Analysis of Food Preservatives Using the Agilent 1290 Infinity II LC

Application Note

Food Testing and Agriculture

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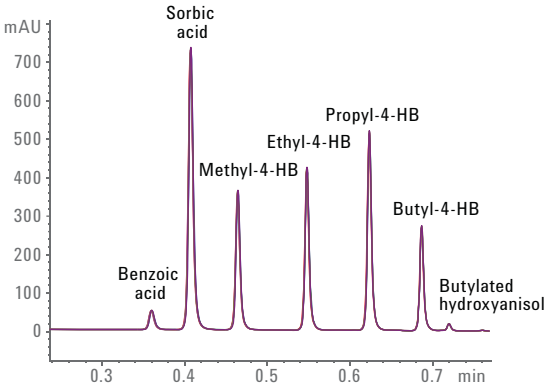
Abstract

This Application Note describes method transfer from HPLC to UHPLC of seven typically used food preservatives using the Agilent 1290 Infinity II LC. An enormous time and solvent savings of about 90 % was achieved with a UHPLC separation optimized for speed. Using a 2.1-mm id column, the amount of injected sample could be reduced by 75 %. Both HPLC and UHPLC methods achieved excellent precision, resolution, and linearity as well as comparable limits of detection and quantification.



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Introduction

Preservatives are widely used to prevent microbiological growth in food, beverages, and cosmetics. The most commonly used preservatives are parabens (parahydroxybenzoates or esters of 4-hydroxybenzoic acid) and benzoic and sorbic acid. However, some preservatives can have negative influences on metabolism after accumulation in the human body. Therefore, their use is subject to strong regulations due to possible health problems caused from preservative overdose. During quality control analysis, the products are monitored regarding their preservative content.

Conventional high performance liquid chromatography (HPLC) methods are routinely used for food monitoring[1] as one of the most reliable and rugged analysis techniques. Recently, an increased need for faster analyses with higher resolving power has been observed. With sub-2 µm (STM) technology particles and high-pressure systems (up to 1,300 bar), ultra high performance liquid chromatography (UHPLC) has been enabled. Based on the variety of new possibilities of UHPLC systems with STM columns, many classical HPLC methods have been transformed to UHPLC methods.

This Application Note shows the method transfer from HPLC to UHPLC for the analysis of typically used food preservatives using the Agilent 1290 Infinity II LC.

Experimental

Instrumentation

The Agilent 1290 Infinity II LC System used for the experiments consisted of the following modules:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B), equipped with a 10-mm Max-Light cartridge cell

Columns

- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
- Agilent ZORBAX RRHT SB-C18, 4.6 × 50 mm, 1.8 µm (p/n 846975-902)
- Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS systems, version C.01.07 [27]

Solvents

A) Water + 20 mM ammonium formate, pH 4.4

B) Acetonitrile

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). The preservative standards were purchased from Sigma-Aldrich, St. Louis, Missouri, USA.

Sample

The sample was a mix of seven typically used food preservatives, each 50 ng/µL.

- Benzoic acid
- Sorbic acid
- Methyl-4-hydroxybenzoate (methyl-4-HB)
- Ethyl-4-hydroxybenzoate (ethyl-4-HB)
- Propyl-4-hydroybenzoate (propyl-4-HB)
- Butyl-4-hydroybenzoate (butyl-4-HB)
- Butylated hydroyanisol

Table 1. Chromatographic conditions for HPLC with a 4.6 × 150 mm, 5-µm column.

Parameter	Value
Mobile phase	A) Water + 20 mM ammonium formate, pH 4.4 B) Acetonitrile
Flow rate	1 mL/min
Gradient	10 %B at 0 minutes 60 %B at 10 minutes 80 %B at 13 minutes 95 %B at 14 minutes
Stop time	17 minutes
Post time	10 minutes
Injection volume	5 µL
Column temperature	40 °C
Detection	Signal 260/40 nm, reference 380/100 nm Peak width > 0.025 minutes (0.5-seconds response time) Data rate 10 Hz

Table 2. Chromatographic conditions for HPLC with a 4.6 × 50 mm, 1.8-µm column.

Parameter	Value
Mobile phase	A) Water + 20 mM ammonium formate, pH 4.4 B) Acetonitrile
Flow rate	1 mL/min
Gradient	10 %B at 0 minutes 60 %B at 3.5 minutes 80 %B at 4.5 minutes 95 %B at 5 minutes
Stop time	5.5 minutes
Post time	5 minutes
Injection volume	5 µL
Column temperature	40 °C
Detection	Signal 260/40 nm, reference 380/100 nm Peak width > 0.025 minutes (0.5-seconds response time) Data rate 10 Hz

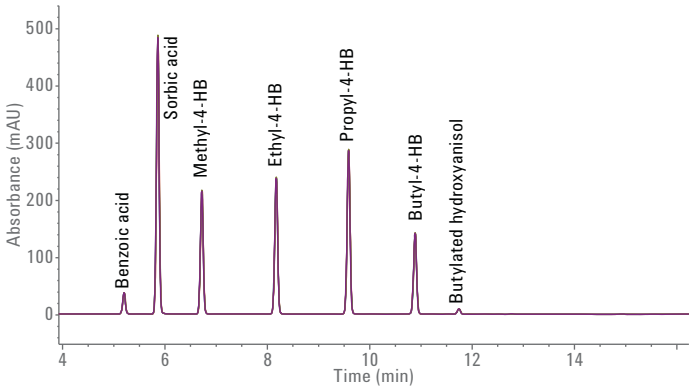
Table 3. Chromatographic conditions for HPLC with a 2.1 × 50 mm, 1.8-µm column, optimized for speed.

