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Use of Supercritical Fluid
Chromatography/Mass
Spectrometry for Rapid
Separation of Fat Soluble
Vitamins, A, D, E and K

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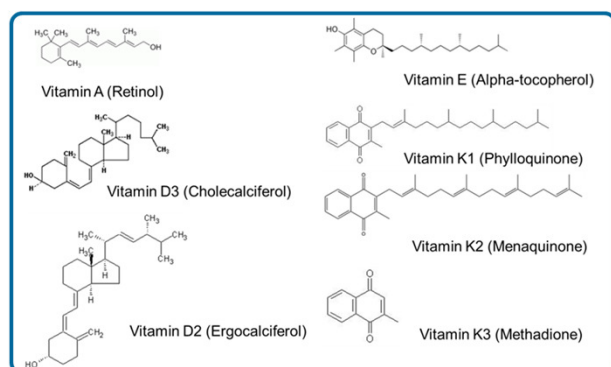
Introduction

Supercritical fluid chromatography mass spectrometry was used to develop a method for rapidly separating the fat soluble vitamins, A, D, E, and K. Several columns and conditions were investigated in order to determine the optimal conditions for the separation the vitamins and their forms. All the fat soluble vitamins are important to sustained health and well being. Vitamin A is necessary for both low-light and color vision. The Egyptians found that night blindness could be cured by eating liver which is high in vitamin A. Vitamin K is important in blood coagulation and is found in three forms. Vitamin K1, found in plants is converted to vitamin K2 by gut bacteria. The K3 form is the synthetic form of the vitamin. Vitamin E is composed of eight forms which includes tocopherols and tocotrienols. The alpha-tocopherol form is the most biologically active form. The delta-tocopherol form is form most commonly found in food.

Vitamin D, in particular, has been of medical interest in prevention of a number of chronic diseases and in the high rate of vitamin D deficiency around the world. A recent article in JAMA(1) indicated that some over the counter (OTC) sources for vitamin D were highly variable and did not meet US Pharmacopeial Convention standards. Vitamin D3 should be the choice for supplements as it is the form synthesized by the skin when exposed to light and it is less toxic than D2. Two OTC Vitamin D3 bottles were tested to determine the concentration of the active ingredient.

In developing methods for SFC, determining the stationary phase that separates the components optimally is the initial task. Once the stationary phase has been determined the modifier choice is optimized, with the initial modifier choice being methanol. Temperature has less of an impact on separations unless compounds are structurally similar.

Structures of the Vitamins



Experimental

Instrumentation:

The Agilent 1260 Infinity Analytical SFC System (G4309A) contains the following components:

- SFC Fusion A5 module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity SFC High Performance Autosampler
- Agilent 1260 Infinity Thermostatted Column Compartment
- Agilent 1290 Infinity Diode Array Detector with high pressure flow cell
- Agilent 1260 Infinity Vacuum Degasser
- Agilent 6150 Quadrupole MS with a Multimode source

Standards

All vitamin standards were purchased from Sigma-Aldrich

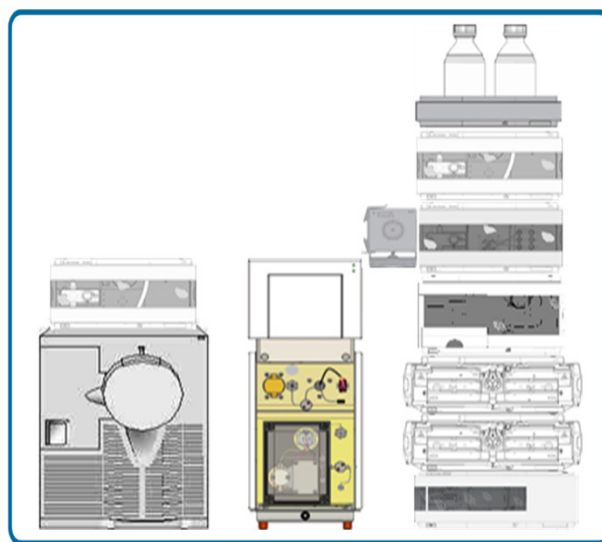
Stationary Phases

Stationary phases used for this study included the following: silica, cyano, amino, and a C18.

Modifiers

The initial modifier for screening all the stationary phases was methanol. Once the optimal stationary phase was determined, the modifier was optimized. The modifiers investigated were alcohols, ethanol and isopropanol.

