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Preface

The *Empower Software System Suitability Quick Reference Guide* provides an overview of Empower™ System Suitability software, troubleshooting information, installation procedures, and equations used by the software to determine system suitability.

You should understand the principles of chromatography and be familiar with acquiring, processing, and reporting data using Empower software.

**Organization**

This guide contains the following:

- **Chapter 1** describes the System Suitability software and its place in an HPLC system.
- **Chapter 2** describes how to install the System Suitability software and how to load the contents of the project included on the System Suitability disk.
- **Chapter 3** describes the equations that Empower software uses to determine system suitability.

**Related Documentation**

*Waters Licenses, Warranties, and Support:* Provides software license and warranty information, describes training and extended support, and tells how Waters handles shipments, damages, claims, and returns.

**Online Documentation**

*Empower Help:* Describes all Empower windows, menus, menu selections, and dialog boxes for the base software and software options. Also includes reference information and procedures for performing all tasks required to use Empower software. Included as part of the Empower software.

*Empower Read Me File:* Describes product features and enhancements, helpful tips, installation and/or configuration considerations, and changes since the previous version.

*Empower LIMS Help:* Describes how to use the Empower LIMS Interface to export results and import worklists.

*Empower Toolkit Professional Help:* Describes how to use the common-object-model, message-based protocol to communicate with the Empower software from a third-party application.
Printed Documentation for Base Product

**Empower Software Getting Started Guide:** Provides an introduction to the Empower software. Describes the basics of how to use Empower software to acquire data, develop a processing method, review results, and print a report. Also covers basic information for managing projects and configuring systems.

**Empower Software Data Acquisition and Processing Theory Guide:** Provides theories pertaining to data acquisition, peak detection and integration, and quantitation of sample components.

**Empower System Installation and Configuration Guide:** Describes Empower software installation, including the stand-alone Personal workstation, Workgroup configuration, and the Enterprise client/server system. Discusses how to configure the computer and chromatographic instruments as part of the Empower System. Also covers the installation, configuration, and use of acquisition servers such as the LAC/E™ module, the busLAC/E™ card, and interface cards used to communicate with serial instruments.

**Empower System Upgrade and Configuration Guide:** Describes how to add hardware and upgrade the Empower software using an import-and-export upgrade method.

**Empower Software System Administrator’s Guide:** Describes how to administer the Empower Enterprise client/server system and Workgroup configuration.

**Empower Software Release Notes:** Contains last-minute information about the product. Also provides supplementary information about specific Empower software releases.

Printed Documentation for Software Options

**Empower System Suitability Quick Reference Guide:** Describes the basics of the Empower System Suitability option and describes the equations used by the System Suitability software.

**Empower PDA Software Getting Started Guide:** Describes the basics of how to use the Empower PDA option to develop a PDA processing method and to review PDA results.

**Empower GC Software Getting Started Guide:** Describes how to use the Empower GC option to develop a GC processing method and to review GC results.

**Empower GPC Software Getting Started Guide:** Describes how to use the Empower GPC option to develop a GPC processing method and to review GPC results.
**Empower GPCV Software Getting Started Guide:** Describes how to use the Empower GPCV option to develop a GPCV processing method and to review GPCV results.

**Empower Light Scattering Software Getting Started Guide:** Describes how to use the Empower Light Scattering option to develop a light scattering processing method and to review light scattering results.

**Empower ZQ Mass Detector Software Getting Started Guide:** Describes installation, configuration, calibration, and tuning methods, as well as how to operate the ZQ Mass Detector with Empower software.

**Empower Chromatographic Pattern Matching Software Getting Started Guide:** Describes how to use the Chromatographic Pattern Matching option to develop a pattern matching processing method and to review pattern matching results.

**Empower Dissolution System Software Quick Start Guide:** Describes how to operate the Alliance® Dissolution System using Empower software.

**Empower Toolkit Programmer’s Reference Guide:** Describes how to use the common-object-model, message-based protocol to communicate with Empower software from a third-party application.

**Waters Integrity System Getting Started Guide:** Describes features of the Waters Integrity® System and provides step-by-step tutorials that guide a user through the use of the Empower Mass Spectrometry (MS) option.

