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應用手冊

Migrating Peptide Mapping Methods from the Arc™ Premier System to the Alliance™ iS Bio System with Gradient SmartStart Technology and Intelligent Method Translator App (iMTA)

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Abstract

The Alliance iS Bio HPLC System is an innovative biocompatible and exceedingly inert HPLC platform designed for biopharmaceutical manufacturing environments. Through low system dispersion and a default 680 µL mixer, the Alliance iS Bio HPLC System is suitable for biopharmaceutical applications where analytes are subjected to non-specific adsorption to metal surfaces and acidic mobile phase are deployed. In this study, we demonstrate how integrated features including Gradient SmartStart Technology and the Intelligent Method Translator App (iMTA) can support seamless transfer of methods from Arc Premier systems with quaternary solvent manager and binary solvent manager configuration while preserving separation selectivity, bypassing the need for time-consuming method re-optimization.

Benefits

- The Intelligent Method Translator App (iMTA) streamlines the method transfer between systems with error

free and time saving method translation

- Gradient SmartStart Technology maintains separation selectivity and increases confidence in retention time-based peak assignment
- The Alliance iS Bio HPLC System delivers consistent and reproducible results well-suited for QC environments

Introduction

As drug candidates enter manufacturing phases, analysis methods are frequently migrated in parallel for continued monitoring of quality indicating attributes. This process is not trivial, as instrument configuration and design may vary in receiving labs which can impact assay results. Ensuring migrated methods can deliver comparable performance with minimal re-optimization saves time and reduces errors. As part of its innovative design, the Alliance iS Bio HPLC System (Figure 1) offers users access to the iMTA app and Gradient SmartStart Technology. These features support a seamless transfer of methods from other HPLC systems, bypassing the need for time-consuming method re-optimization. As an integrated feature, the iMTA can automate the method transcription process between platforms. When used in conjunction with the Gradient SmartStart Technology, which accounts for dwell volume/pump design differences, it enables time-saving and error-free method translation between systems while preserving chromatographic performance for increased confidence in data analysis and peak assignment.

In this study, we evaluate method migration results from an Arc Premier System featuring either a binary solvent manager (BSM) or a quaternary solvent manager (QSM) to the Alliance iS Bio HPLC System. Peptide mapping was chosen as a representative method frequently deployed in the analysis of protein-based biopharmaceuticals. Peak area % and separation selectivity will be used as metrics to evaluate the comparability of the methods between platforms.

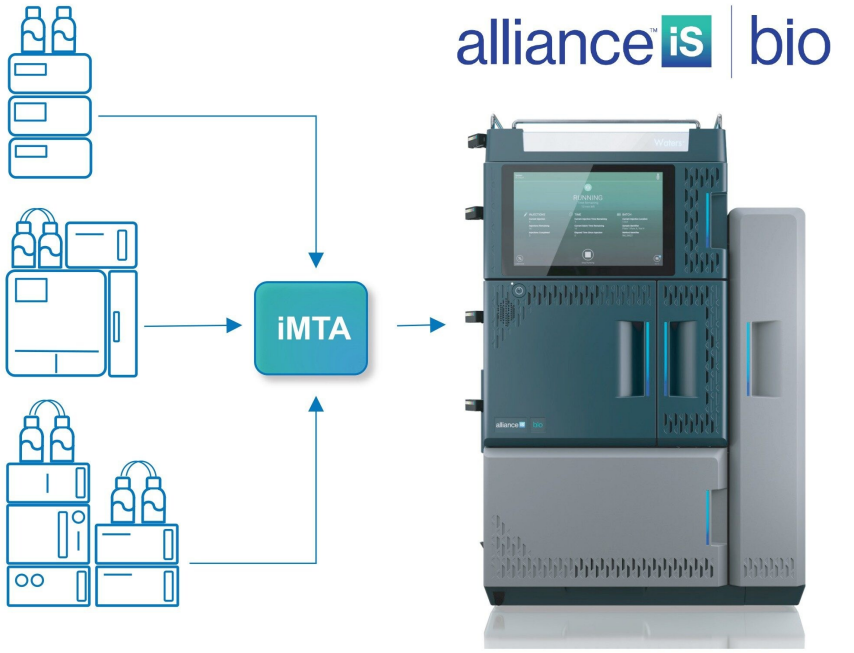

Figure 1. The iMTA application reduces error and saves time by translating method conditions from several legacy HPLC systems and converting the parameters to an Alliance iS Bio HPLC System method.

