A RAPID ASSAY FOR THE DETERMINATION OF SERUM β-CAROTENE, RETINOL AND RETINYL-PALMITATE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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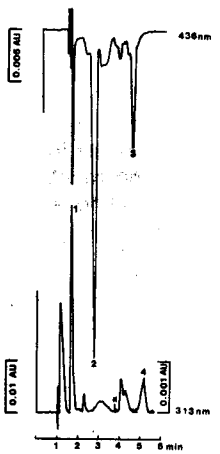
A simple and rapid assay for the quantitation of serum β-carotene, retinol and retinyl-palmitate based on an isocratic reverse-phase HPLC system is presented. The system separates the above nutrients in a single run and detects them using a dual wavelength detector (series 440, Waters Assoc., USA)(Briggs et al, 1985) within six minutes (see figure). Sample preparation includes precipitation of serum proteins by the addition of aqueous ethanol and extraction with hexane which also contains the internal standard (I.S.) β-apo-8'-carotenal. The organic phase is evaporated to dryness under nitrogen and the residue dissolved in the mobile phase before injection onto the column.

The assay is characterised by excellent sensitivity (detection at a signal to noise ratio of 3:1) and good precision (see table), and the inter-assay variability could be improved by temperature control of the column.

This method was developed for the assessment of vitamin A and β-carotene absorption tests but general routine clinical and research surveys can also benefit from this rapid method. The advantages of this system lie in its (i) speed and resultant high sample through-put; (ii) saving of laboratory time, expensive solvents and sample requirement; and (iii) capacity to measure low levels of these nutrients simultaneously.



HPLC Conditions
Column : Hibar C18 (12.5x0.4 cm I.D.)
Mobile phase : acetonitrile : dichloromethane (70 : 30)
Flow rate : 1.0 mL/min
Temperature : ambient
Pressure : 500-600 psi
* : sensitivity increased 10 fold.

Figure : HPLC elution profile of serum extract to which the I.S. has been added. The vertical axis represents detector sensitivity. The horizontal axis represents retention time where peak (1) is retinol; (2) β-apo-8'-carotenal (I.S.); (3) β-carotene, and (4) retinyl-palmitate.

Detection limits, intraassay and interassay coefficient of variation (CV%)

	β-Carotene	Retinol	Retinyl-palmitate
Detection limits μmol/L	0.03	0.08	0.03
Intraassay CV% (n=10)	3.41	2.60	5.39
Interassay CV% (n= 8)	5.53	6.52	8.08

BRIGGS, D.R., KAJADPHAI, A. & JONES, G.P. (1985). Proc.Nutr.Soc.Aust. 10:131.

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