MODERNIZATION OF A LEGACY NORMAL PHASE METHOD ON A MODERN HPLC SYSTEM

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INTRODUCTION

In regulated laboratories, particularly those within the pharmaceutical industry, High-Performance Liquid Chromatography (HPLC) systems play a crucial role in routine analysis. However, some challenges arise due to legacy methods that were developed during the early days of HPLC technology. These methods have not kept pace with recent advancements and modernization in the field and do not fully leverage the latest technological improvements. Advancements in column chemistry and system robustness allow for better separation and detection of analytes.

Modern HPLC systems are more robust, reliable, and capable of handling challenging analyses and can accommodate various chromatographic modes, including Normal Phase chromatography. While legacy methods persist, regulatory agencies such as the United States Pharmacopeia (USP) provide guidance on how to adapt and adjust these methods. This helps bridge the gap between legacy methods and modern HPLC systems.

In this study, we examine the modernization of a legacy normal phase method, USP Propofol Assay, for use with a HPLC system. The original method specified an older L3 column in a non-standardized column configuration (4.6 mm x 200 mm). Given these column specifications, we compared a suitable column to coupling two columns to match the specified column dimensions. We also explored the use of a column that was scaled in accordance with USP Chapter 621[1] that meets the L/dp requirement.

METHOD

Propofol USP Assay[2] System Suitability Requirements:

- Tailing is NMT 1.5 for propofol
- Area relative standard deviation is NMT 1.0% for propofol
- Retention time relative standard deviation is NMT 1.0% for propofol

Sample:

Propofol standard solution is used for system suitability. Standard solution consists of 2.4 mg/mL of propofol in hexanes

Column Connector:

Assembly, rigid connector (p/n WAT022681)

METHOD

Propofol USP Monograph Assay Conditions:

LC System:	Alliance™ iS HPLC System
Detection:	Dual Wavelength UV Detector, 275 nm @ 10 points/second
Columns:	**1**: Spherisorb™ 5µm Silica Column 4.6 × 200mm (p/n PSS830114) **2a**: XBridge™ BEH™ HILIC Column, 130Å, 5-µm, 4.6 × 50mm (p/n 186004451) **2b**: XBridge BEH HILIC Column, 130Å, 5-µm, 4.6 × 150mm (p/n 186004453) **3**: XBridge BEH HILIC Column, 130Å, 5-µm, 4.6 × 250mm (p/n 186004454)
Column Temp:	25.0°C
Sample Temp:	10.0°C
Injection Volume:	10.0 µL
Flow Rate:	2.0 mL/min
Diluent:	Hexanes
Mobile Phase:	Hexanes, acetonitrile, and absolute alcohol (990:7.5:1)
Method:	Assay: Isocratic 15-minute method

RESULTS & DISCUSSION

In this study, we obtained a L3 column, specifically the Spherisorb 5µm Silica Column (4.6 × 200mm, referred to as Column 1), which aligned with the specifications outlined in the USP monograph. Upon configuring the assay analysis, we encountered a challenge: the tailing factor of 2.038 could not meet the requirement of NMT 1.5 for propofol (as indicated in Table 1). Further investigation of the chromatogram revealed detector oversaturation, resulting in poor peak shape and a failed result outcome (see Figure 1).

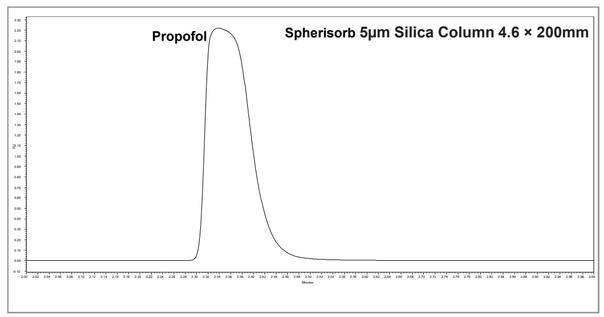

Figure 1. 1 minute window (2min to 3min) chromatogram of propofol assay standard on column 1

RESULTS & DISCUSSION

In our efforts to meet system suitability requirements, we sought alternative L3 columns. Since the only available 200 mm L3 column was unsuitable, we connected two shorter, suitable L3 columns: the XBridge BEH HILIC Column (130Å, 5µm, 4.6 × 50mm, referred to as Column 2a) and the XBridge BEH HILIC Column (130Å, 5µm, 4.6 × 150mm, referred to as Column 2b). These columns were joined using a rigid connector to create a 200 mm L3 column. Following this, we utilized the coupled column for the propofol assay analysis. The results meet system suitability requirements. Notably, we achieved a tailing factor of 1.361 (as indicated in Table 1) and obtained an acceptable chromatogram (refer to Figure 2).