Parameter	Value
Mobile phase	A) Water + 20 mM ammonium formate, pH 4.4 B) Acetonitrile
Flow rate	1.5 mL/min
Gradient	10 %B at 0 minutes 60 %B at 0.5 minutes 80 %B at 0.6 minutes 95 %B at 0.7 minutes
Stop time	1 minutes
Post time	1 minutes
Injection volume	1.25 µL
Column temperature	40 °C
Detection	Signal 260/40 nm, reference 380/100 nm Peak width > 0.0031 minutes (0.063-seconds response time) Data rate 80 Hz

Results and Discussion

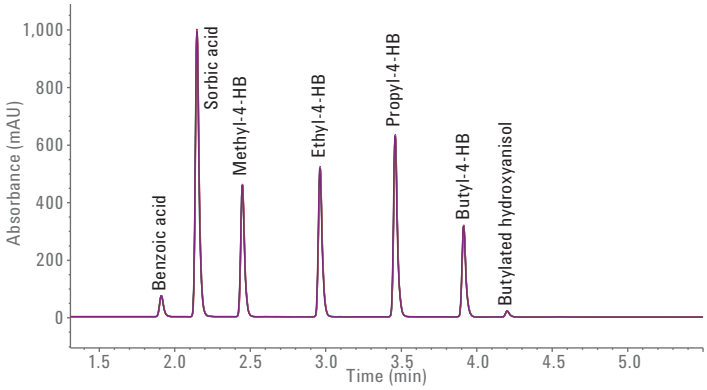
The preservative standards were analyzed under HPLC conditions (Figure 1). Six consecutive runs were analyzed for their precision regarding retention time, area, and resolution. The relative standard deviations (RSD) of retention time and area were found to be excellent, below 0.032 and 0.4 % respectively.

To shorten the analysis time of the preservative standards, the method was transferred to a UHPLC method using an Agilent ZORBAX SB-C18, 4.6 × 50 mm, 1.8-µm column. The total cycle time was shortened from 27 to 10.5 minutes, resulting in a total time and solvent savings of over 60 %. Figure 2 shows the overlay of six consecutive runs on the shorter column together with the RSD values for retention time and area, and the resolution values. The RSDs for retention time and area were still found to be excellent, with values below 0.04 and 0.5 % respectively. Also, the resolution was still comparable to the HPLC method.



Compound	RT RSD (%)	Area RSD (%)	Resolution
Benzoic acid	0.031	0.396	19.0
Sorbic acid	0.021	0.309	6.5
Methyl-4-HB	0.016	0.327	8.4
Ethyl-4-HB	0.01	0.320	13.7
Propyl-4-HB	0.014	0.325	13.1
Butyl-4-HB	0.011	0.294	11.7
Butylated hydroxyanisol	0.008	0.327	7.7

Figure 1. HPLC Analysis of seven food preservatives (overlay of six consecutive runs) using an Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5-µm column.



Compound	RT RSD (%)	Area RSD (%)	Resolution
Benzoic acid	0.039	0.496	13.3
Sorbic acid	0.036	0.445	5.0
Methyl-4-HB	0.034	0.488	6.6
Ethyl-4-HB	0.028	0.435	11.3
Propyl-4-HB	0.023	0.453	10.9
Butyl-4-HB	0.018	0.439	9.9
Butylated hydroxyanisol	0.018	0.497	6.2

Figure 2. UHPLC Analysis of seven food preservatives (overlay of six consecutive runs) using an Agilent ZORBAX SB-C18, 4.6 × 50 mm, 1.8-µm column.

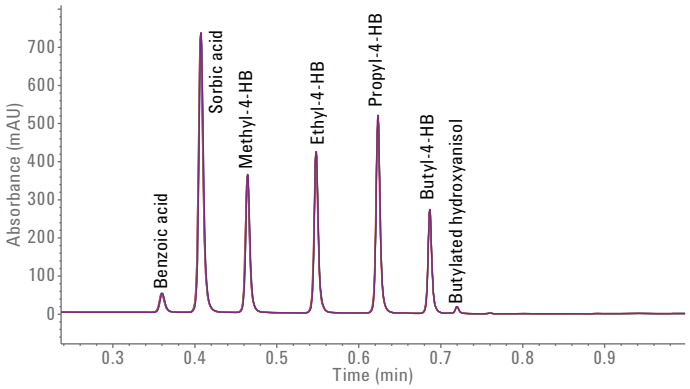
To enable the option of an ultrafast separation and reduce the injected sample volume, an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8 µm column was used. With a UHPLC method optimized for speed, a separation within 0.75 minutes was possible at a flow rate of 1.5 mL/min. Figure 3 shows an overlay of six consecutive runs on the 2.1-mm column together with the values for retention time and area precision and resolution.

