A Sensitive and Validated HPLC-UVMethod for the Quantitative Analysis of Sulfamethoxazole in Human Plasma: Application in TDM for PJP Patients

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Background: Pneumocystis jirovecii pneumonia (PJP) is a critical pulmonary infection predominantly caused by Pneumocystis jirovecii, formerly known as Pneumocystis carinii. This opportunistic fungal infection has seen a marked rise in incidence, largely due to the increased use of immunosuppressive medications and chemotherapy for cancer treatment. The treatment of choice for PJP, as endorsed by numerous authoritative guidelines, is co-trimoxazole. Within this drug regimen, maintaining an optimal blood concentration of sulfamethoxazole (SMZ) is essential for the treatment's success.

Methods: We have developed a highly sensitive and validated high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method for the quantitative analysis of SMZ and its metabolite in human plasma. This method integrates a simple protein precipitation step followed by chromatographic separation on a SinoPak BEH C18 column (250 × 4.6 mm, 5 μm). The mobile phase consists of an isocratic mixture of 0.1% acetate buffer and acetonitrile in a 77:23 (v/v) ratio. The UV detection is optimized at 257 nm. The method underwent a rigorous validation process in accordance with international guidelines, ensuring its compliance with the highest standards for linearity, accuracy, precision, selectivity, lower limit of quantitation (LLOQ), and stability under various conditions.

Results: The HPLC-UV method demonstrates excellent linearity, with correlation coefficients greater than 0.998, indicating a high degree of data fit to the linear model. It is highly specific, with no interference from plasma components or a wide range of potentially co-administered drugs. The sensitivity of the method is commendable, with a lower limit of quantification of 2.11 μg/mL, ensuring the detection of even low levels of SMZ and its metabolite. The reproducibility of the method is evidenced by extraction recovery rates and intra- and inter-day precision, both of which are less than 5% for SMZ and its metabolite.

Conclusions: The study's analyses confirmed that the high-performance liquid chromatography (HPLC) method exhibits excellent linearity, accuracy, and precision. The successful application of this assay in routine therapeutic drug monitoring has provided a robust scientific foundation for the rational clinical use of co-trimoxazole in the management of PJP.

Key Words: Pneumocystis jirovecii pneumonia, Sulfamethoxazole, Therapeutic drug monitoring, HPLC