

Analysis of Fat-Soluble Vitamins and Antioxidants in Supplements by RP-HPLC

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ABSTRACT

Fat-soluble vitamins (FSVs) and fat-soluble antioxidants (FSAs) play essential roles in a wide spectrum of biochemical and physiological processes. Vitamin E (tocopherol) along with other FSAs (e.g., carotenoids and coenzyme Q10 [CoQ10]) are purported to help mitigate the effects of oxidative stress that have been linked to numerous diseases, including cancer, neurodegeneration, and atherosclerosis. These compounds are thought to exert their beneficial effects by acting as chain-breaking antioxidants, inhibiting lipid peroxidation of polyunsaturated fatty acids (PUFAs) contained within biological membranes, thereby preventing the formation of potentially cytotoxic and highly reactive aldehydes. Shown here is a simple, reversed-phase high-performance liquid chromatography (RP-HPLC)-charged-aerosol-detection method for the measurement of 11 FSVs and FSAs in commercially-available supplements including: vitamins A (*trans*-retinol, retinyl acetate, and palmitate), E (α -, δ -, γ -tocopherols and succinate), D, and K1 (phyloquinone); lycopene; lutein; and CoQ10. The analysis was completed in 20 min. Correlation coefficients were > 0.999 . Limit of quantitation (LOQ) values were < 30 ng on column. This method is an enhancement over current analytical approaches (e.g., UV and evaporative-light-scattering detection, ELSD) and offers a simple means of quantifying FSVs and FSAs in supplements and foods. Recovery values from commercial products show the quantitative capacity of this method.

INTRODUCTION

FSVs are typically measured using UV absorbance or fluorescence. However, as they contain a variety of chromophores, several different wavelengths are needed for sensitive measurement. This can significantly increase the complexity of analysis. An alternative normal-phase HPLC method showed that UV absorption at 280 and 295 nm was less sensitive¹ than the HPLC-charged-aerosol-detection method. The fluorescence detection of tocopherol analytes had a wide range of relative response factors, ranging from 1.00 to 2.30.²

Another universal method uses ELSD, but this detector lacks sensitivity and has a sigmoidal calibration curve over a relatively short range of concentrations. A plot of analyte peak area versus amount on column is relatively flat at the upper and lower end of the instrument's range, resulting in poor accuracy and precision. This limits the instrument's use with low-concentration analytes.

Mass spectrometry has the sensitivity for low-level concentrations, but it is a nonlinear detector, which is expensive to operate for simple quantitation.

The Charged Aerosol Detector (CAD[®]) is a sensitive, mass-based detector especially well suited for the determination of nonvolatile analytes. For the quantitation of FSVs and carotenoids, the Corona[®] *ultra*[™] detector offers high precision, typically $< 3\%$ RSD, and high sensitivity, with limit of detection (LOD) values < 10 ng on column.

Using this detector, an RP-HPLC method is shown to successfully resolve 11 FSV and carotenoid standards in < 20 min. Three commercially available products were prepared and quantified, showing good recovery values based on amounts indicated on the product labels. Early data shows that this method is capable of measuring vitamin D (not shown) and α -tocopherol succinate, which is a component of the commercial products.

EXPERIMENTAL

Charged Aerosol Detection Parameters

Gas:	35.0 psi (nitrogen generator)
Filter:	High
Nebulizer Heater:	30 °C

HPLC Parameters

Mobile Phase A:	Methanol/water/acetic acid (750:250:4)		
Mobile Phase B:	Acetonitrile/methanol/tetrahydrofuran/acetic acid (500:375:125:4)		
Gradient:	<i>Time</i>	<i>%A</i>	<i>%B</i>
	0.00	70.0	30.0
	1.00	50.0	50.0
	5.00	40.0	60.0
	10.00	35.0	65.0
	12.00	10.0	90.0
	17.00	0.0	100.0
	17.10	70.0	30.0
	20.00	70.0	30.0
Flow Rate:	1.5 mL/min		
Run Time:	20 min		
HPLC Column:	Halo® C8, 150 × 4.6 mm, 2.7 µm		
Column Temp.:	40 °C		
Sample Temp.:	10 °C		
Injection Volume:	10 µL		

Sample Preparation

Standards were dissolved in ethanol/butylated hydroxyanisole (10 mg/L; BHA) to the appropriate concentrations. The solid products (CoQ10 and Solgar VM-75®) were crushed and extracted in methanol/chloroform (3:5) in a sonicator. The supernatant was centrifuged to remove particulates and then diluted appropriately in the EtOH/BHA solution.

The natural vitamin E product containing 400 IU (268 mg) of naturally based α -tocopherol (gelcap form) was dissolved in warm water, extracted in diethyl ether, and diluted with EtOH/BHA solution.

