Ultrafast Analysis of Food Preservatives Using an Entry-Level Agilent 1290 Infinity II LC

Suitable for Agilent
1290 Infinity III LC

Application Note

Food Testing and Agriculture

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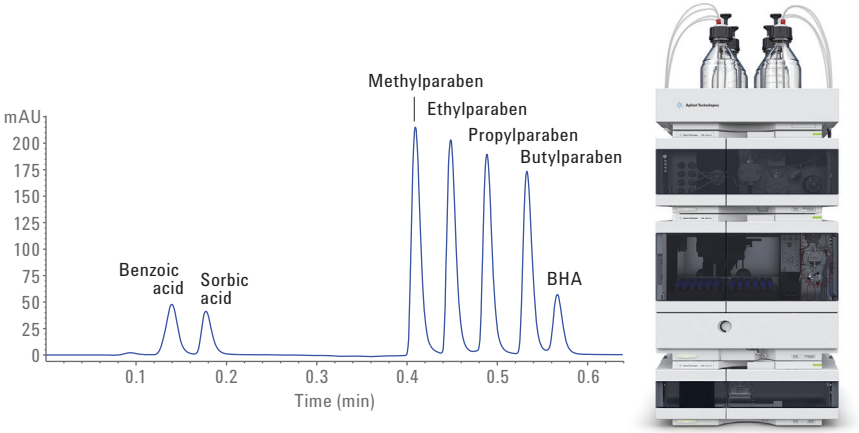
Abstract

Ultrahigh performance LC systems are the preferred choice when analytical separations require short run times. The Agilent 1290 Infinity II LC is a modular system available in different configurations tailored for varying demands. This Application Note shows the ultrafast analysis of seven food preservatives using a 1290 Infinity II LC with a compact, entry-level configuration. A separation method was developed on an Agilent 1290 Infinity LC, and transferred to the 1290 Infinity II LC for comparison. After optimization, a separation and quantification within less than 0.6 minutes was achieved on both systems. Repeatability and linearity were comparable and excellent on both the 1290 Infinity and the 1290 Infinity II LC.



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Introduction

The key benefit of ultrahigh performance LC systems is the ability to use columns with small particles, which requires a system with high-pressure stability. With sufficiently small particle size, the separation of even complex mixtures can be achieved in well below one minute. This is especially useful to speed up routine analyses with large sample quantities.

One approach to increase the efficiency of an analytical system while decreasing analysis time is to replace conventional systems with an ultrahigh performance system such as an Agilent 1290 Infinity II LC. With a compact, entry-level configuration such as listed below, the user can take full advantage of the high-pressure compatibility and low dispersion of an ultrahigh performance LC system:

- Agilent 1290 Infinity II Flexible Pump
- Agilent 1290 Infinity II Vialsampler with integrated sample cooler and column compartment
- Agilent 1290 Infinity II Variable Wavelength Detector (VWD)

This Application Note compares the performance of a 1290 Infinity II LC system in a basic configuration with a 1290 Infinity LC equipped with equivalent modules. The latter system might appear as an affordable choice to enter the world of highly efficient Agilent ultrahigh performance LC systems. However, unlike its successor, the 1290 Infinity LC lacks the possibility of integrating a sample cooler or column compartment. These have to be configured as individual modules, increasing the investment and the height of the system stack. In fact, the 1290 Infinity II LC, configured as described in this Application Note, is as affordable as its predecessor, while providing new and improved features that contribute to higher efficiency and a more compact, less complex design.

A sample of seven commonly used food preservatives was separated in the shortest time possible. The method was developed and optimized on a 1290 Infinity LC, the preceding high-end Agilent system, then transferred to a 1290 Infinity II LC in entry-level configuration. The performance of both systems was evaluated by calculating the relative standard deviations (RSDs) of retention time (RT) and peak area out of 10 consecutive runs. A calibration of all seven compounds was carried out, and linearity as well as limits of detection (LOD) and limits of quantification (LOQ) were determined.

Experimental

Instrumentation

The Agilent 1290 Infinity LC consisted of the following modules:

- Agilent 1290 Infinity Quaternary Pump (G4204A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Variable Wavelength Detector (G1314E), equipped with a 10 mm standard flow cell (Option #018)

The Agilent 1290 Infinity II LC had the following, entry-level configuration:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Vialsampler (G7129B) with integrated sample cooler (Option #100) and column compartment (Option #063)
- Agilent 1290 Infinity II Variable Wavelength Detector (G7114B), equipped with a 10 mm standard flow cell (Option #018)

Column

Agilent ZORBAX Eclipse Plus C18 RRHD 2.1 × 50 mm, 1.8 µm (p/n 959757-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR1 [106]

Solvents and samples

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). Standards of the seven preservatives were purchased from Sigma-Aldrich, St. Louis, Missouri, USA.

