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Note

High-performance liquid chromatographic determination of antioxidants in fats and oils

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Numerous methods are available for the determination of one or more antioxidants in fats and oils, including thin-layer chromatography[1,2], colorimetry[3], gel chromatography[4], gas–liquid chromatography (GLC)[5–9] and high-performance liquid chromatography (HPLC)[10,11]. All these methods require extraction and, in the case of the GLC methods, derivatization of the antioxidants prior to quantification, increasing the analysis time and the likelihood of losses during analysis. Min *et al.*[12] and Yu *et al.*[13] determined *tert.*-butylhydroquinone (TBHQ) directly in oils using GLC with flame ionization detection. Van Niekerk and Du Plessis[14] and Indyk and Woollard[15] determined TBHQ directly in oils using normal-phase HPLC with fluorescence and UV detection respectively.

This paper reports a simple HPLC procedure which requires neither extraction nor derivatization for the direct determination of six antioxidants, *viz.*, BHA (3-*tert.*-butyl-4-hydroxyanisole), PG (propyl gallate), OG (octyl gallate), DG (dodecyl gallate), TBHQ and NDGA (nordihydroguaiaretic acid), in edible oils and fats.

EXPERIMENTAL

Instrumentation

A Model 5000 liquid chromatograph (Varian Associates, Palo Alto, CA, U.S.A.), a Valco loop injector (capacity 50 μl), a Model III UV detector (Laboratory Data Control, Riviera Beach, FL, U.S.A.) and a Model 3390A integrator (Hewlett-Packard, Avondale, PA, U.S.A.) were employed. Separations were achieved using a 25 cm × 0.46 cm column packed with 5-μm LiChrosorb DIOL (Merck, Darmstadt, F.R.G.) at ambient temperature (23 ± 1°C).

Reagents

Hexane and 1,4-dioxane were obtained from Merck. Acetonitrile was redistilled before use. The mobile phase, hexane–1,4-dioxane–acetonitrile (62:28:10), was filtered through a 0.45-μm membrane filter (Millipore Corporation, Bedford, MA, U.S.A.) and degassed before use. The antioxidants were obtained from a number of commercial sources.

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Procedure

Stock solutions of the six antioxidants were freshly prepared in mobile phase at concentrations up to 100 μg/ml. Approximately 1 g of an oil or fat sample was accurately weighed into a 10-ml volumetric flask, dissolved, with the aid of an ultrasonic bath if necessary, in 4–6 ml of mobile phase and diluted to volume in the same solvent.

RESULTS

The chromatogram obtained for a synthetic standard solution is reproduced in Fig. 1, showing the excellent resolution of the six components. A chromatogram of a sample of olive oil spiked with these antioxidants is shown in Fig. 2. A blank oil sample showed no peaks at the corresponding retention volumes. The peak identities were confirmed by co-chromatography, and linear calibration plots were obtained for all six antioxidants over the concentration range 0–200 mg/kg. The coefficients of variation for replicate (n = 6) injections were 0.99 (BHA), 1.63 (DG), 1.22 (TBHQ), 1.53 (OG), 1.56 (PG) and 1.93% (NDGA). Limits of detection (signal-to-noise ratio = 3) were 25 (BHA, DG, TBHQ, OG), 35 (PG) and 100 ng (NDGA).

The procedure may be applied to the analysis of peanut, soya, marula, sunflower, safflower and rapeseed oils, and beef fat, for which chromatograms similar to Fig. 3 are obtained. Interference from co-eluting material restricts the application of the procedure to the determination of PG and NDGA in maize oil and only to NDGA in cottonseed oil.

