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HPLC METHOD FOR THE ANALYSIS OF α-TOCOPHEROL FROM *Myriophyllum alterniflorum*

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Vitamin E, or α-tocopherol, occurs naturally in alternate watermilfoil, *Myriophyllum alterniflorum* (Haloragaceae). This hydrophyte can tolerate environmental stress such as heavy metals [1] because of its antioxidative system, which includes antioxidants such as α-tocopherol, carotenoids, and proline. These compounds can interact with free radicals (e.g., reactive oxygen species), preventing the initiation of potentially lethal process such as lipoperoxidation and subsequent membrane disorganization [2].

The vitamin E molecule, which is composed of a phytol side chain and a chromanol ring, may be incorporated into biological membranes, thus contributing to their physical stability [3]. The pharmacological action of α-tocopherol is not only as a natural antioxidant but also as a preventive against cancer [4]. Moreover, vitamin E is also used in cosmetology as an anti-aging agent [5].

So far, high-proficiency extraction of α-tocopherol in extracts of *M. alterniflorum* has not been reported. Therefore, the objective of this work is the development of **1** an extraction protocol allowing the best conservation (conformation stability, evaporation) of α-tocopherol from chlorophyllian organs and **2** a high-performance liquid chromatography (HPLC) method for accurate value measurement to predict if the alternate watermilfoil could represent an interesting source of natural α-tocopherol for pharmaceutical and cosmetic applications.

M. alterniflorum was collected in the Vienne River near the Fournet Bridge (45°42′42″N 1°52′17″E, Rempnat, Haute-Vienne, France). Fresh plant materials (500 mg) were extracted with 7.5 mL of 96% ethanol (HPLC results are better when extraction is done with ethanol than acetone, methanol, or acetonitrile). Samples were ground at 4°C and then sonicated using a Vibracell 75186 (Sonics, Newtown, CT, USA; 2-s pulses of 30 W and 1-s interpulse intervals during 3 min) at 4°C.

The samples were then filtered on a 0.2 μm syringe filter (Minisart RC 25, Sartorius Stedim Biotech GmbH) and analyzed using a LaChrom HPLC system (Merck-Hitachi, Darmstadt, Germany) equipped with an isocratic HPLC pump (L-7100), an automatic injector (L-7200), and an analogue interface (D-7000) for the fluorescence detector (L-7485). Chromatographic analyses were performed on a 5 μm particle LiChrospher C18 (Merck Chemicals, Darmstadt, Germany) column (250 mm × 4.6 mm) kept in acetonitrile at 25°C. Acetonitrile–methanol (40:60, v/v) with 0.01% (v/v) of trifluoroacetic acid added was employed as mobile phase at a flow rate of 1 mL/min in an isocratic elution. The injection volume was 100 μL, and duplicate injections were used for each sample. The α-tocopherol was detected by absorbance at 288 nm.

Peak identification was performed by comparing the retention time (13.413 min) in the extract matrix with the pure standard for HPLC (10191-41-0, Sigma-Aldrich, St. Louis, MO, USA), and confirmed with characteristic spectra obtained, which also permitted the confirmation of the peak purity (2.063, 8.043, 10.067, 11.673, 13.413).

The equation of the regression line is determined as $Y = 4756227.823X$ for vitamin E. Excellent linearity is obtained for compounds between peak areas and concentrations of 0.210 to 23.536 μg/mL with $r^2 = 0.999$. The LOD (limits of detection) was calculated to be 0.042 μg/mL, and the LOQ (limits of quantification) was calculated to be 0.210 μg/mL.

Quantitative determination of vitamin E in *M. alterniflorum* was carried out by HPLC using the external standard method, and the results based on nine measurements are 118.883 ± 10.324 μg/g fresh weight (FW). The relative precision of the method was about 4%.

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According to our results, *M. alterniflorum* can be considered as a good α-tocopherol source compared to other species commonly used in vitamin E extraction (basil 40.5 µg/g FW, coriander 76.0 µg/g FW, dill 34.2 µg/g FW, spearmint 60.4 µg/g FW, peppermint 49.2 µg/g FW, oregano 79.4 µg/g FW, parsley 51.4 µg/g FW [6]) and is thus of interest for pharmaceutical and cosmetic applications.

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