Agilent 1290 Infinity Quaternary LC – Support of Columns with 2.1 to 4.6 mm ID to 1200 bar

Technical Overview

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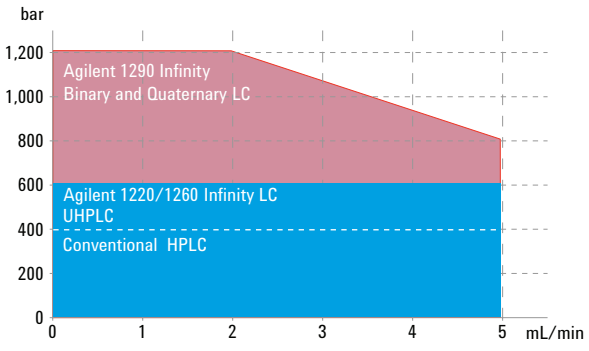
Abstract

Modern LC equipment should be prepared to support conventional HPLC and also UHPLC applications. The power range should include the capability of a high pressure range but also the possibility to apply high flow rates and column compartment temperatures up to 100 °C. This enables the usage of analytical columns of different lengths, different internal diameters, and different particle sizes.

The Agilent 1290 Infinity Quaternary LC System fulfills all these demands. This Technical Overview illustrates, how the 1290 Infinity Quaternary LC systems supports applications up to the edges of its power range of flow rates of 2 mL/min at 1,200 bar and 5 mL/min at 800 bar.



Verified for Agilent
1290 Infinity II LC

Agilent Technologies

Introduction

There is a strong demand to be prepared for more and more UHPLC methods at high pressure, using columns with sub-2-µm particles, and at the same time, to be able to run established conventional methods. In addition, there is a need for a wide flow range to allow ultrafast applications at high flow rates. Modern LC equipment such as the 1290 Infinity Quaternary LC is designed to fulfill all these demands. The 1290 Infinity Quaternary Pump supports flow rates of 2 mL/min and up to 1,200 bar and 5 mL/min up to 800 bar.

This allows the use of analytical columns of different internal diameters and lengths. Typical IDs are 2.1, 3.0, and 4.6 mm, and the column lengths vary between 50 and 250 mm.

The following experiments show that using the 1290 Infinity Quaternary LC System, a wide flow and pressure range is covered using analytical columns of different internal diameter and length applying conventional, fast and ultrafast chromatographic conditions. A sample set of eight compounds was selected, with different hydrophobicity eluting across the whole gradient.

Experimental

Instrumentation

Description	Model number
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 Diode Array Detector	G4212A

Acquisition and Evaluation Software

OpenLAB CDS ChemStation version C.01.04

Chromatographic Conditions

Parameter	Setting
Injection volume	1 to 3 µL depending on the column dimension
Column temperature	45 °C
Mobile phases	A = Water, B = Acetonitrile, C = Methanol, D = 1% TFA in water
Detection	280/10 nm, Ref 400/100 nm, flow cell 10 mm, peak width > 0.013 minutes (20 Hz)

Samples

Antioxidants such as:

PG = Propyl gallate
THBP = 2,4.5-trihydroxybutyrophenone
TBHQ = Tert-butylhydroquinone
BHA = Butylated hydroxyanisole
Ionox 100 = 2,6-DI-tert-butyl-4-hydroxymethylphenol
OG = Octyl gallate
BHT = Butylated hydroxytoluene
LG = Lauryl gallate

Columns

Column type	Dimensions	Part number	Experiment*
Agilent ZORBAX SB C18	4.6 × 150 mm, 5 µm	685975-902	1
Agilent ZORBAX SB C18	3 × 100 mm, 3.5 µm	861954-302	2
Agilent Poroshell 120 SB C18	4.6 × 100 mm, 2.7 µm	685975-902	3
Agilent Poroshell 120 EC C18	4.6 × 50 mm, 2.7 µm	699975-902	4
Agilent ZORBAX RRHD SB C18	3 × 50 mm, 1.8 µm	857700-302	5 and 10
Agilent ZORBAX RRHD SB C18	2.1 × 50 mm, 1.8 µm	857700-902	6 and 7
Agilent ZORBAX RRHD SB C18	2.1 × 100 mm, 1.8 µm	858700-902	8 and 9
Agilent ZORBAX RRHD Eclipse Plus C18	2.1 × 150 mm, 1.8 µm	959759-902	11
Agilent Poroshell 120 SB–C18	4.6 × 100 mm, 2.7 µm	695975-902	12

