HPLC columns

Evaluation of batch-to-batch consistency of reversed phase HPLC columns for long-term method validation

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Abstract

Purpose: The purpose of this study is to evaluate the performance consistency of a widely-used reversed phase High-Performance Liquid Chromatography (HPLC) column over multiple batches and several years of production. The goal is to ensure that the columns maintain reproducible results that support validated methods for pharmaceutical purity analysis across different production sites and over time. Consistency in column performance is critical to maintaining the reliability of pharmaceutical analyses and ensuring the quality and safety of pharmaceutical products.

Methods: Chromatographic data was collected and analyzed to compare the performance of columns from different media batches. Statistical methods were applied to evaluate the consistency of the chromatographic parameters across different column media batches.

Results: The results confirmed that the columns maintained consistent performance, demonstrating product stability suitable to support validated methods over extended periods. This consistency is crucial for maintaining the reliability of pharmaceutical analyses across different laboratories and over time.

Introduction

HPLC columns validated for the analysis of pharmaceutical purity must remain unchanged throughout the drug's lifecycle. These columns are validated using specific methods, and it is crucial that they yield reproducible results across different production sites. Given that HPLC columns have a limited lifespan and require periodic replacement, it is necessary to provide column-to-column and batch-to-batch consistency in their performance. The responsibility falls on column vendors to ensure that the quality and performance of the columns remain consistent despite any changes in production processes.

In this study, the performance consistency of a widely-used reversed phase HPLC column was evaluated over multiple batches and several years of production. The assessment involved measuring common chromatographic parameters to ensure reproducibility.

Materials and methods

Sample Preparation

For the analysis, the sample preparation involved the use of several compounds, including theophylline, p-nitroaniline, methyl benzoate, phenetole, and o-xylene. These compounds were meticulously prepared and handled according to established protocols to ensure the accuracy and reliability of the analytical results.

Analysis method

The Thermo Scientific™ Hypersil GOLD™ C18 analytical columns (Table 1), were evaluated. Each column features C18 (Octadecyl) chemistry, providing robust and reliable separation capabilities. The columns contain 5 µm particle size and 175 Å pore diameter, ensuring a high surface area of 220 m²/g for enhanced interaction with analytes.

Table 1. Analytical column specifications

	Particle size (µm)	Length (mm)	Inner diameter (mm)
Hypersil GOLD C18 column 250 mm x 4.6 mm 5 µm (PN 25005-254630)	5	250	4.6
Hypersil GOLD C18 column 150 mm x 4.6 mm 5 µm (PN 25005-154630)	5	150	4.6
Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm (PN 25005-052130)	5	50	2.1

In the method analysis, three specific columns of different dimentions were tested under isocratic conditions by using acetonitrile (A) and water (B) as the mobile phase.

Table 2. HPLC analysis parameters

	Hypersil GOLD C18 column 250 mm x 4.6 mm 5 µm (PN 25005-254630)	Hypersil GOLD C18 column 150 mm x 4.6 mm 5 µm (PN 25005-154630)	Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm (PN 25005-052130)
Mobile Phase (A:B)	60:40	60:40	50:50
Flow (mL/min)	1.25	1.25	0.2
Sample volume (µL)	2.5	2.5	0.5

Data Analysis

Every media batch underwent a detailed evaluation where the mean values were computed initially. Following this, the standard deviation for each batch was calculated to understand the variability within the data. The Relative Standard Deviation (RSD) was then derived from all the data to quantify the consistency across batches. Finally, the retention window was calculated based on the annual data, providing a comprehensive insight into the retention characteristics over the year.

Results

To evaluate the performance consistency of the HPLC columns, a statistical analysis was conducted focusing on two key chromatographic parameters: average relative retention time (RRT) and average peak asymmetry. A total of 90 different batches of HPLC column media, synthesized over a period of three years, were analyzed. Approximately 17,500 analytical columns were evaluated to ensure a comprehensive assessment of performance consistency. For each batch, the retention time and peak asymmetry for a set of standard pharmaceutical compounds were measured. To ensure the reliability of the measurements, multiple columns were packed from a single media batch, with each column tested individually to confirm consistent and accurate results.

