# Analysis of antioxidants in chewing gum using the Agilent 1120 Compact LC

Application Note

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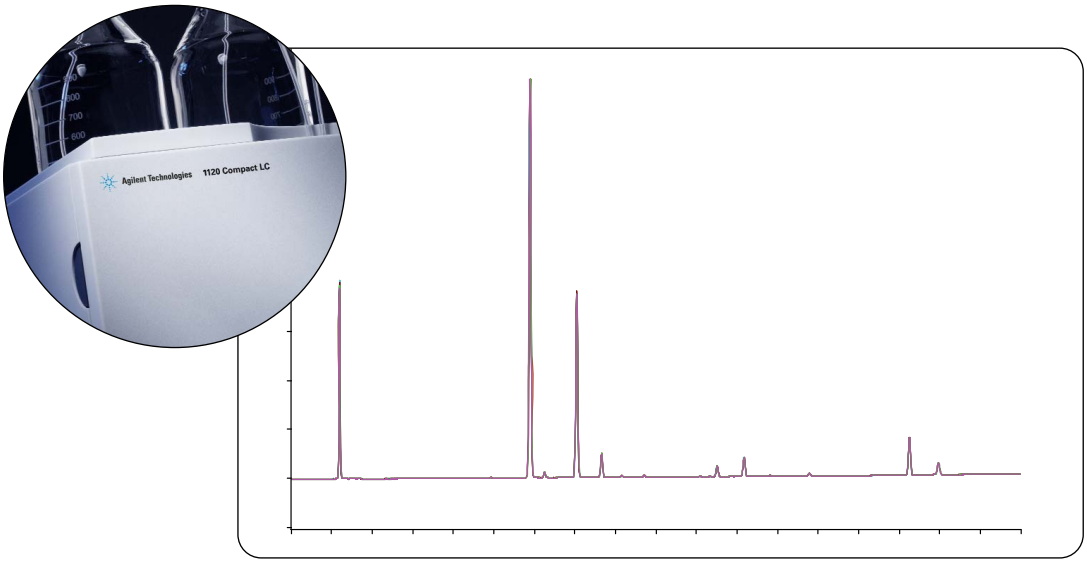



## Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. It is an intergrated LC designed for ease of use, performance and reliability. It is ideally suited for the analysis of antioxidants on account of its capability to achieve highly precise retention times and peak areas, and low detection limits for the analyzed compounds. The data in this Application Note demonstrates:

- Excellent retention time precision of less than 0.07 % RSD
- Excellent peak area precision of less than 0.3 % RSD for baseline separated peaks
- Limit of detection <1 to 75 ng for all antioxidants analyzed



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## Introduction

Antioxidants are widely used in the food industry. For example, butylated hydroxyanisole (BHA) is used in biscuits, fruit cake, candy and walnuts as well as in chewing gum. Estimated and proven risks of these compounds have led to regulations for the maximum allowed concentrations in foodstuffs. Some antioxidants such as BHA are not allowed in baby foods because of their influence on the accelerated digestion of vitamin D. BHA also has a detrimental influence on the blood level of lipids and cholesterol.

In this study, eight antioxidants were analyzed and the precision of retention times and areas was measured. Further, the limit of detection (LOD) of the compounds was determined. As a real-life example, the level of BHA in chewing gum was measured.

## Experimental

### Equipment

- Agilent 1120 Compact LC comprising gradient pump with integrated degasser, autosampler with vial tray, column oven and variable wavelength detector, see figure 1
- Agilent HC-C18(2), high carbon load, 150 x 4.6 mm, 5 µm particle size column
- Agilent EZChrom Elite Compact software

### Chromatographic conditions

- Mobile phase:
  A: Water + 0.045 % TFA
  B: ACN + 0.045 % TFA
- Gradient: 10 to 90 %B in 15 min
- Flow rate: 1.5 mL/min
- Injection volume: 5 µL
- Column temperature: 40 °C
- Detection wavelength: 260 nm
  Peakwidth: > 0.0025 min
  Response time: 0.06 s

### Sample

1. Vitamin C
2. Propyl gallat (PG)
3. 2,4,5-trihydroxy-butyrophenone (THBP)
4. mono-tert-butyl-hydroquinone (TBHQ)
5. Butylated hydroxyanisole (BHA)
6. 4-hydroxymethyl-2.6-di(tert-butyl)phenol (ionox 100)
7. Butylated-hydroxytoluene (BHT)
8. Ascorbyl-palmitate (ACP)

## Results and discussion

Eight antioxidants were chosen for the determination of precision of retention time and areas. The limits of detection were also evaluated. All compounds were separated with excellent resolution, using conventional chromatographic conditions with a flow rate of 1.5 mL/min and a 15 min gradient, see figure 2.