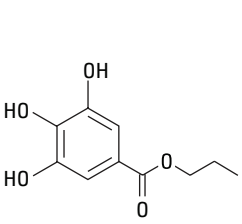
* These numbers are used as experiment identification numbers

Quaternary Gradients

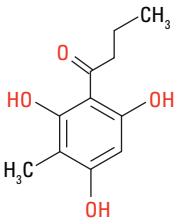
Table 1. Applied quaternary gradients (experiment identification number in parenthesis)

Column, flow rate, (experiment identification number)	Gradient (min)	A (%)	B (%) ACN	C (%) MeOH	D (%) 1% TFA in water
Agilent ZORBAX SB C18, 4.6 × 150 mm, 5 µm, 1.2 mL/min (1)	0	50	20	20	10
	4	30	30	30	10
	15	0	45	45	10
	20	0	45	45	10
Agilent ZORBAX SB C18, 3 × 100, 3.5 µm, 1 mL/min (2)	0	50	20	20	10
	4	30	30	30	10
	12	0	45	45	10
	14	0	45	45	10
Agilent Poroshell 120 EC C18, 4.6 × 50 mm, 2.7 µm, 3.8 mL/min (4)	0	50	20	20	10
	1	30	30	30	10
	2	0	45	45	10
	3	0	45	45	10
Agilent Poroshell 120 SB C18, 4.6 × 100 mm, 2.7 µm, 2 mL/min (3)	0	50	20	20	10
	2	30	30	30	10
	6	0	45	45	10
	9	0	45	45	10
Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm, 1.4 mL/min (7)	0	50	20	20	10
	1.5	30	30	30	10
	4	0	45	45	10
	8	0	45	45	10
Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm, 0.5 mL/min (6) Agilent ZORBAX RRHD SB C18, 3 × 50 mm, 1.8 µm, 1 mL/min (5)	0	50	20	20	10
	2	30	30	30	10
	6	0	45	45	10
	10	0	45	45	10
Agilent ZORBAX RRHD SB C18, 2.1 × 100 mm, 1.8 µm, 0.85 mL/min (9)	0	50	20	20	10
	3	30	30	30	10
	8	0	45	45	10
	10	0	45	45	10
Agilent ZORBAX RRHD SB C18, 2.1 × 100 mm, 1.8 µm, 0.7 mL/min (8)	0	50	20	20	10
	4	30	30	30	10
	10	0	45	45	10
	12	0	45	45	10
Agilent ZORBAX RRHD SB C18, 3 × 50 mm, 1.8 µm, 2.5 mL/min, 60 °C (10)	0	50	20	20	10
	1	30	30	30	10
	3	0	45	45	10
	5	0	45	45	10
Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm, 0.6 mL/min, 25 °C (11)	0	50	20	20	10
	5	30	30	30	10
	10	0	45	45	10
	12	0	45	45	10
Agilent Poroshell 120 C18, 4.6 × 100 mm, 2.7 µm, 3.5 mL/min, 80 °C (12)	0	50	20	20	10
	1.5	30	30	30	10
	4	0	45	45	10
	6	0	45	45	10

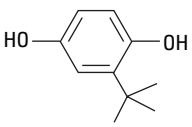
Analyzed Compounds



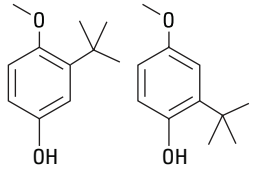
PG



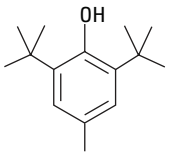
THBP



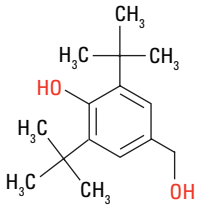
TBHQ



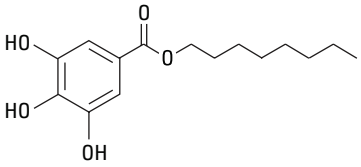
BHA



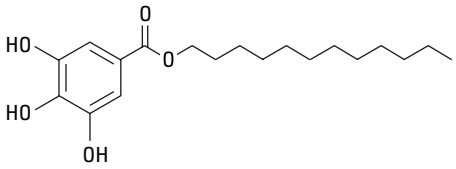
BHT



IONOX 100



OG



LG

Results and Discussion

A quaternary gradient was used to analyze antioxidants on different columns with different flows rates and different resulting backpressures. The column dimensions varied from 2.1 to 4.6 mm for the ID and from 50 to 150 mm for the column length. The gradient times, the flow rates, and the injection volume were adjusted according to the column dimension. Precision of retention times was evaluated for all data sets.