**Empower AutoArchive Software Installation and Configuration Guide:** Describes how to install and configure the Empower AutoArchive option.

Documented on the Web

Related product information and documentation can be found on the World Wide Web. Our address is [http://www.waters.com](http://www.waters.com).

**Related Adobe Acrobat Reader Documentation**

For detailed information about using Adobe Acrobat Reader, see the *Adobe Acrobat Reader Online Guide*. This guide covers procedures such as viewing, navigating, and printing electronic documentation from Adobe Acrobat Reader.

**Printing This Electronic Document**

Adobe Acrobat Reader lets you easily print pages, page ranges, or the entire document by selecting **File > Print**. For optimum print quantity, Waters recommends that you specify a PostScript® printer driver for your printer. Ideally, use a printer that supports 600 dpi print resolution.
**Documentation Conventions**

The following conventions can be used in this guide:

<table>
<thead>
<tr>
<th>Convention</th>
<th>Usage</th>
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<tbody>
<tr>
<td>Purple</td>
<td>Purple text indicates user action such as keys to press, menu selec-</td>
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<td></td>
<td>tions, and commands. For example, “Click <strong>Next</strong> to go to the next</td>
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<td>page.”</td>
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<tr>
<td><em>Italic</em></td>
<td>Italic indicates information that you supply such as variables. It also</td>
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<tr>
<td></td>
<td>indicates emphasis and document titles. For example, “Replace <em>file_name</em></td>
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<td>with the actual name of your file.”</td>
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<tr>
<td><strong>Courier</strong></td>
<td>Courier indicates examples of source code and system output. For</td>
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<td></td>
<td>example, “The <strong>SVRMGR&gt;</strong> prompt appears.”</td>
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<tr>
<td><strong>Courier Bold</strong></td>
<td>Courier bold indicates characters that you type or keys you press in</td>
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<tr>
<td></td>
<td>examples of source code. For example, “At the <strong>LSNRCTL&gt;</strong> prompt,</td>
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<td></td>
<td>enter <strong>set password oracle</strong> to access Oracle.”</td>
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<tr>
<td><strong>Underlined Blue</strong></td>
<td>Indicates hypertext cross-references to a specific chapter, section,</td>
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<td>subsection, or sidehead. Clicking this topic using the hand symbol</td>
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<td>brings you to this topic within the document. Right-clicking and</td>
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<td>selecting <strong>Go Back</strong> from the shortcut menu returns you to the orig-</td>
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<td>inating topic. For example, “**Section 2.2, Restoring the System</td>
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<td>Suitability Sample Project**, summarizes the contents of the System</td>
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<td>Suitability sample project.”</td>
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<td><strong>Screen keys</strong> refer to the keys on the instrument located immedi-</td>
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<td>ately below the screen. For example, “The A/B screen key on the</td>
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<td>2414 Detector displays the selected channel.”</td>
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<td>Three periods indicate that more of the same type of item can</td>
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<td>optionally follow. For example, “You can store <em>filename1</em>,</td>
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<td><em>filename2</em>, ... in each folder.”</td>
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<td>A right arrow between menu options indicates you should choose</td>
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<td>each option in sequence. For example, “Select <strong>File &gt; Exit</strong> means</td>
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<td>you should select <strong>File</strong> from the menu bar, then select <strong>Exit</strong></td>
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<td>from the <strong>File</strong> menu.”</td>
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</table>

**Notes**

Notes call out information that is helpful to the operator. For example:

*Note*: Record your result before you proceed to the next step.
Attentions

Attentions provide information about preventing damage to the system or equipment. For example:

Attention: To avoid damaging the detector flow cell, do not touch the flow cell window.

Cautions

Cautions provide information essential to the safety of the operator. For example:

Caution: To avoid burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.

Caution: To avoid electrical shock and injury, turn off the detector and unplug the power cord before performing maintenance procedures.

Caution: To avoid chemical or electrical hazards, observe safe laboratory practices when operating the system.
Chapter 1
Empower System Suitability Software Overview

This chapter presents an overview of Empower™ System Suitability software. System Suitability software is used for quality control, method validation, and tracking and plotting trends, particularly in laboratories following GMP/GLP or other regulatory protocols.

