# Automated system suitability test with intelligent run control for peptide mapping QC assays

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## Application benefits

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software provides 21 CFR 11 compliant-ready data acquisition and processing for UV-based protein identity testing by peptide mapping, including full audit trail coverage and electronic signatures, for the biopharmaceutical industry.

- Integrated System Suitability Test (SST) within a Chromeleon Processing Method provides automated integration and the option for pre-defined pass/fail criteria, adding consistency while removing potential user bias, as well as improving overall productivity.

- SST can be combined with Intelligent Run Control (IRC) to reduce the need for user intervention between QC check and sample analysis, while still ensuring samples are analysed on a system deemed suitable for the analytical method.
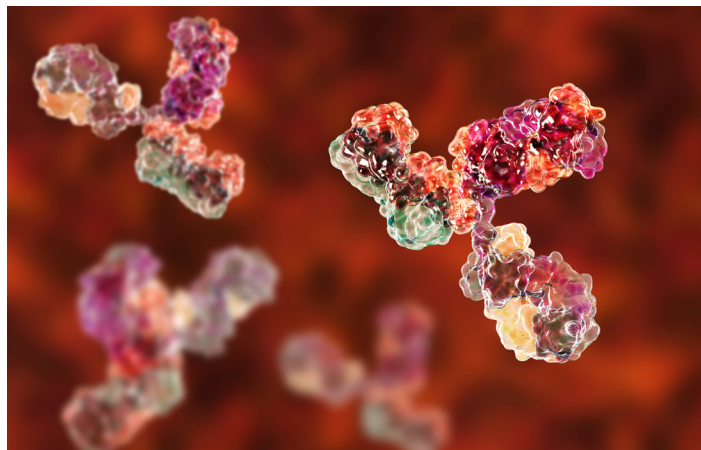
- All aspects of system running, including SST and IRC can be incorporated into a Chromeleon eWorkflow™ procedure, which is a dynamic sequence template that can minimize quality deviations and save analyst time.

- Sample and SST are automatically displayed in a predefined report template; electronic data checking and signature approval is incorporated within Chromeleon CDS, maintaining compliance throughout the process.

## Goal

To demonstrate the simplicity and increased productivity using the compliance-ready automated SST and IRC features in Chromeleon CDS, as part of data acquisition and processing in routine biopharmaceutical peptide mapping applications.

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## Introduction

In a typical biopharma QC laboratory, it is necessary to ensure that an analytical instrument, as part of an integral system, functions within predefined limits to ensure confidence in the data generated and the subsequent patient safety. This is usually achieved by a HPLC system suitability test established for the specific procedure, performed on the day of analysis before committing samples. For regulated environments, USP (<1055>)[1] and ICH (Q2-R1)[2] require an SST to be included as part of the analytical procedures for lot-release.

One of these analytical procedures is peptide mapping, a commonly used identity test method during the biopharmaceutical development/manufacturing cycle. Peptide mapping involves the proteolytic cleavage of a protein and separation of the resulting peptides by liquid chromatography (LC). The principle uses of peptide mapping are for confirmation of protein primary structure and characterization of post-translational modifications (PTMs). In its uncharacterized form, a peptide map provides a "fingerprint", unique to that specific protein, which can be used for identity, and is particularly powerful when combined with concurrent analysis of a well characterized reference sample. A critical consideration in method development is consistent chromatographic reproducibility of the fingerprint, which underpins its ability to be used for protein identity.[3]

A properly designed SST increases confidence in the generated data by confirming that the analytical system is functioning within a specific set of pre-defined parameters. It also aids troubleshooting, when required, by simplifying the identification of the source of aberrant data. For this reason, the use of SST is encouraged as standard practice for both routine analysis and method development activities, even in laboratories that are not required to adhere to current good manufacturing practice (cGMP) or good laboratory practice (GLP) regulations.

