

Appendix:

Preparation of vitamin D solutions

The vitamin D₃ in ethanol solution concentration was prepared in the Clinical Pathology Laboratory at Mt Sinai Hospital, Toronto Canada (RV). US Pharmacopoeia (USP)-grade vitamin D₃ (Cholecalciferol) was purchased in crystalline form from Sigma (St Louis) and dissolved in USP-grade ethanol. Measurement of molar concentration of vitamin D₃ content of both the Vigantol oil, and the Sigma solutions were performed using ultraviolet absorption spectra obtained on a diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA); an extinction coefficient of 18,300 AU/mol/L was used as previously described (34).

Quality Assurance

For the short and long term studies, samples of the vitamin D₃ prepared were assayed in the Clinical Pathology laboratory at Mt Sinai Hospital, Toronto Canada (RV). Concentrations of both Sigma & Vigantol D₃ were measured by directly adding the liquid to mobile phase (acetonitrile:methanol, 1:1) for measurement by HPLC, versus an external calibrator. The HPLC column was Dupont Zorbax ODS column eluted at a flow rate of 0.5 mL/min. The vitamin D concentration was within 10% of that anticipated (data not shown).