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Application Note

Lessons from COVID-19: Verifying Performance of an HPLC-UV Method with MS-Compatible Conditions for Chloroquine Phosphate Analysis

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

Abstract

Like many small molecule drugs, chloroquine phosphate (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 or chloroquine 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 chloroquine phosphate 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 chloroquine phosphate tablets (USP42-NF37). Regardless of the final clinical outcome for chloroquine in the context of COVID-19, these analytical advances are still applicable for chloroquine 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 chloroquine and amodiaquine peaks compared to the USP method
- Quick and accurate peak identification using mass spectral data from an ACQUITY QDa Detector

Introduction

Chloroquine (CQ), like its derivative hydroxychloroquine, has long been prescribed for chemprophylaxis 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] However, current clinical consensus does not appear to support the use of chloroquine or hydroxychloroquine in hospitalized COVID-19 patients, a primary patient population target for clinical investigation.[3] Still, some questions remain about the utility of these drugs earlier in the course of SARS-CoV-2 infection or as a pre-exposure prophylactic.[4,5] While governments and pharmaceutical companies actively pursued clinical investigations during early stages of the COVID-19 pandemic, we examined new analytical methods to support potential needs for faster, higher performance, and MS-compatible methods in pharmaceutical development and manufacturing.

In this application brief we present a fast and reliable HPLC-UV method for chloroquine phosphate analysis with use of MS-compatible buffer, while meeting the USP system suitability requirements.[6]

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

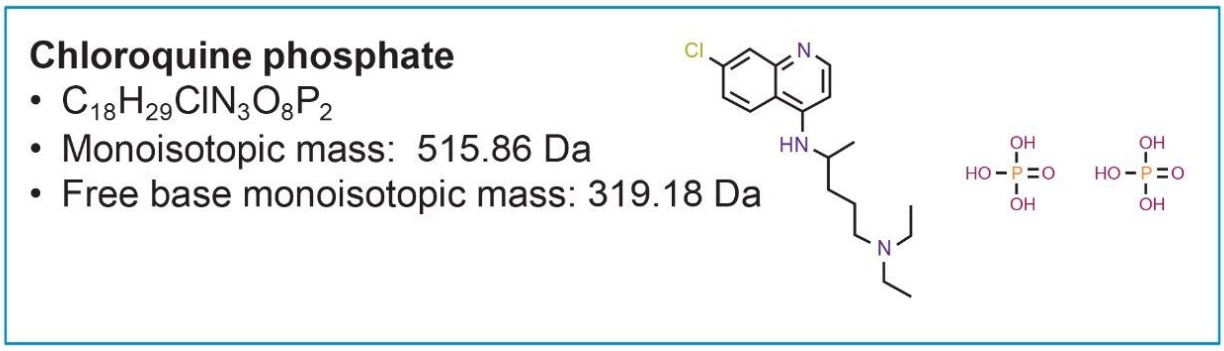

Figure 1. Chloroquine phosphate.

Results and Discussion

The performance of the new HPLC method for analysis for chloroquine phosphate was measured following the system suitability requirements as per the assay procedure listed in the current USP monograph for chloroquine phosphate tablets[6]. The system suitability solution was 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 chloroquine and amodiaquine peaks compared to the current USP method (Figure 2). Furthermore, faster run time was achieved (3 minutes) with the new method 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[4]
LC system	ACQUITY Arc	ACQUITY Arc
Detection	PDA (derived at 224 nm) and ACQUITY QDa	PDA (derived at 224 nm)
Column(s)	XSelect CSH C_{18}, 4.6 × 100 mm, 3.5 µm	Symmetry C_{18}, 4.6 × 100 mm, 5 µm
Column temp.	40 °C	30 °C
Injection volume	10 µL	10 µL
Flow rate	1.5 mL/min	1.2 mL/min
Mobile phase	Acetonitrile/10 mM ammonium formate (10/90) with 0.1% formic acid	Methanol/buffer (22:78) Buffer: 6.8 g/L of monobasic potassium phosphate in water. Add 1 mL of perchloric acid, pH 2.5 adjusted with phosphoric acid
System suitability solution	0.05 mg/mL of amodiaquine HCl and 0.05 mg/mL of chloroquine phosphate in water	0.15 mg/mL of amodiaquine HCl and 0.15 mg/mL of chloroquine phosphate in water

Table 1. Conditions of the new HPLC and USP methods for chloroquine phosphate.

