TECHNICAL NOTE 73337

Global round robin test of thiopental EP method performance on identical HPLC systems

Authors: Sylvia Grosse, Katherine Lovejoy, Frank Steiner
Thermo Fisher Scientific, Germering, Germany

Keywords: Reproducibility, precision, European Pharmacopoeia, system suitability test, UV-Vis, thiopental, variability, small molecule pharmaceutical

Demonstrated benefits

The presented results show that Thermo Scientific™ Vanquish™ Core HPLC systems have excellent system-to-system reproducibility when the sample preparation, eluent preparation, and LC column are identical. Users can rely on the fact that the performance of multiple Vanquish Core HPLC systems will be highly predictable and robust for routine methods in quality control labs when other variables are controlled.

Goals

- Evaluate system-to-system variability, while controlling for different operators, column lots, and solvent grades.
- Present intra- and inter-laboratory precision data for retention time, peak areas, and relative quantification.

Introduction

High system-to-system reproducibility is critical for high performance liquid chromatography (HPLC) systems used for routine analysis in labs where many systems stand side-by-side, such as in quality control and batch testing release laboratories. High reproducibility among systems is also needed for method transfer between labs, such as for transfer of methods from systems in a research and development lab and to identical systems in a quality control lab.

The Vanquish Core HPLC systems are designed for such routine and universal use. Multiple systems must produce identical results. In this technical note, we present the results of a global round robin test designed to evaluate the system-to-system reproducibility. Multiple HPLC instruments of the same model were used to analyze thiopental and its impurities as described by the related substances method in the current monograph published by the European Pharmacopoeia (EP).[1] For that purpose, eight labs and seven operators in four countries on three continents were equipped with identical HPLC instruments but different pumping technologies and UV detector types and were asked to perform the exact same analysis.

EP certified reference standards and new columns from different batches were used. We report inter- and intra-laboratory precision data for retention times, peak areas, relative quantification, and system-to-system variability. General trends in the effect of eluent preparation, sample preparation, and column batch on variability were also explored. The system-to-system reproducibility of the Vanquish Core HPLC systems was found to be excellent, especially when eluent, sample, and column variables were controlled.

Experimental

Chemicals (Germering laboratory)

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific Acetonitrile, Optima™ LC/MS grade (P/N A955-212)
- Fisher Chemical HPLC electrochemical grade ortho-phosphoric acid 85% (P/N O/0515/PB08)
- EP Certified Reference Standard Thiopental for System Suitability CRS,[2] containing impurities A, B, C, and D (P/N Catalogue code Y0001478)

Equipment (Germering laboratory)

- Vials (amber, 2 mL), Fisher Scientific (P/N 03-391-6)
- Cap with Septum (Silicone/PTFE), Fisher Scientific (P/N 13-622-292)

Instrumentation

- Thermo Scientific Vanquish Core Quaternary and Binary HPLC systems were used for the analyses, equipped with:
  - System Base Vanquish Core (P/N VC-S01-A)
  - Quaternary Pump C (P/N VC-P20-A)
    or
  - Binary Pump C (P/N VC-P10-A)
  - Split Sampler CT (P/N VC-A12-A)
  - Column Compartment C (P/N VC-C10-A-03)
  - Diode Array Detector CG with standard flow cell, 13 µL (P/N VC-D11-A with P/N 6083.0510)
    or
  - Variable Wavelength Detector C with standard flow cell, 11 µL (P/N VC-D40-A with P/N 6077.0250)

Sample preparation

The system suitability standard was prepared as 1 mg/mL thiopental for system suitability CRS, containing the impurities A, B, C, and D, in mobile phase. A 2 mg portion of the EP reference standard for system suitability was weighed in a 2 mL volumetric flask. The flask was then filled to 2 mL with mobile phase. The standard dissolved upon vortexing for about 1 minute.

