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Note

Determination of butylated hydroxytoluene in poultry premix by high-performance liquid chromatography

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In the presence of oxygen and the catalytic activity of trace elements in feed, ready oxidization of unsaturated fats occurs, resulting in a decrease in the energy value of the feed and in the destruction of vitamins E, A and D[1]. Butylated hydroxytoluene (BHT = 2,6-di-*tert.*-butyl-4-methylphenol) is often added as an antioxidant. Premixes added to poultry feed in quantities from 0.5 to 2% contain from 5000 to 20 000 ppm BHT.

Several methods for determining BHT are known: spectrophotometry[2], thin-layer chromatography[3], gas chromatography[4] and high-performance liquid chromatography (HPLC)[5,6]. For example, the HPLC analysis of BHT present as a preservative in technical grade resmethrin formulations[5] or in different vitamin A preparations[6] has been reported.

The present paper describes a method for the determination of BHT in poultry premixes by HPLC. It is considerably simpler, less-time consuming and less expensive (not only because of the saving in time but also in reagents) than any previous method. Less than an hour is enough for a complete analysis, from weighing a sample to quantitating the results. BHT is extracted from a sample with methanol, the extract is filtered, separated on an HPLC column and the absorption measured at 280 nm.

EXPERIMENTAL

Apparatus and reagents

Perkin-Elmer Model 601 liquid chromatograph was equipped with a Rheodyne loop injector. Operating conditions: mobile phase, 5% redistilled water in methanol, flow-rate 1 ml/min; pressure 400 p.s.i.; wavelength 280 nm; temperature, ambient; chart speed 12 in./h. A Perkin-Elmer Model LC 55 continuously variable-wavelength UV–VIS absorption spectrophotometer, a Perkin-Elmer Model 123 recorder and a Perkin-Elmer Model M calculating integrator were used. The HPLC column was packed with HIBAR LiChrosorb RP-18 (Merck), 25 cm × 4 mm I.D.

A stock solution containing 1000 ppm BHT (Merck) was prepared in methanol; working solutions containing 200 and 20 ppm BHT were prepared by appropriate dilution in the mobile phase. The poultry premix contained no BHT. Methanol (UVASOL, Merck) and deionized water were used.

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Sample preparation

Amounts of 1–2 g of premix were weighed into laboratory bottles. BHT was then added to 7000, 8000, 10 000, 15 000 and 20 000 ppm. The samples were transferred to 100-ml graduated flasks, made up to the mark with methanol and mixed for 15 min with an electromagnetic mixer. After standing to allow the residue to settle, the supernatant was either filtered through a 0.25-μm Millipore filter and 5 μl injected into the column, or a 1–2 ml aliquot was diluted in the mobile phase to give a solution containing about 20 ppm BHT, filtered through a Millipore filter as previously and 10 μl were injected.

RESULTS AND DISCUSSION

In spite of the fact that the absorption maximum for BHT occurs at 230 nm, we used a wavelength of 280 nm at which there was no interference. However, several kinds of premixes without BHT (from different suppliers) were found to contain substances having similar retention times to that of BHT. In those experiments, methanol was used as a mobile phase. When 5% of water was added the mobile phase polarity increased and the BHT peak was completely separated from adjacent peaks (Fig. 1).

The results of the analyses performed with premixes containing supplemental BHT are presented in Table I. Five samples and five analyses were used for every concentration and the values recorded in the table are the arithmetic means from these experiments.

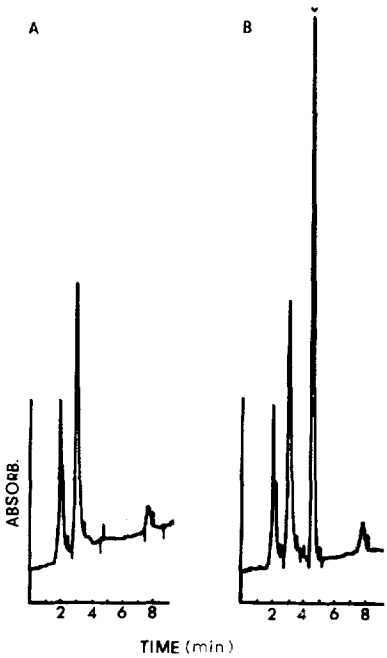

Fig. 1. Chromatogram of premix without BHT (A) and of a premix with 1000 ppm added BHT (B), measured at 280 nm.

TABLE I

ANALYSIS OF BUTYLATED HYDROXYTOLUENE ADDED TO POULTRY PREMIXES

BHT added (ppm)	*Recovery**		*S.D.*	*C.V. (%)*
	ppm	*%*		
7000	6983	99.8	110.36	1.58
8000	8143	101.8	144.34	1.77
10 000	9866	98.7	258.39	2.62
15 000	14 850	99.0	348.52	2.35
20 000	19 744	98.7	602.39	3.05

* Each value is the arithmetic mean for five samples.

The recovery varied from 98.7 to 101.8% and the coefficient of variation from 1.58 to 3.05%. There was no difference in reproducibility over the BHT concentration range injected (20–200 ppm).

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