Experimental

MS grade water and acetonitrile were purchased from Honeywell. MS grade formic acid was purchased from Fisher. The mAb Tryptic Digestion Standard (p/n: 186009126 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009126-mab-tryptic-digestion-standard.html>) purchased from Waters Corporation™ was reconstituted using 0.1% formic acid in water at a concentration of 0.5 mg/mL.

LC Conditions

LC system:	Alliance iS Bio HPLC System (QSM), Arc Premier

	System (BSM or QSM, both with a 680 µL mixer)
Detection:	TUV, λ = 214 nm
Column:	XSelect™ Premier Peptide CSH™ C_{18} Column, 130 Å, 2.5 µm, 4.6 x 100 mm (p/n: 186009908) (+eConnect™ p/n: 186009908RF)
Column temperature:	60 °C
Sample temperature:	10 °C
Injection volume:	50 µL
Flow rate:	0.96 mL/min
Mobile phase:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Chromatography software:	Arc Premier System: Empower™ 3.6.1 Alliance iS Bio HPLC System: Empower 3.8

Time (min)	Flow rate (mL/min)	%A	%B	Curve
initial	0.96	99.0	1.0	initial
1.00	0.96	99.0	1.0	6
51.00	0.96	65.0	35.0	6
61.00	0.96	15.0	85.0	6
67.00	0.96	99.0	1.0	6
80.00	0.96	99.0	1.0	6

Gradient table.[1]

Results and Discussion

Traditionally speaking, method migration often starts with transferring method parameters from the existing system to the new system wherein results are compared to determine system suitability and method equivalency. As a manual activity, this process is time consuming, and can be error-prone due to transcription errors or CDS interface differences across vendors. Waters addresses this challenge elegantly through the iMTA, which can transfer Empower instrument methods in a semi-automated fashion from various legacy systems to the Alliance iS Bio HPLC System.[2,3] As shown in Figure 2, the iMTA can be accessed through the Empower Apps menu allowing users to select a legacy method. It automatically translates key parameters for the solvent manager, sample manager, column manager, and the detector and simplifies the transfer process to a few "clicks". In this example, the peptide mapping method conditions outlined in the experimental section were translated from an Arc Premier System configured with QSM and saved as an Alliance iS Bio HPLC System method. Both the Arc Premier System (QSM) and the iMTA method were run on their corresponding system and compared in terms of retention time reproducibility, peak area %, and separation selectivity.

