Development and Validation of a Sensitive and Selective Method for Hydroxychloroquine Sulfate Drug Products to Address the Underlying Drug Shortage Issues

Jiang Wang, Diaa Shakleya, Daniela Selaya, Adil Mohammad, Patrick Faustino

Division of Product Quality Research, Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration. 10903 New Hampshire Avenue, Silver Spring, Maryland, 20993

Abstract

Background: The COVID-19 pandemic has led to a worldwide effort to identify and develop potential preventatives and treatments, and one of the paths was to test approved medicines. Hydroxychloroquine, which is for the treatment of malaria, lupus and rheumatoid arthritis was evaluated based on its potential therapeutic benefits for COVID-19. Although it was determined to be ineffective against COVID-19, there was a significant increase of new prescriptions for this product. On March 31st, 2020 FDA has posted information regarding shortage of hydroxychloroquine to the drug shortages webpage.

Purpose: The objective was to rapidly develop and implement a sensitive and selective analytical method to evaluate hydroxychloroquine drug products for the product quality of drug products not been approved for the US market to help address the drug shortage.

Methodology: The analysis for hydroxychloroquine and its three impurities were carried out on an UHPLC system with tandem mass spectrometer. The chromatographic separation was achieved on an advanced phenyl column with sub-2 µm core-shell particles. A 10-min gradient elution program was set up to ensure the sufficient resolution while maintaining a high-throughput analytical capability. The tandem mass spectrometer was operated with positive electrospray ionization under the Multiple Ion Monitor mode for all the analytes.

Results: The method was validated according to the requirements of the USP <1225> validation for compendial method. The method was determined to be sensitive and selective and was successfully applied to the evaluation of 200 mg strength hydroxychloroquine sulfate tablets from three different manufacturers.

Conclusion: A UHPLC-MS/MS method using advanced column technology has been developed and validated for the simultaneous quantification of hydroxychloroquine and its three impurities. The method with MRM detection has shown sufficient sensitivity, selectivity and analytical range with the potential to be implemented as a high-throughput method with a 10 min run time. The validated method has been successfully applied to the quality assessment of approved hydroxychloroquine sulfate drug products in the US market. This work is also part of ongoing efforts to develop an advanced analytical platform to establish research readiness and capacity for a rapid regulatory response to emerging quality and public health issues.

Introduction

To develop advanced analytics for potential preventatives and treatments for COVID-19, chloroquine and hydroxychloroquine were tested for potential therapeutic benefit against COVID-19 suggested by in vitro studies, but later they were determined to be ineffective based on multiple clinical trials. However, the significant increase of new prescriptions required FDA to post information regarding drug shortages of chloroquine and hydroxychloroquine. To help prepare for their generic drug products submitted for approval in the US market to help address the drug shortage, This study reports the successful development and validation of an advanced UHPLC-MS/MS method for the quality control of HCQ and its pharmaceutical impurities in hydroxychloroquine sulfate drug products. The method was successfully validated and has been applied in approved hydroxychloroquine sulfate drug products. Products from three different manufactures tested were within USP specifications for both content (98.8-101%) and for all impurities (<0.1%).

Materials and Methods

Three different brands of 200 mg strength hydroxychloroquine sulfate tablets were purchased for the evaluation of potency and impurities. Reference standards for hydroxychloroquine sulfate and chloroquine related compound A were purchased from the USP (Rockville, MD) while reference standards for two impurities, desethyl-hydroxychloroquine dihydrochloride and O-acetyl-hydroxychloroquine were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Information on the properties and structures of these compounds are listed in Table 1 and Figure 1. The compounds were used as received without further purification or modification. Optima LC/MS grade acetonitrile, isopropanol, methanol and formic acid (FA) were purchased from Fischer Scientific (Pittsburgh, PA). Filtered 18 mΩ water was supplied in-house by a Millipore Milli-Q System (Bedford, MA). All other chemicals and reagents were of analytical grade.

The analysis for hydroxychloroquine and its three impurities were carried out on an UHPLC system with tandem mass spectrometer. The chromatographic separation was achieved Waters Cortecs UPLC phenyl column with 1.6 µm solid core particles, which provide excellent selectivity, while reducing the potential for peak broadening. A 10-min gradient elution program starting with 3% organic mobile phase ensures proper retention of the polar impurity HCQ-DES and fast elution for HCQ, HCQ-OA and CQA which allows for a high throughput analysis. Formic acid and ammonium formate were used to replace the buffer triethylamine and phosphoric acid used in the USP method since they are unsuitable for use in MS analyses. A stable isotope labelled internal standard chloroquine-d5 was used monitor MS instrument performance. The tandem mass spectrometer was operated with positive electrospray ionization under the Multiple Ion Monitor mode for all the analytes.

Table 1. Chemical Data on hydroxychloroquine sulfate and related impurities

Reference standard	MW	MW of free base	Free base formula
Hydroxychloroquine sulfate	433.95	335.88	$C_{18}H_{26}ClN_3O$
Chloroquine Related Compound A (CQA)	198.05	198.05	$C_9H_5Cl_2N$
Desethyl-hydroxychloroquine Dihydrochloride (HCQ-DES)	380.74	307.82	$C_{16}H_{22}ClN_3O$
O-acetyl-hydroxychloroquine (HCQ-OA)	377.91	377.91	$C_{20}H_{28}ClN_3O_2$