Mobile phase preparation

The mobile phase was prepared by adding 1 g phosphoric acid (85%) to 900 mL of water in a 1000 mL volumetric flask and filling to 1000 mL with water. A 350 mL portion of acetonitrile was added to 650 mL of the phosphoric acid solution in an eluent bottle, mixed by inverting the bottle several times until a clear solution became visible, and degassed by placement for five minutes in an ultrasonic bath.

Table 1. Chromatographic conditions

Parameter	Value
Column	Thermo Scientific™ Hypersil GOLD™, 150 × 4.6 mm, 5 µm (P/N 25005-154630)
Mobile phase	65:35 1 g/L phosphoric acid (85%) in water:ACN (v:v) (isocratic, channel A)
Run time	20 min
Flow rate	1 mL/min
Mixer volume	350 µL + 50 µL
Column temperature	25 °C with passive pre-heater (forced air with fan speed 5)
Autosampler temperature	4 °C
UV wavelength	225 nm
UV data collection rate	10 Hz
UV response time	0.5 s
Injection volume	10 µL

Chromatography Data System

The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.3 was used for data acquisition and analysis.

Results and discussion

Results from ten repeated runs of the EP compendial method for thiopental[1] on each of the eight systems with different columns, eluent brands, eluent grades, sample preparations, operators, and locations around the world were compared. In addition, results from three systems were compared when all these variables were controlled.

Systems are equal when sample, eluent, and column are identical

Three systems were compared under highly controlled conditions. The same sample, eluent bottle, and column were moved from system to system. Ten runs were performed on each system. The results showed that when the sample preparation, eluent preparation, column, operator, and site are all identical, the systems tend to produce equal retention times, peak areas, and peak resolution.

The retention times for thiopental on three systems under identical conditions are shown in Figure 1. The relative standard deviation (RSD) for the retention time of thiopental is 0.4% for the three systems. Because of the controlled conditions and the high intrinsic system-to-system reproducibility of the Vanquish Core HPLC system, this value is nearly an order of magnitude better than the 3.5% RSD obtained for the eight systems in the global test.

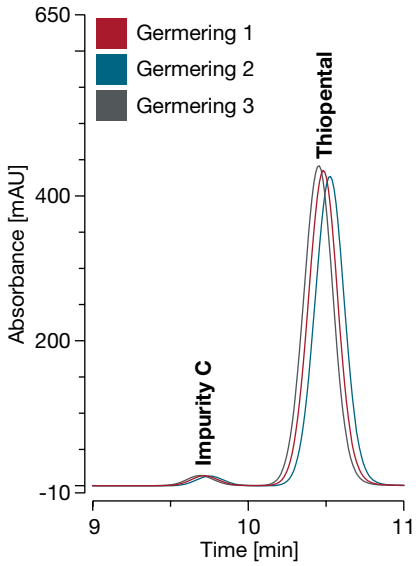

Figure 1. Retention times for thiopental are nearly identical on three different systems when the sample preparation, eluent preparation, column, operator, and site are identical.

The peak areas under the controlled case also show excellent system-to-system reproducibility. Peak area reproducibility data for all five peaks are shown in Figure 2. The peak areas were very similar under the controlled conditions, resulting in peak area RSDs of less than 2.3% for four of the five peaks. The special case of impurity D is discussed in the next section. During the multi-site test, although the peak area measurements were very precise in each lab, the peak areas differed widely between labs. As presented in the next section, this difference was attributed to different sample preparations. The superb system-to-system reproducibility of the Vanquish Core HPLC system provides for nearly identical peak areas when all other conditions are controlled.

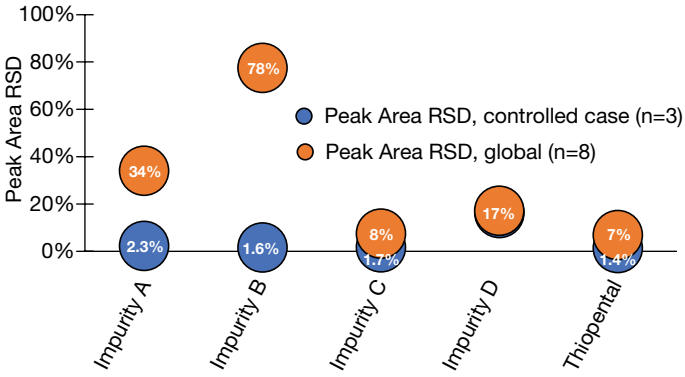

Figure 2. Peak area RSDs are much lower for the controlled case (n = 3) when the sample preparation, eluent preparation, column, operator, and site are identical, than for the global round robin test (n = 8) where none of these variables was controlled.