The standard selected for the SST should ideally have physicochemical similarities to the samples being analyzed, but equally should not be so complex that the results become challenging to interpret. The main function of the SST is to test that the analytical system is fit for purpose for the samples under interrogation. However, interpreting the acquired data for the SST can still be a laborious task, as there is a specific set of acceptance criteria that must be met,[4] and it is essential to determine a pass or fail before proceeding with sample analysis.

Where possible, it is advised to avoid the ambiguity of manually integrating peaks in a chromatogram to ensure regulatory compliance and to prevent incorrectly and inconsistently calculating pass or fail results. Nonetheless, manual integration is commonplace and acceptable when associated with a suitable standard operating procedure (SOP). Analysts should also refrain from taking data outside of a compliant software environment to manually calculate SST results against acceptance criteria. Automated processing methods within chromatography data system software should ideally be utilized to automate integration of chromatographic peaks, to provide analysts with an unbiased summary of results, comparing them to predefined SST limits to determine pass/fail.

In this application note, an automated SST solution is presented along with control of system suitability test parameters and Intelligent Run Control using Chromeleon CDS software. Using Chromeleon CDS, all SST calculations are automatically performed to determine a system pass/fail.[5] The SST results are compared to criteria defined by the user to determine the next steps in the analysis, i.e., continue with samples, stop run, or reinject SST standard. This process can also be automated using the IRC feature. IRC defines specific actions to enable the system to automatically respond to SST parameters falling outside of the accepted range. This automated approach ensures consistent data quality and allows the analysts to focus on other tasks as minimal supervision is required, saving valuable time and money and boosting productivity.

## Experimental

| Recommended consumables | Part number |
|---|---|
| Deionized water, 18.2 MΩ/cm resistivity or higher | N/A |
| Fisher Scientific™ Acetonitrile Optima™ LC/MS grade | A955-212 |
| Fisher Scientific™ Methanol Optima™ LC/MS grade | A456-212 |
| Thermo Scientific™ SMART Digest™ Trypsin Kit, Magnetic Bulk Resin option | 60109-101-MB |

| Instrumentation | Part number |
|---|---|
| Thermo Scientific™ Vanquish™ Duo system consisting of: | |
| Vanquish System Base | VF-S02-A-02 |
| Vanquish Dual Pump F | VF-P32-A-01 |
| Dual Split Sampler FT | VF-A40-A-02 |
| 2× Vanquish Column Compartment H | VH-C10-A-02 |
| 2× Vanquish Variable Wavelength Detector | VF-D40-A |

## Sample preparation

- Thermo Scientific™ Pierce™ BSA Protein Digest Standard, LC-MS grade (P/N 88341)

- Thermo Scientific™ Dionex™ Cytochrome C Digest (P/N 161089)

- Lysozyme protein standard from Thermo Scientific™ Pierce™ Glycoprotein Carbohydrate Estimation Kit (P/N 23260)

- Golimumab (SIMPONI™, Johnson & Johnson)

- Nivolumab (Opdivo™, Bristol-Myers Squibb)

Proteins requiring proteolysis for analysis were digested using the magnetic trypsin bead Thermo Scientific SMART Digest Kit, for 45 min at 75 °C using a Thermo Scientific™ KingFisher™ Duo Prime Purification system. Resulting peptides were separated by reversed phase chromatography using a Thermo Scientific™ VANQUISH™ Acclaim UHPLC column (2.1 × 250 mm, 2.2 µm) at 60 °C over a 40 min linear gradient of 2–40% mobile phase B with a flow rate of 0.3 mL/min (Figure 1). Each flow path of the Vanquish Duo UHPLC system contained its own UHPLC column and Variable Wavelength Detector. The detection wavelength was set to 214 nm, at a data collection rate of 10 Hz.

