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Application News

No. LC-199

Liquid Chromatograph Nexera™ Series

Application of HPLC in Quality Analysis of Hydroxychloroquine Sulfate

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Abstract: In this study, methods were established for analysis of hydroxychloroquine sulfate and related substances following European Pharmacopoeia (EP10.0) and for determination of hydroxychloroquine sulfate content in tablet following US Pharmacopoeia (USP 43). Using Shimadzu UHPLC system, well separation was achieved between hydroxychloroquine sulfate and impurity C, as well as between impurity B and impurity C referring to the EP assay. Using Shimadzu HPLC system with reference to the USP assay, the content of hydroxychloroquine sulfate in tablet was determined with achieving the desired peak separation and reproducibility described in the USP.

Keyword: hydroxychloroquine sulfate, HPLC, European Pharmacopoeia (EP), US Pharmacopoeia (USP)

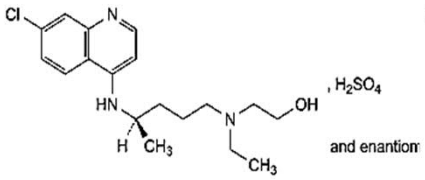
❑ Introduction

Hydroxychloroquine sulfate (CAS#: 747-36-4) was on the market first in 1955 in the USA. To date, it has been approved in more than 70 countries including Canada, France, German, Australia, China and Japan etc.. Hydroxychloroquine sulfate was used initially in the treatment of malaria. The drug was found to exhibit mild immunosuppression and immunomodulation effects in clinical use. Therefore, it has been used widely in the treatments of various diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), connective tissue disease (CTD) etc. Amid the pandemic of novel coronavirus disease (COVID-19) at present, some existing drugs come to public attention and hydroxychloroquine sulfate is one of such drugs.

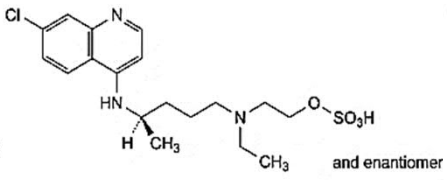
Cell biology screening studies indicate that hydroxychloroquine sulfate exhibits significant suppression effect to coronavirus SARS/MERS. Molecular biochemistry evidences also provide primary support on its significant effect to the glycosylation level of receptor ACE2 of coronavirus. Recently, clinical trials on the evaluation of the safety and effectiveness for coronavirus disease with hydroxychloroquine sulfate were started urgently in many countries in the world.

The quality of a drug is a fundamental factor to guarantee the results of clinical trials. The EP and USP monographs have described the method and criteria for investigation of hydroxychloroquine sulfate and related substances, and for determination of hydroxychloroquine sulfate content in tablets.

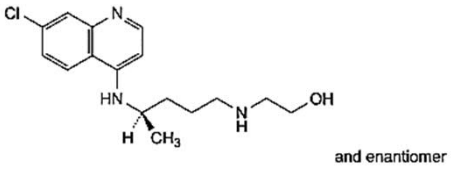
In this work, hydroxychloroquine sulfate and related substances were analyzed following EP assay on Shimadzu UHPLC. The content of hydroxychloroquine sulfate in tablets was determined in refereeing to USP assay in order to provide comprehensive monitoring and evaluation on the quality of hydroxychloroquine sulfate tablet products.



Hydroxychloroquine sulfate
$C_{18}H_{28}ClN_3O_5S$ MW 433.95
CAS#: 747-36-4



EP Impurity B
$C_{18}H_{26}ClN_3O_4S$ MW 415.93
CAS#:103152-84-7



EP Impurity C
$C_{16}H_{22}ClN_3O$ MW 307.82
CAS#: 4298-15-1

❑ Experimental

1.1 Instrument

Shimadzu UHPLC system LC-30A and HPLC system LC-2040C 3D were used in this work.

The s/w used is LabSolutions™ DB ver 6.87, a chromatography workstation.

