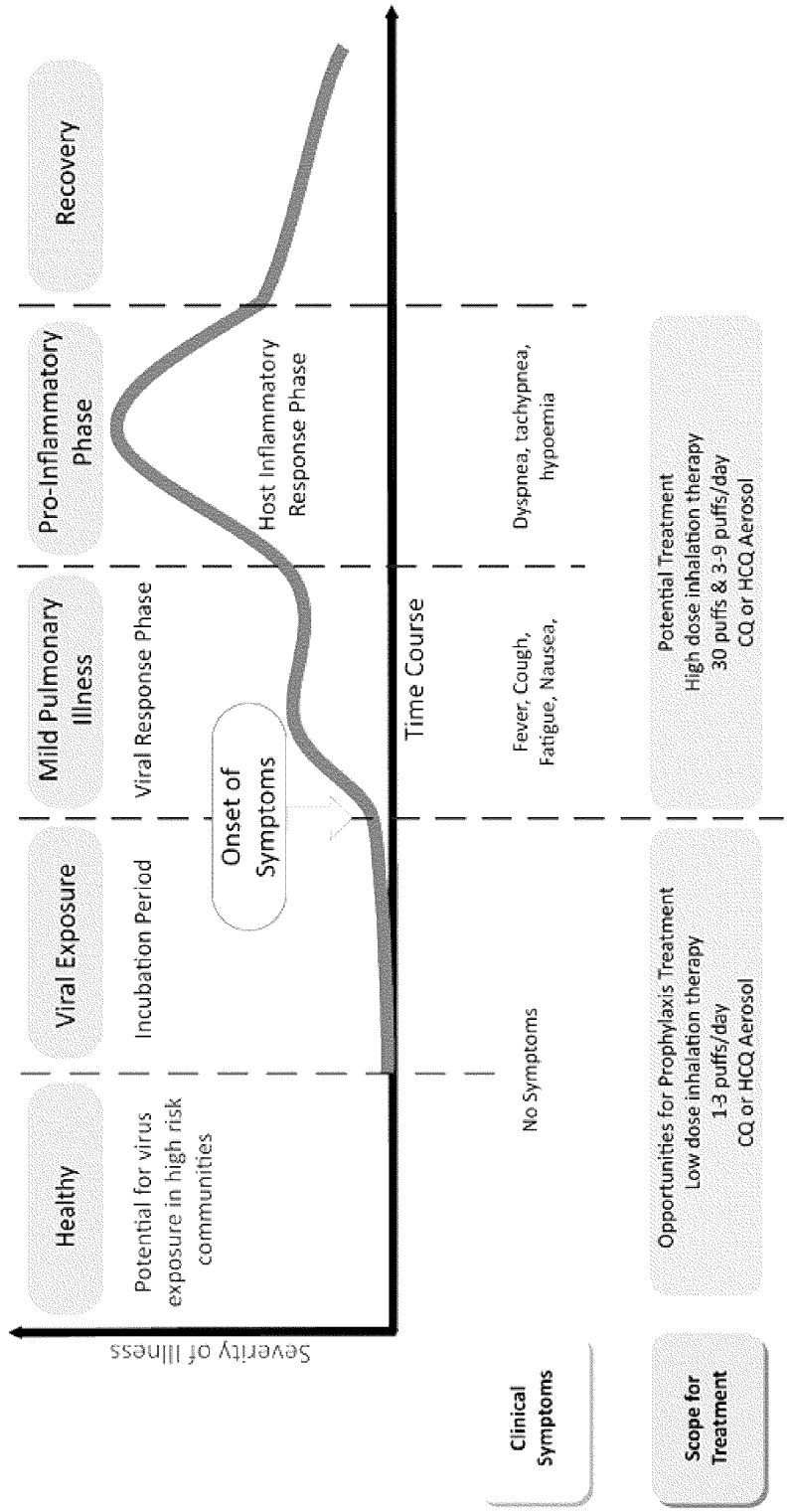

Healthy
Viral Exposure
Mild Pulmonary Illness
Pro-Inflammatory Phase
Recovery
Potential for virus exposure in high risk communities
Incubation Period
Viral Response Phase
Onset of Symptoms
Host Inflammatory Response Phase
Severity of Illness
Time Course
No Symptoms
Fever, Cough, Fatigue, Nausea,
Dyspnea, tachypnea, hypoemia
Opportunities for Prophylaxis Treatment
Low dose inhalation therapy
1-3 puffs/day
CQ or HCQ Aerosol
Potential Treatment
High dose inhalation therapy
30 puffs & 3-9 puffs/day
CQ or HCQ Aerosol
Clinical Symptoms
Scope for Treatment

Figure 1

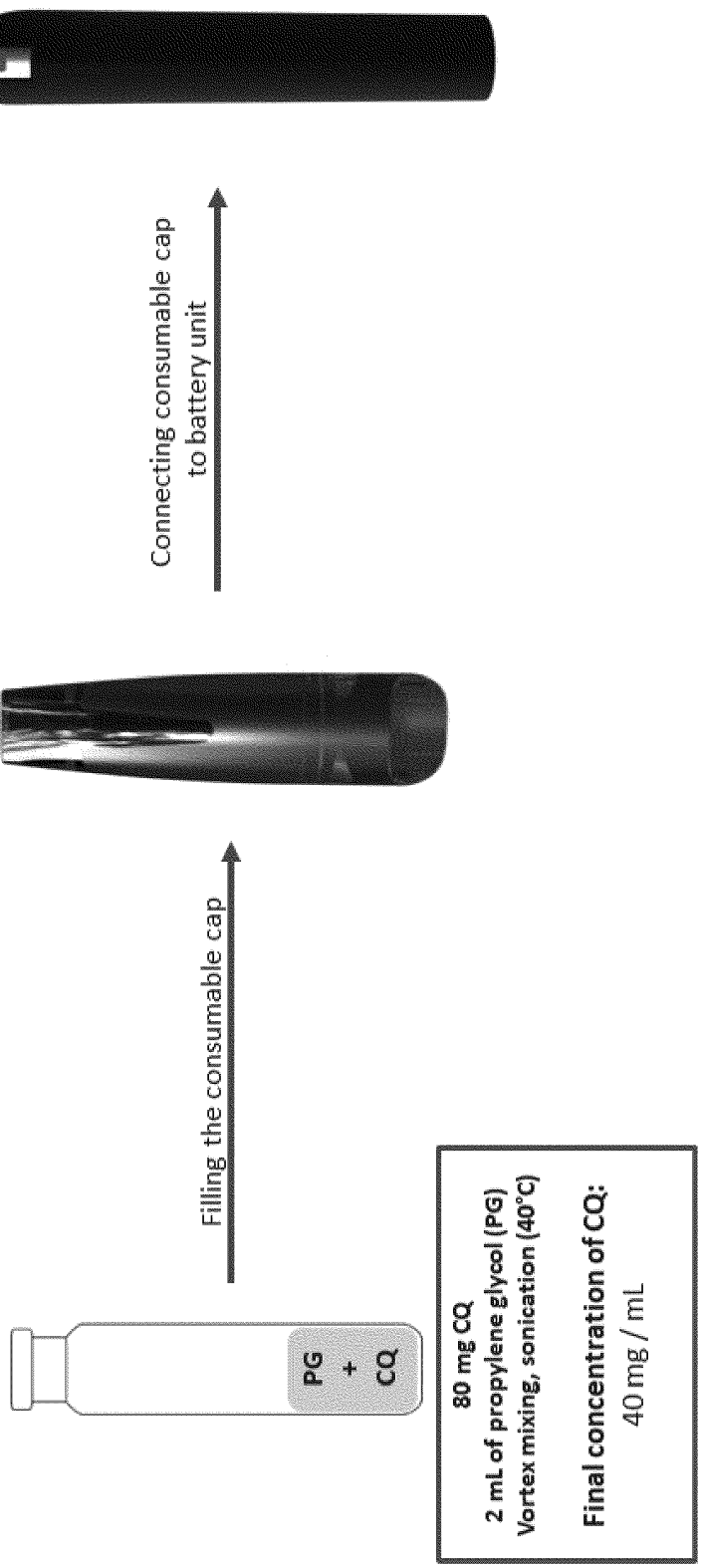

Connecting consumable cap
to battery unit
Filling the consumable cap
PG + CQ
80 mg CQ
2 mL of propylene glycol (PG)
Vortex mixing, sonication (40°C)
Final concentration of CQ:
40 mg / mL

