Determination of butylated hydroxytoluene in chewing gum using GC-MS

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Abstract

Butylated hydroxytoluene is an antioxidant used in food products. The concentration of BHT was determined in commercial chewing gums using GC-MS. The analysis was performed using butylated hydroxyanisole (BHA) as the internal standard. The concentration of BHT in each gum sample was found in μg BHT per gram of chewing gum. The percent recovery was calculated for each sample.

Introduction

Butylated hydroxytoluene, or BHT, is a widely used preservative in food products which prevents the oxidation of lipids. Since BHT is cheap, stable and easy to obtain, it is a commonly used anti-oxidant in food products. Fats must be preserved by anti-oxidizing agents and BHT acts as an anti-oxidant in order to maintain the proper flavor and odor in food including chewing gum. In general it is believed that the formation of a lipid free radical initiates lipid oxidation. This free radical then reacts with oxygen and forms a lipid peroxy radical. This lipid peroxy radical then reacts with hydrogen from a lipid molecule, which makes a new free radical.

\[ L \rightarrow L^\cdot \quad \text{Chain- initiating step} \]
\[ L^\cdot + O_2 \rightarrow LOO^\cdot \quad \text{Chain- propagating step} \]
\[ LOO^\cdot + L \rightarrow LOOH + L^\cdot \quad \text{Chain- propagating step} \]

However, it is believed that BHT blocks lipid oxidation by reacting with the lipid alkyl or peroxy radical. This prevents the formation of a new radical.

\[ L^\cdot + BHT^\cdot \rightarrow L–BHT^\cdot \quad \text{Chain-terminating step} \]
\[ LOO^\cdot + BHT^\cdot \rightarrow LOO–BHT \quad \text{Chain-terminating step} \]

Proper research on the toxicity of BHT in food has not yet been conducted, even though a few foods have recommended concentrations of BHT including dehydrated potato shreds (50 ppm), potato granules (10 ppm), and sweet potato flakes (50 ppm). The FDA also has a recommended amount of 0.1% of BHT in chewing gum. BHT can be hazardous if used at high concentrations, but the current levels of BHT used in products today are not toxic. Even though these levels are not toxic, manufacturers must still be cautious when adding synthetic chemicals to food products. Future restrictions may be made on the use of synthetic anti-oxidants because of food sanitation.

Chewing gum generally contains antioxidants such as BHT and butylated hydroxyanisole (BHA). In this experiment BHT will be analyzed in commercial chewing
gums by GC-MS. BHA was used as an internal standard, and 3,5-di-tert-butylphenol was used as a surrogate standard to recover any lost BHT in the gum sample. Percent recovery is used to determine the concentration of the analyte while considering any losses of analyte.

**Experimental**

**Sample Preparation**

The sample was prepared by grinding sticks of chewing gum in an Osterizer Blender until it became a fine powder. A 5-g sample of the gum was weighed using an analytical balance and placed in a 250-mL Erlenmeyer flask with a rubber stopper. A 100-mL aliquot of acetonitrile was added to the flask, followed by 250 μL of 3,5-di-tert-butylphenol and a stir bar. The flask was placed on a hot plate in the hood and left to stir for an hour. After an hour, the samples were filtered by gravity into a 500-mL round bottom flask using disposable Buchner funnels that had been rinsed with acetonitrile. The sample was rinsed three times with fresh 10-mL volumes of acetonitrile. After filtration, the filter was rinsed with an additional 50-mL aliquot of acetonitrile. A rotary evaporator was then used to evaporate the acetonitrile until almost complete dryness at 30°C. The residue was collected using a small amount of ethyl acetate and transferred to a 5-mL volumetric flask. A 250-μL aliquot of BHA was added to the flask, followed by dilution to the line with ethyl acetate. The final concentration is 50 μL/mL for the BHA.

**Preparation of Standards**

Three stock solutions were prepared at concentrations of 1mg/mL each of BHA, 3,5-di-tert-butylphenol, and BHA in ethyl acetate. The BHT stock solution was prepared by dissolving 0.0150 grams of BHT in a 10-mL volumetric flask. Five standards were prepared in 10-mL volumetric flasks using 0.1 mL, 0.2 mL, 0.5 mL, 1.0 mL, and 2.0 mL of BHT, followed by 0.5 mL of BHA and 3,5-di-tert-butylphenol. A small amount of each standard and the sample was then transferred to a mini injector vial.

