Vitamin A and vitamin E in human serum

Illustration of analytical performance for vitamin A and vitamin E in human serum.

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze vitamin A and vitamin E in human serum matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and methods

The Citrine MS/MS system was controlled, and data processed using Analyst MD software, version 1.6.3. Serum calibrators, controls and samples were processed using the following conditions:

Sample preparation: Sample preparation was performed using Diagnotix's vitamin A and E reagents set [https://www.diagnotix.com/en/products/vitamine-a-e-reagens-set] according to the manufacturer's specifications. A 25 µL serum sample spiked using the set of calibrators was used for the procedure.

Liquid chromatography conditions: Chromatographic separation was achieved using a Phenomenex Kinetex C18 column. Mobile phases A and B from the reagents set were used. The total run time was 6.5 minutes at a flow rate of 600 µL/min. The injection volume was 15 µL.

Mass spectrometry conditions: Mass spectrometry analysis was performed using the Citrine Triple Quad MS/MS system, operating in positive electrospray mode. Compound-dependent parameters were optimized by infusion.

Results

Analytical performance statistics including the concentration range evaluated, accuracy and precision (n=6 replicates), as well as signal-to-noise ratio (S/N) and linearity (r^2) are shown in Table 1. Chromatograms of the compounds evaluated utilizing the described method are shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Table 1. Performance statistics for the analysis of vitamin A and vitamin B in human serum. Measured range (µmol/L), % accuracy, %CV, S/N ratio and linearity for vitamin A and E. Values for the lowest calibrator (0.41 µmol/L for vitamin A and 5.04 µmol/L for vitamin E) and over the measured range (0.41-4.51 µmol/L for vitamin A and 5.04-49.5 µmol/L for vitamin E) were used, as appropriate.

Compound	Range (pg/mL)	% Accuracy	%CV	S/N*	Linearity (r^2)
Vitamin A	0.41-4.51	105.37	2.7	53.0:1*	0.9999
Vitamin E	5.04-49.5	102.94	3.4	753.3:1*	0.9995

*S/N ratio calculated using a peak-to-peak algorithm for lowest matrix calibrator measured.

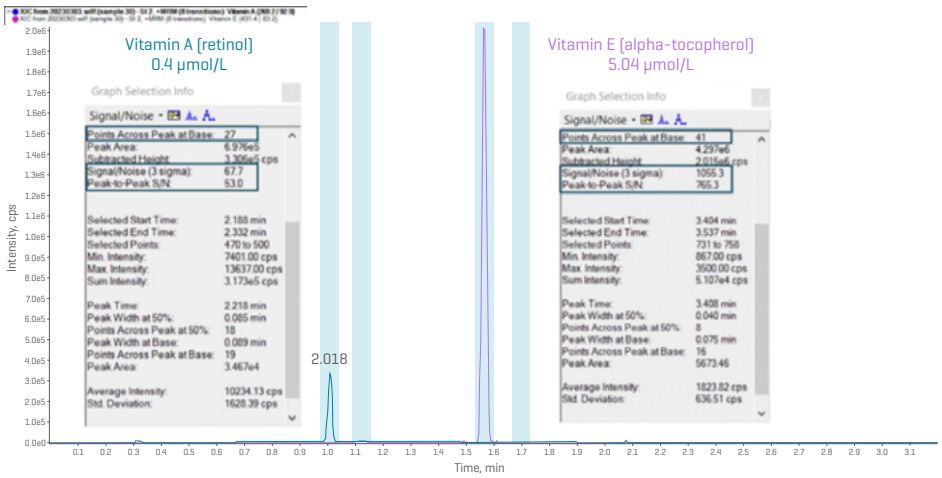

Figure 1. Chromatogram of vitamin A (blue) and vitamin E (pink) extracted from serum matrix. Chromatogram of calibration standards in matrix for vitamin A (retinol) at 0.41 µmol/L and vitamin E (alpha-tocopherol) at 5.04 µmol/L shows a S/N of 53.0:1 for vitamin A and 753.3:1 for vitamin E based on a peak-to-peak algorithm.

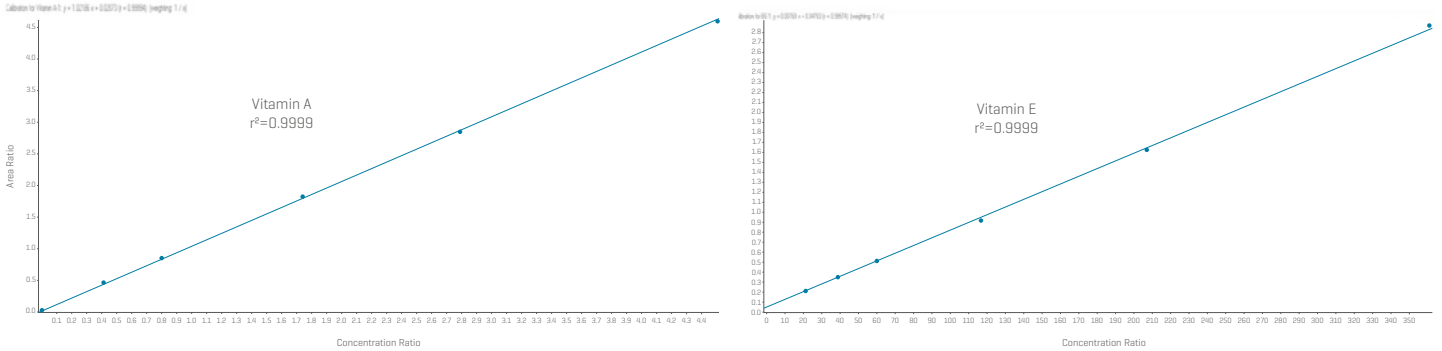

Figure 2. Linear calibration curves for vitamin A (left) and vitamin E (right) extracted from serum matrix. The calibration curves were run across the following concentration ranges (0.41–4.51 µmol/L for vitamin A and 5.04–49.5 µmol/L for vitamin E). The curves were generated using linear regression and 1/x weighting for vitamin B1 and vitamin B6 in serum, resulting in r^2 values of 0.9999 for both analytes.

Conclusions

Based on the above performance testing, the following results were obtained:

Sensitivity: Analytical sensitivity was investigated with a series of calibration standards prepared as described and showed a S/N of 53.0:1 for vitamin A and 753.3:1 for vitamin B, at the lowest matrix calibrator measured (0.41 µmol/L for vitamin A and 5.04 µmol/L for vitamin E), calculated using a peak-to-peak algorithm.

Assay linearity: Linearity was assessed in matrix over the following concentration ranges: 0.41–4.51 µmol/L for vitamin A and 5.04–49.5 µmol/L for vitamin E. The r^2 values were 0.9999 for both analytes.

Accuracy: At the lowest matrix calibrator measured, (0.41 µmol/L for vitamin A and 5.04 µmol/L for vitamin E), the % accuracy was 105.37% for vitamin A and 102.94% for vitamin E, determined by 6 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

Reproducibility: At the lowest measured calibrator (0.41 µmol/L for vitamin A and 5.04 µmol/L for vitamin E), the precision (%CV) was 2.7% for vitamin A and 3.4% for vitamin E, determined by 6 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

In these experiments, the Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for the quantitation of vitamin A and vitamin E in serum matrix.

Acknowledgements

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