**Figure 1**
**Agilent 1120 Compact LC**

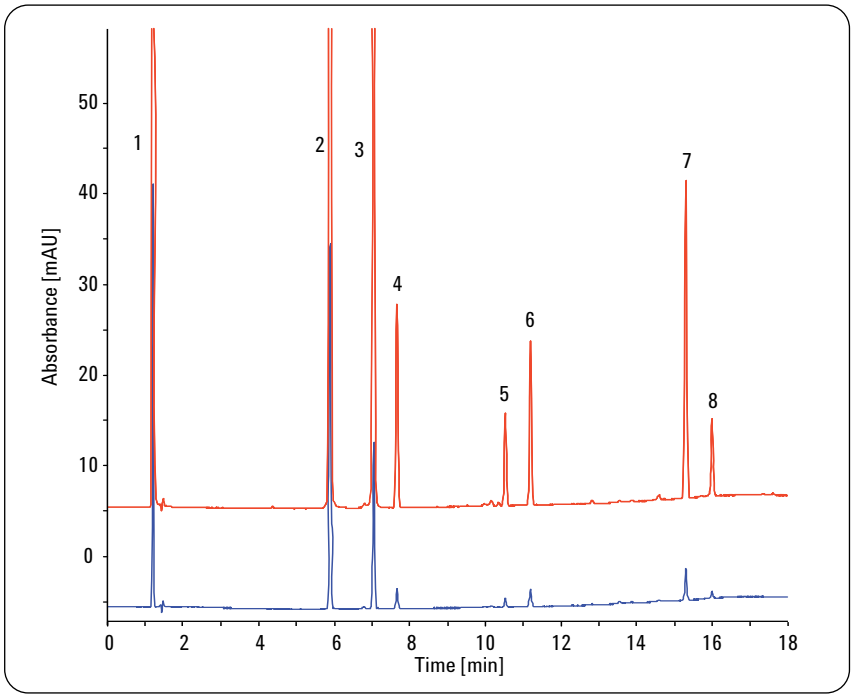


**Figure 2**
**Upper trace–Antioxidant standards, 1350-200ng, 1:10 dilution**
**Lower trace–Antioxidant standards, 135-20ng, 1:100 dilution**

The concentration levels used in this study are listed in table 1. The precision of retention times and areas was determined using the 1:10 dilution. The results are shown in table 2.

Even for peaks with heights less than or equal to 20 mAU, the precision of areas was less than 0.3 %, which is an excellent result. The area precision for vitamin C (peak 1) was affected by the continuous decomposition of this compound in solution. The precision of ACP (peak 8) was influenced by a small broad peak that eluted just before the ACP peak. Both peaks were not completely separated and quantification at about 12 mAU was not as good as for the other fully separated compounds.

The precision of retention times for all compounds was less than 0.05 % relative standard deviation (RSD). Figure 2 shows the overlaid chromatograms six consecutive runs.

The limit of detection was calculated based on the chromatogram of the 1:100 dilution, see figure 1. The results are shown in table 3.

| Peak | Compound | Stock Solution mg/10 mL | 1:10 dilution 5 µL inj. vol. (ng per inj.) | 1:100 dilution 5 µL inj. vol. (ng per inj.) |
|---|---|---|---|---|
| 1 | Vitamin C | 20 | 1000 | 100 |
| 2 | PG | 21 | 1050 | 105 |
| 3 | THBP | 15 | 750 | 75 |
| 4 | TBHQH | 21 | 1050 | 105 |
| 5 | BHA | 11 | 500 | 50 |
| 6 | Ionox100 | 14 | 700 | 70 |
| 7 | BHT | 27 | 1350 | 135 |
| 8 | ACP | 4 | 200 | 20 |