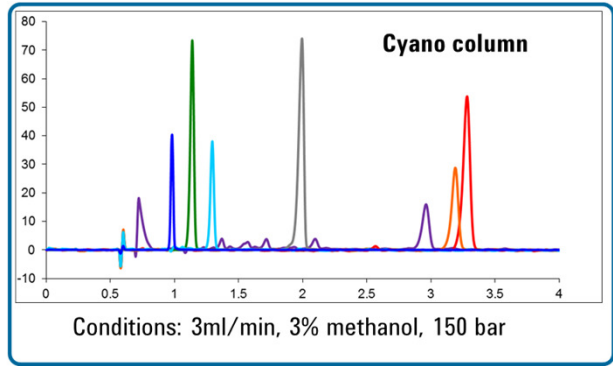
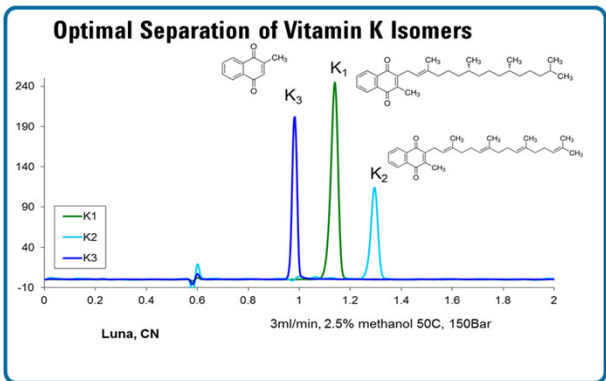
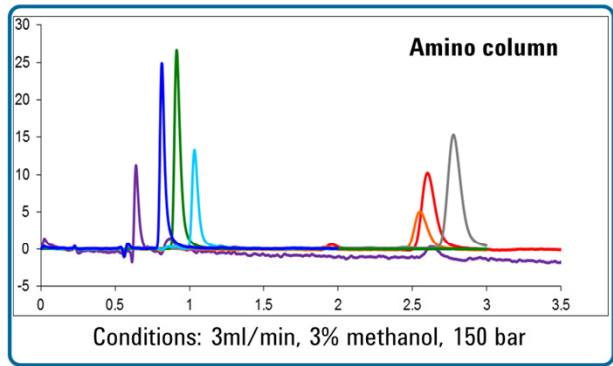
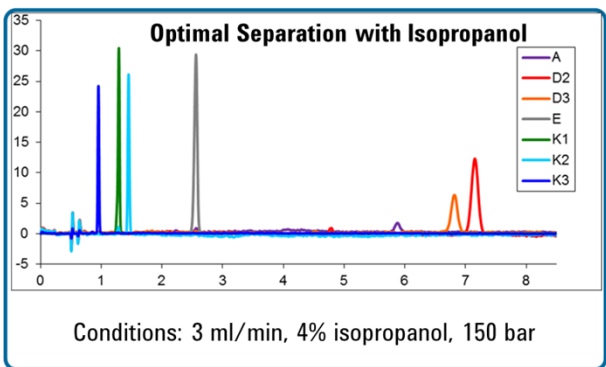
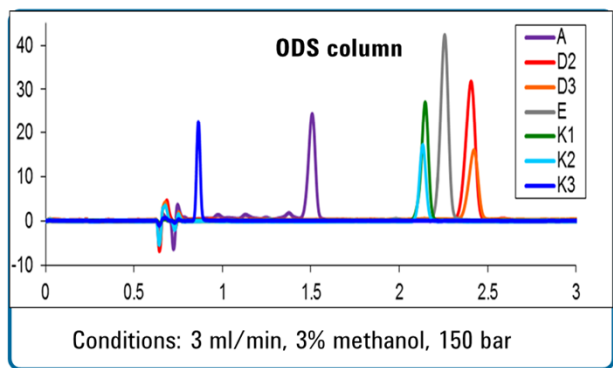
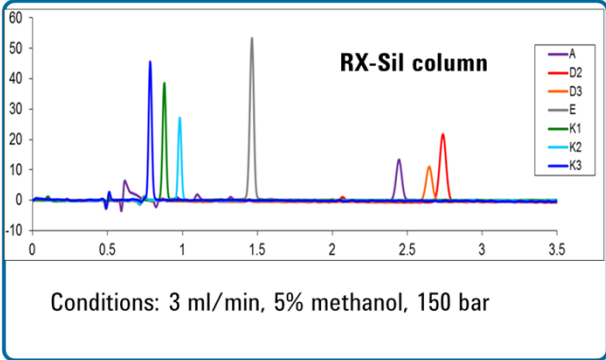
SFC System