## Chromatography data system
The Chromeleon 7.2.10 CDS software was used for data acquisition, data processing, and creating relevant reports.
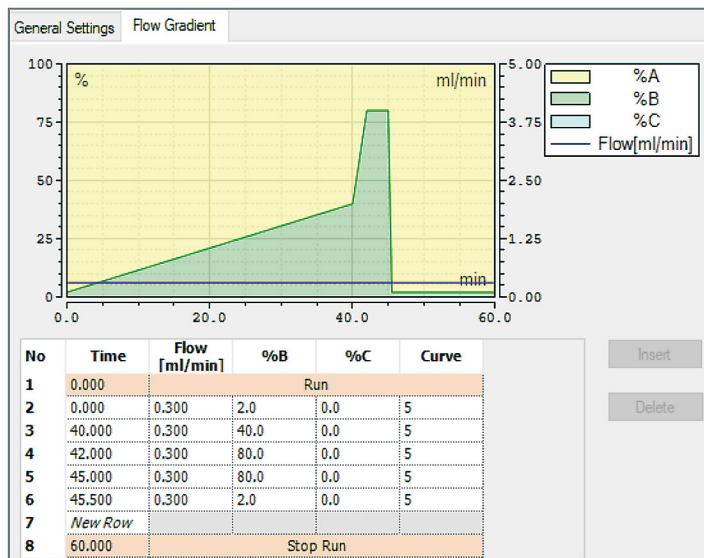


**Figure 1. LC gradient conditions.** Mobile phase A: water with 0.1% formic acid. Mobile phase B: acetonitrile with 0.08% formic acid.

## Results and discussion
Within the processing method for each sample type, peaks were integrated using the Cobra™ Peak Detection Algorithm with SmartPeaks™ Integration Assistant,[6] and relevant peaks of interest named in the component table. A set of test case criteria was determined using the automated wizard tool within the SST/IRC tab (Figure 2). Predefined test conditions for recommended SST specifications, such as resolution, RSD of peak areas, and tailing factor, as well as custom specifications such as peak area and peak height can be added as required. Each criterion can be applied to specific samples within an analytical sequence, i.e., those identified as check standards or with a particular sample name, and also to specific peaks named within the component table. The result of each test will be displayed within the SST/IRC table in the Chromeleon Processing Method (Figure 3).
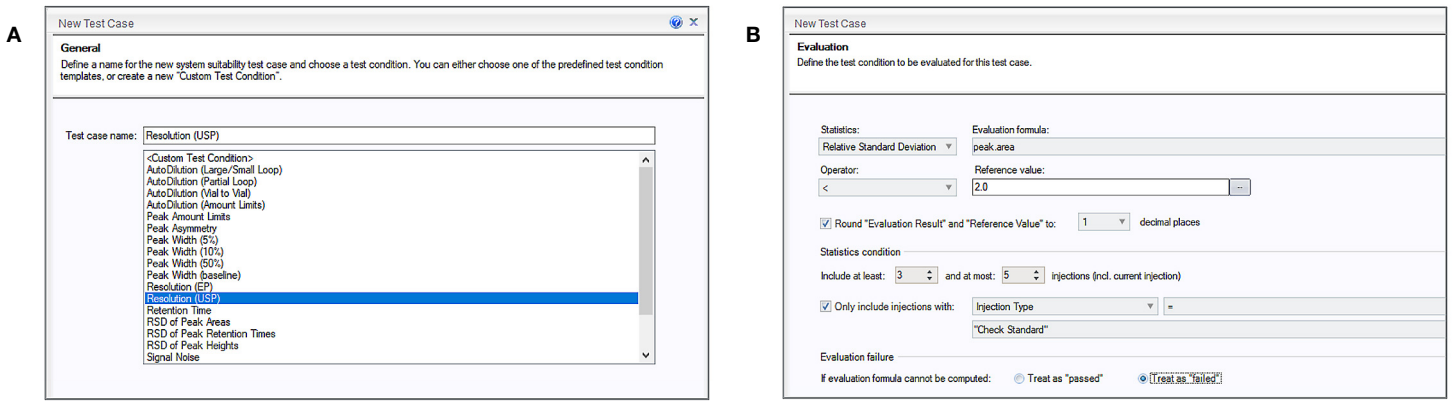
**Figure 2. Wizard for creating SST and IRC New Test Case.** (A) List of recommended SST parameters is included; alternatively, customized SST parameters can be created using <Custom Test Condition>. (B) The following windows of the New Test Case Wizard provides options for precise definitions of samples to be tested, peaks to be assessed, and the pass/fail criteria associated.
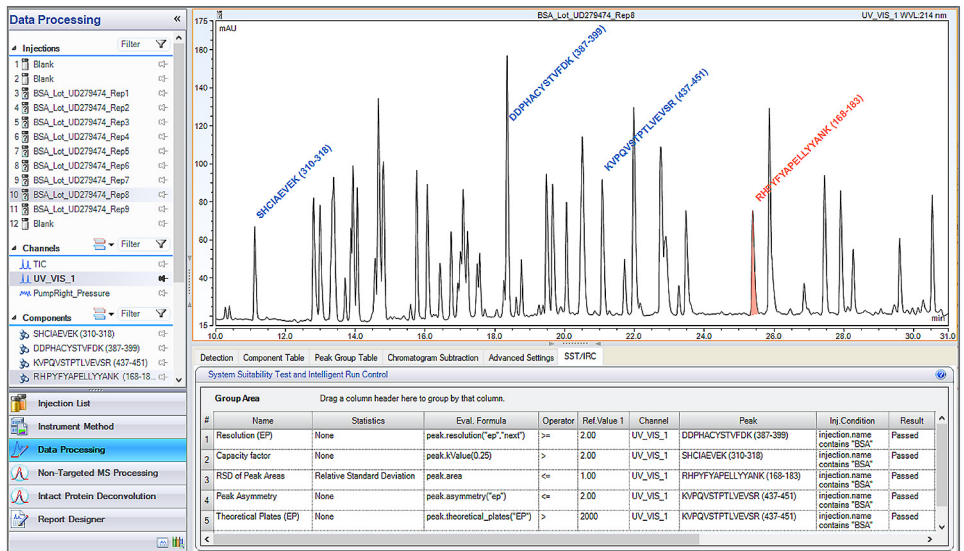


