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Application News

No. AD-0095

Food Additives / Nexera X2

Fast and High Sensitivity Analysis of Six Preservatives in Beverages by UHPLC with Photodiode Array Detection

❑ Introduction

Food preservatives are additives to inhibit, retard or prevent mould, acidification or other deterioration of foodstuffs caused by microbial contamination. The most commonly used preservatives in beverages are benzoic acid, sorbic acid and four para-hydroxybenzoic acid esters (parabens). However, excess amounts of these additives can be harmful to consumer health. In this regard, the minimum permissible concentrations of preservatives are regulated in most countries to ensure safety for consumer [1]. Therefore, quantitative analysis of these preservatives in food is not only required for food quality assurance but also important for consumer interest and protection. High performance liquid chromatography (HPLC) has been used for analysis of the preservatives in beverage [2-4]. In this Application News, a new rapid and high sensitivity UHPLC method for simultaneous determination of the six preservatives in beverages is described. A gradient elution was optimized for separation and quantitation of the six preservatives with a photodiode array detector. A capillary flow cell with extra long optical path of 85 mm was employed to achieve high sensitivity for a very small injection amount of sample (1μL) which was not cleaned up except filtration.

❑ Experimental

Preparation of standards and samples

Benzoic acid, sorbic acid and parabens were obtained from chemicals suppliers. A mixed stock solution of 1.0 g/L of benzoic acid, sorbic acid and methyl, ethyl, propyl, butyl parabens were prepared with ethanol/water (70/30) solvent as the diluent. A set of nine working standards was prepared from the stock solution using the same diluent at the concentrations shown in Table 1. Soft drink, mango juice and cocoa drink were purchased at the local supermarket. The soft drink and mango juice were diluted 20 times and 2 times with diluent respectively while cocoa drink was not diluted. All the samples were filtered through a 0.45 μm syringe filter prior to injection to UHPLC.

Table 1: Concentrations of working standards of six preservatives for setting calibration curves

No.	Working Standard	Benzoic acid (mg/L)	Sorbic acid (mg/L)	Parabens (mg/L)
1	S1	0.2	0.008	0.01
2	S2	2.0	0.08	0.1
3	S3	4.0	0.16	0.2
4	S4	20.0	0.8	1.0
5	S5	60.0	2.4	3.0
6	S6	80.0	3.2	4.0
7	S7	100.0	4.0	5.0
8	S8	150.0	6.0	7.5
9	S9	200.0	8.0	10.0

Instrumental and analytical conditions

A Nexera X2 UHPLC system (Shimadzu Corporation, Japan) was used in this work. The system is consisted of a high pressure binary gradient solvent delivery unit (LC-30AD pumps) and an UHPLC autosampler (SIL-30AC) coupled to a photodiode array detector (SPD-M30A) with a high sensitivity capillary flow cell (85mm optical path length) featured as total reflection and low dispersion. A YMC Triart C18 column of 1.9μm particle size (150mmL. x 2.0mm I.D.) was used for the separation of preservatives (benzoic acid, sorbic acid and methyl, ethyl, propyl, butyl parabens) with an optimized linear gradient program developed. The details of the LC conditions are shown in Table 2.

Table 2: Analytical conditions of preservatives in beverages on Nexera X2 UHPLC

Column	YMC Triart C18 1.9 μm 150 x 2.0mm I.D.
Flow Rate	0.45 mL/min
Mobile Phase	A: 1.5% acetic acid+1.5% ammonium acetate in H2O B: 1.5% ammonium acetate in MeOH
Elution Mode	Gradient elution: 40% B (0.01 to 4.0 min) ➔ 80% B (4.01 to 5.5 min) ➔ 40% B (5.51 to 8.5min)
Oven Temp.	45ºC
Injection Volume	1 μL
Detection (PDA)	Wavelength 240~600nm; Ref: 720nm Quant, 240nm for benzoic acid, 260nm for other compounds

❑ Results and Discussion

Method Development

The six preservatives were well-separated as sharp peaks between 1.7 min and 5.1 min as shown in Figure 1. The total run time of the UHPLC method is 8.5 mins, which is several times faster than the HPLC method reported [2-4]. It is worth to note that two wavelengths were selected for quantitative data processing, i.e., 240 nm for benzonic acid and 260 nm for the rest five compounds [4].

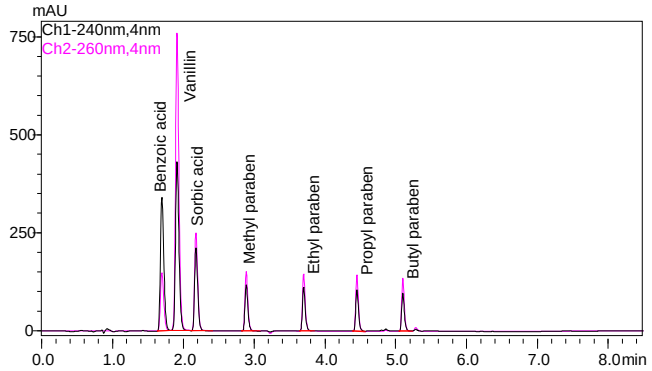

Figure 1: Chromatograms of mixed standard (S3) with 1µL injection volume on Nexera X2.

