Ultrafast Quantitation of Fat Soluble Vitamins A and E in Human Serum Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Clinical Research

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Abstract

Analysis of vitamins A and E in serum by clinical research laboratories requires a reliable analytical method and accurate detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional analytical technologies. An analytical method has been developed using an SPE/MS/MS system to analyze vitamins A and E in serum with much faster sample cycle times and similar analytical results compared to traditional assays. A simple protein crash followed by dilution, then analysis using the Agilent RapidFire High-Throughput Mass Spectrometry System allows for the accurate and precise measurement of these analytes in human serum over a linear range of 0.1–5 µg/mL for vitamin A and 0.4–20 µg/mL for vitamin E. Samples were analyzed on the RapidFire/MS/MS system at 15 seconds per sample, providing a much higher throughput method of analysis compared to traditional protocols.

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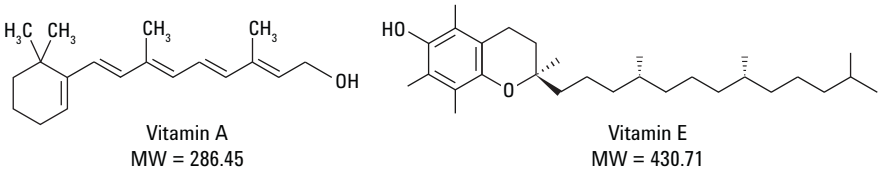
Introduction

Vitamins A (retinol) and E (α-tocopherol) are fat soluble vitamins stored in the body. Chromatographic methods, such as HPLC and, recently, LC/MS/MS are used to measure these vitamins (Figure 1) in serum. Some of these analytical methods use complicated extraction instruments or long and tedious extraction procedures, requiring large amounts of solvents or biological fluids. The main objective of this Application Note was to develop a rapid, selective, and sensitive online SPE/MS/MS method that has a short and simple extraction procedure, consumes small amounts of solvent and biological fluid for extraction, and has an ultrafast turnaround. An analytical method using the Agilent RapidFire/MS/MS system to analyze vitamins A and E in serum has been developed, with much faster sample cycle times and similar analytical results compared to traditional assays.

Using a 96-well plate based extraction system (dilute-and-shoot) allows for automation of the entire sample preparation protocol to increase efficiency and laboratory throughput. The Agilent RapidFire High-throughput Mass Spectrometry System is an ultra-fast SPE/MS/MS system capable of analyzing samples with cycle times of less than 15 seconds. This method, using RapidFire/MS/MS, allows for the rapid, accurate, and precise measurement of Vitamin A and E over a reasonable linear range for both analytes.

Experimental

Analytes



Vitamin A
MW = 286.45

Vitamin E
MW = 430.71

Figure1. Chemical structures of vitamins A and E.

The Agilent RapidFire/MS/MS system consisted of the following modules: Agilent RapidFire 365, Agilent 6460 Triple Quadrupole Mass Spectrometer equipped with APCI source using Agilent MassHunter Triple Quadrupole Acquisition Software (B.06.01) with Qualitative Analysis (B.06.00), Quantitative Analysis (B.06.00), and Agilent RapidFire Acquisition Software.

Samples were analyzed at a rate of 15 seconds per sample. Quantitative and qualitative ions for vitamins A and E, and internal standards vitamin A_D5 and vitamin E_D6 were monitored simultaneously in all experiments (Table 1). Agilent MassHunter Quantitative Software automatically calculated qualifier ion ratios.

Instrumental conditions

Agilent RapidFire/MS/MS conditions	
Buffer A	5 mM Ammonium formate in LC/MS grade water + 0.1 % formic acid; 1.5 mL/min flow rate
Buffers B and C	5 mM Ammonium formate in LC/MS grade methanol + 0.1 % formic acid; 1.25 and 0.7 mL/min flow rate
Aqueous wash	HPLC grade water
Organic wash	100 % Methanol or ACN
SPE cartridge	Agilent RapidFire cartridge E (reversed-phase C18 chemistry, G9203-80105)
RF state 1	600 ms
RF state 2	2,500 ms
RF state 3	0 ms
RF state 4	8,000 ms
RF state 5	1,000 ms
Agilent 6460 Triple Quadrupole conditions	
Source	APCI
Gas temperature	325 °C
Vaporizer	350 °C
Gas flow	10 L/min
Nebulizer	40 psi
Capillary voltage	4,500 V
Corona current	10 µ

Table 1. MRM transitions.

Compound	Q1	Q3	Dwell	Fragmentor	CE	CAV
Vitamin E_D6	437.5	171.1	20	114	21	2
Vitamin E (α-tocopherol)	431.4	165.2	20	146	25	2
Vitamin E (α-tocopherol)	431.4	137.2	20	146	49	2
Vitamin A _D5	291.3	162.2	20	142	5	2
Vitamin A (retinol)	269.4	105.1	20	114	33	2
Vitamin A (retinol)	269.4	93	20	114	21	2

Chemicals and reagents

Vitamin A was purchased from Sigma-Aldrich; retinol_D5 was purchased from Santa Cruz Biotechnology. Vitamin E was purchased from Cerilliant, Round Rock, TX. Vitamin E_D6 was purchased from Toronto Research Chemicals. The quality controls and the blank synthetic serum were purchased from UTAK Laboratories, Valencia, CA. All other solvent and reagents were purchased from Sigma-Aldrich.

