MINI REVIEW

Instrumental analysis of chloroquine and hydroxychloroquine in different matrices

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Sebaiy MM, Abdelazeem AI, Aboulfoutouh A. et al Instrumental analysis of chloroquine and hydroxychloroquine in different matrices.Cur Res Integr Med 2022; 7(2):1-9.

ABSTRACT

Currently, the emergence of a novel human corona virus, COVID- 19, has become a global burden causing severe symptoms and death. In this literature review, we will introduce most of up-to- date reported methods that have been developed for determination of two important antimalarial and anti-COVID-19 drugs which are Chloroquine and Hydroxychloroquine in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples.

Key Words: *COVID-19; Chloroquine; Hydroxychloroquine; degradation products; biological samples.*

INTRODUCTION

Quinoline derivatives such as Chloroquine (CQ) and Hydroxychloroquine (HCQ) are used to treat malaria. These drugs have not yet been licensed for the treatment of viral infections, and there are no well-controlled, prospective, randomized clinical trials or evidence to support their use in patients with coronavirus disease (COVID-19) [1].

Due to the rapidly spreading pandemic caused by extreme acute respiratory syndrome-coronavirus-2, dubbed coronavirus disease (COVID-19) in 2019, CQ and HCQ are now gaining worldwide attention in treatment strategy. The use of CQ/HCQ in the treatment of COVID-19 clinical syndrome has been restricted thus far [2].

Due to the current importance of these drugs in the treatment of pandemic COVID-19, this literature focuses on different analytical methods that have been developed for the determination of these drugs in different pharmaceutical and biological samples.

ANALYTICAL METHODS

Various techniques were used for the analysis of CQ and HCQ in pure forms, in their pharmaceutical formulations and biological fluids [Table 1-5]. The available reported methods in the literature can be summarized as follows:

TABLE 1

Spectroscopic methods

Drugs	Matrix	Method or Reagents	λ_{max} (nm)	Linearity	LOD	Ref
HCQ	Pharmaceutical formulation	UV Spectrophotometry using 0.1N HCl as solvent	343	Jan-20 µg/mL	------	[3]
HCQ	Pharmaceutical formulation	UV Spectrophotometry using 0.01M HCl as solvent	342	08-Dec µg/ mL	0,38 µg/mL	[4]
HCQ	Raw material	UV Spectrophotometry using dil. HCl as solvent	343	10 µg/mL	------	[5]
HCQ	Tablets	UV Spectrophotometry (AUC method)	251 - 261	02-Dec µg/mL	0,37 µg/mL	[6]
CQ	Tablets	UV Spectrophotometry	343	10,88 - 30,56 µg/mL	0,99 µg/mL	[7]
	Tablets, injections and syrup formulations.	Frist derivative spectrophotometry		2,5-50		

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Received:- 01 March 2022, Manuscript No. PULCRIM-22-4110; Editor assigned:- 03 March 2022, Pre-QC No. PULCRIM-22-4110 (PQ); Reviewed:- 19 March 2022, QC No. PULCRIM-22-4110 (Q); Revised:- 22 March 2022, Manuscript No. PULCRIM-22-4110 (R); Published:- 28 March 2022, DOI: 10.37532.2022.7.2.1-9

CQ			349	µg/mL	------	[8]
CQ, Pyrimethamine and Trimethoprim	Pharmaceutical formulation and urine	Ion pair complex with bromocresol purple	420	1,25-8,75 µg/mL	0,125 µg/mL	[9]
CQ and it's metabolite	Urine samples	Ion pair complex with bromothymol blue	410	Up to 120 mg/L	3 mg/L	[10]
CQ and Pyrimethamine	Tablets	Ion-pair complex with molybdenum and thiocyanate	467	Feb-42 µg/mL	------	[11]
CQ	Urine samples	Ion-pair complex with methyl orange	420	Up to 32 µg/mL	0,3 µg/mL	[12]
CQ	Urine samples	Colorimetric test, Dill-Glazko's test, and UV/Visible absorbance spectrometry	329 & 343	------	15 mg/land	[13]
CQ and its degradation products	Pharmaceutical formulation	UV Spectrophotometry	220	01-Oct µg/mL	0,13 µg/mL	[14]
CQ	Tablets, suspension and injections	Ion pair complex with bromocresol green and bromocresol purple	420	Jan-20 µg/mL -0,5-12 µg/mL	0,27 µg/mL-0,15 µg/mL	[15]
CQ	Tablets	UV Spectrophotometry using 0.06 M monosodium phosphate buffer pH 6.8	343	7,2-19,2 µg/mL	------	[16]
CQ	Tablets	Difference spectrophotometry	285 & 345	50-250 µg/mL	------	[17]
CO	Tablets	UV spectrometry	254	10-64µg/mL	-------	[18]