Results and Discussion

Optimization of Stationary Phase and Modifier

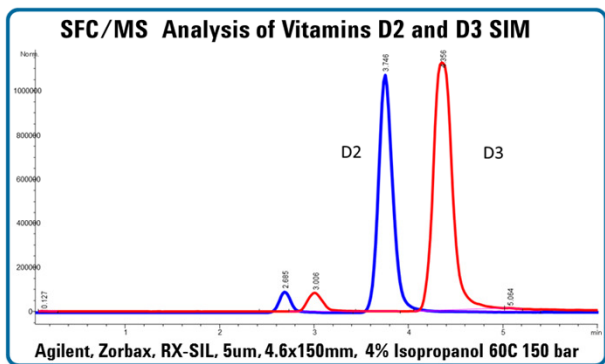
Initial studies focused on the selections of a stationary phase for optimal separation of the fat soluble vitamins. The Agilent RX-Sil column proved to be the best choice for the stationary phase. Subsequently, the use of isopropanol as the modifier provided the best overall separation of the vitamins. The optimization of the vitamin K forms was achieved by decreasing the flow rate and the use of a Cyan column.



Results and Discussion

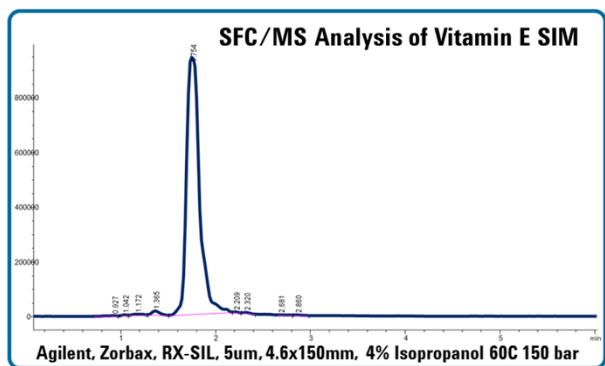
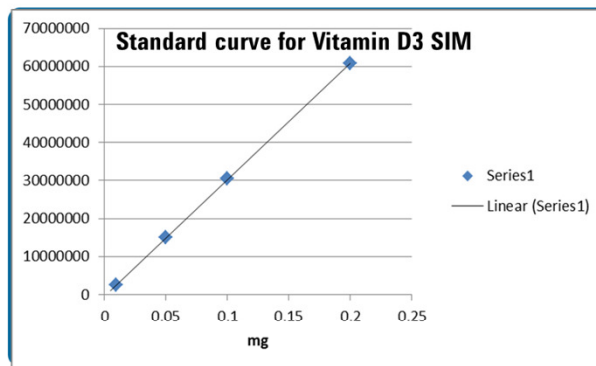
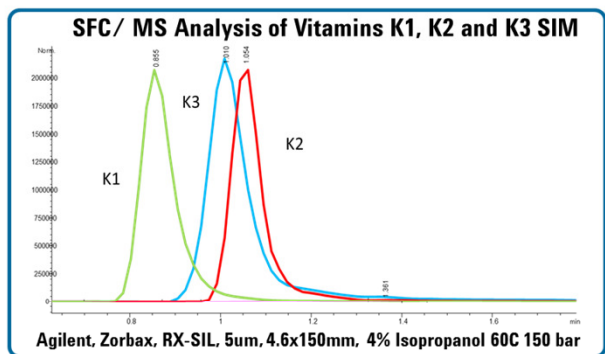
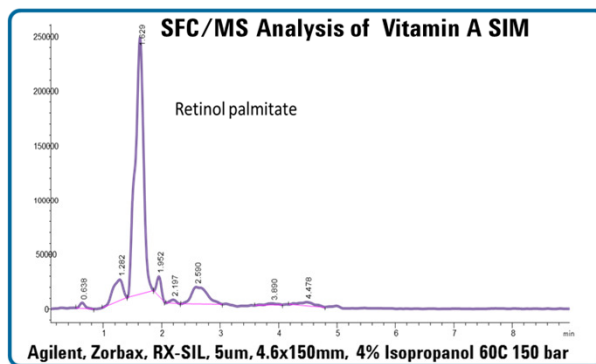
SFC/MS studies

Once the initial conditions for the optimal separation were determined, the separation using mass spec detection for the analysis of the vitamins was investigated. Each of the vitamins in SIM mode are shown below.



Quantitation of Vitamin D3

To determine the quantity of vitamin D3 in two over the counter vitamin D3 supplements a standard curve was developed covering a range of vitamin D3 found in the two supplements chosen for the study. A high dose of 2000 IU, 0.05 mg, and a lower dose of 400 IU, 0.01 mg, were chosen.



Conclusions

Initial studies were done to determine the optimal conditions for the separation of the fat soluble vitamins. Typically the column achieving the best separation is selected. Subsequently, the choice of modifier and the optimal temperature are determined.

In order to determine the concentration of vitamin D3 in two over the counter supplements a standard curve was developed covering the concentration range of the two supplements. The results showed that the concentration of vitamin D3 was within the range for each of the samples.