APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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# Analysis of Butylated Hydroxytoluene in Food with Headspace Trap-GC/MS

## Introduction

Butylated hydroxytoluene (BHT, 2,6-di-tert-butyl-4-methylphenol) is a common food additive. BHT is found in many types of food including butter, meats, cereals, chewing gum, baked goods, snack foods, dehydrated potatoes and beverages. It is used to preserve food odor, color and flavor. BHT is oxidized preferentially in fats or oils, protecting the foods from spoilage.

Concern exists that long-term human consumption of BHT may have potential health risks. It has undergone the additive application and review process required by the U.S. Food and Drug Administration (FDA); the committee concluded that no evidence in the available information on BHT demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, uncertainties exist requiring that additional studies should be conducted.[1] The chemical properties which make BHT an excellent preservative may also be implicated in health effects. The oxidative characteristics and metabolites of BHT may contribute to carcinogenicity. Some people may have difficulty metabolizing BHT, resulting in health and behavioral changes.

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This application note will demonstrate a fast and easy analytical technique to determine the amount of BHT in foods. Headspace (HS) sample introduction is used because it provides a means to analyze food without any sample preparation. Headspace eliminates the need for solvents and other sample-preparation steps to reduce cost and complexity of extraction. In this application note, an adsorbent trap is used to concentrate the headspace sample and increase sensitivity, allowing for low-level detection or small sample sizes.

The analysis is carried out with gas chromatography mass spectrometry (GC/MS) – this will allow us to resolve the BHT from other volatile compounds in the food matrices and provide positive identification of the BHT with mass spectral data. Calibration of the system and analysis of food samples will be demonstrated.

## Experimental

The instrumental platform for this application is the TurboMatrix™ HS Trap coupled to a Clarus® 680 GC/MS, both platforms from PerkinElmer. The transfer line of the HS was directly connected to the Elite™-17ms column with a universal butt connector. The samples are heated in a sealed vial at 80 ˚C for 30 minutes to drive the BHT from the food into the headspace. Using automated headspace technology, the gas is extracted from the vial, concentrated on an adsorbent trap (PerkinElmer® Air Toxics), and injected into the GC/MS system. Table 1 shows the detailed instrumental setup parameters for the HS Trap-GC/MS system.

***Table 1.*** **Instrument Parameters.**

| **Sample Introduction** | **PerkinElmer TurboMatrix HS-40 Trap** |
|---|---|
| Needle Temperature | 90 ˚C |
| Transfer Line Temperature | 110 ˚C |
| Oven Temperature | 80 ˚C |
| Trap Low Temperature | 40 ˚C |
| Trap High Temperature | 280 ˚C |
| Dry Purge (Helium) | 5 min |
| Trap Hold Time | 6 min |
| Desorb Time | 0.5 min |
| Thermostatting Time | 30 min |
| Pressurization Time | 1 min |
| Decay Time | 2 min |
| Column Pressure | 17 psi |
| Vial Pressure | 35 psi |
| Desorb Pressure | 10 psi |
| Universal Capillary Column Connector | Part No. N9302149 |
| Transfer Line | Fused Silica 2 m x 320 µm |

| **Gas Chromatograph** | **PerkinElmer Clarus 680 GC** |
|---|---|
| Headspace Connector | Universal Connector |
| Inlet Temperature | 150 ˚C |
| Oven Program Initial Temp | 50 ˚C |
| Hold Time 1 | 1 min |
| Ramp 1 | 25 ˚C/min to 280 ˚C |
| Hold Time 2 | 1.8 min |
| Vacuum Compensation | On |
| Headspace Control | On |
| Column | Elite-17ms<br>30 m x 0.25 mm x 0.25 µm |
| Carrier Gas | Helium |
| **Mass Spectrometer** | **PerkinElmer Clarus 600 MS** |
| Mass Range | 45-300 u |
| Solvent Delay Time | 0.1 min |
| Scan Time | 0.20 sec |
| InterScan Delay Time | 0.02 sec |
| Transfer Line Temperature | 240 °C |
| Source Temperature | 200 °C |
| Multiplier | 500 V |

### Calibration-Standards Preparation

A 10 ng/µL standard stock solution was prepared by diluting 0.1 mL of a 1000 µg/mL BHT standard to 10 mL with methanol. 1 ng/µL, 2 ng/µL and 5 ng/µL standard working solutions were prepared by diluting 0.1 mL, 0.2 mL and 0.5 mL of a 10 ng/µL BHT standard to 1 mL with methanol. 20 ng/µL, 50 ng/µL and 100 ng/µL standard working solution was prepared by diluting 0.02 mL, 0.05 mL and 0.1 mL of a 1000 µg/mL BHT standard to 1 mL with methanol.