TABLE 2

Spectrofluorimetric methods

Drugs	Matrix	Fluorogenic Reagent (Method	λex (nm)	λem(nm)	Linearity Range	LOD	Ref.
CQ	Tablets	0.05 M H_2S0_4	339	368	01-Oct µg/mL	0.77 µg/mL	[19]
CQ	Tablets and spiked human plasma	M 4.0 sodium dodecyl sulfate (pH 10)			5-0.003 µg/mL	0- 3.3×1 ^{3}µg/mL	[20]
CQ	suspension	10 mg/ml in distilled water	330 300	369 330–400	Up to 0.625 mg/mL	------	[21]

TABLE 3

Chromatographic methods

Drugs	Matrix	Column	Mobile phase	Detector	Linearity	LOD	Ref
HCQ and its metabolites	Human plasm a	Poly (styrene divinylbenene) packing	Methanol (80): Water (20) with triethylamine, (pH 11)	Fluorescen ce detect ion at 337 nm	-4 0 ug/mL	1 ng/mL	[22]
HCQ and its major metabolites	Plasma	CN (cyano-bonded silica)	(79:20: 1) 0.03 M sodium phosphate buffer, pH 7.0:ethanol : acetonitrile	UV at 320 nm.	144 ng/mL	14 ng/mL	[23]
HCQ and three major metabolites	Blood and urine	ODS	Hexane: tert butyl ether: 0.5% n-butylaminin methanol (1:1:1)	Fluorescen ce detect ion at 380 nm	Upto 1000 ng/mL	5- 10 ng/mL	24]

			Phosphate buffer-methanol: ethanol: Triethanolamine (78:22:1:0.0		–2		
HCQ and three major metabolites	Plasma	ODS (cyano column - chiral-AGP column)	8, v/v)	Fluorescence detection at 385 nm	,24 –24	1 < ng/mL	[25]
quinine, HCQ, CQ, and desethylchloroquine	Serum, and whole blood	CN (sulfophenylpropyl-modified silica)	0.015M K_2HPO_4buffer: Methanol: ACN (20:30:50)	Fluorimetry (excitation wavelength, 215 nm)	244 and 200–2000 ng/mL 24-Feb mg/L	4.4 40 mg/mL	[26]
HCQ, CQ and some corticosteroids	Serum	Nova-Pak C_{18} (150 x 3.9 mm, 0µm)	4.705 g NH4ClO4 Methanol: water (98.5:1.5)+	UV at several wavelengths	25- 2000 nmol/L		[27]
Enantio-selective analysis of the metabolites of HCQ	Rat liver microsomes	Chiralpak AD-RH column protected with an RP-8 guard column	hexane:isop ropanol (92:8, v/v) plus 0.1% diethylamine	UV at 343 nm	–04 444 ng/mL	10 ug/mL	[28]
HCQ and its active metabolites	Blood	Hypersil Gold aQ column	A gradient of water and methanol acidified with 0.1% formic acid	MS/MS	–20 2444 ng/mL		[29]
HCQ	Blood	ODS Hypersil C18 column (250×6 mm, 5 µm)	Water and (acetonitrile :methanol: 50:50, v/v) in 75:25 v/v ratio, with sodium 1-pentane sulfonate and phosphoric acid (pH 3)	UV at 343 nm	0.1 - 20.0 µg/mL	4.2 0 ug/mL	[30]
HCQ, minocycline and doxycycline	Blood	Acquity BEH Phenyl (2.1 × 50 mm 1.7 µm)	1% Triethylamine and 1 mM oxalic acid in water adjusted to pH 2.4 with orthophosphoric acid at 85%	UV at 343 nm	– 1.0 14 µg/mL	4 ug/mL	[31]
HCQ and its metabolites	Blood	octadecyl silane Luna C_{18}	A water–methanol–acetonitrile (02:14:02, v /v/v) mixture containing 3.2 mM SDS	Fluorescence detection at 370 nm	-1 2044 ng/mL	1 ng/mL	[32]
HCQ and its metabolites	Mouse blood and tissues	Thermo Aquasil C_{18} (50 × 4.6 mm, 2 µ)	A gradient elution using 0.2% formic acid and 0.1% formic acid in methanol	MS/MS	to 1 2000 ng/m	1 ng/mL	[33]

