SHIMADZU

Application News

Gas Chromatography Mass Spectrometry No.M252

Analysis of Food Additives – Food Antioxidants –

■ Introduction

Food antioxidants serve to prevent the oxidation of food ingredients by becoming oxidized themselves instead. BHT (dibutylhydroxytoluene) and BHA (butylhydroxyanisol), which had become widely used as food antioxidants, are almost never used now because of concern related to their reported carcinogenicity. Instead, vitamin C and vitamin E have recently become widely used. The use of some food additives is not permitted in Japan, but many such substances are permitted in some countries. For example, the antioxidant TBHQ (t-butylhydroquinone) is not permitted to be used in Japan; however, since it may be used in many other countries, there is a possibility of it being included in food imports to Japan. This Application News introduces the results of analysis of the antioxidants BHA, BHT and TBHQ using a gas chromatograph mass spectrometer (GC/MS). The antioxidants were added to a butter sample, and then extracted using a simple pretreatment process. In this analysis, the backflush technique was employed to prevent high-boiling point substances in the actual sample from being introduced into the detector.

■ Results and Discussion

Analysis of Standard Sample

A sample solution consisting of 10 mg/L each of BHA, BHT and TBHQ in acetone was analyzed using the analytical conditions shown in Table 1. The results are shown in Fig. 1. The TIC chromatogram is shown at the top, and the mass spectra of the respective constituent peaks and mass chromatograms at the characteristic *m/z* values are shown below. Each constituent peak was clearly detected.

Recovery Analysis of Spiked Components in Actual Sample

Recovery analysis was conducted for substances added to an actual sample of commercially available butter. After dissolving 100 mg of butter in 1 mL acetone, centrifugal separation (1000 rpm, 5 minutes) was conducted to precipitate the extract, and supernatant was collected and analyzed. For recovery testing, BHA, BHT and TBHQ were added to the butter sample to attain a final concentration of 5 mg/kg each (concentrations of BHA, BHT and TBHQ in sample following pretreatment were 0.5 mg/L, respectively).
The resulting chromatogram is shown in Fig. 2. As in Fig. 1, the TIC chromatogram is shown at the top, and the mass spectra of the respective constituent peaks and mass chromatograms at the characteristic *m/z* values are shown below. In the actual sample as well, peak detection could be conducted selectively for the target constituents of the mass chromatograms at the characteristic *m/z* values. Mass spectra similar to those obtained using the standard solution were also obtained. The analysis was conducted 3 times to determine the repeatability of the quantitation values. The repeatability results and recoveries based on the average quantitation value with n=3 are shown in Table 2. Excellent repeatability and recovery were obtained for the actual sample.

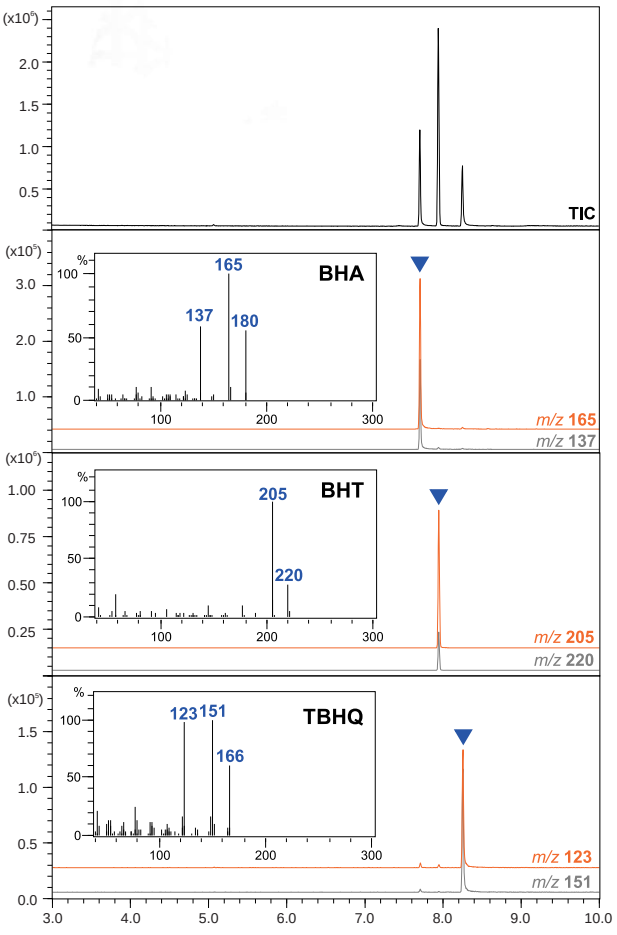

Fig. 1 Chromatograms of BHA, BHT and TBHQ (10 mg/L)

Table 1 Analytical Conditions

Model	: GCMS-QP2010 Plus
-GC-	
Column	: Rtx-5MS (30 m × 0.25 mm I.D. df = 0.25 µm)
Column Temp.	: 60 ˚C-15 ˚C/min-280 ˚C (20 min)
Carrier Gas	: He (45 cm/sec,174.3 kPa)
Carrier Gas Mode	: Constant Pressure Mode
Injection Temp.	: 250 ˚C
Injection Method	: Split Injection
Split Ratio	: 5:1
Injection Volume	: 1 µL
-MS-	
I.F. Temp.	: 260 ˚C
I.S. Temp.	: 230 ˚C
Ionization	: EI
Scan Range	: *m/z* 40-300
Scan Interval	: 0.3 sec

