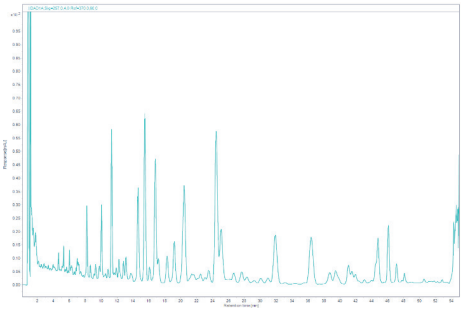
Technical Overview

Performance Comparison to Determine LC Method Compatibility

Comparative study between Agilent 1260 Infinity II Prime LC System and Agilent 1260 Infinity II Prime Bio LC System



Author

Jochen Strassner
Agilent Technologies, Inc.

Abstract

The new Agilent 1260 Infinity II Prime Bio LC System is equipped with a quaternary pump with an 800-bar pressure rating, facilitating a huge number of applications. Suitable applications are the small-molecule-dominated reversed-phase LC application space, as well as the biomolecular space with native protein and nucleic acid applications. The performance of this completely iron-free, robust, and versatile liquid chromatography system is compared to its stainless-steel-based equivalent, the Agilent 1260 Infinity II Prime LC System.

Isocratic, fast gradient, and shallow gradient applications were used to address performance comparison in terms of pump mixing precision and also method compatibility. All applications demonstrated very good reproducibility on both systems. Excellent method compatibility was found for isocratic and fast gradient applications, whereas the shallow gradient method needs some adjustment due to the high impact of small mobile phase composition changes on retention times (RT) of the sample constituents.

Introduction

The number of applications using biomolecules like proteins and nucleic acids as samples in (U)HPLC is steadily increasing. This raises concerns about the usefulness and robustness of the LC hardware, especially when it comes to high-salt buffers as mobile phases and harsh cleaning-in-place solvents like hydrochloric acid, sodium hydroxide, and urea. There is a clear need for robust and reliable instrumentation that can withstand these run and cleaning conditions to give highest performance for both bio-applications and standard reversed-phase applications.

The 1260 Infinity II Prime Bio LC System is one of a family of universal systems consisting of an 800-bar quaternary pump as the core solvent delivery module. Its counterpart in the standard reversed-phase application space is the 1260 Infinity II Prime LC System. Both systems share the same performance specifications.

Method compatibility is a strong argument for customers when buying new LC instrumentation, possibly as a replacement for an older Agilent LC system. Since the performance specifications for both 1260 Infinity II Prime systems are the same, method compatibility is generally possible.

This technical overview compares the pump performance of the iron-free and biocompatible 1260 Infinity II Prime Bio LC System and the stainless steel (SST)-based 1260 Infinity II Prime LC System in a number of different applications (i.e., in isocratic, fast gradient, and shallow-gradient mode).

Experimental

Equipment

The Agilent 1260 Infinity II Prime Bio LC System comprised the following modules:

- Agilent 1260 Infinity II Bio Flexible Pump (G7131C)
- Agilent 1290 Infinity II Bio Multisampler (G7137A) with Sample Thermostat (option #101)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with the standard flow biocompatible heat exchanger
- Agilent 1290 Infinity II DAD (G7117B), equipped with a biocompatible InfinityLab Max-Light Cartridge Cell, 10 mm

The Agilent 1260 Infinity II Prime LC System contains the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1290 Infinity II Multisampler (G7167B) with Sample Thermostat (option #101)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II DAD (G7117B)

Software

Agilent OpenLab CDS version 2.5 or later versions.

Columns

- **Isocratic method:** Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (part number 959993-902)
- **Fast gradient method:** Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (part number 699775-902)
- **Shallow gradient method:** Agilent InfinityLab Poroshell 120 SB-C18, 2.1 × 150 mm, 2.7 µm (part number 683775-902)

Chemicals

All solvents 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, Merck-Millipore, Billerica, MA, USA). Acetonitrile, Lichrosolv and methanol, Lichrosolv were obtained from VWR International, Darmstadt, Germany.