The working curve was prepared by injecting 1 µL of each working standard solution into headspace vials. Working calibration standards at 1, 2, 5, 10, 20, 50, and 100 ng were prepared fresh each day.

One gram of each food sample purchased at local Shanghai markets were placed into the headspace vials. All headspace vials were sealed immediately and transferred to the headspace-trap vial tray.

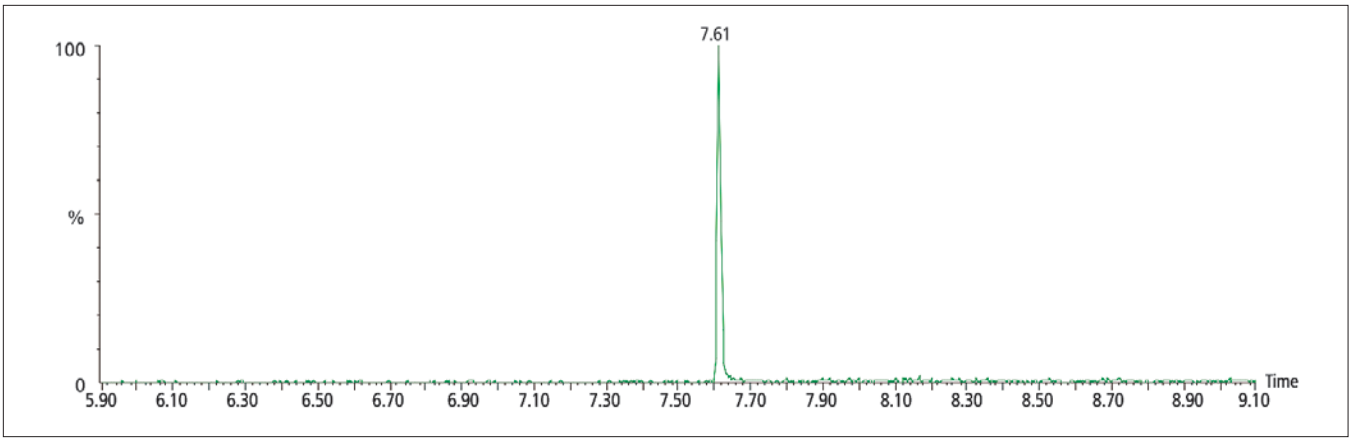


*Figure 1.* Example chromatogram of a 100 ng standard injection of BHT.

## Results and Discussion

The instrument calibration included seven calibration levels in the working curve; the response of this calibration curve was linear (Table 2). Additionally, the method is precise throughout the calibration range, as demonstrated by the relative standard deviation of 3.2% at the calibration limit (1 ng, n=5) and 1.9% at 10 ng (n=5).

Figure 1 is an extracted ion chromatogram, of *m/z* 205, from the analysis of a 100 ng BHT standard. Figure 2 demonstrates the spectral data of BHT which matches exactly the fragmentation of BHT in the NIST® spectral library.

Following the calibration of the system, five food samples were analyzed: a cracker, powdered coffee creamer, instant noodles, sausage, and tea leaves. The BHT concentrations are quantified (Table 3). BHT concentration in the food samples analyzed here was below the quantitation limit of 1 ng/g. It can be seen in Figure 3 (Page 4) that the BHT peak is easily identified in the sample analysis. Each sample was analyzed in triplicate – the area reproducibility achieved (Table 3) demonstrates that the method remains very precise, even below the quantitation limit.

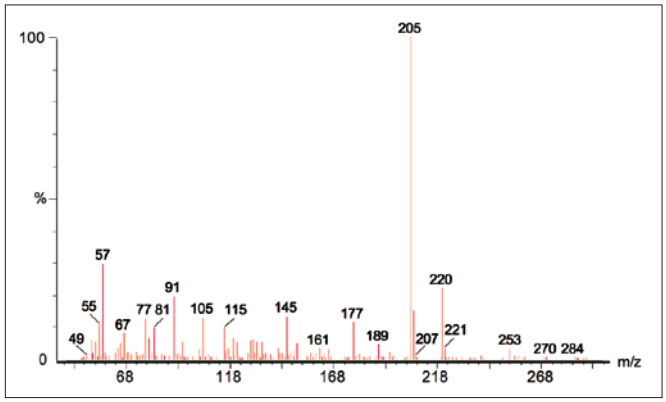


*Figure 2.* Background subtracted spectra from the analysis of a BHT standard.

