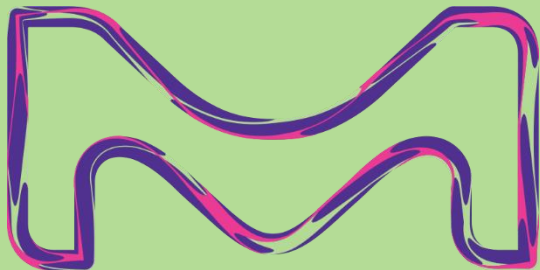


The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

chloroquine phosphate

**HPLC Assay and Impurity Profiling Methods
United States Pharmacopeia (USP43-NF38) Monograph
May 2020**

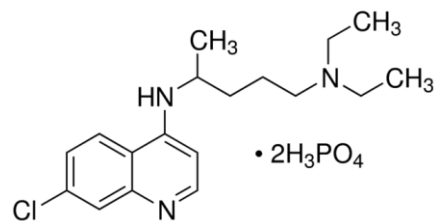


**Millipore
Sigma**

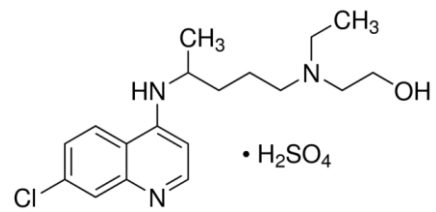
Chloroquine Phosphate – Assay and Impurity Profiling Method

Introduction

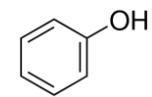
A rapid, accurate, and simple method was implemented for the total chromatographic purity analysis of Chloroquine Phosphate by High Performance Liquid Chromatography with a Diode Array Detector. The experimental conditions follow guidelines, with minor modifications, from the USP43-NF38 monograph methods for Chloroquine Phosphate Assay and Impurity Profiling. Chloroquine Phosphate, Chloroquine Related Compound A, Chloroquine Related Compound D, Chloroquine Related Compound E, Chloroquine Related Compound G, Hydroxychloroquine, and Phenol can be resolved with baseline separation within 16 minutes using an Ascentis Express C18 column (250 x 4.6 mm, 5 μm). A 1.4 g/L Dibasic sodium phosphate solution (pH 3.0) in water: 0.4% triethylamine in methanol (30:70 v/v) were employed as the mobile phase for the isocratic elution. Under applied conditions, system suitability criteria are met, and the method demonstrates good resolution/selectivity, reproducibility, and sensitivity.