**Figure 3. BSA digest and associated processing method within the data processing category of Chromeleon CDS.** Named peaks are labeled within the chromatogram. Selected SST specifications are listed within the SST/IRC tab with the result in the final column of the table.

## Using SST specifications and IRC within Chromeleon CDS

As an SST is performed and assessed prior to sample analysis, any failure to meet the predefined acceptance criteria requires the user to manually intervene by stopping the running sequence immediately to prevent injection of samples on a system that is not deemed suitable for analysis. This is necessary to ensure compliance with standard operating procedures (SOPs), to prevent wasting precious samples, and to avoid performing a retest. Should an SST fail, the analyst must diagnose the problem and, if possible, make the necessary adjustments before performing the SST again, assuming the SOP permits.

It is possible to add IRC actions into the processing method to control how the system responds to pass or fail results. It may be beneficial for the system to pause after running a set of initial SST injections, to allow preparation of critical samples to be added into the autosampler, or a pass result can be set to have no action necessary and for the run to continue. Various actions can be set for a failed result, including pause, abort, or reinjection of the SST samples (Figure 4A).

The results from the SST/IRC tab in the processing method are automatically presented in the report template of Chromeleon CDS, along with the associated chromatogram. This provides a clear visualization of the test cases conducted, the test value, and the outcome of the test (Figure 4B). The same processing method, including IRC actions, can be applied to additional bracketing check standards interspaced throughout any analytical run to monitor any changes in performance. This automated approach allows analysts to focus on other tasks as no supervision or monitoring of the LC system is required, saving valuable time and money and boosting productivity.

