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Application Note

BEH C_{18} Batch-to-Batch Robustness for the Analysis of Rosuvastatin and Impurities

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Abstract

The main goal of this study is to investigate the long-term batch-to-batch reproducibility of 10 different lots of XBridge BEH C_{18} Columns.

Benefits

Batch-to-batch reproducibility of the XBridge BEH C_{18} Column

Introduction

The goal of a method development process is to find a well-working and robust method that consistently delivers the expected performance throughout its lifecycle. This process can be very complex and timeconsuming, especially if multiple chromatographic parameters need to be explored.[1] Column reproducibility is a key parameter that has a critical impact on the long-term reliability and robustness of such analytical methods. This is because column-to-column and batch-to-batch variability can result in unacceptable chromatographic performance that could require the method to be revalidated for regulatory acceptance.[2] As such, it is crucial to select columns that are rugged and reproducible when developing analytical methods to reduce the risk of having out-of-specification and out-of-trend results throughout the method life.

The main goal of this study is to investigate the long-term batch-to-batch reproducibility of 10 different lots of XBridge BEH C_{18} Columns. Lots were selected between 2005 and 2019, representing nearly 15 years of reproducibility data on separating rosuvastatin and impurities. Structures of these compounds are shown in Figure 1. XBridge BEH C_{18} Columns are one of the most popular LC columns with a wide pH range (1–12) allowing for suitable separations for a diverse range of neutral, acidic, and basic compounds.[1,3,4] The trifunctional bonding and end-capping provides stability, reproducibility, and efficiency to be used under wide chromatographic conditions.

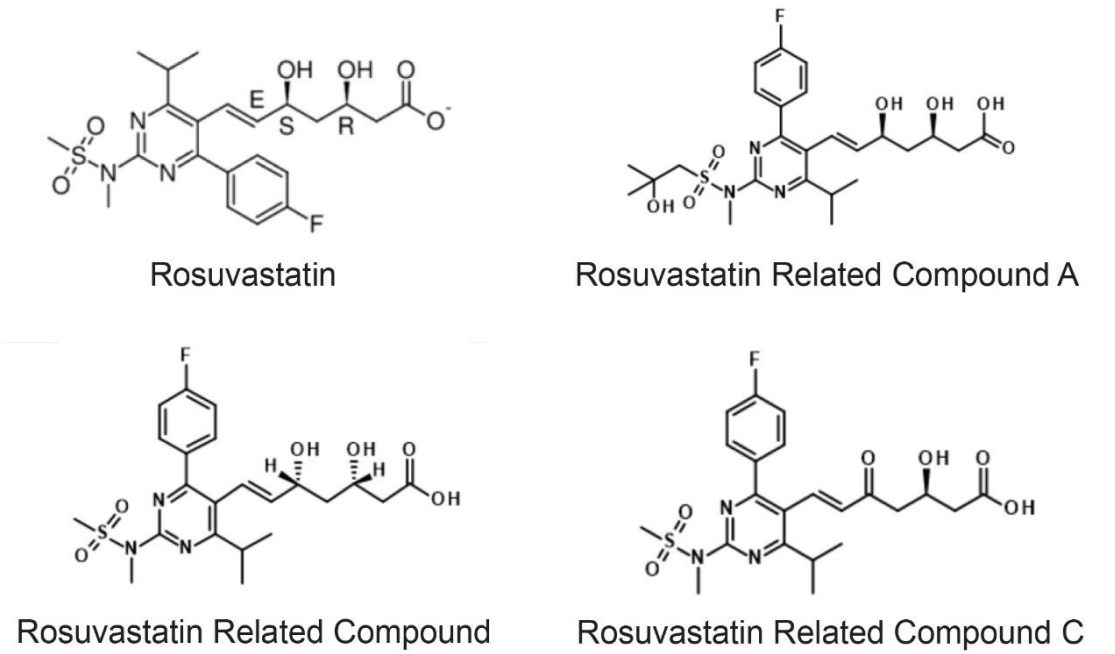

Figure 1. Chemical structures of rosuvastatin and its related compounds A, B, and C.