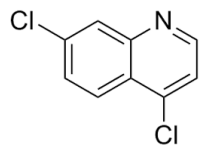
Chloroquine Phosphate



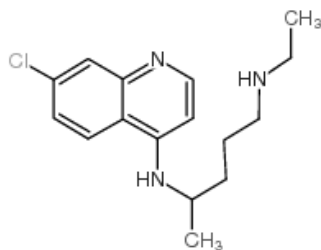
Hydroxychloroquine sulfate



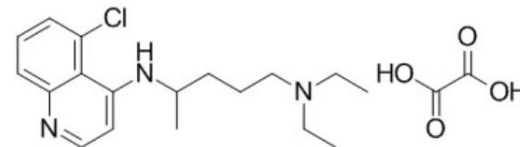
Phenol



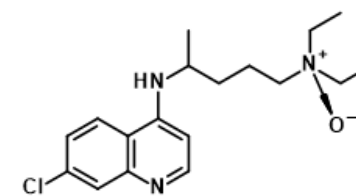
Chloroquine Related Compound A



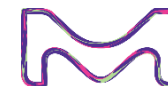
Chloroquine Related Compound D



Chloroquine Related Compound E



Chloroquine Related Compound G

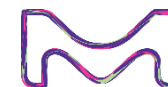
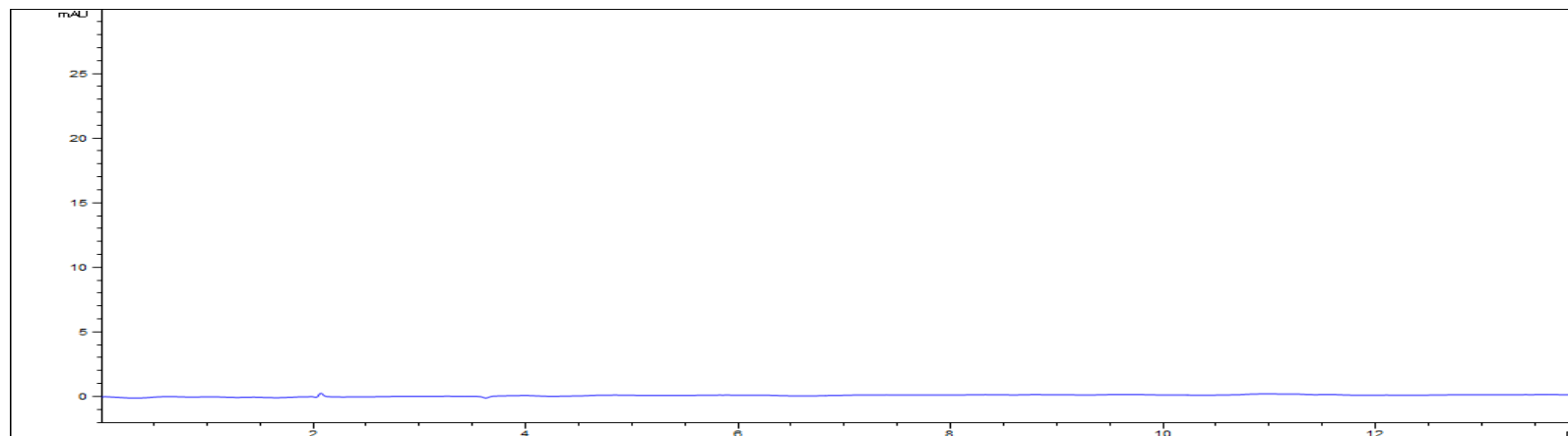


Chloroquine Phosphate – Assay and Impurity Profiling Method

Experimental Conditions

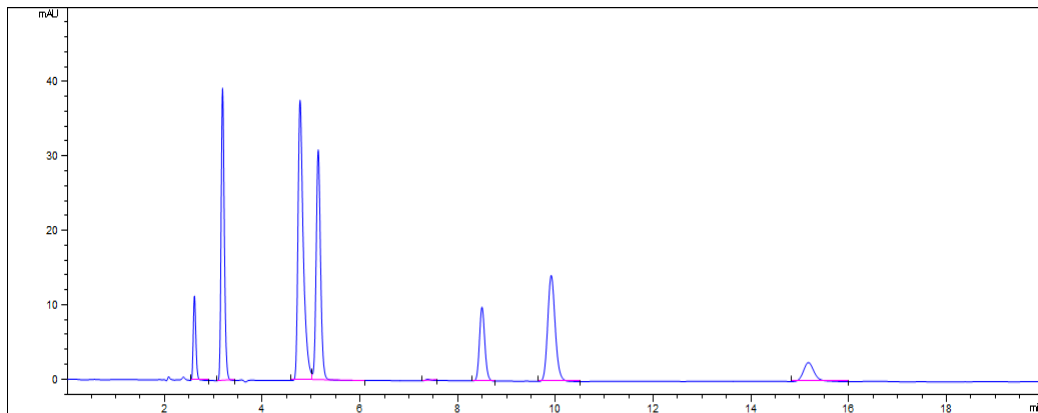
Column	Ascentis Express C18 column (25cm x 4.6mm, 5µm)	Injection volume	20 µL
Detection	UV @ 260 nm (analytical flow cell; 10 µL)	Flow rate	1 mL/min
Buffer	Dissolve 1.4 g of K ₂ HPO ₄ in 1000 mL Milli-Q water and adjust to pH 3.0 using H ₃ PO ₄ .	Temperature	Column: 30°C Autosampler: 16°C
Mobile phase	Buffer and 0.4% triethylamine in Methanol (30:70) v/v.	Pressure	237 bar
Diluent	Mobile Phase		
Test solution	Dissolve 50 mg of Chloroquine Phosphate in 25 mL mobile phase (2.0 mg/mL).		
System suitability solution (SST)	Dissolve 5.0 mg of each Chloroquine Phosphate, Phenol, Hydroxychloroquine, Chloroquine Phosphate Related Compound A, Chloroquine Phosphate Related Compound D, Chloroquine Phosphate Related Compound E, and Chloroquine Phosphate Related Compound G into 25 mL mobile phase. Then, further take 1.0 mL of this solution and dilute to 100.0 mL using diluent (5.0 µg/mL).		

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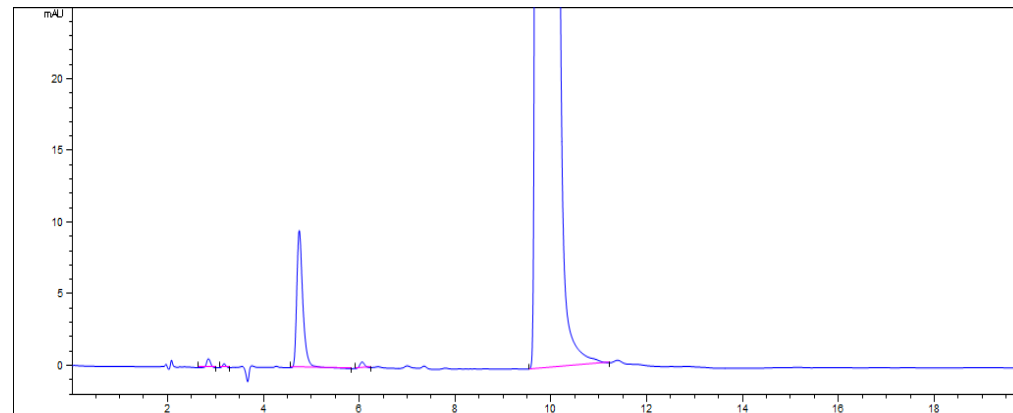