Figure 2A

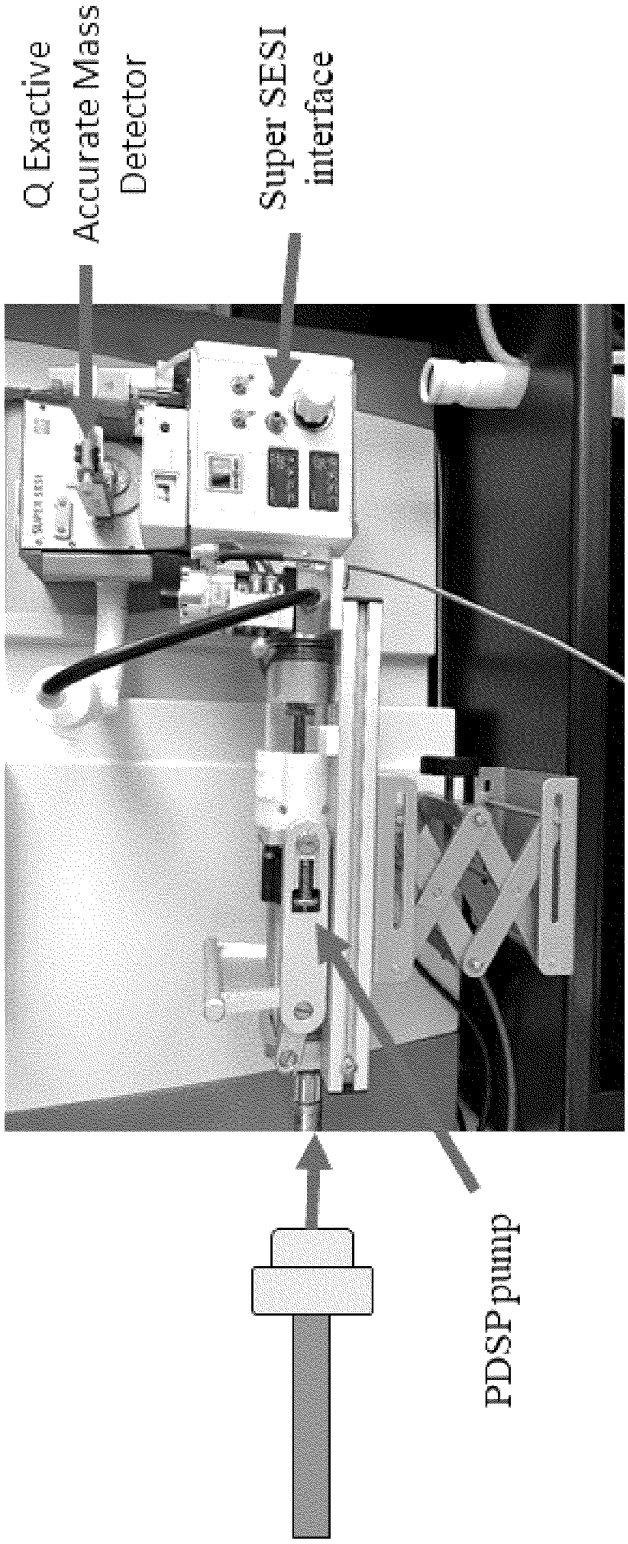

Q Exactive
Accurate Mass
Detector
Super SESI
interface
PDSP pump

Figure 2B

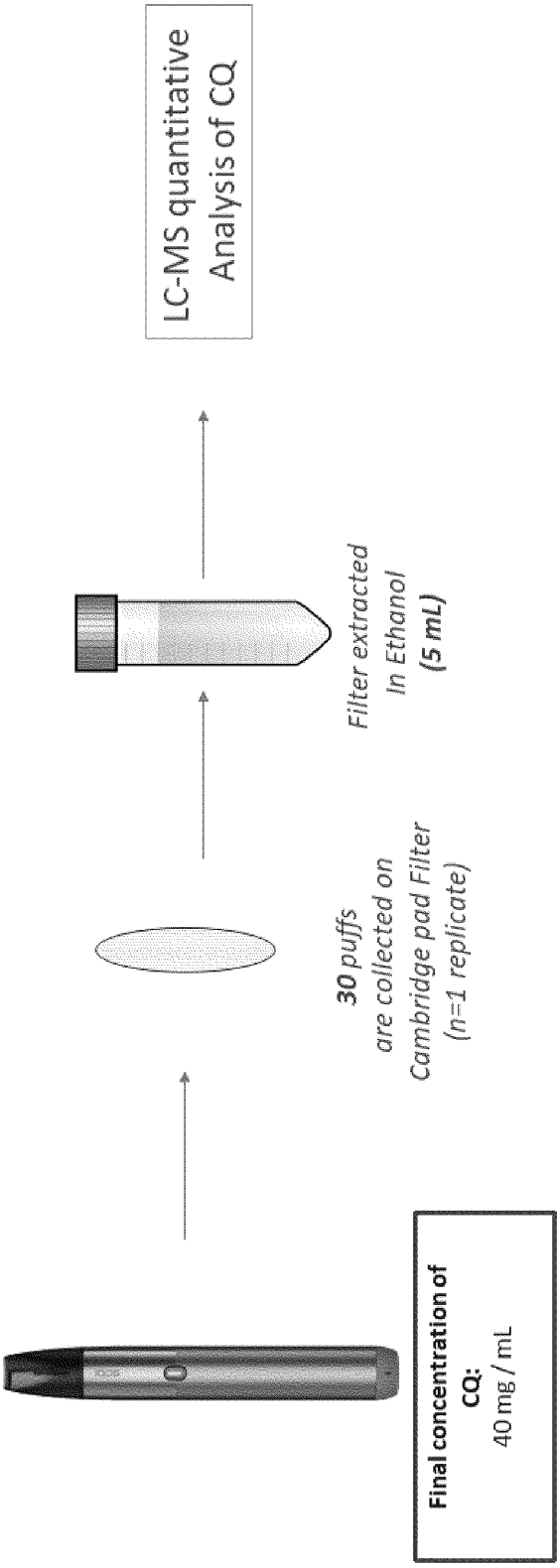

LC-MS quantitative Analysis of CQ
Filter extracted In Ethanol (5 mL)
30 puffs are collected on Cambridge pad Filter (n=1 replicate)
Final concentration of CQ:
40 mg / mL

Figure 2C

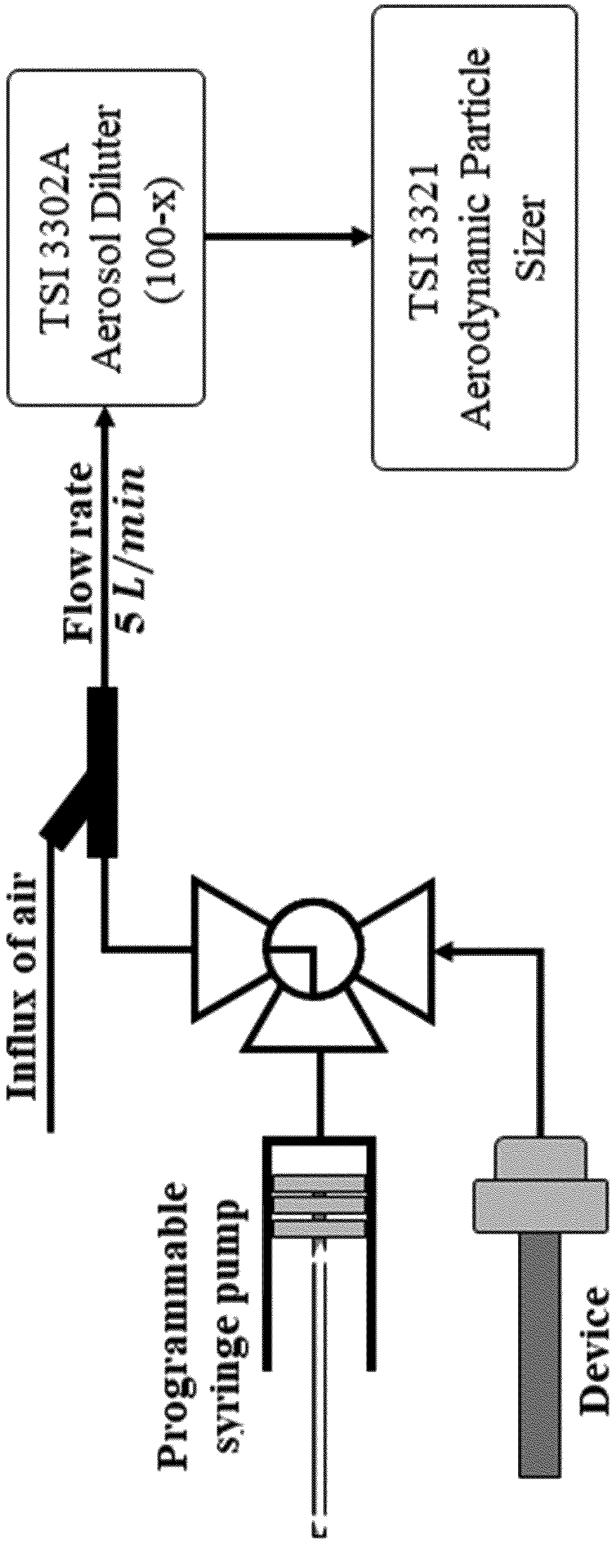

TSI 3302A
Aerosol Diluter
(100-x)
TSI 3321
Aerodynamic Particle
Sizer
Flow rate
5 L/min
Influx of air
Programmable
syringe pump
Device

Figure 3

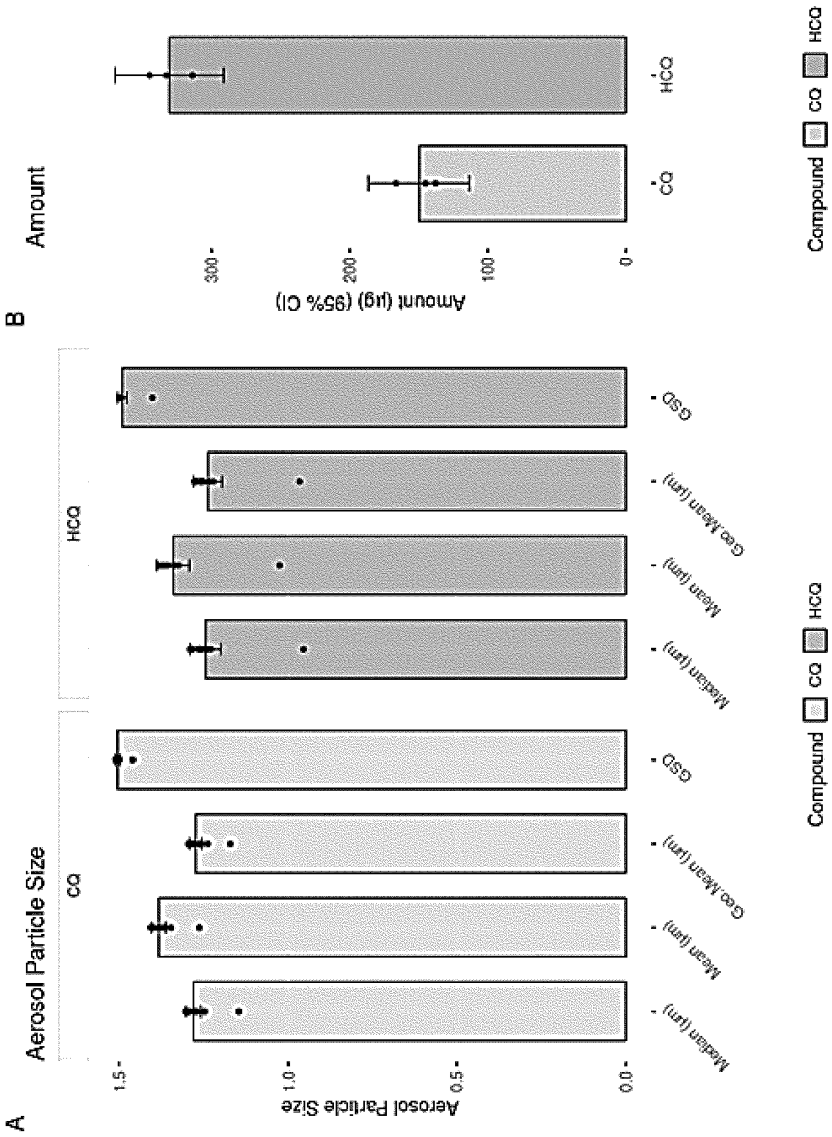

A
Aerosol Particle Size
CQ
HCQ
Median (µm)
Mean (µm)
Geo.Mean (µm)
GSD
Aerosol Particle Size
0.0
0.5
1.0
1.5
B
Amount
Amount (µg) (95% CI)
0
100
200
300
CQ
HCQ
Compound CQ HCQ
Compound CQ HCQ

