

A novel methodology for the dosage of hydroxychloroquine and its metabolites in whole blood of patients affected by autoimmune diseases

1) Charlier B. 2) Pingeon M. 3) Dal Piaz F. 4) Romano M. 5) De Bonis E. 6) Valentini G. 7) Filippelli A.

University of Salerno

Hydroxychloroquine (HCQ) is an ancient antimalarial drug that has proven to be a safe and effective treatment for systemic lupus erythematosus (SLE) and other autoimmune pathologies, such as rheumatoid arthritis (RA). Hematic levels of HCQ are closely related to the therapeutic response in autoimmune disease treatment (Costedoat-Chalumeau N et al., 2006). Several studies in the last decade have shown a link between drug concentration and efficacy in diseases such as SLE and RA, highlighting the importance of maintaining plasmatic concentration of HCQ consistently above certain values (1,000 ng / ml) in order to reduce SLE flares (Costedoat-Chalumeau N, et al, 2013). In addition, the importance of monitoring the main HCQ metabolites in the blood (DHCQ, DCQ, BDCQ) to identify additional predictor markers of clinical outcomes of SLE has also been recently underlined (Munster T. et al., 2002). Therefore, the need for a simple, fast and reliable methodology to use in the clinical routine to continuously monitor the blood concentration of HCQ and allow a personalized adjustment of therapy, is quite evident.

Various techniques for HCQ dosage are currently available; however, few of these show a good separation of the analytes and even fewer are those able to identify the individual metabolites; moreover, most of them rely on measurements performed on plasma or serum.

Our research group has developed a methodology for the simultaneous dosage of HCQ and its three major metabolites DHCQ, DCQ, BDCQ, based on ion pairing RP-HPLC, where SDS was used as ion-pairing reagent. The analytes were extracted from whole blood by means of a simple organic extraction. Chromatographic separation was performed on a C18 column and analytes were identified with a fluorescence detector; excitation and emission wavelengths were set respectively at 320 and 370 nm. We tested our method on a selected subset of 20 human samples, comparing the concentrations evaluated with our HPLC methodology with those obtained by using LC/MSMS spectrometry. The concentration values obtained by the two different techniques are comparable; the lower limit of detection (LLOD) of our method is of 1 ng/mL and the linearity was verified in the range 100-1,500 ng/mL. The technique that we have developed is well suited for use in clinical practice, since it does not require sophisticated equipment and the whole process is fast, economical, and easy to apply also by non-expert users.

Costedoat-Chalumeau N et al. (2006) *Arthritis Rheum*; 54:3284-90

Costedoat-Chalumeau N, et al (2013) *Ann Rheum Dis*; 72: 1786-1792

Munster T. et al., (2002), *Arthritis Rheum* 46(6):1460-9.