Validated Reversed Phase-High Performance Liquid Chromatography Method for the Simultaneous Determination of Tocopherols and Tocotrienols in Whole Grain Barley

E.D. Tsochatzis, M. Papageorgiou

1 Analytical Chemistry Laboratory, Department of Chemical Engineering, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, tsohatzi@chem.auth.gr
2 National Agricultural Research Foundation (N.AG.RE.F), Cereal Institute, P.O. Box 60411, GR-57001, Thermi, Thessaloniki, Greece

The vitamers of vitamin such as α-, β-, γ- and δ-tocotrienol (T3’s) and α-, β-, γ- and δ- tocopherol (T’s) are important phytochemical compounds with antioxidant activity and with potential benefits for human health. In the present study an HPLC method was developed and validated for the determination of tocotrienols and tocopherols (tocols) in barley whole grain samples with RP-HPLC. The analytical column was an Eclipse XDB C18 column (150 x 4.6 mm, 5 μm and it was operated at 30°C. Mobile phase was consisted of methanol-acetonitrile-isopropanol (55:40:5 % v/v) and the elution was isocratic at a flow rate of 0.8 mL/min. Total analysis time was 12 min and the detection of the tocols was performed in a fluorimetric detector where the excitation and emission wavelengths set at 295 nm and 335 nm respectively. Method validation was performed by means of intra-day (n=5) and inter-day accuracy and precision (n=8), sensitivity and linearity. The repeatability was very good with the relative standard deviation varied from 2.7 to 7.2%. Limits of detection (LOD) and limits of quantification (LOQ) varied from 0.05 to 0.2 ng/μL and 0.1 to 0.6 respectively. The linear regression coefficient ($R^2$) was higher than 0.9974. A direct solvent extraction with acetonitrile was performed. It has been used a 1:30 sample/extraction solvent ratio as to succeed the maximum extraction capacity. The recoveries of the tocols from barley samples with the proposed extraction method were respectively good (86-105%) where the RSD was less than 7.4%.

Figure 1. Chromatogram of a whole grain barley sample followed direct solvent extraction with acetonitrile (FLD: excitation at 292 nm, emission 335 nm)

References