Another measure of system-to-system reproducibility is performance on the method's system suitability test. The system suitability test for thiopental states that a resolution of at least 1.5 must be obtained for both the impurity C and thiopental peak pair and the impurity A and impurity B peak pair. This condition was easily met by all three systems in the controlled case, as shown in Figure 3, and by all eight systems in the global test, as discussed in the next section.

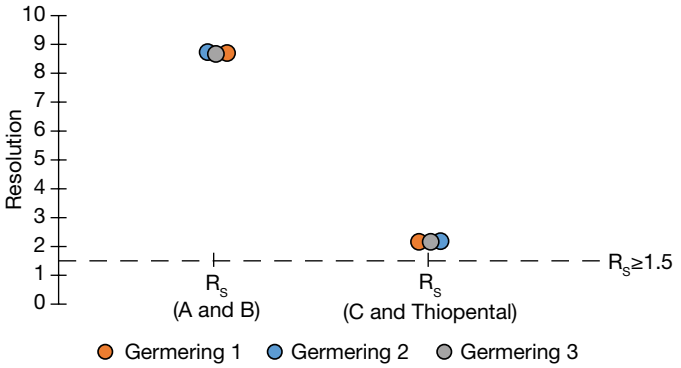

Figure 3. The system suitability test for the thiopental method, which states that the resolution between impurity C and thiopental and between impurity A and impurity B must be at least 1.5, was easily met by all three systems in the controlled case. The Hypersil GOLD column is known for excellent resolution.

Global test results and effect of sample, eluent, and column

The global system-to-system test, carried out on eight different systems in four countries, showed remarkable system-to-system reproducibility. All eight systems easily passed the system suitability test in the EP compendial method for thiopental,[1] as shown in Figure 4. The compendial method also provides approximate relative retention times (RRTs) for the purpose of peak identification. The suggested approximate RRTs and RRTs found in the global test are shown in Table 2. Because C18 columns vary in hydrophobicity, polarity, silanol activity, and metal activity, the matches are not exact, as expected, but were sufficient to allow identification of peaks in the chromatograms. C18 column properties have been tabulated elsewhere[3] and the RRTs of the Hypersil GOLD column in this application are not unexpected based on its characteristics relative to other C18 columns on the market. All thiopental impurities on all systems in the global test could be identified based on the estimated RRTs provided in the compendial method.

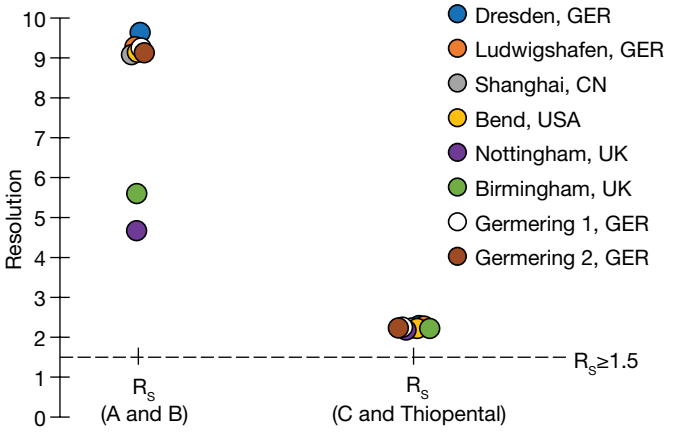

Figure 4. All eight systems passed the system suitability test, which required a resolution of at least 1.5 between impurity C and thiopental.