Samples

- Agilent Isocratic sample (part number 01080-68704)
- Agilent RRLC mix (part number 5188-6529)
- Ganoderma Lucidum Fruiting Body Dry Extract (USP Reference Standard, Catalog No. 1288372)
  15.5 mg Ganoderma Lucidum Fruiting Body Dry Extract was dissolved in 7.75 mL MeOH, vortexed, and sonicated. An approximately fourfold concentration step in a vacuum concentrator, followed by centrifugation and filtration through a 0.22 µm syringe filter, led to the final sample solution.

Methods

Isocratic method

Table 1. Isocratic method conditions.

Parameter	Value
Solvent	A) Water B) Acetonitrile (ACN) C) ACN D) Water
Gradient	Isocratic, 65% B (channel A/B) or 65% C (channel C/D) Stop time: 20 minutes Post-time: off
Pump Settings	Minimum stroke: automatic Compressibility: 60 × 10^{-6}/bar Primary channel: automatic
Flow Rate	1.000 mL/min
Temperature	40 °C
Detection	254 nm 10 Hz
Injection	Injection volume: 5 µL Sample temperature: 10 °C Needle wash: 3 s in ACN/water (50/50)

Shallow gradient method

Table 3. Shallow gradient method conditions.

Parameter	Value
Solvent	A) 0.075% phosphoric acid in water B) ACN
Gradient	0 min 20% B 3 min 26.5% B 34 min 26.5% B 52 min 38.5% B 54 min 100% B Stop time: 55 min Post-time: 15 min
Pump Settings	Minimum stroke: automatic Compressibility: 50 × 10^{-6}/bar Primary channel: automatic
Flow Rate	0.400 mL/min
Temperature	30 °C
Detection	257 nm 20 Hz
Injection	Injection volume: 5 µL Sample temperature: 10 °C Needle wash: 3 s in ACN/water (50/50)

Fast gradient method

Table 2. Fast gradient method conditions.

Parameter	Value
Solvent	A) water B) ACN
Gradient	0 min 20% B 5 min 90% B Stop time: 6 min Post-time: 3 min
Pump Settings	Minimum stroke: automatic Compressibility: 55 × 10^{-6}/bar Primary channel: automatic
Flow Rate	1.000 mL/min
Temperature	40 °C
Detection	245 nm 40 Hz
Injection	Injection volume: 5 µL Sample temperature: 10 °C Needle wash: 3 s in ACN/water (50/50)

Results and discussion

Isocratic method

With this application, the capability of the pumps to mix the mobile phase for separating the components of the isocratic sample in an accurate, stable, and precise manner was addressed. Repeatability and method compatibility was tested by running 10 injections of isocratic sample, where one sequence was executed with solvent channels A/B and the second sequence with C/D, as solvent lines for mixing the mobile phase. The last seven consecutive chromatograms from each sequence were used for evaluation (see Figure 1).

The same method was run on both systems. The overlay of corresponding chromatograms demonstrates excellent repeatability in terms of peak retention times on both channel combinations with both systems. Small differences in peak shape are related to tiny variances between the instruments.