Table 2 combines the conditions for all experiments. The first column mentions the used analytical column, and the second column numbers the experiments. These numbers are used in all relevant tables and figures.

Agilent Poroshell 120 columns are packed with core shell particles and allow high flow rates at moderate backpressures. These columns are best suited for fast and ultrafast analysis using high flow rates without exceeding the 600-bar limit while providing high separation efficiency, see Figure 2.

Table 2. Overview of power range experiments, with experiment identification number, flow rates, gradient times, resulting maximum pressure and RSD of retention times.

Column	Experiment	Column temperature	Flow (mL/min)	Gradient time (min)	Max pressure (bar)	RSD RT (%)
Agilent ZORBAX SB C18, 4.6 × 150 mm, 5 µm	1	45 °C	1.2	15	118	0.04-0.011
Agilent ZORBAX SB C18, 3 × 100 mm, 3.5 µm	2	45 °C	1	12	192	0.023-0.078
Agilent Poroshell 120 SB C18, 4.6 × 100 mm, 2.7 µm	3	45 °C	2	6	440	0.019-0.034
Agilent Poroshell 120 EC C18, 4.6 × 50 mm, 2.7 µm	4	45 °C	3.8	2	522	0.012-0.075
Agilent ZORBAX RRHD SB C18, 3 × 50mm, 1.8 µm	5	45 °C	1	6	420	0.02-0.186
Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm)	6	45 °C	0.5	6	435	0.033-0.185
Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm	7	45 °C	1.4	4	1030	0.052-0.321
Agilent ZORBAX SBC18, 2.1 × 100 mm, 1.8 µm	8	45 °C	0.7	8	750	0.051-0.229
Agilent ZORBAX SBC18, 2.1 × 100 mm, 1.8 µm	9	45 °C	0.85	8	1008	0.021-0.364
Agilent ZORBAX RRHD SB C18, 3 × 50 mm, 1.8 µm (60 °C)	10	60 °C	2.5	3	970	0.075-0.263
Agilent ZORBAX RRHD Eclipse plus C18, 2.1 × 150 mm, 1,8 µm (25 °C)	11	25 °C	0.6	10	1106	0.008-0.086
Agilent Poroshell 120 SB C18, 4.6 × 100 mm, 2.7 µm, (80 °C)	12	80 °C	3.5	4	585	0.011-0.074

The resulting maximum backpressures varied between 118 and 1,106 bar, the flow rates varied between 0.5 and 3.8 mL/min. Excellent precision for the retention times was obtained in all the experiments. Figure 1 includes the maximum obtained pressures and flow rate combinations of all experiments into the power range diagram of the 1290 Infinity Quaternary Pump. Conventional conditions were applied up to 400 bar. Between 600 and 1,200 bar, UHPLC conditions were used applying low flow rates with long columns and high flow rates with short columns.

Flow Range

The 1290 Infinity Quaternary pump comprises a practical flow range from 100 µL/min to 5 mL/min up to 800 bar. The flow range is linearly decreasing to 2 mL/min up to 1,200 bar. Figure 2 shows chromatograms representing flow rates from 0.5 up to 3.8 L/min.

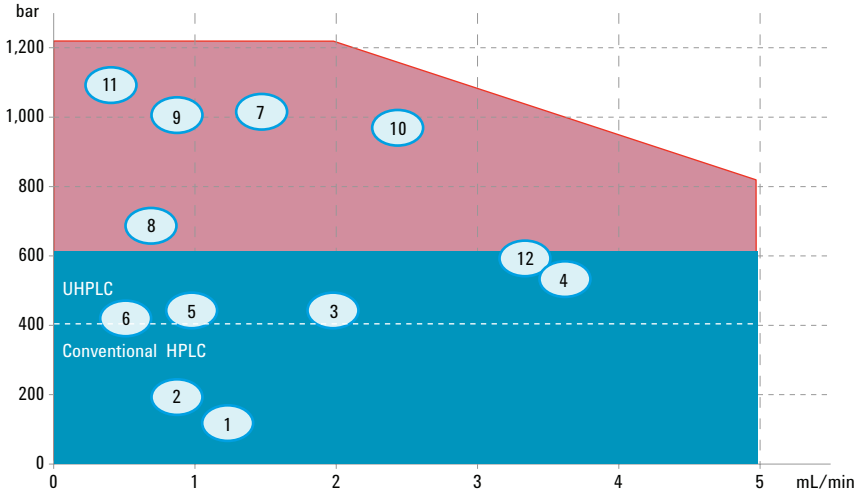


Figure 1. Overview of all experiments added to the power range diagram of the Agilent 1290 Infinity Quaternary Pump (the numbered circle refer to the identification number of the experiments).