Figure 1. Batch-wise average RRT, normalized by o-Xylene for Hypersil GOLD C18 column 250 mm x 4.6 mm 5 µm (83 Batches)

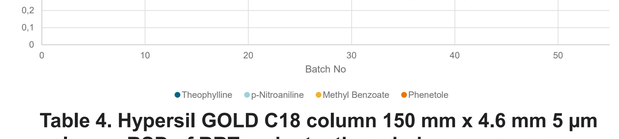


Table 3. Hypersil GOLD C18 column 250 mm x 4.6 mm 5 µm columns RSD of RRT and retention window summary

	RSD of RRT, %	Retention window, s
Theophylline	2.0	7.8
p-Nitroaniline	1.27	12
Methyl Benzoate	0.75	15.6
Phenetole	0.34	25

RRT for Hypersil GOLD C18 column 250 mm x 4.6 mm 5 µm columns was conducted across 83 batches synthesized media over a period of three years. RRT of theophylline, p-nitroaniline, methyl benzoate, and phenetole were assessed by normalizing them against the retention time by o-xylene. In the compound data (Table 3) RSD and retention window in seconds for all 4 compounds. For Theophylline, the RSD is 2% with a retention window of 7.8 seconds, indicating moderate precision. p-Nitroaniline has an RSD of 1.27% and a retention window of 12 seconds, showing higher precision. Methyl Benzoate exhibits an even lower RSD of 0.75% and a retention window of 15.6 seconds, reflecting very high precision. Phenetole, with the highest precision, has an RSD of 0.34% and a retention window of 25 seconds.

Figure 2. Hypersil GOLD C18 column 150 mm x 4.6 mm 5 µm : Normalized (by o-xylene) average RRT in 53 Batches

Table 4. Hypersil GOLD C18 column 150 mm x 4.6 mm 5 µm columns RSD of RRT and retention window summary

	RSD of RRT, %	Retention window, s
Theophylline	2.11	5.7
p-Nitroaniline	1.32	4.7
Methyl Benzoate	0.77	6.7
Phenetole	0.35	10.3

An evaluation of the average mean of RRT for Hypersil GOLD column C18 150 mm x 4.6 mm 5 µm was conducted across 53 different media batches. The results indicated that RRT of theophylline exhibited RSD of 2.11%, with a corresponding retention window of 5.7 seconds. For p-nitroaniline, the RSD was observed to be 1.32%, and the retention window measured at 4.7 seconds. Methyl benzoate demonstrated an RSD of 0.77% with a retention window of 6.7 seconds. Lastly, phenetole showed an RSD of 0.35%, with a retention window of 10.3 seconds.

Figure 3. Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm: average RRT normalized by o-Xylene in 29 Batches

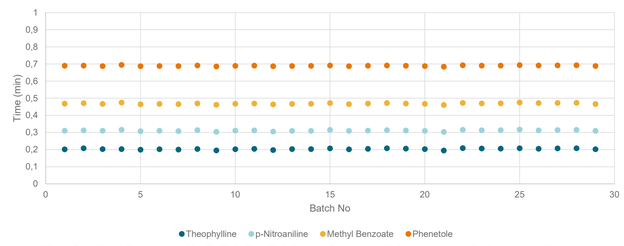


Table 5. Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm columns RSD of RRT and retention window summary

	RSD of RRT, %	Retention window, s
Theophylline	1.7	3
p-Nitroaniline	1.08	6.4
Methyl Benzoate	0.74	9.9
Phenetole	0.32	16.8

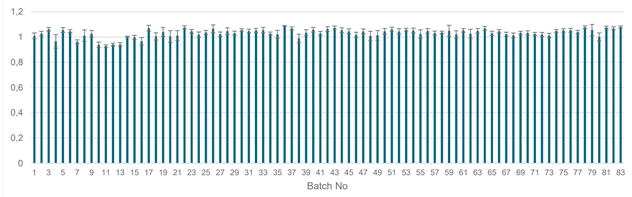
The average RRT for Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm was calculated for 29 media batches synthesized over three years. Normalizing the retention times by o-xylene, the consistency and variability of several compounds were assessed: theophylline, p-Nitroaniline, methyl benzoate, and phenetole. The results demonstrated that theophylline exhibited an RSD of 1.7% and a retention window of 3 seconds. For p-Nitroaniline, the RSD was 1.08% with a retention window of 6.4 seconds. Methyl Benzoate showed an RSD of 0.74% and a retention window of 9.9 seconds. Phenetole, the final compound, had an RSD of 0.32% with a retention window of 16.8 seconds.

These evaluations provide a comprehensive understanding of RRT and their consistency across multiple media batches for different product numbers. The results indicate low variability in retention times, as reflected by the RSD values, suggesting high reproducibility in the media synthesis processes. The retention window calculations highlight differences in retention behavior among the compounds, with Phenetole consistently showing the longest retention window.

These findings are crucial for quality control and optimization, offering insights into the stability and reliability of retention times for key compounds. By normalizing against o-xylene, the data remains comparable across different media batches and conditions, providing a robust framework for future studies and applications.