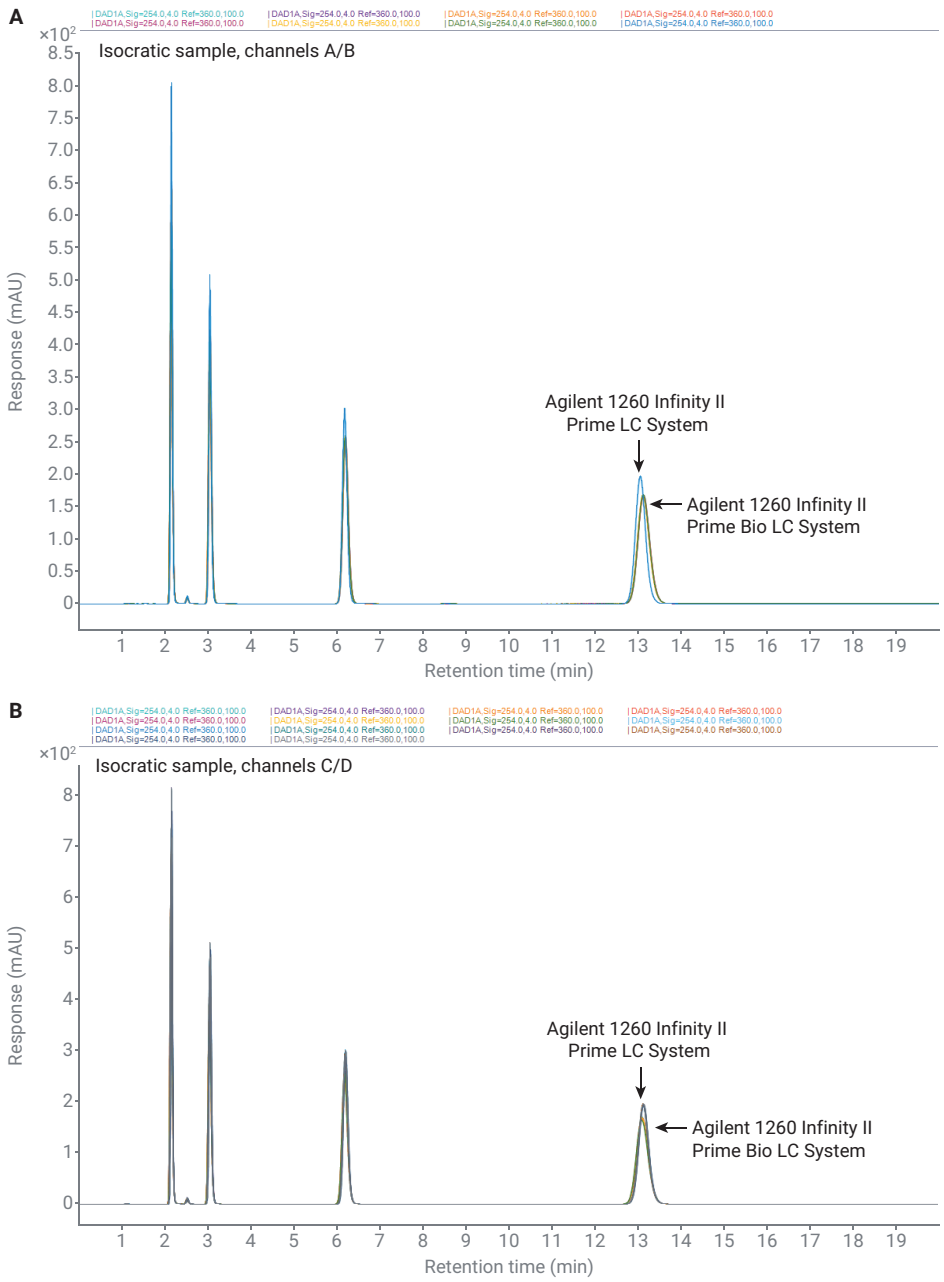


Figure 1. Overlay of seven subsequent chromatographic runs of the isocratic sample on the Agilent 1260 Infinity II Prime LC System and the Agilent 1260 Infinity II Prime Bio LC System. (A) data for channels A/B; (B) data for channels C/D.

The solvent composition precision specification for both systems is defined as <0.15% RSD, or 0.02 minutes SD, whatever is greater. Table 4 shows the composition precision RSD values for the isocratic sample at the given retention times of its constituents. High pump performance could be confirmed, no matter which solvent channel combination was used. The method compatibility is outstanding, with RT deviations far below the ±1% range, thereby demonstrating a high degree of performance match between the two instruments.

Fast gradient method

UHPLC methods generally feature faster and steeper solvent composition gradients using small particle columns running at higher backpressures. In this case, a 5-minute gradient starting from 20% B ranging up to 90% B and a run time of 6 minutes was applied. Overlaying seven UV traces generated by both systems revealed very good reproducibility of pump mixing precision (Figure 2, Table 5).

Retention time differences between the two systems become visible, especially at the later eluting peaks. However, the relative retention time deviation (%RT) of eluting sample components is well below 2% between the two instruments, demonstrating valuable method compatibility.

Table 4. Method compatibility and retention time (RT) precision evaluation for both solvent channels. Average RT values out of seven sample injections from both LC systems were used. For method compatibility, the values for the Agilent 1260 Infinity II Prime LC System were used as a reference.

