Transferring a Method for the Characterization of PTMs from the UltiMate 3000 BioRS System to the Vanquish Flex UHPLC System

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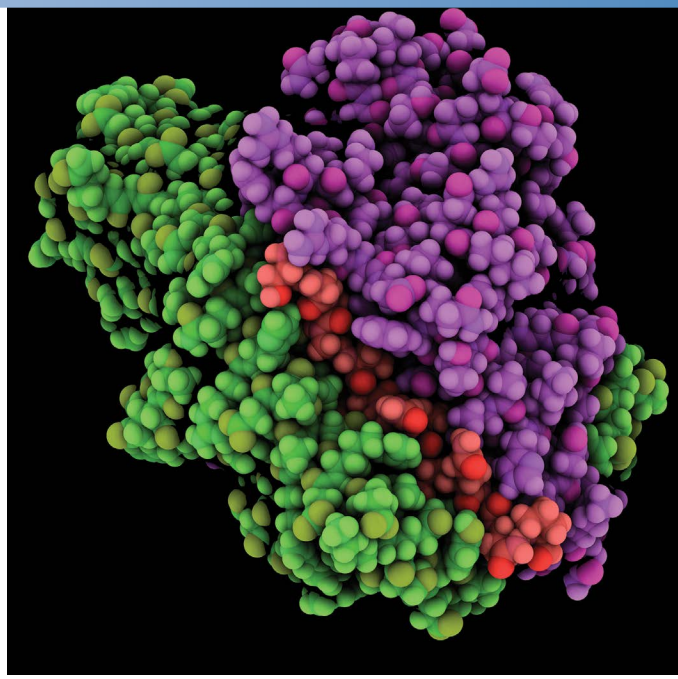
Key Words
Method Transfer, Thermo Scientific ProPac WCX-10, Biocompatible UHPLC, Protein Digest, Biotherapeutics Characterization, Biopharma, Deamidation

Goal
Demonstrating a successful transfer of an HPLC protein deamidation monitoring method from the Thermo Scientific™ UltiMate™ 3000 BioRS UHPLC to the Thermo Scientific™ Vanquish™ Flex UHPLC system.

Introduction
Therapeutic proteins play a key role in today's health care methods. Antibodies are especially suited for complex tasks in biological medication. The majority of biologics registered for therapeutic use in the last 15 years are antibodies. Antibodies have a molecular weight of about 150 kDa and are composed of approximately 500 amino acids. Due to their high molecular complexity, they can be affected by post-translational modifications (PTMs) like N- and O-glycosylation, deamidation, disulfide bonds, or oxidation. These can impair the biological functionality and reduce the efficacy. Deamidation is one of the most common PTMs resulting in a conversion of asparagine to aspartic acid. The change in the amino acid composition can cause allergy and affects the therapeutic protein in half-life, stability, and pharmacological activity.[1-3] According to regulatory guidelines, pharmaceutical companies have to ensure consistency and quality of their products. Therefore, checking for amino acid changes is mandatory.

Ion-exchange chromatography is a frequently used technique for the separation of deamidated therapeutic proteins and charge variants in general.[4] Further, enhanced loading capacity in comparison to reversed-phase chromatography favors the use of cation-exchange chromatography. As an additional benefit, the separation can be performed under native conditions for the evaluation of biological activity.

Analytical method transfer is one of the most discussed topics in regulated laboratories. After a developed method is validated and implemented in an industrial workflow, the method can be transferred considering specified criteria and regulatory guidelines to other systems.[5]



Depending on the acceptance criteria, the receiving unit has to achieve the same validated results as in the original method.

A challenge in method transfer is to mimic different instrumental types with respect to gradient delay volume, hydrodynamic behavior, and thermostatting. The Vanquish Flex UHPLC system provides two different thermostatting modes as well as active and passive mobile phase pre-heating and an adjustable gradient delay volume. These instrumental features substantially support and facilitate method transfer, leading the best match of retention times, resolution, and peak area in compliance with validation criteria.

