Waters
Lab Highlights
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TANDEM RCSS SIMPLIFIES THE HPLC DETERMINATION OF VITAMIN D IN MILK PRODUCTS

The analysis of Vitamin D in foods has proved to be quite difficult. Thompson, et al, have described a method employing Radial-PAK™ C_{18} cartridges to quantitate vitamin D in dairy products (1,2) but preliminary HPLC cleanup on silica is required to remove interfering substances.

Recently Indyk and Woollard have published a paper (3) describing the use of two 5µ Radial-PAK™ C_{18} cartridges in RCM-100® holders in series to achieve the high degree of resolution required to analyze Vitamin D_3 in extracts of saponified, fortified milk powders without preliminary chromatography, thereby simplifying and speeding the analysis by at least a factor of two.

Figure 1 illustrates chromatograms of standards using one and two cartridges.

Figure 1. Single and tandem column reverse-phase chromatography of a vitamin A, D_2, D_3 and E mixture.
Column: Radial-PAK, 5 microns, spherical, C-18.
Mobile Phase: 100% methanol.
Flow Rate: 0.6 ml/min (single), 1.0 ml/min (tandem).
Detection: 280 nm, 0.005 AUFS.
1: trans-Retinol.
2: ergocalciferol (D_2).
* 3: cholecalciferol (D_3).
4: α-tocopherol.

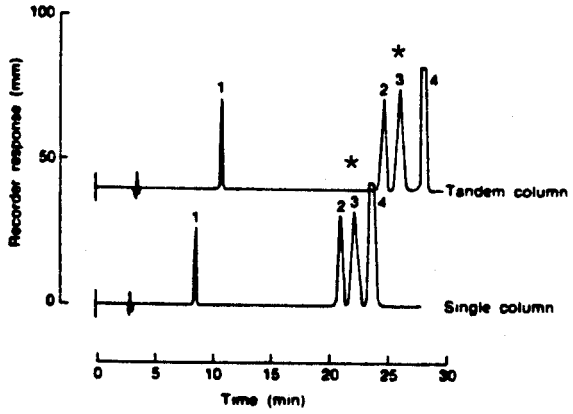
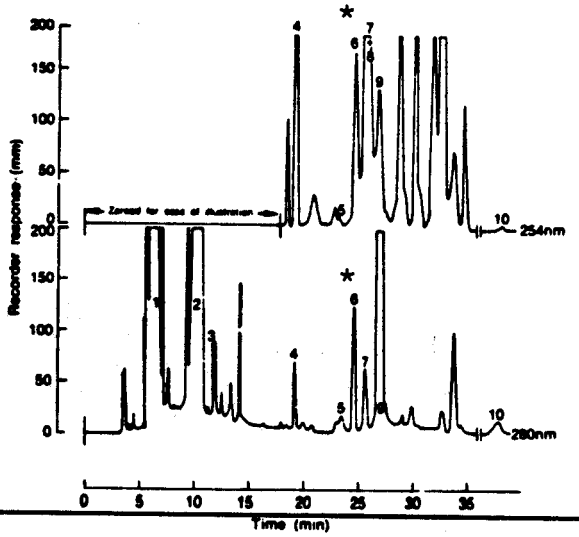


Figure 2 shows a chromatogram of a typical milk powder extract. Note the baseline separation of Peaks 5 (γ-tocopherol), 6 (Vitamin D_3), and 7 (unknown) in the lower (280 nm) trace.

Figure 2. Dual wavelength chromatograph of a typical fortified* whole milk powder extract.
Column: tandem Rad-PAK.
Mobile Phase: methanol:THF (99:1 v/v).
Flow Rate: 1 ml/min.
Detection: top, 254 nm; bottom, 280 nm; 0.005 AUFS.
1, solvent; 2, vitamin A; 3, solvent; 4, unknown; 5, γ-tocopherol;
*6, vitamin D_3; 7, unknown; 8, unknown; 9, α-tocopherol; 10, carotene (at elevated flow rate); other peaks not identified.
*: Fortified with vitamins A and D_3.



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This work was brought to our attention by Dr. Woollard, who sent us a reprint of his paper and a letter expressing his high regard for the Radial-PAK™ cartridges.

1. Thompson, J. N., Hatina, G., Maxwell, W. B., and Duval, S., J. Assoc. Off. Anal. Chem., 65, 624-631 (1982).
2. Maxwell, W., Waters Lab Highlight 009.
3. Indyk, H. and Woollard, D. C., New Zealand Journal of Dairy Science and Technology, 19, 19-30 (1984).