Solvent Channels	Retention Time 1260 Agilent Infinity II Prime LC System (min)	RT % RSD	Retention Time 1260 Agilent Infinity II Prime Bio LC System (min)	RT % RSD	RT Deviation (min)	RT Deviation (%)
A/B	2.1533	0.0408	2.1487	0.0164	0.0046	-0.2134
A/B	3.0430	0.0361	3.0431	0.0771	0.0000	0.0012
A/B	6.1627	0.0226	6.1780	0.0417	-0.0153	0.2478
A/B	13.0088	0.0236	13.0808	0.0235	-0.0720	0.5537
C/D	2.1579	0.0536	2.1494	0.0385	0.0085	-0.3920
C/D	3.0534	0.0632	3.0435	0.0523	0.0099	-0.3244
C/D	6.1957	0.0349	6.1791	0.0380	0.0165	-0.2668
C/D	13.1101	0.0190	13.0814	0.0232	0.0287	-0.2191

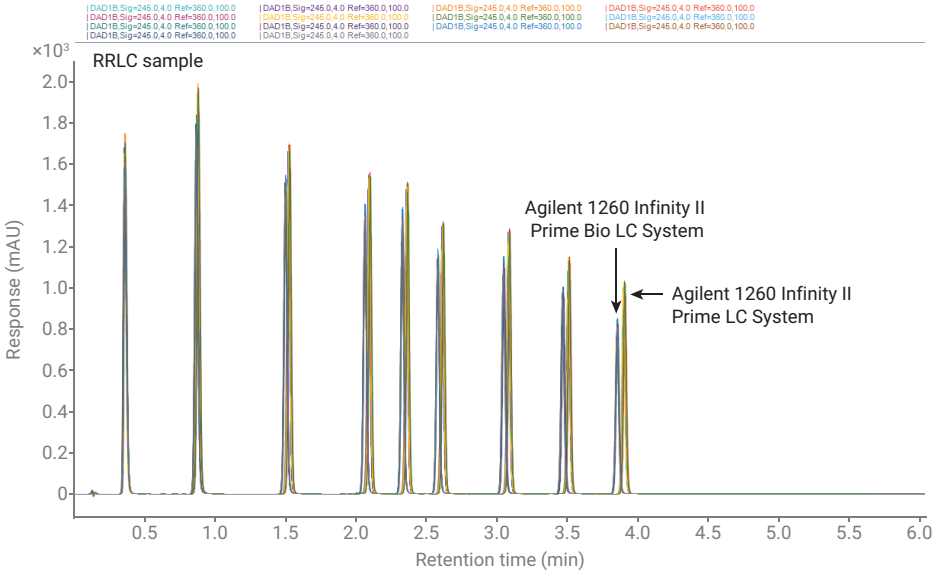


Figure 2. Overlay of seven subsequent chromatographic runs of the RRLC sample on the Agilent 1260 Infinity II Prime LC System and the Agilent 1260 Infinity II Prime Bio LC System.

Table 5. Method compatibility and retention time (RT) precision evaluation with RRLC sample. Average RT values out of seven sample injections from both LC systems were used. For method compatibility estimation, the values for the Agilent 1260 Infinity II Prime LC System were used as a reference.

Compound ID RRLC Sample	Retention Time Agilent 1260 Infinity II Prime LC System (min)	RT %RSD	Retention Time Agilent 1260 Infinity II Prime Bio LC System (min)	RT %RSD	RT Deviation (min)	RT Deviation (%)
1	0.3604	0.5275	0.3593	0.0766	0.0012	-0.3233
2	0.8738	0.3954	0.8625	0.0578	0.0113	-1.2879
3	1.5150	0.3013	1.4900	0.0514	0.0250	-1.6513
4	2.0798	0.2418	2.0486	0.0252	0.0312	-1.5021
5	2.3447	0.1989	2.3117	0.0327	0.0331	-1.4106
6	2.5960	0.1765	2.5610	0.0238	0.0350	-1.3463
7	3.0619	0.1427	3.0236	0.0617	0.0384	-1.2531
8	3.4828	0.1268	3.4395	0.0684	0.0434	-1.2448
9	3.8720	0.1170	3.8227	0.0369	0.0492	-1.2719

Shallow gradient method

In the third application, a complex sample was chosen for analysis, which required a rather shallow gradient for separation. The Ganoderma Lucidum Fruiting Body Extract is a triterpenoid-enriched extract supplied by USP as a standard reagent (USP, Rockville, MD, USA). The separation method is based on the USP published chromatographic method[1], and the assignment of the obtained peaks was done according to Ganoderma Lucidum Fruiting Body Dry Extract Lot Certificate F012B0, USP Catalog No. 1288372.[2] As presented in Figure 3, good mobile phase composition precision and an identical elution pattern of the sample were achieved with both systems.