The RSDs of retention time and area were found to be below 0.1 and 0.4 % respectively for six consecutive runs. Regarding the more than 20-times shortening of the analysis time, the RSDs of the UHPLC method were still superb. In addition, the peak resolution was still represented by baseline-separated peaks.

In comparison to the HPLC method using a 4.6 × 150 mm, 5-µm column, an enormous time savings was possible, from 27 to 2 minutes total cycle time. With a flow rate of 1.5 mL with the 2.1-mm UHPLC column (instead of 1 mL with the 4.6-mm column), a total solvent savings of nearly 90 % was achieved based on the short run time. In addition, 75 % of the sample could be saved.

All three methods were evaluated regarding linearity, limit of detection (LOD), and limit of quantification (LOQ). Ten different concentration levels (from 100 µg/mL to 0.195 µg/mL, at a dilution of 1:2) were prepared from the stock solutions, and the linear relationship was determined between the peak area and the corresponding concentrations. LOD and LOQ were defined as the signal-to-noise (S/N) ratio of 3:1 and 10:1 respectively.

Table 4 shows the results of the evaluation. All three methods showed high linearity with correlation coefficients over 0.9999 for all standards except butylated hydroxyanisol.



Compound	RT RSD (%)	Area RSD (%)	Resolution
Benzoic acid	0.099	0.144	9.1
Sorbic acid	0.071	0.082	4.3
Methyl-4-HB	0.049	0.168	5.7
Ethyl-4-HB	0.037	0.106	8.9
Propyl-4-HB	0.031	0.370	8.2
Butyl-4-HB	0.022	0.074	7.2
Butylated hydroxyanisol	0.017	0.359	3.9

Figure 3. UHPLC Analysis of seven food preservatives (overlay of six consecutive runs) using an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8-µm column.

Table 4. Comparison of linearity between the HPLC and the two UHPLC methods.

Linearity	4.6 × 150 mm, 5 µm	4.6 × 50 mm, 1.8 µm	2.1 × 50 mm, 1.8 µm
Benzoic acid	0.99996	1.0	0.99997
Sorbic acid	0.99997	0.99999	0.99996
Methyl-4-HB	0.99996	1.0	0.99995
Ethyl-4-HB	0.99997	1.0	0.99995
Propyl-4-HB	0.99996	0.99999	0.99994
Butyl-4-HB	0.99997	1.0	0.99997
Butylated hydroxyanisol	0.99998	0.99915	0.99883

For five out of seven preservative standards, LOD and LOQ were improved using the UHPLC conditions (Table 5 and Table 6). For the first and last peak, the values remained about the same under HPLC and UHPLC conditions.

Conclusion

This Application Note shows the analysis of seven typically used food preservatives with an Agilent 1290 Infinity II LC. The HPLC method was transferred to UHPLC from a standard HPLC column (4.6 × 150 mm, 5 µm) to two short UHPLC columns (4.6 and 2.1 × 50 mm, 1.8 µm). The first transfer to the 4.6 × 50 mm, 1.8-µm column enabled a time and solvent savings of about 60 %. The separation on the 2.1 × 50 mm, 1.8-µm column was optimized for speed with a flow rate of 1.5 mL/min, resulting in a total cycle time of 2 minutes. Hence, an enormous time savings was achieved, and a 75 % reduction of injected sample. Ultimately, a total solvent savings of nearly 90 % was achieved based on the short run time. The evaluation of both HPLC and UHPLC methods revealed excellent precision, resolution, and linearity as well as comparable limits of detection and quantification.

Reference

1. Gratzfeld-Huesgen, A; Schuster, R. HPLC for Food Analysis, *Agilent Technologies Primer*, publication number 5988-3294EN, September **2001**.

Table 5 Comparison of LOD between the HPLC and the two UHPLC methods.

LOD (pg)	4.6 × 150 mm, 5 µm	4.6 × 50 mm, 1.8 µm	2.1 × 50 mm, 1.8 µm
Benzoic acid	180.3	48.3	183.1
Sorbic acid	20.5	2.1	13.8
Methyl-4-HB	46.5	11.5	29.3
Ethyl-4-HB	41.9	13.3	23.6
Propyl-4-HB	34.1	9.0	19.8
Butyl-4-HB	84.7	7.5	24.4
Butylated hydroxyanisol	781.3	293.7	651.3

Table 6 Comparison of LOQ between the HPLC and the two UHPLC methods.

LOQ (pg)	4.6 × 150 mm, 5 µm	4.6 × 50 mm, 1.8 µm	2.1 × 50 mm, 1.8 µm
Benzoic acid	601.0	161.1	610.3
Sorbic acid	68.3	7.1	46.1
Methyl-4-HB	155.0	38.3	97.6
Ethyl-4-HB	139.5	44.4	78.7
Propyl-4-HB	113.5	29.9	66.0
Butyl-4-HB	282.4	25.0	81.4
Butylated hydroxyanisol	2604.2	979.0	2170.8

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