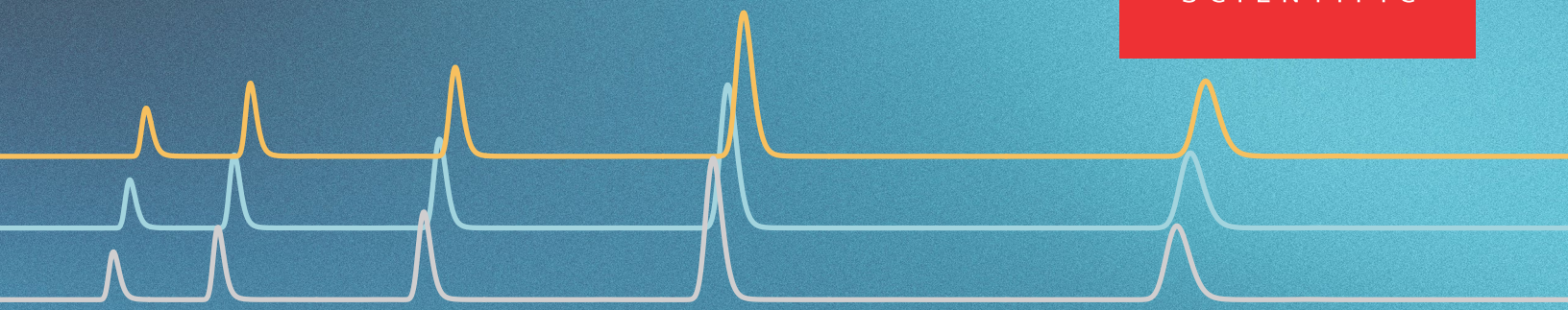
Technical note | 004127



Pharma

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

Authors

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Keywords

HPLC columns, reproducibility, Hypersil GOLD column, column-to-column, batch-to-batch

Benefits

- This technical note showcases the consistent manufacturing of Thermo Scientific™ Hypersil GOLD™ Columns in different formats, ensuring reliable performance and reproducible results across multiple media batches.
- The column packing quality is demonstrated to be highly consistent and reliable, as evidenced by the analysis of relative retention time and peak asymmetry across multiple media batches.

Goal

The goal of this technical note is to prove the long-term batch-to-batch consistency of Hypersil GOLD reversed-phase HPLC columns by analyzing relative retention time and peak asymmetry over a three-year period.

Introduction

High-performance liquid chromatography (HPLC) methods are integral to the pharmaceutical industry, ensuring the purity and quality of drug products. For these methods to remain reliable and effective, it is essential that the analytical columns used demonstrate consistent performance over time. This technical note focuses on the reproducibility of analytical columns tested with pharmaceutical compounds over several years.

Maintaining consistent column performance is critical for various reasons. It ensures that HPLC methods remain valid throughout the lifecycle of a drug product, allowing for accurate and reliable purity analysis. Additionally, the ability to directly compare purity characterization data over a multi-year period facilitates long-term trend analysis and supports continuous quality monitoring, ensuring that pharmaceutical products consistently meet stringent purity standards. The HPLC methods should be transferable and reproducible across different laboratories, for example, to serve different production sites.

This technical note explores the reproducibility of analytical columns over the years. By examining data from QC certificates, it demonstrates how consistent column performance contributes to the integrity of analytical methods and the overall quality control process in the pharmaceutical industry.

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm, Millipore™ Milli-Q™ Academic Water Purification System, Cat. No. ZMQS50001
- Fisher Scientific™ Acetonitrile, HPLC grade, Cat. No. A998-4
- Fisher Scientific™ 2-Propanol (IPA) (HPLC), Cat. No. A451-4
- Fisher Scientific™ Water with 0.1% formic acid (FA) (v/v), Optima™ LC/MS grade, Cat. No. LS118-4
- Sigma-Aldrich™ Theophylline HPLC grade, Cat. No. T1633
- Sigma-Aldrich™ 4-Nitroaniline ≥99%, Cat. No. 185310
- Sigma-Aldrich™ Methyl benzoate 99%, Cat. No. M29908
- Sigma-Aldrich™ Phenetole 99%, Cat. No. 241989
- Supelco™ *o*-Xylene anhydrous, ≥99%, Cat. No. 95660

Sample handling

- Thermo Scientific™ SureSTART™ 2 mL Glass Screw Top Vials, Level 2 High-Throughput Applications, 100/pack, Cat. No. 6ASV9-1P
- Thermo Scientific™ SureSTART™ 9 mm Screw Caps, Level 2 High-Throughput Applications White Silicone/Red PTFE, 100/pack, Cat. No. 6ASC9ST1

*The Fisher Scientific product codes can be unique to different countries; the codes given above should be compatible across the EU and USA.

