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Phase Switching SPE for Faster 1,25-dihydroxyvitamin D Analysis

Bruce W. Hollis[1]

Feature Article: Hollis BW. Assay of circulating 1,25-dihydroxyvitamin D involving a novel single-cartridge extraction and purification procedure. Clin Chem 1986;32:2060–63.[2]

This paper described a simplified method for extracting and purifying $1,25(OH)_2D$ from serum or plasma before quantification by radioreceptor assay (RA). Following precipitation of plasma proteins with acetonitrile and centrifugation, the supernatant was removed, combined with pH 10.5 buffer, and applied to a C18 nonendcapped solid-phase extraction cartridge. The cartridge was washed with various solvent combinations, and the $1,25(OH)_2D$-containing fraction was selectively eluted. After evaporation the residue was dissolved in ethanol and analyzed by RA for $1,25(OH)_2D$ content.

In 1982 I was awarded an NIH RO1 grant to study the vitamin D requirement of newborn infants. One of the specific aims, measuring the circulating $1,25(OH)_2D$ in our study participants, presented a problem because in the early 1980s, the measurement of circulating $1,25(OH)_2D$ required a minimum of 2 mL of serum, a blood volume that an institutional review board would not have approved for a study on infants. The assay was a radioreceptor-based procedure that required the raising of rachitic chicks as a source of the labile vitamin D receptor (VDR), which was isolated from them on a routine basis. Further, the assay required exhaustive preparative chromatography, including hplc, to isolate the $1,25(OH)_2D$ before its quantification by RA. With this procedure we could process 25 samples/week, a number that was not adequate for the clinical study I was about to undertake.

During this same period, colleagues of mine at the USDA, Tim Reinhardt and Ron Horst, had identified a VDR from calf thymus that was quite stable and demonstrated excellent potential for use in the RA for $1,25(OH)_2D$ as a replacement for the labile chick intestinal VDR *(1)*. However, the requirement for exhaustive preparative chromatographic isolation of $1,25(OH)_2D$ before RA remained. We all worked diligently on this project, and in 1983 we achieved a solution to the problem, which was subsequently published in 1984 *(2)*. The solution was to extract serum or plasma samples with acetonitrile and isolate $1,25(OH)_2D$ by using newly developed C18 cartridges. This solid-phase extraction step was followed by a 2nd silica cartridge purification of $1,25(OH)_2D$ using hexane/propanol mixtures followed by RA. This method allowed us to assay up to 50 samples/day using as little as 0.5 mL of sample. The new procedure was met with the usual skepticism, but was ultimately accepted and widely used. In fact, it was the basis for this first commercial assay kit for $1,25(OH)_2D$, which was offered in 1985 by Immunonuclear Corporation, known today as DiaSorin.

As good as this procedure was for that period in time, it still contained a time-consuming step in the extraction, the drying of 5 mL of acetonitrile after solid-phase extraction and before silica chromatography. In 1985 I set out to eliminate that cumbersome evaporation step with the idea of eluting the $1,25(OH)_2D$ from the C18 cartridge with hexane and directly applying that fraction to the silica cartridge to isolate $1,25(OH)_2D$ before RA. To my amazement, hexane would not elute $1,25(OH)_2D$ from the C18 cartridge at all! Over the next week, I manipulated the C18 cartridge system with different solvent mixtures of H_2O/methanol to hexane/isopropanol to selectively elute $1,25(OH)_2D$ from all interfering substances and vitamin D metabolites, and I achieved a more robust RA procedure for $1,25(OH)_2D$ quantification.

I termed this separation technique to be "phase-switching" by moving from an aqueous to a nonpolar separation on the same cartridge. Subsequently, the RA was modified into a RIA with an ^{125}I-reporter *(3)*. It was also the first $1,25(OH)_2D$ test to gain FDA clearance for use in clinical diagnosis. It is gratifying that this system of purification is still widely used, more than 20 years after it was developed, in many clinical and research laboratories to assess circulating $1,25(OH)_2D$ concentrations.

[1] Department of Pediatrics and Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC.
[2] This paper has been cited more than 250 times since publication.
Address correspondence to the author at: Departments of Pediatrics, Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425. E-mail hollisb@musc.edu.
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