HCQ	Human blood	Agilent ZORBAX Eclipse XDB – C_8	0.10% formic acid–acetonitrile (94:6, v/v)	MS/MS	–0 L 2444 ng/mL	2 ng/mL	[34]
HCQ	Tablets	Agilent Zorbax C_8	0.01M of 1-pentane sulfonic acid and 0.02% of Ortho phosphoric acid in purified water with acetonitrile and methanol (800: 100: 100 v/v)	UV at 254 nm	-20 244 µg/mL		[35]
HCQ and its metabolites	Blood	U-HPLC RP_{18}	Piperazine buffer (46.4 mM, pH = 9.8) and acetonitrile (68:32, v/v)	Fluorescence detection at 390 nm	125 - 4000 ng/mL	2.2 ng/mL	[36]
HCQ enantiomers	Blood	Chiralpak AD-H column 4.6 mm × 100 mm, 5 µm))	n-hexane-isopropanol (93:7, v/v) plus 0.5% DEA into hexane	UV at 343 nm	Jan-25 µg/mL	4.4 2 µg/mL	[37]
HCQ and its metabolites	Blood	XTerra phenyl® column (250 × 4.6 mm, 0 µm; Waters, MA, USA)	Glycine buffer/sodium chloride (pH 9.7, 100 mM) and methanol (46:54; v/v	Fluorescence detection at 380 nm	-4 444 ng/mL	20 ng/mL	[38]
HCQ	Tablets	X-terra phenyl column (204 × 0. 6 mm, 5 µm)	Acetonitrile and buffer in the ratio 70:30 v/v respectively	UV at 220 nm	-4.24 2 µg/mL	4.4 22 µg/mL	[39]
HCQ and Its two metabolites	Blood	YMC-Triart C_{18} column (250 × 4.6 mm, 0 µm)	mM 24 sodium phosphate buffer solution containing 0.25% triethylamine (pH 8.0)— acetonitrile (60:40, v/v)	Fluorescence detection at 405 nm	–2 2444 ng/mL	10 ng/mL	[41]
CQ and HCQ	Human plasma	Agilent Zorbax SB-C_{18}	%4.1 %20 trifluoroacetic acid and 5% methanol, evolved in 8 minutes to 50% methanol	UV at 343 nm			[42]
HCQ and its metabolites & Azithromycin	Human plasma	PFP column (2.0 × 50 mm, 3 µm)	0.1% TFA in acetonitrile	MS/MS	to 2 1000 ng/mL	4.2 ng/mL	[43]
HCQ	Blood	Kinetex C_8 (2.1 × 50 mm, 2.2 µm, Phenome nex, Torrance, USA)	0.10% formic acid and 0.01 and 0.01% triethylamine in water (or acetonitrile).	MS/MS	10 - 2000 ng/mL	0 ng/mL	[44]
HCQ and its	Human		Hexane/methanol/ethanol (96:2:2, v/v/v) plus 0.2%	UV at 343	–04		

major metabolites	urine						
		Chiralcel OD-H	diethylamine	nm	1444 ng/mL		[45]
					-4.42	4.4	
CQ and primaquine	Tablets	Hypersil C18	Acetonitrile and 0.1% aqueous triethylamine	UV at 260 nm	4.21 mg/mL	1 mg/mL	[46]
CQ, mono-desethyl chloroquine, diazepam, and nordiazepam	Blood	Bondapak C_8 column (30 cm x 3.9 mm, 14 µm)	0.0125M potassium dihydrogen phosphate solution and 1N phosphoric acid (99.90:0.10 v/v): acetonitrile	UV at 343 nm	-4 1444 ng/mL	14 ng/mL	[47]
CQ and desethyl chloroquine	Blood	Thermo Hypersil Gold C_{18}, 250 mm × 2.1 mm, 5 µm	1% diethylamine, acetonitrile and methanol (20:55:25, v:v:v)	UV at 256 nm	25 - 1500 ng/mL	0.2 ng/mL	[48]
CQ and quinine	Human plasma, erythrocytes and urine	Inertsil silica (250 mm x 4 mm, 0 µm)	acetonitrile and methanol—25% ammonia solution (92.7:7.5, v/v)	Fluorescence detection at 375 nm	-20 1444 ng/mL	0 ng/mL	[49]
CQ, Pyrimethamine and Cetirizine	Human serum	Purosphe r C_{18}	methanol: water (70:30) at pH of 2.8	UV at 230 nm	144-1 µg/mL		