The Vanquish Duo UHPLC system for Dual LC contains two separate flow paths in one integrated system, further improving productivity. This enables two analytical sequences to be analyzed at the same time, within a single LC platform. Due to the inherent consistency of separation between both flow paths of the LC system, if identical conditions and UHPLC columns are used, the same processing method, including identical SST and IRC criteria, can be applied to the corresponding samples analyzed on each side of the Vanquish Duo UHPLC system (Figure 5).
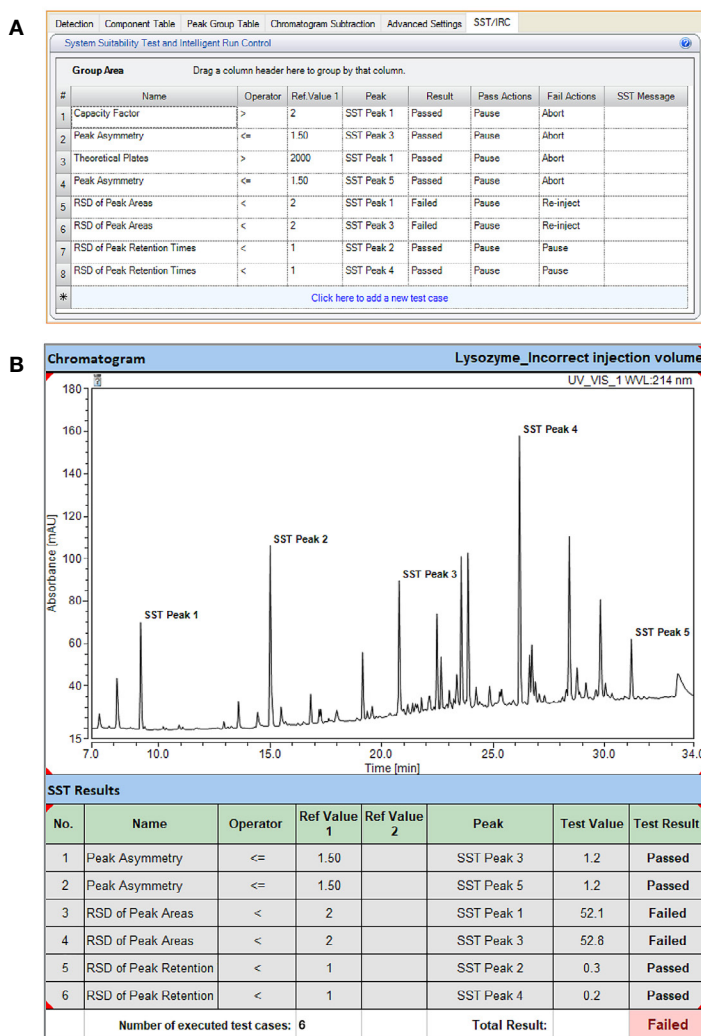
Figure 4. Analysis of lysozyme digest for SST using the SST/IRC feature in Chromeleon CDS. (A) SST/IRC tab of the processing method, including IRC actions for individual test cases. (B) Chromeleon results for SST indicating an overall fail due to incorrect RSD of peak areas caused by an incorrect injection volume.
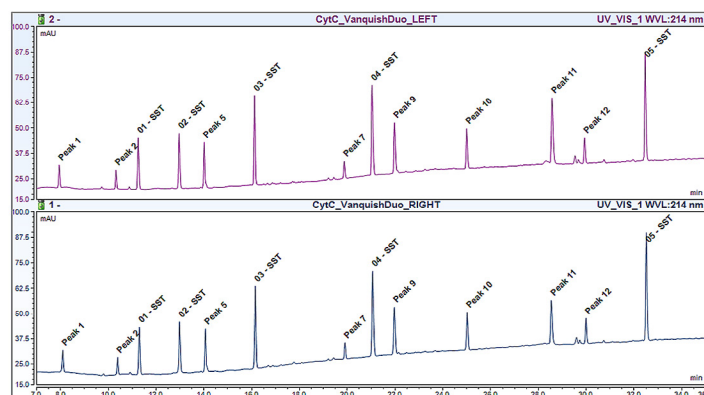


Figure 5. Chromatograms of Cytochrome C Digest SST standard acquired simultaneously with the Vanquish Duo UHPLC system for Dual LC. Both chromatograms, from left (upper) and right (lower) sides of the Vanquish Duo system, can be analyzed with the same Chromeleon Processing Method.

