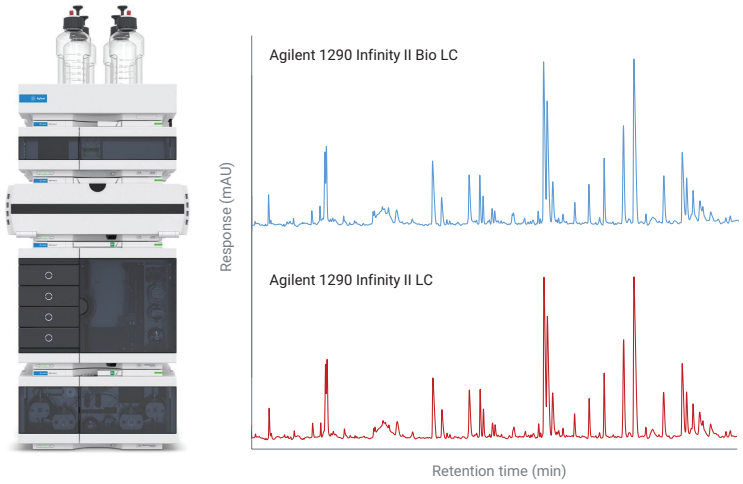
Application Note

Biopharma

Seamless Method Transfer to the Agilent 1290 Infinity II Bio LC System

Peptide-mapping analysis shows excellent performance and high method compatibility compared to the Agilent 1290 Infinity II LC System



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Abstract

Peptide mapping requires reliable and robust methods with high precision for analyzing the primary structure and post translational modifications (PTMs) of biopharmaceuticals. However, method transferability and compatibility can be an issue for validated methods. This application note shows that method transfer can be easy and convenient with the new Agilent 1290 Infinity II Bio LC System. Building on the excellent average relative retention time deviation of 0.039% for 12 selected peptides, it was discovered that the retention times only deviated by 0.17% between the 1290 Infinity II Bio LC System and the Agilent 1290 Infinity II LC System. By combining the LC systems with the Agilent 6545XT AdvanceBio LC/Q-TOF, additional comparative statistical analysis of peak abundances and in-depth analysis of the PENNY peptide revealed no significant differences between both systems, rendering the new 1290 Infinity II Bio LC the ideal choice for UV- or MS-based peptide-mapping workflows.

Introduction

Method transfer and compatibility from one instrument to another are important for laboratories across different industries.[1] Especially in the biopharmaceutical industry, instrument-to-instrument method transfer is highly important for validated methods. To demonstrate the seamless method transfer from the 1290 Infinity II LC to the 1290 Infinity II Bio LC, the peptide-mapping workflow was chosen because of its considerable relevance in the evaluation of biological products as described in ICH Guideline Q6B.[2] Using a tryptic digest of the NISTmAb, this application note will show that method transfer can be straightforward thanks to the 1290 Infinity II Bio LC.

Experimental

Equipment

The Agilent 1290 Infinity II Bio LC System and the Agilent 1290 Infinity II LC System coupled to the Agilent 6545XT AdvanceBio LC/Q-TOF comprised the following modules:

Agilent 1290 Infinity II Bio LC:

- Agilent 1290 Infinity II Bio High-Speed Pump (G7132A)
- Agilent 1290 Infinity II Bio Multisampler (G7137A) with Sample Thermostat (option 101)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116A) equipped with a Standard Flow Quick Connect Bio Heat Exchanger (G7116-60071) and two Agilent Thermal Equilibration Devices (G7116-60013)
- Agilent 1290 Infinity II Variable Wavelength Detector (VWD) (G7114B), equipped with a Bio Micro Flow Cell VWD, 3 mm, 2 µL, RFID.
- Agilent 6545XT AdvanceBio LC/Q-TOF (G6545XT)

