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## LC-UV Conditions

**Column:** Luna<sup>®</sup> 5  $\mu$ m C18(2)  
Kinetex<sup>®</sup> 5  $\mu$ m C18

**Dimension:** 150 x 4.6 mm

**Mobile Phase:** 0.4 % Triethylamine in Methanol and Buffer (70:30)

**Buffer:** 1.4 g/L of anhydrous dibasic sodium phosphate in water.  
Adjust with 10 % phosphoric acid to a pH of 3.0

**Flow Rate:** 1.0 mL/min

**Temperature:** Ambient (26 °C)

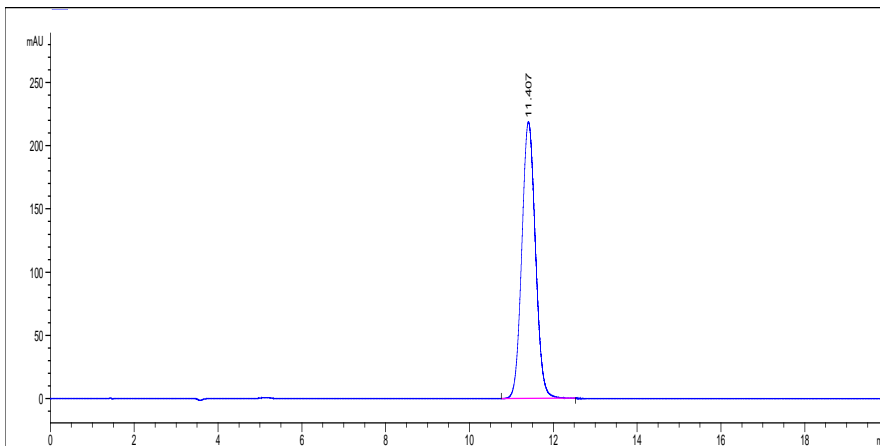
**Detector:** UV @ 260 nm

**Injection:** 20  $\mu$ L

**Run Time:** 20 minutes

**Sample:** Chloroquine Phosphate

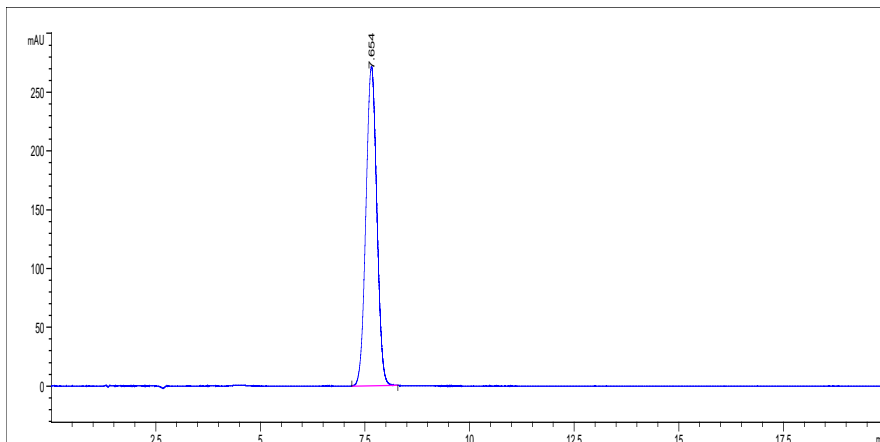
### Luna 5 $\mu$ m C18(2) (00F-4252-E0)



System Suitability		
Compound	Tailing Factor NMT 2.0	RSD (peak area, 5 replicates) NMT 2.0%
Chloroquine Phosphate	1.07	0.13%

App ID 25922

### Kinetex 5 $\mu$ m C18 (00F-4601-E0)



System Suitability		
Compound	Tailing Factor NMT 2.0	RSD (peak area, 5 replicates) NMT 2.0%
Chloroquine Phosphate	1.02	0.09%

