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Short communication

A preparative chiral separation of hydroxychloroquine using supercritical fluid chromatography

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ABSTRACT

A robust supercritical chromatography (SFC) method using an Enantiocel C2–5 column was developed for the multigram separation of the enantiomers of hydroxychloroquine (HCQ), affirming its use as a scalable technology and ability to provide quantities of each enantiomer for clinical evaluation. The enantiomers of HCQ were collected on a gram scale with greater than 99% enantiomeric excess. The S and R enantiomer elution order was confirmed using optical rotation determinations with comparison to previously determined assignments.

1. Introduction

Hydroxychloroquine (HCQ) as the racemic sulfate salt (Plaquenil®) is used widely in the treatment of malaria, rheumatoid arthritis, and systemic lupus erythematosus [1]. It is currently under consideration as a possible treatment for Covid-19 caused by SARS-CoV-2 infection. As summarized in a recent review [1], HCQ can have serious side effects such as retinal damage, muscle weakness and heart arrhythmia [1]. There also are numerous reports over the past thirty years that the enantiomers of HCQ (and its close relative, chloroquine) are metabolized differently when the racemic drug is administered [2]. The differential biological activity of the enantiomers has been reported after administration of the racemate of HCQ to rats [3], rabbits [4] and humans [5]. The effect of each individual enantiomer has not been reported however, which may be attributed to the difficulty in obtaining quantities of the pure enantiomers of HCQ. The enantiomers have been prepared using a chemical resolution at an early stage in a synthesis procedure [6]. There is no published asymmetric synthesis of either enantiomer nor of a scalable chromatographic resolution [7].

To optimize the therapeutic value of a drug, it is necessary to determine whether one enantiomer has lower therapeutic value and/or significant undesirable adverse effects. Early drug development research relies heavily on chromatographic techniques such as supercritical fluid chromatography (SFC) for the rapid resolution of the stereoisomers of drug candidates [8,9]. This technology, considered scalable, has not previously been applied to the separation of HCQ. The drug is currently administered as a racemate for a variety of therapeutic targets. It is well documented that HCQ undergoes extensive metabolism in humans to produce significant levels of three chiral metabolites leading to up to eight distinct chemical entities each with their own pharmacokinetic and pharmacodynamic properties [2,10]. Due to a current interest in drug repurposing strategies, researchers have emphasized that a critical next step is the determination of the risk-to-benefit profile of the R and S enantiomers of HCQ [11].

2. Experimental

2.1. Materials and chemicals

Carbon dioxide (bone dry grade) was obtained from Praxair (Pittsburgh, PA, USA). HPLC grade solvents were obtained from EMD chemicals (Gibbstown, NJ, USA) except for ethanol, which was obtained from Thermo Fisher Scientific (Allentown, PA, USA), diethylamine (DEA) was obtained from Sigma-Aldrich (St. Louis. MO, USA). An Enantiocel C2–5 preparative column (3 × 25 cm) was obtained from ColumnTek (State College, PA, USA).

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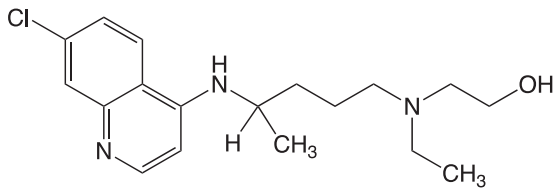

Fig. 1. Hydroxychloroquine (HCQ).

2.2. Instrumentation

Samples were analyzed on a Berger Analytical SFC system equipped with dual pump (FCM-1200), an autosampler (ALS-3100), a column oven (TCM-200) and a diode array detector (DAD-4100) (Mettler-Toledo, Newark, DE, USA). Samples were preparatively separated on a Berger Multigram II SFC equipped with two SD-1 Varian pumps, a Knauer K-2501 Spectrophotometer set at 220 nm,