1.1 System Suitability and Empower Software

System Suitability is fully integrated into Empower software and provides testing capabilities to ensure that your chromatography system is working within acceptable limits. As described in The United States Pharmacopeia (USP) guidelines, suitability testing is a concept which holds that the electronics, equipment, specimens, and analytical operations constitute a single analytical system, which is amenable to an overall test of system function.

The Empower System Suitability software option provides system testing and method validation for LC, GC, IC, CIA, CE, GPC, PDA, and MS chromatography applications. System Suitability performs statistical calculations on results and summarizes the information in graphical or tabular formats.

With System Suitability, you can produce reports that show the statistical accuracy and reproducibility of your chromatographic system data. Your reports can also include control charts that monitor user-specified error and warning parameter limits on the individual components in a chromatogram. Empower software bases its system suitability tests on standard laboratory calculations, including The United States Pharmacopeia¹, European Pharmacopeia², and Japanese Pharmacopeia³ guidelines and calculations.

1.2 What Is System Suitability Testing?

System Suitability testing provides a means of checking that an entire chromatographic system is working within acceptable limits. Empower System Suitability software monitors your chromatographic system automatically and provides a graphical summary of system performance based on parameters and limits you set up within Empower software. If all your parameters fall within the specified relative standard deviation (RSD) criteria, your system is suitable to run unknowns. Empower software produces reports showing statistical accuracy and reproducibility of the chromatographic system data.

System Suitability tests the following:
- Method validation
- System performance
- Reproducibility
- Tracking and plotting trends
- Processing and reporting

You can define System Suitability limits as a range of allowable values for each component involved in system suitability testing. The System Suitability limits you set are used to determine the limits shown as minimum and maximum values in summary charts, and faulted, out-of-range values in summary tables and in printed reports. You identify the components and set the limits for the appropriate fields in the Components table and the Suitability Limits table (using the Components tab and the Limits tab in the Processing Method window of Review).

Method Validation

Method validation is the process of determining the precision, accuracy, limit of detection, limit of quantitation, selectivity, range, linearity, and ruggedness of your method. Usually, you perform method validation once for each method. You can use Empower System Suitability software to calculate:
- Precision – % RSD
- Accuracy – % Deviation
- Limit of detection
- Limit of quantitation – derived from the noise and drift measurements
- Selectivity – calculated directly by the software
- Reproducibility – % RSD for a component over a series of runs
- Range or linearity – from calibration curve(s)
- Ruggedness – % RSD when method conditions are varied
System Performance

System Suitability software enables you to monitor instrument operation and calibration in your HPLC system. You can use System Suitability to determine if an instrument is functioning properly (for example, flow rate, UV wavelength, injection accuracy, A/D accuracy, or software integration routine) and when an HPLC component (for example, column, mobile phase, or detector lamp) should be replaced.

System Suitability software measures performance by analyzing the ability of the system to separate components using the following parameters:

- Plate count (N)
- Tailing or symmetry factor
- Resolution ($R_S$)
- Relative resolution
- Selectivity ($\alpha$)
- Capacity factor ($k'$)

Reproducibility

System Suitability enables Empower software to measure system reproducibility by analyzing the consistency of the separation from injection to injection using the following peak parameters (among others):

- Area
- Height
- Amount
- Retention time

Tracking and Plotting Trends

System Suitability allows Empower software to track and plot trends in performance of a chromatography system. For example, to detect column aging, you can track and plot trends for plate count values from the results of sample sets acquired over time.

Processing and Reporting

As a completely integrated part of Empower software, System Suitability processing and reporting parameters are included in:

- Processing methods – System Suitability parameters and limits are part of a processing method. You set them during method development using the Empower Review window.
  
  For details, see the “Using System Suitability” topic in the Empower Help Find tab.
• **Report methods** – System Suitability report groups and fields are part of a report method. You can create System Suitability report methods in the Report Publisher window, or you can use one of the report methods included in the System Suitability default project.

