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應用手冊

Successful Method Migration of USP Insulin Assay to the Alliance™ iS Bio HPLC System

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Abstract

Within the pharmaceutical industry, compendial LC methods are used to assess whether the regulatory specifications for finished products are met. Insulin, a polypeptide hormone consisting of two peptide chains, requires numerous liquid chromatography (LC) methods to accurately measure insulin, insulin degradation, and potency of insulin. This includes the USP Monograph for Insulin Assay, which measures the potency of the drug product.[1] or method transfer or migration, the methods may be moved across systems with the goal of demonstrating equivalent performance.

In this work, the USP Monograph for Insulin Assay will be tested across multiple systems, evaluating both system suitability requirements and % API potency of the substance, USP Human Insulin RS. This work will demonstrate the key factors for successful method transfer of a regulated method across multiple systems.

Benefits

- The USP monograph for Insulin Assay method can be successfully migrated between multiple HPLC systems
- All suitability requirements for the USP monograph were successfully met across multiple systems which included USP resolution, USP tailing, and standard peak area %RSD
- Ability to successfully migrate the USP monograph for Insulin Assay to the Alliance iS Bio HPLC System and

obtain better precision %RDSs in comparison to the other systems

Introduction

Method migration can be affected by many factors. System differences can affect many chromatographic parameters, and result in retention time shifts, peak distortion due to strong solvent effects, differences in resolution, and band broadening to name a few.

In this study, method migration of the USP monograph for Insulin Assay analysis, will be performed. The analysis specifically contains Insulin and its degradant product, A–21 desamido insulin. Challenging method conditions for this assay include column temperature, mobile phase pH, mobile phase preparation and composition (organic modifier %). All of which may impact the analysis and the acceptance criteria, including: USP tailing, USP resolution, % area for A–21 Desamido Insulin degradation and % potency will be measured using the USP monograph for Insulin Assay acceptance criteria. This study will focus on the migration of the method from older HPLC technology, including the Alliance e2695 HPLC System, and other comparable HPLC systems to the Alliance iS Bio HPLC System.

Experimental

Solution A, System Suitability, Standard and Sample Preparation

Solution A was prepared by dissolving 28.4g of anhydrous sodium sulfide into 1L of water. Phosphoric acid was added at 2.7 mL. The solution was adjusted to a pH of 2.3 using ethanolamine. Solution A is then added to acetonitrile to make the mobile phase (74:26 Solution A: Acetonitrile).

USP Reference Standard Insulin Human (1342106) and USP Reference Standard Insulin (Pork) (1342300) were purchased from Sigma. System suitability was prepared by preparing a 1.5 mg/mL USP Human Insulin solution and allowing the solution to sit for no less than 72 hours (3 days) to obtain a solution containing the A–21 desamido insulin degradant at no less than 5%. The standard was weighed up at 1.5 mg/mL using the USP Insulin (Pork). The sample solution was weighed up at 1.5 mg/mL using the USP Human Insulin.

LC Conditions

LC systems and detection:	Alliance e2695 HPLC System – W2489 UV/Vis Detector Alliance iS Bio HPLC System – TUV Detector System Y HPLC – TUV Detector System X Bio HPLC – DAD Detector
Wavelength:	214 nm
Sampling rate:	10 points/sec
Vials:	LCGC Certified Clear Glass, 12 x 32 mm, Screw Neck Vial, with Cap and preslit PTFE/Silicone Septum, 2 mL Volume, 100/pk (p/n: 186000307C)
Columns:	XBridge™ Peptide C_{18} Column, 5 µm 4.6 x 150 mm (p/n: 186003580)
Column temparature:	40°C
Sample temparature:	23°C
Injection volume:	20 µL
Flow rate:	1.0 mL/min
Mobile phase A:	Acetonitrile:Solution A (26:74)
Mobile phase B:	Acetonitrile:Solution A (26:74)
Mobile phase C:	10:90 Water:Acetonitrile

Mobile phase D:	10:90 Water:Acetonitrile
Sample manager wash:	10:90 Water:Acetonitrile
Sample manager needle wash:	10:90 Water:Acetonitrile
Separation:	Isocratic, 1 mL/min for 25 mins

Data Management

Chromatography data system:	Empower™ 3, FR 3.8.0.1 (Alliance iS Bio HPLC System) Empower 3, FR 3.7.0 (Alliance e2695 HPLC System)

Results and Discussion

Method Migration and Assessing System Characteristics

Method migration or replicating a method across systems in a single laboratory, typically is straight forward for robust methods. However, moving a method from one system to another can be impacted by system characteristics, particularly if the method is sensitive to small changes in methods conditions. For example, temperature sensitive methods can be impacted by differences in column heater performance. For this study, we will evaluate the method migration of the USP monograph for insulin assay from HPLC systems to the Alliance iS Bio System, and assess the impact, if any, of system characteristics on system suitability criteria.

