Application Brief

Clinical research

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Analysis of Hydroxychloroquine and Metabolites in Human Whole Blood Using the Agilent Captiva EMR—Lipid by LC/TQ

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Abstract

This study developed and validated a research method for the quantitative analysis of hydroxychloroquine (HCQ) and its metabolites, in human whole blood. Human whole blood samples were prepared by protein precipitation extraction followed with Agilent Captiva EMR—Lipid cleanup, then analyzed by LC/TQ. The method provided a reliable solution with excellent quantitation accuracy (100 ±15%) and precision (≤15%) for this emerging application.

Experimental

Target analytes

The four target analytes in this application are HCQ, desethylhydroxychloroquine (DHCQ), desethylchloroquine (DCQ), and bis-desethylchloroquine (BDCQ). Table 1 shows the targets of interest, structures, chemical formulas, and molecular ions.

Instrument method

The samples were run on an LC/TQ system under the conditions shown in Table 2.

Sample extraction

The following products were used for sample preparation.

- Agilent Captiva EMR—Lipid, 96-well plate, 40 mg (part number 5190-1000)
- Agilent positive pressure manifold 96 processor (PPM-96) (part number 5191-4116),
- Agilent square 96-well 2 mL collection plate (part number 5133009)
- Agilent square 96-well sealing caps (part number 5133005)

Table 1. Molecules of interest.

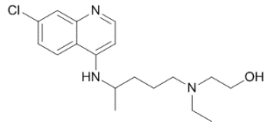
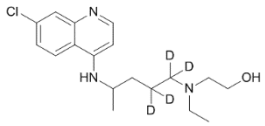
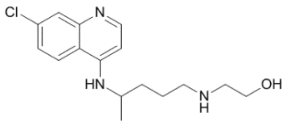
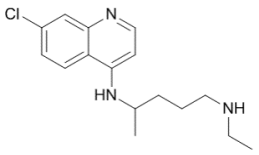
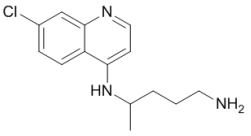
Molecule	Structure	Chemical Formula	$[M+H]^+$ (*m/z*)
Hydroxychloroquine (HCQ)		$C_{18}H_{26}ClN_3O$	336.1837
Hydroxychloroquine-d4 (HCQ-D4)		$C_{18}H_{22}D_4ClN_3O$	340.2088
Desethylhydroxychloroquine (DHCQ)		$C_{16}H_{22}ClN_3O$	308.1524
Desethylchloroquine (DCQ)		$C_{16}H_{22}ClN_3$	292.1575
bis-Desethylchloroquine (BDCQ)		$C_{14}H_{18}ClN_3$	264.1262

Table 2. Instrumental conditions.

HPLC Conditions	
Column	Agilent ZORBAX Eclipse XBD-C8, 2.1 × 50 mm, 3.5 µm (p/n 971700-906)
Flow Rate	0.3 mL/min
Column Temperature	40 °C
Injection Volume	10 µL
Mobile Phase	A) water with 0.1% formic acid B) ACN with 0.1% formic acid
Gradient	Time (min) %B Flow rate (mL/min) 0 5 0.3 1.0 5 0.3 2.0 8 0.3 5.0 10 0.3 5.1 70 0.3 7.0 70 0.3
Post Time	3.0 minutes
QQQ Conditions	
Gas Temperature	350 °C
Gas Flow	7 L/min
Nebulizer	35 psi
Sheath Gas Heater	350 °C
Sheath Gas Flow	11 L/min
Capillary	3,500 V (POS)
Data Acquisition	MRM as shown in Table 3.

The procedure is shown in Figure 1.

Table 3. Target analytes MRM conditions.

Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)	RT (min)
HCQ	336.2	247.0	19	3.17
		158.2	19	
HCQ-D4 (IS)	340.2	251.0	19	3.16
		162.2	19	
BDCQ	264.1	179.0	20	2.55
		247.0	15	
DHCQ	308.1	179.0	20	2.70
		130.2	17	
DCQ	292.2	179.0	18	3.10
		114.3	16	