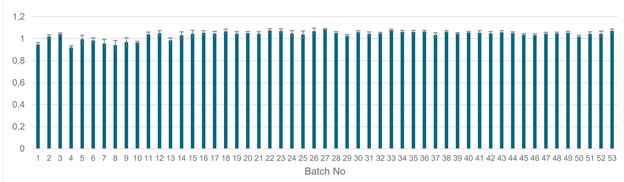
In addition to assessing RRT, a detailed analysis of the average peak asymmetry for Hypersil GOLD C18 column 250 mm x 4.6 mm 5 µm across 83 batches was conducted (Figure 4). Peak asymmetry is a key parameter for evaluating the quality and performance of chromatographic peaks. The average peak asymmetry values were calculated for each media batch, and error bars were included to illustrate the variability within the batches. These findings highlight the consistency and reliability of the media synthesis process, as well as the importance of monitoring peak asymmetry to ensure optimal chromatographic performance. O-xylene average asymmetry was evaluated for each media batch individually. Each media batch was packed with multiple columns, and each column was tested separately to evaluate o-xylene peak asymmetry. Afterward, the standard deviation was calculated for every media batch. The use of error bars provides a visual representation of the variability, allowing for a more comprehensive understanding of the data.

Figure 4. Hypersil GOLD C18 column 250 mm x 4.6 mm 5 µm: o-Xylene peak asymmetry analysis with standard deviation in 83 Batches



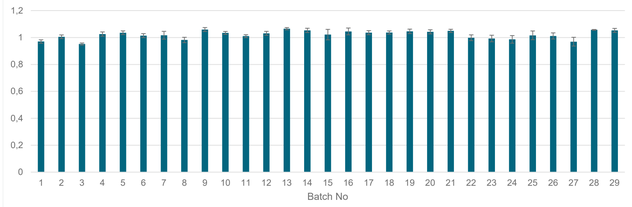
A detailed evaluation of the average peak asymmetry for Hypersil GOLD C18 column 150 mm x 4.6 mm 5 µm was conducted across 53 batches to assess the chromatographic performance of o-xylene. The analysis revealed a range of peak asymmetry values, with corresponding standard deviations reflecting the precision of the measurements. These results provide valuable insights into the consistency and reliability of the chromatographic peaks, emphasizing the need for continuous monitoring and optimization. The use of error bars in Figure 5 allows for a clear visualization of the variability, aiding in the identification of any possible inconsistencies in the column packing process for the Hypersil GOLD C18 150 mm x 4.6 mm 5 µm column.

Figure 5. Peak asymmetry evaluation of o-Xylene for Hypersil GOLD C18 column 150 mm x 4.6 mm 5 µm: average values and standard deviations



For Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm, an evaluation of the average peak asymmetry of o-xylene across 29 batches was conducted to assess the chromatographic performance and consistency. This analysis revealed a range of peak asymmetry values, with each batch accompanied by its standard deviation to highlight the precision of the packing column consistency (completing the packing of the columns). Each column was carefully packed and evaluated individually. The variation in peak asymmetry values across the batches, along with the associated standard deviations, provides a detailed understanding of the reproducibility and stability of the media synthesis process. This information is crucial for ensuring the quality and reliability of the chromatographic peaks, and the use of error bars in the figure allows for a clear visualization of the data. Overall, these findings underscore the importance of continuous monitoring and optimization in maintaining high-quality chromatographic performance for Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm.

Figure 6. Evaluation of o-Xylene peak asymmetry in Hypersil GOLD C18 column 50 mm x 2.1 mm 5 µm: mean and standard deviation values



The comprehensive analysis of peak asymmetry across different product numbers and batches underscores the importance of maintaining high-quality standards in chromatographic processes. By evaluating the average peak asymmetry and incorporating error bars to visualize variability, we can identify potential areas for improvement in the synthesis and production processes.

This approach allowed for a comprehensive evaluation of the retention time data, providing insights into the precision and reproducibility of the chromatographic process for these compounds.

Conclusions

The study confirmed that the evaluated HPLC columns exhibited consistent performance across multiple batches and years of production, with low variability in relative retention times and peak asymmetry. This consistency is crucial for maintaining the reliability of pharmaceutical purity analyses and ensuring the quality and safety of pharmaceutical products.

- The evaluation of ~17,500 analytical columns and 90 different media batches provided a comprehensive assessment of performance consistency.
- The evaluation of Hypersil GOLD C18 columns with different lengths showed consistent relative retention times, with all RSD values being lower than 2.11%.
- The column packing quality is demonstrated to be highly consistent and reliable, as evidenced by the detailed analysis of peak asymmetry across multiple media batches
- The HPLC columns assessed in this study showed stable and consistent performance across numerous batches over several years, guaranteeing dependable outcomes for pharmaceutical purity analysis.

References

1. Lloyd R. Snyder, Josep H. J. Kirkland, John W. Dolan “Introduction to Modern Liquid Chromatography” third Edition

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