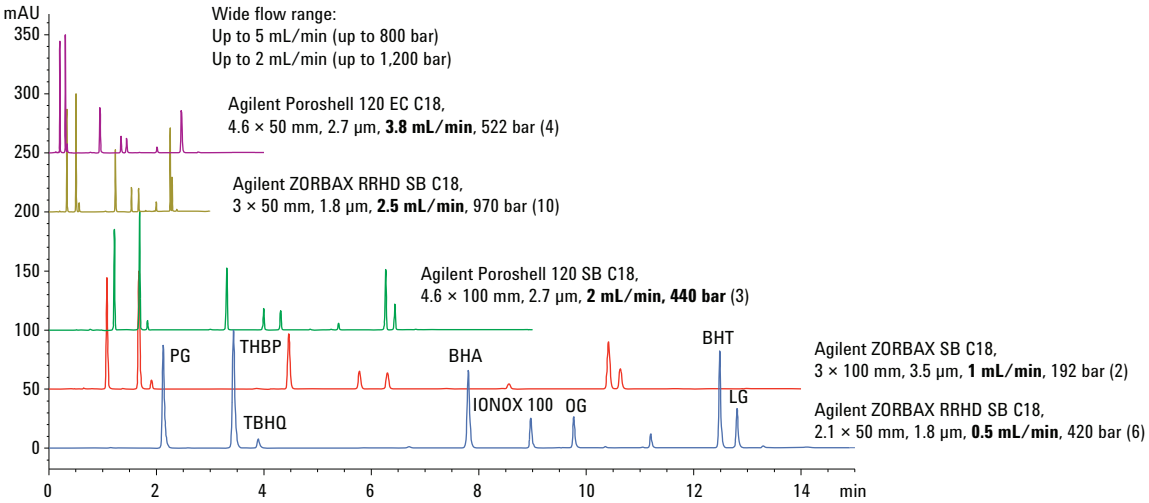


Figure 2. Overlay of chromatograms obtained using different flow rates.

Pressure Range

The allowed maximum pressure is determined by the instrument's analytical column. Columns packed with 5 or 3.5-µm particles are stable up to 400 bar. Columns with smaller particle size such as 2.7-µm and 1.8-µm are usable up to 600 bar. Examples are the Agilent Poroshell 120 with 2.7-µm particles and the Agilent RRHT columns packed with 1.8-µm particles. Dedicated columns packed with particles smaller than 2 µm are usable up to 1,000 or even up 1,200 bar. Examples are Agilent ZORBAX RRHD columns packed with 1.8-µm particles.

The 1290 Infinity Quaternary pump covers the complete range from low pressure to high pressure. Figures 3 and 4 show example chromatograms.

Experiments 1 and 12 showed no separation of the last two compounds. In all other experiments, the separation of the eight compounds was possible. The fastest separation was obtained on the Poroshell 120SB C18, 4.6 × 50 mm, 2.7 µm. Within 3 minutes, the run was completed. Figure 4 shows overlaid chromatograms obtained at backpressures above 600 bar.

All peaks were separated, except for Experiment 10 at a backpressure of 970 bar.