Agilent 1290 Infinity II LC:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167A) with Sample Thermostat (option #101)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116A) equipped with a Standard Flow Quick Connect Heat Exchanger (G7116-60015) and two Agilent Thermal Equilibration Devices (G7116-60013)
- Agilent 1290 Infinity II Variable Wavelength Detector (VWD) (G7114B), equipped with a Micro Flow Cell VWD, 3 mm, 2 µL, RFID.
- Agilent 6545XT AdvanceBio LC/Q-TOF (G6545XT)

Software

- Agilent MassHunter workstation data acquisition (B.09.00)
- Agilent MassHunter Qualitative Analysis (B.10.00)
- Agilent MassHunter Mass Profiler (B.10.00)

Columns

- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (part number 959759-902)
- Agilent ZORBAX RRHD Eclipse Plus C18 Fast Guards, 2.1 × 5 mm, 1.8 µm (part number 821725-901)

Chemicals

LC-grade acetonitrile, ammonium bicarbonate, *tris*(2-carboxyethyl) phosphine, and 2-iodoacetamide were purchased from Merck (Darmstadt, Germany). 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). Formic acid was purchased from VWR (Darmstadt, Germany). Trypsin (porcine, mass spectrometry-grade) was obtained from G-Biosciences (St. Louis, USA).

Sample preparation

0.8 mg of the Agilent-NISTmAb (part number 5191-5744) in 100 µL of ammonium bicarbonate (100 mM) was denatured and reduced by the addition of 2 µL of *tris*(2-carboxyethyl)phosphine (TCEP, 200 mM) and incubated at 60 °C for 1 hour. After the alkylation with 4 µL of 2-iodoacetamide (IAM, 200 mM, 1 hour at RT), quenching of excess IAM with 2 µL of TCEP (1 hour at RT), and subsequent dilution with 0.8 mL of 25 mM ammonium bicarbonate, the enzyme trypsin was added (20:1, NISTmAb to trypsin w/w). After the overnight digestion at 37 °C, the pH of the resulting suspension was decreased below pH 4 by the addition of 2 µL of formic acid.

Results and discussion

To show the excellent performance and method transfer between the 1290 Infinity II Bio LC and the 1290 Infinity II LC, a tryptic digest of the NISTmAb was analyzed with UV and MS detection. Both systems were equipped with capillaries of the same length and diameters to have similar extra column volumes. However, the 1290 Infinity II Bio LC featured a completely iron-free flow path especially suited for sticky biomolecules. For both analyses, the same ZORBAX RRHD Eclipse Plus column and method parameters were used (Table 1). Figure 1 shows the chromatograms of the peptide maps acquired by both systems. Excellent similarities between the peptide patterns are visible, with almost no detectable differences.

Table 1. Peptide-mapping method for the Agilent 1290 Infinity II LC and Bio LC.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm + Fast Guard 2.1 × 5 mm
Solvent	A) Water + 0.1% formic acid B) Acetonitrile + 0.1% formic acid
Gradient	0.00 min – 2% B 44.00 min – 45% B 44.01 min – 97% B 50.00 min – 97% B 50.01 min – 2% B 60.00 min – 2% B
Flow Rate	0.300 mL/min
Temperature	40 °C with thermal equilibration devices installed
UV Detection	VWD: 214 nm, 10 Hz/MS: see Table 2
Injection	Injection volume: 15 µL Sample temperature: 4 °C Wash: 3 s with water (flush port)

Table 2. Source and MS parameters for the All Ions MS/MS analysis of peptides.

Parameter	Value
Instrument	Agilent 6545XT AdvanceBio LC/Q-TOF
Gas Temperature	300 °C
Drying Gas Flow	13 L/min
Nebulizer	40 psig
Sheath Gas Temperature	350 °C
Sheath Gas Flow	12 L/min
Vcap	4,000 V
Nozzle Voltage	500 V
Fragmentor	175 V
Skimmer	65 V
Oct 1 RF Vpp	750 V
Acquisition Mode	Positive, extended dynamic range (2 GHz)
Mass Range	*m/z* 100 to 1,700
Acquisition Rate	6 spectra/sec
Collision Energy	All ions MS/MS—0 V, 10 V, 25 V

