



Normal Phase Analysis of Tocopherols in Margarine using HPLC

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Food

Abstract

Tocopherols cannot be separated completely using reversed-phase chromatography. However, normal-phase chromatography can separate isocratically all eight tocopherols (T) and tocotrienols (T₃) naturally occurring in fats, oils, and other foodstuffs. Fluorescence detection is recommended for the analysis of total lipid extraction because UV absorbance detection is not selective enough to prevent detection of coeluting peaks.

Chromatographic conditions

The HPLC method presented here was used in the analysis of margarine.

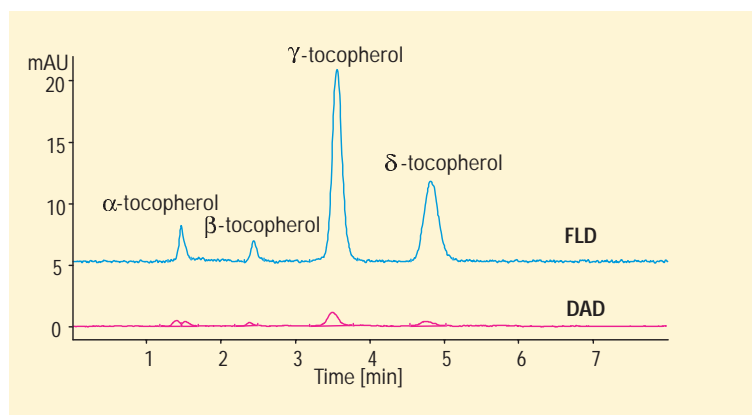


Figure 1
Analysis of tocopherols on normal phase using UV and fluorescence detection

Conditions

Column

100 ~ 2.1 mm Hypersil SI 100, 5 µm

Mobile phase

hexane + 2 % isopropanol

Stop time 8 min

Flow rate 0.3 ml/min

Column compartment 25 °C

Injection vol 0.5 µl

Detector UV-DAD 295/80 nm

Fluorescence

excitation wavelength 295 nm,
emission wavelength 330 nm

Sample preparation

20 g sample dissolved in 15 ml hexane



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HPLC method performance

Limit of detection for diode-array
10–20 ng, S/N = 2

Limit of detection for fluorescence
0.5–2 ng S/N = 2

Repeatability of
RT over 10 runs <2 %
areas over 10 runs <2 %

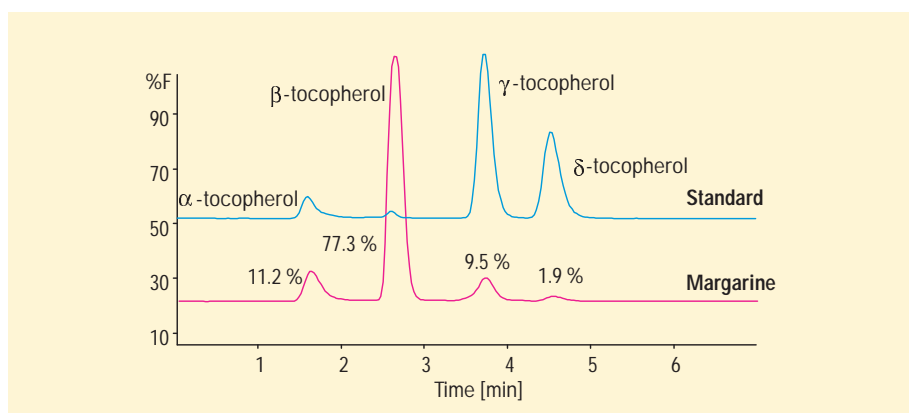
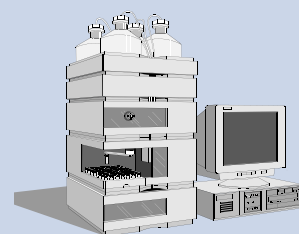


Figure 2
Analysis of tocopherol concentration in margarine fat extract with
fluorescence detection

Equipment

Agilent 1100 Series

- vacuum degasser
 - isocratic pump
 - autosampler
 - thermostatted column compartment
 - electrochemical detector
- Agilent ChemStation + software



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