Table 2. Relative retention times found for the global round-robin test compared to those described by the EP. Relative retentions of the early eluting impurities A and B were greater than described. Those of the late-eluting impurities C and D matched the EP description. Differences are attributed to the characteristics of the packing of the Hypersil GOLD column. Because C18 columns vary in properties such as hydrophobicity, polarity, silanol activity, and metal activity between brand and manufacturer, reference tables of C18 column characteristics are available.[3]

Impurity	EP-defined relative retention ($RT_{impurity}/RT_{thiopental}$)	Found relative retention, average, n=8 (min, max)
A	about 0.3	0.53 (0.51, 0.54)
B	about 0.4	0.70 (0.62, 0.73)
C	about 0.9	0.93 (0.92, 0.93)
D	about 1.3	1.27 (1.24, 1.36)

Peak area reproducibility as related to sample preparation

Sample preparation was affected by the non-homogeneous distribution of solids inside the vials that are sold as the EP system suitability standard. Although all the sites were provided with the product as purchased from the EP, every scoop of the spatula brought up different amounts of each solid. Amounts of each impurity were therefore different in every sample preparation, and these differences could not be controlled. The chromatograms in Figure 5 show variation due to in-vial heterogeneity for two different sample preparations on the same system and for the same sample preparation on three different systems.

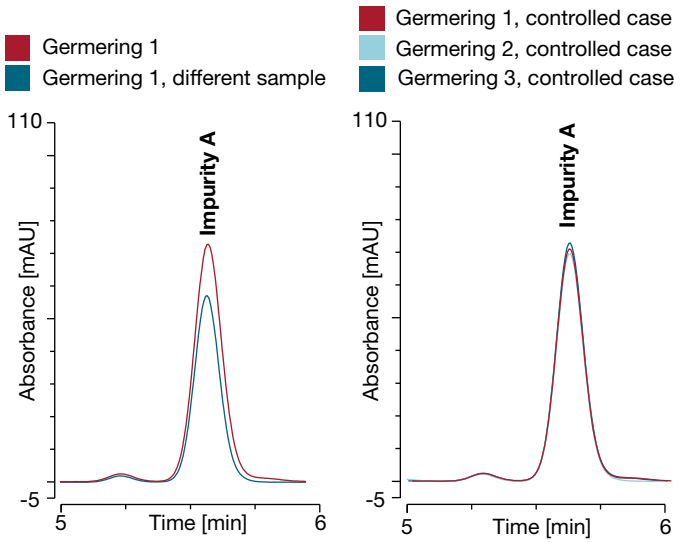

Figure 5. Example of changes in peak area for different sample preparations. The peak for impurity A is shown. On the left side, the same system is shown with two different sample preps. On the right side, three different systems are shown with the same sample prep. The system-to-system difference is much smaller than the difference between two sample preparations.

The sample inhomogeneity had the greatest effect on the determination of impurity B levels. Impurity A levels also showed inhomogeneity. The signal-to-noise ratios (S/N) given in Table 3 show the differences in sample preparations. Signal-to-noise was determined using a fixed one-minute region late in the chromatogram in which no peaks were present.

Table 3. Peak area RSD for ten injections and average S/N for each analyte, reported as the minimum and maximum values provided by the eight global test sites

Peak name	Peak area RSD (min and max of eight sites)	S/N (min and max of 8 sites)
Impurity A	0.06%–0.23%	1654–11159
Impurity B	0.09%–4.4%	16–724
Impurity C	0.08%–0.22%	757–2033
Impurity D	0.75%–2.0%	32–58
Thiopental	0.03%–0.08%	23427–62738

Larger peaks had excellent peak area precision, as shown in Table 3 and Figure 6 for impurity C, impurity A, and thiopental. Small peaks had a lower signal-to-noise ratio, which was related to worse peak area precision. Specifically, a signal-to-noise ratio of less than 60 was associated with worse peak area precision (above 1% RSD). For example, the peak area precision of the small impurity D peak was consistently worse than those of the other impurities, with RSDs ranging from 0.75 to 2.0%, and S/N ratios ranging from 32 to 58. For the impurity B peak, which was present in widely varying amounts in the samples, the relationship of S/N and peak area precision is shown in Figure 7. The differences in amount of impurity B only reflect variation in sample preparation and do not indicate system instability.