## Using SST specifications for peptide mapping samples

As part of a peptide mapping sequence, chromatographic peaks of mAb tryptic peptides can be automatically integrated within Chromeleon CDS using the Cobra Peak Detection Algorithm with SmartPeaks Integration Assistant, and assessed automatically using the SST/IRC tab of the processing method, improving consistency by limiting analyst interaction. The pass/fail criteria for the mAb tryptic peaks can be built into the SST/IRC in the same way as for SST samples (Figure 6A). Typically, a known reference of the biotherapeutic will be compared to samples from new batches for QC testing prior to lot-release via peptide mapping of chromatographic peaks (Figure 6B). Pass/fail results of the chromatographic test cases are presented in the Chromeleon Report Template (Figure 6C), allowing for easy spotting of mismatching protein sequence and applied sample.
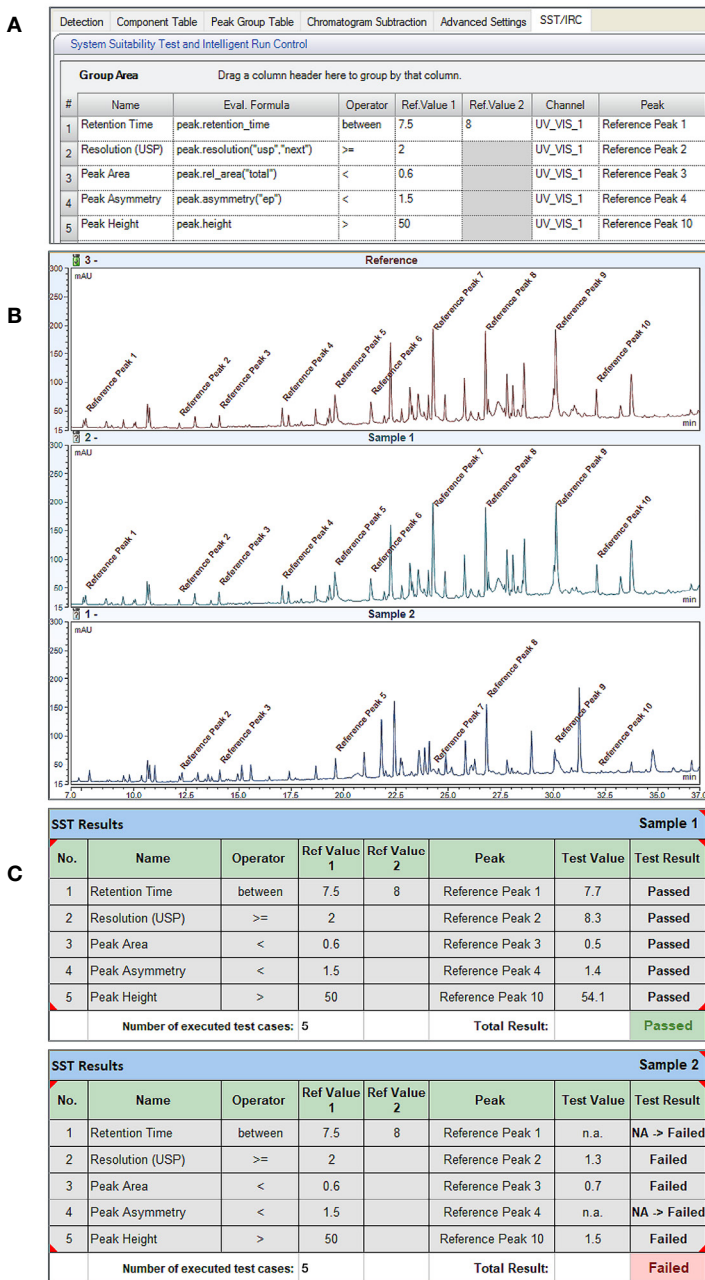
**A**

| | Detection | Component Table | Peak Group Table | Chromatogram Subtraction | Advanced Settings | SST/IRC | |

System Suitability Test and Intelligent Run Control

Group Area — Drag a column header here to group by that column.

