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Note d'application

Differentiation of Natural Vitamin E from Synthetic Vitamin E for Food and Dietary Supplement Nutrition Labeling

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

Abstract

To ensure true nutrition label claims for vitamin E, the source of vitamin E (natural or synthetic) needs to be verified. This application brief demonstrates the capability of Waters ACQUITY UPC^2 System and Trefoil Columns for the differentiation between natural and synthetic vitamin E by separating the stereoisomers of α-tocopherol and its acetate into 2 or more peaks. Sample preparation for these analyses is simple with no derivatization required and the chromatographic analysis run time is 35 min for the α-tocopherols, and 15 min for the α-tocopheryl acetate, respectively. For the first time, the differentiation between natural and synthetic vitamin E can be run routinely for food, dietary supplements, infant formula and other related products.

Benefits

- Reliable differentiation between natural and synthetic Vitamin E
- Simple and fast solution
- Enables true nutritional label claims
- Methodology designed for routine QC environments

Introduction

Vitamin E is an essential vitamin that functions as a chain-breaking antioxidant in the body by preventing the spread of free-radical reactions. In 2016, the US FDA amended the regulations for nutritional labeling of conventional foods and dietary supplements to include updated Daily Values (DVs), or Reference Daily Intakes (RDIs) for several vitamins. The new RDI for vitamin E is 15 milligrams (mg) α-tocopherol. 1 mg α-tocopherol (label claim) is equivalent to 1 mg *RRR*-α-tocopherol or 2 mg *all-rac*-α-tocopherol (Ref 1). The International Unit (IU) of vitamin E is no longer used. European Union has adopted a similar regulation (Ref 2). Since α-tocopherol has three chiral centers, it has eight stereoisomers. Naturally occurring α-tocopherol contains exclusively the *RRR*-α-tocopherol, while the synthetic α-tocopherol contains all eight stereoisomers in equal proportions. To ensure true nutrition labeling for vitamin E, the source of vitamin E (natural or synthetic) needs to be verified. However, there is no standard method available for the food industry to differentiate between natural vitamin E and synthetic vitamin E. An analytical method that is suitable for the

differentiation between natural and synthetic vitamin E in a routine QC environment is highly desired in order to ensure correct vitamin E label claims.

Results and Discussion

Waters ACQUITY UPC^2 System and Waters Trefoil Columns offer an excellent solution for the differentiation between natural and synthetic vitamin E. The ACQUITY UPC^2 System is an advanced supercritical fluid chromatography platform that offers excellent performance in efficiency, resolution, and speed. Waters Trefoil Columns are polysaccharide-based chiral columns that provide a broad range of selectivity for separating enantiomers and stereoisomers. Figure 1 shows chromatograms of *all-rac*-α-tocopherol standard and samples. The samples include three dietary supplements and one infant formula sample. The peak ID of the *RRR*-α-tocopherol and the δ- and γ-tocopherol are shown in the chromatograms. These peak IDs have been confirmed with individual standards by the RT under the same conditions. Due to the lack of standards, the other stereoisomer peaks of α-tocopherol have not been identified. In Fig. 1, the *RRR*-α-tocopherol is almost baseline separated from the other α-tocopherol stereoisomers within a 35 min isocratic elution with CO_2 (with a small amount of co-solvent) on two Trefoil AMY1 Columns (2.5 mm, 3 x 150 mm). All samples showed a single *RRR*-α-tocopherol peak, which indicate only natural vitamin E is present in these samples. The sample preparation for the dietary supplements was simply a dilution with iso-octane. For the infant formula sample, the procedure included a saponification, extraction and reconstitution. There was no derivatization involved. The α-tocopheryl acetate is another common vitamin E that is used in dietary supplements and foods. Figure 2 shows chromatograms of an *all-rac*-α-tocopheryl acetate and a *RRR*-α-tocopheryl acetate. The stereoisomers of *all-rac*-α-tocopheryl acetate were separated into two main peaks while the *RRR*-α-tocopheryl acetate showed a single sharp peak under a 15 min isocratic elution of CO_2 (with a small amount of co-solvent) on two Trefoil CEL1 Columns (2.5 mm, 3 x 150 mm). The difference in the chromatographic pattern is good enough to differentiate the source of α-tocopheryl acetate. Further separation of the stereoisomers of α-tocopherol or its acetate is possible, but it would require additional columns and need longer run time, which are not deemed suitable for the routine analysis environment.