App ID 25924



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## LC-UV Conditions

**Column:** Luna<sup>®</sup> 5 µm C18(2)  
Kinetex<sup>®</sup> 5 µm C18

**Dimension:** 150 x 4.6 mm

**Mobile Phase:** 0.4 % Triethylamine in Methanol and Buffer (70:30)

**Buffer:** 1.4 g/L of anhydrous Dibasic Sodium phosphate in Water.  
Adjust with 10 % Phosphoric Acid to a pH of 3.0

**Flow Rate:** 1.0 mL/min

**Temperature:** Ambient (26 °C)

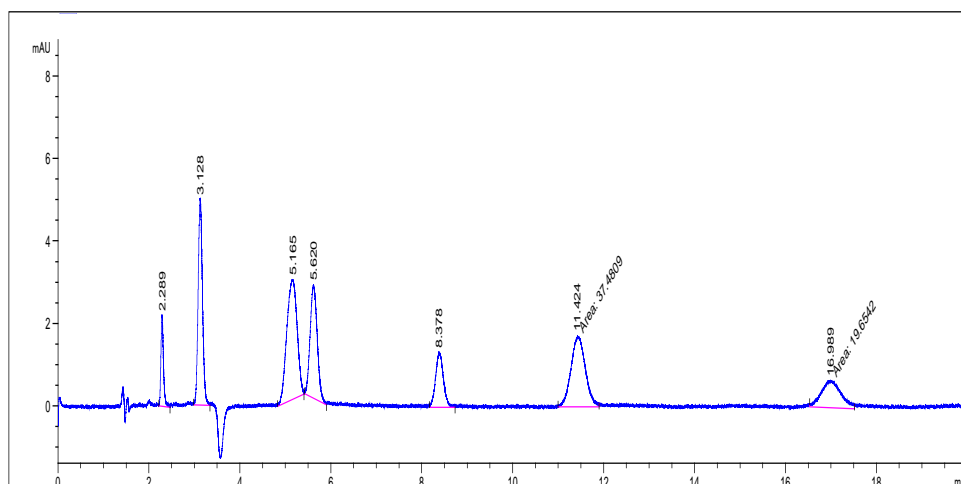
**Detector:** UV @ 260 nm

**Injection:** 20 µL

**Run Time:** 20 minutes

**Sample:** (see chromatograms for peak identification)

### Luna 5 µm C18(2) (00F-4252-E0)

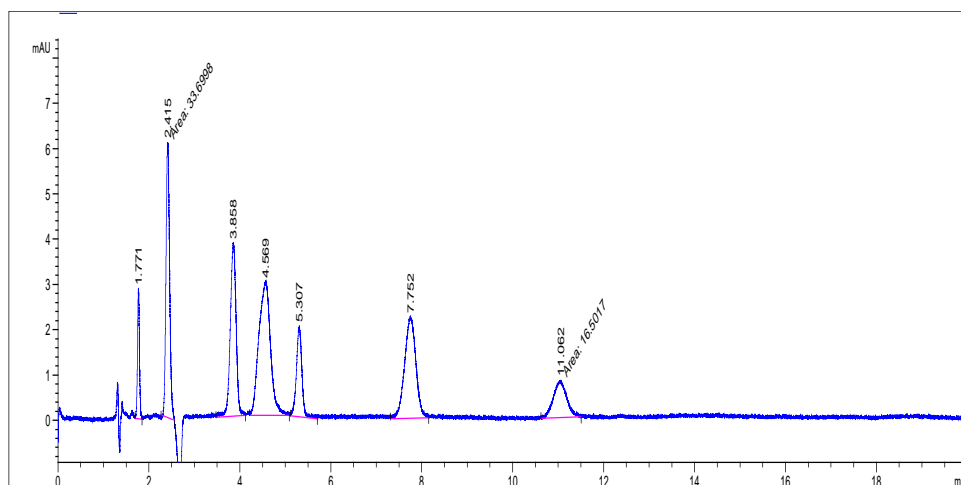


App ID 25923

1. Phenol
2. Related Compound G
3. Related Compound D
4. Hydroxychloroquine sulfate
5. Related Compound A
6. Chloroquine phosphate
7. Related Compound E

System Suitability	
Compound	Resolution NLT 2.0
Related Compound A	
Chloroquine Phosphate	6.93

### Kinetex 5 µm C18 (00F-4601-E0)



App ID 25925

1. Phenol
2. Related Compound G
3. Hydroxychloroquine sulfate
4. Related Compound D
5. Related Compound A
6. Chloroquine phosphate
7. Related Compound E

System Suitability	
Compound	Resolution NLT 2.0
Related Compound A	
Chloroquine Phosphate	7.39

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