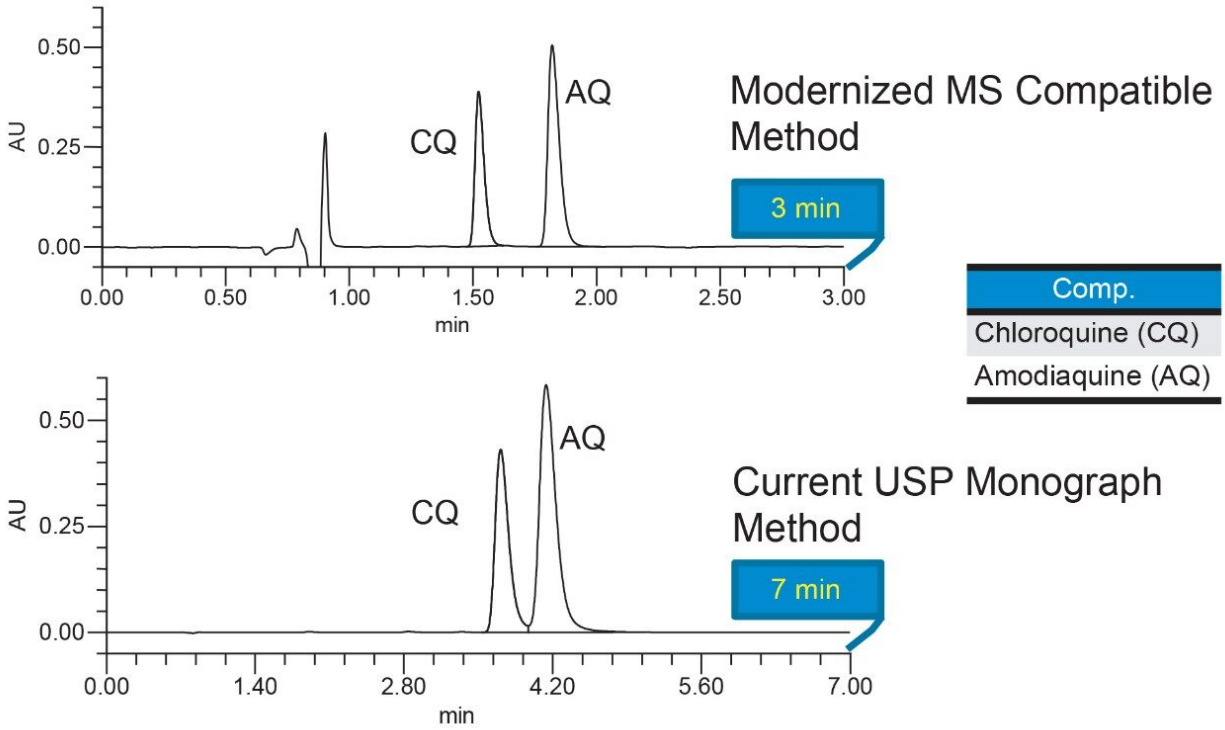

Figure 2. Analysis of the system suitability solution using new HPLC and USP methods, UV 224 nm.

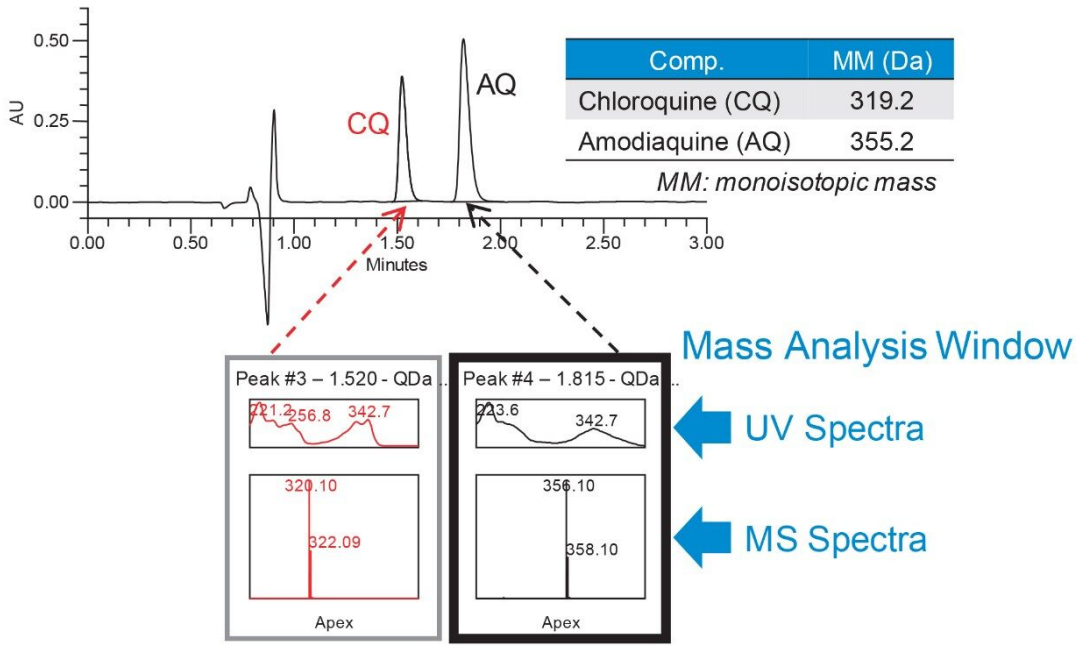

Figure 3. Mass analysis window from the Empower 3 Software for peak identity confirmation.

The system suitability results of the method operated under new HPLC-MS compatible conditions successfully met the USP criteria (Table 2). Resolution between chloroquine and amodiaquine 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 system suitability solution were ≤0.4%. Furthermore, the new method provided a slight improvement in USP tailing for chloroquine and the same USP tailing for amodiaquine.

Parameter	USP requirement[4]	Modernized method	USP method
Resolution between AQ and CQ	Not less than (NLT) 1.5	3.8	1.7
Tailing factor for the AQ and CQ peaks	Not more than (NMT) 1.5	1.4 for CQ 1.5 for AQ	1.5 for CQ 1.5 for AQ
Relative standard deviation (RSD) for the AQ and CQ	Not more than (NMT) 2.0%	▪ Chloroquine peak –RSD of areas: 0.4% –RSD of retention times: 0.1% ▪ Amodiaquine peak –RSD of areas: 0.1% –RSD of retention times: 0.3%	▪ Chloroquine peak –RSD of areas: 0.0% –RSD of retention times: 0.1% ▪ Amodiaquine peak –RSD of areas: 0.1% –RSD of retention times: 0.1%

Table 2. System suitability results for the new MS-compatible HPLC and USP methods for chloroquine phosphate in tablets.

Conclusion

This application brief provides an MS-compatible method for chloroquine phosphate tablets. By enabling MS analysis, this method enhances the analytical tool-kit available for chloroquine characterization and development. MS analysis enables qualitative compound identification without the need for individual standards. Furthermore, this new method exhibits faster run time and improved resolution compared to the current USP Monograph method. Regardless of whether chloroquine 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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