CALIBRATION PLOTS

Calibration plots are presented here, ranging from 5–333 ng on column (Figure 1) and from 4.8–323 ng on column (Figure 2). All RSDs were < 5% for all concentrations > 20 ng on column (with an exception of lycopene which exhibited fronting, thereby reducing its response relative to other FSVs).

As seen in Table 1, LOD values (2–4 ng on column) were lower than those for tocopherols by HPLC-UV (10–20 ng on column).¹

The range of relative response factors for tocopherols varied from 1.00 to 1.43 at 333 ng on column. This is 60% lower than for variance found using fluorescence detection,² indicating that the charged aerosol detector responses are more uniform across this range of compounds.

Table 1. LOD and LOQ Values

FSV or Carotenoid	LOD (ng)	LOQ (ng)
<i>trans</i> -Retinol	2	7
Retinyl acetate	7	23
Lutein	4	15
δ -Tocopherol	2	6
α -Tocopherol succinate	2	6
γ -Tocopherol	2	5
Phylloquinone	3	8
α -Tocopherol	4	12
Lycopene	12	40
Retinyl palmitate	3	9
CoQ10	4	13

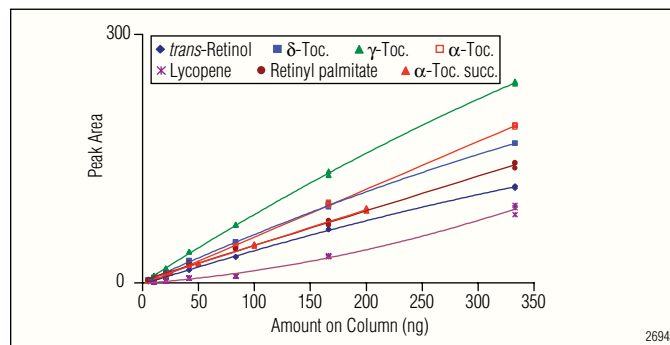


Figure 1. Calibration curves for seven FSVs by RP-HPLC (5 to 333 ng on column).

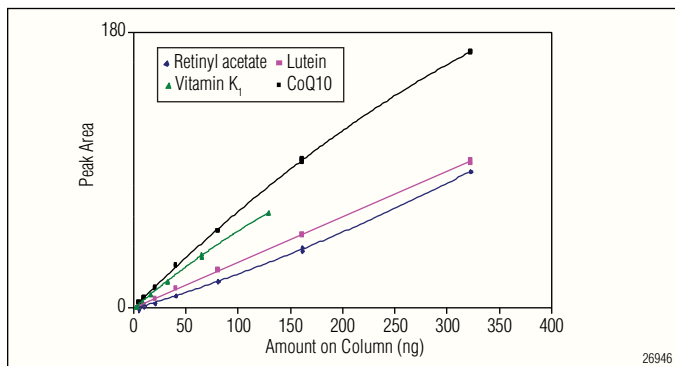


Figure 2. Calibration curves for four FSVs by RP-HPLC (4.8 to 323 ng on column).

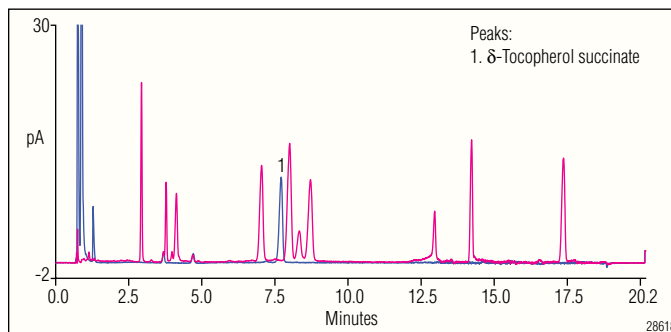


Figure 5. RP-HPLC chromatogram of Solgar VM-75 product (blue) overlaid with FSVs standard chromatogram (pink; 166 ng on column, vitamin K₁ at 66 ng).

CHROMATOGRAMS

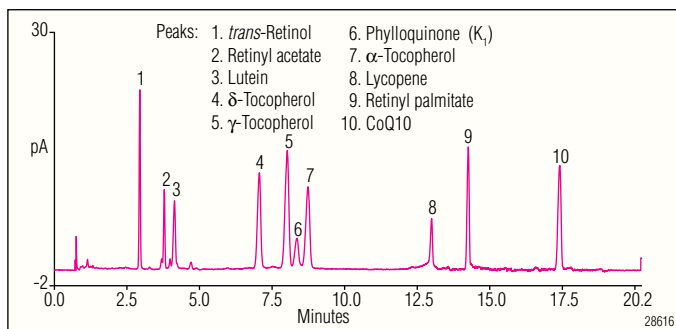


Figure 3. RP-HPLC chromatogram of FSV standards (166 ng on column, vitamin K₁ at 66 ng).