Figure 4

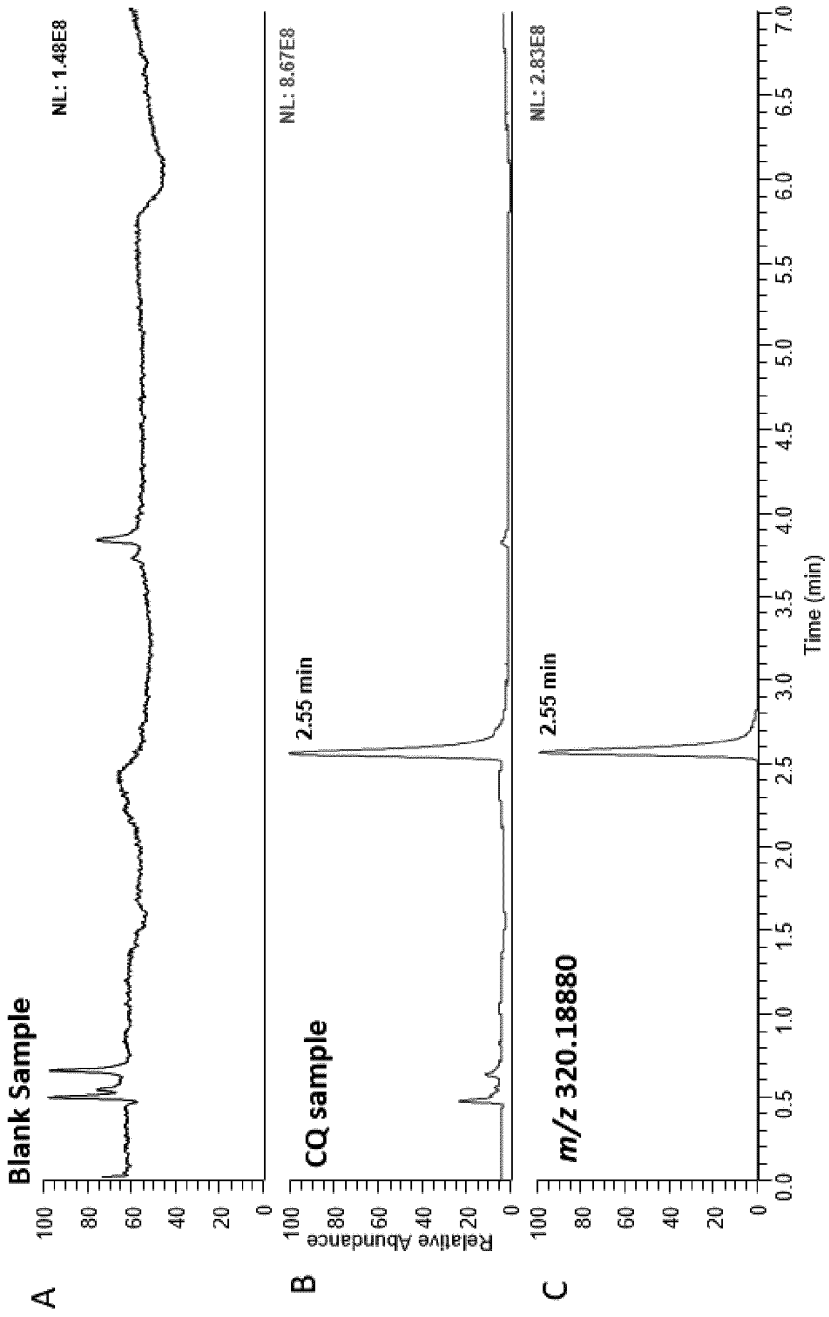

A
Blank Sample
NL: 1.48E8
B
CQ sample
NL: 8.67E8
2.55 min
C
m/z 320.18880
NL: 2.83E8
2.55 min
Relative Abundance
Time (min)

Figure 5

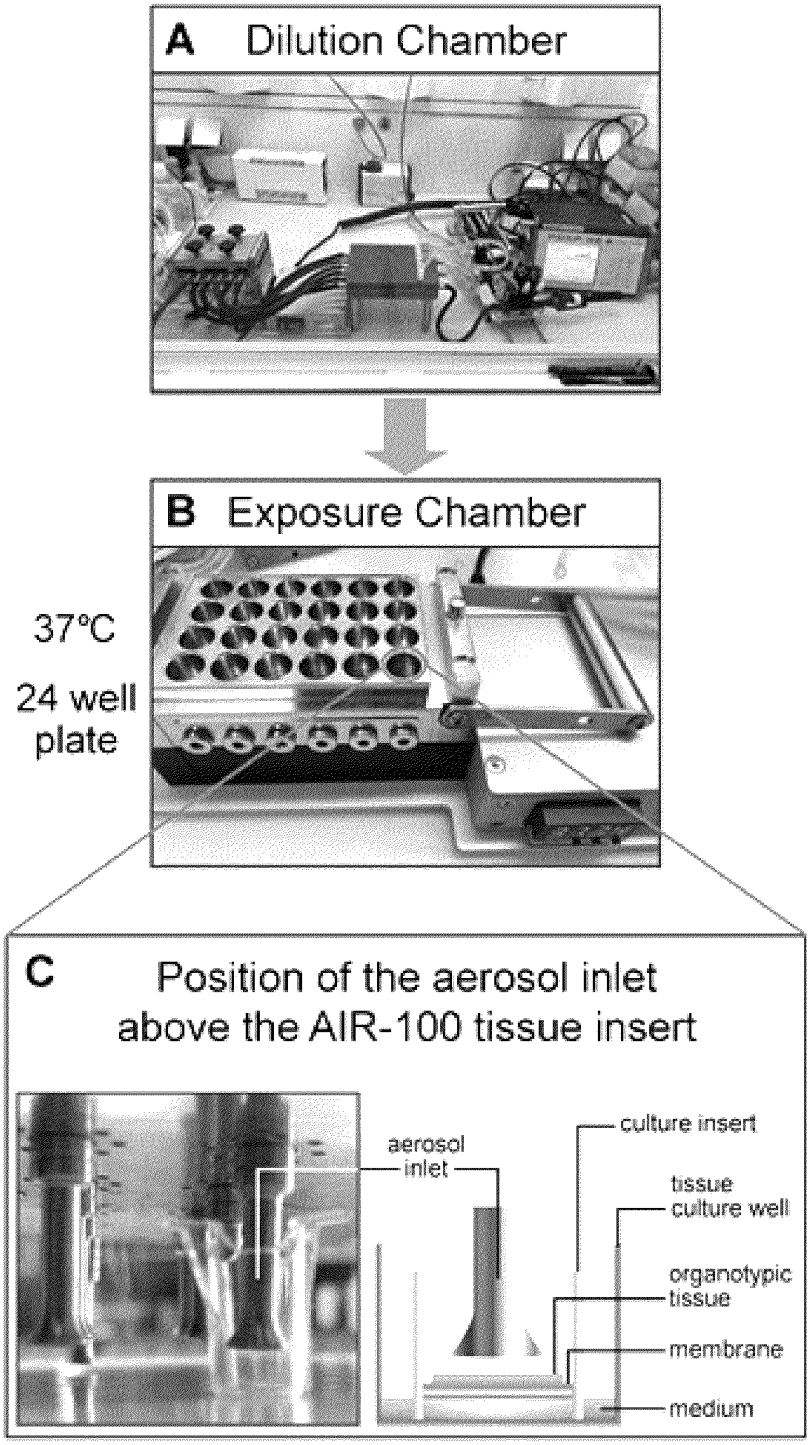

A Dilution Chamber
B Exposure Chamber
37°C
24 well plate
C Position of the aerosol inlet above the AIR-100 tissue insert
aerosol inlet
culture insert
tissue culture well
organotypic tissue
membrane
medium

Figure 6

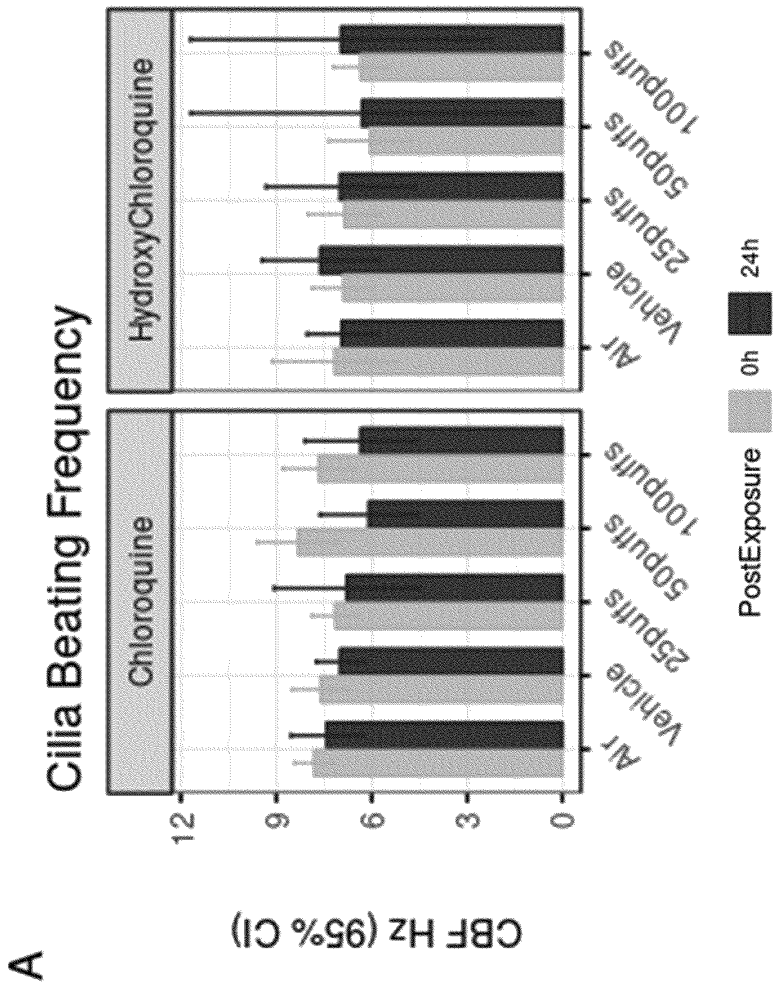

A
Cilia Beating Frequency
CBF Hz (95% CI)
0
3
6
9
12
Chloroquine
HydroxyChloroquine
Air
Vehicle
25puffs
50puffs
100puffs
PostExposure
0h
24h