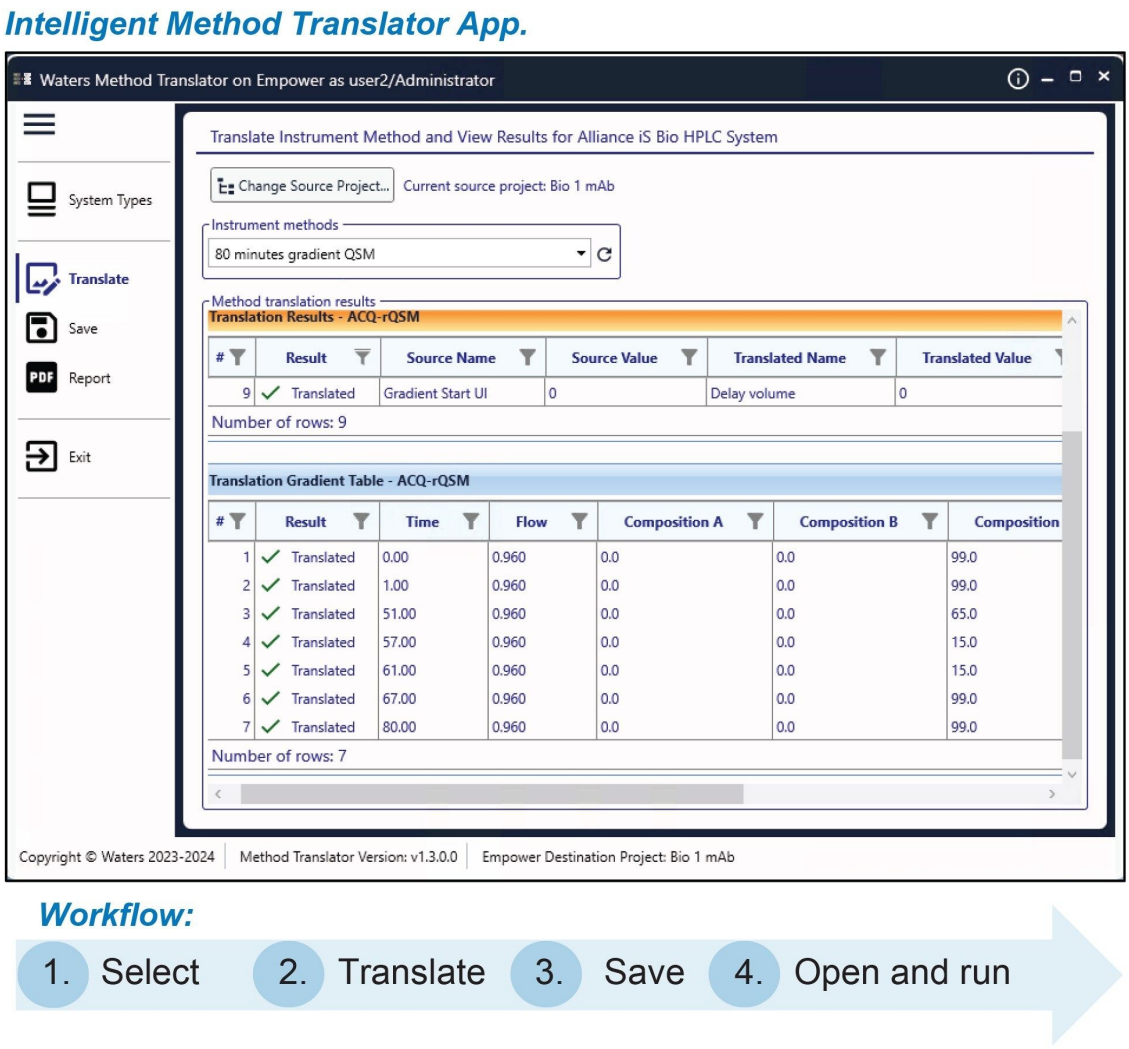

Figure 2. The Intelligent Method Transfer application seamlessly translates the key parameters to an Alliance iS Bio HPLC System method which is ready to be saved and used.

