

The simultaneous analysis of Everolimus, Tacrolimus and Cyclosporin-A in Dried Blood Spots Using LC/MSMS

*Lorraine Jacobs, Jos van den Elshout, Robert van der Wegen & Rudi Segers
Eurofins Medinet B.V., Bergschot 71, 4817 PA Breda, the Netherlands*

Pharmacokinetic studies typically require large volumes of blood (generally 100–500 µl per subject per time-point) in order to provide sufficient plasma for quantitative bioanalysis. Here we present the use of small blood volumes in the form of dried blood spots (DBS) for the simultaneous analysis of several drugs used for transplantation patients, namely everolimus, tacrolimus and cyclosporine-A. After spotting 30µL blood on IDBS Bioanalysis cards the spot was dried, a predefined amount was punched from the paper and compounds were extracted using a mixture of methanol, water and 0.1M ZnSO₄. After extraction 50 µL was injected onto the LC-MS/MS system.

The DBS analysis was optimized and validated in the range of 0.20 to 100 ng/mL for everolimus and tacrolimus and 4.0 to 2000 ng/mL for cyclosporine-A. The method showed a good repeatability with a relative standard deviation (RSD) lower than 15% based on six different concentration levels ranging from LLOQ up to the ULOQ. Stability testing revealed that moist and ambient temperature conditions decreased sample quality, so the cards are best stored in controlled dry conditions. The impact of blood quality (hematocrit values) was also tested. Spot appearance changed due to hematocrit variation, but no significant effect was observed regarding measured compound concentrations.

The described DBS method for the analysis of everolimus, tacrolimus and cyclosporine-A had comparable performance as our existing whole-blood method, but offers the advantages of a DBS approach.