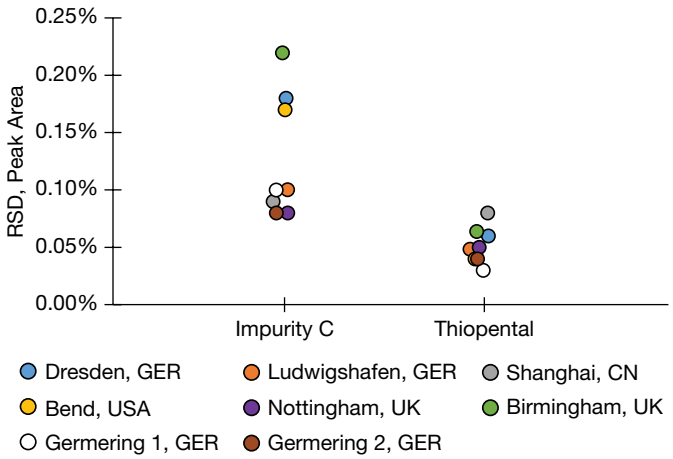

Figure 6. Peak area reproducibility for impurity C and thiopental in the global system-to-system test

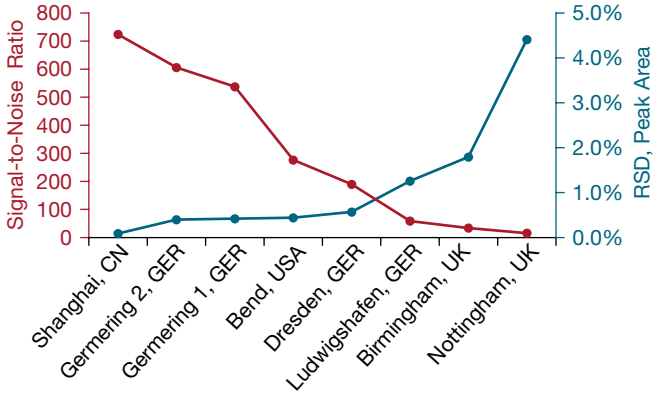

Figure 7. For the peak of impurity B, a clear relationship between peak area precision and peak size relative to the baseline is observed. Peak area RSD is inversely related to signal-to-noise of a given peak. Data for smaller peaks with very low S/N ratios show very poor precision, in other words, high peak area RSDs.

Retention time reproducibility as related to eluent preparation

Every site prepared eluents by adding phosphoric acid (85%) by weight and adding the water and acetonitrile by volume. Even within the same lab, as shown in Figure 8, slight differences in eluent preparation had more influence on retention times than column lot or system-to-system variability. In other words, the retention time of thiopental differs more with different eluent preparations on the same system than on different systems with the same eluent preparation.