1.2 Analytical conditions

- EP assay (LC-30A UHPLC system)

Column: EP10.0 01/2017:2849, C18 50 X 2.1 mm I.D., 1.7 µm
Mobile phase: A - Methanol / Buffer (10:90 v:v); B Methanol / Buffer (85:15 v:v) *
Flow rate: 0.7 mL/min
Injection volume: 4 µL
Column Temperature: 40 °C
Autosampler temperature: 6 °C
Elution mode: gradient solution, B initial conc. 0%, see time program in Table 1
*Buffer is described in the monograph in the EP 10.0

Table 1. Gradient elution time program

Time (min)	Module	Command	Value (%)
1.00	Pumps	Pump B.Conc	0
11.00	Pumps	Pump B.Conc	100
11.10	Pumps	Pump B.Conc	0
12.50	Controller	Stop	

- USP assay (LC-2040C 3D HPLC system)

Column: Shim-pack™ GIST C18 250×4.6mmI.D., 5 µm (PN: 227-30017-08)
Mobile phase: Water/MeOH/CAN/phosphoric acid (800mL/100mL/100mL/2mL), containing 96 mg Sodium 1-pentanesulphonate
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Column Temperature: 40 °C
Autosampler temperature: 6 °C
Elution mode: isocratic gradient

1.3 Sample preparation

As mentioned in EP 10.0 and USP 43.

❑ Results and Discussion

2.1 Determination of hydroxychloroquine sulfate related substances following EP method

In EP10.0 monograph, it requires peak resolution greater than 3.0 for Impurity C and hydroxychloroquine sulfate as well as for Impurity B and Impurity C.

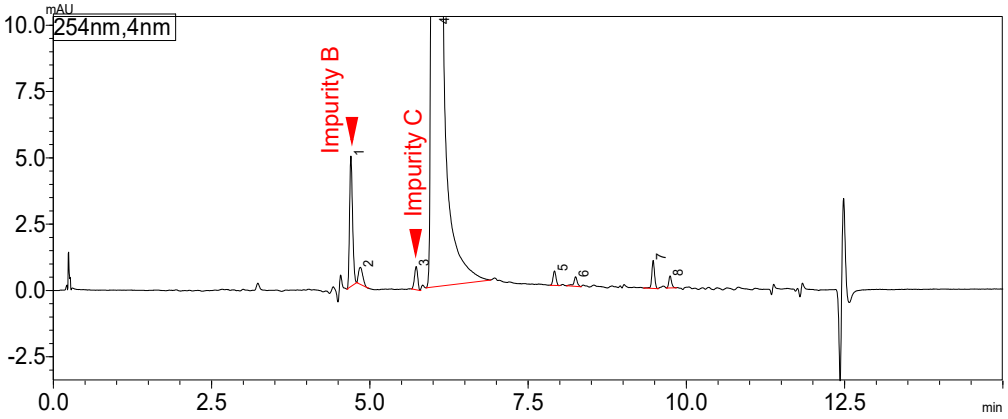


Figure 1. Chromatogram of hydroxychloroquine sulfate related substances

Table 2. Chromatographic information of hydroxychloroquine sulfate (HCQ) and related substances

Peak#	Ret. Time	Area	Height	Area%	Resolution (EP)
1（Impurity B）	4.701	16191	4885	0.251	/
2	4.849	3007	642	0.047	1.337
3（Impurity C）	5.732	3041	869	0.047	7.769
4（HCQ）	6.050	6421680	1570520	99.537	3.050
5	7.917	1770	542	0.027	18.378
6	8.251	1377	355	0.021	3.624
7	9.475	3153	1040	0.049	13.984
8	9.742	1333	449	0.021	3.206
Total	8.242	6451552	1579303	100.000	3.889

The result indicates that the resolutions obtained are greater than 3.0 for hydroxychloroquine sulfate and Impurity C, as well as for Impurity B and Impurity C. This result meets the criteria stated in the EP monograph.

2.2 Determination of hydroxychloroquine sulfate content in tablet following USP assay

2.2.1 Result of resolution

In the USP method, it requires that the resolution must be not less than 1.8 for chloroquine phosphate (CQ) and hydroxychloroquine sulfate (HCQ). The resolution obtained is 3.728, which meets fully the criteria of R greater than 1.8.