| # | Name | Eval. Formula | Operator | Ref.Value 1 | Ref.Value 2 | Channel | Peak |
|---|------|--------------|----------|-------------|-------------|---------|------|
| 1 | Retention Time | peak.retention_time | between | 7.5 | 8 | UV_VIS_1 | Reference Peak 1 |
| 2 | Resolution (USP) | peak.resolution("usp","next") | >= | 2 | | UV_VIS_1 | Reference Peak 2 |
| 3 | Peak Area | peak.rel_area("total") | < | 0.6 | | UV_VIS_1 | Reference Peak 3 |
| 4 | Peak Asymmetry | peak.asymmetry("ep") | < | 1.5 | | UV_VIS_1 | Reference Peak 4 |
| 5 | Peak Height | peak.height | > | 50 | | UV_VIS_1 | Reference Peak 10 |

**B**

**C**

**SST Results** — Sample 1

| No. | Name | Operator | Ref Value 1 | Ref Value 2 | Peak | Test Value | Test Result |
|-----|------|----------|-------------|-------------|------|------------|-------------|
| 1 | Retention Time | between | 7.5 | 8 | Reference Peak 1 | 7.7 | Passed |
| 2 | Resolution (USP) | >= | 2 | | Reference Peak 2 | 8.3 | Passed |
| 3 | Peak Area | < | 0.6 | | Reference Peak 3 | 0.5 | Passed |
| 4 | Peak Asymmetry | < | 1.5 | | Reference Peak 4 | 1.4 | Passed |
| 5 | Peak Height | > | 50 | | Reference Peak 10 | 54.1 | Passed |
| | Number of executed test cases: 5 | | | | Total Result: | | Passed |

**SST Results** — Sample 2

| No. | Name | Operator | Ref Value 1 | Ref Value 2 | Peak | Test Value | Test Result |
|-----|------|----------|-------------|-------------|------|------------|-------------|
| 1 | Retention Time | between | 7.5 | 8 | Reference Peak 1 | n.a. | NA -> Failed |
| 2 | Resolution (USP) | >= | 2 | | Reference Peak 2 | 1.3 | Failed |
| 3 | Peak Area | < | 0.6 | | Reference Peak 3 | 0.7 | Failed |
| 4 | Peak Asymmetry | < | 1.5 | | Reference Peak 4 | n.a. | NA -> Failed |
| 5 | Peak Height | > | 50 | | Reference Peak 10 | 1.5 | Failed |
| | Number of executed test cases: 5 | | | | Total Result: | | Failed |

**Figure 6. Using SST parameters for processing peptide mapping samples.** (A) SST & IRC tab of the Chromeleon Processing Method for comparison of mAb peptide mapping samples to reference. (B) UV chromatograms for reference mAb (golimumab), plus two test mAbs. (C) Chromeleon results for SST indicating a pass for the correct mAb (Sample 1—golimumab) and fail for the incorrect mAb (Sample 2—nivolumab).

## Conclusion

- Peptide mapping elution profiles in liquid chromatography can be complex, and therefore consistency of peak integration and interpretation of the data can be challenging.

- Automated data processing ensures that samples are analyzed in a consistent manner, eliminating the potential for analyst-to-analyst variation, integration bias, or calculation errors that can occur during manual processing.

- Incorporation of a well designed SST procedure provides confidence that the analytical system is fit for purpose prior to analysis of precious samples. Automation of this procedure safeguards against potential inter-analysis variation.

- IRC uses consistently unbiased, data-driven decision making to react and adapt to potential changes or problems, which may occur during an analytical run to maintain data quality and adherence to SOPs.

- The wide variety of SST specifications within Chromeleon CDS provide additional flexibility in the way the SST/IRC calculations can be utilized not limiting its use to routine QC environments but allowing for implementation by all laboratories at all stages of the biotherapeutic method development cycle.

- Automation of system suitability testing, combined with the incorporation of IRC, can save time and money while increasing laboratory productivity without impacting data quality or compliance.

- The Vanquish Duo UHPLC system for Dual LC can be used to run multiple samples simultaneously with identical LC separation and processing methods and SST criteria.

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