Concomitantly determination of mycophenolic acid (MPA), tacrolimus (TAC) and creatinine (CRE) in saliva using LC-MS/MS– analytical method development, validation and clinical application

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Background: Tacrolimus (TAC) and mycophenolate mofetil (MPA) are widely used in immunosuppressive pharmacotherapy. Creatinine (CRE) is the most common biomarker to monitor renal function. Typically, whole blood for TAC and serum/plasma for MPA, CRE are used for these analytes determination. However, for the pediatric population, where the volume of collected blood is limited, the oral fluid (OF, saliva) presents a promising alternative matrix. Therefore, this study aimed to develop and optimize a novel LC-MS/MS (liquid chromatography-tandem mass spectrometry) method for the simultaneous determination of TAC, MPA, and CRE in OF from patients with renal diseases or after renal transplantation, a significant advancement in our field.

Methods: The blank OF samples were collected using the Salivette® system with a cotton swab. The blank OF contained endogenous creatinine; therefore, background subtraction was applied. The 50µL saliva sample was purified with acetonitrile and methanol (9:1, v/v) mixture and evaporated under nitrogen steam to dryness. The reconstituted residue was detected in positive mode under previously established conditions. As mobile phase water and acetonitrile with formic acid and ammonium formate were used in gradient mode. The method has been validated according to EMA (European Medicines Agency) guidelines.

Results: The method was successfully validated in analytical ranges: 5-500 pg/mL (TAC), 1-400 ng/mL (MPA) and 5-3000 ng/mL (CRE). The accuracy and precision were outstanding, ranging from 92.34 to 109.99%, while the coefficients of variation were impressively low, ranging from 2.13% to 12.78%. The samples remained stable during two weeks of storage at $4^{O}C$, and for a minimum of 3 months during storage at $-20^{O}C$. Importantly, no matrix or carry-over effects were observed, further confirming the reliability of the method.

Conclusions: The rapid, sensitive, and selective LC-MS/MS method for simultaneous determination of TAC, MPA, and CRE has been optimized and validated, marking a significant step forward in our research. In the next phase, we will explore the correlation between analyte levels determined in clinical samples (blood or plasma/serum versus OF), a promising avenue for future studies.

Key Words: tacrolimus, mycophenolic acid, creatinine, saliva, LC-MS/MS