**Table 1**
**Concentration levels of the analyzed antioxidants.**

| Peak | Compound | % RSD Ret. Times | % RSD Areas | Peak height (mAU) |
|---|---|---|---|---|
| 1 | Vitamin C | 0.04 | 2.01* | ~200 |
| 2 | PG | 0.03 | 0.08 | ~400 |
| 3 | THBP | 0.02 | 0.12 | ~200 |
| 4 | TBHQH | 0.01 | 0.23 | <25 |
| 5 | BHA | 0.02 | 0.21 | <15 |
| 6 | Ionox 100 | 0.01 | 0.10 | <20 |
| 7 | BHT | 0.01 | 0.26 | <50 |
| 8 | ACP | 0.01 | 1.74** | ~12 |

**Table 2**
**Precision of retention times and areas (*decomposition; **integration problem with front peak).**

| Peak | Compound | LOD with S/N = 3 1:100 dilution (ng) |
|---|---|---|
| 1 | Vitamin C | <1* |
| 2 | PG | <1 |
| 3 | THBP | 75 |
| 4 | TBHQH | 6 |
| 5 | BHA | 3 |
| 6 | Ionox100 | 2.3 |
| 7 | BHT | 2.5 |
| 8 | ACP | 2 |

**Table 3**
**Limits of detection for the antioxidants (*rapid decomposition made determination of traces difficult).**

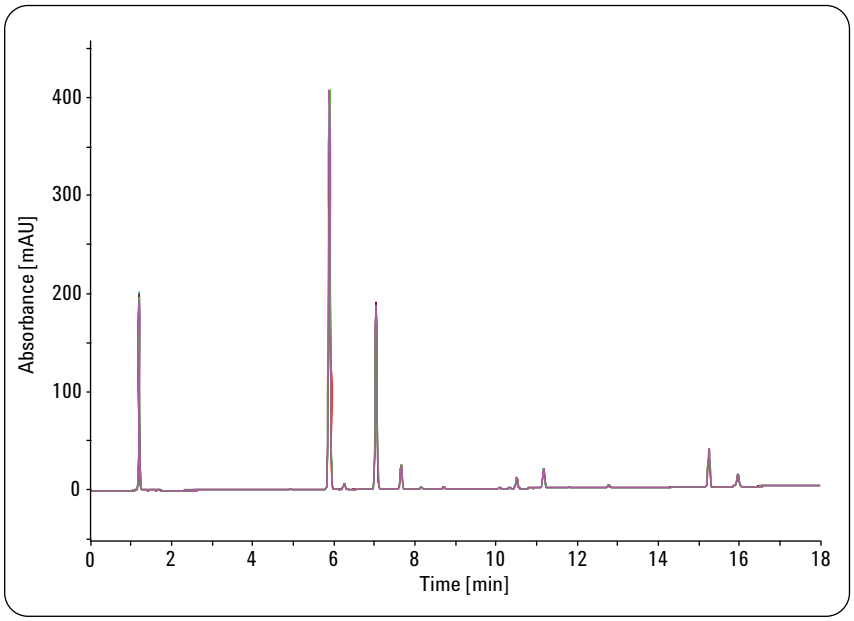


**Figure 3**
**Overlay of six consecutive runs.**

The BHA content in chewing gum was determined using the method developed in this study. Figure 4 shows complete chromatograms of standard and chewing gum extract. Figure 5 shows the part of the chromatogram between 9 and 13 minutes enlarged for more detailed. The sample was prepared by cutting 14.1 g of sugar-free chewing gum in small pieces and extracting with acetonitrile in an ultrasonic bath for 30 min. See figure 1 for other conditions. About 25 ppb BHA could be determined in 1 g of chewing gum. This is a relative value because recovery rates were not evaluated.

