

Serum Extraction for Carotenoids, Retinoids, and Tocopherols.

Serum is extracted and quantitated using previously published methods (Tang et al, 1993, Yeum et al, 1998). Serum is prepared for extraction using 200 μ L sample and 0.5 mL 0.9% saline. Echinenone, retinyl acetate and tocopherol, in ethanol, are added as internal standards. The mixture is extracted by using 2 mL chloroform:methanol (2:1, v:v). The reverse phase HPLC system consists of a 616 LC pump (Waters Corp., Milford, MA), Waters 717 plus autosampler (Waters Corp., Milford, MA), a C30 carotenoid column (3 μ m, 150 x 4.6 mm, YMC, Wilmington, NC), and Waters 994 programmable photodiode array. The data is collected and analyzed using Millennium32 Software (version 3.05.01, Windows NT, Waters Corp. 1998). The carotenoids are quantitated at 455 nm. Retinoids (retinol and retinyl palmitate) and tocopherols (delta, alpha and gamma) are quantitated at 340 and 292 nm, respectively. Carotenoids, retinoids and tocopherols are quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. Concentrations are corrected for extraction and handling losses by monitoring the recovery of the internal standards. The lower limit of detection is 0.2 pmol for carotenoids, 2.0 pmol for retinoids, and 2.7 pmol for tocopherols.