

# APPLICATIONS

## Rapid Sample Extraction of Chloroquine, Hydroxychloroquine, and Desethylchloroquine from Serum for LC-MS/MS Analysis

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### Overview

In this fast analysis, a direct, in-plate protein precipitation and filtration is applied to increase the throughput of LC-MS/MS measurement for chloroquine, hydroxychloroquine and the metabolite desethylchloroquine from serum. The automatable sample extraction procedure produced excellent recovery values.

### Sample Preparation

<b>96-Well Plate:</b>	Impact <sup>TM</sup> Protein Precipitation Plate
<b>Part No.:</b>	<a href="#">CE0-7565</a>
<b>Dispense:</b>	450 µL of Methanol into each well
<b>Add:</b>	150 µL serum into each well
<b>Vortex:</b>	2 minutes
<b>Filter:</b>	Place an empty collection plate below the Impact protein precipitation plate and pass sample through by applying vacuum, positive pressure, or centrifuge
<b>Dilute:</b>	Dilute filtrate 1:5 with aqueous 0.1 % Formic acid
<b>Inject:</b>	Inject onto LC-MS/MS

### LC-MS/MS Conditions

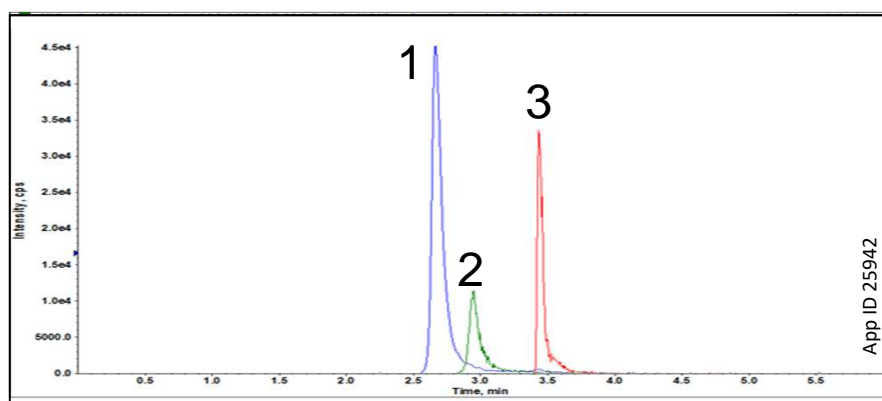
<b>Column:</b>	Kinetex <sup>®</sup> 2.6 µm F5														
<b>Dimension:</b>	50 x 2.1 mm														
<b>Part No.:</b>	<a href="#">00B-4723-AN</a>														
<b>Mobile Phase:</b>	A: 0.1 % Formic Acid in Water B: Methanol														
<b>Gradient:</b>	<table> <tr> <th>Time (min)</th><th>%B</th></tr> <tr><td>0.0</td><td>2</td></tr> <tr><td>2.0</td><td>7</td></tr> <tr><td>3.0</td><td>15</td></tr> <tr><td>3.2</td><td>40</td></tr> <tr><td>4.2</td><td>95</td></tr> <tr><td>6.0</td><td>2</td></tr> </table>	Time (min)	%B	0.0	2	2.0	7	3.0	15	3.2	40	4.2	95	6.0	2
Time (min)	%B														
0.0	2														
2.0	7														
3.0	15														
3.2	40														
4.2	95														
6.0	2														
<b>Flow Rate:</b>	0.5 mL/min														
<b>Temperature:</b>	20 °C														
<b>Detection:</b>	MS/MS (MRM, SCIEX <sup>®</sup> 4000 QTRAP <sup>®</sup> )														
<b>Sample:</b>	1. Desethylchloroquine 2. Hydroxychloroquine 3. Chloroquine														

### Recovery Value (%) and CVs (%)

	Recovery	CV (n=6)
Chloroquine (ClQ)	97.1	10.6
Hydroxychloroquine (HClQ)	95.8	16.9
Desethylchloroquine (DesClQ)	103.3	16.2

### Advantages of the Workflow

1. High-throughput
  - a. 96-well format allows for rapid, 10 minutes sample preparation of 96 samples at once
  - b. Analyze samples by LC-MS/MS in 6 minutes
2. Easily automatable sample preparation
  - a. Effectively hold the organic solvent prior to and during the protein precipitation for easy sample cleanup
  - b. Remove precipitated proteins while sample and Chloroquine and its metabolites pass through and into a collection plate, no transfer steps required



# APPLICATIONS

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