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응용 자료

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Abstract

USP monographs provide guidelines for quality assurance of a specific drug product. These guidelines usually outline the active ingredients' identity, potency, purity and may include other specific tests like impurity analysis or dissolution. Once these tests are created and validated, the guidelines can be used to test all sorts of pharmacological samples and solutions. USP monographs are excellent for evaluating medicines, providing universal methods for the pharmaceutical industry to evaluate their products. These methods are very rigid and the slightest change in sample preparation, mobile phase preparation, or column functionality and performance can have a massive impact on the results. This could potentially lead to severe consequences, ranging from a failed batch of material to an investigation by a governing body. Using reliable long-lasting columns during testing is essential to the process to avoid those potential complications. MaxPeak Premier High-Performance Surfaces (HPS) Technologies, featured in MaxPeak Premier Columns are designed to reduce variability, and boost confidence in results by reducing the non-specific adsorption interactions that analytes can have with the metal surface of the columns.

In this application note, the USP monograph for evaluation of acetaminophen concentration in cold and cough medicine was modernized following General Chapter <621> guidelines. A MaxPeak Premier Column and a Stainless-Steel column were used in comparison after modernization. All requirements of the monograph were met and passed using both types of columns, however, the MaxPeak Premier Column showed a significant decrease in variability across all injections.

Benefits

- Increased reproducibility across injections using MaxPeak Premier Columns
- Eliminated doubt in peak identification due to highly reproducible results

Introduction

The United States Pharmacopeia (USP) sets the quality, strength, purity, and identity standards for drug products, food ingredients, and other dietary supplements. USP monographs are developed so that there is a procedure for and regulations around testing new generic formulations. Most over-the-counter medications that are used daily have been tested using USP monographs that were developed when the medicine originally lost its patent protection. These USP monographs most likely use older column technology based on when they were first developed. The column technology may use longer column configurations (*i.e.*, 4.6 x 250 mm) and/or large particle size (*i.e.*, 5 µm). Using these kinds of columns usually translates to longer run times with more solvent used per injection. Thankfully, newer column technology with smaller particle sizes (*i.e.*, 2.5 µm) provides higher efficiency, leading to shorter run times and a decrease in mobile phase consumption. However, modernizing a USP monograph can be complicated if not properly done, which is one reason why there is such a hesitancy to modernize. The USP General Chapter <621> provides guidelines and procedures to make modernization more accessible[1] These guidelines cover the parameters that are allowed to be changed to properly modernize the monograph, including run time, flow rate, and column specifications.[1]

Furthermore, given that USP monograph system suitability requirements are strict, there needs to be a sense of confidence and reliability in the equipment being used. This can be difficult when outside factors such as non-specific adsorption between analyte and metal surfaces may be present.[2] This interaction can lead to irreproducibility as well as poor peak shape for the analytes in question. MaxPeak Premier Columns were specifically developed to mitigate the metal-adsorption of analytes leading to improved peak shape and recovery along with greater reproducibility.[2]

In this application note, the benefits a Waters™ MaxPeak Premier Column provides compared to a Stainless-Steel column are shown. The importance of mitigating non-specific metal adsorption that could affect the results and reproducibility of a specific test, even when the analyte in question may not at first glance be affected by the presences of metals is discussed.

Experimental

Sample Description

Three separate solutions were created as outlined in the USP monograph method. The standard solution and system suitability standard contained 0.2 mg/mL acetaminophen from a purified standard. The sample solution contained 0.2 mg/mL of acetaminophen. The acetaminophen sample was taken from OTC cough syrup. All three solutions were prepared in 45/55 Methanol/Water as the diluent.

LC Conditions

LC systems:	ACQUITY™ H-Class Plus with PDA Detector
Detection:	UV @ 254 nm
Columns:	XBridge™ BEH™ C_{18}, 2.5 µm. 2.1 x 100 mm (p/n: 186006031) XBridge Premier BEH C_{18}, 2.5 µm, 2.1 x 100 mm (p/n: 186009828)
Sample temperature:	10 °C
Injection volume:	0.8 µL
Flow rate:	0.42 mL/min
Mobile phase A:	100% Methanol
Mobile phase B:	100% Water
Isocratic conditions:	45/55 Methanol/Water

Data Management

Chromatography software:	Empower™ 3 Feature Release 5 Hotfix 1

Results and Discussion

After modernization of the monograph, following USP General Chapter <621> guidelines, five injections of the standard solution, system suitability standard, and sample solution (n=15) were completed using both a Stainless-Steel column and a MaxPeak Premier Column. The last injection of all three solutions run on both columns are shown in Figure 1. The peaks outlined in blue are the results of the MaxPeak Premier Column while the peaks in orange are the results of the Stainless-Steel column. Both system suitability and standard solutions have the same USP tailing, retention time relative standard deviation, peak area relative standard deviation and acetaminophen percentage requirements.[3] USP tailing cannot exceed that of 2.0 for the acetaminophen peak and relative standard deviation (%RSD) cannot exceed 2.0%.[3] The percentage of acetaminophen found in the samples must range between 90–110%.[3] For each column used, the criterion of the monograph is met. The criteria for the strength of acetaminophen in formulated cough syrups are listed in the USP monograph. At first glance it is clear the MaxPeak Premier Column provides improved accuracy and more reproducible results.

