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應用手冊

Comprehending COVID-19: Assessing System Suitability of a Modernized MS Compatible USP Monograph for Chloroquine Phosphate Tablets

Margaret Maziarz

Waters Corporation

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

Abstract

As a result of the ongoing COVID-19 pandemic, numerous small molecule drugs are being re-investigated as potential therapies to the novel coronavirus. Chloroquine, like its derivative hydroxychloroquine, long prescribed as an anti-malarial, is a small molecule drug receiving an extended look. Plans for large scale global clinical trials are underway to study the ability of chloroquine to prevent or reduce the severity of SARS-CoV-2 infection in front-line health-care workers.[1] To support analytical characterization during therapeutic development, this application brief offers a modernized MS compatible method 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 method.

Benefits

- MS compatible method
- Improved resolution between chloroquine and amodiaquine
- Faster run-times using a shorter column and higher flow rate
- Reduced tailing for chloroquine

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.[2] Interestingly, in vitro studies have shown that this active pharmaceutical ingredient might inhibit SARS-CoV-2 infection.[3] CQ is believed to prevent viral infection through interference of ACE2.[3] As a result of *in vitro* studies and the proposed mechanism of action, the COVID-19 Research Outcomes Worldwide Network (CROWN) is preparing to test the effectiveness of chloroquine against novel coronavirus infection in front-line health workers across the globe.[1]

In this application brief we present a modern, improved method for the assay of chloroquine phosphate in tablets with use of MS compatible buffer, while meeting USP system suitability requirements.

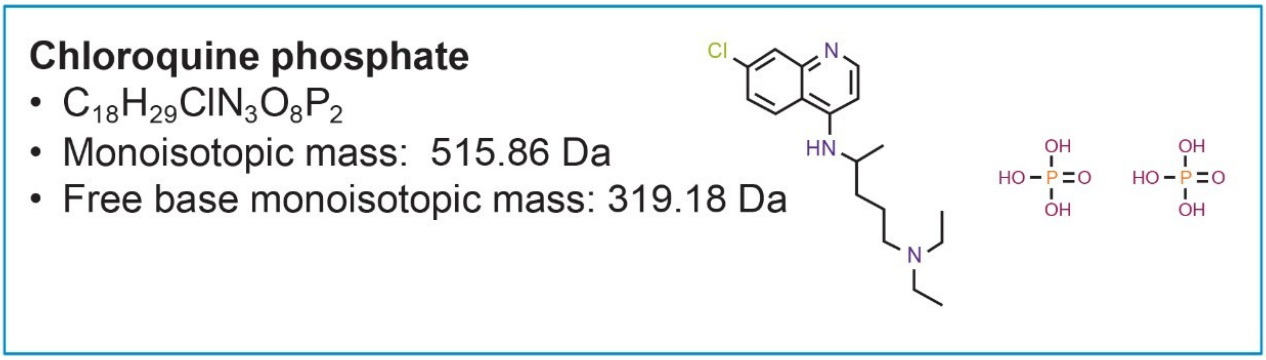

Figure 1. Chloroquine phosphate.

Results and Discussion

Conditions for the modernized MS compatible and current USP methods for the assay of chloroquine phosphate in tablets are summarized in Table 1.

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 for modernized MS compatible and USP methods.

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 with the improved method (3 minutes) 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).