Instrumentation

Thermo Scientific™ Vanquish™ Flex UHPLC System, including the following modules:

- Thermo Scientific™ System Base Vanquish™ Horizon/Flex, Cat. No. VF-S01-A-02
- Thermo Scientific™ Vanquish™ Quaternary Pump F, Cat. No. VF-P20-A
- Thermo Scientific™ Vanquish™ Split Sampler FT, Cat. No. VF-A10-A-02
- Thermo Scientific™ Vanquish™ Column Compartment H, Cat. No. VH-C10-A-02
- Thermo Scientific™ Vanquish™ Variable Wavelength Detector F, Cat. No. VF-D40-A
- Thermo Scientific™ Vanquish™ Semi-Micro Flow Cell, 2.5 µL, 7 mm, SST, Cat. No. 6077.0360

Standard preparation

To prepare the standard mixture, 0.005 g of theophylline was weighed, followed by 0.015 g of *p*-nitroaniline. Then, 100 µL of methyl benzoate, 185 µL of phenetole, and 330 µL of *o*-xylene were measured. The diluent 50/50 (v/v) acetonitrile (ACN) and water (H_2O) was prepared by measuring 45 mL of the ACN/H_2O diluent and dissolving the weighed theophylline and *p*-nitroaniline in the diluent. Then, the measured methyl benzoate, phenetole, and *o*-xylene were added to the solution. The solution was mixed thoroughly to ensure all components were fully dissolved and the solution was homogeneous.

Chromatographic conditions

See Table 1.

Chromatography Data System

The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) was used for data acquisition and analysis.

Table 1. Chromatographic conditions.

Parameter		
Column	Hypersil GOLD 250 mm × 4.6 mm, 5 µm (Cat. No. 25005-254630) Hypersil GOLD 150 mm × 4.6 mm, 5 µm (Cat. No. 25005-154630)	Hypersil GOLD 50 mm × 2.1 mm, 5 µm (Cat. No. 25005-052130)
Solvent A	Water	
Solvent B	Acetonitrile	
Isocratic	60:40 (ACN:H_2O) (v/v)	50:50 (ACN:H_2O) (v/v)
Flow rate	1.25 mL	0.2 mL
Column temperature	Ambient	
Needle wash solution	75/25 IPA/H_2O + 0.1% FA (v/v)	
Injection volume	2.5 µL	0.5 µL
Detector settings	254 nm, data collection rate 20.0 Hz, response time 0.20 s	254 nm, data collection rate 50.0 Hz, response time 0.10 s

Results and discussion

To evaluate the consistency of the HPLC columns, a statistical analysis was conducted on approximately 17,500 analytical columns, focusing on average relative retention time (RRT) and average peak asymmetry.

After packing columns from a media batch, a quality control test was performed for each column individually. For every set of columns packed with a specific media batch, the mean values for relative retention time were calculated, along with standard deviation.

Next, the relative standard deviation (RSD) for RRT was calculated to compare the degree of variation across different media batches.

Relative retention time assessment

The RRT for each compound in the standard mixture—namely theophylline, *p*-nitroaniline, methyl benzoate, and phenetole—was calculated by dividing its retention time by the retention time of *o*-xylene. Data was averaged to obtain the RSD for RRT values, allowing for a robust statistical analysis and ensuring the performance consistency of the HPLC columns across multiple batches over the years of production.

These RSD were determined across 83 media batches for 250 mm × 4.6 mm, 5 µm columns; 53 media batches for 150 mm × 4.6 mm, 5 µm columns; and 29 media batches for 50 mm × 2.1 mm, 5 µm columns. Overall, the RRT RSD was below 2.11%, demonstrating excellent batch-to-batch consistency regardless of the column format used (Table 2).