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In this application note we show an easy method transfer from the UltiMate 3000 BioRS UHPLC system to the Vanquish Flex UHPLC system. Both have entirely biocompatible fluidics providing high robustness and reliability, especially under harsh conditions like high salt concentrations. The Thermo Scientific™ ProPac™ WCX-10 column was used to provide high resolution, even for samples that differ only in one charged residue, and to minimize secondary (nonionic) interactions.

Experimental

Sample Preparation

The deamidation was forced by using a 1% ammonium bicarbonate solution. First, 15 mg of ribonuclease A (bovine pancreas) was dissolved in 1 mL starting conditions to get a protein concentration of 15 mg/mL. Then, 334 µL of the protein solution, 100 µL of 10% ammonium bicarbonate (w/v), and 566 µL of deionized water were combined in a 2 mL tube to create a final ribonuclease A concentration of 5 mg/mL. The tubes were placed in a thermo shaker for 10 minutes at 37 °C and aliquots were taken afterwards.

Instrumentation

Vanquish Flex UHPLC system consisting of:

- System Base (P/N VF-S01-A)
- Quaternary Pump F (P/N VF-P20-A)
- Split Sampler FT with 25 µL Sample Loop (P/N VF-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater, 0.1 × 380 mm, VH-C1 (P/N 6732.0110)
- Diode Array Detector HL (P/N VH-D10-A) equipped with LightPipe™ Standard Flow Cell (P/N 6083.0100)

UltiMate BioRS 3000 system consisting of:

- LPG-3400RS Pump (P/N 5040.0036)
- WPS-3000TBRS Well Plate Autosampler with 25 µL Sample Loop (P/N 5841.0020)
- TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
- Passive Pre-heater (P/N 6722.0540)
- DAD-3000RS Detector (P/N 5082.0020)
- Semi-micro Flow Cell for DAD, PEEK 2.5 µL volume, 7 mm pathlength (P/N 6082.0500)

Default Thermo Scientific™ Viper™ capillary fittings were used for flow connections of the devices.

Chromatographic Conditions

Column	ProPac WCX-10 Analytical, 4 × 250 mm (P/N 054993)
Mobile Phase A	10 mM sodium phosphate in water, pH 6.0
Mobile Phase B	10 mM sodium phosphate, 1 M sodium chloride in water, pH 6.0
Gradient	0–30 min: 4–70% B; 30–40 min: 70–75% B; 40–42 min: 75–4% B; 42–55 min: 4% B
Flow Rate	1.00 mL/min
Temperature	30 °C (Forced air mode for Vanquish Flex system)
Maximal Pressure	152 bar (2204 psi)
Injection Volume	10 µL
Detection	280 nm
	Data Collection Rate: 10 Hz
	Response Time: 0.4 s

Data Processing

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2 SR3, was used for data analysis.

Results and Discussion

A salt-based gradient for a cation-exchange chromatography method was used to separate the deamidation products in a generic gradient program. The deamidation monitoring for both systems is shown in Figure 1. The chromatogram shows a match for all seven peaks. Comparing the two systems, the average relative deviation in retention time is 0.34% and there is an excellent standard deviation for the internal system reproducibility with three runs (Table 1). Moreover, the peak area ratio between the main variant of ribonuclease A and the two deamidation products (Peaks 2 and 3) match in this analytical method transfer with a deviation of 0.25% on average. The deamidation products as a percentage of the main peak area were 1.2% for Peak 2 and 3.3% for Peak 3 on the UltiMate 3000 BioRS system, and 1.4% and 3.6%, respectively, on the Vanquish Flex system. The resolution between the two deamidation products was 1.7 for both systems.