When looking at the repeatability of chromatographic parameters using different batches of columns, variances may occur in retention times, peak symmetries, efficiencies, and various other parameters. Lot-to-lot reproducibility of the retention and several other profile characteristics of the peaks obtained with 10 columns from 10 different batches of XBridge BEH C_{18} will be examined in this work.

Experimental

Materials and standard preparations

Rosuvastatin reference standard was purchased from USP (Rockville, USA). Rosuvastatin related compounds A, B, and C were also purchased from USP (Rockville, USA). A stock solution of rosuvastatin standard at a concentration of 0.7 mg/mL was prepared in a mixture of acetonitrile and water (1:1). Stock solutions of rosuvastatin related compounds A, B, and C at concentrations of 0.1 mg/mL were also prepared in a mixture of acetonitrile and water (1:1). These stock solutions were next diluted in a mixture of acetonitrile and water (1:1) to make the test mixture. The final concentrations for these analytes in this mixture were 0.014 mg/mL-1 rosuvastatin and 0.0028 mg/mL rosuvastatin related compounds A, B, and C.

Method conditions

An HPLC method of rosuvastatin was described in the US Pharmacopoeia with an analysis time of about 70 min was used in this study.

Data management

Empower 3 Chromatographic Data System (CDS)

LC Conditions

System:	ACQUITY Arc with Quaternary Solvent Manager (rQSM), Sample Manager (rFTN), Column Heater, ACQUITY UPLC PDA Detector, ACQUITY QDa Mass Detector
Detector:	ACQUITY PDA and QDa
Columns:	10 different batches of XBridge BEH C_{18}, 3.5 µm, 3.0 × 150 mm
Batch Dates:	6/2/2005, 10/21/2005, 4/3/2006, 12/5/2007, 9/22/2009, 1/6/2011, 17/10/12, 20/11/15, 25/06/17, 18/03/19
Flow rate:	0.75 mL/min
Mobile phase A:	70% water:29% acetonitrile: 1% trifluoroacetic acid 0.1%
Mobile phase B:	25% water:74% acetonitrile: 1% trifluoroacetic acid 0.1%
Profile:	Isocratic at 0% organic for 30.0 min Gradient from 0–40% organic for gradient times ranging from 30–50 min

	Gradient from 40–100% organic for gradient times ranging from 50–60 min Isocratic at 100% organic for 10.0 min Ramp down from 100% to 0% organic for 1.0 min Isocratic at 0% organic for 9 min
Column temp.:	Constant 40 °C
UV detection:	242 nm
Injection volume:	10 µL working solution

MS conditions

System:	ACQUITY QDa Mass Detector
Ionization mode:	ESI+
Capillary voltage:	0.8 kV
Con voltage:	15 V
Source temp.:	600 °C

Results and Discussion

Relative Retention Time

A paramount parameter to consider when evaluating batch-to-batch reproducibility of columns is the relative retention time of compounds. Table 1 summarizes the relative retention times of rosuvastatin related compounds, A, B, and C when analyzed on 10 different batches of XBridge BEH C_{18} Column using the USP method described above. Results have shown that the relative retention time is very reproducible on the 10

batches for all the analytes. For example, the %RSD for the relative retention times of the rosuvastatin related compounds B and C were only 3.3 and 3.6 on these 10 columns. Such results are referred to as "highly precise" when different batches of columns are studied.[5] It should be noted that the relative retention time reproducibility is normally analyte dependent and it can be more reproducible for some analytes than others. For example, the relative retention time for rosuvastatin related compound A was remarkably reproducible with a %RSD value of 0.35 over the 10 different column batches, as can be seen in Table 1.

