Waters™

应用纪要

Method Migration of a Normal Phase HPLC Method for Tocopherols in Dietary Supplements

Lise Gauthier, Kimberly Martin, Paula Hong

Waters Corporation

Abstract

With the increasing popularity of dietary supplements, there is a need to ensure that products meet label claims. However, many multi-vitamin formulations contain both water- and fat-soluble vitamins posing challenges for a single mode of chromatography for analysis. Since vitamins have a wide range of chemical characteristics, analysis is typically performed using multiple modes of chromatography. The highly polar water-soluble vitamins are typically analyzed by reversed phase (RP) or hydrophilic interaction chromatography (HILIC), while for the non-polar, fat-soluble vitamins the preferred mode of chromatography is normal phase (NP).[1]

For normal phase methods that are isocratic, the ability to migrate methods between HPLC systems is essential as systems are updated and replaced. Due to the particular characteristics of normal phase chromatography including non-polar solvents and differences in instrument design, set up and operation can present challenges when performing method migration; therefore, when migrating methods between systems it is important that these differences are identified and controlled.

This work describes the migration of a method from legacy HPLC systems to the modern Alliance™ iS

HPLC System. The normal phase method for the separation of four vitamin E tocopherols was migrated between the systems. The results obtained met the defined method migration acceptance criteria, specifically the system suitability requirements for peak area %RSD and resolution. Following migration, the method was used for determination of tocopherols in dietary supplements demonstrating the ability of the method to quantify tocopherols.

Benefits

- Legacy normal phase HPLC methods can easily be migrated to the Alliance iS HPLC System
- The normal phase method detailed in this application note provides a means for quantitation of tocopherols in vitamin supplements

Introduction

Normal phase HPLC is a valuable technique for the separation of polar and hydrophilic compounds and for isomer separation. Normal phase HPLC requires a polar stationary phase combined with non or moderately polar mobile phases. Normal phase HPLC is frequently used for the analysis of compounds that have limited water solubility. These compounds can be extracted directly into non-polar solvents and analyzed making the sample preparation compatible with normal phase. In contrast, for reversed phase (RP) separation these compounds require additional, often time-consuming sample preparation steps including evaporation and reconstitution into a solvent that is compatible with reversed phase separations.

Normal phase and reversed phase chromatography can often be performed on the same HPLC system however some system modifications may be required to convert systems in the standard configuration to the normal phase configuration. This is due to the incompatibility of materials of construction with normal phase solvents. Specifically, hardware modifications (*e.g.,* check valves, tubing, pump seals) may be required. Additionally, a solvent changeover process is needed when switching between modes to avoid solvent immiscibility issues.

Vitamin E is a name assigned to a class of fat-soluble compounds that exhibit distinctive antioxidant properties. There are four tocopherols (alpha, beta, gamma, delta) that display vitamin E activity.

Dietary supplements from natural sources contain a distribution of these four tocopherols, however alpha-tocopherol is the only form that is recognized to meet human nutrition requirements.[2]

A normal phase method for the determination of tocopherols in dietary supplements was developed. The method was successfully run on two legacy HPLC systems (System 1=Waters Alliance e2695 System; System 2=System Y from a different vendor). The method was then successfully migrated to a modern system, the Alliance iS HPLC System. The criteria for successful method migration was defined as the ability to meet the established system suitability requirements of the method. Dietary supplement samples were prepared and analyzed on the Alliance iS HPLC System and the results compared against the label claims.

Experimental

Standard Preparation

A working standard containing four tocopherols (alpha, beta, gamma, delta) was prepared using standards purchased from MilliporeSigma. The tocopherols were diluted with hexane to a final concentration of 0.2 mg/mL.