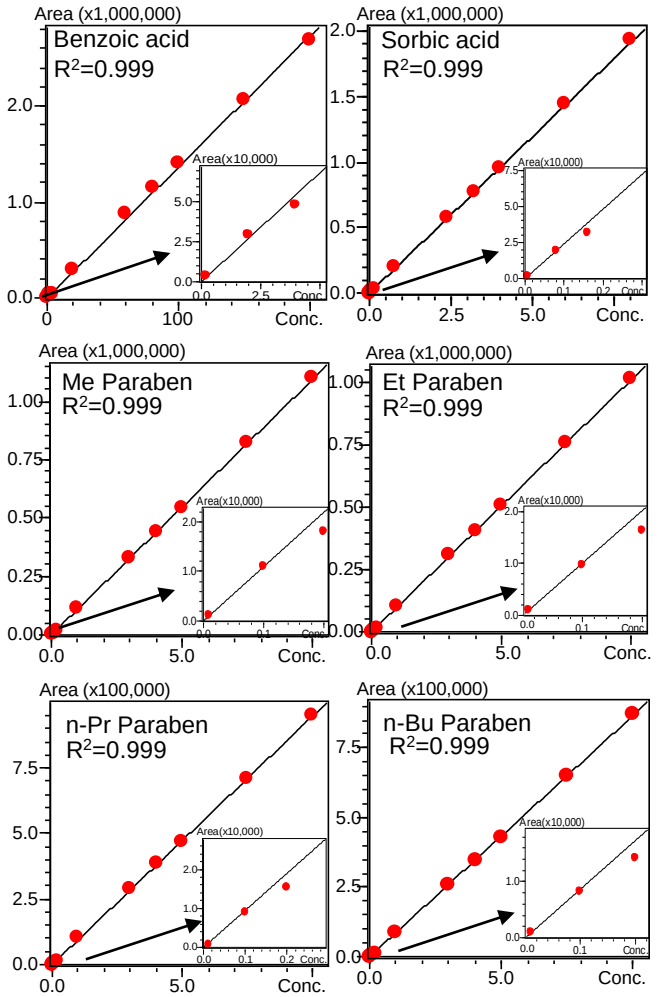

Figure 2: Calibration curves of six preservatives at concentration S1~S9 (see Table 1)

Figure 2 shows the calibration curves of the six compounds established with 1µL injection volume. The linearity with correlation coefficient (R^2) greater than 0.999 across the wide calibration range of 0.008~200 mg/L was obtained for the six compounds.

The repeatability of the method was evaluated at the levels S2 and S5. The peak area %RSD for the six compounds were lower than 5.1% and 0.3% respectively (Table 3).

Table 3: Results of repeatability evaluation using working standard S2 and S5 (n=6, 1µL injection)

Compound	Conc. (mg/L)	RSD%	Conc. (mg/L)	RSD%
Benzoic acid	2.0	1.1	60.0	0.2
Sorbic acid	0.08	1.5	60.0	0.2
Methyl paraben	0.1	1.2	2.4	0.2
Ethyl paraben	0.1	3.8	3.0	0.2
Propyl paraben	0.1	3.2	3.0	0.2
Butyl paraben	0.1	5.1	3.0	0.3

The LOD and LOQ of the method, and peak identification criteria (RT & λ_{Max}) are summarized in Table 4. The results were obtained from the mixed standard S1 (Figure 3). The high sensitivity achieved, i.e., LOQs ranging at 8~10 µg/L of the compounds except benzoic acid (280 µg/L), is attributed partially to the use of a high sensitivity SPD-M30A detector with using a capillary cell of 85mm optical path.

Table 4: LOD (S/N=3), LOQ (S/N=10) and peak identification criteria of UHPLC method obtained from S1 chromatogram

Compound	Conc. (µg/L)	RT	λ_{Max}	LOD (µg/L)	LOQ (µg/L)
Benzoic acid	200	1.702	238	90	280
Sorbic acid	8	2.183	257	2.7	8
Methyl paraben	10	2.866	258	3	10
Ethyl paraben	10	3.687	257	3	10
Propyl paraben	10	4.445	258	3	10
Butyl paraben	10	5.091	256	3	10

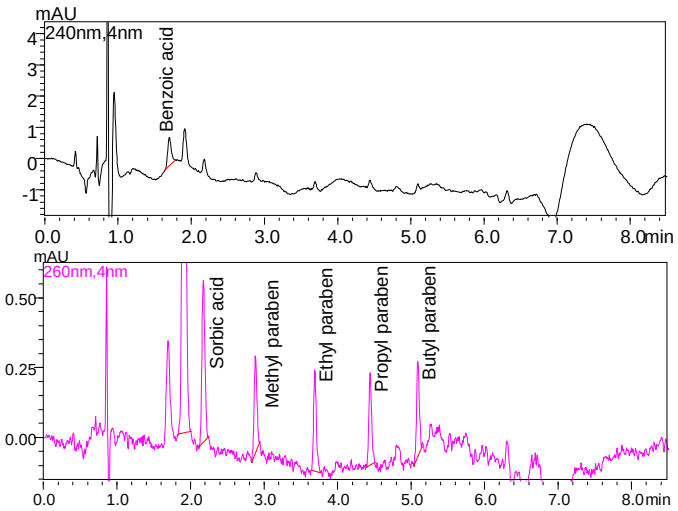

Figure 3: Chromatogram of mixed standard S1 (1µL).

