Development and Validation of a GC-MS Method for the Quantitation of Nanoformulated Primaquine in Whole Blood and Plasma of Mouse Model

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A Gas chromatography-mass spectrometry (GC/MS) method was developed and validated for the quantitation of the antimalarial drug, nanoformulated Primaquine (PQ), in whole blood and plasma. The analyte was extracted using a protein precipitation method followed by chromatographic separation on a Waters Xterra, RP C8, 2.5µm, 50mm x 4.6mm analytical column with a mobile phase consisting of A: 0.5% Formic acid in 20mM NH4COOH, B: Methanol pH adjusted to 3.0 with FA at a ratio of 3:7 (v/v), delivered at a constant flow rate of 0.5 ml/min. Mefloquine (MEF) was used as the internal standard. Compound reaction monitoring was performed using 260.4 Da for precursor ion and 175. 2 and 379.2 Da for product ions for the quantification of PQ and 379.2 Da for precursor ion and 175.2 and 379.2 Da for product ions for the quantification, respectively. Calibration curves were constructed over the concentration range 16.7–4300 ng/ml. The mean intra- and inter-assay accuracy values for the analysis of PQ in WB was 104% (%CV = 5.6) and 98.6% (%CV = 5.7), respectively. The mean intra- and inter-assay accuracy values for the analysis of PQ in plasma was 92.7% (%CV = 3.7) and 93.7% (%CV = 5.4), respectively. No significant matrix effect was observed during the method validation. The validated method was applied to an absorption study in mice, to determine and compare PQ concentrations in whole blood and plasma samples. Results of the statistical analysis using a linear mixed effects growth curve model concluded that there was no significant difference (p-value = 0.688) between WB and plasma PQ concentrations. This method utilizes a small sample volume of 20 µl, facilitating low blood collection volumes and a short chromatographic run time of 3 min which allows for high sample through put analysis.

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