Method Conditions

LC Conditions

LC systems:	Alliance e2695 System Legacy System Y Alliance iS HPLC System
Detection:	2489 UV/Vis Detector Variable Wavelength Detector (VWD) TUV Detector

Wavelength:	295 nm
Sampling rate:	10 *Hz*
Vials:	LCGC certified clear glass 12 x 32 mm screw neck vial, total recovery with cap and Preslit PTFE/Silicone septum 2 mL Volume (p/n: 186000307C)
Columns:	XBridge™ BEH™ HILIC Column, 5 µm 4.6 x 150 mm (p/n: 186004453)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	30 µL
Flow rate:	1.3 mL/min
Mobile phase:	95:5 Hexane:Ethyl Acetate with 0.05% Acetic Acid
Needle wash:	90:10 Hexane:2–Propanol

Data Management

Chromatography data system:	Empower™ 3.8.0

Results and Discussion

Prior to running the method, each of the systems was transitioned from the reversed phase to the normal phase mode. Differences in instrument design were considered and controlled. This involved some hardware changes required to run normal phase solvents on select systems. Check valves and waste tubing were easily replaced on the Alliance iS HPLC System, while System Y required a more extensive replacement of pump seals in addition to check valves and tubing. All systems were flushed with isopropanol to remove aqueous solvents before normal phase solvents were introduced.

System suitability criteria were selected based on the needs of the quantitative method, and included resolution and peak area precision for each of the tocopherols. System suitability results were calculated for the analysis on each system by making six replicate injections of the working standard and calculating the peak area precision and USP resolution for each tocopherol. Representative chromatograms and the results obtained from each system are seen in Figures 1 & 2 and Table 1.

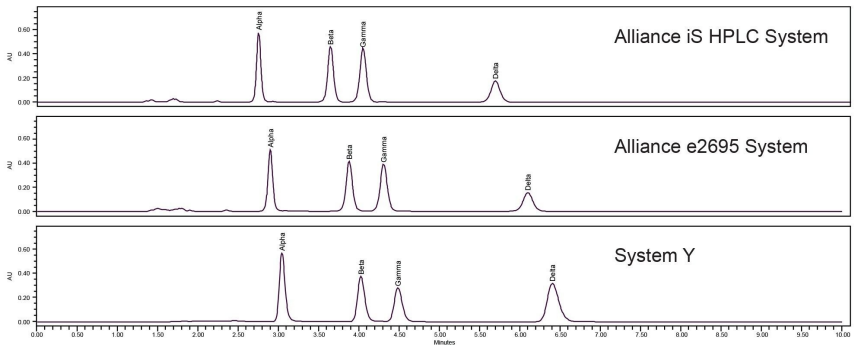

Figure 1. Representative chromatograms of the standard preparation on the Alliance iS HPLC System, the Alliance e2695 System and System Y.

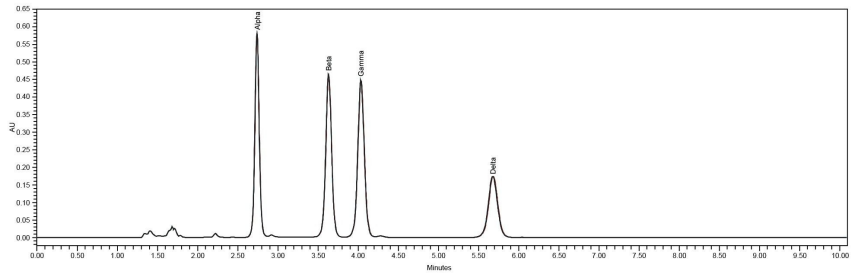

Figure 2. Overlay of six system suitability injections on the Alliance iS HPLC System.

Parameter	Acceptance criteria	Alliance e2695 System	System Y	Alliance iS HPLC System
alpha peak area %RSD	≤2.0%	0.1	0.2	0.1
beta peak area %RSD	≤2.0%	0.1	0.2	0.1
gamma peak area %RSD	≤2.0%	0.1	0.2	0.1
delta peak area %RSD	≤2.0%	0.0	0.1	0.0
USP resolution (beta – alpha)	≥5.0	7.0	6.7	7.8
USP resolution (gamma – beta)	≥1.5	2.7	2.7	2.9
USP resolution (delta – gamma)	≥7.0	8.5	8.9	9.2

Table 1. System Suitability Results.

Comparable chromatography was obtained from each of the systems. System suitability criteria were also met on each of the systems. Peak area precision was similar across the systems with the Alliance iS HPLC System and the legacy Alliance e2695 having equivalent precision for all tocopherols in the standard. USP Resolution was slightly improved on the Alliance iS HPLC System. Since the results obtained met the specified acceptance criteria, the method migration to the Alliance iS HPLC System was successful.