**Instrument Preparations and Analysis**

Each standard and the sample were then subjected to GC-MS analysis on an Agilent 6890 gas chromatograph with a 5973N mass selective detector. The model of the column was HP 5MS 30 m × 0.25 mm by × 0.25 μm. The temperature of the column was held at 100°C for 5 minutes and programmed to ramp from 100°C to 300°C at 20°C/minute. The injection port and ion source were at temperatures of 250°C and 280°C, respectively, and the ionization voltage was 70 eV. The carrier gas was helium at an inlet pressure of 7.7 psi at 50°C. Ions used for selected ion monitoring were m/z 220 for BHT, m/z 165 for BHA and m/z 191 for 3,5-di-tert-butylphenol. The retention time and peak areas were recorded.
Results and Discussion

Calibration plots

The aim of this experiment was to determine the overall concentration of BHT in chewing gum. This was achieved by extracting BHT from the gum sample and comparing the sample to BHT standards using a calibration plot. Figure 1 displays the response ratio vs. the concentration of BHT. The calibration plot was prepared using the raw data provided by GC-MS. The x-axis is simply the known concentration (ppm) of BHT in the standardized solutions. The y-axis is the response factor, defined as the peak area of BHT over the peak area of the BHA standard, which was given by GC-MS.

![Graph of Area Response Ratio versus BHT concentration in standards](image)

Chromatograms of standards and samples

The chromatograms below show the peaks for BHT, the internal standard (BHA) and the surrogate standard (3,5-di-tert-butylphenol). Figure 2 shows the chromatogram for a BHT standard and Fig. 3 is the chromatogram for the Juicy Fruit sample. The internal standard peaks at 4.98 minutes. BHT peaks at 5.16 minutes. The surrogate standard peaks at 5.29 minutes.
Percent recovery

In order to account for analyte loss, a surrogate standard was added to the sample at the beginning of the extraction process. The percent recovery method was used to make up for any sample lost during the experiment. The use of percent recovery ultimately results in concentration numbers that are closer to the true value of analyte in the sample. The average relative response factor (RRF_{ave}) was determined from the standard solutions and the data from GC-MS. The RRF_{ave} was calculated using BHA concentrations and peak areas as well as the surrogate standard concentrations and peak areas found in the experiment. The equation used to determine the RRF_{ave} can be seen below:

\[
\text{Percent recovery} = \frac{\text{RRF}_{\text{ave}} \times \text{Surrogate Standard Concentration}}{\text{Sample Concentration}} 
\]

Percent recovery was determined using the following equation:

Table 1 displays the percent recovery of the six samples, the final concentration of BHT in the total gum sample, and the amount of BHT in micrograms per gram of gum.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>% recovery</th>
<th>Final [BHT]</th>
<th>μg BHT /g gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrigley's Juicy Fruit</td>
<td>31.2357</td>
<td>92.7346</td>
<td>92.3008</td>
</tr>
<tr>
<td>Wrigley's Winterfresh</td>
<td>6.2738</td>
<td>129.7534</td>
<td>128.4153</td>
</tr>
<tr>
<td>Orbit Peppermint</td>
<td>32.0487</td>
<td>152.0503</td>
<td>150.2563</td>
</tr>
<tr>
<td>Orbit Bubblemint</td>
<td>57.1267</td>
<td>136.4725</td>
<td>136.0807</td>
</tr>
<tr>
<td>Stride Sweetberry</td>
<td>54.8955</td>
<td>133.5133</td>
<td>131.8883</td>
</tr>
<tr>
<td>Stride Winterblue</td>
<td>36.0792</td>
<td>174.7181</td>
<td>174.3833</td>
</tr>
</tbody>
</table>

The values obtained for the concentrations of BHT were analyzed a week after sample extraction. BHT concentrations may have been different had the analysis been performed the same day the analyte was extracted from the gum sample. The determined concentrations are dependent on the stability of BHT over long time periods. More samples need to be run on each sample of gum in order to determine the accuracy and precision of the results.

Conclusions

The purpose of this experiment was to determine the concentrations of butylated hydroxyl toluene (BHT) in six different samples of chewing gum. These concentration of BHT in one gram of gum was 92.30, 128.41, 150.25, 136.08, 131.88, 174.38 μg for Juicy Fruit, Winterfresh, Orbit Peppermint, Orbit Bubblemint, Stride Sweetberry, and Stride Winterblue, respectively. The percent recovery for each sample was 31.23%, 6.27%,
32.05%, 57.13%, 54.89%, and 36.08% for Juicy Fruit, Winterfresh, Orbit Peppermint, Orbit Bubblemint, Stride Sweetberry, and Stride Winterblue, respectively.

Acknowledgements

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References