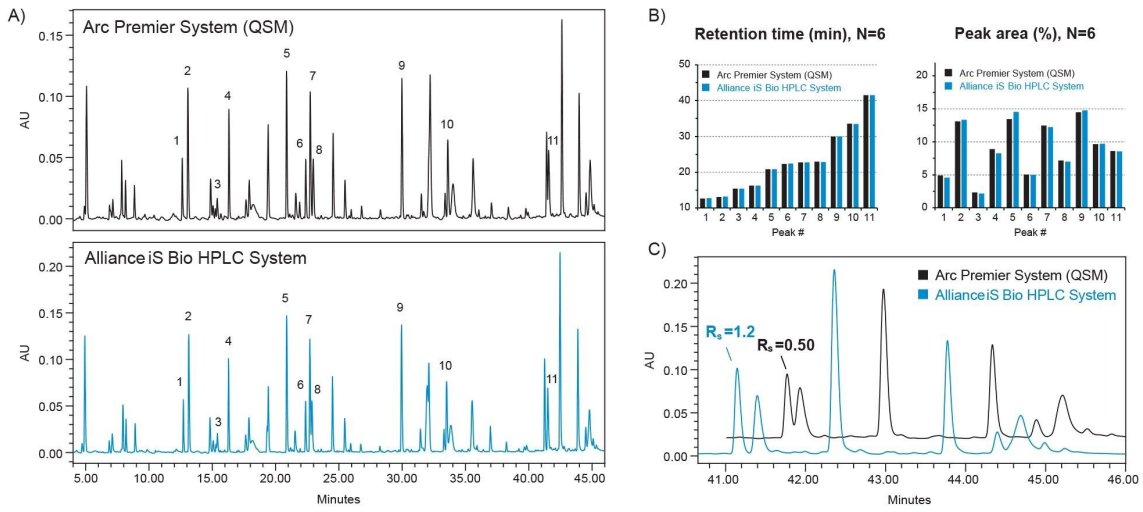

Figure 3. UV absorption spectra (blank subtracted) A) of NIST mAb trypsin digested peptides on Arc Premier System (QSM) and the Alliance iS Bio HPLC System. B)Both systems delivered comparable peak area % and retention time reproducibility of each peptide, N=6. C) For peak 11, the Alliance iS Bio HPLC system offers better resolution and better peak area % consistency.

As shown in Figure 3A, a total of 11 peaks equally sampled across the peptide map were selected to assess chromatographic performance. As LC systems configured with a quaternary solvent manager exhibit similar system dwell volumes, the Alliance iS Bio HPLC System produces comparable chromatograms to those of the Arc Premier System (QSM) with minimal or no modification to the method (Figure 3A). Both systems delivered similar retention time (RT) and peak area % for all 11 peaks (Figure 3B). Retention time reproducibility for both systems was within specifications for a set of six replicate injections with the Alliance iS Bio HPLC System performing more consistently with overall mean retention time standard deviation (SD) ≤ ±1.0 sec in comparison to the Arc Premier System (QSM) with RT SD ≤ ± 1.8 sec. The Alliance iS Bio HPLC System outperformed the Arc Premier System in terms of peak area reproducibility with %RSD ≤ 3.3% compared to 7.2% on the Arc Premier System (QSM). The improved performance observed in the Alliance iS Bio HPLC System resulted in higher consistency in resolution and peak area reproducibility, an example of which is shown in Figure 3C for peak 11 (peak area % RSD = 1.7 % vs peak area % RSD = 5.1%).

To challenge the system, the same method was run on an Arc Premier System configured with a binary solvent manager, which is often preferred for its ability to deliver accurate gradient compositions under demanding

conditions. As shown in Figure 4A, the Alliance iS Bio HPLC System is able to deliver comparable results in terms of peak profile; retention time reproducibility with both systems exhibiting RT SD ≤ 1.0 sec (Figure. 5). Peak area % was highly similar between systems with peak area % RSD ≤ 3.0% for both systems (Figure. 4B). Differences in pump designs, tubing configuration, and column temperature (passive or active pre-heating) can impact dwell volume and gradient delivery timing, which can result in a change in separation selectivity. In this case, the observed difference in peak retention time between systems (ΔRT=0.64 minute) as shown in Figure 4C is due to the increased dwell volume of the Alliance iS Bio HPLC System (1669 µL) compared to the Arc Premier System (1080 µL).

