ABSTRACT

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR DETERMINATION OF ANTIMALARIAL DRUGS USED IN FALCIPARUM IN THEIR PHARMACEUTICAL DOSAGE FORMS AND BULK

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Key Words: Analytical Method development, Analytical method validation, RP-HPLC, HPTLC, LC-MS, Artemether, Lumefantrine, Arteether, Mefloquine hydrochloride, Artesunate, Hydroxychlorquine, Process related impurities, SIAMs

Abstract:
Background: Several data are published explaining the wide spread of disease. Due to such severity, it is causing deaths about 3 million per year. So quality of antimalarial agents is the most important aspect in terms of delivering effective therapy and almost all drugs are suffering from problems of stability. Major drawback found is unavailability of proper methods for determination of certain antimalarial agents as well as their combinations. Certain available official as well as nonofficial method suffers from problem of cost effectiveness and are time consuming. They cannot even maintain the stability during the analysis. So there is a need of proper Chromatographic method which can maintain the stability and can be confirmed by mass studies. Available methods for certain combinations like Artemether and Lumefantrine are totally unsuitable for the mentioned purpose. So it’s quite essential to have a method which can justify the stability and can easily identify as well as separate parent drug in presence of their related impurities.
Aim: To develop and validate various analytical methods like RP-HPLC/Stability Indicating RP-HPLC, HPTLC, LC-MS for determination of antimalarial agents in their combined pharmaceutical formulation or bulk in such a way that they ensure the stability during the analysis.

Material and Method:
A new HPTLC method was performed and was validated for estimation of artemether (ART) and Lumefantrine (LUM) from its combined established formulation on CAMAG HPTLC instrument. Artemether (ARM) and arteether (ARE) were separated and estimated from bulk by reverse phase liquid chromatography and stability was assured by LC-MS determination. Combination of artesunate and Mefloquine hydrochloride was estimated by newly developed HPTLC and RP HPLC method which was superior to available method. Finally method indicating the stability was validated for quantification of hydroxychlorquione from its potential process related impurities by incorporation of ion pairing reagent in mobile phase.

Result:
ART and LUM were successfully quantified with assay values of 98.34 ± 1.89 for and 101.58 ± 0.43 respectively in range of 1200-7200 ng/spot of LUM and 200-1200 ng/band of ART with 0.997 and 0.995 as value of $r^2$ respectively. Validation of method was successful as per ICH Q2R1 guidelines. Artemether and arteether were quantified from bulk with assay values of 98.29 ± 1.07 for and 98.31 ± 0.80 respectively, method was extremely quick as elution time was found to be 2.70 and 1.52 for ARM and ARE. Confirmation of stability was achieved by LC-MS studies. Artesunate and Mefloquine hydrochloride were estimated by developed and validated HPTLC with assay values of 100.08 ± 1.69 and 98.22 ± 1.72 respectively. RI value of eluting components was 0.235 ± 0.007 and 0.547 ± 0.016. An expeditious RP-HPLC method for establishing concentrations of ARS and MEFLO yielded good assay values of 100.55 ± 0.99 and 101.35 ± 0.11. Elution time was found to be 3.10 and 5.02 minutes for ARS and MEFLO. Linearity of method was in between range of 2-10 and 1.6-8 µg/ml for MEFLO and ARS with 0.997 as value of $r^2$. HCQ along with impurities were separated and quantified in short run time with incorporation of sodium pentane sulphonate. Elution time was found to be 2.15, 3.88, 5.01,
12.96 minutes for IMP-2, HCQ, IMP-3, IMP-4. Stability of method can be assured as % degradation was within the limits. All the components showed linearity between 10-50 µg/ml. When quantified, assay value were found to be 100.21 ± 1.01, 99.01 ± 1.15, 98.96 ±1.43 and 100.73 ±1.57 % for IMP 2, HCQ, IMP 3 and IMP 4 respectively.