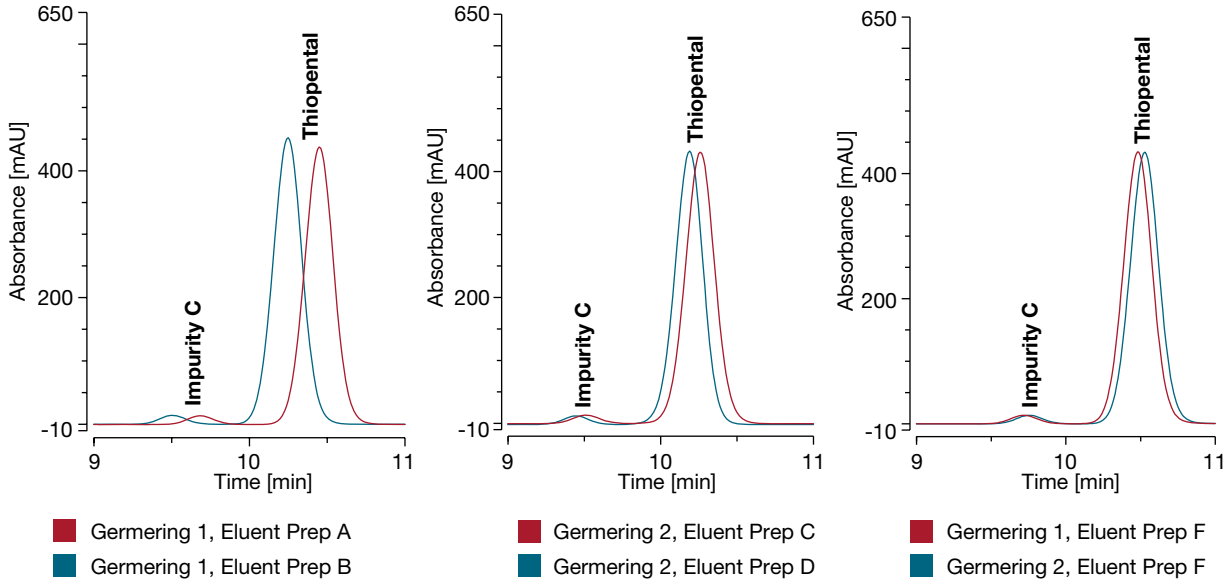

Figure 8. Three examples of changes in retention time are shown for five different eluent preparations and two different systems. The peaks for thiopental and impurity C are shown. The best retention time reproducibility is found when the eluent preparations are identical. For example, the retention time of the thiopental peak on system 1 (left pane) and system 2 (middle pane) shows greater differences between eluent preps than between systems (right pane). The retention times differ more with different eluent preps than with different systems.

Retention time is largely independent of column packing material lot

In an effort to consider the effects of column packing lot, retention times from the global system-to-system tests were examined. Figure 9 allows comparison of retention times for all analytes as a function of column lot. Three column lots were used in the global test and are identified as Lot A, B, or C. The retention times vary somewhat, but do not strongly correlate with column lot differences. Because the column-to-column difference is so minor, differences in retention time were attributed to eluent preparation, as discussed above.

Data on individual system components

Autosampler performance

The peak area precision for the controlled case with three systems side-by-side in the same lab demonstrates the excellent performance of the autosampler (Figure 10). The RSDs from ten injections per system show that this autosampler easily delivers a 0.05%–0.15% RSD for peak area precision when peak areas are large and signal-to-noise is above 1000, as observed for thiopental, impurity A, and impurity C. Minor variations in peak integration affect smaller peaks more than larger ones and these variations impact peak area precision.