Chromatographic conditions

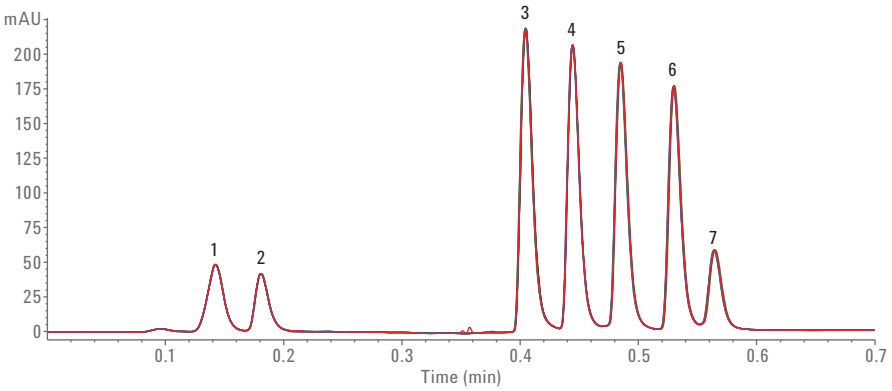
Parameter	Value
Mobile phase	A) Water + 20 mM ammonium formate, pH = 4.4 B) Acetonitrile
Flow rate	1.9 mL/min
Gradient	0.0 minutes – 8 %B 0.1 minutes – 60 %B 0.5 minutes – 60 %B 0.6 minutes – 98 %B
Stop time	0.7 minutes
Post time	1.0 minutes
Needle wash mode	3 seconds standard wash
Injection volume	5 µL
Sample temperature	8 °C
Column temperature	60 °C
Detection	254 nm Peak width > 0.0063 minutes (0.13 seconds response time) (80 Hz)

Results and Discussion

A mix of seven commonly used food preservatives was separated on a 1290 Infinity LC with focus on high throughput. The method was first optimized to achieve baseline separation of all compounds within less than 0.6 minutes, then transferred to a 1290 Infinity II LC with an equivalent system configuration. System performance was evaluated by calculating the RSDs of RT and peak area for each compound based on 10 consecutive runs. Calibrations of all compounds were carried out in triplicate on both systems, and evaluated for linearity, LODs, and LOQs.

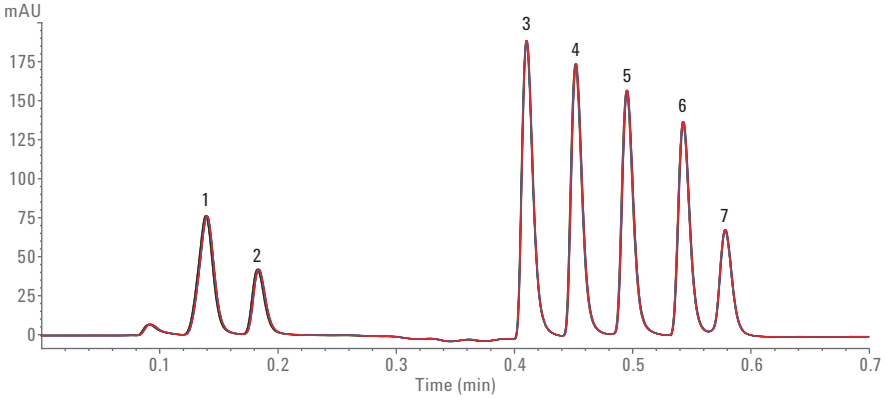
Figure 1 shows a chromatogram overlay of 10 consecutive runs conducted with a high-throughput optimized method on a 1290 Infinity LC. All compounds were baseline separated, which is considered to be achieved with a resolution of 1.6 or higher[1]. RT RSDs were excellent, and below 0.09 %. Injection volume repeatability was precise, yielding area RSDs below 0.18 % for all compounds.

The method was transferred to a 1290 Infinity II LC, and the analysis was repeated using the same settings as the 1290 Infinity LC. Figure 2 shows a chromatogram overlay of 10 consecutive runs on the 1290 Infinity II LC, along with peak properties for each analyte. As on the preceding system, all compounds were baseline separated. Resolution of the different analytes was 1.9 or greater, which was slightly better than on the 1290 Infinity LC. RT precision was excellent, with RT RSDs below 0.07 % for all but two compounds. The absolute deviation of RTs between the 1290 Infinity LC and 1290 Infinity II LC was 0.013 minutes or less (< 3.9 %). This is well within the specifications of the Agilent Intelligent System Emulation Technology (ISET), a software-based emulation of earlier Agilent and non-Agilent LC systems available for the 1290 Infinity II LC[2]. Injection precision (area RSD) of the 1290 Infinity II LC was excellent, and better than 0.18 % for all compounds. Both instruments delivered an equivalent and excellent separation of the food preservatives sample.