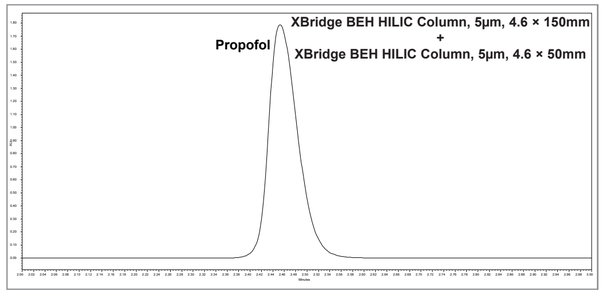

Figure 2. 1 minute window (2min to 3min) chromatogram of propofol assay standard on connected columns 2a and 2b

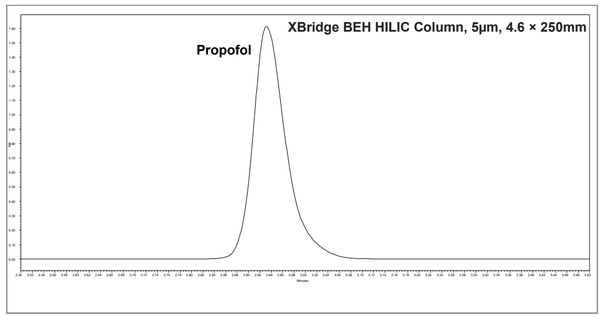

Figure 3. 1 minute window (2.5min to 3.5min) chromatogram of propofol assay standard on column 3

Propofol standard: system suitability			
Column	RT %RSD (NMT 1.0%)	Area %RSD (NMT 1.0%)	Tailing (NMT 1.5)
1	0.061	0.168	2.038
2a + 2b	0.112	0.275	1.361
3	0.192	0.109	1.385

Table 1. System suitability requirement for propofol assay from the 3 different column setups. Results are derived from 5 replicate injections

RESULTS & DISCUSSION

While connecting two columns is a viable option, it does come with cost considerations. Purchasing two columns and a connector adds to the expenses. Additionally, this approach introduces the possibility of new issues by introducing more variables and also leading to additional time for system setups.

In accordance with USP Chapter 621, adjustments to column length and/or particle size are permissible as long as the L/dp ratio remains within specified limits. To illustrate this, we introduced a new L3 column—the XBridge BEH HILIC Column (130Å, 5µm, 4.6 × 250mm, referred to as Column 3). Leveraging the Waters Columns Calculator, we determined the L/dp ratio for both Column 1 and Column 3. Notably, the L/dp value of 50,000 for Column 3 falls within the USP requirement, which allows a range of –25% to +50% relative to the L/dp ratio of Column 1 (40,000)(see figure 4). Despite having the same particle size, Column 3's different length satisfies the L/dp ratio.

By incorporating this scaled column into the assay analysis, we successfully met system suitability requirements. Notably, we achieved a tailing factor of 1.385 (as indicated in Table 1) and obtained an acceptable chromatogram (refer to Figure 3). This approach enables users to utilize a single column while still achieving comparable results and benefiting from advancements over older column technologies.

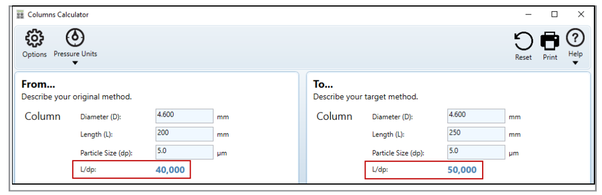

Figure 4. Columns calculator used to scale from column 1 to column 3.

CONCLUSION

- **Legacy columns, like the L3 used for column 1, has an older packing technology and is unable to provide a suitable chromatography.**
- **The use of a newer packing as in BEH Columns helps provide a successful analysis**
- **Coupling two columns, albeit successful, may prove to be a much more expensive option and may be introducing more opportunities for issues to arise.**
- **The third column derived from the use of the columns calculator may be the more suitable route since it adheres to the USP guidance as well as not being more expensive and time consuming as the coupling of two columns.**

References

1. General Chapter: USP. 621 Chromatography, In: USP-NF. Rockville, MD: USP; 01 Oct 2023. DOI: https://doi.org/10.31003/USPNF_M99380_08_01
2. Monograph: USP. Propofol. In: USP-NF. Rockville, MD: USP; 01 Mar 2024. DOI: https://doi.org/10.31003/USPNF_M70460_05_01

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