ABSTRACT

Artemisinin a representative of Endoperoxide class of drugs and its derivatives particularly artesunate are the most important class of antimalarial drugs used in clinical practice. They are recommended as the first-line treatment of malaria in combination with other longer-acting antimalarial drugs (lumefantrine, piperaquine).

As the main skeleton of these compounds lacks UV visible or fluorescent chromophore, earlier methods of detection have used post-column on-line derivatisation or electrochemical detection in the reductive mode. However, these methods suffer from poor sensitivity and selectivity.

Within this thesis the whole LC-MS method development for the analysis of artesunate and its major metabolite dihydroartemisin in biological samples from the very beginning was performed. It included tuning of ESI - triple quadrupole MS detector and optimization of chromatographic conditions, particularly mobile phase pH.

Artesunate (ARST) and dihydroartemisin (DHA) were assayed in human plasma using artemisisnin as an internal standard. Different approaches of plasma sample treatment, (protein precipitation and liquid-liquid extraction) were optimized and compared. Pre-validation data for liquid-liquid extraction revealed LLOQ as 2.5 and 3.0 ng/ml for DHA and ARST, respectively using 400 μl of plasma. Pre-validation data for protein precipitation revealed LLOQ for both DHA and ARST 8.0 ng/ml using 300 μl of plasma.

Further study focused on influence of free iron and haem iron as the source of vast degradation of compounds within process of assay was performed and possible solutions of this issue were suggested. This study is essential to overcome difficulties regarding analysis of these compounds in blood/plasma samples taken from patients infected by malaria parasite, where products of haemolysis are presented in large amount. Moreover, this study was of great importance for the analysis of homogenized rat´s embryo samples which contain large amount of haemoglobin and myoglobin.

The assay of these compounds in rat´s embryo is crucial for further investigation of potential risks associated with the using of artemisinin derivatives in pregnancy, particularly within the first trimester. (The safety of usage Artemisinins during pregnancy is EU collaborative project; Coordinator: Professor S. A. Ward; Liverpool School of Tropical Medicine).