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## Note

# High-performance liquid chromatographic separation of the isomers of butylated hydroxyanisole

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Butylated hydroxyanisole (BHA) is widely used in the pharmaceutical and food industries as an antioxidant. Commercially available BHA is a mixture of two positional isomers: 3-(1,1-dimethylethyl)-4-hydroxyanisole (3-BHA, major isomer) and 2-(1,1-dimethylethyl)-4-hydroxyanisole (2-BHA, minor isomer). While the two isomers have similar oxidation potentials their antioxidant capacities are markedly different with the 3-BHA being some two to four times more effective than the 2-BHA[1]. The oxidation products differ too[2] due to the differences in the position of the *tert.*-butyl group. The levels of 2-BHA found in commercial material vary from 5 to 37%[3]. Current British (B.P.)[4] and United States (U.S.P.)[5] pharmacopoeias require that the levels of 2- and 3-BHA be determined: the B.P. requires the level of 2-BHA to be less than 15% (w/w). Current spectroscopic[5] or chromatographic[4,6–9] methods are time consuming or lack sensitivity but a recent publication[10] described a normal phase high-performance liquid chromatographic (HPLC) method for the separation of the isomers. While this method is rapid and fairly sensitive it requires the use of a high coast, chiral column packing of limited stability and the 2-BHA isomer is eluted after the 3-BHA, which can make determination of low levels of the 2-BHA difficult. It was also reported[10] that attempts at resolving the isomers by reversed-phase chromatography were unsuccessful. This report describes a rapid, sensitive, reversed-phase HPLC method for the separation of BHA isomers. The method will detect less than 0.5 ng of the isomers injected and the results are compared with those obtained by the U.S.P. method.

## EXPERIMENTAL

HPLC was carried out using a Constametric III pump and Spectromonitor D variable wavelength detector (Laboratory Data Control, Stone, U.K.) with a 10 × 0.5 cm I.D. stainless-steel column slurry packed with Hypersil ODS of 3 μm particle size (Shandon Southern, Cheshire, U.K.). 20-μl samples were injected using a 60-position autosampler with a Rheodyne 7010 valve (Laboratory Data Control). The mobile phase was acetonitrile–water (40:60, v/v) and the flow-rate was 2.0 ml $min^{-1}$.

Infrared spectra were recorded on a Model 957 spectrophotometer (Perkin-Elmer, Beaconsfield, U.K.).

BHA was purchased from May and Baker (Dagenham, Essex, U.K.): reference

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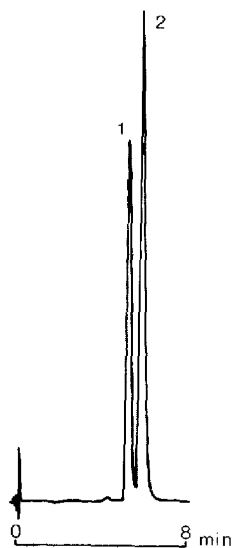


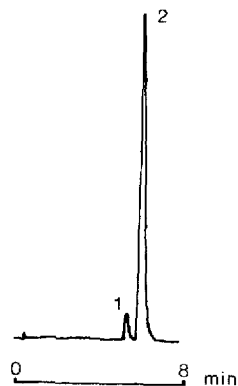


Fig. 1. HPLC chromatogram of 2-BHA (1) and 3-BHA (2) standards. For conditions see text.

Fig. 2. HPLC chromatogram of commercial batch of BHA. Conditions as for Fig. 1.

samples were U.S.P. reference standards. Samples were dissolved in the chromatographic mobile phase. Acetonitrile was HPLC grade (Rathburn Chemicals, Peebles, U.K.).

## RESULTS AND DISCUSSION

Preliminary studies showed that BHA isomers were difficult to resolve on a 5-$\mu$m Hypersil ODS column but were easily resolved on a 3-$\mu$m Hypersil ODS column using acetonitrile–water (2:3) as mobile phase. By monitoring at 228 nm, the lowest detectable concentration, defined as twice the signal-to-noise ratio, was 25 ng/ml for 20 $\mu$l injected (equivalent to 500 pg on-column). The detector response to both the 2-BHA and 3-BHA was linear over the range 0.5–500 ng on-column (2-BHA: $r$ = 1, $n$ = 6: 3-BHA: $r$ = 0.9998, $n$ = 6). Fig. 1 is a typical chromatogram of a mixture of standards.

The determination of 2-BHA levels in a commercial batch of BHA (Fig. 2) was carried out by comparison with an external reference standard, the peak height due to the 2-BHA in the test sample solution being compared with that of the reference standard solution. Using solutions prepared at 8 $\mu$g ml$^{-1}$, the level of 2-BHA was determined to be 7.0% (w/w) with a coefficient of variation of 1.6% (six determinations): this compares with the level determined according to the U.S.P. XX procedure[5] at 7.8% (w/w).

Thus BHA isomers may be separated and quantified without prior derivatisation by reversed-phase chromatography. The procedure is reliable and robust: the column used in this work has been in routine use for determining BHA levels in formulated products for more than a year and has required only occasional maintenance to the top of the column.

## ACKNOWLEDGEMENTS

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