Therapeutic drug monitoring of apixaban using volumetric-absorptive microsampling and LC-MS/MS technique – method development, validation and clinical application

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Background: Apixaban (APX; Eliquis®) is a direct oral anticoagulant (DOAC) agent for brain stroke and thrombosis prophylaxis. Pharmacologically, it is an oral, direct and selective inhibitor of Xa factor. Routinely, DOACs does not require therapeutic drug monitoring (TDM) despite to wide therapeutic range. On the other hand, specific clinical situations, such as toxic side effects may generated the necessity of quantification of APX concentration in the blood. The presented study aimed to develop and validate an analytical method for APX determination in capillary blood. Additionally, the correlation between capillary blood and serum has been done as a preliminary clinical assessment.

Methods: The two different microsampling devices were used in the validation – Capitainer™ (Capitainer AB, Sweden) and Mitra™ (Trajan, Australia). For analyte extraction, the mixture of methanol with water was used for dried spot/tip reconstitution. An isotope labelled apixaban was used as an internal standard, and the sample was purified using simple precipitation with acetonitrile. Sample was injected into LC-MS/MS system, and detected using MRM (multiple reaction monitoring) transitions in positive mode ionization. The method was assessed by analysing samples from patient under APX pharmacotherapy.

Results: Calibration for APX was established in 5 – 500 ng/mL range with average correlation R^2= 0.999. The accuracy ranged from 96.31 to 110.63%, while average intra- and inter-run CV% ranged from 1.89 to 9.65%. Interestingly, the stability of the analyte in both microsampling technique was up to 3 weeks in ambient, dark conditions. The analytical validation results was similar for both devices, but sightly better for Mitra™ device.

Conclusions: The simple, reliable LC-MS/MS method for APX determination in capillary blood samples has been successfully validated and preliminary implemented into clinical practice. The next step is full clinical validation and cross-validation with reference LC-MS/MS technique and immunochemical assays.

Key Words: tacrolimus, ciclosporine, proficiency testing, VAMS, LC-MS/MS