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Nota de aplicación

Lessons from COVID-19: Verifying Performance of an HPLC/UV method with MS Compatible Conditions for Hydroxychloroquine Sulfate Analysis.

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Like many small molecule drugs, hydroxychloroquine sulfate (Figure 1), an effective anti-malarial, was investigated as a potential therapy to the novel coronavirus. While current clinical evidence does not appear to support the use of hydroxychloroquine for hospitalized SARS-CoV-2 infected patients, Waters began examining improved analytical methods during the early stages of the COVID-19 pandemic. This application brief offers a quick HPLC/UV method with MS compatible conditions for hydroxychloroquine sulfate analysis. Mass spectroscopy (MS) allows the investigator to accurately identify new or unknown components that may develop during the formulation process or routine testing. The new method offers higher resolution, less tailing, and faster run times compared to the current USP Monograph for hydroxychloroquine sulfate tablets (USP42-NF37). Regardless of the final clinical outcome for hydroxychloroquine in the context of COVID-19, these analytical advances are still applicable for hydroxychloroquine analysis generally. Additionally, this work may be transferrable in part for other small molecule therapies.

Benefits

- Fast and reliable HPLC/UV method using ACQUITY Arc System with MS compatible conditions
- Improved resolution and reduced peak tailing for hydroxychloroquine and chloroquine peaks compared to the USP method
- Quick and accurate peak identification using mass spectral data from an ACQUITY QDa Detector

Introduction

Hydroxychloroquine (HQ) has long been prescribed for chemoprophylaxis against malaria and, more recently, to help in the treatment of chronic autoimmune diseases.[1] Early in vitro studies showed that this active pharmaceutical ingredient might inhibit SARS-CoV-2 infection.[2] Current clinical consensus, however, does not appear to support the use of hydroxychloroquine in hospitalized COVID-19 patients, a primary patient population target for clinical investigation.[3,4,5] Regardless, some questions remain about the utility of the drug early in the course of SARS-CoV-2 infection or as a pre-exposure prophylactic.[6] While governments and pharmaceutical companies actively pursued clinical investigations into hydroxychloroquine during early stages of the COVID-19 pandemic, we examined new analytical methods to support potential needs for faster, higher performing, MS-compatible methods in pharmaceutical development and manufacturing.

In this application brief we present a fast and reliable HPLC/UV method for hydroxychloroquine sulfate analysis with use of MS compatible buffer, while meeting the USP system suitability requirements.[7]

Regardless of whether hydroxychloroquine will be used in the context of COVID-19, this new analytical method is still useful for hydroxychloroquine in general and may be transferrable in part for similar small molecule therapeutics.

Hydroxychloroquine sulfate

- $C_{18}H_{28}ClN_3O_5S$
- Monoisotopic mass: 433.14 Da
- Free base monoisotopic mass: 335.18 Da

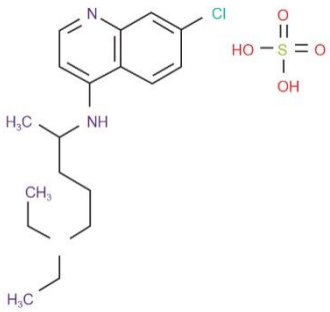

Figure 1. Hydroxychloroquine sulfate.

Results and Discussion

The analysis for hydroxychloroquine sulfate was performed following the assay procedure in the current USP monograph for hydroxychloroquine sulfate tablets.[6] The standard and system suitability solutions were run on the ACQUITY Arc System using both the HPLC/UV with MS compatible conditions and the USP method conditions (Table 1). The ACQUITY QDa Mass Detector was only used with the new MS compatible method to collect mass spectral data information for the analytes. Analysis of the system suitability solution performed using the new method resulted in a higher resolution between hydroxychloroquine and chloroquine peaks compared to the current USP method (Figure 2). Furthermore, a faster run time was achieved (3 minutes) with compared to the USP method (7 minutes). The mass spectral data from the ACQUITY QDa Detector enabled quick and accurate peak identification by mass detection (Figure 3).