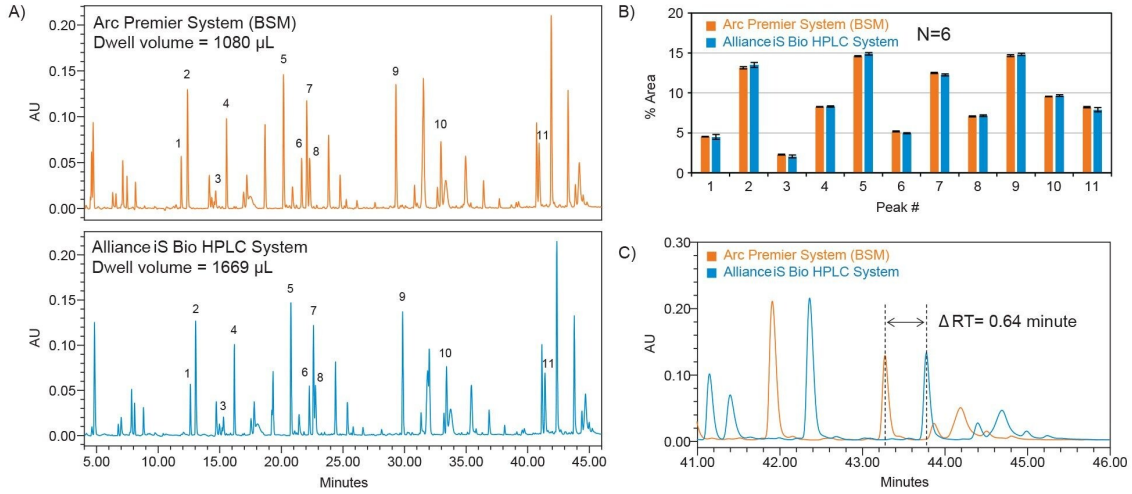

Figure 4. UV absorption spectra (blank subtracted) A) of NIST mAb trypsin digested peptides on Arc Premier System (BSM) and the Alliance iS Bio HPLC System. B) Both systems delivered comparable peak area % of each peptide. C) Due to differences in pump design and dwell volume, the retention time difference across systems reached 0.64 minute.

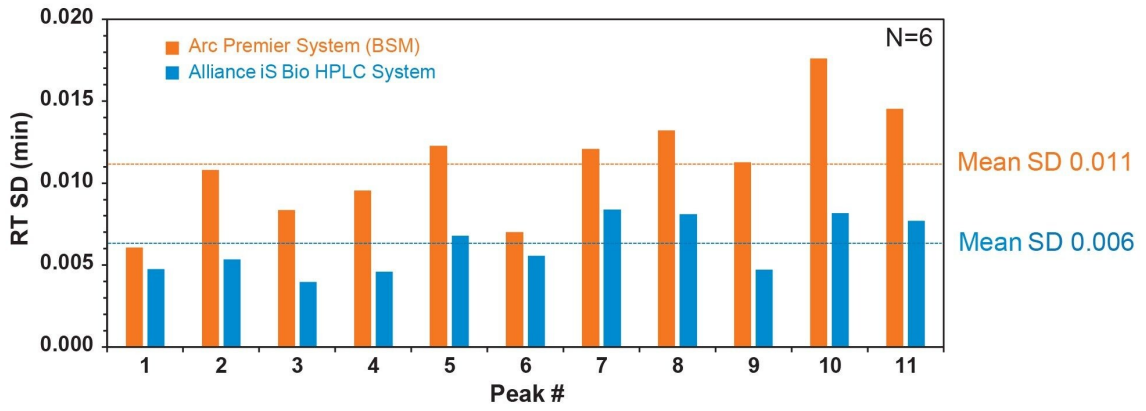

Figure 5. Both the Arc Premier System (BSM) and the Alliance iS Bio HPLC System delivered retention time reproducibility within specifications for a set of 6 replicate injections with the Alliance iS Bio HPLC System performing more consistently.