However, a retention time shift can be observed, especially during the isocratic hold step during the method (Figure 3). This is further exhibited in Table 6, where the retention times of several peaks deviate more than 5%, indicating worse method compatibility for this particular method compared to the two former applications.

To further evaluate the root cause for the higher RT deviation, the method was slightly modified such that the isocratic hold step for B was adjusted to ±0.2% of the original method. This simulated the change in retention time for the separated compounds due to slight composition accuracy differences between the two pumps, which were still performing within the specified range of ±0.4%. As shown in Figure 4, this very small composition change resulted in a significant retention time shift of the eluting peaks, indicating that the method is too sensitive to composition changes and thereby overestimates the differences in composition accuracy between the two pumps. This effect generally needs to be considered when testing method compatibility.

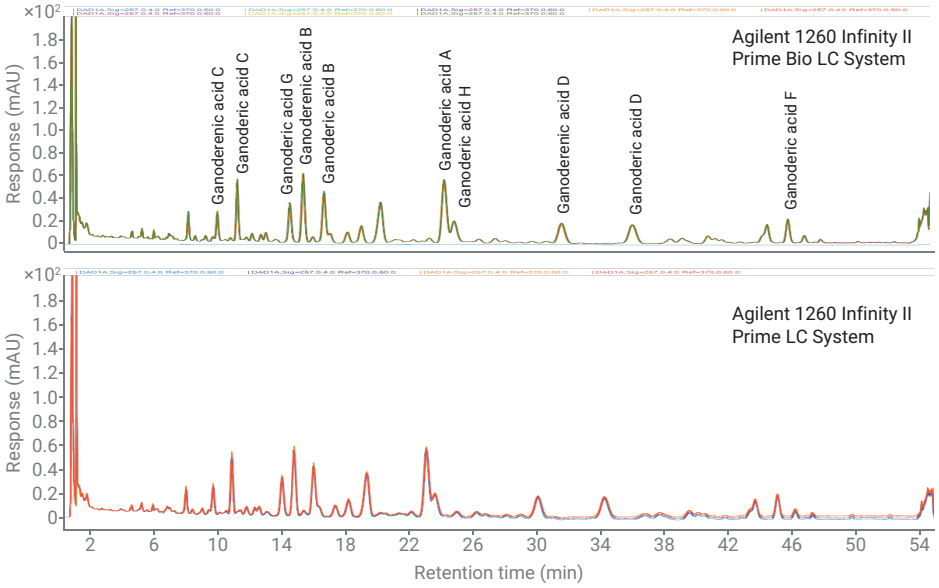


Figure 3. Overlay of seven subsequent chromatograms of Ganoderma Lucidum Fruiting Body Extract separations on the Agilent 1260 Infinity II Prime LC System and Agilent 1260 Infinity II Prime Bio LC System. Assignment of peaks as mentioned above.