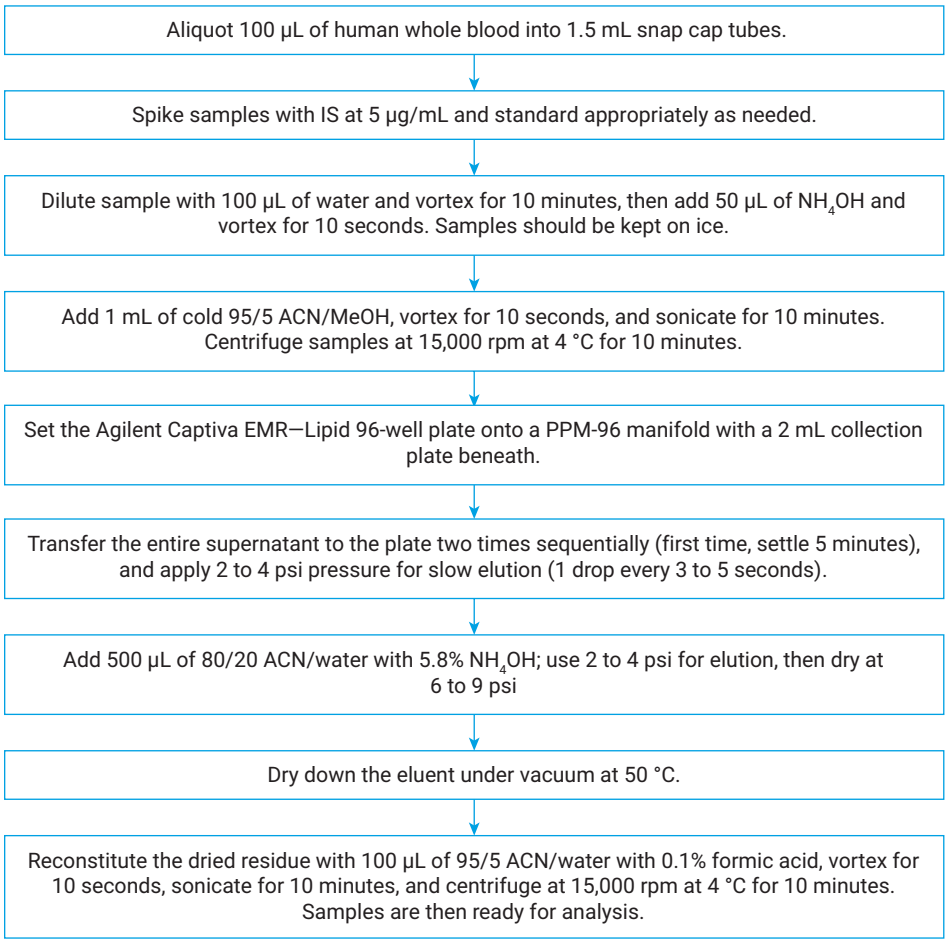


Figure 1. Sample preparation workflow chart.

Results and discussion

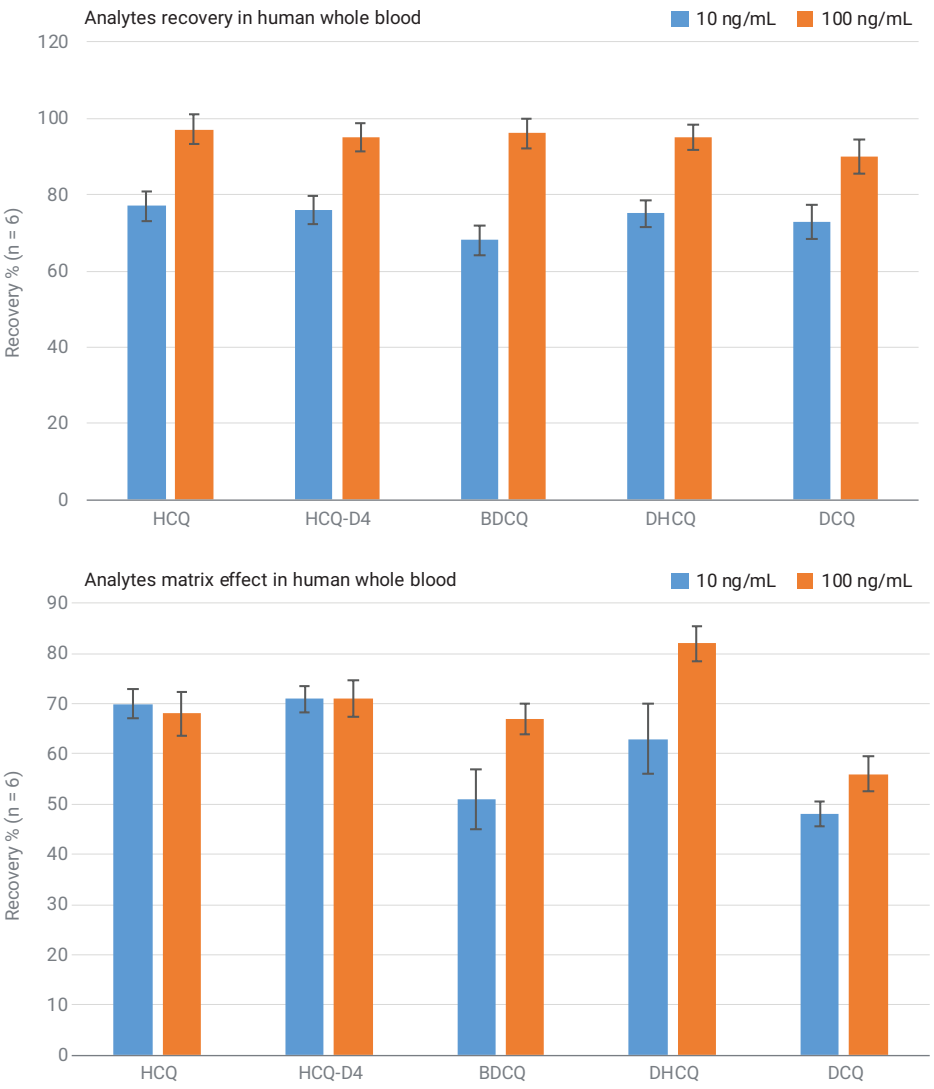


Figure 2. Method recovery and matrix effect in whole blood.