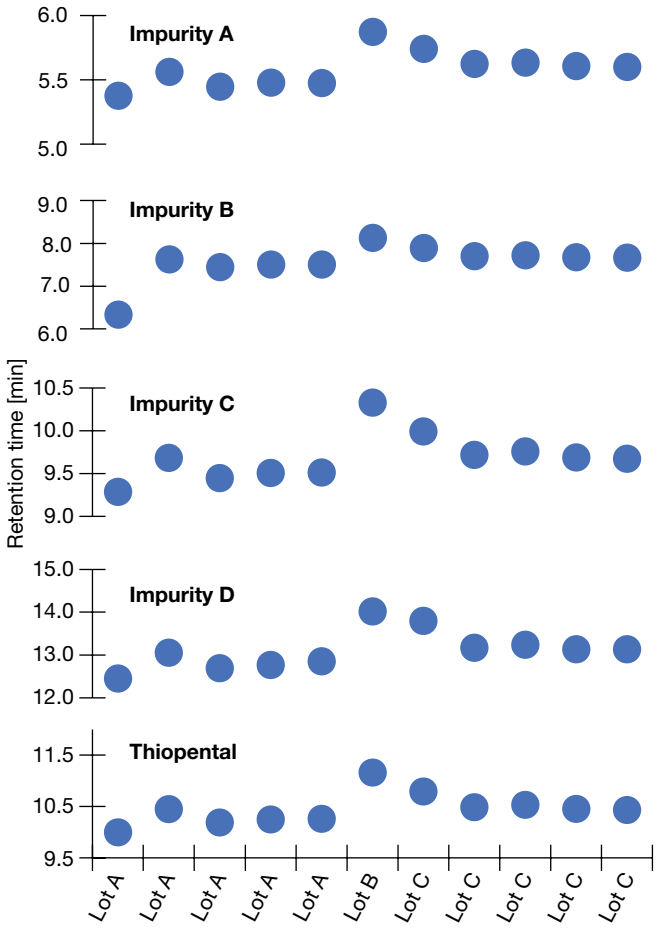

Figure 9. Comparison of retention times for all analytes measured on columns packed with three different solid phase lots. Data from the global tests are included. The general trend in retention times is independent of column lot and suggests more of a dependence on eluent preparation.

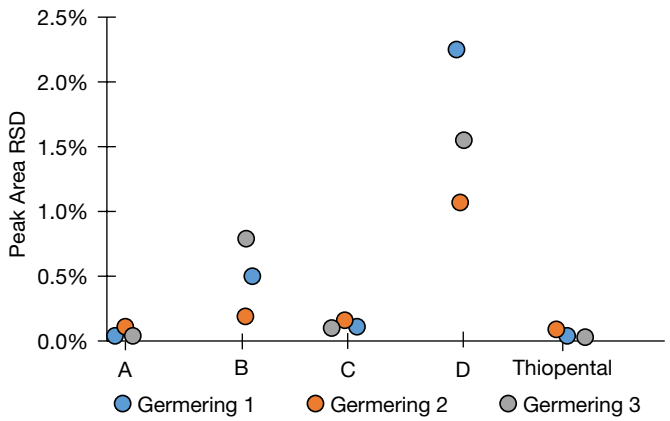

Figure 10. The peak area precision for the largest peaks of the controlled case showcases the performance of the autosampler. The average of the three systems for RSDs of peak areas from ten injections per system are shown. The peak area precision of 0.05% RSD for thiopental, 0.06% for impurity A, and 0.12% for impurity C show outstanding sampling precision. The average RSDs for the smaller peaks of impurity B and impurity D were 0.49% and 1.6%. Smaller peaks show worse peak area precision than larger peaks because of differences in peak integration, which was done automatically by the Chromeleon processing method.

Pump type

The Vanquish Core HPLC system offers both binary and quaternary pumps and the results of both pump types were compared. The same excellent retention time and peak area precision were found, regardless of pump type. Relative areas for thiopental were also identical, as was signal-to-noise ratio for thiopental.

Differences based on pump type were not expected. The biggest difference between the two pump types is the gradient production, but this method required no gradient and used a pre-mixed solvent in channel A.

Conclusions

- The Vanquish Core HPLC systems have excellent system-to-system reproducibility for retention time and peak area, as shown on eight systems in four countries on three continents.
- Retention time reproducibility is largely governed by eluent preparation. Column lot is less important. When identical eluents are used, the RSD for the retention time of thiopental and impurities on three different systems is never more than 0.4%.
- Peak area reproducibility depends largely on the sample preparation, which was inhomogeneous due to peculiar characteristics of the EP standard product used. When identical samples are used, the RSD for the peak area of thiopental and four of five impurities on three different systems is never greater than 2.3%.
- The system suitability test criteria for thiopental are easily met, no matter where in the world the Vanquish Core HPLC system operates.

References

1. "Thiopental sodium and sodium carbonate." European Pharmacopoeia 9.0, 07/2012:0212.
2. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.); 7, Allée Kastner CS 30026, F-67081 Strasbourg (France).
3. "Comparison Guide to C18 Reversed Phase HPLC Columns." MacMod Analytical Inc., Fourth Edition, June 2008, available at http://www.mac-mod.com/pdf/technical-report/036-ColumnComparisonGuide.pdf . Accessed November 27, 2019.

Find out more at **thermofisher.com/vanquishcore**

ThermoFisher SCIENTIFIC