Quantitative analysis of vitamin A and E in human serum

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This technical note demonstrates the sensitive and accurate quantitation of vitamins A and E in human serum using a rapid protein precipitation sample preparation procedure with analysis using the SCIEX QTRAP 4500 system. Excellent linearity was observed across clinically relevant concentrations. Low-µmol/L level sensitivity was achieved at the lowest calibrator with signal-to-noise ratios (S/N) of 72:1 for vitamin A at 0.76 µmol/L and 105:1 for vitamin E at 6.76 µmol/L. In addition, the method showed excellent precision and accuracy at low-level concentrations, highlighting the assay's strong quantitative performance.

Key benefits of vitamin A and E analysis from human serum using the QTRAP 4500 system

Rapid sample preparation. The two fat-soluble vitamins were extracted from human serum samples using a fast protein precipitation procedure

Excellent linearity. Calibration curves for vitamin A and vitamin E showed r^2 values above 0.99 across the calibration range (0.76-3.76 µmol/L for vitamin A and 6.76-44.20 µmol/L for vitamin E)

Low-µmol/L level sensitivity and excellent quantitative performance. Sensitive quantitation of vitamin A and vitamin E was achieved with excellent precision (1.3% for both vitamin A and vitamin E) and accuracy (99.5% for vitamin A and 101.4% for vitamin E) at the lowest calibrator levels (0.76 µmol/L for vitamin A and 6.76 µmol/L for vitamin E)

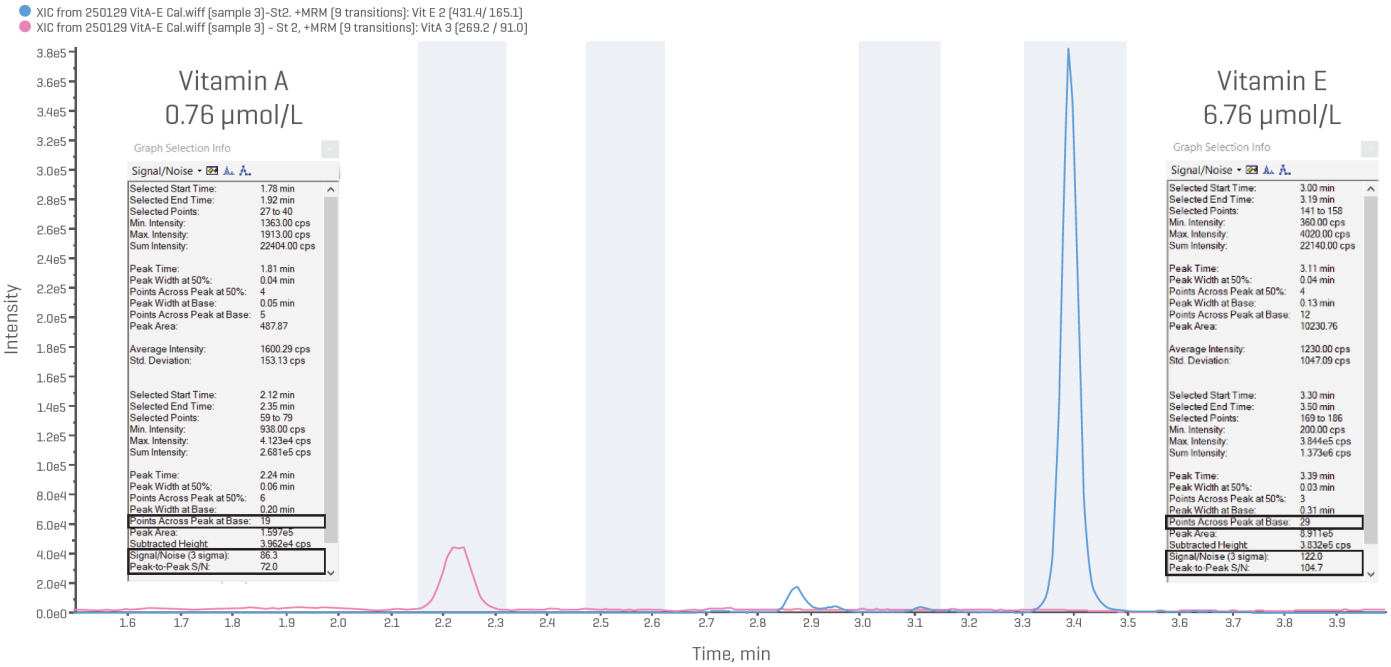

Figure 1. Chromatogram of vitamin A (pink) and vitamin E (blue) extracted from serum matrix. Chromatogram of calibration standards in matrix for vitamin A (retinol) at 0.76 µmol/L and vitamin E (alpha-tocopherol) at 6.76 µmol/L shows a S/N of 72:1 for vitamin A and 105:1 for vitamin E, respectively, based on a peak-to-peak algorithm.

