The overall aim of this project is to apply pharmacokinetic-pharmacodynamic (PK-PD) models in a longitudinal trial of repeated treatment with chloroquine combinations to learn how best to combine antimalarial drugs to deter the emergence and spread of resistance. Selection of drug resistant P. falciparum is proposed to occur in a drug concentration range that extends from the minimum inhibitory concentration (MIC) of susceptible parasites to the MIC of the resistant parasites. We term this concentration range the ‘window of selection’. No antimalarial drug has a window of selection defined, other than pyrimethamine. Using a combination of pharmacokinetic data from Professor Plowe’s chloroquine combination efficacy study and pharmacodynamic data from the clinical trial and in vitro studies, we shall identify PK-PD characteristics of drug combinations that will deter emergence and spread of resistance. The initial part of this project involves measurement of whole blood chloroquine concentrations in patients treated with chloroquine alone or in combination with either artesunate, azithromycin or atovaquone-proguanil. We report progress on an HPLC assay validation for chloroquine concentration measurement.

A reversed-HPLC method has been developed for the analysis of chloroquine in 100 μL fingerprick whole blood. Chloroquine was eluted with a 2.5 ml Hexane / Methyl tert Butyl Ether (MTBE) mixture (ratio1:1, v/v) and was assayed on a Phenomenex Spherclone BDS C18 5µ 15cm x 4.6mm analytical column. The mobile phase comprised 0.1% Triethylamine in Acetonitrile, 85:15 (pH adjusted to 3.0 with orthophosphoric acid). Chloroquine recovery was 84%. The CV between 10 ng/ml and 25 ng/mL was 13 % and the limit of quantitation was 25 ng/mL with a CV= 5%. The limit of chloroquine detection was 10 ng/mL. The assay uses a smaller volume of sample, and can more accurately measure low blood chloroquine concentrations than published assays which use UV detection. The assay will be used to measure blood chloroquine level in children who were given a standard oral dose of chloroquine as treatment for uncomplicated malaria. A preliminary HPLC analysis of 300 specimens is due to start.