For details, see the “Interpreting System Suitability Data Plots in Reports” topic in the Empower Help Find tab.

Once System Suitability processing and report methods are created, you can:

• Run and report System Suitability analysis on a set of samples during data acquisition.

• Combine processing and reporting on multiple sets of samples after data acquisition.

### 1.3 Using System Suitability

You use Empower System Suitability software to determine if a system is suitable to report results on unknowns by testing:

• Separation criteria

• Reproducibility criteria

You begin by setting system suitability processing parameters (to define the calculations to use and the range of allowable peak values) and reporting parameters (to define the reports used to monitor results), and then saving the associated methods in a method set. For details, see the “Defining System Suitability Processing Method Parameters” topic in the Empower Help Find tab.

After you set processing parameters and limits and the criteria for reporting system suitability data, you can begin using System Suitability software to test your system.

**Verifying System Suitability**

To verify that the system is suitable for acquiring and processing samples, perform the actions described in the following Empower Help Find tab topics:

• “Acquiring a Sample Set”

• “Reviewing the Results”

• “Printing a Report from the Project Window”

If results fall outside specifications, enter **System Suitability, troubleshooting** in the Empower Help Index tab to access the “Using System Suitability to Troubleshoot a Chromatographic System” topic.
Validating Your Methodology

To validate your methods, first create an instrument method in the Project window. For details, see the “Creating a New Instrument Method” topic in the Empower Help Find tab.

Next, follow the instructions in the Empower Help for validating your methods. For details, see the “Performing Method Validation” topic in the Empower Help Find tab.

System Suitability software can assist you in validating a chromatographic methodology. There are eight factors measured in method validation:

- Precision
- Accuracy
- Limit of detection
- Limit of quantitation
- Selectivity
- Reproducibility
- Range or linearity
- Ruggedness

1.4 Troubleshooting

System Suitability allows you to perform troubleshooting procedures on a chromatography system by viewing peak data for:

- Retention time
- Resolution (R_s)
- Capacity factor (k’)
- Selectivity (α)
- Column efficiency (plate count, N)
- Tailing or symmetry factor
- Baseline noise and drift
To quickly determine whether your system is performing correctly, create a report for a sample set that includes the overall percent RSD for selected fields. If the replicate system suitability injections in your sample set vary by more than a given value (such as 2%), you can examine data from each injection until you locate the problem.

For details, see the following topics in the Empower Help Find tab:

- “Setting System Suitability Limits”
- “Defining System Suitability Report Group Properties”
- “Creating a Report Method”

Or you can enter System Suitability,troubleshooting in the Empower Help Index tab to access the “Using System Suitability to Troubleshoot a Chromatographic System” topic.
Chapter 2
Installing System Suitability

This chapter contains instructions for installing Empower System Suitability software. You install System Suitability software from a key disk (3.5-inch diskette) to the workstation or server where Empower software resides. A CD-ROM (Empower System Suitability Option) contains the System Suitability sample project files, which you need to restore after installing the System Suitability software. Section 2.2, Restoring the System Suitability Sample Project, summarizes the contents of the System Suitability sample project.

You must install the Empower System Suitability software to use the Empower System Suitability application.

You can install System Suitability on a Personal stand-alone workstation, an Enterprise client/server system, or a Workgroup system.

- In a Personal stand-alone configuration, you can install System Suitability on only one workstation per license. If you remove the System Suitability option from one stand-alone workstation, you can install it on another.
- In an Enterprise client/server or Workgroup environment, the System Suitability option is locked to the database node on which it is installed, not the client computer.

You cannot install System Suitability for an Enterprise client/server or Workgroup node on a Personal stand-alone workstation, nor can you install System Suitability for a Personal stand-alone workstation on an Enterprise client/server or Workgroup node.

Once you have installed System Suitability using the procedures in this section, the System Suitability software is enabled for all projects. You can disable System Suitability for specific projects. For details on disabling an option for a specific project, see the “Using the Configuration Manager” and “Project Properties” topics in the Empower Help Find tab.

2.1 Installing System Suitability Software

To install Empower System Suitability software on your system:

1. If you have not already done so, install the Empower software and database (see the Empower System Installation and Configuration Guide).