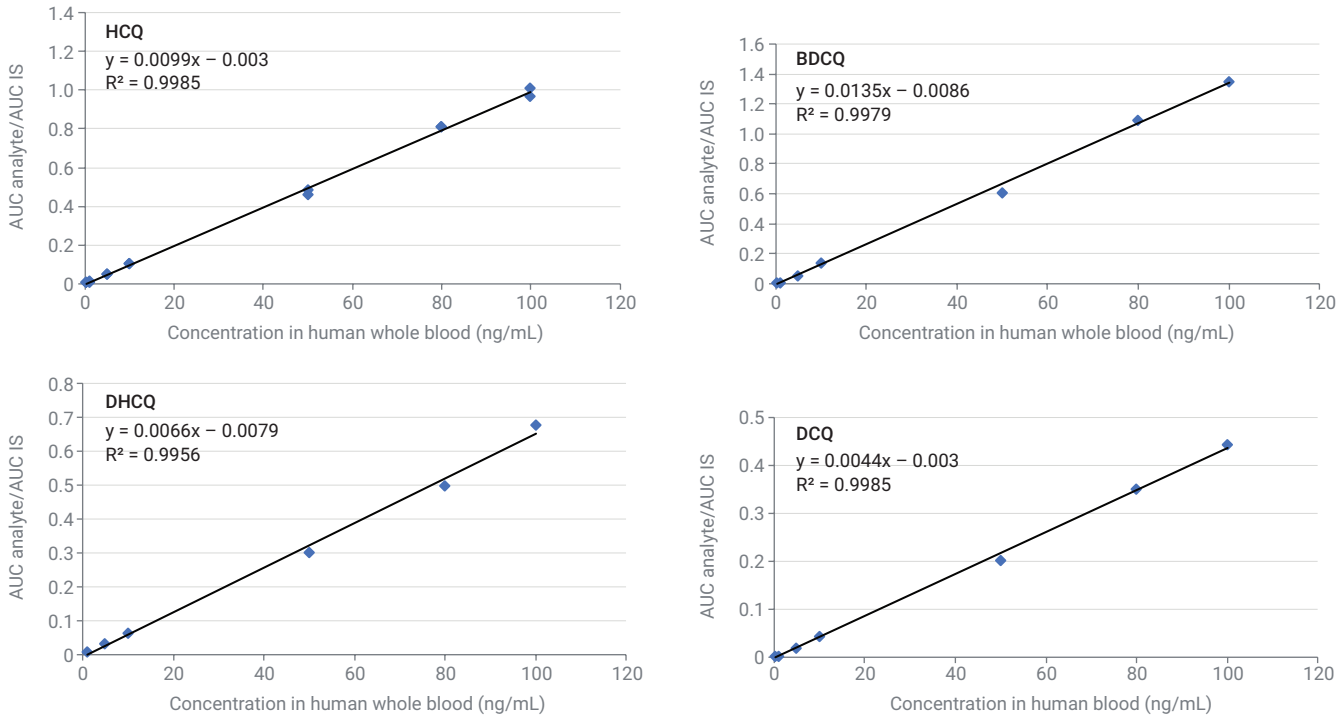


Figure 3. Calibration curves in sample matrix.

Table 1. Method accuracy and precision.

Spiking Concentration		HCQ	DHCQ	DCQ	BDCQ
1 ng/mL (n = 6)	Calculated concentration (ng/mL)	0.98	1.03	1.00	1.04
	Accuracy %	98	103	100	104
	C.V %	3.7	6.3	6.3	9.7
10 ng/mL (n = 6)	Calculated concentration (ng/mL)	10.19	9.63	10.05	9.69
	Accuracy %	102	96	101	97
	C.V %	2.2	4.0	3.5	6.7
100 ng/mL (n = 6)	Calculated concentration. (ng/mL)	105.25	108.05	108.13	106.36
	Accuracy %	105	108	108	106
	C.V %	5.1	4.3	5.3	3.7

Conclusion

A robust research method using protein precipitation extraction followed by Agilent Captiva EMR—Lipid cleanup, was established for fast and reliable analysis of hydroxychloroquine and its metabolites in human whole blood using LC/TQ. The method provided excellent analyte accuracy and precision, efficient recovery and matrix removal, and a simplified workflow.

Acknowledgment

The authors want to thank Nathalie Bourgeois for the initiation of this collaboration work, as well as Dr. Jean-Claude, Dr. Das, and Dr. Kremer for their support.

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Printed in the USA, October 15, 2020
5994-2420EN

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