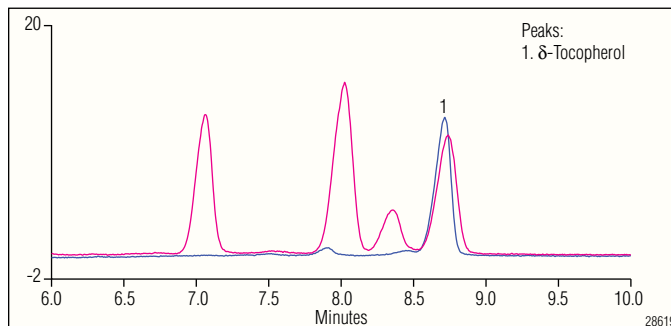


Figure 6. RP-HPLC chromatogram of natural vitamin E product (blue) overlaid with FSVs standard chromatogram (pink; 166 ng on column, vitamin K₁ at 66 ng) on different days.

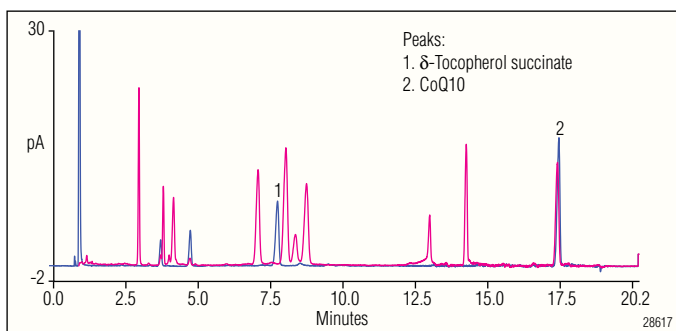


Figure 4. RP-HPLC chromatogram of the CoQ10-vitamin E product (blue) overlaid with FSVs standard chromatogram (pink; 166 ng on column, vitamin K₁ at 66 ng).

DISCUSSION AND CONCLUSIONS

The method presented here shows good selectivity for many different fat-soluble vitamins and antioxidants, resolving a total of 10 different compounds (in order of elution): *trans*-retinol, retinyl acetate, lutein, δ -tocopherol, γ -tocopherol, phylloquinone, α -tocopherol, lycopene, retinyl palmitate, and CoQ10 (seen in Figure 3). Other FSV standards (analyzed by this method but not shown here) include cholecalciferol (vitamin D₃), α -tocopherol succinate, and β -carotene. Vitamin D, both D₂ and D₃, was found to elute before *trans*-retinol. α -Tocopherol succinate was found to elute before γ -tocopherol as seen in Figures 4 and 5. β -Carotene was found to coelute with retinyl palmitate under these conditions.

Standards prepared directly from solids were diluted into FSV solutions and used for this study. From these solutions, calibration curves of each FSV were generated. The resulting calibration plots are presented in Figure 1 (5–333 ng on column) and Figure 2 (4.8–323 ng on column) showing second-order polynomial fits. Correlation coefficients ranged from 0.994 (lycopene) to 0.999, indicating sufficient accuracy for quantitative work.

The LOQ values for all analytes evaluated were < 25 ng, excluding lycopene. Precision was acceptable for all FSVs, with RSD values < 5% (n = 3) across all amounts above 20 ng on column. All FSV analytes showed acceptable peak shapes, with the exception of lycopene, which exhibited fronting on this column.

A chromatogram containing 166 ng of standards (Figure 3) is overlaid with each of the three products prepared and analyzed, as seen in Figures 4–6.

Three commercially available FSV products were processed to quantify FSV content and results were compared to those indicated on the label. The percent recovery values based on label claim are seen in Table 2.

The method presented here provides a means for the determination of FSVs in commonly available products.

REFERENCES

1. Agilent (U.S.) HPLC for Food Analysis- A Primer. 2001 <http://www.chem.agilent.com/Library/primers/Public/59883294.pdf> (accessed August 19, 2010).
2. Garcia-Moreno, M.J.; Vera-Ruiz, E.M.; Fernández-Martínez, J.M.; Velasco, L.; Pérez-Vich, B. Genetic and Molecular Analysis of High Gamma-Tocopherol Content in Sunflower. *Crop Sci.* **2006**, *46*, 2015–2021.

Table 2. Label Claim % Recoveries for Products

Product	α -Tocopherol Succinate	α -Tocopherol	CoQ10
CoQ10-Vitamin E	Label: 112 mg, 100 IU Found: 113 mg, 101%	N/A	Label: 200 mg Found: 145 mg, 73%
Solgar VM-75	Label: 169 mg, 150 IU Found: 153 mg, 98%	N/A	N/A
Natural Vitamin E	N/A	Label: 268 mg, 400 IU Found: 262 mg, 98%	N/A

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LPN 2818-01 3/11
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