Rapid Quality Assessment for COVID-19 Therapeutics Following Emergency Use Authorization

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Abstract

As COVID-19 rapidly spread across the US, in March 2020, FDA issued an Emergency Use Authorization (EUA) for chloroquine phosphate and hydroxychloroquine sulfate, two drugs indicated for the treatment or prevention of malaria and other conditions. The EUA allowed chloroquine and hydroxychloroquine donated to the Strategic National Stockpile (SNS) and distributed to the states to be used by licensed healthcare providers to treat certain hospitalized patients with COVID-19. However, some of the products to be donated to the SNS were manufactured and stored outside the US in facilities not subject to FDA inspection or approved for use in the US market. As a result, tablet formulations and drug substances were required to undergo quality testing at CDER/OPQ/OTR. No standardized testing protocol existed for such situations, and circumstances demanded rapid response. Thus, OTR was tasked with organizing and assessing the quality of chloroquine and hydroxychloroquine substances and products for the emergency treatment of COVID-19 to assist the Agency's response. High demand for supplies as a result of the EUA greatly complicated the selection and implementation of methods. Methods for assay, impurity, and dissolution were evaluated from US, British, and European pharmacopeias, as well as from US firms. To ensure safety, OTR opted to include advanced analytical techniques to assess non-standard quality attributes. These included analyses for residual solvents using gas chromatography, metals analysis using X-ray fluorescence (XRF) and inductively coupled plasma mass spectrometry (ICP-MS), and nitrosamine testing using liquid chromatography with mass spectrometry (LC/MS). It was also discovered that chloroquine tablets were manufactured in a facility that made β-lactam antibiotics, which can cause severe allergic reactions at trace levels, so additional LC/MS studies were conducted to evaluate possible cross-contamination. In the end, OTR scientists determined that chloroquine drug products and hydroxychloroquine drug substances were safe for public use. As a result of these efforts, products tested were temporarily added to the SNS and could be used consistent with the conditions of authorization. These analyses were the first of their kind within the Agency for both speed and complexity. These procedures established an emergency testing protocol for future public health emergencies should the need arise.

Introduction

Note: *OTR's testing represented a part of FDA's activities to evaluate the quality of these samples and their suitability for the US market*

- Chloroquine Phosphate Tablets
  - Product donated to the SNS for distribution under the EUA was not approved in the US
  - Three lots of 1 million tablets were received as a donation
  - Tablets were received from Indian and Pakistani storage facilities
  - Product was manufactured in facility containing β-lactam antibiotics
  - Sample information:

FACTS #	Product	Facility Location	Dosage (mg)
1142404	Chloroquine phosphate tablets	India	250
1142537	Chloroquine phosphate tablets	Pakistan	250
1142538	Chloroquine phosphate tablets	Pakistan	250

- Hydroxychloroquine Sulfate Substance
  - Many products are approved in USA
  - Drug shortage resulted in the need to accept imports from manufacturers lacking current FDA inspections
  - Drug substance (DS) used in tablet manufacture were acquired for testing from the manufacturer (FACTS# 1142628)

Materials and Methods

- Chloroquine Phosphate Tablets
  - Assay, Impurities, and Dissolution tests were performed as minimal quality assessments
  - The USP monograph for the DP does not contain the required tests, so impurity methods from the DS monograph were used instead.

	USP Drug Product[1]	USP Drug Substance[2]
Assay	HPLC-UV	HPLC-UV
Impurities	None	HPLC-UV
Dissolution	Dissolution, HPLC-UV	-

Table 1: USP methods for chloroquine phosphate tablets and substance

- To ensure product safety, OTR performed additional testing beyond minimal standards. These included analyses for residual solvents, metals, β-lactam antibiotics, and nitrosamines.