Parameter	Modernized MS compatible method	USP monograph method[5]
LC system	ACQUITY Arc	ACQUITY Arc
Detection	PDA (derived at 254 nm) and ACQUITY QDa	PDA (derived at 254 nm)
Column(s)	XSelect CSH C_{18}, 4.6 × 100 mm, 3.5 µm	Symmetry C_{18}, 4.6 × 250 mm, 5 µm
Column temp.	40 °C	30 °C
Injection volume	10 µL	20 µL
Flow rate	1.5 mL/min	1.0 mL/min
Mobile phase	Acetonitrile/10 mM ammonium formate (10/90) with 0.1% formic acid	Methanol/acetonitrile/water/ phosphoric acid (100:100:800:2). Add 96 mg of sodium 1-pentasulfonate.
Sample	Chloroquine phosphate (CQ) and hydroxychloroquine sulfate (HCQ) at 0.05 mg/mL in water	Chloroquine phosphate (CQ) and hydroxychloroquine sulfate (HCQ) at 0.05 mg/mL mobile phase

Table 1. Conditions of the new HPLC and USP methods for hydroxychloroquine sulfate.

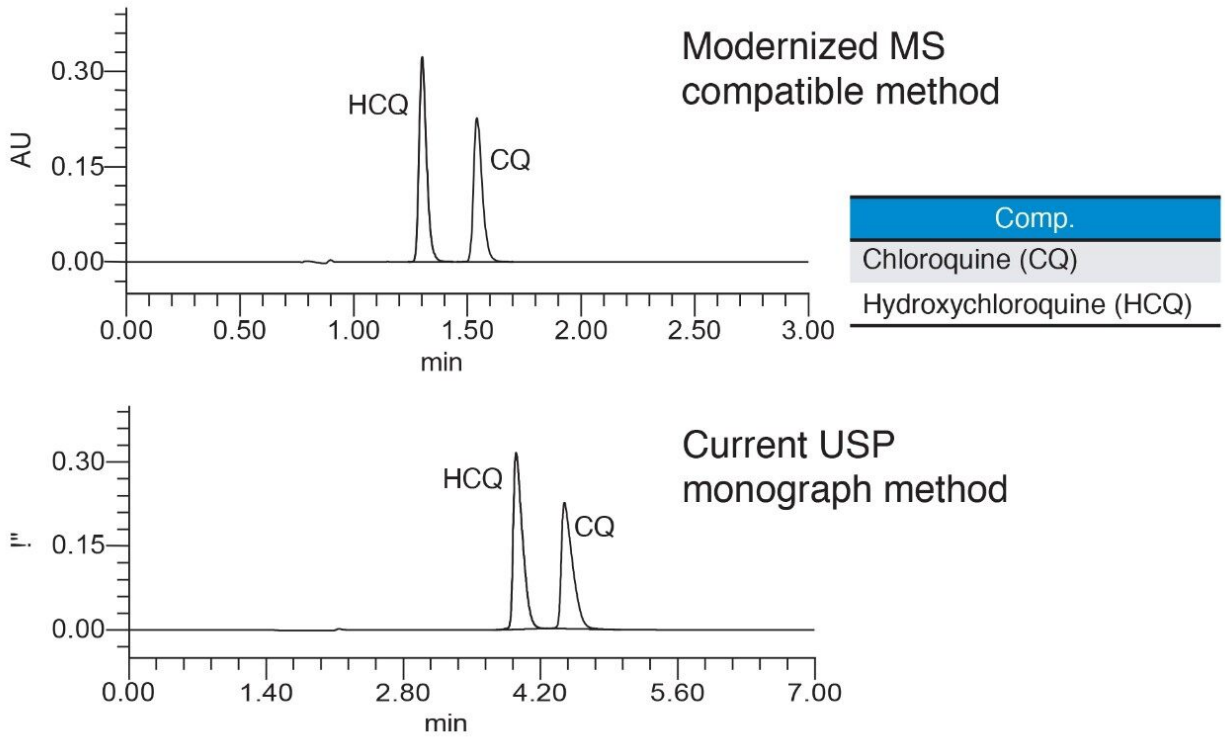

Figure 2. Analysis of the system suitability solution using new HPLC and USP methods, UV at 254 nm.

