Improved quantification of retinol, tocopherol and carotenoid in human plasma by HPLC using retinol acetate as internal standard

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Background - Previously, we reported a gradient HPLC procedure for simultaneous quantification of retinol, tocopherols and carotenoids in human plasma based on both retinol acetate (RA) and tocopherol acetate (TA) as internal standards (IS) (1).

Objective - To simplify and improve imprecision of assay using only RA internal standard.

Outcomes - In a series of 3229 plasma samples assayed over 10 months, including 129 plasma matrix quality controls. The assay coefficients of variation (CV) were less than 6% for all analytes, except □-cryptoxanthin.

Analyte µg/dl	Within run concentration[1]	CV (%)	Between run concentration[2]	CV (%)
□-tocopherol	1526 ± 34	2.2	905 ± 43	4.8
retinol	76.4 ± 1.5	2.0	46.6 ± 2.0	4.2
□-carotene	23.2 ± 0.6	2.5	41.3 ± 1.9	4.7
□-carotene	4.8 ± 0.2	3.2	6.4 ± 0.3	5.4
□-cryptoxanthin	14.1 ± 0.4	2.7	18.5 ± 1.6	8.7
lutein/zeaxanthin	21.2 ± 0.4	1.7	14.4 ± 0.8	5.4
total lycopene	28.3 ± 0.7 .	2.5	28.9 ± 1.6	5.4

[1]mean ± SD n=8; [2]mean ± SD n=129

Conclusions – This precision is somewhat better than observed previously using TA as an IS, where CV% ranged from 8.1% to 5.4 and 9.2 to 5.4 for □-carotene and lycopene (1).

1. Su Q, Rowley KG, Balazs NDH. Carotenoids: separation methods applicable to biological samples. J Chromatogr B – Biomed Sci Appl 2002; 781: 393-418.