Prior to performing the analysis, system differences were evaluated and measured. These included characteristics such as dwell volume and extra column dispersion for the (Table 1). All systems used were quaternary systems.

System	Dwell volume/Gradient delay (V_D) (mL)	Extra column dispersion (4 σ)
Alliance iS Bio HPLC System	1.757	21
System X Bio HPLC System	1.397	35
System Y HPLC System	0.556	21
Alliance e2695 HPLC System	1.145	78

Table 1. System dwell volume and extra column dispersion measurements across the systems.

Analysis of the method conditions allows us to assess which system characteristics may impact the USP insulin assay results. With the method column temperature setting at 40°C, system to system variability and the use of a pre-heater can impact retention times. Mobile phase preparation may also contribute to differences. Mobile phase should be pre-made and split between systems to eliminate any additional variation.

Based on these measurements differences across the systems include dwell volume and dispersion. Dwell volume can be impacted by pump design and mixer configuration, while dispersion can be impacted by tubing, including preheaters. For the USP monograph of insulin assay, an isocratic method, dwell volume does not impact retention times, while extra column dispersion can impact band broadening, resolution and efficiency.

Method Migration of Insulin System Suitability to Alliance iS Bio System

The USP monograph for Insulin Assay, a widely used monograph, is an isocratic method that contains two analytes for the system suitability solution, insulin and insulins degradant product, A–21 desamido insulin. For laboratories that are moving from older instrumentation to new instrumentation, maintaining comparable results during a method migration is crucial. To ensure only system characteristics on method performance were evaluated, the same sample/standard preparation and mobile phase preparations were pooled and split across the systems. This assay was tested on legacy systems in the lab. Results showed that all systems meet the USP criteria with the Alliance iS Bio HPLC System displaying the best results for %RSD for peak area precision for the sample and standard solution. The Alliance iS Bio HPLC System also had some of the lowest results for %RSD for retention time precision for the sample and standard solution.

The stacked chromatograms for the system suitability solution is shown in Figure 1. While retention time shifts are observed, the method was successfully migrated to all the systems tested. The Alliance iS Bio HPLC System

displayed comparable results, along with improved results for %RSD's for standard and sample retention time and peak area. Additional contributors to retention time shift could be related to differences in temperature control of the column.[2]

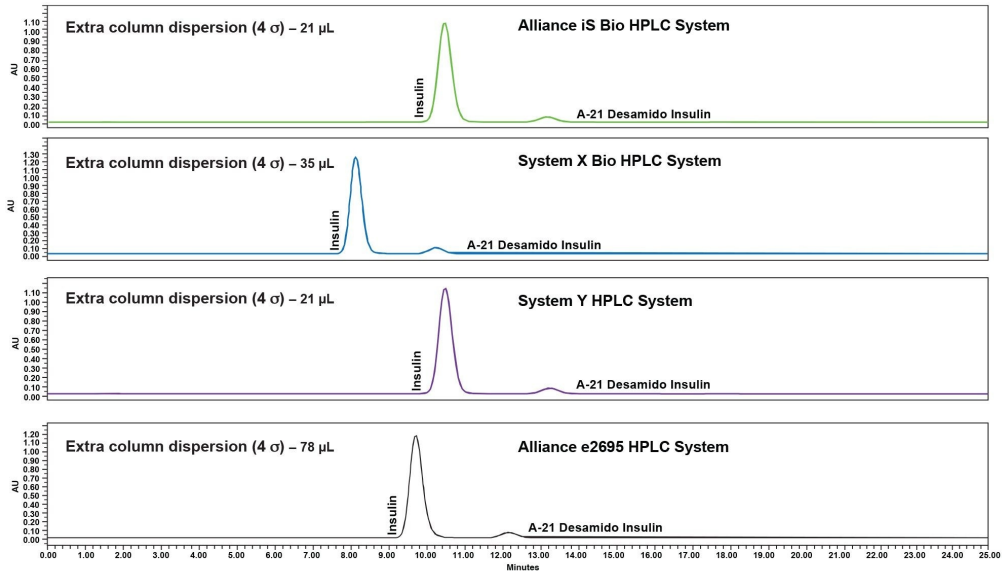

Figure 1. Stacked Overlay of Insulin Assay System Suitability Chromatograms for all systems.

