# **Application Note**

620018X

### High-Speed Analysis of Food Additives in Functional Beverage by Ultra High-performance Liquid Chromatography with Photodiode Array Detection

#### Introduction

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There are a number of food additives used in beverage to add good flavor and taste, good appearance, long shelf life, and nutritional enrichment. However, those food additives may have a bad effect on health if not properly controlled. Therefore, they are regulated by the Food Sanitation Act.

In this report, food additives in functional beverage are measured and analyzed by Ultra High-performance Liquid Chromatography with Photodiode Array Detection, which enables high speed data acquisition rate at 100 spectra/sec.

Keyword : UHPLC, Functional beverage, Food additives, 1.8 mm, C18 column, PDA detector

#### Experimental

| Equipment.   |                 | Conditions.         |   |
|--------------|-----------------|---------------------|---|
| Pump:        | X-LC 3185PU x 2 | Column:             | ZORBAX Eclipse Plus C18 (3 mmID x 50 mmL, 1.8 mm)   |
| Degasser:    | X-LC 3080DG     | Eluent A:           | 0.3% Phosphoric acid solution   |
| Mixer:       | X-LC 3180MX     | Eluent B:           | Acetonitrile  |
| Column oven: | X-LC 3067CO     | Gradient condition: | (A/B), $0 \min(95/5) \rightarrow 1.2 \min(82/18) \rightarrow 1.5 \min(82/18) \rightarrow$ |
| Autosampler: | X-LC 3159AS     |                     | $2.0 \min(77/23) \rightarrow 2.3 \min(77/23) \rightarrow 5.0 \min(35/65) \rightarrow$     |
| Detector:    | X-LC 3110MD     |                     | $5.05 \min(10/90) \rightarrow 5.05 \min(10/90) \rightarrow 6.0 \min(10/90)$               |
|              |                 |                     | 6.05 min(95/5), 1 cycle 8.0 min   |
|              |                 | Flow rate:          | 0.8 mL/min  |
|              |                 | Column temp.:       | 40°C  |
|              |                 | Wavelength:         | 200-650 nm  |
|              |                 | Injection volume:   | 1 mL  |
|              |                 | Standard sample:    | 12 Food additive standards  |

#### Results

The chromatogram and contour plot of the standard mixture of food additives are shown in Fig. 1 and chromatograms at 4 different wavelengths are shown in Fig. 2. 12 components are clearly separated within 5 min.



Fig. 1. Chromatogram of standard mixture of food additives.

1: Acesulfame K (0.2 mg/mL), 2: p-Hydroxybenzoic acid (0.05 mg/mL), 3: Caffeine(0.05 mg/mL), 4: Vitamin B2 sodium phsphate(Riboflavin sodium phsphate) (0.1 mg/mL), 5: Vitamin B2(Riboflavin) (0.1 mg/mL), 6: Aspartame (0.1 mg/mL), 7: Benzoic acid (0.05 mg/mL), 8: Methyl p-hydroxybenzoate (0.05 mg/mL), 9: Propyl gallate (0.05 mg/mL), 10: Ethyl p-hydroxybenzoate (0.05 mg/mL), 11: Propyl p-hydroxybenzoate (0.05 mg/mL), 12: Butyl p-hydroxybenzoate (0.05 mg/mL)

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620018X



**Fig. 2.** Chromatogram of the standard mixture of food additives at different wavelengths. The peak numbers and corresponding compounds are the same as in Fig. 1.

In Fig. 3, the chromatogram and contour plot of a commercial functional beverage are shown and the chromatograms at 4 different wavelengths are shown in Fig. 4.



Fig. 3. Chromatogram of a functional beverage. The peak numbers and corresponding compounds are the same as in Fig. 1.

Preparation. The commercial functional beverage was filtered with 0.2 mm membrane filter.

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620018X



**Fig. 4.** Chromatograms of the functional beverage at 4 different wavelengths. The peak numbers and corresponding compounds are the same as in Fig. 1.

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