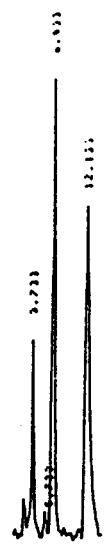
Proc. Nutr. Soc. Aust. (1992) 17

MEASUREMENT OF ALPHA-TOCOPHEROL IN HUMAN PLATELETS BY HPLC

N. GHANI and M. A. ALLMAN

Studies have shown that fish oil is beneficial to decrease the risk of cardiovascular disease. However, because of its highly unsaturated status it increases the body's requirement for vitamin E. As part of a study to examine the effect of fish oil supplements on platelet vitamin E, we developed a HPLC method for alpha-tocopherol. Blood (20ml) was collected from six healthy volunteers. Alpha-tocopherol was extracted from washed platelets following a modification of the method of Caye-Vaugien et al. (1990).

A HPLC system equipped with a Hewlett Packard 1050 pump, a model ETP- Kortec K 95 autosampler and a fluorescence detector (Shimadzu RF 535) was used. The HPLC separations were performed on a Si 60 Lichrosorb column, 25cm x 4.6mm ID, 5µm particle size. The mobile phase was n-heptane (96.7%), tert-butylmethylether (3%) and propan-2-ol (0.3%). Flow rate was 1 ml/min with fluorescence detection EX 295 nm, EM 325 nm. Alpha-tocopherol acetate (12.2 µg/ml) was used as an internal standard.



A typical chromatogram of the internal standard solution and extract from the platelets is shown in the Figure. Under these analytical conditions the alpha-tocopherol and alpha-tocopherol acetate were well-separated. The assay was linear between 1.60 µg/ ml and 12.88 µg/ml (r=0.99) which was the expected physiological range. The within run co-efficient of variation as determined by 10-fold analysis of samples was 4%.

The alpha-tocopherol concentrations in human platelets are measurable in physiological concentrations using this method. The method is simple, specific and highly sensitive.

CAYE-VAUGIEN, C., KREMPF, M., LAMARCHE, P., CHARBONNET, B. and PIERI, J. (1990). Int. J. Vit. Res. 21: 114.

Faculty of Health Science, University of Sydney, and Human Nutrition Unit, University of Sydney, New South Wales 2006