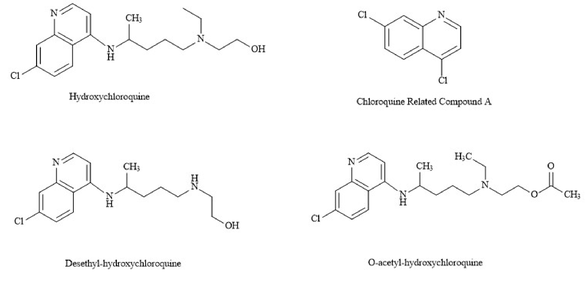

Figure 1. Chemical structures of hydroxychloroquine and related impurities

Results and Discussion

The method was validated according to the requirements of the USP <1225> for linearity, accuracy, precision, specificity, and analytical range. The calibration curve was validated over the range of 100-10000 ng/mL for HCQ and 10-1000ng/mL for HCQ-DES, HCQ-OA and CQA. The correlation coefficient was greater than 0.99. Intra- and inter-day accuracy and precision results have also been evaluated. The acceptance range of accuracy was 80-120% for LLOQ, and 85-115% for the remaining QCs. The allowable limits for the RSD were 20% and 15% for the LLOQ. Both accuracy and precision were found to be acceptable. Specificity of the method was demonstrated by the absence of endogenous peaks at the retention times of hydroxychloroquine, its three impurities and the internal standard chloroquine-d5 in blank mobile phase. (Figure 2). Carryover was evaluated by injecting mobile phase blank immediately following injection of sample representing the upper limit of quantitation. The results showed no carryover (Data not shown). HCQ and its impurities in the extracted samples were found to be stable up to 12 hours in the autosampler at 4°C. The results are listed in Table 2. Because blank matrices for all three test drug products were not available, the matrix effect of hydroxychloroquine and its three impurities, as detected with MS/MS, was evaluated using a spiking and recovery method. The results are shown in Table 3. The validated method was successfully applied to the quantitation of hydroxychloroquine and its three impurities in 200 mg strength hydroxychloroquine sulfate tablets from three different manufacturers. On a dried basis, the hydroxychloroquine sulfate tablets tested from different manufacturers contained 98.8-101 % hydroxychloroquine sulfate and all impurities met the USP criteria which the content of each impurity is less than 0.1%.

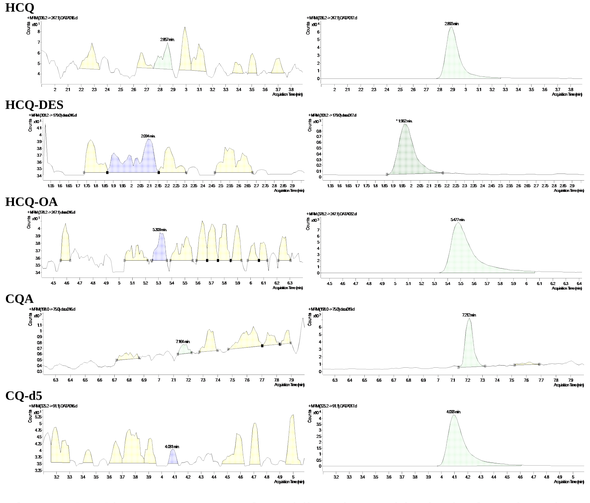

Figure 2. Chromatograms of mobile phase blank (Left column) and calibration standard of four analytes at LLOQ level and internal standard (Right column).

Table 2. Autosampler stability (Post-extraction, up to 12 hours)

Analytes	Autosampler Stability (ng/mL)			
	10/100	20/200	100/1000	1000/10000
HCQ	100%	96.4%	100%	99.2%
HCQ-DES	101%	97.5%	101%	99.2%
HCQ-OA	101%	91.6%	100%	97.3%
CQA	104%	106.5%	102%	99.2%

Table 3. Recovery of standard solution at (100/1000 ng/mL) added to extracted tablet samples (n=6)

Analyte	Product 1		Product 2		Product 3	
	AVE	RSD%	AVE	RSD%	AVE	RSD%
HCQ-DES	104%	2.04%	105%	1.25%	105%	1.27%
HCQ	100%	3.99%	99.7%	7.01%	102%	4.72%
HCQ-OA	99.4%	2.30%	96.9%	0.98%	96.3%	1.50%
CQA	98.5%	4.00%	98.5%	4.98%	103%	2.35%

Conclusion

A selective and reproducible UHPLC-MS/MS method has been developed and validated for the quantitation of HCQ and its three impurities, desethyl-hydroxychloroquine (HCQ-DES), O-acetyl-hydroxychloroquine (HCQ-OA) and Chloroquine Related Compound A (CQA) in hydroxychloroquine sulfate tablets (Figure 1). The method features a simple sample preparation procedure followed by a 10-min chromatographic run. The chromatographic column was a Waters Acquity UHPLC Cortecs Phenyl column with 1.6 µm solid core silica particles which allows for the simultaneous determination of HCQ and its three impurities. The validated method has been successfully applied to the quality assessment of approved hydroxychloroquine sulfate drug products in the US market. This work documents the successful development and validation of a new analytical method for the evaluation of the quality of hydroxychloroquine sulfate drug products for use in the United States.

This work is also part of OTR efforts to develop an advanced analytical platform to establish research readiness and capacity for a rapid regulatory response to emerging quality and public health issues. The work also represents our continuing efforts to implement advanced UHPLC column technologies and highly sensitive and selective MRM detection procedures to rapidly implement, increasingly necessary innovative analytical methods. These on-going efforts to develop advanced analytics will help to ensure research readiness for a rapid and effective regulatory response to emerging emergency public health issues such as unapproved hydroxychloroquine sulfate drug products.

(Disclaimer: This poster reflects the views of the authors and should not be construed to represent FDA's views or policies)