Chloroquine Phosphate – Assay and Impurity Profiling Method

System Suitability Solution (SST)
(Standard)

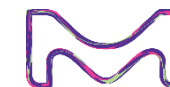


Chloroquine phosphate
(Test solution)



Chromatographic Data (System Suitability Solution)

Peaks	Compound	Retention Time (min)	RRT	Resolution	Theoretical plates	Tailing factor
1	Phenol	2.60	0.26	-	11484	1.1
2	Chloroquine related compound G (RCG)	3.18	0.32	5.4	11444	1.2
3	Chloroquine related compound D (RCD)	4.77	0.48	10.5	10437	1.6
4	Hydroxychloroquine sulfate	5.14	0.52	2.2	16090	1.1
5	Chloroquine related compound A (RCA)	8.49	0.86	18.8	28913	1.1
6	Chloroquine Phosphate	9.91	1.00	6.0	20259	1.1
7	Chloroquine related compound E (RCE)	15.18	1.53	15.9	23609	1.2



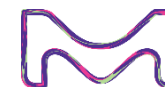
Chloroquine Phosphate – Assay and Impurity Profiling Method

Specificity (System Suitability Solution)

Peaks	Compound	Retention Time (min)	RRT	RRT Reference per USP43-NF38	Resolution	Requirement per USP43-NF38	Theoretical plates	Tailing factor
1	Phenol	2.60	0.26	0.2	-		11484	1.1
2	Chloroquine related compound G (RCG)	3.18	0.32	0.27	5.4		11444	1.2
3	Chloroquine related compound D (RCD)	4.77	0.48	0.42	10.5		10437	1.6
4	Hydroxychloroquine sulfate	5.14	0.52	0.49	2.2		16090	1.1
5	Chloroquine related compound A (RCA)	8.49	0.86	0.73	18.8		28913	1.1
6	Chloroquine Phosphate	9.91	1.00	1.0	6.0	> 2.0 (to RCA)	20259	1.1
7	Chloroquine related compound E (RCE)	15.18	1.53	1.5	15.9		23609	1.2

Repeatability (System Suitability Solution)

Peaks	Compound	Area Response (n=3)	Standard Deviation	RSD (%)
1	Phenol	42.42	0.13	0.3
2	Chloroquine related compound G (RCG)	178.92	1.12	0.6
3	Chloroquine related compound D (RCD)	283.39	0.90	0.3
4	Hydroxychloroquine sulfate	195.04	0.64	0.3
5	Chloroquine related compound A (RCA)	75.60	0.22	0.3
6	Chloroquine Phosphate	152.47	0.70	0.5
7	Chloroquine related compound E (RCE)	37.97	1.57	4.1



Chloroquine Phosphate – Assay and Impurity Profiling Method

Product list	PN
Chloroquine phosphate United States Pharmacopeia (USP) Reference Standard	1118000
Chloroquine Related Compound A United States Pharmacopeia (USP) Reference Standard	1118328
Chloroquine Related Compound D United States Pharmacopeia (USP) Reference Standard	1118350
Chloroquine Related Compound E United States Pharmacopeia (USP) Reference Standard	1118361
Chloroquine Related Compound G United States Pharmacopeia (USP) Reference Standard	1118394
Hydroxychloroquine sulfate United States Pharmacopeia (USP) Reference Standard	1327000
Phenol United States Pharmacopeia (USP) Reference Standard	1524806
Ascentis® Express C18, 5 µm HPLC Column (25 cm x4.6 mm)	50538-U
Methanol isocratic grade for liquid chromatography LiChrosolv®	1.06018
Water for chromatography (LC-MS grade) LiChrosolv®	1.15333
or tap fresh from an appropriate Milli-Q® water purification systems	
Potassium phosphate dibasic (K ₂ HPO ₄) anhydrous for analysis EMSURE®	1.05104
Ortho-phosphoric acid EMSURE®	1.00573
Triethylamine for HPLC, LiChropur	81101
Millex® syringe filter units, disposable, Durapore® PVDF, pore size 0.45 µm, non-sterile	SLHVX13NK

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