TABLE 4

HPLC methods

Drug	Matrix	Mobile phase	Stationary phase	Detection	Linearity range	LOD	Ref
HCQ, Methotrexate, and Sulfasalazine.	Serum and urine	Ethyl acetate : methanol 25% : ammonia (8:2:3 v/v)	Silica gel 60 F 254 plates	UV at 340 nm	120 – 100 4 ng/mL	46 84 ng/mL	[50]
CQ and primaquine	Tablets and capsules	Hexane: diethyl ether: methanol: diethyl anime (37.5 :37.5:25:0.5 v/v).	Silica gel 60 F 254 plates	UV at 254 nm.	4- 33 µg/ mL	1.6 µg/mL	[51]
CQ and Desethyl chloroquine.	Plasma and urine sample.	Toluene : diethyl anime (9:1 v/v).	Silica gel plates	Fluorescence	----- -	1 0 mumol/L	[52]
CQ and desethyl chloroquine	Urine	Ammonia : methanol in methyl tert butyl ether (MTBE) 18:8 v/v .	Silica gel 60 plates with impregnated fluorophore	Colorimetric and UV 254 nm	01-May µg/ mL	0.2 5 µg/mL	[53]
CQ , Azethromycin and paraceta	Tablets	Methanol 25%:amonia (100 : 1.5 v/v) .	Silica gel 60 F plates	UV at 254n m	0.1- 10 mg/ mL	--- ---	[54]

TABLE 5

Electrochemical determination

Drugs	Matrix	Electrode	Linearity	LOD	Ref.
		Cathodically pretreated (CPT) boron-			

		doped diamond (BDD) electrode			
HCQ	Tablets and urine	Polymeric membrane (PMEs) and modified carbon paste (CPEs) electrodes which are based on sodium tetraphenyl borate (NaTPB) or ammonium reineckate (Rt) as sensing materials	mu 1.2 - 4.1 mol/L	0.06 mu mol/L	[55]
HCQ	Tablets and urine		1.0 × 10^{-2} - 1.0 x 10^{-6} mol/L	5.0 × 10−2 mol/L	[56]
HCQ	Tablets	Glassy carbon electrode	2 × 10^{-5} - 5 × 10^{-4} mol/L	11.2 µg/mL	[57]
CQ	Tablets and human serum	Glassy carbon electrode modified with reduced graphene oxide on WS 2 quantum dots	0.5 - 02 µM	4.10 µM	[58]
CQ	Pharmaceutical formulation	Boron-doped diamond (BDD) electrode	0.01 - 0.25 µmol/L	2 $nmol^{-1}$	[59]
CQ	Tablets and biological fluids	Poly(vinyl chloride) membrane electrodes	0.01 - 100 mM	0.02 mM	[60]
CQ	Tablets	Carbon paste electrode modified with $Cu(OH)_2$ nano -wire	0.068 - 6.88 µg/mL	0.01 µg/mL	[61]
CQ	Pharmaceutical Preparations	Two types of polyvinyl chloride (PVC)	1.0 × 10^{-1} - 1.0 × 10^{-6} M	3.2 × 10^{-6} M	[62]
CQ	Serum	carbon paste and dsDNA-modified carbon paste electrodes	1.0 × 10^{-2} - 1.0 × 10^{-0} mol/L	3.0 × 10−0 mol/L	[63]
CQ	Tablets and	glassy carbon electrode modified with electrochemically polymerised methyl orange (PMO) and multi-walled carbon nanotubes (MWCNT)	1.0×10-7-3.5×10-6 mol/L	8.9×10^{-8} mol/L	[64]
HCQ	In the presence of Uric Acid	covalent modification of glassy carbon electrode (GCE) by self-assembly of a novel Schiff base	2×10-5 to 5×10-4 M	11.2 µg/mL	[64]

CONCLUSION

This literature review represents an up to date survey about all reported methods that have been developed for the determination of Chloroquine and Hydroxychloroquine in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as spectrophotometry, Spectrofluorimetry, liquid chromatography, electrochemistry, etc

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