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ORIGINAL ARTICLES

A Liquid Chromatography/Tandem Mass Spectrometry Method for Determination of 25-Hydroxy Vitamin D_2 and 25-Hydroxy Vitamin D_3 in Dried Blood Spots: A Potential Adjunct to Diabetes and Cardiometabolic Risk Screening

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Abstract

Background:

Now emerging as an important risk factor for type 1 diabetes, vitamin D deficiency is also associated with obesity, metabolic syndrome, and type 2 diabetes and has been identified as a potential cardiometabolic risk factor. A simple, accurate screening test for 25-hydroxy vitamin D [25(OH)D] deficiency is needed. We developed a liquid chromatography/tandem mass spectrometry assay for 25-hydroxy vitamin D_2 [25(OH)D_2] and 25-hydroxy vitamin D_3 [25(OH)D_3] in dried blood spots.

Method:

Blood spots were collected by finger stick simultaneously with serum samples obtained by venipuncture from healthy volunteers. Disks punched from the dried blood spots were sonicated with an internal standard solution of deuterated 25(OH)D_3 (26,26,26,27,27,27-d_6). Methanol was added to precipitate proteins prior to extraction with hexane. The extracted samples were dried and reconstituted in 50:50 methanol:H_2O before injection into a Varian 320-MS TQ mass spectrometer.

Results:

Blood spot assay precision was good over the reportable range: interassay coefficients of variation were 13, 13, and 11% at concentrations of 14, 26, and 81 ng/ml, respectively, for 25-hydroxy vitamin D_3 and 12% at 23 ng/ml for 25(OH)D_2. The 25(OH)D_3 assay was linear from 3.5 to 75 ng/ml ($R > 0.99$). Blood spot and serum values showed excellent correlation for 25(OH)D_2 ($R = 0.90$, $n = 54$) and 25(OH)D_3 ($R = 0.91$, $n = 83$).

Conclusions:

This blood spot assay for 25(OH)D_2 and 25(OH)D_3 provides a convenient and cost-effective alternative to serum assays and can be automated. This may be valuable in large-scale screening for risk of type 1 diabetes, for cardiometabolic risk screening, and for monitoring vitamin D supplementation.

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Abbreviations: (CE) collision energy, (CI) confidence interval, (IGF) insulin-like growth factor, (IS) internal standard, (LC-MS/MS) liquid chromatography/tandem mass spectrometry, [25(OH)D] 25-hydroxy vitamin D, [25(OH)D_2] 25-hydroxy vitamin D_2, [25(OH)D_3] 25-hydroxy vitamin D_3

Keywords: blood spot, cardiometabolic risk, diabetes, liquid chromatography/tandem mass spectrometry, screening, vitamin D

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