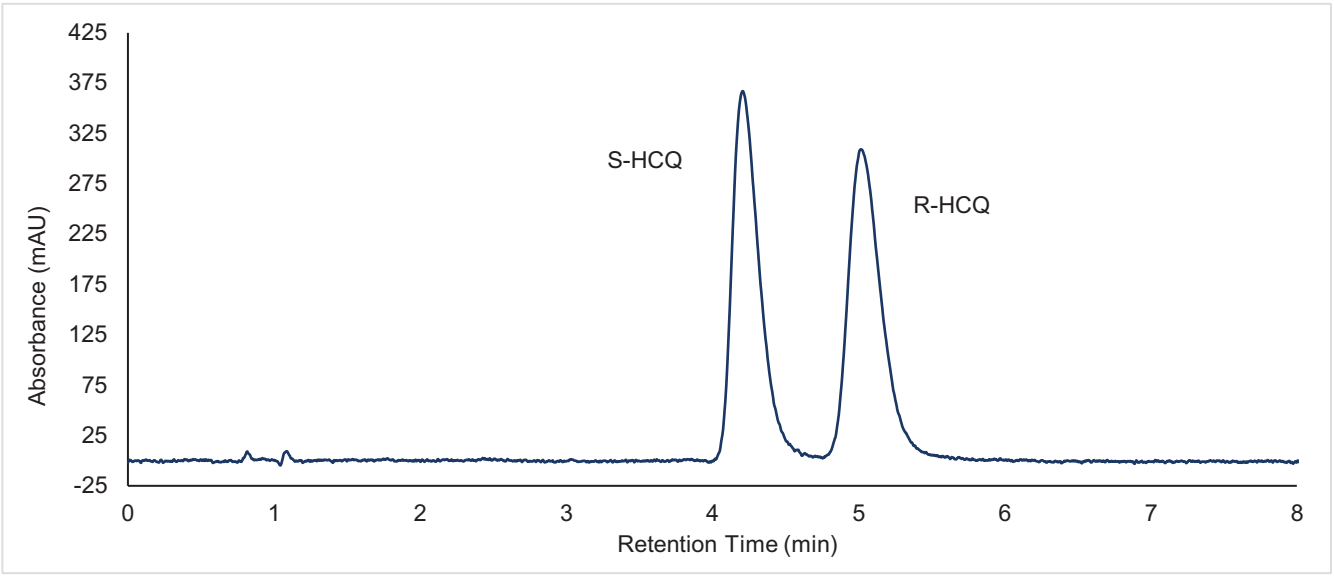

Fig. 2. Analytical SFC chromatogram of the enantiomers of HCQ on an Enantiocel C2–5 column (0.46 × 25 cm) using 40% methanol (0.1% DEA)/CO_2 at 4 mL/min and detection at 220 nm.