Batch #	t_R Rosuvastatin (minutes)	Relative t_R (Rosuvastatin related A/rosuvastatin)	Relative t_R (Rosuvastatin related B/rosuvastatin)	Relative t_R (Rosuvastatin related C/rosuvastatin)
1	13.26	0.89	3.93	4.90
2	13.69	0.89	3.80	4.71
3	12.72	0.89	4.01	4.99
4	12.72	0.90	3.98	4.95
5	12.79	0.89	3.92	4.87
6	12.68	0.89	3.95	4.91
7	13.20	0.89	3.81	4.73
8	13.81	0.89	3.67	4.52
9	13.83	0.90	3.66	4.51
10	13.26	0.89	3.80	4.70
% RSD	3.50	0.35	3.30	3.60

Table 1. Relative retention time of rosuvastatin related compounds A, B, and C when analyzed using the USP method described in the text. Each value represents the average of three-replicate injections. The RSD value represents 30 injections made on 10 columns, packed with 10 different batches of packing material.

Critical Pair Resolution

Another chromatographic parameter that is important to consider when evaluating columnto- column reproducibility is the resolution of the critical pair. A critical pair represents the two components of the chromatogram with the lowest calculated resolution between them. In this case, the critical pair was rosuvastatin related compound A and rosuvastatin. The ability of the different columns to reproducibly resolve the critical pair was also evaluated here. Results have shown that all columns were able to resolve these two components with an average USP resolution of 3.3 and with a good reproducibility of 5% RSD. Representative separation of rosuvastatin and impurities is depicted in Figure 2.

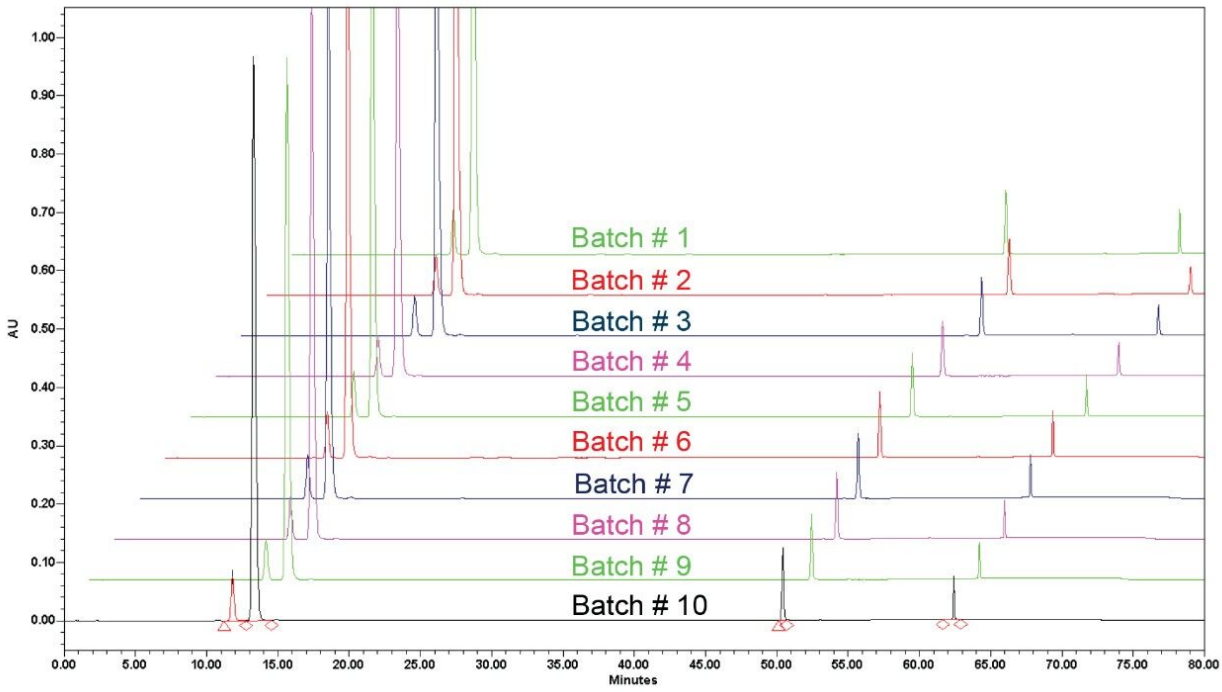

Figure 2. Chromatograms of the USP method described on XBridge BEH C_{18} Columns from 10 different batches. The retention order is impurity A, rosuvastatin, impurities B and C.