Peak	Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
1	Benzoic acid	0.140	0.085	0.052	—
2	Sorbic acid	0.177	0.068	0.112	1.66
3	Methylparaben	0.409	0.054	0.100	12.09
4	Ethylparaben	0.448	0.037	0.132	2.20
5	Propylparaben	0.488	0.058	0.141	2.24
6	Butylparaben	0.533	0.062	0.172	2.46
7	Butylated hydroxyanisole	0.566	0.066	0.113	1.82

Figure 1. High-throughput separation of seven food preservatives on an Agilent 1290 Infinity LC. Chromatogram overlay and peak properties of 10 consecutive runs.



Peak	Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
1	Benzoic acid	0.140	0.387	0.172	—
2	Sorbic acid	0.184	0.314	0.094	2.04
3	Methylparaben	0.411	0.053	0.072	12.63
4	Ethylparaben	0.452	0.065	0.083	2.44
5	Propylparaben	0.495	0.063	0.038	2.50
6	Butylparaben	0.543	0.053	0.062	2.71
7	Butylated hydroxyanisole	0.579	0.043	0.081	1.97

Figure 2. High-throughput separation of seven food preservatives on an Agilent 1290 Infinity II LC. Chromatogram overlay and peak properties of 10 consecutive runs.

Calibrations of each of the seven food preservatives were carried out on both systems described in the Instrumentation section. Calibration curves were constructed using linear regression. All compounds were calibrated between 1.56 and 200 ng/µL, except sorbic acid, which was calibrated between 0.04 and 5 ng/µL. On both the 1290 Infinity and the 1290 Infinity II LC, correlation coefficients were 0.999 or greater for each analyte. LODs and LOQs were extrapolated based on a signal-to-noise ratio of 3 and 10, respectively, determined by USP method. LODs were below 0.5 ng, and LOQs below 1.7 ng for all compounds except butylated hydroxyanisole (Table 1). Sensitivity and linearity were at excellent levels, and comparable on both systems.

Conclusion

This Application Note describes the ultrafast analysis of a mix of seven food preservatives on an Agilent 1290 Infinity II LC with entry-level configuration. A method was developed and optimized on an Agilent 1290 Infinity LC, an earlier Agilent high-end LC system. Baseline separation was achieved within less than 0.6 minutes, while RT and peak area precision were on excellent levels, with RSDs below 0.09 and 0.18 %, respectively. A calibration of the sample yielded LOQs below 1.5 ng for six of the seven compounds. When the method was transferred to a 1290 Infinity II LC with equivalent configuration sensitivity, linearity and RT precision were on comparable, excellent levels. Area precisions were slightly better for six of the seven compounds analyzed. These data show that even an entry-level 1290 Infinity II LC delivers the excellent performance and full benefits of an ultrahigh performance LC system. When sophisticated features of a high-end 1290 Infinity II LC, such as multiple-solvent needle wash, dual needle injection, 16 microtiter-plate sample capacity, or full UV-Vis spectra acquisition, are not crucial for the analysis, a 1290 Infinity II LC in a basic configuration offers the best value. Since all 1290 Infinity II modules are compatible and interchangeable, a system upgrade towards a high-end configuration to meet future demands always remains possible.

References

1. The LC Handbook, *Agilent Technologies Primer*, publication number 5990-7595EN, **2016**.

2. Agilent 1290 Infinity with ISET, *Agilent Technologies User Manual*, publication number G4220-90313, **2014.**

Table 1. Calibration data of seven food preservatives separated on an Agilent 1290 Infinity LC or an Agilent 1290 Infinity II LC: Calibrated concentration range, linear regression correlation coefficients, and LODs and LOQs.

Compound	c_{low} (ng/µL)	c_{high} (ng/µL)	Agilent 1290 Infinity LC			Agilent 1290 Infinity II LC		
			Correlation	LOD (ng)	LOQ (ng)	Correlation	LOD (ng)	LOQ (ng)
Benzoic acid	1.56	200	1.000	0.44	1.47	1.000	0.49	1.62
Sorbic acid	0.04	5	1.000	0.10	0.33	1.000	0.02	0.06
Methylparaben	1.56	200	1.000	0.25	0.83	0.999	0.05	0.17
Ethylparaben	1.56	200	1.000	0.27	0.91	0.999	0.06	0.21
Propylparaben	1.56	200	1.000	0.27	0.91	0.999	0.07	0.23
Butylparaben	1.56	200	1.000	0.31	1.05	0.999	0.08	0.26
Butylated hydroxyanisole	1.56	200	1.000	5.16	17.19	0.999	5.37	17.90

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