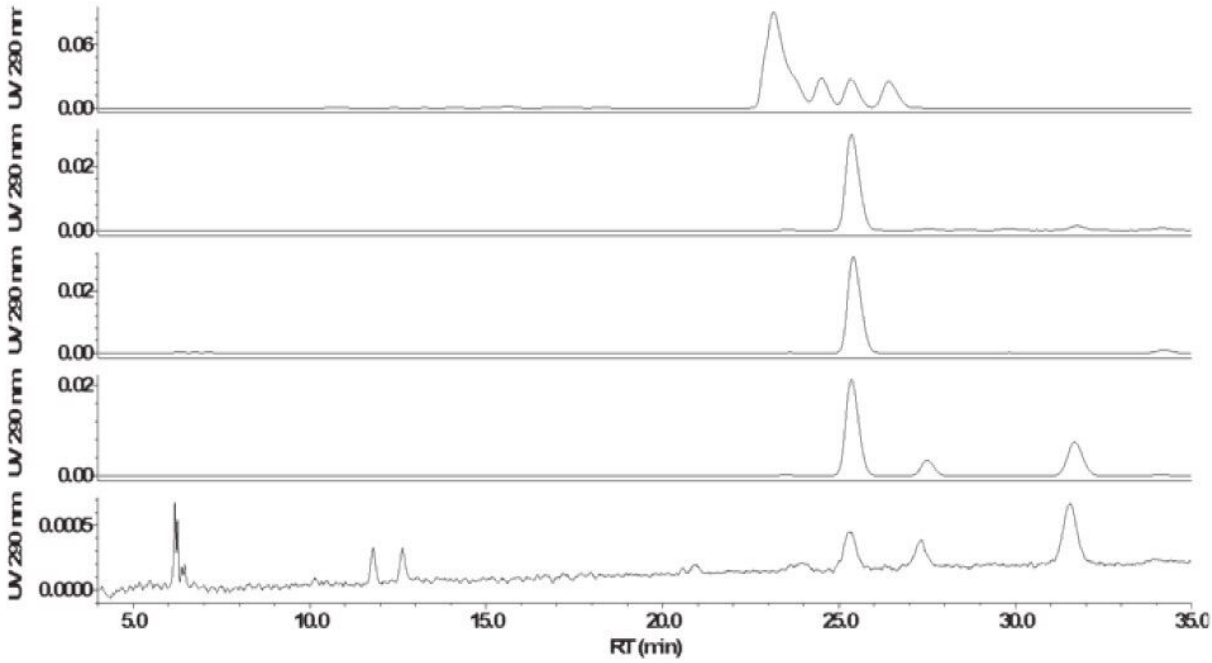

Figure 1. Chromatograms of all-rac-α-tocopherol standard and dietary supplement and food samples. Sample P, M, and G are dietary supplements. Sample IF is infant formula powder. Column: 2 Trefoil AMY1 2.5 um 3 x 150 mm. System: ACQUITY UPC^2 System with ACQUITY PDA Detector. Peak ID: 1) RRR-α-tocopherol; 2) δ-tocopherol; 3) γ-tocopherol.

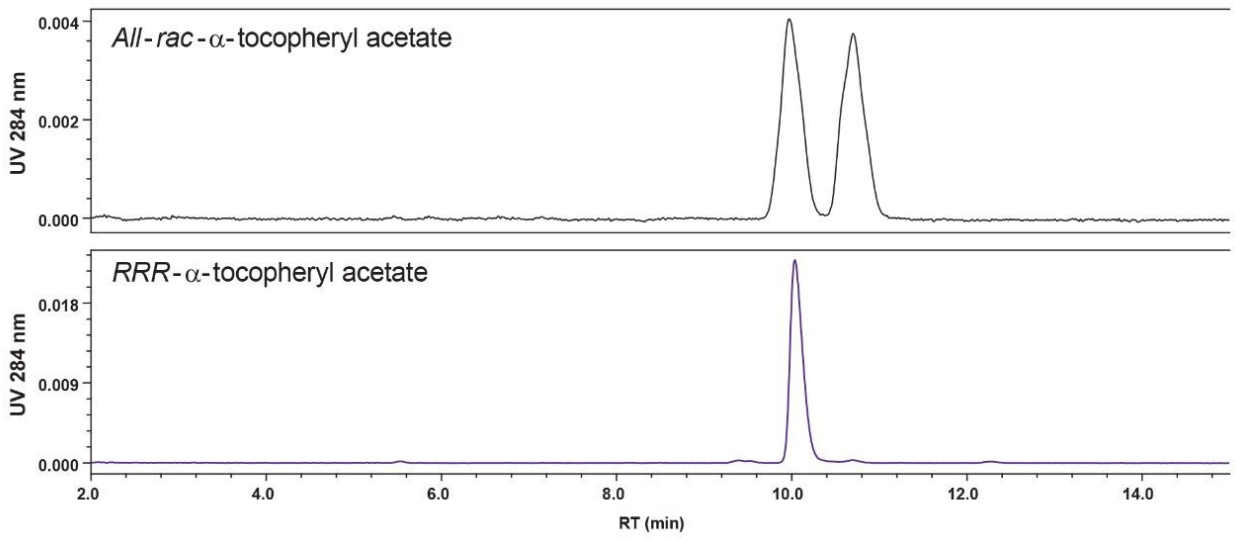

Figure 2. Chromatograms of all-rac-α-tocopheryl acetate and RRR-α-tocopheryl acetate standards. Column: 2 Trefoil CEL1 2.5 um 3 x 150 mm. System: ACQUITY UPC^2 System with ACQUITY PDA Detector.

Conclusion

Waters ACQUITY UPC^2 System with Trefoil AMY1 and CEL1 Columns provide a simple and fast solution for differentiation between the natural vitamin E and the synthetic vitamin E. The stereoisomers of α-tocopherol and its acetate can be separated into 2 or more peaks. Based on the chromatographic profile, natural vitamin E can be easily differentiated from synthetic vitamin E. The sample preparation for these analyses is simple. There is no derivatization required. The chromatographic analysis run time is 35 min for the α-tocopherols, and 15 min for the α-tocopheryl acetate, respectively. For the first time, the differentiation between natural and synthetic vitamin E can be easily conducted. These analytical methods are suitable for the routine analysis of vitamin E in foods, dietary supplements, and other related products.

References

1. Food Labeling: Revision of the Nutrition and Supplement Facts Labels, FDA, HHS, Federal Register/Vol.

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2. Commission Delegated Regulation (EU) 2016/127, Official Journal of the European Union, Vol. 59 (2.2.2016), L25/1

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ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

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