For better evaluation, three generic resolution (R_s) values were calculated for both separations (Figure 1) and also showed exceptionally good comparability. To analyze the performance of the 1290 Infinity II Bio LC and 1290 Infinity II LC regarding retention time precision, 12 peptides were chosen, and the corresponding relative standard deviations (RSD) of the retention times were calculated based on 10 consecutive injections. Figure 2 depicts that all RSD values, irrespective of the system, are below 0.1%, showcasing the excellent performance of the Agilent 1290 Infinity II Bio High-Speed Pump and 1290 Infinity II High-Speed Pump. The average RSD value of the 12 peptides even gets as low as 0.039% for the 1290 Infinity II Bio LC, rendering this system an excellent choice for robust and reliable peptide mapping. However, besides high performance, method compatibility between different LC systems is also very important for numerous labs.

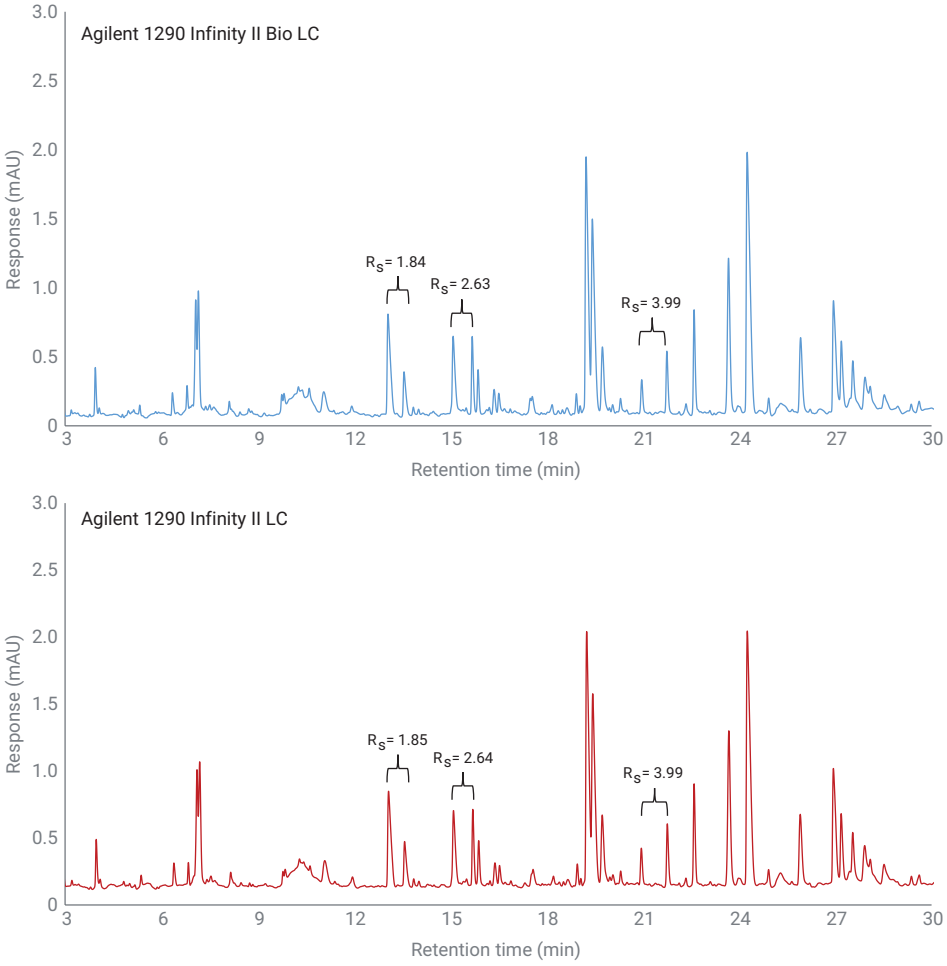
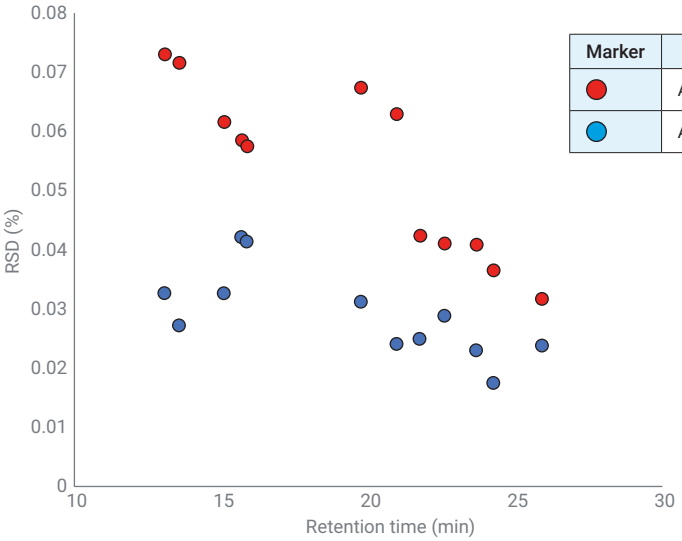