Figure 7A

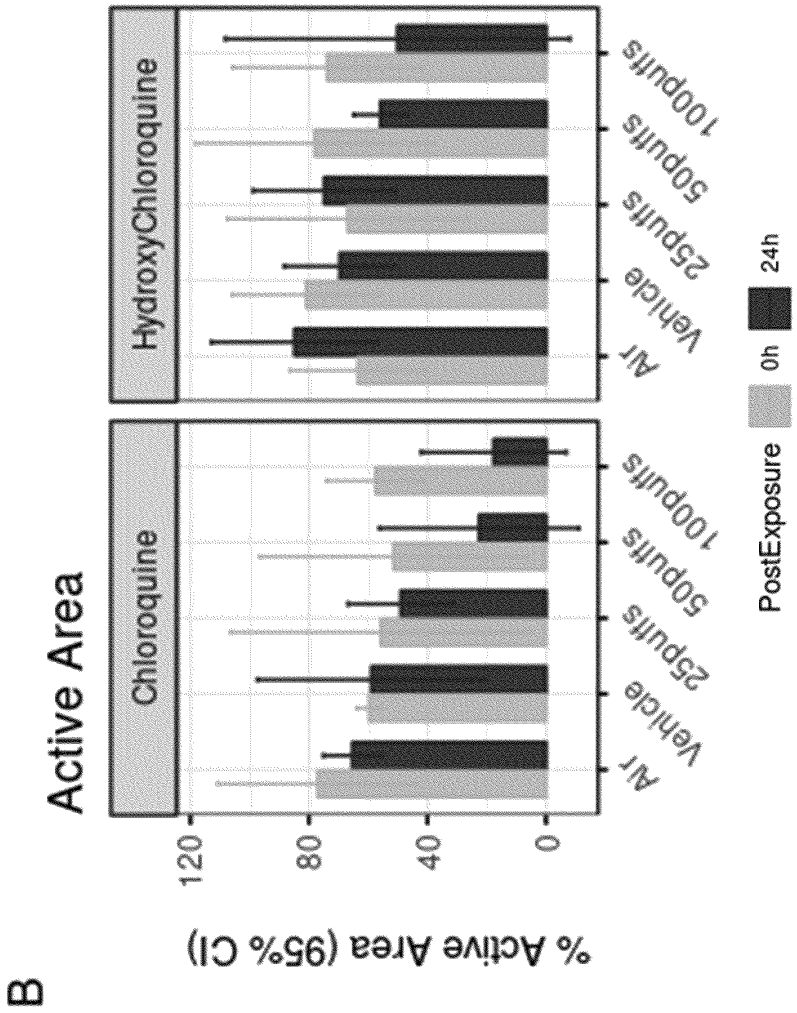

B
Active Area
% Active Area (95% CI)
120
80
40
0
Chloroquine
HydroxyChloroquine
Air
Vehicle
25pulls
50pulls
100pulls
PostExposure
0h
24h

Figure 7B

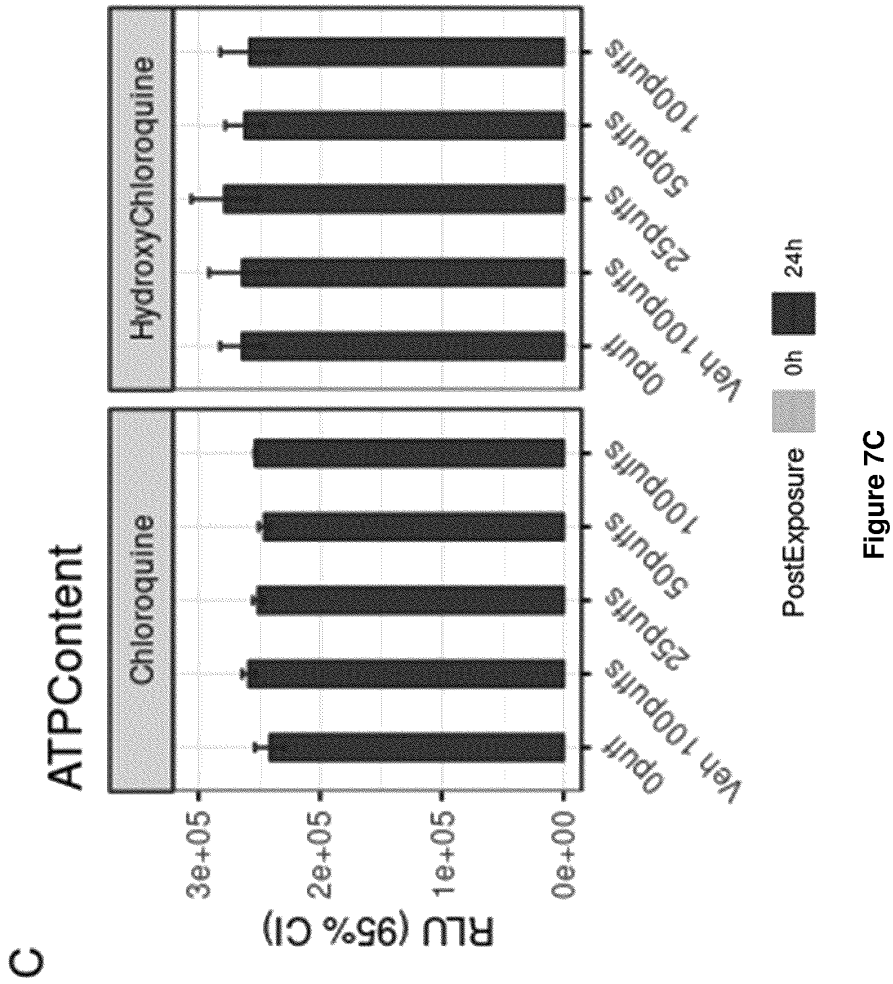

C
ATPContent
Chloroquine
HydroxyChloroquine
RLU (95% CI)
0e+00
1e+05
2e+05
3e+05
0µM
Ver 100µM
25µM
50µM
100µM
PostExposure
0h
24h

Figure 7C

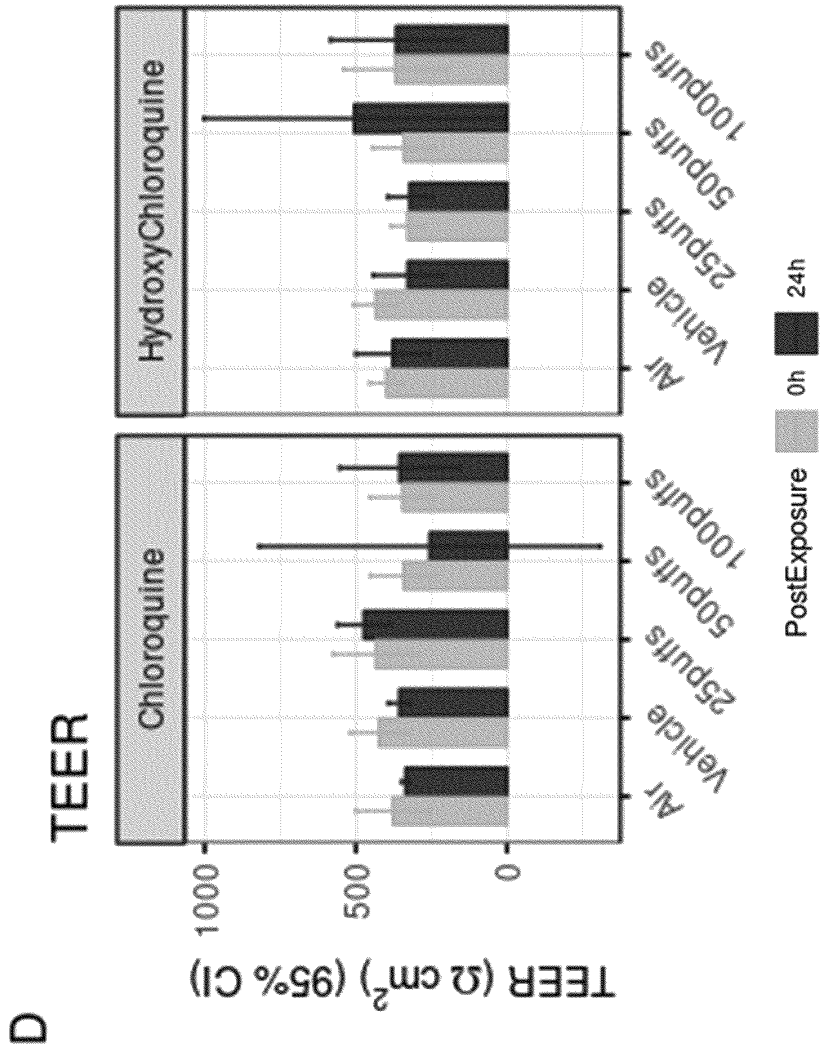

D
TEER
Chloroquine
HydroxyChloroquine
TEER (Ω cm²) (95% CI)
1000
500
0
Air
Vehicle
25puffs
50puffs
100puffs
PostExposure
0h
24h