Introduction

Vitamins A and E are essential fat-soluble vitamins that support various bodily functions, including vision, antioxidant defence, and immune system health. Deficiencies in these vitamins often result from malnutrition or underlying health issues. Therefore, accurately measuring these vitamins is crucial for diagnosing deficiencies.

Methods

Sample preparation: Vitamin A and vitamin E were extracted from human serum using a protein precipitation procedure. In short, 25 µL of blank serum spiked with vitamin A and vitamin E at six concentration levels were added to a centrifuge tube to which 975 µL of a protein precipitation solution spiked with deuterated vitamin A and vitamin E internal standards. The tube was vortexed and centrifuged for 5 minutes at 10,000 x g. The supernatant was transferred to a UPLC vial for analysis.

Liquid chromatography: Chromatographic separation was achieved using a Phenomenex Kinetex Luna Omega Polar C18 column (50 x 2.1 mm, 1.6 µm, 00B-4748-AN). Mobile phase A was water and mobile phase B was methanol. The total run time was 6.5 minutes at a flow rate of 600 µL/min. The injection volume was 15 µL. The LC gradient program is presented in **Table 1.**

Table 1: Chromatographic gradient for the analysis of vitamin A and E in human serum.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	35	65
1.4	5	95
1.9	5	95
2.0	0	100
3.7	0	100
3.71	35	65
5.5	35	65

Mass spectrometry: Data was collected using a QTRAP 4500 system with an IonDrive Turbo V ion source and operated in electrospray ionization (ESI) positive mode. The Scheduled MRM algorithm was used in SCIEX OS software (version 3.1.6) to collect sufficient data points to produce robust, quantifiable data. Source and gas conditions are presented in **Table 2.** Compound-dependent parameters were optimized by infusion.

Table 2: Source and gas parameters for the analysis of vitamin A and E in human serum using the QTRAP 4500 system.

Parameter	Value
Polarity	Positive
Ion source gas 1	60 psi
Ion source gas 2	70 psi
Curtain gas	30 psi
Source temperature	550 °C
Ion spray voltage	5500 V
CAD gas	9

Data processing: Data processing was performed using SCIEX OS software (version 3.1.6). Peak integration was achieved using the MQ4 algorithm. Quantitative analysis was conducted in the Analytics module of SCIEX OS, where calibration curves, concentration calculations, assay precision, and accuracy statistics were automatically generated.

Results and discussion

Figure 1 shows the chromatographic separation of vitamin A and vitamin E in a control human serum sample at a final concentration of 0.76 µmol/L for vitamin A and 6.76 µmol/L for vitamin E, respectively. The extracted ion chromatograms showed a S/N of 72:1 for vitamin A and 105:1 for vitamin E, at the lowest matrix calibrator measured (0.76 µmol/L for vitamin A and 6.76 µmol/L for vitamin E), calculated using a peak-to-peak algorithm.

Figure 2 shows the representative extracted ion chromatograms (XICs) for A) vitamin A and B) vitamin E across their respective concentration ranges (0.76-3.76 µmol/L for vitamin A and 6.76-44.20 µmol/L for vitamin E). The signals shown for the lowest matrix calibrators measured (0.76 µmol/L for vitamin A and 6.76 µmol/L for vitamin E) are well above the blank signal for both analytes.