Analysis of beverage samples

The UHPLC method established was applied for quantitation of preservatives in three kinds of beverages: soft drink B1, fruit juice B2 and cocoa drink B3. The chromatograms of the samples are shown in Figure 4 and the results are summarized into Table 5. No preservatives was detected in cocoa drink. Benzoic acid and sorbic acid were detected in the soft drink and fruit juice. The identification of both benzoic acid and sorbic acid peaks were confirmed by UV spectra.

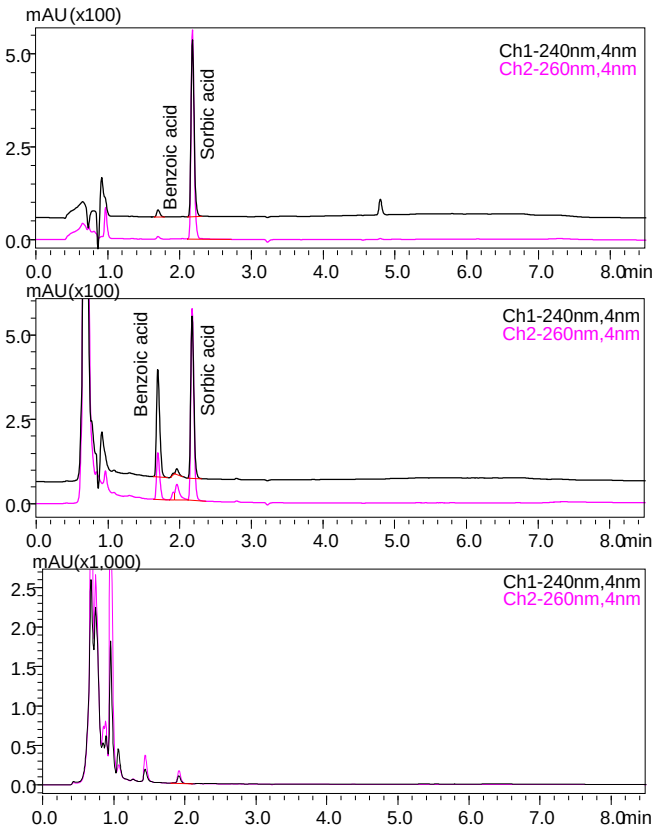

Figure 4: Chromatogram of beverage samples (injection: 1μL): Soft drink with 20 times dilution (top); Fruit drink with 2 times dilutions; Cocoa drink without dilution (bottom).

Table 5: Quantitative results of six preservatives in three beverages, each with duplicate injections

Sample Name	Benzoic acid		Sorbic acid		Parabens	
	RT (min)	Conc (mg/L)	RT (min)	Conc (mg/L)	RT (min)	Conc (mg/L)
B1	1.70	82.4	2.18	137.4	ND	
B2	1.70	142.4	2.18	13.8		
B3	ND					

❑ Conclusions

A rapid and high sensitivity UHPLC method for quantitation of six preservatives, benzoic acid, sorbic acid and four para-hydroxybenzoic acid esters (parabens), in beverages was established using a reversed phase UHPLC column (1.9μm particle size). A capillary flow cell with extra long optical path of 85 mm was employed in the photodiode array detector. The method achieves LOQs ranging 8-10 μg/L for the compounds except benzoic acid (280 μg/L), with 1μL injection volume. The very small injection volume minimizes the contamination of beverage samples to the column and system, as such suitable for direct analysis of beverage samples without need for clean up procedure.

❑ References

1. Singapore Agri-Food and Veterinary Services website: Food Regulations – AVA (2014)
2. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science: *Determination of Benzoic acid, Sorbic acid and Methyl, Ethyl, Propyl and Butyl Parabens by HPLC* (2004)
3. Tzu-Yun Chu, Chine-Lin Chen and Hsueh-Fang Wang, *A Rapid Method for the Simultaneous Determination of Preservatives in Soy Sauce,* Journal of Food and Drug Analysis, Vol. 11, No. 3, Pages 246-250 (2003)
4. Shu-Xiang Sun, Shu-Bai Li and Pei Yao, *Simultaneous Determination of Six Preservatives in Beverage by HPLC,* 3rd International Conference on Material, Mechanical and Manufacturing Engineering (2015)

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