Figure 7D

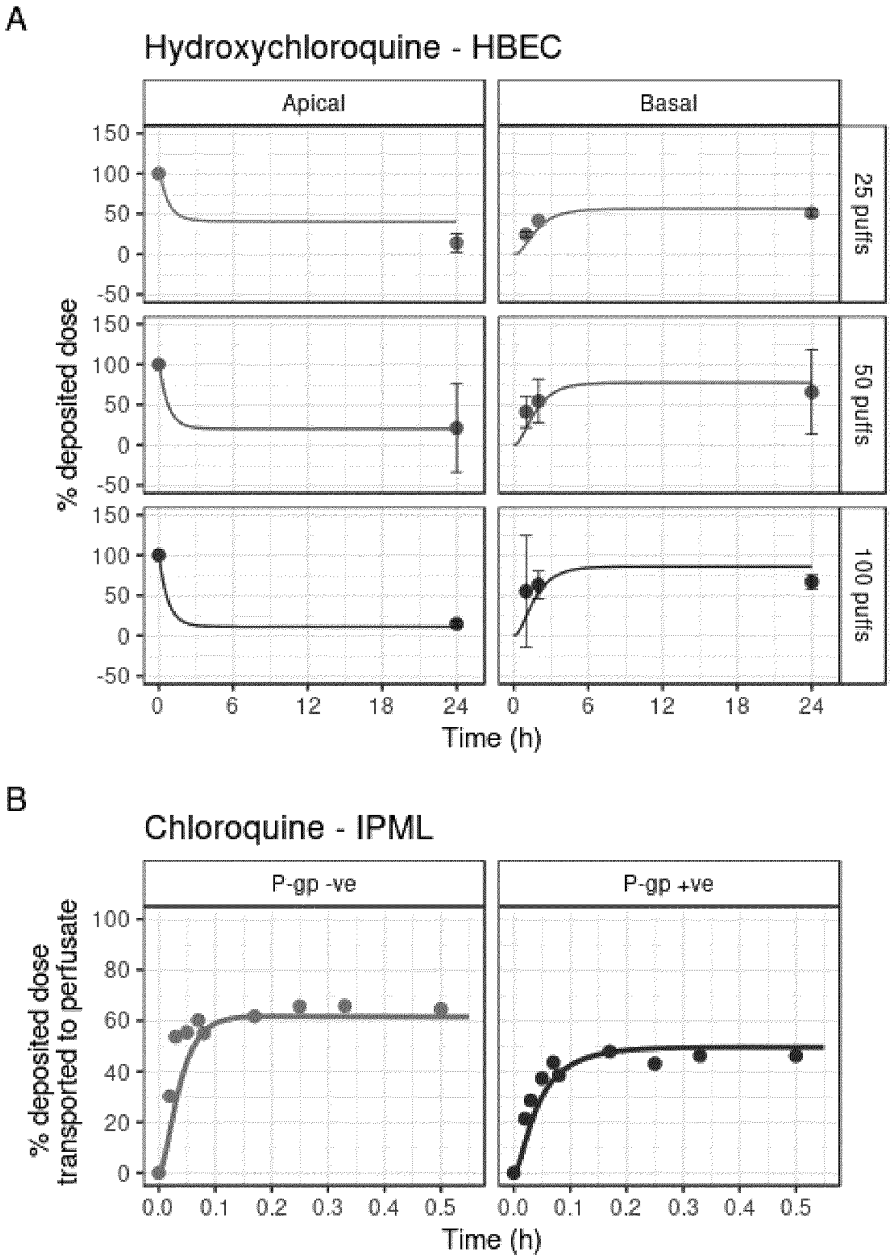

A
Hydroxychloroquine - HBEC
Apical
Basal
25 puffs
50 puffs
100 puffs
% deposited dose
Time (h)
B
Chloroquine - IPML
P-gp -ve
P-gp +ve
% deposited dose transported to perfusate
Time (h)

Figure 8

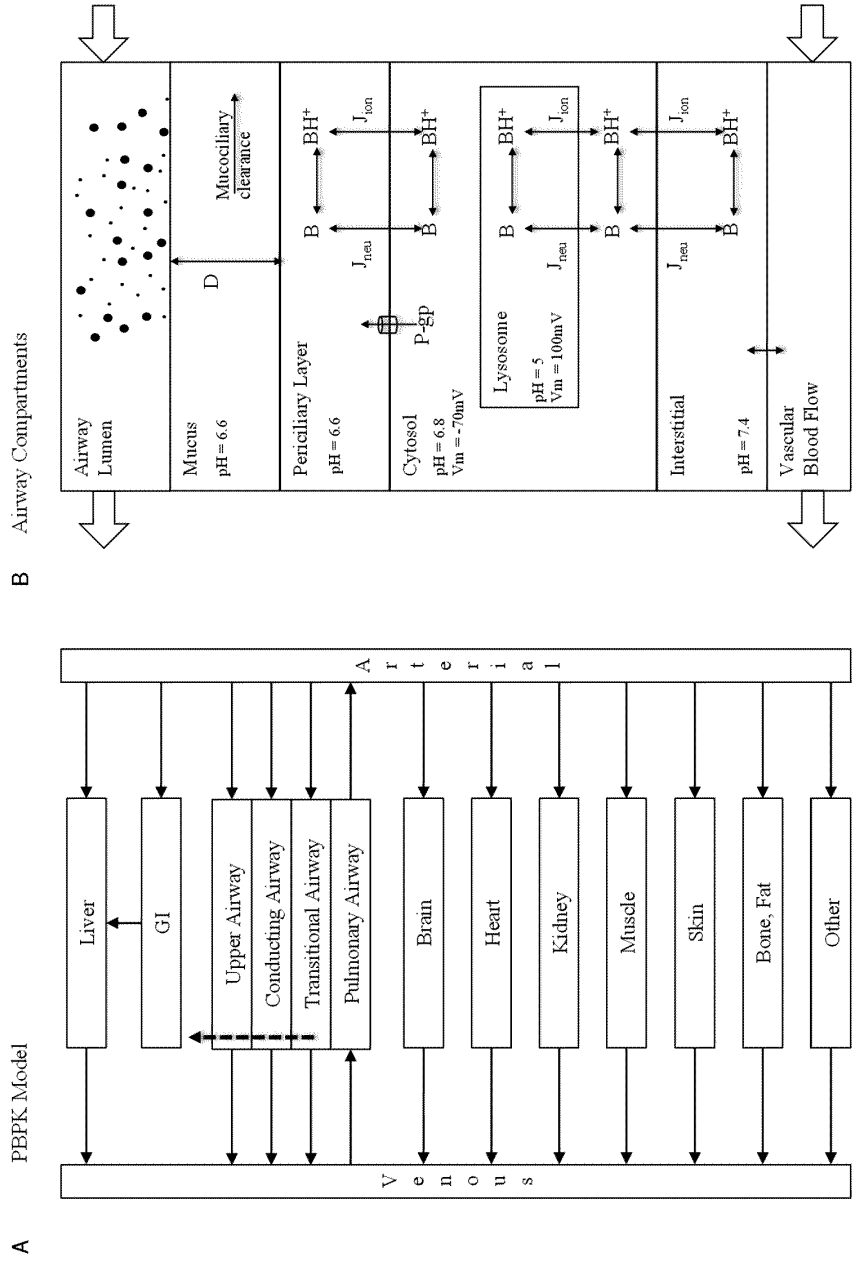

A
PBPK Model
Arterial
Venous
Liver
GI
Upper Airway
Conducting Airway
Transitional Airway
Pulmonary Airway
Brain
Heart
Kidney
Muscle
Skin
Bone, Fat
Other
B
Airway Compartments
Airway Lumen
Mucus
pH = 6.6
D
Mucociliary clearance
Periciliary Layer
pH = 6.6
Cytosol
pH = 6.8
Vm = -70mV
P-gp
Lysosome
pH = 5
Vm = 100mV
Interstitial
pH = 7.4
Vascular Blood Flow
B
BH+
J_ion
J_neu

Figure 9A

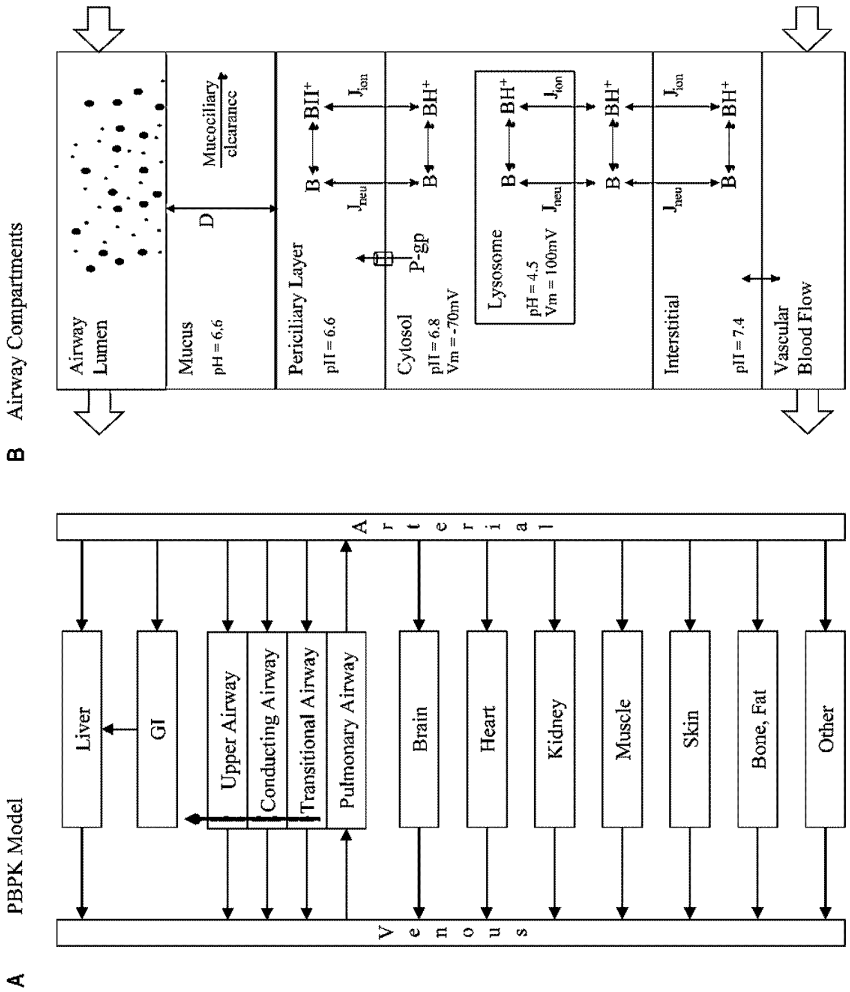

A PBPK Model
Liver
GI
Upper Airway
Conducting Airway
Transitional Airway
Pulmonary Airway
Brain
Heart
Kidney
Muscle
Skin
Bone, Fat
Other
Arterial
Venous
B Airway Compartments
Airway Lumen
Mucus
pH = 6.6
Mucociliary clearance
D
Periciliary Layer
pH = 6.6
Cytosol
pH = 6.8
Vm = -70mV
P-gp
Lysosome
pH = 4.5
Vm = 100mV
Interstitial
pH = 7.4
Vascular Blood Flow
B
BH+
BH2+
Jneu
Jion

