Saliva-based assay to measure the concentration of pyrazinamide using a mobile UV Spectrophotometer

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Background: Tuberculosis is a leading infectious disease worldwide with higher prevalence rates in less-resourced settings. Pyrazinamide is an effective first-line drug for susceptible tuberculosis treatment. Treatment response is variable even when using a weigh-based dosing regimen due to factors such as gender, age, and body mass index. Simple and accessible strategies to optimise pyrazinamide dosing, even in less-resourced settings are therefore warranted to facilitate adequate treatment response. This study aimed to develop and validate an assay to quantify pyrazinamide concentration in saliva using a mobile UV spectrophotometer.

Methods: All reference materials were obtained from reliable manufacturers with ≥ 98% in purity. All measurements were conducted using the nano-volume drop function on the Implen NP80 mobile UV nanophotometer. Assay development involved applying second derivative spectroscopy with the Savitzky-Golay filter between wavelengths of 200 to 300 nm to measure saliva samples of spiked drug concentrations. Assay validation as per EMA and FDA guidelines included assessing selectivity, specificity, linearity, accuracy, precision, carry-over and matrix effects. Specificity was also analysed by evaluating the impact of co-administered medications on pyrazinamide results. The effect of filtration on reducing interferences of saliva samples was assessed using 0.22µm Millex-GP and GV filters. Sample stability was measured after seven days in cold (2 – 8°C), room temperature (20°C) and warm (40°C) storage conditions whereas, freeze-thaw stability was evaluated after three cycles.

Results: The calibration curve from nine data points (7.5, 10, 15, 25, 50, 75, 100, 150, 200 mg/L) was linear (R^2 = 0.9991). The overall accuracy and precision ranged from – 0.66% to 5.15%, and 0.56% to 4.95% respectively. Within-day precision was 0.91% whereas between-day precision was 7.01% at a LLOQ of 7.5 mg/L. Carry-over and matrix effects were both acceptable with an accuracy of < ± 4% and precision of < 7.5%. All co-administered medications displayed negligible interferences except levofloxacin which resulted in an accuracy bias of – 36% at an expected therapeutic concentration of 15 mg/L. This analytical interference of levofloxacin was limited to pyrazinamide concentrations < 25 mg/L and hence did not have an impact on the assay's clinical applicability. Both Millex-GP and GV filters had similar results with an acceptable < 5% in precision and a range of – 10.45% to 5.35% in accuracy for all quality control (QC) concentrations. Pyrazinamide was considered stable in saliva after seven days in all storage conditions with an acceptable precision < 6.5% and accuracy < ± 11% for both low and high QCs. Freeze-thaw stability after three cycles resulted in a precision < 1% and an accuracy < 11% for both low and high QCs.

Conclusions: A novel point-of-care saliva-based assay for pyrazinamide has been successfully developed and validated using the mobile UV spectrophotometer to facilitate the personalised dosing of pyrazinamide in less-resourced settings. We recommend using the assay in combination with a limited sampling strategy at two and six hours, to estimate the overall drug exposure and aid clinicians in making informed dosing decisions to improve treatment outcomes.