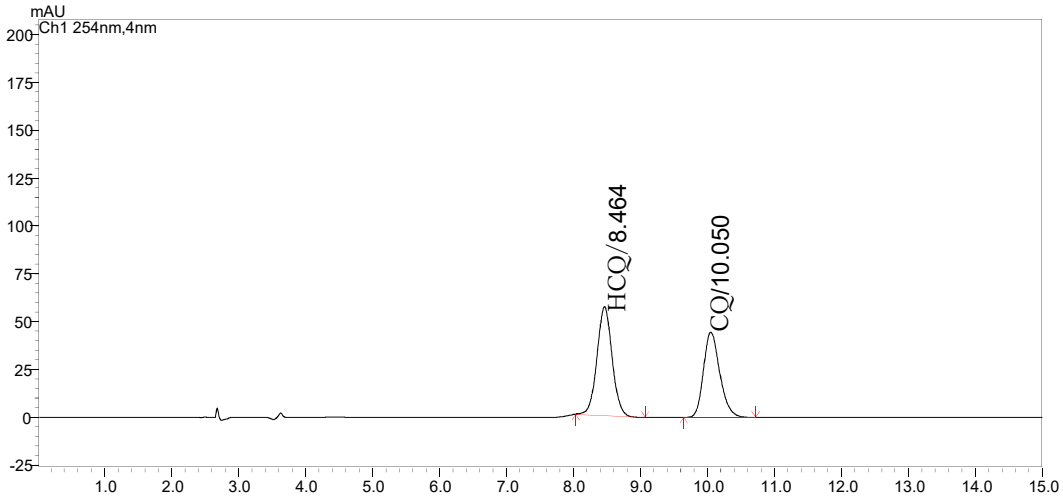


Figure 2. Chromatogram of hydroxychloroquine sulfate (HCQ) and chloroquine phosphate (CQ)

Table 3. Result of resolution of hydroxychloroquine sulfate (HCQ) and chloroquine phosphate (CQ)

Peak#	Ret. Time	Area	Height	Theoretical Plate Number	Width (USP)/min	Resolution (USP)
HCQ	8.464	888,481	56,853	8107	0.410	/
CQ	10.050	751,613	44,490	8332	0.441	3.728 (>1.8)

2.2.2 Reproducibility

The USP assay requires that the peak area RSD% is lower than 1.5% (n=5). The standard solution of hydroxychloroquine sulfate (50 μg/mL) was injected for 5 times. The retention times and peak areas obtained were used to calculate RSD%. The results are shown in Table 4.

Table 4. Results of Reproducibility Tests (n=5)

Sample concentration	Retention Time (min)	Peak Area
hydroxychloroquine sulfate (50 µg /mL)	8.233	888,721
	8.234	888,407
	8.237	888,412
	8.240	888,213
	8.244	888,879
Average	8.238	888,526
SD	0.0047	268.15
RSD%	0.06	0.03

The results show that the peak area RSD% of hydroxychloroquine sulfate is 0.03% (n=5), which fully meets the USP criteria of less than 1.5%.

2.2.3 Analysis of actual sample

Twenty tablets of hydroxychloroquine sulfate (content 0.1 g per tablet) were obtained from a drugstore. Following USP procedure, the tablets were pre-treated and analysed. The results are shown in Table 5. The content is calculated using below equation:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = Peak area of sample r_S = Peak area of standard
C_S= Conc. of standard (mg/mL) C_U = Conc. of sample (mg/mL)

Table 5 Result of HCQ content (%) as compared to the labelled content

Sample Name	Peak Area of Standard	Peak Area of Sample	Conc. of Std. (mg /mL)	Conc. of Sample (mg /mL)	Content (%)
hydroxychloroquine sulfate	888,526	888,481	0.05000	0.05002	99.95
		888,904		0.05000	100.04
		888,879		0.05003	99.98
Average		888,754		0.05002	99.99
SD		237.3715		0.000015	0.036864
RSD%		0.03		0.03	0.04

The result shows that the content of hydroxychloroquine sulfate of the tablet is 99.99% as compared to the labelled content. This result meets the USP criteria (not lower than 93% and not higher than 107.0%).

❑ Conclusion

Following EP10.0 assay, hydroxychloroquine sulfate and their related substances were analysed on Shimadzu UHPLC system. The results indicate that hydroxychloroquine sulfate and Impurity C, Impurity B and Impurity C are well separated. The resolution of the separation measured meet the requirement desribed in the EP monograph. Using Shimadzu HPLC, the content of hydroxychloroquine sulfate in a tablet product was determined following USP 43 method. The resolution of the separation for hydroxychloroquine sulfate and chloroquine phosphate measured is 3.728 and the analysis reproducibility RSD% based on peak area is 0.03% (n=5). These results meet fully the criteria stated in the USP monograph for determination of hydroxychloroquine sulfate content in tablet.

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