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Note

The separation of butylated hydroxyanisole isomers on Sephadex LH-20

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Butylated hydroxyanisole (BHA) is an antioxidant used to prolong the shelf life of many fats and fatty foods. Commercial BHA consists of two isomers (3-*tert.*-butyl-4-hydroxyanisole and 2-*tert.*-butyl-4-hydroxyanisole), and it may contain 5–37% of the 2-isomer[1,2].

Current research studies of various food additives require pure samples of each BHA isomer in sufficient quantity for biological testing. The two isomers differ in their reactivity due to the difference in degree of steric hindrance of the phenolic group by the *tert.*-butyl group. It has been reported[3] that Sephadex LH-20 retards hydroxyl compounds in chloroform; therefore, the chromatographic separation of BHA isomers was attempted using LH-20 with a chloroform–cyclohexane eluent.

EXPERIMENTAL

Thirty grams of Sephadex LH-20 gel (Pharmacia, Piscataway, N.J., U.S.A.) were swollen in chloroform–cyclohexane (1:1, v/v) overnight. The chloroform contained 0.75% alcohol as a stabilizer. The gel was transferred to a Sephadex SR-25 column and allowed to settle under gravity. The resulting column was 16.5 cm long, had a calculated volume of 84 ml, and was stabilized with the appropriate flow adapters. After continued use, the gel had a tendency to compress approximately 1 cm. The column was incorporated into a total chromatography system, which is outlined in Fig. 1.

BHA and BHT (2,6-di-*tert.*-butyl-4-methylphenol) were obtained from ICN Pharmaceuticals (Cleveland, Ohio, U.S.A.). Pure samples (98–100%) of the isomeric forms of BHA were kindly supplied by Eastman (Kingsport, Tenn., U.S.A.). The isomeric standards were used to calibrate the chromatographic column, thin-layer chromatography (TLC) system, and ultraviolet (UV) and infrared (IR) spectrophotometers.

Samples (10–200 mg) were dissolved in 0.5–1 ml of eluting solvent and transferred onto the column with 1-ml washings of eluting solvent. The effluent was monitored for UV absorption at 280 nm, and fractions of 5 ml were collected at a flow-rate of 30 ml/h. Fractions were pooled according to their UV absorption, and the solvent was removed under reduced pressure. The composition of the peaks was determined by TLC on silica gel[1] and IR internal reflection spectrometry.

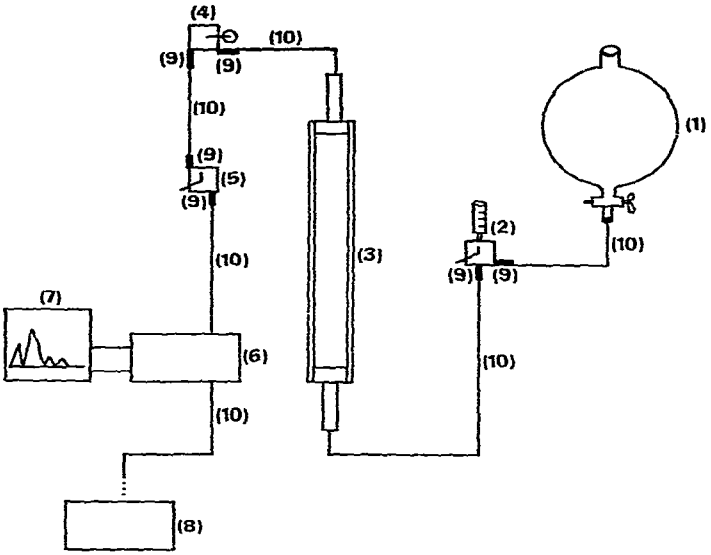

Fig. 1. Total chromatography system. 1 = Reservoir, 500 ml; 2 = 3-way valve with glass syringe; 3 = Sephadex SR-25 column; 4 = metering-type valve; 5 = on/off-type valve; 6 = UV monitor; 7 = recorder; 8 = fraction collector; 9 = 18-gauge stainless-steel needles ground to blunt point; 10 = PTFE tubing (I.D. 1.2 mm, O.D. 1.8 mm).

RESULTS AND DISCUSSION

Table I summarizes the elution peak volumes and the elution fractions containing the BHA isomers and BHT. BHT was included because it is a food antioxidant used in conjunction with BHA and contains a hydroxyl group sterically hindered by two *tert.*-butyl groups. Its early elution suggests hydrogen bonding between hydroxyl groups of the compounds, and Sephadex LH-20 is responsible for the adsorption observed in this solvent mixture.

Fig. 2 illustrates a typical separation of 200 mg of BHA. The separation of the two isomers is excellent. Three unknown peaks were also observed, but no attempt has been made to identify these trace impurities. The isomer peaks showed single spots in TLC[1], and their IR spectra compared identically to the spectra of known compounds and to published spectra[4].

TABLE I

SEPARATION OF BHA AND BHT ON SEPHADEX LH-20 WITH CHLOROFORM–CYCLOHEXANE (1:1) AS THE SOLVENT SYSTEM

Column height, 16.5 cm; flow-rate, 30 ml/h.

Compound	*Approx. elution peak (ml)*	*Elution fraction (ml)*
BHT	41– 45	36– 50
3-BHA	376–380	346–430
2-BHA	476–480	441–530

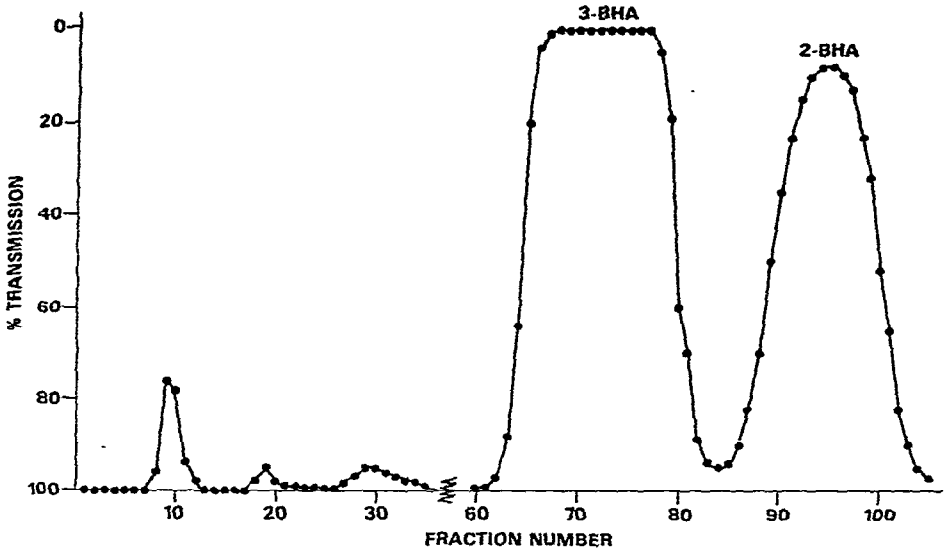

Fig. 2. Separation of BHA isomers on Sephadex LH-20 in cyclohexane–chloroform (1:1, v/v). Sample, 200 mg. Flow-rate, 30 ml/h. Ratio of 3-BHA to 2-BHA, 12:1.

The system described is applicable for the separation and purification of BHA isomers. Its advantage is that BHA remains stable, whereas in preparative TLC, the compounds discolor on silica gel after brief exposure to air.

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