Analysis	Technique	Method Source	Method Type
Assay	HPLC-UV	USP Chloroquine Phosphate Tablets	Compendial
Impurities	HPLC-UV	USP Chloroquine Phosphate	Compendial
Dissolution	HPLC-UV	USP Chloroquine Phosphate Tablets	Compendial
Residual Solvents	GC– FID	<467> USP Residual Solvents	Compendial
Metals	XRF	Original	In-house
	ICP-MS	Original	In-house
β-Lactam Antibiotics	LC/MS/MS	Original	In-house
Nitrosamines	LC/MS	Original	In-house

Table 2: Analysis methods for chloroquine phosphate tablets

- Hydroxychloroquine Sulfate Substance
  - The USP DS and DP monographs lacked adequate testing procedures
  - European (EP) and British (BP) Pharmacopeias contained adequate methods, but necessary standards required additional time to synthesize
  - Methods from the firm which manufactured the substance under analysis contained acceptable methods that did not require unavailable standards

	USP Drug Substance[3]	USP Drug Product[4]	EP Drug Substance[5]	BP Drug Substance[6]	Firm
Assay	Inadequate	HPLC-UV	HPLC-UV	HPLC-UV	HPLC-UV
Impurities	Inadequate	None	HPLC-UV	HPLC-UV	HPLC-UV
Standards Available?	-	-	No	No	Yes

Table 3: Pharmacopeia and firm methods for hydroxychloroquine sulfate tablets (drug product) and substance

- Additional testing beyond minimal standards was performed by OTR as outlined below. Analysis for β-lactam antibiotics was not needed since this class of products was not manufactured in the same facility.

Analysis	Technique	Method Source	Method Type
Assay	HPLC-UV	Firm	Firm
Impurities	HPLC-UV	Firm	Firm
Residual Solvents	GC–FID	<467> USP Residual Solvents	Compendial (USP)
Metals	XRF	Original	In-house
	ICP-MS	Original	In-house
Nitrosamine	LC/MS	Original	In-house

Table 4: Analysis methods for hydroxychloroquine sulfate substance

Results and Discussion

Note: For brevity, results are only shown for one lot of chloroquine phosphate tablets (sample 1142404), but results were similar for all three of the lots analyzed.

- Assay and Impurities (HPLC)

Chloroquine phosphate tablets

	Compound	Avg (%)	Spec (%)	Result
Assay	Chloroquine	98.0	93-107%	PASS
Impurities	Phenol	0.00	NMT 0.10	PASS
	RC G	0.03	NMT 0.10	PASS
	RC D	0.20	NMT 0.50	PASS
	Hydroxychloroquine	0.00	NMT 0.10	PASS
	RC A	0.00	NMT 0.10	PASS
	RC E	0.00	NMT 0.10	PASS
	Unidentified	0.05	NMT 0.10	PASS
	Unidentified	0.06	NMT 0.10	PASS
	Unidentified	0.03	NMT 0.10	PASS
	Unidentified	0.03	NMT 0.10	PASS
	Total Impurities:	0.39	NMT 2.0	PASS

Table 5: Assay and impurity results for lot chloroquine phosphate tablets (Sample 1142404)

Hydroxychloroquine sulfate substance

	Compound	Average (%)	Spec (%)	Result
Assay	Hydroxychloroquine	99.4	98-102	PASS
Impurities	Desethyl hydroxychloroquine	0.16%	NMT 0.20	PASS
	Hydroxychloroquine-O-acetate	0.12%	NMT 0.20	PASS
	Hydroxychlroquine-O-sulfate	0.00%	NMT 0.15	PASS
	4,7-Dichloroquinoline	0.00%	NMT 0.10	PASS
	Unspecified	None	NMT 0.10	PASS
	Total Impurities:	0.28%	NMT 0.60	PASS

Table 6: Assay and impurity results for hydroxychloroquine sulfate substance (Sample 1142628)

- Dissolution (HPLC)

Chloroquine phosphate tablets

% Dissolved (n=6)						
Low (%)	High (%)	Mean (%)	SD (%)	%RSD	Specifications	Pass/Fail
96.9	101.0	98.8	1.5	1.5	NLT 80%	Pass

Table 7: Dissolution results for chloroquine phosphate tablets (Sample 1142404)

- Residual solvents (GC/MS)
  - Chloroquine phosphate tablets and hydroxychloroquine sulfate substance did not contain any detectable level of Class 1 residual solvents (most severe)

Solvent	Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 8: USP Class 1 residual solvents

  - Chloroquine phosphate tablets (Sample 1142404) were determined to contain toluene (Class 2) and ethyl acetate (Class 3) at levels below USP specifications
  - Hydroxychloroquine sulfate substance (Sample 1142628) did not contain any detectable Class 2 or Class 3 residual solvents
- Metals (XRF and ICP-MS)