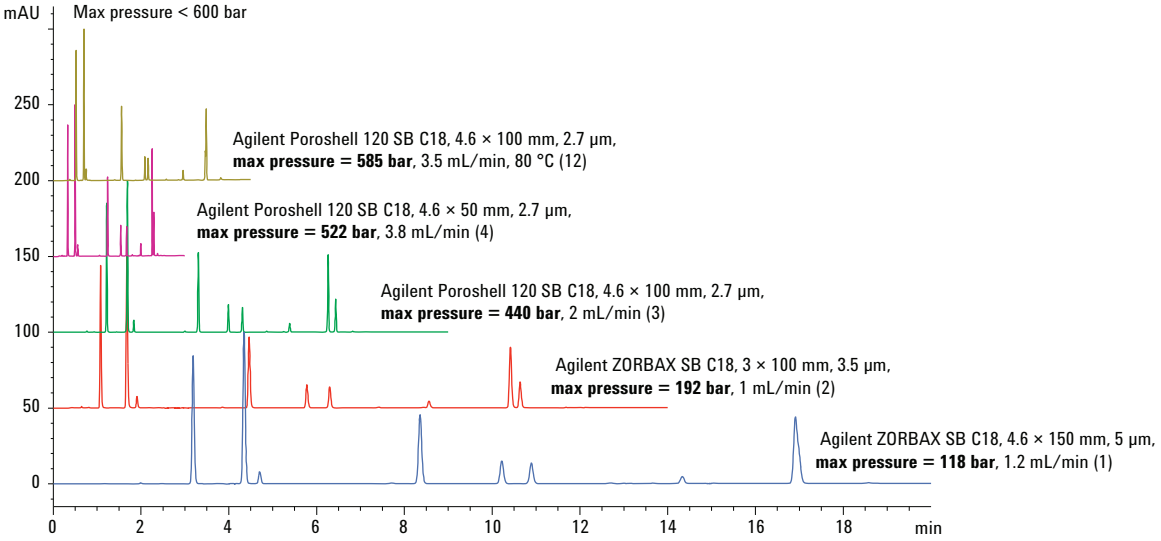


Figure 3. Overlay of chromatograms obtained using conventional and UHPLC runs up to 600 bar.

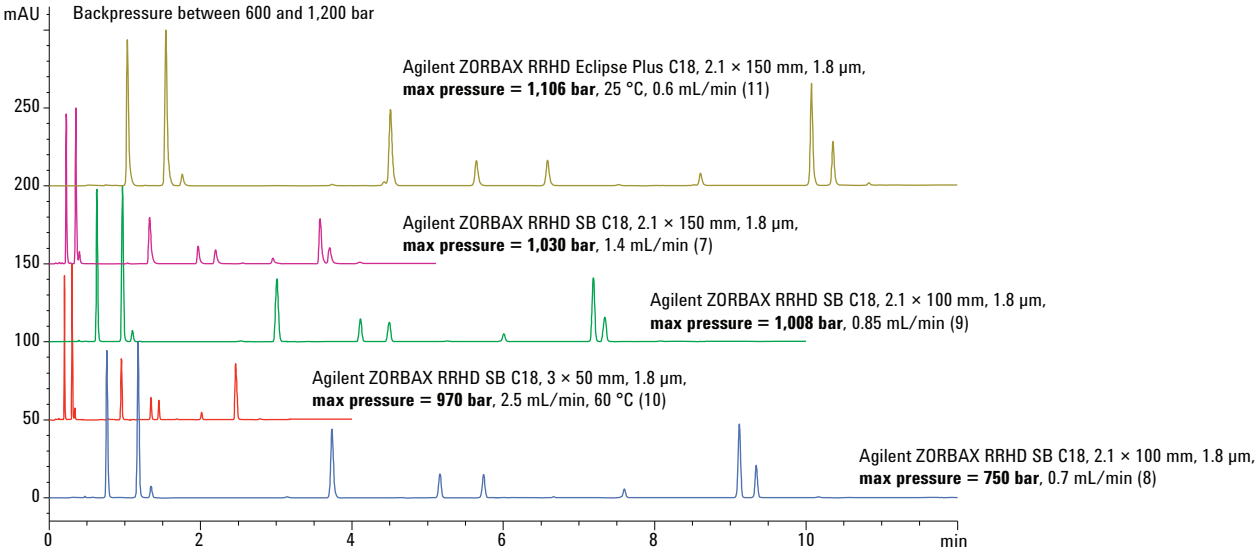


Figure 4. Overlay of chromatograms obtained using runs with maximum pressures between 600 and 1,200 bar.

Conclusion

To demonstrate the power range of the Agilent 1290 Infinity Quaternary LC System, applications at backpressures between 118 and 1,106 bar and flow rates between 0.5 and 3.8 mL/min were applied. Columns of different length from 50 to 150 mm, ID from 2.1 to 4.6 mm and filled with particles between 5 and 1.8-µm were used. According to the column dimensions, different flow rates, gradients, and injection volumes were applied. For all applied conditions, the retention time precision was evaluated. Typically, the relative standard deviation (RSD) was between 0.015 and 0.35%.

www.agilent.com/chem/1290

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