After the method was migrated to the Alliance iS HPLC System, it was used for the determination of tocopherols in two dietary supplement dosage forms - naturally sourced multivitamin tablets and in vitamin E softgel capsules. Sample preparation was facilitated through the use of normal phase HPLC for analysis. The tocopherols were extracted from the sample matrix into a non-

polar solvent (hexane) and injected directly onto the system eliminating the need for additional laborious sample preparation steps. The analysis utilized a single point external standard for quantitation. Chromatograms of sample preparations are shown in Figures 3 and 4.

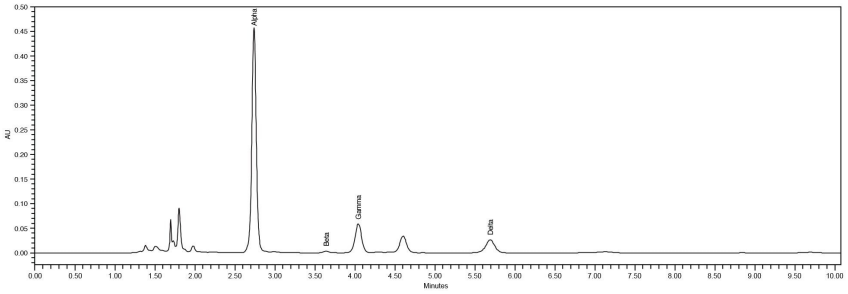

Figure 3. Multi-vitamin sample preparation on the Alliance iS HPLC System.

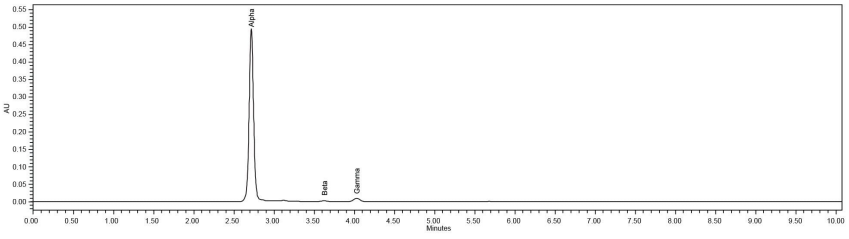

Figure 4. Vitamin E softgel capsule sample preparation on the Alliance iS HPLC System.

Quantitative results for alpha-tocopherol were compared against the label claim of each product. The results demonstrate the ability to quantify tocopherols (as alpha-tocopherol) in the dietary supplements. Results are shown in Table 2.

Sample	Label claim per dosage form (mg)	Result per dosage form (mg)	Percent of label claim
Multivitamin tablet	2	1.8	91%
Vitamin E softgel capsule	268	309	115%

Table 2. Sample results for alpha-tocopherol from the Alliance iS HPLC System.

Conclusion

The ability to migrate methods across HPLC systems is essential as systems are updated and replaced with newer systems. A normal phase method for the determination of tocopherols in dietary supplements was successfully migrated from legacy systems to the Alliance iS HPLC System. The method was then used for the determination of tocopherols in two dietary supplement samples.

The method migration acceptance criteria (which was defined as meeting system suitability requirements) was met on the Alliance iS HPLC System with comparable chromatography and results to that of the legacy systems. Sample results demonstrate the ability of the method to quantify tocopherols in dietary supplements. Overall, the results show the ease at which methods can be migrated to the Alliance iS HPLC System and the performance benefits of keeping instrumentation assets up to date.

References

1. Eric S. Grumbach and Kenneth J. Fountain. Comprehensive Guide to HILIC Hydrophilic Interaction Chromatography. <https://www.waters.com/nextgen/global/shop/education/715002531-comprehensive-guide-to-hilic-hydrophilic-interaction-chromatogra.html> Waters Corporation, 2010.
2. National Institutes of Health Office of Dietary Supplements. (2021). Vitamin E [Fact Sheet].

https://ods.od.nih.gov/factsheets/VitaminE-HealthProfessional for health professional/ <https://ods.od.nih.gov/factsheets/VitaminE-HealthProfessional%20for%20health%20professional/> .

Featured Products

Alliance iS HPLC System <https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/alliance-is-hplc-system.html>

2489 UV Visible (UV Vis) Detector <https://www.waters.com/1001362>

Empower Chromatography Data Software (CDS) <https://www.waters.com/10190669>

720008232, February 2024