Class	Element	PDE (ug/day)	30* of CQP PDE (ug/g) *	30% of HCS PDE (ug/g) **	CQP Results (µg/g)***	HCS Results (µg/g)***
1	Cadmium	5	3.90	1.18	Below LOQ	Below LOQ
	Lead	5	3.90	1.18	Below LOQ	Below LOQ
	Arsenic	15	11.80	3.53	Below LOQ	0.2±0.006
	Mercury	30	23.50	7.06	Below LOQ	Below LOQ
2A	Vanadium	100	78.5	23.55	0.22	Below LOQ
	Palladium	100	78.5	23.55	Below LOQ	Below LOQ
	Nickel	200	157.00	47.10	4.54	0.18±0.03
2B	Ruthenium	100	78.5	23.55	Below LOQ	Below LOQ
	Iridium	100	78.5	23.55	Below LOQ	Below LOQ
	Platinum	100	78.5	23.55	Below LOQ	Below LOQ
3	Molybdenum	3000	2355	706	Below LOQ	Below LOQ
	Copper	3000	2355	706	0.28	Below LOQ
	Chromium	11000	8634	2590	13.24	0.32±0.04

Table 9: ICP-MS metals results for chloroquine phosphate DP (Sample 1142404) and hydroxychloroquine DS

* based on maximal daily dose of 1.0 g/day
** based on maximal daily does of 0.6 g/day
***LOQ = 0.001 µg/g (1.0 ppb)

- β-Lactam Antibiotics (LC/MS/MS)
  - Chloroquine phosphate tablets were manufactured in a facility that also produced β-lactam antibiotics
  - No cross-contamination was detected

Compound	Result	LOD
Ceftriaxone*	Not detected	0.62 ppm
Cefoperazone*	Not detected	0.32 ppm
Sulbactam*	Not detected	4 ppm
Cefixime	Not detected	0.16 ppm
Cefpodoxime Proxetil	Not detected	0.16 ppm
Cefuroxime Axetil	Not detected	0.62 ppm
Cefuroxime	Not detected	2.5 ppm
Ofloxacin	Not detected	0.62 ppm
Aztreonam	Not detected	25 ppm
Imipenem Monohydrate	Not detected	25 ppm
Cefuroxime Sodium	Not detected	25 ppm
Ampicillin	Not detected	25 ppm
Loracarbef	Not detected	25 ppm
Amoxicillin	Not detected	25 ppm
Penicillin G Potassium	Not detected	25 ppm
(+)-6-Aminopenicillanic acid	Not detected	<25 ppm

* Produced at facility where tablets were manufactured

Table 10: β-Lactam results for chloroquine phosphate tablets

For a complete summary, see Hongbin Zhu's poster *"LC-MS/MS Screening for β-Lactam Contamination in Chloroquine Phosphate Tablets"*

- Nitrosamines (LC/MS)
  - No nitrosamines were found in chloroquine phosphate tablets or the hydroxychloroquine sulfate substance

Compound	Abbreviation	Result
N-nitrosodimethylamine	NDMA	< 0.033 ppm
N-nitroso-N-methyl-4-aminobutyric acid	NMBA	< 0.013 ppm
N-nitrosodiethylamine	NDEA	< 0.013 ppm
N-nitrosoethylisopropylamine	NEIPA	< 0.013 ppm
N-nitrosodiisopropylamine	NDIPA	< 0.013 ppm
N-nitrosodipropylamine	NDPA	< 0.013 ppm
N-nitrosomethylphenylamine	NMPA	< 0.013 ppm
N-nitrosodibutylamine	NDBA	< 0.013 ppm

Table 11: Nitrosamine results for chloroquine phosphate tablets

Conclusion

- Chloroquine phosphate tablets and hydroxychloroquine sulfate substance passed all standard quality attribute specifications.
- No other contaminants identified for analysis were detected using advanced analytical techniques.
- FDA issued an EUA for these products, but later revoked it because the legal criteria for authorization were no longer met.
- These procedures established an emergency testing protocol for future public health emergencies should the need arise.

References

1. USP42-37, Chloroquine Phosphate Tablets
2. USP42-37, Chloroquine Phosphate
3. USP42-37, Hydroxychloroquine Sulfate
4. USP42-37, Hydroxychloroquine Sulfate Tablets
5. EP 10.0, Hydroxychloroquine Sulfate
6. BP 2020, Hydroxychloroquine Tablets