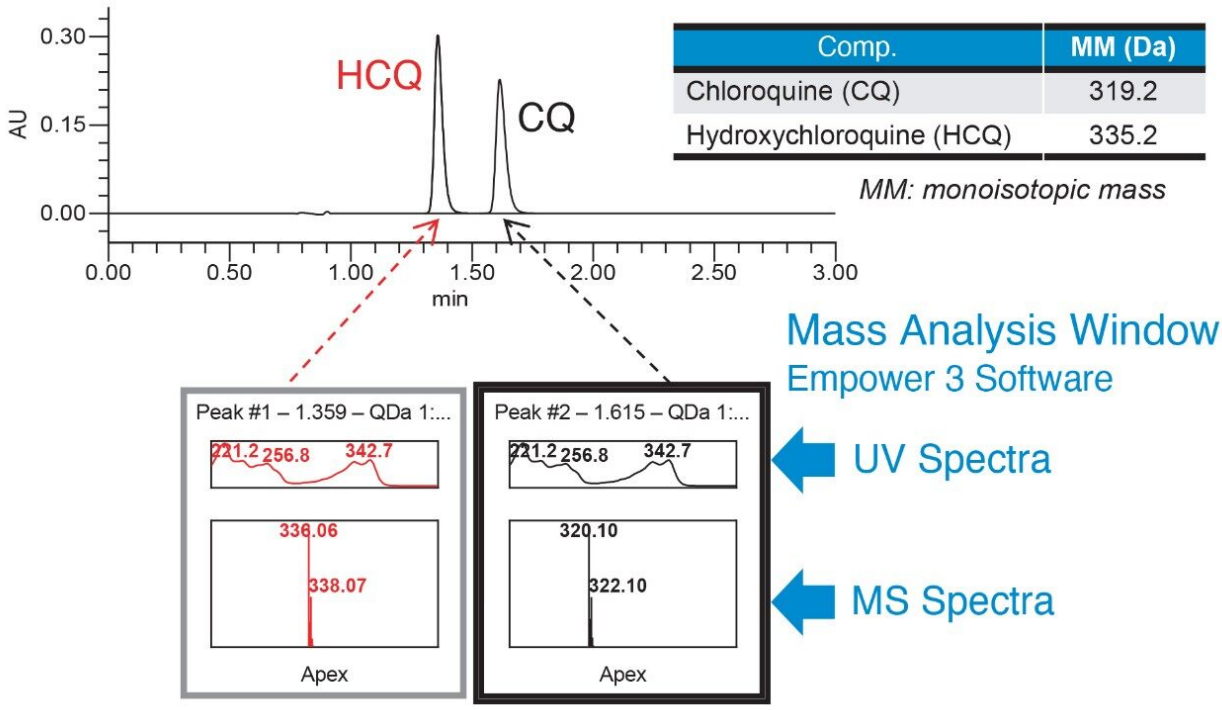

Figure 3. Mass analysis window from the Empower 3 software for peak identity confirmation. Analysis performed using new HPLC method with MS compatible conditions, UV at 245 nm.

Performance of the new HPLC method was verified according to the system suitability requirements as per the assay procedure in the current USP monograph for hydroxychloroquine sulfate tablets.[6] The system suitability results of the method operated under new HPLC MS compatible conditions successfully met the USP criteria (Table 2). Resolution between hydroxychloroquine and chloroquine improved significantly when run under new conditions compared to the USP method. Relative standard deviations of peak areas and retention times for 5 replicate injections of the standard solution were less than 0.1%. Furthermore, the new method provided improvements in USP tailing for both hydroxychloroquine and chloroquine.

Parameter	USP requirement[2]	New HPLC method with MS compatible conditions	USP Method
Resolution between CQ and HCQ	Not less than (NLT) 1.8	3.7	2.5
Relative standard deviation (RSD) for replicate injections of HCQ standard	Not more than (NMT) 1.5%	▪ RSD of peak areas: 0.0% ▪ RSD of retention times: 0.1%	▪ RSD of peak areas: 0.2% ▪ RSD of retention times: 0.2%
Peak tailing for CQ	N/A	1.4	2.1
Peak tailing for HCQ	N/A	1.4	1.9

Table 2. System suitability results of the methods operated under new HPLC and USP conditions.

Conclusion

This application brief provides an MS compatible method for assay of hydroxychloroquine sulfate. By enabling MS analysis, this method enhances the analytical tool-kit available for hydroxychloroquine characterization and development. MS analysis enables qualitative compound identification without the need for individual standards. Furthermore, this new method exhibits faster run times, improved resolution, and less peak tailing compared to the current USP Monograph method. Regardless of whether hydroxychloroquine serves as a potential treatment for COVID-19, improved speed may prove to be important during time-sensitive pharmaceutical manufacturing. Furthermore, more robust analytical performance may provide enhanced confidence in critical quality control environments.

References

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