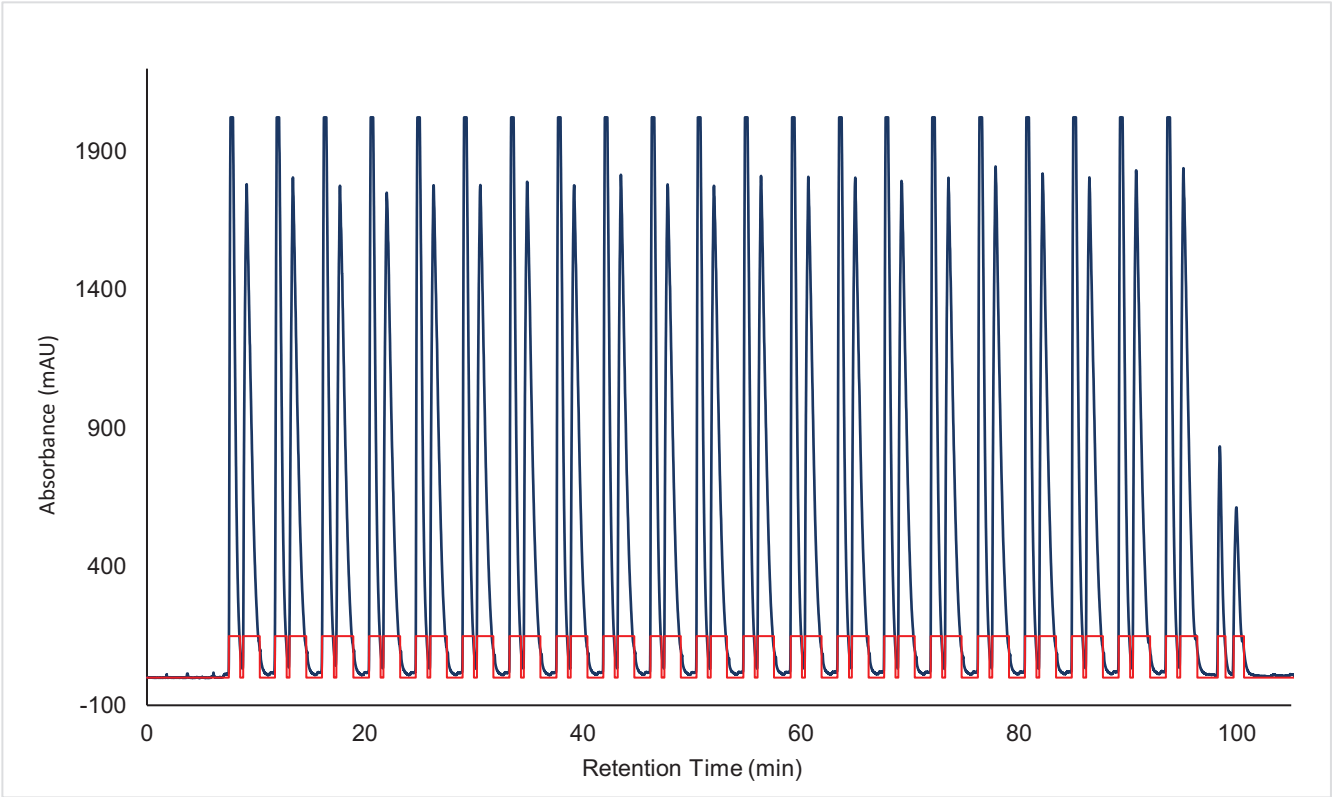

Fig. 3. Preparative SFC chromatogram of the separation of the enantiomers of 1 g of HCQ using 'stacked injections' on an Enantiocel C2–5 column (3 × 25 cm) using 40% methanol (0.1% DEA)/CO_2 at 80 mL/min and detection at 220 nm.

a 6-ton bulk CO2 tank with a built-in chiller and heater and G700 compressor (Mettler-Toledo, Newark, DE, USA). Optical rotation was performed on a JASCO P1010 polarimeter.

2.3. Preparative SFC separation

Hydroxychloroquine sulfate (1.03 g, 2.35 mmol) was dissolved in 20 mL of water and 2 mL of diethylamine. The aqueous layer was washed with dichloromethane (3 × 15 mL). The combined organic layers were dried over MgSO4 and concentrated in vacuo to yield a pale yellow oil (732 mg, 2.18 mmol, 92% yield). The sample was separated on a ColumnTek Enantiocel C2–5 (3 × 25 cm) cellulose-derivatized column, using 40% methanol (0.1% DEA)/CO_2 at a flow rate of 80 mL/min and detection at 220 nm. A total of 380 mg/3 mL were injected and collected within 3 min intervals.

2.4. Absolute configuration assignment

Optical rotation of freebase peak-1$[\alpha]^{22}_D = +114.8°$ ($c = 0.73$, MeOH), peak-2 $[\alpha]^{22}_D = -93.5°$ ($c = 0.93$, MeOH). Sample standards of S(+) HCQ and R(-) HCQ were analyzed by SFC and confirmed that peak-1 is the 'S' enantiomer and peak-2 is the 'R' enantiomer.

3. Results and discussion

A literature search reveals two methods for the chiral chromatographic separation of hydroxychloroquine sulfate enantiomers [10,12,13]. The separations rely on the use of macrocyclic antibiotic stationary phases known to have broad enantioselectivity with limited scalability: a Chiral AGP (100 × 4 mm) column using a buffer consisting of 94:5:1 0.05 M ammonium phosphate (pH adjusted to 7 with 3 N sodium hydroxide):isopropanol:acetonitrile and a flow rate of 1 mL/min [2] and a CHIROBIOTIC V (25 × 0.46 cm) column using 98:1:1 methanol:acetic acid:triethylamine and a flow rate of 2 mL/min [14]. Here we report a robust method applicable to large scale preparative separations. Analysis of HCQ sulfate on 19 scalable chiral stationary phases (Chiralpak AD-H, AS-H, IA, IC, ID, IG and Chiralcel OD-H, OJ-H, OZ-H, Whelk-01 (S,S), LUX-AMY-2, LUX-CEL-3, LUX-CEL-4 and Enantiocel A2–5, A5–5, A6–5, C2–5, C6–5, C9–5) using three supercritical fluid mobile phases consisting of CO_2 in methanol, ethanol, or isopropanol; each with 0.1% DEA produced 'hits' on only one stationary phase with methanol or ethanol as the alcohol modifier (Fig. 1, Fig. 2).

Following minimal optimization, the method was translated from an analytical to a semi-preparative scale. Although the chiral stationary phase resolves both the sulfate salt and the free base

