Determination of 25-hydroxyvitamin D3 in dried blood spots: are we there yet?

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Background: Since vitamin D deficiency has been linked to a multitude of diseases, the interest in population data on vitamin D status has increased. To achieve this, several microsampling-assisted methods have been set up focusing on the determination of either 25-hydroxyvitamin D3 (25OHD3) alone or in combination with other derivatives such as 25-hydroxyvitamin D2 (25OHD2). However, although of utmost importance, none of these reports describes the evaluation of the robustness of the extraction procedure. Indeed, in dried blood microsample-based methods, the internal standard (IS) is typically added to the extraction solvent and hence, cannot compensate for variability in analyte recovery.

Methods: An LC-MS/MS method was developed for the determination of 25OHD3 and 25OHD2 starting from a single 6 mm dried blood spot (DBS – collected on filter paper) sub-punch. Sample preparation included spraying the IS onto the DBS before wetting the punch with water and extraction in methanol. Finally, liquid-liquid extraction with n-hexane was performed, followed by drying down the supernatant and reconstitution in LC-MS/MS solvent.

Results: Pre-validation experiments showed that – as could actually be expected in microsampling-based analysis – the IS could not correct for differences observed in the extractability of 25OHD3 and D2 in DBS. Different extraction solvents/procedures as well as different ways of adding the IS were evaluated. Robustness of the method could only be achieved when the IS was sprayed onto the DBS prior to the two-step extraction. Results of a thorough optimization of extraction conditions will be presented, as well as the results of the application of the optimized methodology in the context of an epidemiological study determining the vitamin D status in a representative sample of the Belgian population aged 3 to 65+ years old.

Conclusion: Although several reports on the determination of 25OHD3 in dried blood microsamples have been published, we showed here that a thorough (pre)validation is of utmost importance to ensure correct and trustworthy analytical results. Furthermore, the applicability and feasibility of a dried blood microsampling-based method for the purpose of collecting population data on vitamin D status will be demonstrated.

Key Words: 25-hydroxyvitamin D, microsampling, extractability, epidemiology