Low sample consumption for Vitamin D analysis of infants

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Introduction

Traditional immunoassay-based analysis of 25-OH Vitamin D requires a large sample volume, typically 1 - 1.5 ml of serum. Such volume is always easily available for analysis of adults. In contrast, for infants and babies, sample volume is always a bottleneck. Clinical analysis is always performed for critical target analytes. Indicative, but non-essential analytes can sometimes be skipped due to very limited sample availability.

We successfully overcame this constrain by combination of a highly sensitive triple quadrupole mass spectrometer 6490 Agilent Technologies for detection and quantitation, use of a fused-core chromatographic column (Supelco) for a fast, 6 min separation and supported liquid-liquid extraction (Biotage) as a simple and automatable sample preparation step. That combination allowed to use only 40 microliter of serum per assay and maintains LLOQ of 2 ng/mL. The original sample protocol was modified due to our finding of a differential binding of 25(OH)D3 and it’s stable labeled Internal Standard to albumin.

Results

An original, suggested by manufacturer sample preparation procedure was: mix of 50 μL HPLC grade water and 50 μL isopropanol (propan-2-ol), add 100 μL of sample (plasma) and mix for 10 seconds. Load pre-treated plasma (200 μL total volume) onto the ISOLUTE SLE+ plate, leave the samples to absorb for 5 minutes under gravity and elute analyte of interest by 1 ml of heptane. Evaporate eluate to dryness, reconstitute and analyze by LC/MS.

During initial development we noticed that analyte to internal standard ratio for 25(OH)D3 was approx. 10% higher in neat calibrators, compared to samples prepared in stripped serum. For Vitamin D2 this effect was less expressed (data not shown).

We reproduced this adverse side effect by addition of stripped serum to pure, neat solution of the vitamin D calibrators for understanding of the hidden pitfalls in sample preparation. Vitamin D3 is mostly bound to carrier proteins. In contrast, the internal standard in our assay was prepared as a neat, matrix-free solution in 50% Isopropanol. We chose matrix-free IS solution due to its long-term stability at 4C. Supported liquid-liquid extraction process is a less denaturative, compared to a plasma crash approach. As result, at certain conditions, some part of the analyte could be remain bound. It shouldn’t be assumed, that IS spiked in the sample will have the same degree of the binding by definition! Observed ratio must be confirmed with the ratio in neat sample. As we can see in Fig 2, the order of the matrix addition into the sample has a distinct impact on the analytic/IS ratio for 25(OH)D3.

Conclusions

Using SLE-based sample preparation we successfully used only 40 μl of serum while appropriate LLOQ of 2 ng/ml was achieved. We found that in this cohort, babies (3-12 month) were not Vitamin D deficient, compare to an adults. The infant’s average 25(OH)D3 was 57.3 ng/ml compared to the 38.0 ng/ml for the adults in Bronx, NY.