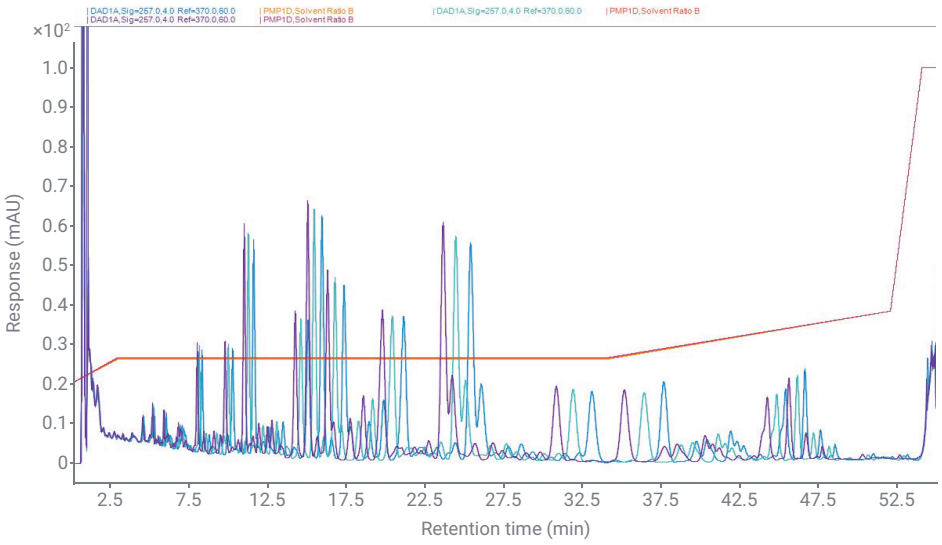


Figure 4. Effect of slight changes in isocratic hold step on retention time. Isocratic hold step was modified by ±0.2% B. Results for the Agilent 1260 Infinity II Prime Bio LC System are shown. Green: 26.5% B isocratic hold step (original); blue: 26.3% B; magenta: 26.7% B.

Table 6. Calculation of retention time deviation between the Agilent 1260 Infinity II Prime LC System and Agilent 1260 Infinity II Prime Bio LC System. Retention times for the 1260 Infinity II Prime LC System act as a reference for calculating deviation. RT precision for each instrument is shown accordingly. Each RT and RT %RSD value is an average of seven measurements.

Compound ID Ganoderma L. Extract	Retention Time Agilent 1260 Infinity II Prime LC System (min)	RT %RSD	Retention Time Agilent 1260 Infinity II Prime Bio LC System (min)	RT %RSD2	RT Deviation (min)	RT Deviation (%)
1. Ganoderenic acid C	9.6596	0.1357	9.9924	0.0111	-0.3327	3.4446
2. Ganoderic acid C	10.8330	0.1216	11.2505	0.0112	-0.4176	3.8546
3. Ganoderic acid G	13.9869	0.0977	14.5709	0.0123	-0.5839	4.1749
4. Ganoderenic acid B	14.7444	0.0811	15.4070	0.0138	-0.6627	4.4943
5. Ganoderic acid B	15.9688	0.0868	16.7174	0.0136	-0.7486	4.6878
6. Ganoderic acid A	23.0435	0.1056	24.2967	0.0136	-1.2532	5.4386
7. Ganoderic acid H	23.5823	0.1021	24.9284	0.0082	-1.3461	5.7080
8. Ganoderenic acid D	30.0558	0.0992	31.7005	0.0223	-1.6447	5.4721
9. Ganoderic acid D	34.2341	0.1031	36.1752	0.0187	-1.9411	5.6700
10. Ganoderic acid F	45.1035	0.0405	45.9687	0.0108	-0.8652	1.9182

Conclusion

In this technical overview, the performance of the new Agilent 1260 Infinity II Prime Bio LC System was compared to the standard stainless steel-based Agilent 1260 Infinity II Prime LC System by running three different applications. With a high degree of reproducibility for each system for all three applications, good method compatibility could be demonstrated for the isocratic and fast gradient method. Retention time deviations remained below ±2% relative to the retention times of the corresponding eluting compounds.

The third application consisting of a shallow gradient, including a long-lasting isocratic hold step, gave higher RT deviations for a number of eluting compounds. It could be demonstrated that the substantial deviation could derive from slight differences in mobile phase composition between the systems. Pumps that perform within their composition accuracy specifications can still reveal RT shifts larger than 5%, exhibiting the importance of a suitable method to be used for evaluating method compatibility.

References

1. Ganoderma lucidum Fruiting Body Monograph, Source URL: https://hmc.usp.org/monographs/ganoderma-lucidum-fruiting-body-1-0, The United States Pharmacopeial Convention, **2015**.
2. Ganoderma lucidum Fruiting Body Dry Extract Lot Certificate F012B0, USP Catalog No. 1288372, The United States Pharmacopeial Convention, **2014**.

www.agilent.com/chem

DE44314.0505092593

Printed in the USA, May 20, 2021
5994-3533EN

Agilent
Trusted Answers