Figure 1. Chromatograms of a tryptic digest of the NISTmAb separated by the Agilent 1290 Infinity II Bio LC and the Agilent 1290 Infinity II Bio LC with the same method (Table 1).



Marker	LC System	RSD (%)
●	Agilent 1290 Infinity II LC	0.073
●	Agilent 1290 Infinity II Bio LC	0.039

Figure 2. Relative retention time precision (RSD) values for the Agilent 1290 Infinity II Bio LC and the Agilent 1290 Infinity II LC.

Absolute retention times need to be in certain windows to identify analytes in a validated or compliance environment. By determining the average retention times of the 12 peptides for both LC systems and calculating the deviation of the retention times between the 1290 Infinity II Bio LC and 1290 Infinity II LC, the performances were evaluated. Average peptide retention times are depicted in the table of Figure 3 and corresponding deviations are shown as bar plots. Minimal deviations of up to 0.17% between the LC systems were calculated, showing seamless method transfer between the 1290 Infinity II Bio LC and 1290 Infinity II LC.

To further investigate the method compatibility, both systems were coupled to the Agilent 6545XT AdvanceBio LC/Q-TOF. In an untargeted approach, the MS detector was used in All Ions mode (Table 2), periodically fragmenting all precursor ions in the collision cell. These information-rich data sets were then evaluated with the Agilent MassHunter Mass Profiler (B.10.00) software for both LC systems to get a holistic view of the differences in the abundance of identified peaks. Ten consecutive injections of a tryptic digest of the NISTmAb on both LC systems were the basis for subsequent statistical analysis. The 250 most abundant peaks were evaluated by correlation analysis, and the corresponding log-fold changes are depicted in Figure 4A. If a peak does not differ in both systems, it will cluster around the 1x line in Figure 4A, signaling no significant difference in the peak area. However, if there is a peak with a two-times higher abundance in one system, it would be located around the 2x line. Looking at the graphical data results, it becomes clear that there are no significant differences for most peaks. Up to 75% of the peaks varied with 10% or less in abundance. Even more

LC System	Average Peptide Retention Times (min)											
	1	2	3	4	5	6	7	8	9	10	11	12
Agilent 1290 Infinity II LC	13.082	13.577	15.104	15.704	15.887	19.751	20.968	21.769	22.599	23.684	24.261	25.907
Agilent 1290 Infinity II Bio LC	13.062	13.559	15.084	15.677	15.860	19.743	20.961	21.742	22.585	23.663	24.249	25.907