Peak Symmetry Factor

Peak symmetry is an important chromatographic parameter that should be considered when looking at batch-to-batch reproducibility of columns. The peak symmetry parameter that was studied in this work is the USP tailing factor. It is determined from the peak width at 5% of the peak height. Results can be seen in Table 2 which shows the tailing factors for all analytes when analyzed on the 10 different columns. The %RSD values of the tailing factor on the 10 columns from the 10 different batches were in the range of 2.1 to 3.2. These results indicate excellent batch-to-batch column reproducibility.

Batch #	USP Tailing (Rosuvastatin related A)	USP Tailing (Rosuvastatin)	USP Tailing (Rosuvastatin related B)	USP Tailing (Rosuvastatin related C)
1	1.14	1.37	1.09	1.12
2	1.12	1.37	1.08	1.11
3	1.1	1.31	1.07	1.09
4	1.15	1.45	1.12	1.17
5	1.1	1.36	1.08	1.12
6	1.05	1.29	1.04	1.07
7	1.09	1.34	1.05	1.1
8	1.06	1.33	1.05	1.09
9	1.1	1.38	1.07	1.12
10	1.08	1.33	1.06	1.11
%RSD	3.2	3.6	2.2	2.8

Table 2. USP tailing results of rosuvastatin standard and its impurities. Each value represents the average of three-replicate injections. The RSD value represents 30 injections made on 10 columns, packed with 10 different batches of packing material.

Column Efficiency (Plate Count)

Column efficiencies, represented by the USP plate count of rosuvastatin and its related compound peaks on the 10 different batches, were also studied in this work. Results have shown very good plate count reproducibility for these compounds. For example, as can be seen in Table 3, the %RSD values for the plate count of rosuvastatin and its related compound A were 7.9 and 8.1, respectively. These results indicate very good plate count reproducibility given that column efficiencies have always been more difficult to measure with precision than retention data.5 For this study, the USP plate counts were calculated for rosuvastatin and its related compound A only because these are the only two analytes that elute during the isocratic segment of the chromatographic profile.

Batch #	Plate Count (Rosuvastatin)	Plate Count (Rosuvastatin Related A)
1	11 400	12 400
2	12 500	13 300
3	11 400	12 000
4	14 200	15 500
5	14 000	15 000
6	13 400	14 000
7	13 400	14 300
8	14 300	15 100
9	13 500	14 600
10	13 700	14 700
% RSD	7.9	8.1

Table 3. USP plate count of rosuvastatin and its related compounds A, B, and C. Each value represents the average of three-replicate injections. The RSD value represents 30 injections made on 12 columns, packed with 12 different batches of packing material.

Batch-To-Batch Consistency

Perhaps not as paramount as retention time, it is still worth noting that batch-to-batch consistency over such an extended time period supports the precision of the resulting data, highlighting the importance of quality and consistency during the LC column manufacturing process. This is control is critical when mitigating column performance risks as a supporting tool during method lifecycle management (MLCM).

Conclusion

- This application note demonstrates the robustness of XBridge BEH C_{18} Columns when used for the analysis of rosuvastatin and impurities.
- LC column batch-to-batch reproducibility is an example of a key control strategy to mitigate column performance risks and to further ensure good method performance throughout a method's lifetime.
- This application note also demonstrates the importance of robustness and control of the column temperature, the mobile phase flow rate, and the mobile phase composition obtained with the Waters equipment

References

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720006739, January 2020