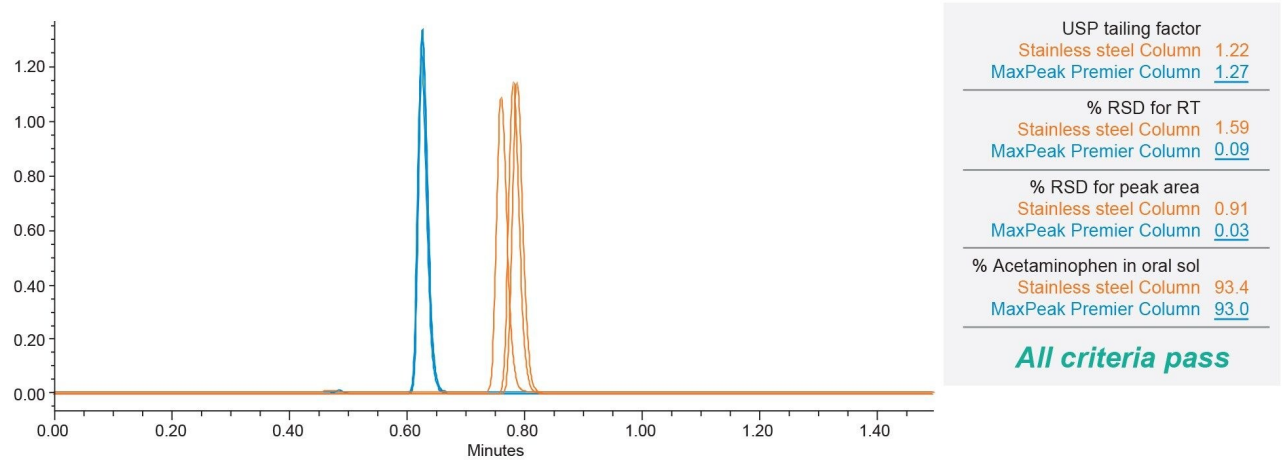

Figure 1. Chromatograms showing overlays of the standard solution, system suitability standard, and sample solutions of acetaminophen, using the modernized USP monograph conditions with 2.1 x 100 mm, 2.5 µm XBridge BEH C_{18} Premier Columns and Stainless-Steel columns using an ACQUITY H-Class System. Values for tailing and relative standard deviation are average values across all injections (n=15).

The %RSD for retention time on the MaxPeak Premier Column is 0.09% across 15 injections while the retention time %RSD on the Stainless-Steel column is 1.59%. As seen in Figure 1, the overlaid chromatograms show this with all three samples overlapping perfectly on the MaxPeak Premier Column, while at least one sample is off-center on the Stainless-Steel column. In this case the peak that is most different on the Stainless-Steel column is the sample solution, which contains not only acetaminophen but other active ingredients and excipients as well from the oral solution. Similarly, the %RSD for peak area using the MaxPeak Premier Column is 0.03% while the %RSD for peak area with the Stainless-Steel is higher at 0.91%. Lastly the percentage of acetaminophen found in the sample solutions does slightly differ from the two columns tested by 0.4%. The MaxPeak Premier Column shows the percent acetaminophen found in the sample to be 93.0% while the Stainless-Steel %acetaminophen was calculated to be 93.4%. Since both columns are well within passing requirements, the difference is negligible. Given all 15 injections of three different samples on the MaxPeak Premier Column were consistent indicates that there is no solvent mismatch between sample and mobile phase runs. There is, likely, a synergistic interaction between the acetaminophen and active ingredients of the oral solution with the metal surface of the column resulting in retention time shifts and variability as shown on the Stainless-Steel column.

MaxPeak Premier Columns improve the reproducibility of this assay across multiple samples by mitigating the

secondary interactions between sample and metal hardware. While acetaminophen itself may not interact with the metal surface column, the other components of the sample may cause a slight variation in acetaminophen retention. The Stainless-Steel column does pass all requirements; however, the MaxPeak Premier Column shows definitive improvements in terms of reproducibility across sample types eliminating any doubt that the results are accurate and appropriate for batch release testing.

Conclusion

The standards set by the United States Pharmacopeia are widely used to ensure medicinal products are meeting safety and potency requirements. However, these methods often use older LC technology with columns that are less efficient. Newer column technology and smaller particle size can be used to reduce analysis time and solvent usage due to recent changes to USP General Chapter <621>. These changes also enable the use of newer column hardware, such as MaxPeak Premier Columns.

This application note demonstrates that while Stainless-Steel columns provide passing results upon modernization of a USP monograph, using a MaxPeak Premier Column will ultimately eliminate the doubt in accuracy and reproducibility during testing. The use of MaxPeak Premier Columns greatly reduced retention time and peak area variability across samples tested, without adversely affecting assay results or peak tailing. This demonstrates that MaxPeak Premier Columns are suitable for a variety of analyses.

Acknowledgements

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References

1. USP General Chapter <621>. Accessed April 25th 2022. https://www.usp.org/sites/default/files/usp/document/harmonization/gen-chapter/harmonization-november-2021-m99380.pdf <https://www.usp.org/sites/default/files/usp/document/harmonization/gen-chapter/harmonization-november-2021-m99380.pdf>

2. Berthelette K., Turner J., Kalwood J., Haynes K. Improving Separation Efficiency with CORTECS™ Premier Column that Feature Solid-Core Particles and MaxPeak Premier HPS Technology. Waters Application Note. 720007872, 2023.

3. USP monograph: Acetaminophen, Dextromethorphan Hydrobromide, Doxylamine Succinate, and Pseudoephedrine Hydrochloride Oral Solution. Accessed April 25th 2022. https://online.uspnf.com/uspnf/document/1_GUID-36376BA5-52C8-469D-AC2E-E17BD8223113_1_enUS?source=Search%20Results&highlight=acetaminophen < https://online.uspnf.com/uspnf/document/1_GUID-36376BA5-52C8-469D-AC2E-E17BD8223113_1_enUS?source=Search%20Results&highlight=acetaminophen>

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