Sample preparation

The samples, calibrators (0.1, 0.25, 1.25, 2.5, and 5.0 µg/mL for vitamin A, 0.4, 1.0, 5.0, 10.0, and 20.0 µg/mL for vitamin E), and QC standards (0.6 and 1.8 for vitamin A, 6.0 and 28.0 for vitamin E) were prepared using the following procedure.In a 2.2-mL deep well collection plate; 200 µL of sample, calibrators 5 points, 2 point QCs for vitamin A and vitamin E, and 25 µL of internal standard mixture (vitamin A_D5 and vitamin E_D6 at 2.0 and 5.0 µg/mL respectively prepared in LC/MS grade methanol) were mixed, then 10 µL of (1:1) glacial acetic was added. Proteins were crashed with the addition of 500 µL of acetonitrile followed by centrifugation for 10 minutes at 3,500 rpm. Then, 55 µL of the sample supernatants were transferred to each corresponding well in a 96-well black plate. After a (1:10) dilution with LC/MS grade water, the plate was sealed with an Agilent PlateLoc Thermal plate sealer, and mixed gently prior to RapidFire/MS/MS analysis.

We recommend adding EDTA and BHT (anti-oxidizing agent) to the synthetic serum to improve stability and binding characteristics of vitamin A. Also, because vitamin A is light-sensitive, it is recommended that one perform the analysis in a black or opaque plate.

Data analysis

Data analysis was performed using MassHunter Triple Quadrupole Quantitative analysis software. Calibration curves were constructed using linear least squares regression with 1/X weighing for the multiple reactions monitoring (MRM). The quantitation was performed by comparing spectral peak area ratio to a known concentration of the internal standards.

Results and Discussion

Samples were prepared by spiking vitamin A, vitamin E, and the internal standards into synthetic serum followed by a protein crash, then diluting the supernatant 10-fold with water. Samples were then analyzed through SPE/MS/MS using the RapidFire/MS/MS system and a hydrophobic C18 cartridge at 15 seconds per sample (Figure 2). This RapidFire/MS/MS methodology is capable of throughputs greater than 240 samples per hour, providing a high-throughput and very efficient mode of analysis.

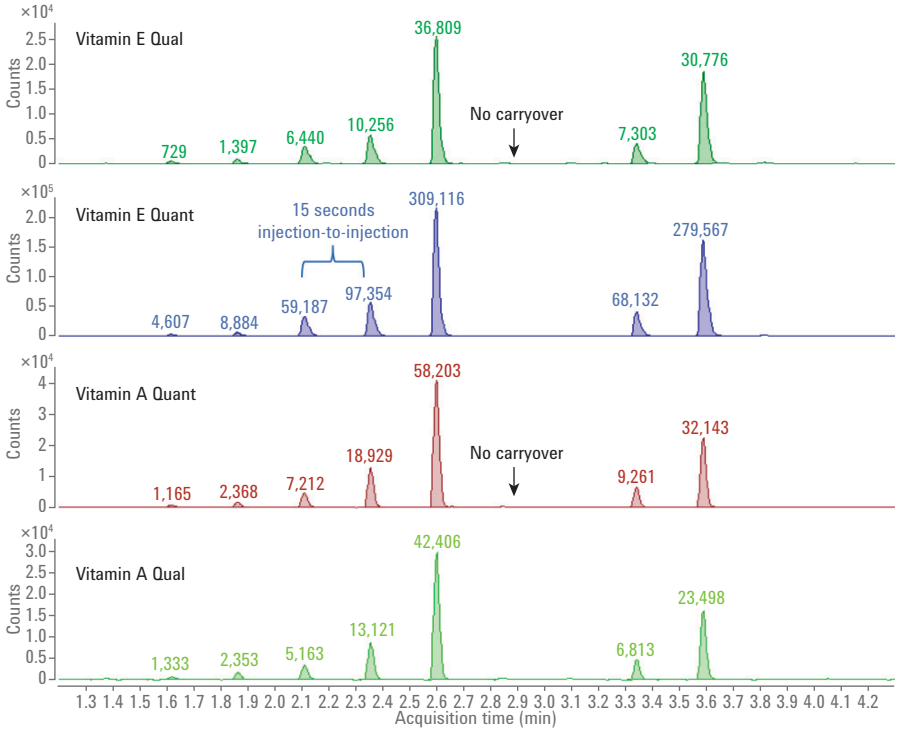


Figure 2. Representative calibration curve data for each of the analytes showing the injection-to-injection interval of 15 seconds. No significant carryover is seen in the matrix blank injections after the highest calibrator.