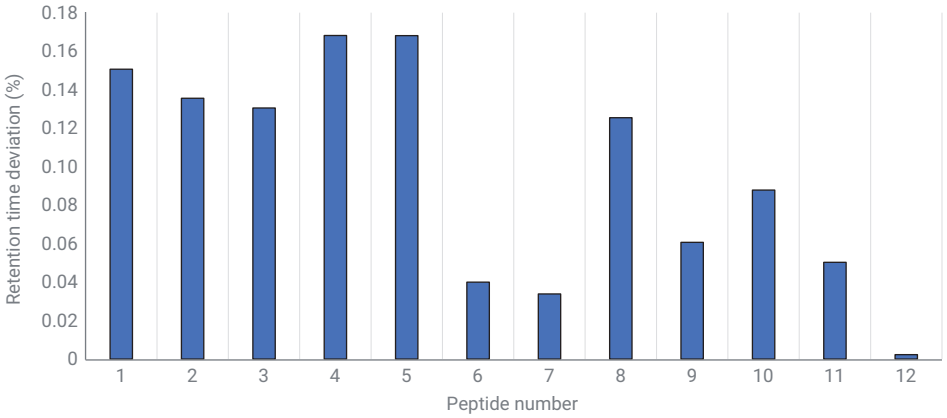


Figure 3. Average retention times for the 12 chosen peptides and their deviations between the two LC systems.

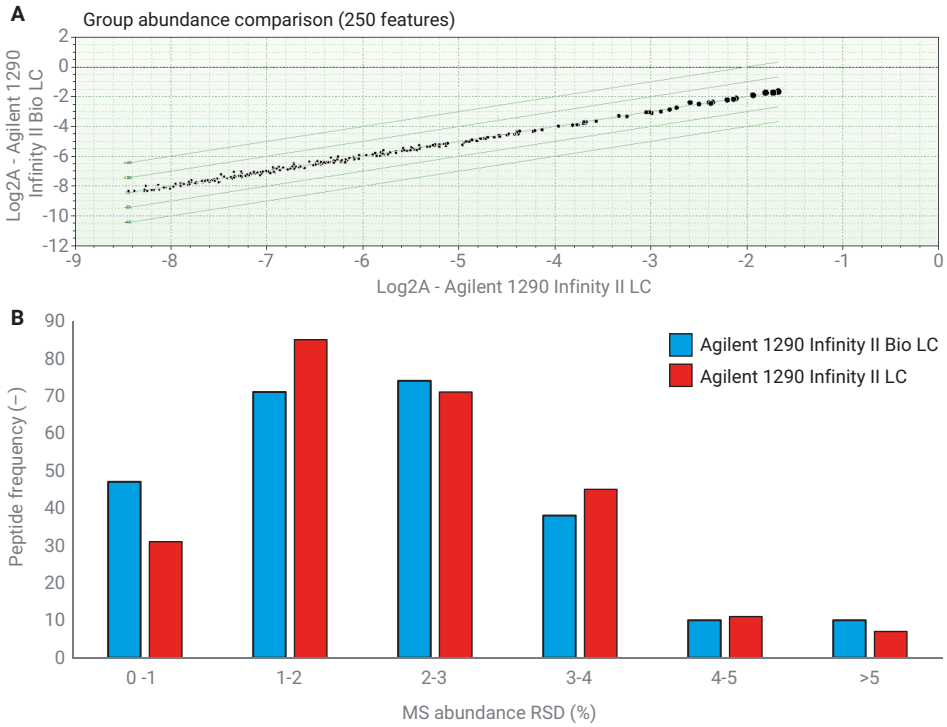


Figure 4. (A) Statistical correlation analysis based on 10 consecutive injections. Log fold values are depicted as black dots for the 250 highest abundance peaks identified by Agilent MassHunter Mass Profiler (B.10.00). (B) Histogram for the RSD values of the peptide abundances for the Agilent 1290 Infinity II Bio LC and 1290 Infinity II LC.

strikingly, the RSD for the abundances over 10 injections were nearly the same for the 1290 Infinity II Bio LC and 1290 Infinity II LC (Figure 4B). Over 90% of the peptide peaks had an area RSD value of 4% or less.

