

Simultaneous Separation of Fat-Soluble Vitamins by Reversed-Phase HPLC Using a Cholesteryl Group Bonded Stationary Phase.

Toshi Ono¹, Tsunehisa Hirose², Kazuhiro Kimata²

¹Nacalai USA, Inc., San Diego, United States, ²Nacalai Tesque, Inc., Kyoto, Japan

Abstract

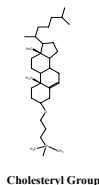
Vitamins are essential nutrients for human health, and can be classified as either water-soluble vitamins (B-complex and C) or fat-soluble vitamins (different forms of A, D, E and K). Several methods for the analysis of vitamins have described in the past. Reversed phase C18 columns are often used for the separation of water-soluble vitamins and the baseline separation can be achieved. However, the structural similarities between different forms of fat-soluble vitamins, such as vitamin D2 and D3, have posed chromatographic challenges to analysts interested in quantifying these vitamins within a single run. A cholesteryl group bonded stationary phase demonstrates superior selectivity of the analogs and allows simultaneous separation of 9 fat-soluble vitamins including D2 and D3.

Introduction

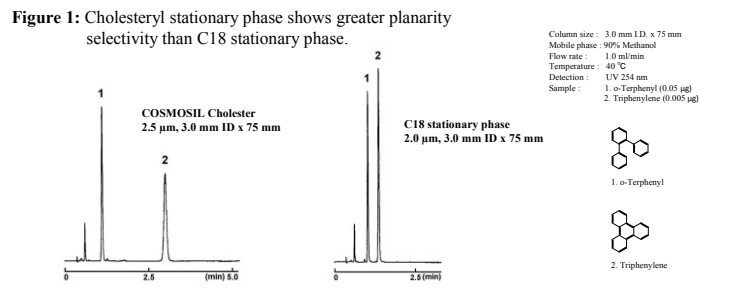
Although small amounts of fat-soluble vitamins are needed for human health, they generally pose a great risk for toxicity when consumed at very high levels. Therefore, it is important to assure the levels of fat-soluble vitamins in food products are accurate. This study demonstrated the characteristics and advantages of a novel cholesteryl group bonded stationary phase for simultaneous determination of fat-soluble vitamins, compared to the most commonly used sub-2 μm C18 stationary phases.

Stationary phase characteristics

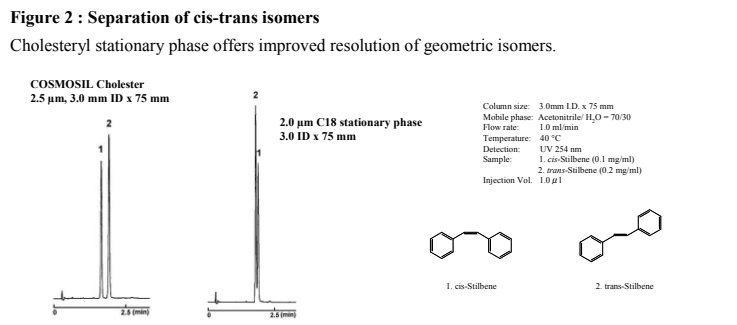
Silica Gel	High Purity Spherical Silica
Stationary Phase	Cholesteryl group
Average Particle Size	2.5 μm
Average Pore Size	130 Å
Surface Area	330 m ² /g
Carbon content	21%



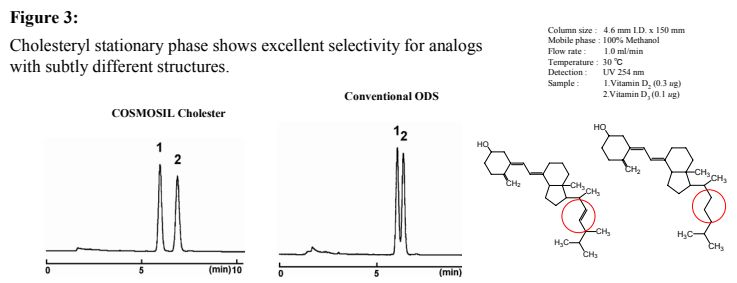
Stationary phase characteristics: Shape selectivity 1



Stationary phase characteristics: Shape selectivity 2



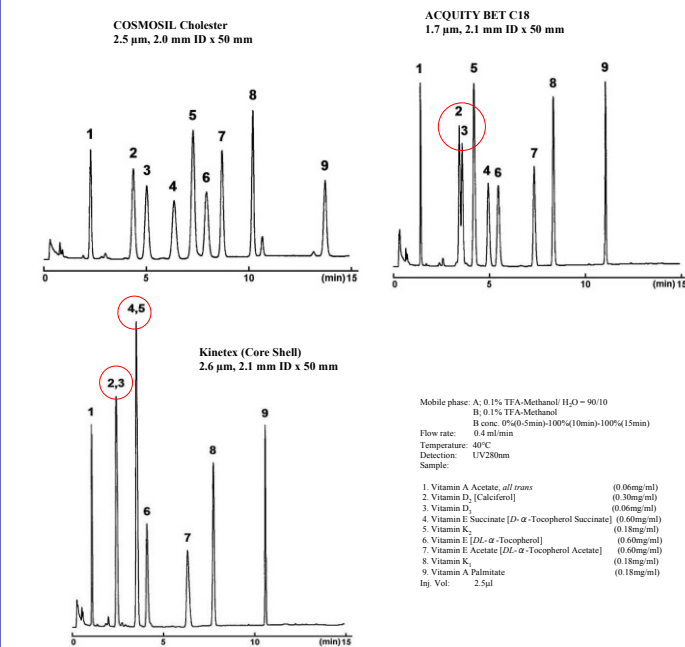
Stationary phase characteristics: Analog selectivity



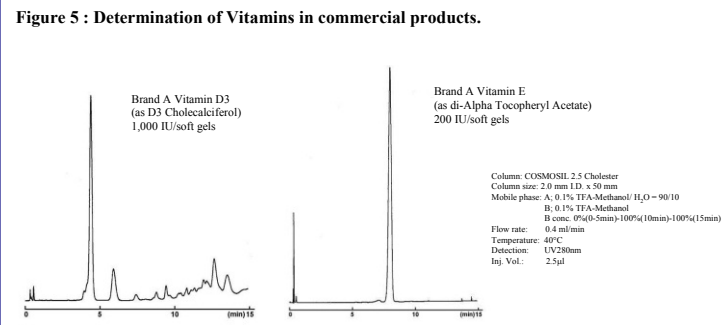
Simultaneous Determination of 9 Fat-soluble Vitamins

Figure 4: Separation of fat-soluble vitamins mixture

Cholesteryl stationary phase shows excellent resolution for all these 9 fat-soluble vitamins compared with 1.7 μm UHPLC C18 stationary phase and 2.6 μm core shell column.



Determination of Vitamin D and E in commercial supplement



Conclusions

- COSMOSIL Cholester shows greater shape selectivity and better resolution for analogs with subtly different structures.
- Although sub-2 μm and core shell particles are used for very fast and high efficiency separation, conventional phase chemistries may not provide optimal separations for some compounds with structural similarities.
- COSMOSIL Cholester provides excellent resolution for the simultaneous separation of fat-soluble vitamins.