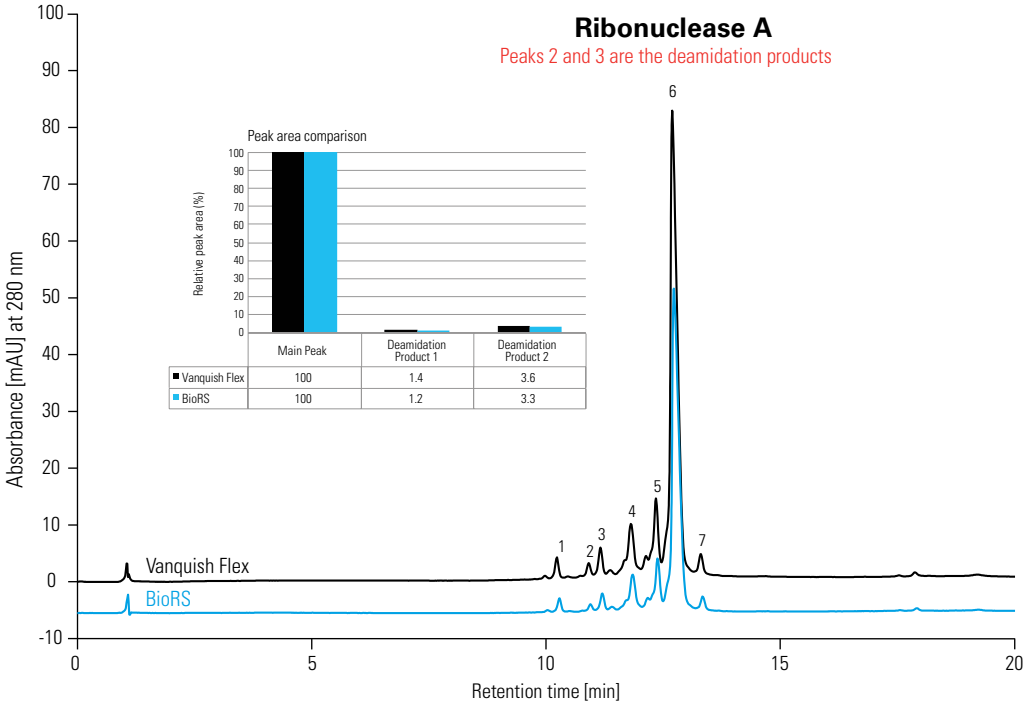

Figure 1. Overlay of a deamidation monitoring measured on the Vanquish Flex (black trace) and the UltiMate 3000 BioRs (blue trace) systems.

Table 1. Retention time data and deviations for both UHPLC systems. The relative deviation in retention time was calculated by dividing the delta in retention time by the average in retention time between both UHPLC systems (N=3).

Peak	Average Retention Time Vanquish Flex System [min]	Standard Deviation Vanquish Flex System [min]	Average Retention Time UltiMate 3000 BioRS System [min]	Standard Deviation UltiMate 3000 BioRS System[min]	Δ Average Retention Time [min]	Relative Deviation Retention Time Between Systems [%]
1	10.231	0.002	10.284	0.001	0.053	0.52
2	10.909	0.001	10.947	0.003	0.038	0.34
3	11.159	0.001	11.201	0.001	0.041	0.37
4	11.811	0.001	11.848	0.005	0.037	0.31
5	12.344	0.001	12.378	0.004	0.034	0.28
6	12.690	0.000	12.726	0.003	0.036	0.28
7	13.302	0.002	13.339	0.001	0.037	0.28

Conclusion

Easy method transfer of a post-translational modification characterization from the UltiMate 3000 BioRS system to the Vanquish Flex system was shown. The average relative deviation in retention time between the methods on both UHPLC systems was 0.34%. Furthermore, the relative peak area ratios of the deamidation products deviate by only 0.25% between the sending unit (UltiMate 3000 BioRS system) and the receiving unit (Vanquish Flex system). Different thermostatting options and an adjustable gradient delay volume in the Vanquish Flex system provide the ability to physically recreate hardware conditions for successful method transfer.

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