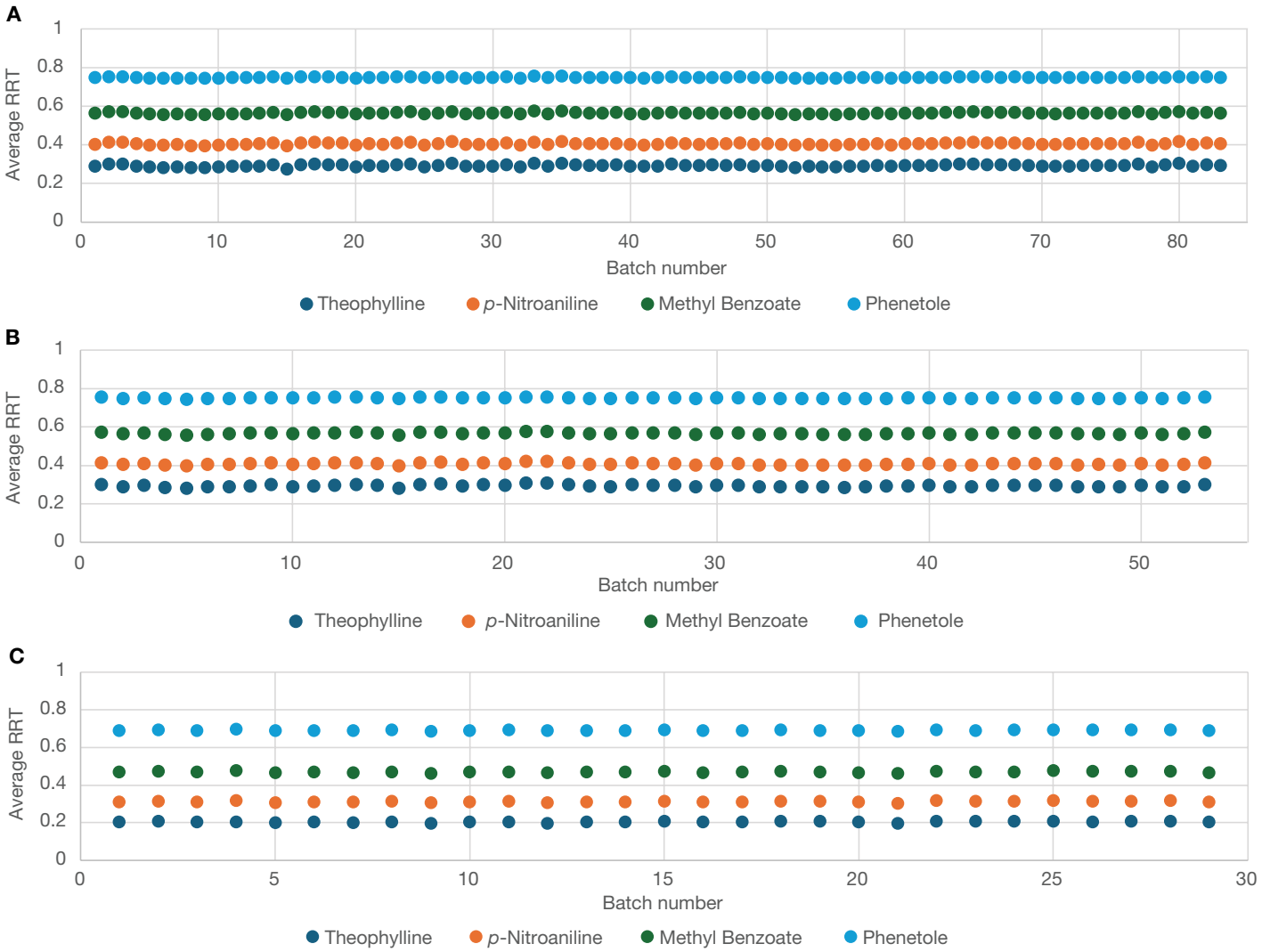

Figure 1. Average RRT, normalized by *o*-xylene for (A) 250 mm × 4.6 mm, 5 µm columns (83 batches), (B) 150 mm × 4.6 mm, 5 µm columns (53 batches), and (C) 50 mm × 2.1 mm, 5 µm columns (29 batches).

Peak asymmetry assessment

o-Xylene was chosen as it is the last eluting compound, least affected by system effects, and provides a clear indication of column packing quality. A well-packed column produces a symmetrical and sharp peak for *o*-xylene. The data analysis involved calculating the average peak asymmetry of *o*-xylene for each media batch, followed by the standard deviation. Error bars representing the standard deviation were incorporated into the graphical analysis (Figure 2).

Each Hypersil GOLD column format shows average peak asymmetry between 0.92 and 1.09 with a maximum standard deviation of ±0.04.

Table 2. Average RRT, RSD of RRT summary for all tested column formats.