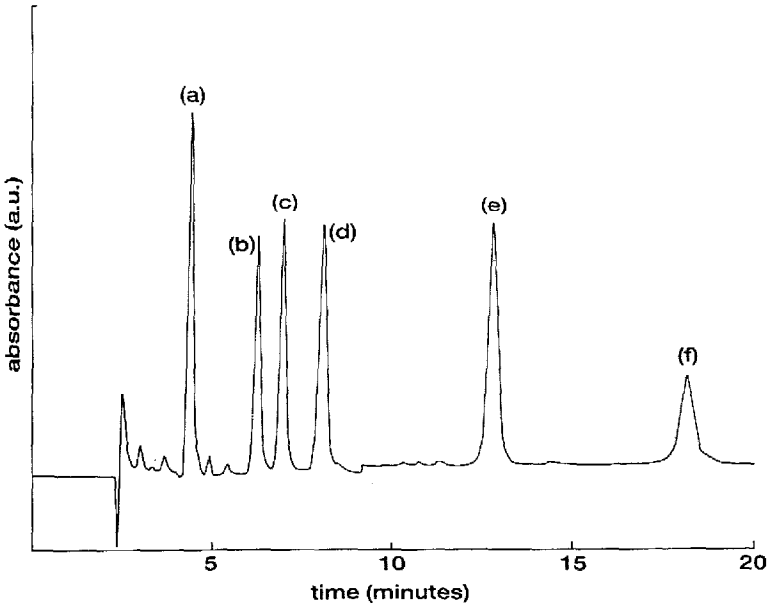

Fig. 1. Chromatogram of antioxidant standards, each approximately 5 μg/ml. Conditions: 25 cm × 0.46 cm LiChrosorb DIOL (5 μm) column; mobile phase, hexane–1,4-dioxane–acetonitrile (62:28:10) at 1.0 ml/min; detection, 0.016 a.u.f.s. at 280 nm; injection 50 μl. Peaks: a = BHA; b = DG; c = TBHQ; d = OG; e = PG; f = NDGA.

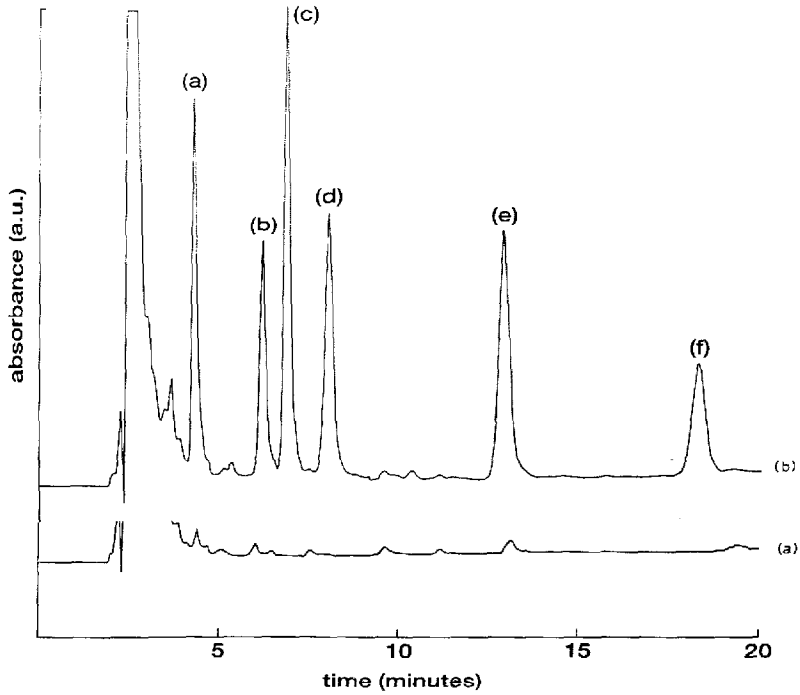

Fig. 2. Curve (a) shows a commercial sample of olive oil (approximately 10%, w/v) and curve (b) the oil spiked with BHA (116), DG (106), TBHQ (206), OG (104), PG (139) and NDGA (144 mg/kg). Conditions and peak identities as in Fig. 1.

Under the HPLC conditions described, BHT (3,5-di-*tert.*-butyl-4-hydroxytoluene), another widely used antioxidant, is not resolved from the neutral lipids in the sample. The method is useful for the rapid determination of BHA, DG, TBHQ, OG, PG and NDGA at relatively low levels in numerous types of oils and fat.

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