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In serum, the primary indicator of vitamin D nutritional status is 25-hydroxyvitamin D (25(OH)D), whereas 1,25-dihydroxyvitamin D (1,25(OH)2D) reflects mineral and skeletal homeostasis. Essential for a reliable assay of 1,25(OH)2D in serum is its separation from 25(OH)D, because the latter circulates in a 1000-fold greater concentration and significantly cross-reacts with the receptors used in radioassays of 1,25(OH)2D. Hollis (1) used a “Bond Elut” C18 column (Analytech International, Harbor City, CA) to separate the two in an acetonitrile extract of serum. Selecting the proper C18 column is critical for this type of separation (1).

Here we describe a procedure involving use of an “SPE-Octadecyl (C18)” column (J.T. Baker Chemicals, Phillipsburg, NJ) to separate 25(OH)D and 1,25(OH)2D before radioassay. To monitor recovery, we added to three 1-mL serum samples 1000 Bq of [3H]25(OH)D, [3H]1,25(OH)2D, and [3H]24,25-dihydroxyvitamin D, respectively. A serum extract prepared as described by Hollis (1) was applied to an SPE-Octadecyl (C18) column (500 mg in a 3-mL column) that had been successively prewashed with 3 mL of isopropanol, 3 mL of methanol, and 3 mL of water. After applying the sample, we washed the column with 6 mL of water and 6 mL of methanol/water (70/30, by vol), then eluted the 25(OH)D with 10 mL of hexane/methylene chloride (95/5, by vol). Analytical recoveries of tritium-labeled 25(OH)D and 1,25(OH)2D through the entire extraction and separation procedure were 97% (SD 3.3%) and 96% (SD 5.4%), respectively. Similar results were obtained for several batches of SPE-Octadecyl (C18) columns.

To remove residual 25(OH)D, Hollis (1) applied an additional wash of hexane/isopropanol (99/1, by vol) before eluting the 1,25(OH)2D. We found that this wash eluted a substantial fraction of 1,25(OH)2D from the SPE-Octadecyl (C18) column, and we omitted this step in our procedure. Consequently we recovered 2.1% (SD 0.7%) of 25(OH)D in the 1,25(OH)2D fraction. This is acceptable because the apparent increase of the 1,25(OH)2D concentration in serum resulting from this recovery, combined with the 0.1% cross-reactivity of 25(OH)D in the thymus receptor assay (2), corresponds to only 0.002% of the 25(OH)D in serum. A small proportion (12.0% (SD 1.4%)) of the 24,25-dihydroxyvitamin D [24,25(OH)2D] was recovered in the 25(OH)D fraction, the remainder in the 1,25(OH)2D fraction. This does not significantly affect the determination of 1,25(OH)2D and 25(OH)D in the serum, because 24,25(OH)2D cross-reacts by only 0.02% with 1,25(OH)2D in the thymus receptor assay (2) and the concentration of 24,25(OH)2D in serum is low compared with that of 25(OH)D.

We determined 25(OH)D and 1,25(OH)2D concentrations in 40 patients’ sera, using this extraction procedure and applying a radioimmunoassay and a radioreceptor assay (both from Incstar Corp., Stillwater, MN) to quantify 25(OH)D and 1,25(OH)2D, respectively, in the appropriate column fractions, the solvents having been evaporated (under nitrogen) and the residues dissolved in the buffers supplied by Incstar Corp. Results obtained by HPLC chromatography (3) correlated well for 25(OH)D (range of values = 10–300 pmol/L, r = 0.97, slope = 0.87, intercept = 0.76, and S.D. = 12.8), and 1,25(OH)2D (range of values = 3–240 pmol/L, r = 0.98, slope = 1.06, intercept = 1.47, and S.D. = 8.8).

Evidently an SPE-Octadecyl (C18) column effectively separates vitamin D metabolites, permitting reliable radioassay of 25(OH)D and 1,25(OH)2D in serum.

References

Evaluation of the Olympus PK310 Automated Enzyme Immunoassay System, Masakazu Miura, Yachiyo Fukuyama, Tetsuo Hirano, and Hiroko Matsuzaki (Clin. Labs., Tokyo Metropolitan Police Hospital, 2-10-41 Fujimi, Chiyoda-ku, Tokyo 102, Japan)

The PK310 Analyzer System (Olympus Co., Tokyo), a random-access instrument for one- and two-step heterogeneous enzyme immunoassays, has an assay menu of 16 on-line items. Test throughput is 60 tests per hour. Our evaluation of the PK310 included studies, for seven different analytes, of precision, carryover, and correlation of results with those obtained by other EIA or FPIA methods in routine use in this laboratory. The reagents were supplied by the manufacturer (Wako Pure Co., Osaka), except for CA19-9 reagent (Fujirebio Inc., Tokyo). We evaluated precision and accuracy for seven different analytes measured in the PK310. Immunoassay control sera, levels 1 and 2 (Bio-Rad Labs., Anaheim, CA), were used for six analytes, and level 3 for CA19-9. For the within-run precision study, controls were run in replicates of 20. In the between-run