Figure 9B

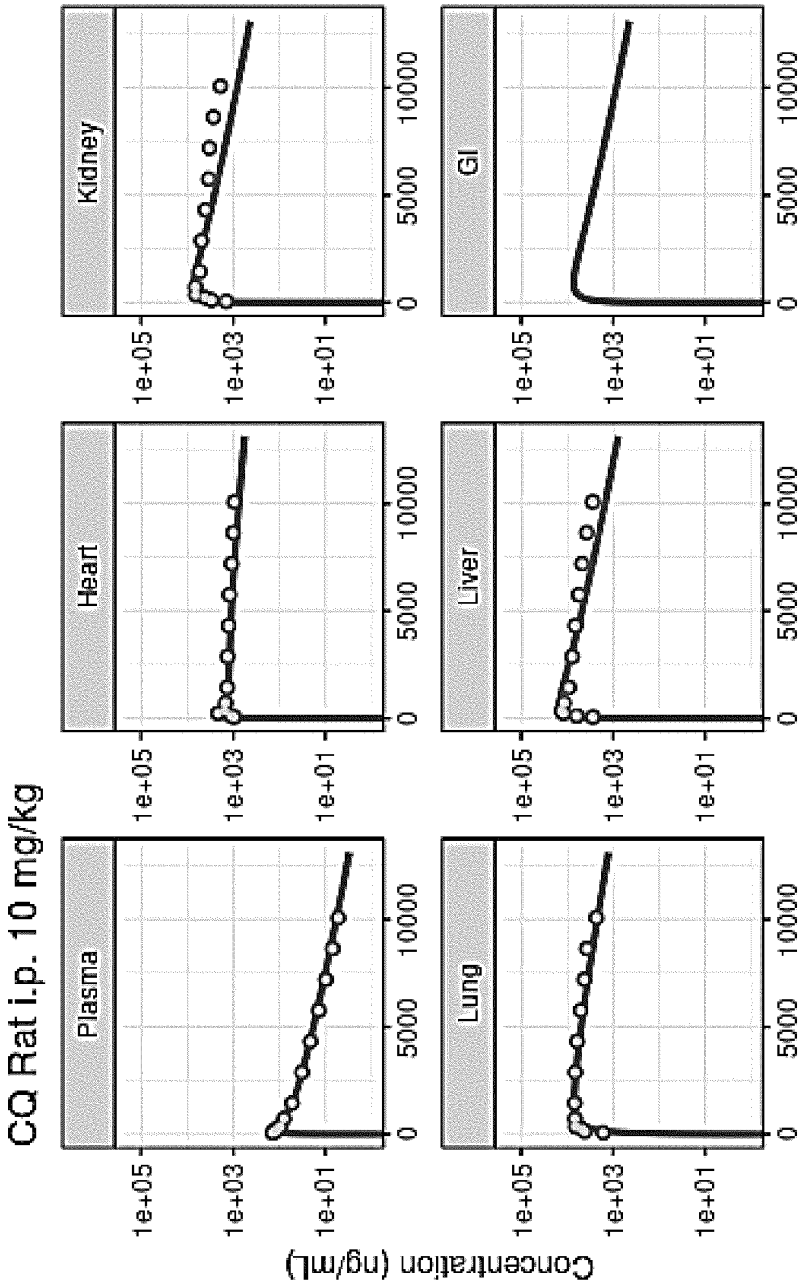

CQ Rat i.p. 10 mg/kg
Plasma
Heart
Kidney
Lung
Liver
GI
1e+05
1e+03
1e+01
0
5000
10000
Concentration (ng/mL)
Time (min)

Figure 10

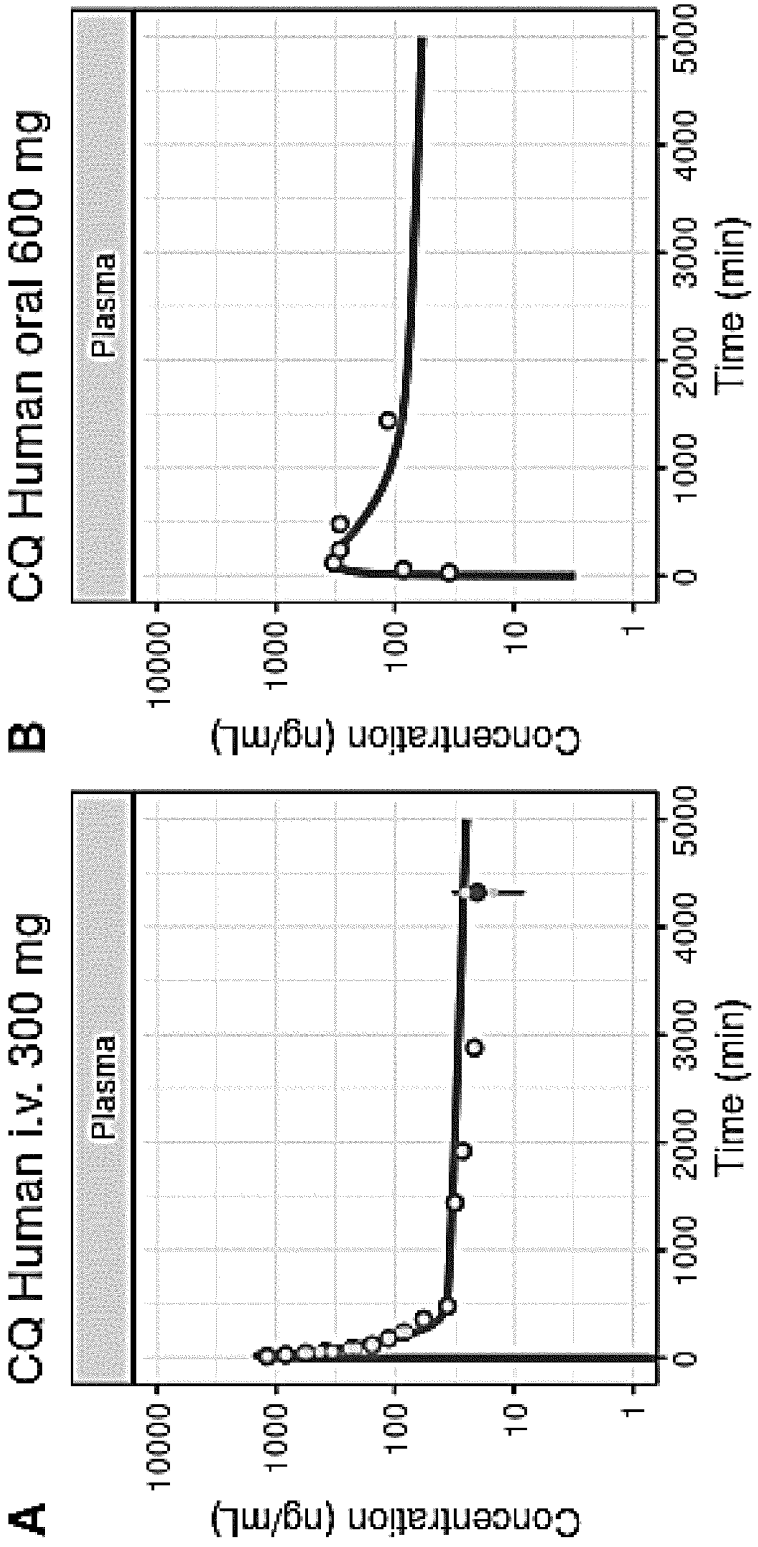

A
CQ Human i.v. 300 mg
Plasma
Concentration (ng/mL)
10000
1000
100
10
1
0
1000
2000
3000
4000
5000
Time (min)
B
CQ Human oral 600 mg
Plasma
Concentration (ng/mL)
10000
1000
100
10
1
0
1000
2000
3000
4000
5000
Time (min)

Figure 11

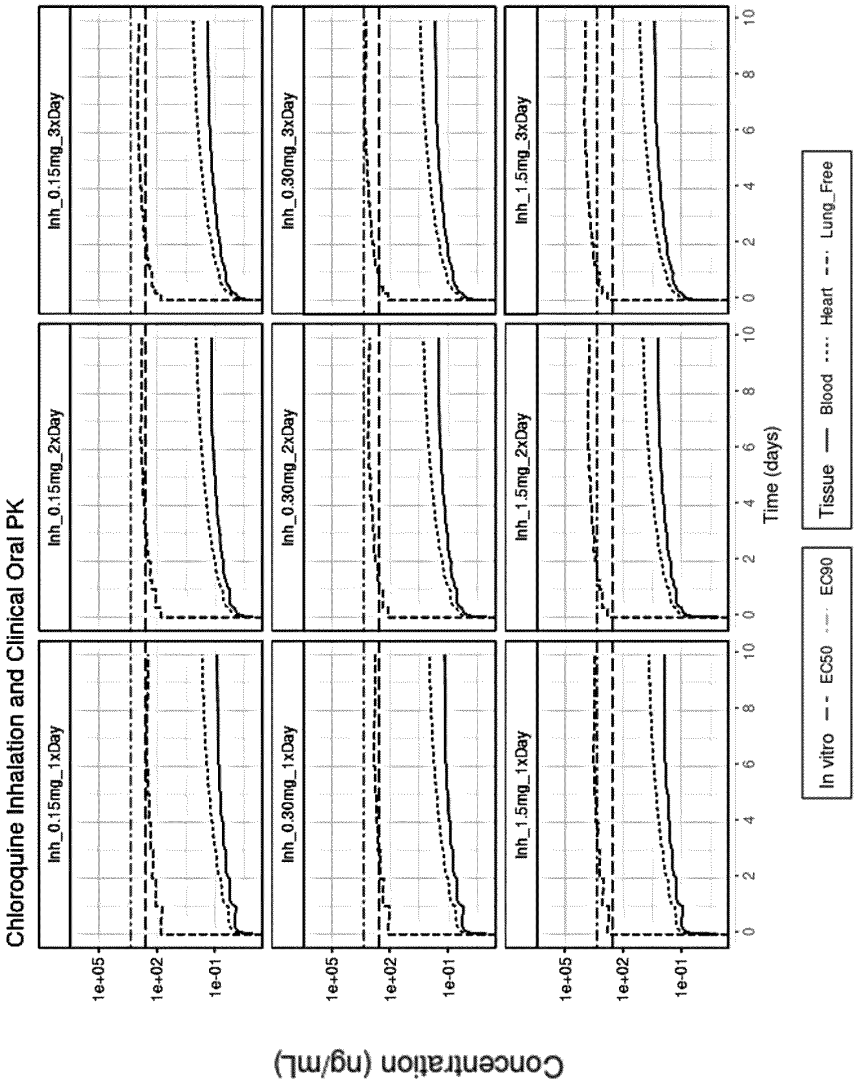

Chloroquine Inhalation and Clinical Oral PK
Inh_0.15mg_1xDay
Inh_0.15mg_2xDay
Inh_0.15mg_3xDay
Inh_0.30mg_1xDay
Inh_0.30mg_2xDay
Inh_0.30mg_3xDay
Inh_1.5mg_1xDay
Inh_1.5mg_2xDay
Inh_1.5mg_3xDay
Concentration (ng/mL)
1e+05
1e+02
1e-01
0
2
4
6
8
10
Time (days)
In vitro EC50 EC90
Tissue Blood Heart Lung_Free