The quantitative performance of the method was investigated by injecting a series of calibrator samples spiked at concentrations ranging from 0.76-3.76 µmol/L for vitamin A and 6.76-44.20 µmol/L for vitamin E, respectively. Linearity, accuracy and precision were assessed across the calibration ranges for each of the two analytes. **Figure 3** shows the

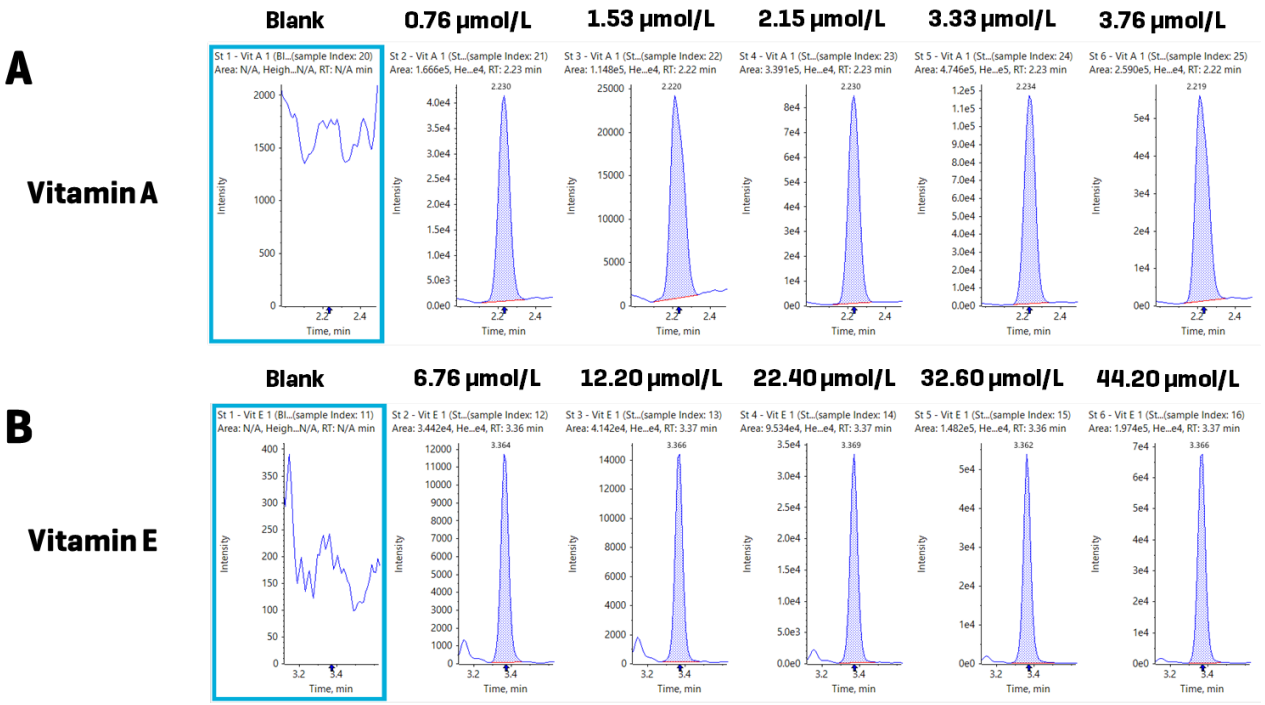

Figure 2. Extracted ion chromatograms for the two analytes targeted in this method. The XICs show the signal for A) vitamin A and B) vitamin E across their respective concentration ranges (0.76–3.76 µmol/L for vitamin A and 6.76–44.20 µmol/L for vitamin E).

calibration curves for vitamin A (top) and vitamin E (bottom) over the analytes' respective calibration ranges. The plots show excellent linear responses across the calibration series, with r^2 values greater than 0.99 for both analytes.

The accuracy and precision values were calculated by 3 replicates in matrix at the lowest matrix calibrators measured (0.76 µmol/L for vitamin A and 6.76 µmol/L for vitamin E). The accuracy was 99.5% for vitamin A and 101.4% for vitamin E, respectively. The precision (%CV) was 1.3% for both vitamin A and vitamin E.