The suitability requirements for this system suitability solution includes USP resolution (HH) of no less than (NLT) 2.0 between insulin and A–21 desamido insulin, and USP tailing of no more than (NMT) 1.8 for the insulin peak for the system suitability solution (n=5). The additional suitability requirement for the standard solution (n=5) is NMT 1.6% for the % RSD for insulin peak area (n=5). Internal criteria was also set for % API of +/-2% between systems.

System	USP tailing	USP resolution (HH)	STD peak area %RSD for insulin	STD retention time % RSD for insulin
Alliance iS Bio HPLC System	1.08	3.62	0.10	0.13
System X Bio HPLC	1.08	3.28	0.60	0.55
System Y HPLC	1.08	3.73	0.27	0.06
Alliance e2695 HPLC System	1.16	3.45	0.12	0.22
Acceptance criteria	**NMT 1.8**	**NLT 2.0**	**NMT 1.6%**	**N/A**

Table 2. USP monograph of Insulin Assay suitability requirement results (n=5) across systems.

The results of the suitability requirements are detailed in Table. 2. The suitability requirements were met for all the systems for this monograph. The results were consistent between all the systems. The Alliance e2695 had an increase in USP tailing when compared to the other systems. When migrating the method from the Alliance e2695 HPLC System to the Alliance iS Bio HPLC System the results for USP tailing, USP resolution and standard peak area and retention time %RSD for the insulin peak were all improved.

Lastly, the standard peak area %RSD for n=5 was calculated for all the systems. The Alliance iS Bio HPLC System had the lowest standard peak area %RSD at 0.10. The remaining three systems showed results of 0.12 to 0.60 for standard peak area %RSD, which are comparable and within the set criteria of NMT 1.6%. This demonstrates that the method can be successfully migrated to all the systems. For the USP resolution (HH) requirement of NLT 2.0, minimal variation in the results was observed.

Evaluation of Sample Analysis Across Systems

In the Table 3 results, the measured API %, sample retention time %RSD for insulin and the sample retention time %RSD were measured. The results showed close agreement for the measured API %, with the largest difference at 0.7% which is within our internal criteria requirement of +/-2%.

System	Measured API %	Sample retention time %RSD for insulin	Sample peak area %RSD for insulin
Alliance iS Bio HPLC System	97.0	0.18	0.20
System X Bio HPLC	97.4	0.69	0.22
System Y HPLC	96.7	0.25	0.26
Alliance e2695 HPLC System	96.9	0.28	0.15

Table 3. Additional parameters (n=5) were measured for method migration comparison.

The retention time %RSD for insulin in the standard and the sample solutions were comparable with System X Bio HPLC reporting out the highest % RSD at 0.55 for the standard retention times and 0.69 for the sample retention times. Retention time %RSD measures precision of the instrument.

The results shown in Tables 2-3 showed very good agreement and demonstrate that the USP monograph for Insulin Assay can be successfully from the Alliance e2695 HPLC System to the Alliance iS Bio HPLC System.

Conclusion

While laboratories are phasing out older systems to update their laboratories with newer systems, it is very important that suitability requirements and acceptance criteria can be met for compendial methods and that any differences in results are minimal. In addition to the set suitability requirements for this method, many additional criteria parameters were evaluated to ensure that there were no significant differences between the systems.

In this method migration, the results for all the systems showed good agreement. The Alliance iS Bio HPLC System demonstrated improved %RSDs for standard peak area and sample and standard retention times over older HPLC systems. Suitability requirements were all within acceptance ranges. The method migration of USP monograph for Insulin Assay showed comparable results indicating a successful method migration to the Alliance iS Bio HPLC System.

References

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