Vitamins A and E standard curves in serum had excellent linearity within the measured range (0.1–5 µg/mL) for vitamin A, and (0.4–20 µg/mL) for vitamin E with an R^2 value greater than 0.995 (Figure 3). The QCs were analyzed to obtain intra- and interday precision and accuracy values. Intra- and interday accuracies determined were within 10 %, and coefficient of variation values were all less than 10 % for concentrations within the measured range (Table 2). Carryover was assessed by analyzing the AUC of the matrix blank calculated as % of the mean peak area of the 0.1 and 0.4 µg/mL samples for vitamins A and E respectively. No significant carryover was observed for either analyte (Figure 2).

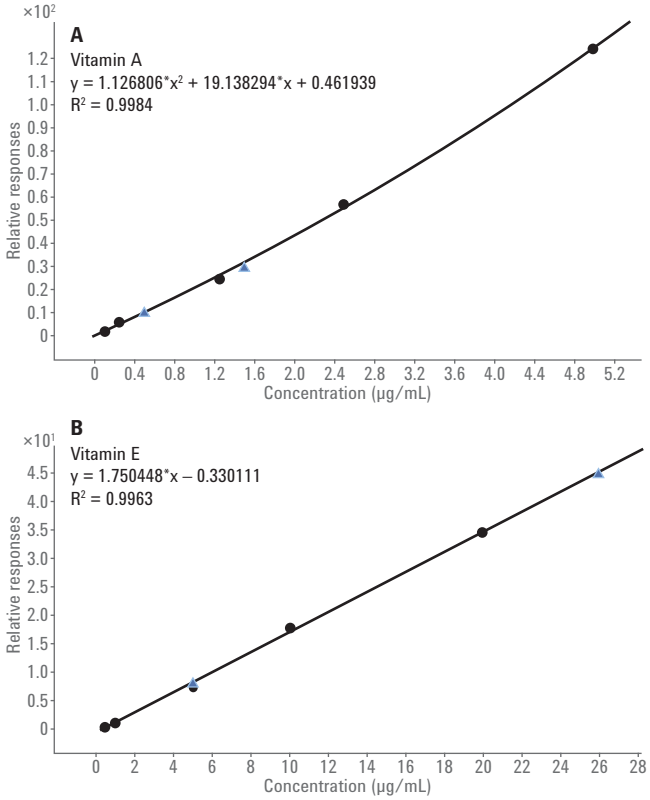


Figure 3. Representative calibration curves showing linear range from 0.1–5 µg/mL and 0.4–20 µg/mL for vitamins A and E respectively. Black circles are calibrators, blue triangles are QC samples.

Table 2. Intraday and interday accuracy and precision data for the QC standards.

Vitamin A concentration (µg/mL)	Intraday % accuracy (n = 6)	Intraday % precision (n = 6)	Interday % accuracy (n = 6)	Interday % precision (n = 6)
0.6	98.6	7.2	104.6	9.2
1.8	95.4	7.9	107.7	4.4
Vitamin E concentration (µg/mL)	**Intraday % accuracy (n = 6)**	**Intraday % precision (n = 6)**	**Interday % accuracy (n = 6)**	**Interday % precision (n = 6)**
6.0	97.5	9.2	102.1	6.8
28	102.6	3.2	102.5	5.3

The reproducibility of the analytical method was evaluated by measuring > 2,000 sequential injections of both analytes spiked into blank human serum. The instrument response was stable for both analytes, with a coefficient of variation of 9.0 % and 8.0 %, showing the robustness of the RapidFire system, SPE cartridge lifetime, and consistency of quantitation for the analytes in the panel. As an example, the data for vitamin E can be found in Figure 4, where the CV was 8.0 %.

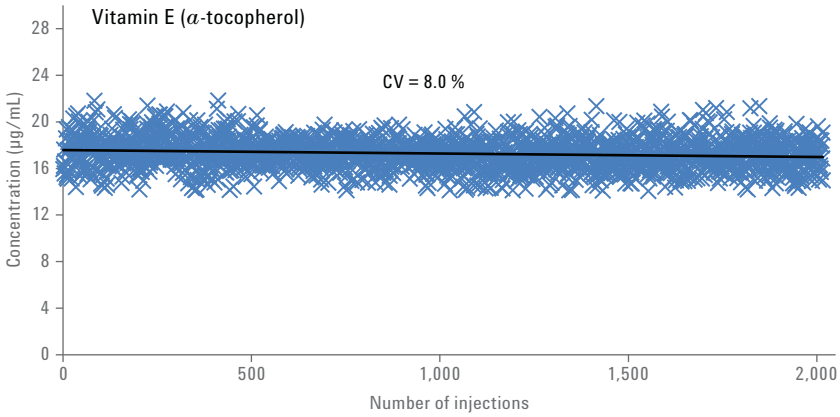


Figure 4. Reproducibility evaluation using sequential injections of vitamin E.

Conclusions

The fat-soluble vitamins A and E in human serum were rapidly, accurately, and precisely measured using a simple protein crash and dilution method with the Agilent RapidFire/MS/MS System. Samples were analyzed with injection-to-injection cycle times of 15 seconds, providing a high-throughput analytical method for these analytes. This methodology is capable of throughputs of 240 samples per hour, with comparable results to LC/MS/MS, but at > 10x the speed and efficiency of LC/MS/MS methods.

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Published in the USA, November 7, 2014
5991-5293EN

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