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應用手冊

Normal-Phase Separation of Tocopherols with the ACQUITY UPLC H-Class System featuring Auto▪Blend Technology

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief describes the normal-phase UPLC chromatographic separation of tocopherols in Vitamin E dietary supplements was achieved in less than 1 minute with excellent repeatability.

Benefits

Fast separation of tocopherols in vitamin E dietary supplements.

Introduction

There are four tocopherols that exhibit vitamin E activity. These four forms differ by the degree of methylation on the chromanol ring. The higher the degree of methylation, the greater the vitamin activity. Dietary supplements that are created from naturally occurring sources of vitamin E will have a distribution of these four tocopherols; whereas, synthetic supplements will generally only have α-tocopherol present (the most active form). Analysis of vitamin E supplements can be accomplished by either reversed-phase or normal phase chromatography. However, since vitamin E is a fat soluble vitamin, extraction must be carried out with an apolar solvent. Therefore, to analyze by reversed-phase chromatography, a secondary sample prep step is often necessary to replace the extraction solvent with one that is compatible with reversed phase solvents. If normal-phase chromatography is used, this extra sample prep step can be avoided. Typically, HPLC run times for this analysis can range from 10 minutes to 30 minutes to achieve baseline resolution of all four components. For a QC laboratory that is screening numerous lots of natural vitamin E supplements and/or raw materials, these run times can lead to process bottlenecks. Reducing the run time for this normal-phase application can dramatically reduce QC analysis times and ultimately the production time line.

Results and Discussion

The normal-phase chromatographic separation of the four vitamin E active tocopherols was achieved in less than one minute on the ACQUITY UPLC H-Class System as shown in Figure 1. For this application, the THF/Hexane kit (p/n 205000661) was installed. The Auto▪Blend capabilities of the ACQUITY UPLC Quaternary Solvent Manager (QSM) allowed for the isocratic mobile phase to be blended on-line. This eliminated the need to

manually prepare a pre-mixed mobile phase each day prior to analysis. Solvent A was n-hexane and Solvent B was ethyl acetate with 1% acetic acid (added to reduce secondary surface interactions). The separation was achieved on an ACQUITY UPLC BEH HILIC Column. Typically, silica-based normal-phase columns do not generate as repeatable retention results as reversedphase columns due to surface contamination and secondary interactions. However, the results for this normal-phase UPLC separation on the BEH particle yielded highly repeatable results shown in Table 1 for both retention time and peak area. Two vitamin E supplements, one natural and one natural/synthetic blend, were analyzed as shown in Figure 2. The supplements were quantitatively dissolved in 100% n-hexane and directly injected onto the ACQUITY UPLC H-Class System. With the four tocopherols baseline resolved in less than one minute, the relative amount of each of the components can easily be determined and compared to the total labeled vitamin E amounts.

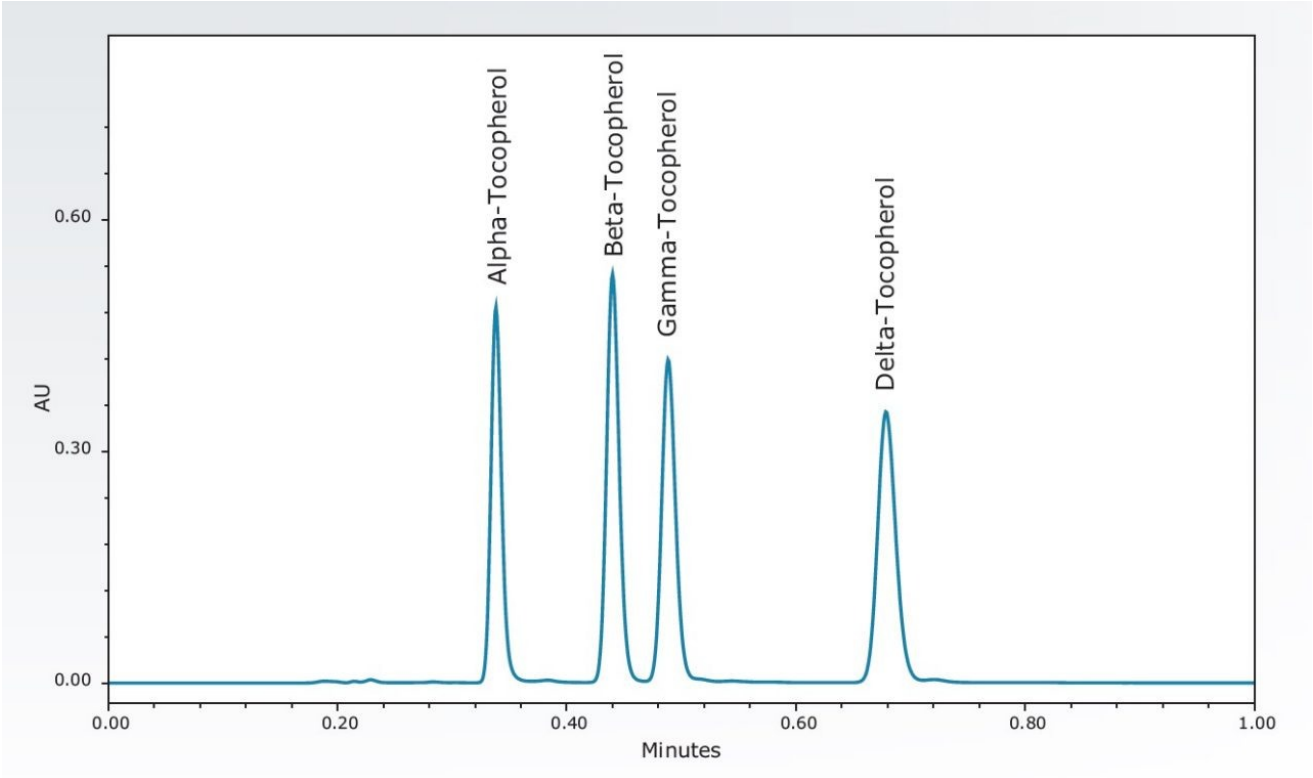

Figure 1. Separation of the four tocopherol standards by normal-phase UPLC on the ACQUITY UPLC H-Class System with an ACQUITY UPLC BEH HILIC Column.