To further evaluate both systems, the PENNY peptide (GFYPSDIAVEWESNGQPENNYK) was analyzed by extracting the EIC graphs for both systems (see Figure 5). This peptide is part of the conserved region (Fc) shared by nearly all human or humanized mAbs, which can be used as a decent indicator for induced deamidation and contains four acidic amino acid residues.[3] Previous reports described the adsorption and peak tailing of small acidic molecules containing phosphate or multiple carboxylate moieties on stainless steel surfaces.[4] Strikingly, there is no apparent tailing of the PENNY peptide on the 1290 Infinity II LC, despite the acidic residues. The PENNY peptide has a similar tailing factor (see Figure 5, T_f) on both LC systems. Both systems have excellent relative retention times (Bio LC = 0.02%, LC = 0.04%, n = 3) and area precision values (Bio LC = 1.26%, LC = 1.41%, n = 3). These findings underline the excellent performance of the 1290 Infinity II Bio LC for peptide mapping with the added benefit of a biocompatible flow path. A biocompatible flow path is mandatory for sticky compounds such as phosphorylated peptides.

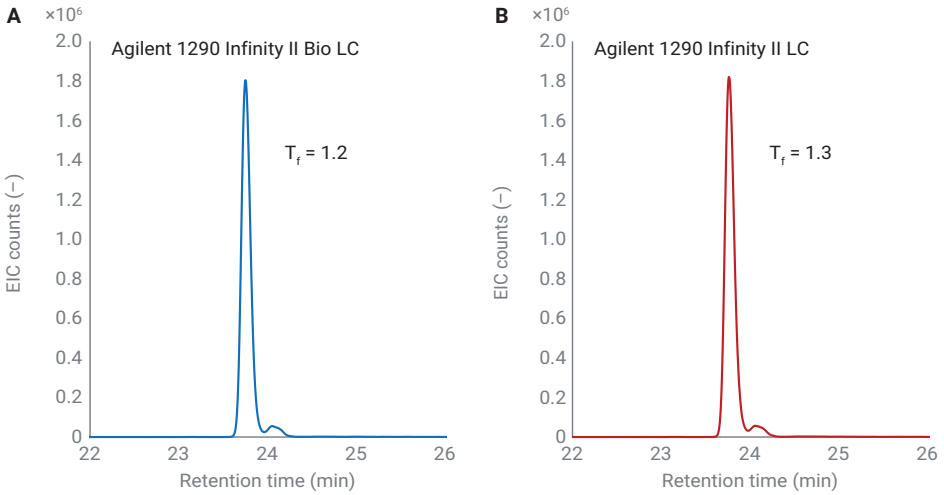


Figure 5. Extracted ion chromatogram (EIC) for the PENNY peptide (GFYPSDIAVEWESNGQPENNYK) on the Agilent 1290 Infinity II Bio LC and Agilent 1290 Infinity II LC. Both systems show excellent tailing factors (T_f).

Conclusion

Method transfer can sometimes be a laborious and difficult process for many labs when configuring and installing a new LC system. This application note showed that this is not the case for the Agilent 1290 Infinity II Bio LC. By running the same NISTmAb peptide-mapping method on the 1290 Infinity II Bio LC and 1290 Infinity II LC, it was shown that the method could be seamlessly transferred with retention time deviations of only up to 0.17% between the systems. Thanks to the 1290 Infinity II Bio High-Speed Pump, the average relative retention time deviations after 10 injections also showed an excellent value with 0.039%. By coupling both systems with the 6545XT AdvanceBio LC/Q-TOF, a comprehensive statistical analysis of peak abundances showed no significant differences with excellent average RSD of 2.8% and excellent performance analyzing the PENNY peptide. Combining these findings, it is clear that efficient and convenient method transfer between the 1290 Infinity II Bio LC and 1290 Infinity II LC can easily be achieved.

The 1290 Infinity II Bio LC is therefore the ideal choice for peptide-mapping workflows regardless of the detection method, with the benefit of an iron-free flow path.

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