To address the challenge of adjusting the gradient start relative to the injection without altering the gradient table, Waters has developed the Gradient SmartStart Technology that provides the opportunity to adjust the gradient start relative to the injection without the need for altering the gradient table.[4] This technology introduces Gradient Start Options in the instrument method, which allows adjustment of the gradient start to occur at, before, or after injection to compensate for the dwell volume differences between systems when migrating methods (Figure. 6B). As shown in Figure 6A, when using a volume of 589 µL (1669 µL–1080 µL) for the adjusted gradient, the chromatograms are aligned in terms of peak retention time when comparing the Arc Premier System (BSM) and Alliance iS Bio HPLC System. The selectivity is calculated as $\alpha = (t_{R2} - t_0)/(t_{R1} - t_0)$, where t_0 is the time representing an unretained species or the void volume, t_{R1} is the retention time of the target peak, and t_{R2} is the retention time of a reference peak eluted at around 42 minutes selected for its high intensity. As shown in Figure 6C, peak selectivity was in better agreement between methods when using the gradient start adjustment when comparing slopes of the fitted data using orthogonal plots. These results demonstrate that iMTA and Gradient SmartStart Technology can be utilized to successfully migrate methods from LC platforms of different designs while preserving separation selectivity, thereby reducing the need for method re-optimization, operating costs, and potential errors for the end users.

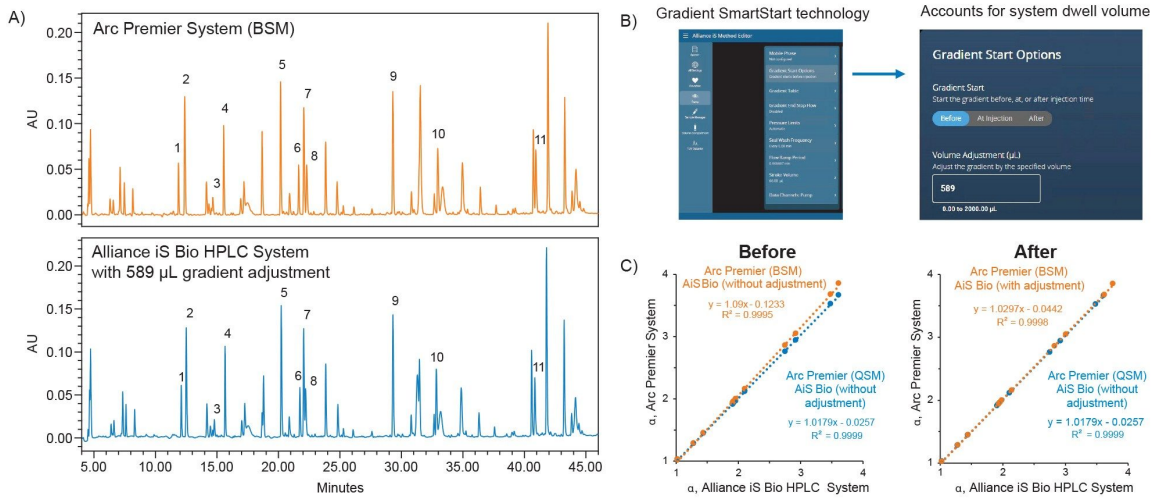

Figure 6. UV absorption spectra (blank subtracted) A) of NIST mAb trypsin digested peptides on Arc Premier System (BSM) and the Alliance iS Bio HPLC System with gradient adjustment. B) The Gradient SmartStart Technology accounts for the system dwell volume difference and use a two-parameter feature to adjust gradient time to match the injection time to preserve the selectivity of gradient. C) The selectivity of the Alliance iS Bio HPLC System inherently matched the performance with the Arc Premier System (QSM) because of the shared pump design. Through the gradient adjustment, the selectivity of the Alliance iS Bio HPLC System matched the performance with the Arc Premier System (BSM) as shown in the slope closer to 1.

Conclusion

The Alliance iS Bio HPLC System is an innovative HPLC platform with features that ease the method migration burden from legacy HPLC systems. Through the Intelligent Method Translator App (iMTA), it can automatically translate instrument parameters and generate ready-to-use methods for the Alliance iS Bio HPLC System. Another innovative feature, the Gradient SmartStart Technology, can accommodate the dwell volume and pump design differences and preserve gradient performance for shallow gradient peptide mapping methods. This eliminates the need for time-consuming method re-optimization and increases the confidence to assign peak identity based on retention time and likelihood of success in gradient-based method migration.

References

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