Tocopherol	Retention Time %RSD	Peak Area %RSD
Alpha	0.14	0.19
Beta	0.13	0.19
Gamma	0.13	0.17
Delta	0.09	0.23

Table 1. Retention time and peak area repeatability of six replicate injections for the four tocopherol standards.

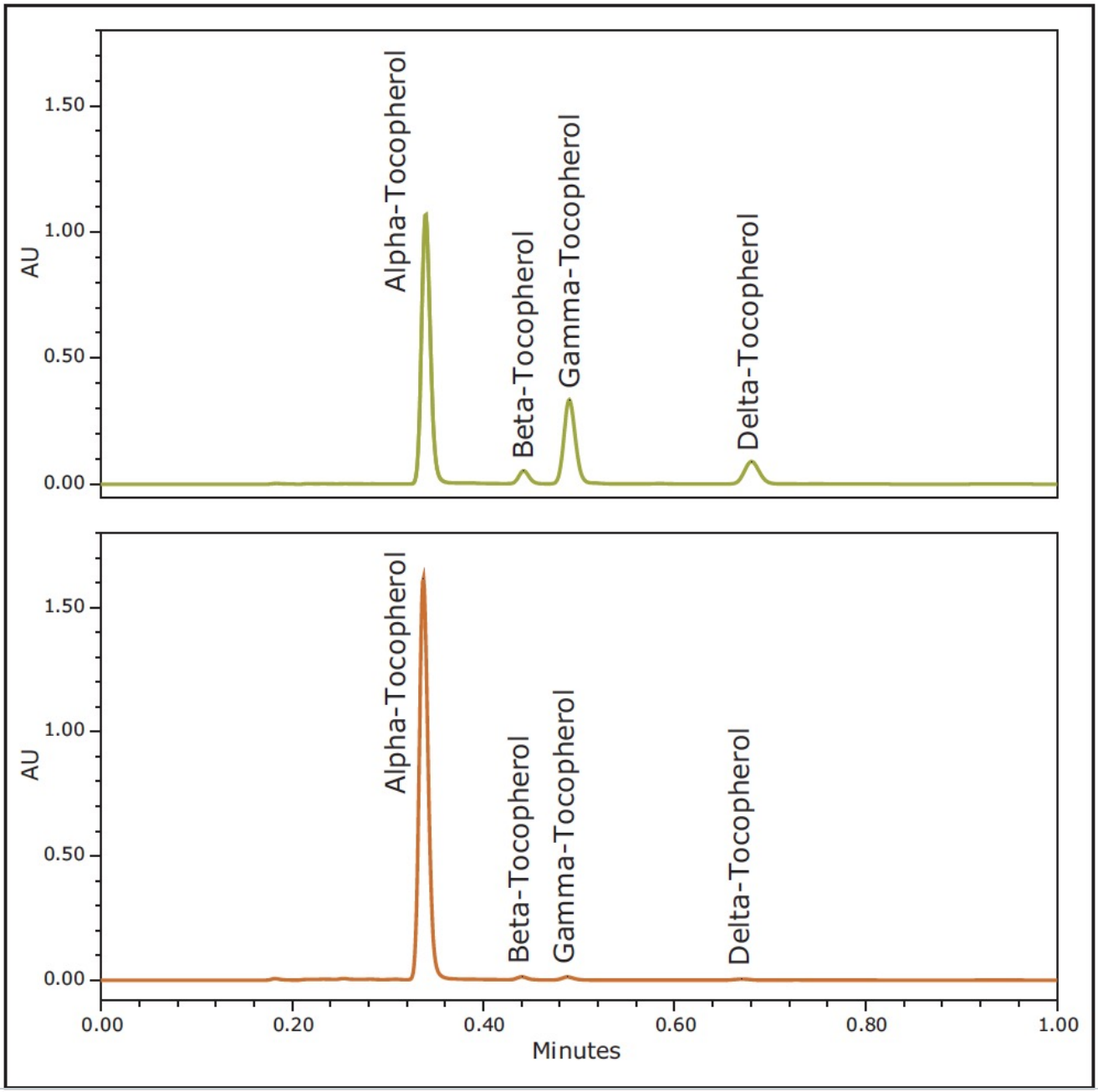

Figure 2. Analysis of two vitamin E dietary supplements.

Conclusion

Choosing the chromatographic separation mode that is best suited for the application will always yield the best resolution in the fastest analysis time. The analysis of vitamin E active tocopherols by normal-phase chromatography yields a fast, repeatable method with a significantly shorter analysis time than reversed-phase methods, especially when factoring in sample preparation times. The ACQUITY UPLC H-Class System was ideally suited for this normal-phase application. The system's Auto▪Blend Technology reduced mobile phase preparation times and delivered excellent injection-to-injection repeatability.

Featured Products

- ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>
- Auto▪Blend Plus <https://www.waters.com/134623262>

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