## Conclusion

The Agilent 1120 Compact LC was used for the analysis of antioxidants. This instrument was able to analyze these compounds with high precision. Retention time precision was less than 0.05 % RSD and area precicion of baseline separated peaks was less than 0.3 % RSD. The limit of detection was between 1 and 75 ng. In a real-life example, 25 ppb of BHA were found in 1 g of chewing gum after extraction with acetonitrile in an ultrasonic bath.

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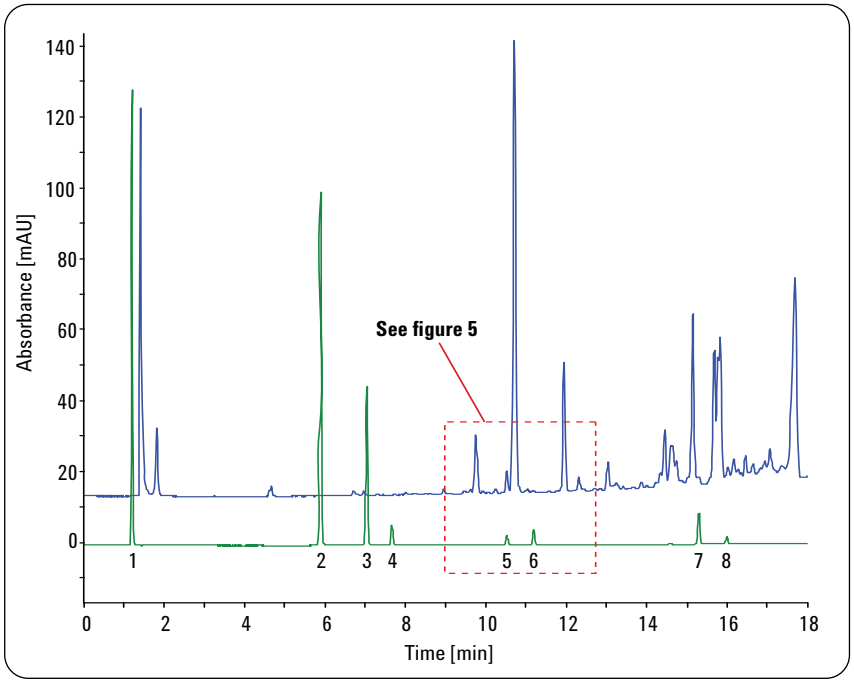


**Figure 4**
**Upper trace–chewing gum extract, lower trace–antioxidant standards.**

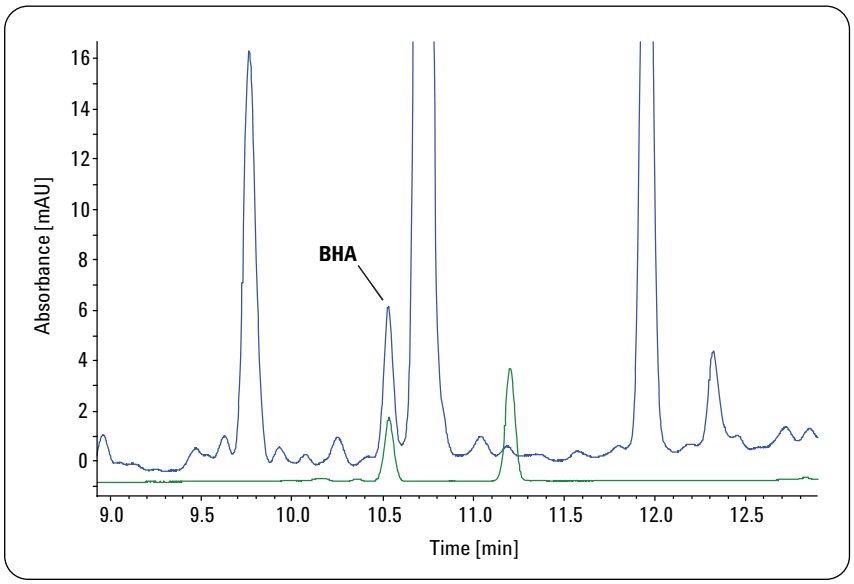


**Figure 5**
**Enlarged view of figure 4, showing the chewing gum extract (upper trace) and the BHA peak at 10.55 min, equivalent to about 25 ng/g or 25 ppb.**

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