Figure 12A

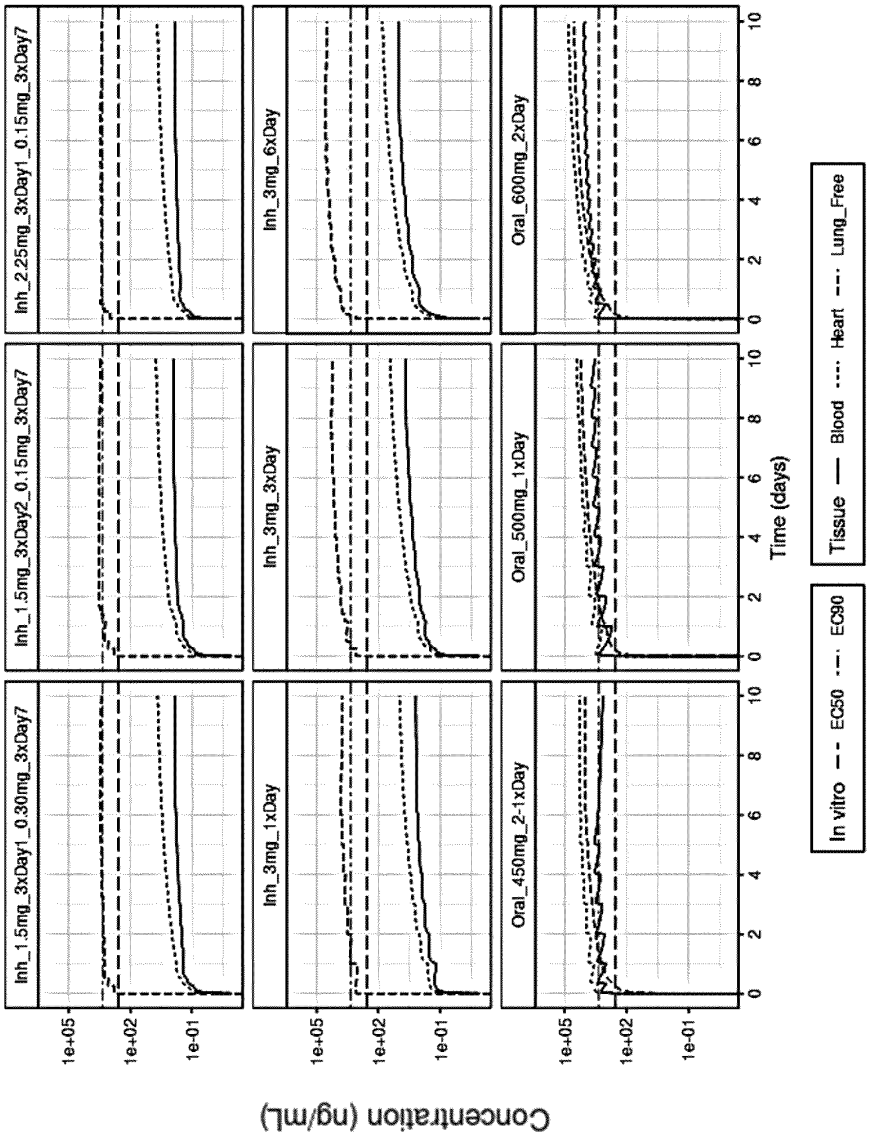

Inh_1.5mg_3xDay1_0.30mg_3xDay7
Inh_1.5mg_3xDay2_0.15mg_3xDay7
Inh_2.25mg_3xDay1_0.15mg_3xDay7
Inh_3mg_1xDay
Inh_3mg_3xDay
Inh_3mg_6xDay
Oral_450mg_2-1xDay
Oral_500mg_1xDay
Oral_600mg_2xDay
Concentration (ng/mL)
1e+05
1e+02
1e-01
0
2
4
6
8
10
Time (days)
In vitro EC50 EC90
Tissue Blood Heart Lung_Free

Figure 12B

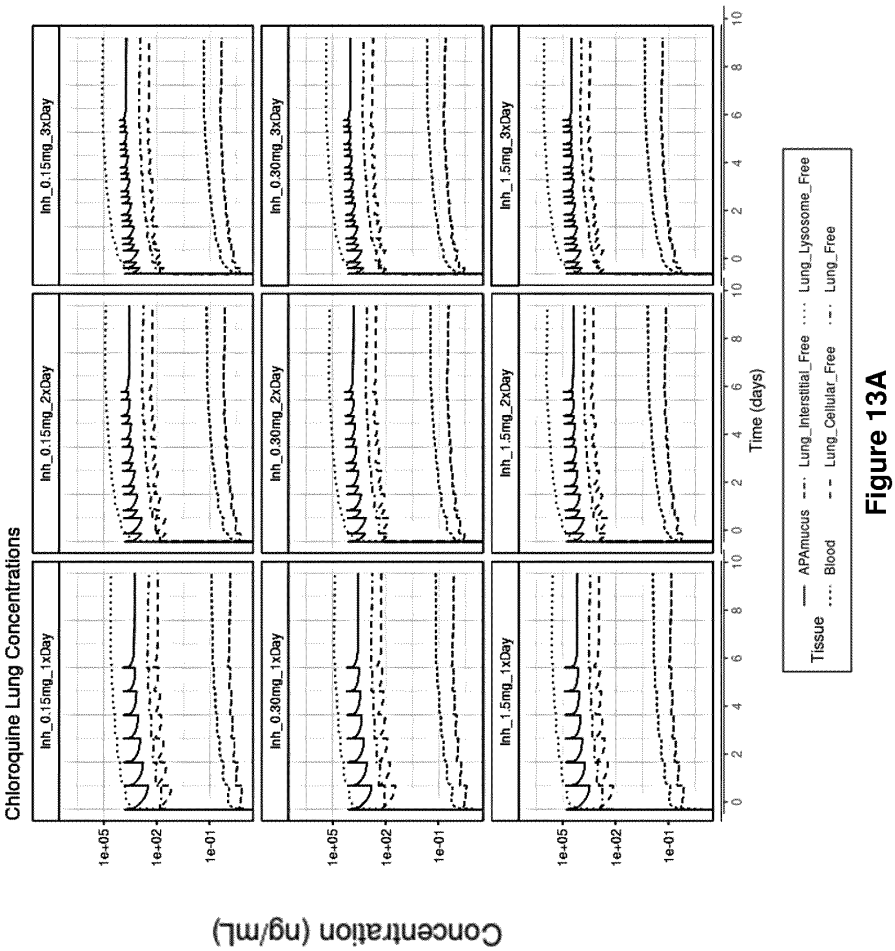

Chloroquine Lung Concentrations
Inh_0.15mg_1xDay
Inh_0.15mg_2xDay
Inh_0.15mg_3xDay
Inh_0.30mg_1xDay
Inh_0.30mg_2xDay
Inh_0.30mg_3xDay
Inh_1.5mg_1xDay
Inh_1.5mg_2xDay
Inh_1.5mg_3xDay
1e+05
1e+02
1e-01
0
2
4
6
8
10
Time (days)
Concentration (ng/mL)
Tissue
APAmucus
Blood
Lung_Interstitial_Free
Lung_Cellular_Free
Lung_Lysosome_Free
Lung_Free

Figure 13A

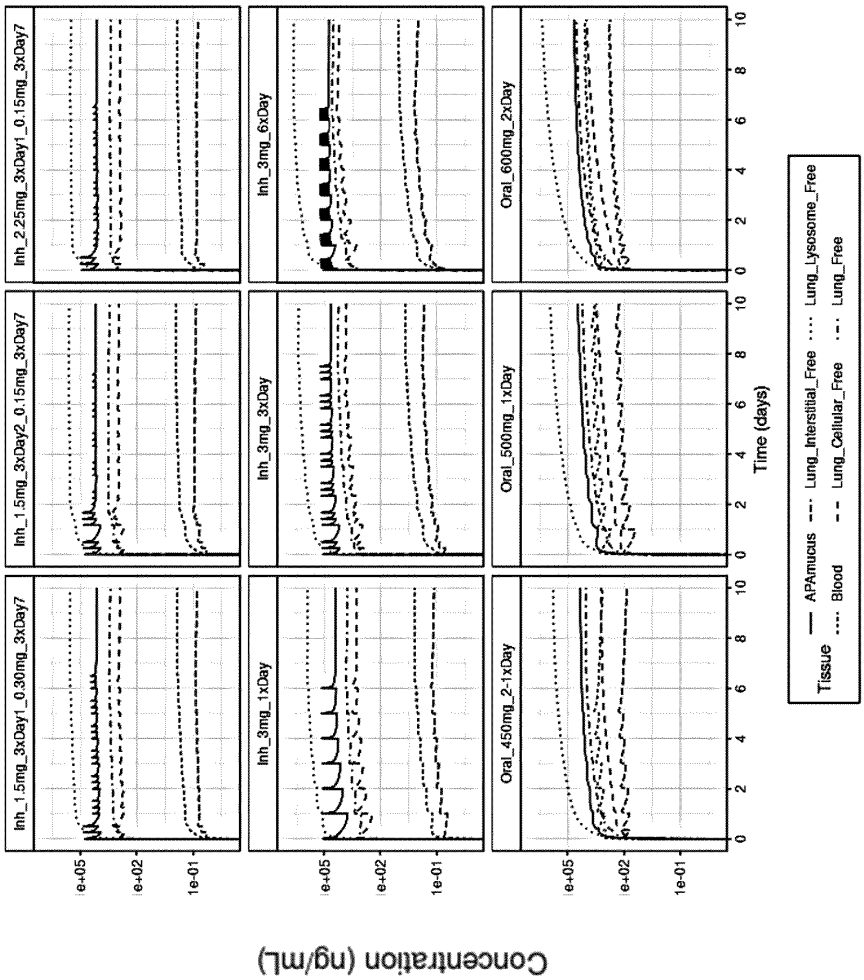

Inh_1.5mg_3xDay1_0.30mg_3xDay7
Inh_1.5mg_3xDay2_0.15mg_3xDay7
Inh_2.25mg_3xDay1_0.15mg_3xDay7
Inh_3mg_1xDay
Inh_3mg_3xDay
Inh_3mg_6xDay
Oral_450mg_2-1xDay
Oral_500mg_1xDay
Oral_600mg_2xDay
Concentration (ng/mL)
1e+05
1e+02
1e-01
0
2
4
6
8
10
Time (days)
Tissue
APAmucus
Blood
Lung_Interstitial_Free
Lung_Cellular_Free
Lung_Lysosome_Free
Lung_Free