Conclusions

A fast and sensitive LC-MS/MS method for the detection of vitamin A and E extracted from human serum samples was developed using the 4500 system. The method demonstrated:

- Fast sample preparation which consisted of a simple protein deproteination, requiring only 25 µL of human serum sample
- Good sensitivity at the lowest calibrator level, resulting in S/N of 72:1 for vitamin A at 0.76 µmol/L and 105:1 for vitamin E at 6.76 µmol/L
- Excellent linear responses across the calibration series consisting of 5 calibrators, with r^2 values greater than 0.99 for both analytes
- High quantitation performance of the method, resulting in excellent precision (1.3% for both vitamin A and vitamin E) and accuracy (99.5% for vitamin A and 101.4% for vitamin E) at the lowest calibrator levels (0.76 µmol/L for vitamin A and 6.76 µmol/L for vitamin E)

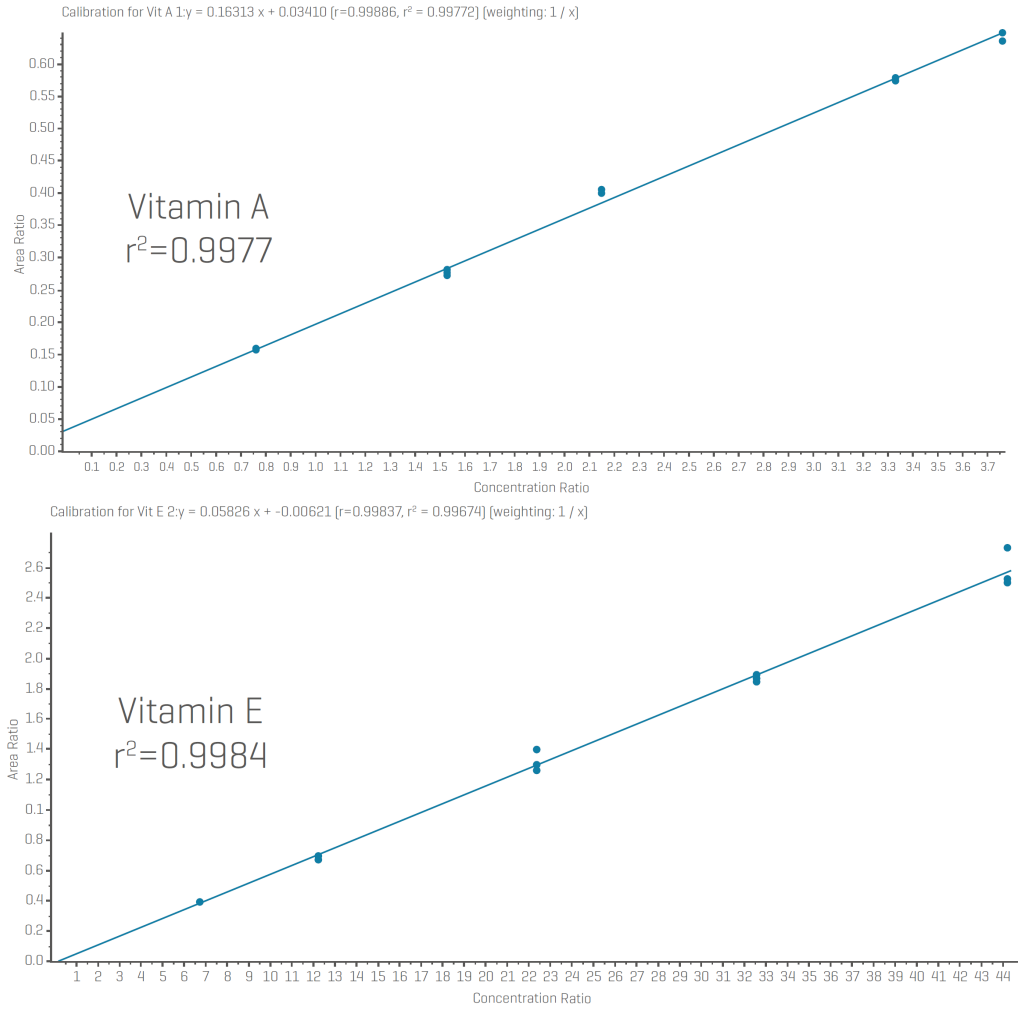

Figure 3. Linear calibration curves for vitamin A (top) and vitamin E (bottom) extracted from serum matrix. The calibration curves were evaluated across the following concentration ranges: 0.76–3.76 µmol/L for vitamin A and 6.76–44.20 µmol/L for vitamin E. The curves were generated using linear regression and 1/x weighting for vitamin A and vitamin E in serum, resulting in r^2 values of 0.9977 and 0.9984, respectively.

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