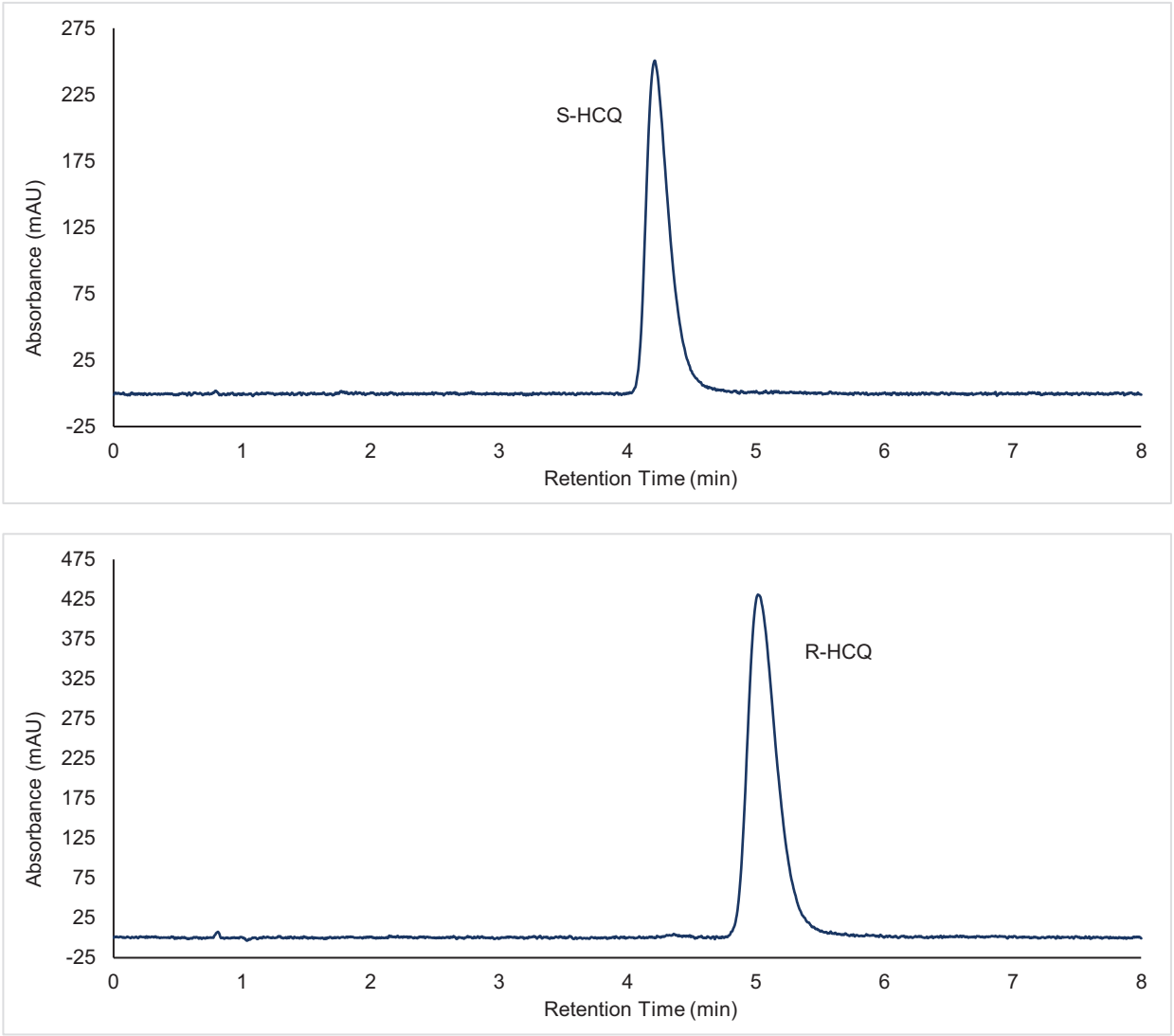

Fig. 4. Analytical SFC chromatograms of the combined collected fractions of each enantiomer of HCQ on an Enantiocel C2–5 column (0.46 × 25 cm) using 40% methanol (0.1% DEA)/CO_2 at 4 mL/min and detection at 220 nm.

of the molecule with equal resolution and selectivity, it is important to convert the sulfate salt of the molecule to the free base in advance of the preparative separation. Most salts can be processed directly by SFC, in this case however, the sulfate salt form of the compound causes a gradual loss of resolution with increased number of injections. This effect was attributed to the binding of the strong acid to the stationary phase and gradually reducing the number of active sites involved in the separation. Resolution can be re-established after washing the column in a solution of aqueous alcohol (methanol:isopropanol:water 10:10:1). To avoid these complications, the sample was converted to the free base and dissolved readily in methanol to a concentration of 126 mg/mL. An added benefit of converting the sample to the free base was the increase in solubility. A 'stacked' injection of the preparative separation (Fig. 3) of 1.0 g of HCQ was readily achieved on a 3 by 25 cm column in less than two hours.

An Enantiocel C2–5 column (ColumnTek) and methanol/CO2 mobile phase with a 0.1% DEA additive produced a selectivity of 1.22 and resolution of 2.13 for the HCQ enantiomers. A total of 363 mg and 338 mg of each enantiomer was collected with greater than 99% enantiomeric excess in less than two hours (Fig. 4).

4. Conclusion

A rapid and robust chromatographic method using an Enantiocel C2–5 column was developed for the preparative separation of the two enantiomers of HCQ with potential commercial application. The process enables the independent evaluation of each enantiomer in early stages of drug discovery and development.

Credit author statement

Laura Wilson: Conceptualization, Methodology, Validation, Investigation, Writing-Review and Editing **Charles Mi**: Resources **Christina Kraml**: Conceptualization, Writing-Original Draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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