Table 2 Results of Butter Sample Analysis

	Quantitation Values (mg/kg)				RSD (%)	Average Recovery Rate (%)
	1	2	3	Average		
BHT	6.4	6.2	5.8	6.1	4.7	123
BHA	4.7	4.6	4.6	4.6	1.3	93
TBHQ	5.9	5.9	5.3	5.7	6.4	114

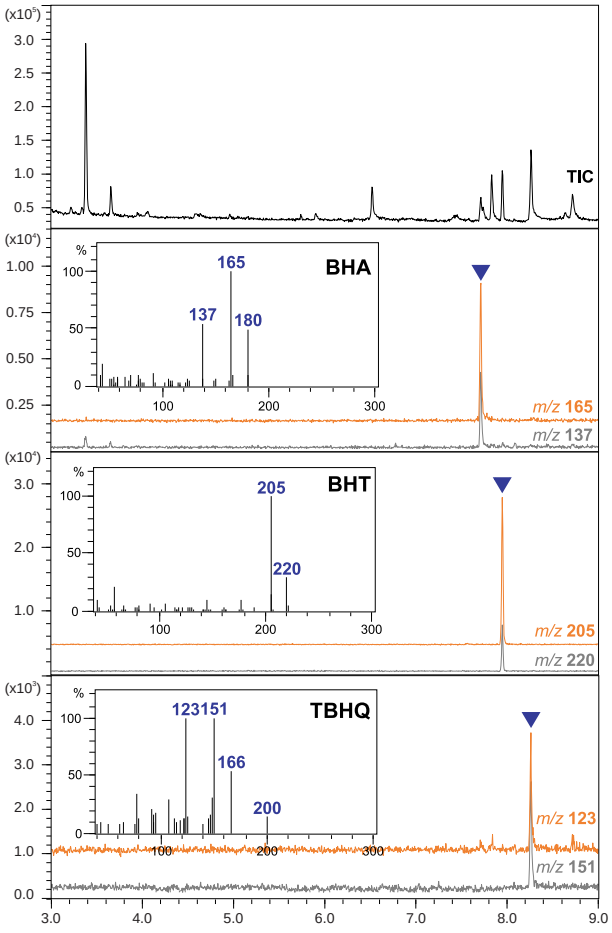

Fig. 2 Chromatograms of Butter Sample (5 mg/L)

Capillary Backflush System

In this investigation, actual sample analysis was conducted using a simple sample preparation process (minimal sample cleanup was performed after extraction). Therefore, most of the high-boiling point substances would typically be introduced into the GC/MS. Backflushing reverses the flow of carrier gas in the column to discharge high-boiling point substances from the split vent of the injection port. One of the benefits of backflushing is the reduced frequency of maintenance, since the high-boiling point substances are not introduced into the detector. In addition, high-boiling point substances may not be completely discharged from the column during the analysis. Residual high-boiling point substances in the column can cause such problems as reduced sensitivity and poor repeatability, so discharging them via the injection port provides an efficient means of removing them from the column while extending the life of the column. The backflush settings were implemented via time program, so that following elution of the target components (in this analysis, at 9 minutes), the split vent pressure was raised from 20 kPa to 200 kPa, and at the same time the column pressure was reduced to 20 kPa. This program can be set automatically using the backflush software utility.

Fig. 3 shows a comparison of TIC chromatograms of butter analysis with and without the use of the backflush technique. The zoomed chromatograms in Fig. 3 display only the data acquired up to the completion of elution of BHA, BHT and TBHQ. Similar chromatograms were obtained when backflush was used and when it was not used. When backflushing was conducted, however, no peaks were detected after the 9 minutes corresponding to the time setting. Implementing the backflush prevented introduction of the high-boiling point substances into the detector, and provided a means of discharging them efficiently from the column.

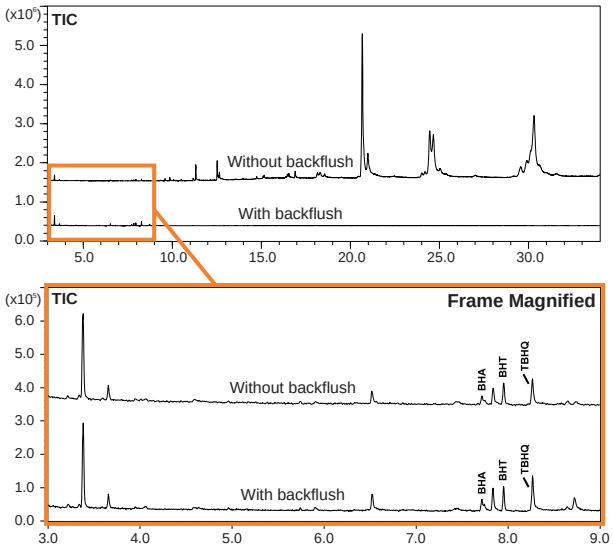

Fig. 3 Comparison of Butter Sample Analysis with/without Backflush

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