Figure 13B

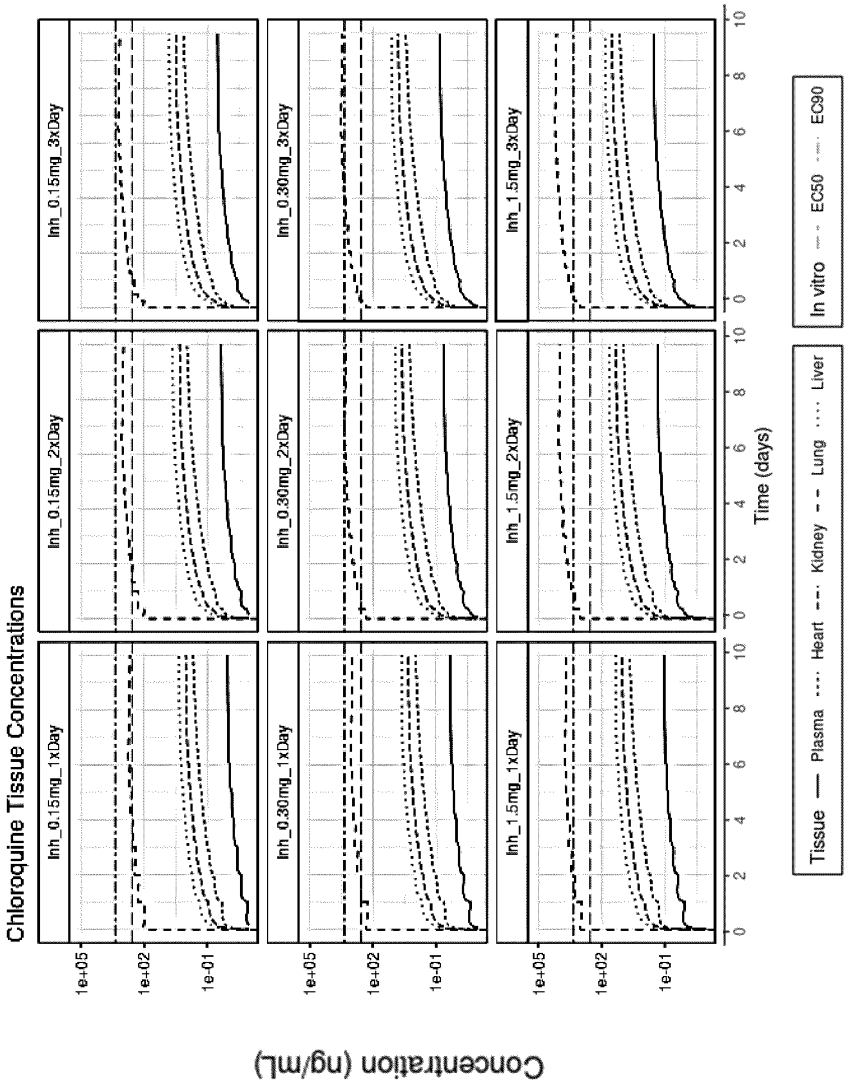

Chloroquine Tissue Concentrations
Inh_0.15mg_1xDay
Inh_0.15mg_2xDay
Inh_0.15mg_3xDay
Inh_0.30mg_1xDay
Inh_0.30mg_2xDay
Inh_0.30mg_3xDay
Inh_1.5mg_1xDay
Inh_1.5mg_2xDay
Inh_1.5mg_3xDay
1e+05
1e+02
1e-01
0
2
4
6
8
10
Time (days)
Concentration (ng/mL)
Tissue
Plasma
Heart
Kidney
Lung
Liver
In vitro
EC50
EC90

Figure 14A

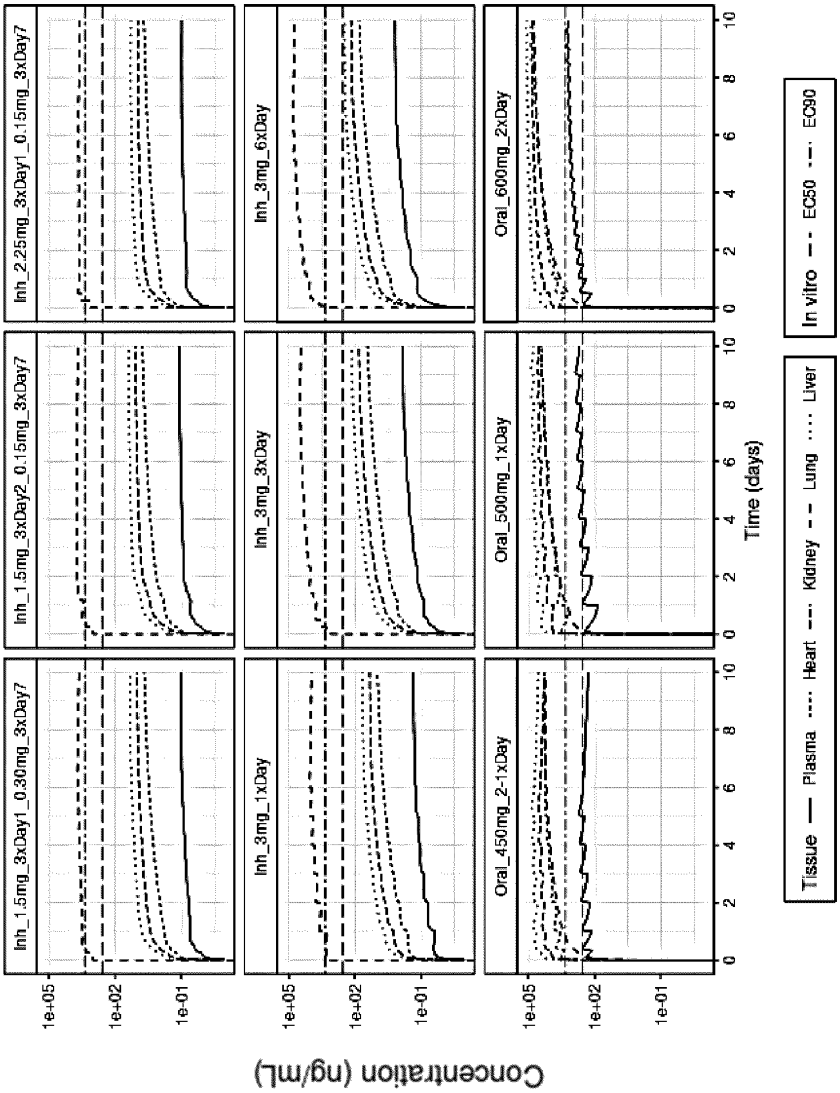

Inh_1.5mg_3xDay1_0.30mg_3xDay7
Inh_1.5mg_3xDay2_0.15mg_3xDay7
Inh_2.25mg_3xDay1_0.15mg_3xDay7
Inh_3mg_1xDay
Inh_3mg_3xDay
Inh_3mg_6xDay
Oral_450mg_2-1xDay
Oral_500mg_1xDay
Oral_600mg_2xDay
Concentration (ng/mL)
1e+05
1e+02
1e-01
0
2
4
6
8
10
Time (days)
Tissue
Plasma
Heart
Kidney
Lung
Liver
In vitro
EC50
EC90

Figure 14B

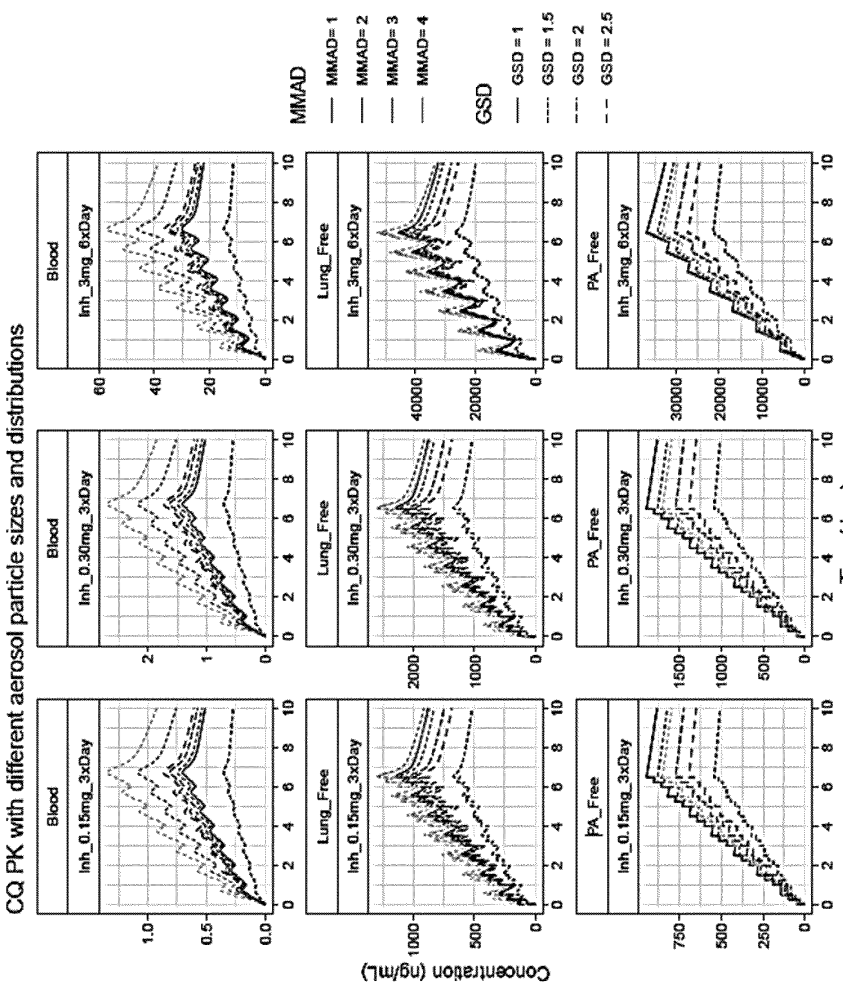

CQ PK with different aerosol particle sizes and distributions
Blood
Lung_Free
PA_Free
Inh_0.15mg_3xDay
Inh_0.30mg_3xDay
Inh_3mg_6xDay
Concentration (ng/mL)
Time (days)
MMAD
MMAD= 1
MMAD= 2
MMAD= 3
MMAD= 4
GSD
GSD = 1
GSD = 1.5
GSD = 2
GSD = 2.5

Figure 15