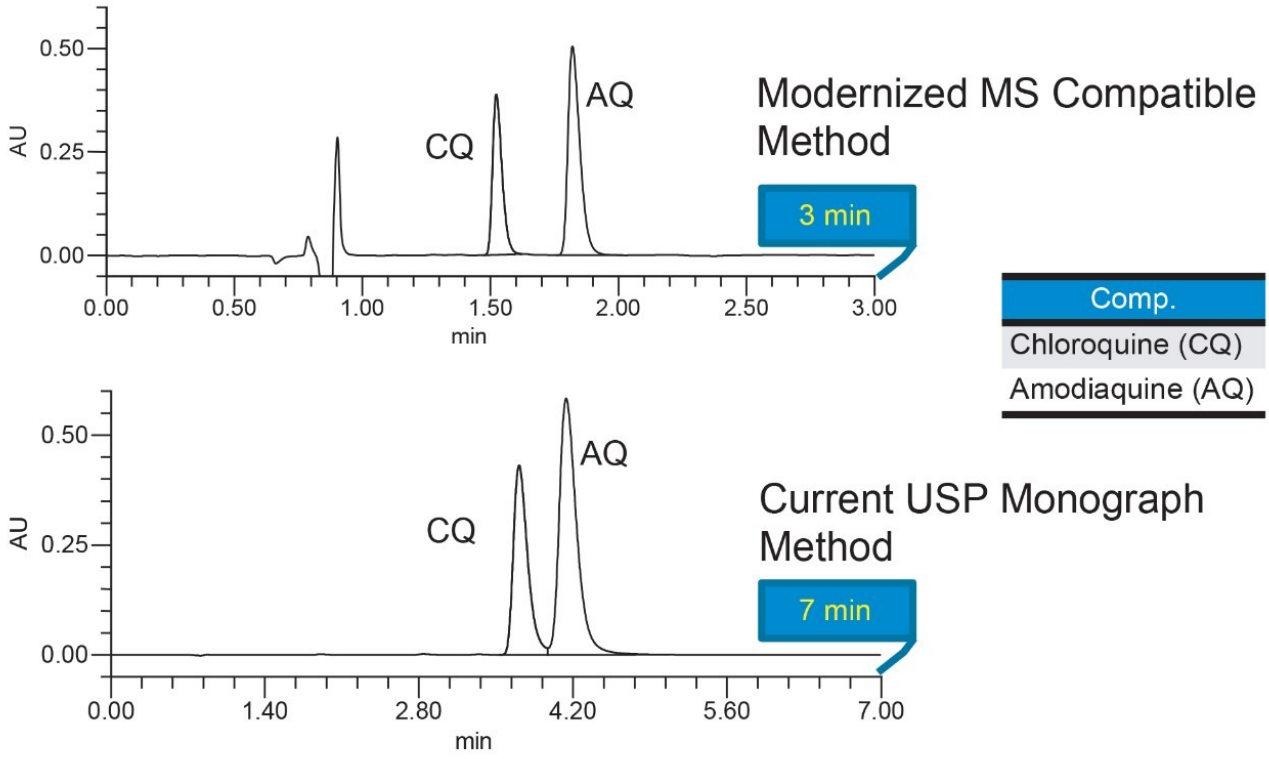

Figure 2. S*ystem suitability solution acquired using modernized MS compatible and USP methods for assay of chloroquine phosphate in tablets.*

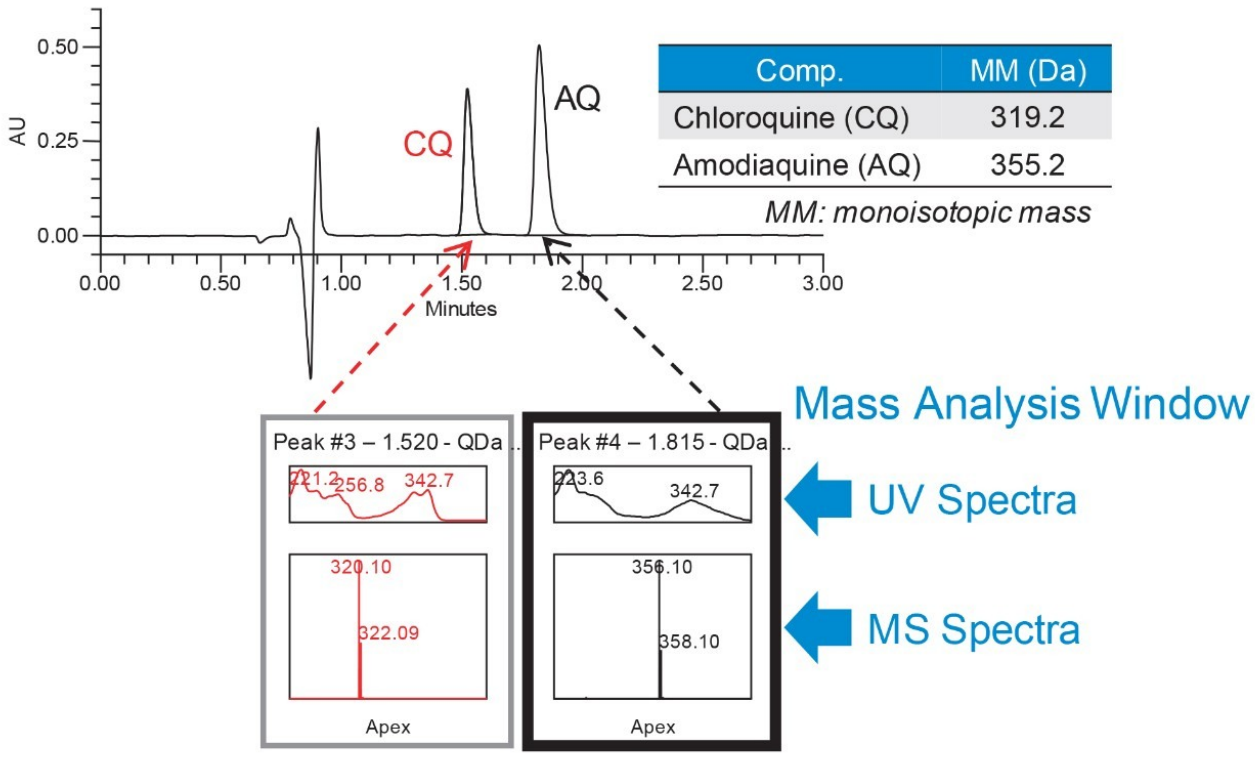

Figure 3. Mass analysis window from the Empower 3 software for peak identity confirmation.

Performance of the modernized MS compatible method was measured using 5 replicate injections of the system suitability solution against the requirements defined in the USP monograph for chloroquine phosphate in tablets.[4] The results showed that system suitability for the modernized MS compatible method met the USP criteria (Table 2). Resolution between chloroquine and amodiaquine improved significantly when run under modernized MS compatible 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 modernized MS compatible 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 modernized MS compatible and USP methods for chloroquine phosphate in tablets.

Conclusion

This application brief provides an MS compatible method for assay of chloroquine phosphate in tablet formulation. 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 modernized MS compatible method exhibits faster run time, improved resolution, and less peak tailing compared to the current USP Monograph method. As chloroquine is investigated as a potential treatment for COVID-19, improved speed may prove to be important during time-sensitive drug development and subsequent manufacturing. Furthermore, more robust analytical performance may provide enhanced confidence in critical development and quality control environments during the novel coronavirus outbreak.

References

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