

THE DETERMINATION OF CAROTENES IN PLASMA

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Information about specific provitamins A in plasma is of importance for assessing the role of β -carotene and other carotenoids in the aetiology of cancer. Plasma provitamins A levels can also be used as an indicator of vitamin A status and in the clinical assessment of hypercarotenaemia. Analytical procedures for determining provitamins A have been of varying specificity, involving colorimetry or separation by open column or thin-layer chromatography, followed by spectrophotometric quantification. More recently high-performance liquid chromatography (HPLC) procedures have been reported for the estimation of β -carotene in plasma (Peng et al. 1983; Driskell et al. 1983) and serum (Broich et al. 1983).

We have developed a HPLC procedure, for the estimation of α - and β -carotene in plasma, which uses a readily available internal standard (β -apo-8'-carotenal) combined with the technique of standard additions. To 200 μ L of plasma was added 1.0 mL of 50% ethanol in water and 1.0 mL of the internal standard (β -apo-8'-carotenal) in hexane. After mixing and centrifugation, most of the organic phase was removed. This was evaporated under nitrogen and the residue dissolved in the HPLC solvent. Chromatography was performed on a Spherisorb ODS 2 column (Alltech Assoc.) with a mobile phase of methanol : acetonitrile : chloroform (46 : 46 : 8) at 2.0 mL/min and detection using a 436 nm filter. A standard curve was prepared by adding aliquots of α - and β -carotene standard solutions to the plasma. Peak height ratios (peak height of α - and β -carotene \div peak height of β -apo-8'-carotenal) were plotted against concentration of the standards. Extrapolation of peak height ratios allows the amount of endogenous α - and β -carotene in the plasma sample to be determined. The standard curves for α - and β -carotene were linear for the concentration range 20 to 70 μ g/mL, with correlation coefficients of 0.99 and 0.99 respectively. Reproducibility was determined on five aliquots of the pooled plasma (six determinations per aliquot) and the coefficients of variation for α -carotene and β -carotene were 6.5 and 10.3%, respectively. The concentration of α -carotene in the plasma pool was 6.0 μ g/100 mL and that of β -carotene was 20.0 μ g/100 mL.

Previous procedures using HPLC for determining α - and β -carotene have the disadvantages of no internal standardisation (Peng et al. 1983) or two separate injections (Broich et al. 1983) for quantification. Driskell et al. (1983) use a synthetic derivative of β -carotene dimethyl- β -carotene as an internal standard. None of these procedures use a standard addition technique for additional quality control.

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