HAMILTON | HPLC Application Note

Vitamin E(xtraordinary), Isolation of Tocopherols by PRP-1

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Application

Tocopherols are highly important antioxidant vitamins found mainly in the chloroplasts of plants. Their extreme lipophilicity can make isolation of the various vitamers challenging in certain reversed-phase chromatography instances where water is omitted from the eluent section altogether. The various vitamers of tocopherols (α, ß, γ, δ) differ according to methylations on the fused phenoltetrahydropyran core. Tocopherols have been found to participate in the regulation of cellular signaling, cell proliferation, proper gene expression, and serum cholesterol reduction.[1,2] It is believed that tocopherols protect polyunsaturated lipids from oxidation and are generally thought of as lipid peroxyl radical scavengers, which can react with both reactive oxygen and reactive nitrogen species that, if left unchecked, lead to disease.[3]

Tocopherols are most notably found in various plant seed oils, grains, meats, and dairy products.[4] Purification of the vitamers was achieved by utilizing the unique adsorption profile associated with the PRP-1 HPLC column by Hamilton Company. The pore structure and lipophilicity associated with this column was effective for the isolation of three of the vitamers while still achieving baseline resolution and good peak shape. The key aspect of this separation is the use of tetrahydrofuran with acetonitrile. The use of THF as an eluent facilitates better mass transfer kinetics when used in polymeric stationary phases like PRP-1, which is especially important when there is a high degree of lipophilic interaction with the solute. The PS-DVB core provides great reproducibility and its longevity is unparalleled, making analysis easy time and time again.

1. *Traber, M.G. and Atkinson, J. Free Rad. Biol. Med., 43, 4-15 (2007).*
2. *Sen, C.K., Khanna, S. and Roy, S. Mol. Aspects Med., 28, 692-728 (2007).*
3. *Niki, E. Free Rad. Biol. Med., 49, 503-515 (2010).*
4. *Ruperez, F. J., Martin, D., Herrera, E. and Barbas, C. J. Chromatogr. A, 935, 45-69 (2001).*

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Chromatogram and Compound Results

Column Information

Packing Material	PRP-1, 5 µm
Dimensions	150 x 4.1 mm
P/N	79444

Chromatographic Conditions

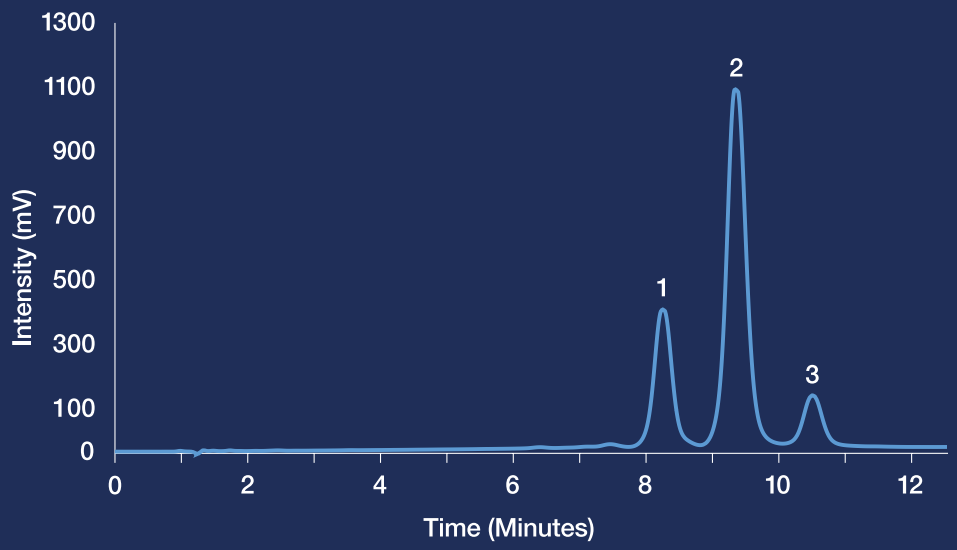
Gradient	0.0–10.00 min. 80–100%B 10.01–12.00 min 100%B
Temperature	Ambient
Injection Volume	5 µL
Detection	UV at 292 nm
Eluent A	20 mM NH_4OAc
Eluent B	Acetonitrile/Tetrahydrofuran (1:1)
Flow Rate	1.0 mL/min

Compounds:

1: Δ-tocopherol

2: ß+γ-tocopherol

3: α-tocopherol



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Hamilton Company is a global manufacturer and supplier of world-class analytical components, medical instrumentation, temperature control systems, laboratory robotics and automated liquid handling equipment. For more than 35 years, Hamilton Company has developed and manufactured pressure-stable, polymeric polystyrene-divinylbenzene (PS-DVB) HPLC columns that are used in most of the world's top chromatography labs. With a wide range of particle sizes, pore sizes, pH stability from 1 to 14, temperature resistance over 100°C, and chemistries to match most analyte types, Hamilton polymeric columns are the chromatographer's choice for challenging separations.

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