***Table 2.* Calibration Table for BHT.**

| Name | Retention Time (min) | Quantifier Ion | Qualifier Ion 1 | Qualifier Ion 2 | %RSD (n=5 at 1 ng) | %RSD (n=5 at 10 ng) | $r^2$ |
|---|---|---|---|---|---|---|---|
| BHT | 7.60 | 205 | 220 | 57 | 3.2 | 1.9 | 0.9980 |

***Table 3.* %RSD of BHT in Food Samples.**

| Sample | BHT (ng/g) in 1 g of Sample | BHT (ng/g) in 1 g of Duplicate | BHT (ng/g) in 1 g of Triplicate | Values Mean (ng/g) | %RSD |
|---|---|---|---|---|---|
| Crackers | 0.65 | 0.55 | 0.54 | 0.58 | 10.5 |
| Coffee Creamer | 0.66 | 0.73 | 0.69 | 0.69 | 5.1 |
| Instant Noodles | 0.67 | 0.67 | 0.70 | 0.68 | 2.5 |
| Sausage | 0.67 | 0.53 | 0.56 | 0.59 | 12.6 |
| Tea Leaves | 0.62 | 0.54 | 0.53 | 0.56 | 8.8 |

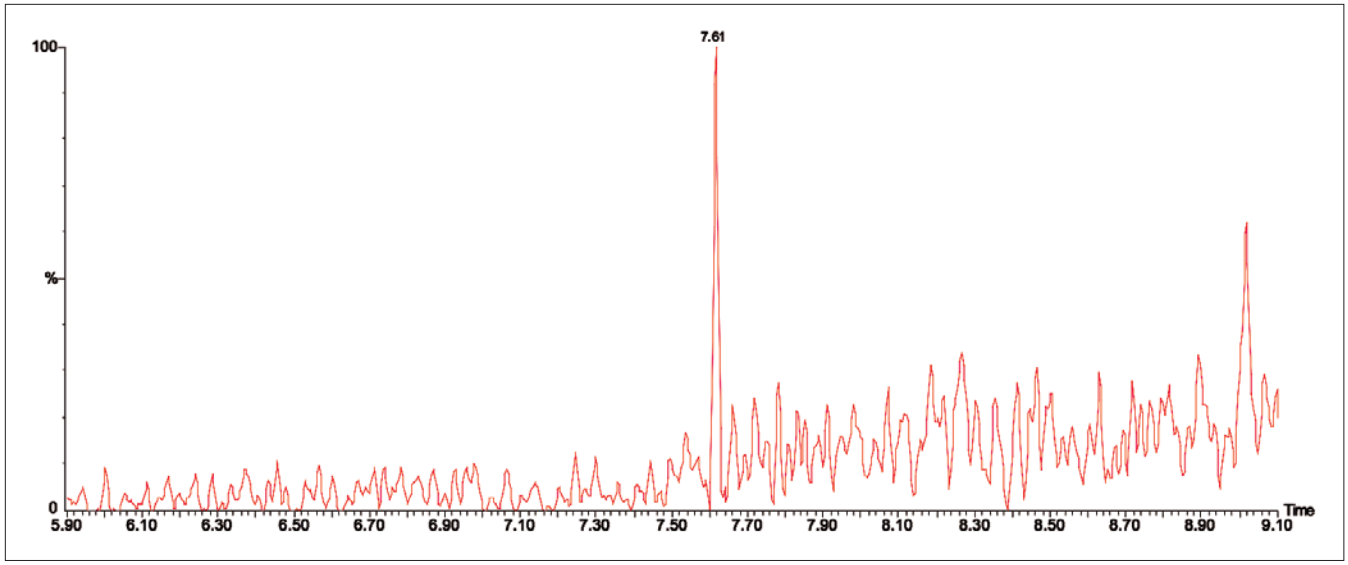


*Figure 3.* Resultant chromatogram from the analysis of instant noodles for BHT.

## Conclusion

BHT is a common food additive used to prevent spoilage. Analysis of BHT is needed for both food quality and safety reasons. Food is often a complicated sample matrix which is time consuming to prepare and analyze. This method uses headspace technology to virtually eliminate sample preparation and reduce the cost and labor of the analysis. In addition to eliminating sample preparation, the method is both sensitive and precise as demonstrated by the analysis of standard reference materials and a variety of food samples. The throughput of the system is further improved by the Clarus 680 GC/MS with a fast-cooling GC oven, further improving throughput and productivity. The MS data provides positive confirmation of BHT in sample matrices.

## References

1. Database of Select Committee on GRAS Substances (SCOGS) Reviews-Butylated Hydroxytoluene (BHT), available from http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogsListing&id=41

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