	Hypersil GOLD 250 mm × 4.6 mm, 5 µm column		Hypersil GOLD 150 mm × 4.6 mm, 5 µm column		Hypersil GOLD 50 mm × 2.1 mm, 5 µm column	
	Average RRT	RSD of RRT, %	Average RRT	RSD of RRT, %	Average RRT	RSD of RRT, %
Theophylline	0.29	2.00	0.29	2.11	0.20	1.70
p-Nitroaniline	0.41	1.27	0.41	1.32	0.31	1.08
Methyl benzoate	0.56	0.75	0.57	0.77	0.47	0.74
Phenetole	0.75	0.34	0.75	0.35	0.69	0.32

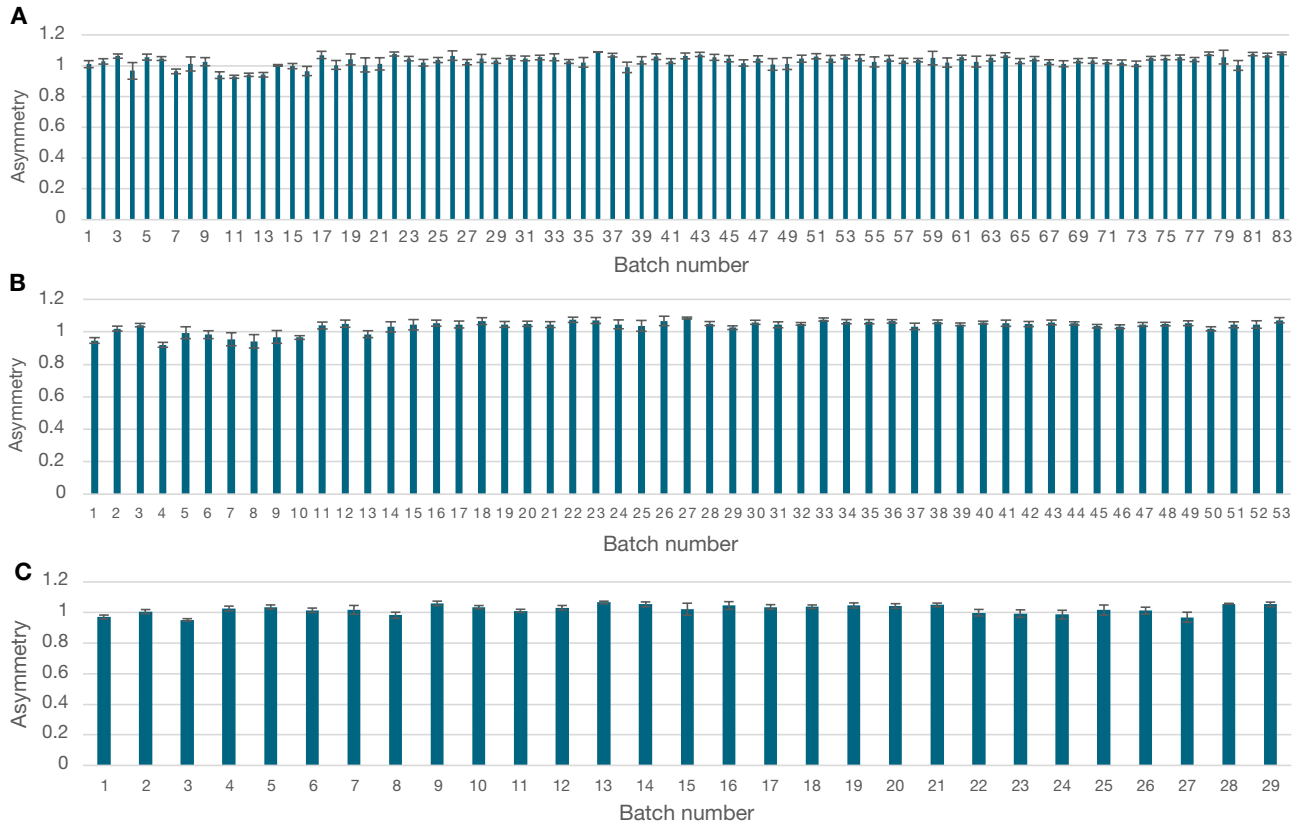

Figure 2. Average peak asymmetry evaluation of *o*-xylene for (A) 250 mm × 4.6 mm, 5 µm columns (83 batches), (B) 150 mm × 4.6 mm, 5 µm columns (53 batches), and (C) 50 mm × 2.1 mm, 5 µm columns (29 batches); with error bars showing the standard deviation.

Conclusion

The study proves that the evaluated HPLC columns exhibit 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 approximately 17,500 analytical columns provided a comprehensive assessment of performance consistency.
- The evaluation of Hypersil GOLD columns with different formats showed consistent relative retention times, with all RSD values below 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.

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