

THE SIMULTANEOUS ANALYSIS OF PLASMA VITAMIN A AND CAROTENOIDS  
BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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We have previously reported a high performance liquid chromatography (HPLC) procedure for the simultaneous determination of retinol, retinyl acetate, retinyl palmitate,  $\alpha$ -carotene and  $\beta$ -carotene by monitoring the eluent at 313 and 436 nm (Briggs et al. 1985).

We have now adapted this procedure for the estimation of these substances in plasma. Our procedure uses an isocratic elution, a reverse phase column at a temperature of 28°C, flow rate at 2.0 ml/min with two internal standards, N-octadecyl-naphthylurethan and  $\beta$ -apo-8'-carotenal, for the use at 313 and 436 nm respectively. N-octadecyl-naphthylurethan was synthesised from 1-octadecanol and  $\alpha$ -naphthyl isocyanate according to the method of Vogel 1961 to provide an internal standard with a retention time between retinol and retinyl palmitate with absorption at 313 nm. A 200  $\mu$ l aliquote of plasma pool obtained from 7 volunteers was treated with 50% ethanol and extracted with hexane (Briggs et al. 1983) containing the two internal standards. The results of the plasma analysis are shown in Figure 1.

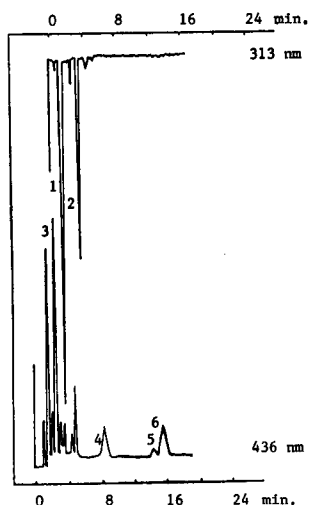


Figure 1 HPLC chromatogram of human plasma pool extract.

The numbered peaks correspond to 1) retinol; 2) N-octadecyl-naphthylurethan, internal standard at 313 nm; 3)  $\beta$ -apo-8'-carotenal, internal standard at 436 nm; 4) lycopene; 5)  $\alpha$ -carotene and 6)  $\beta$ -carotene.

HPLC CONDITIONS

Flow rate: 2.0 ml/min,  
Chart speed: 0.25 cm/min,  
Injection amount: 30  $\mu$ l  
Elution solvent: Methanol:Acetonitrile (30:70)  
Column: steel NOVA-PAK C18  
Temperature: 28°C  
Absorption was read at 313 and 436 nm, simultaneously.

This method allows the simultaneous determination of retinol, lycopene,  $\alpha$ -carotene and  $\beta$ -carotene in plasma. It has the added advantage of internal standardisation for each of the detectors operating at 313 and 436.

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