   Note: To install System Suitability software on your workstation or network server, the Empower software must be installed on the same workstation or database node.
To ensure successful installation, Waters® strongly recommends that the Empower software not be running during installation.

2. Insert the Empower System Suitability key disk (3.5-inch diskette) into the diskette drive.

3. Click **Start**, then click **Run**. The Run dialog box appears (Figure 2-1).

![Run Dialog Box](image)

4. Type `A:\Setup.exe` (or the appropriate disk drive letter in which the System Suitability key disk is inserted, followed by `:\Setup.exe`).

5. Click **OK**. The Empower Option Setup dialog box appears (Figure 2-2).

   **Note:** Because the key disk is being read onto your workstation hard disk or server node, it can take a few minutes for the Empower Option Setup dialog box to appear on your screen.
As Figure 2-2 shows, the name of the Empower option on the disk inserted in the drive appears. Ensure the name displayed is “System Suitability” before proceeding.

6. Click OK to install the System Suitability software (or click Cancel to cancel the action). The system displays one of the following messages:

- If the System Suitability option has not been previously installed on your workstation or network server, the following message box appears (Figure 2-3). Click OK to close this message box.

![Empower Option Setup Dialog Box](image)

Figure 2-2 Empower Option Setup Dialog Box

![Option Successfully Added Message Box](image)

Figure 2-3 Option Successfully Added Message Box

*Installing System Suitability Software* 18
If the System Suitability option has already been installed on your workstation or network, the following query box appears (Figure 2-4). Click OK to remove the option, or click Cancel to dismiss the query box without removing the option.

![Figure 2-4 Option Already Installed Query Box](image)

If the System Suitability key disk has already been used to install System Suitability on another stand-alone workstation, the following message box appears (Figure 2-5). Click OK to dismiss the message box. You need to uninstall System Suitability from one workstation to use it on another workstation (see the next discussion, "Uninstalling the System Suitability Option").

![Figure 2-5 Option Already Installed on Another Workstation Message Box](image)

### Uninstalling the System Suitability Option

To install the System Suitability option on another stand-alone workstation, you must uninstall it from its current location.

To uninstall System Suitability:

1. Insert the Empower System Suitability key disk into the disk drive.
2. Click Start, then click Run. The Run dialog box appears (Figure 2-1).
3. Type A:\Setup.exe (or the appropriate disk drive letter in which the System Suitability disk is inserted, followed by :\Setup.exe).
4. Click OK. The Empower Option Setup dialog box appears (Figure 2-2).
5. Click OK. The Option Already Installed message box appears (Figure 2-4).
6. Click OK. The following message box appears (Figure 2-6).

![Figure 2-6 Option Successfully Removed Message Box](image)

7. Click OK to close the message box.

Once you have uninstalled System Suitability from one workstation, you can eject the System Suitability key disk and install the software on another workstation.

### 2.2 Restoring the System Suitability Sample Project

After you install System Suitability software from the key disk, you can restore the System Suitability sample project from the System Suitability CD-ROM. You can use the System Suitability sample project as a template for testing your chromatographic system. The project name is SysSuit_Default.

For details on logging in to Empower software, see the “Logging in Manually” or “Logging in Automatically” topic in the Empower Help Find tab.

For details on restoring a project, see the “Restoring a Project Using the Wizard” topic in the Empower Help Find tab.

**Contents of the System Suitability Sample Project**

The System Suitability sample project contains:

- Four sample sets, each containing six raw data files
- Four results sets, each containing six results files
- A processing method with System Suitability enabled and typical parameter settings
- Report methods
Chapter 3
System Suitability Equations

This chapter describes the equations used by Empower System Suitability software for the following test parameters:

- Plate count (N)
- Tailing or symmetry
- Resolution ($R_s$)
- Capacity factor ($k'$)
- Selectivity ($\alpha$)
- Baseline noise and drift
- Statistical quantities

3.1 System Suitability Results

Table 3-1 summarizes all Empower System Suitability results using the terminology from The European Pharmacopeia, The Japanese Pharmacopeia, and The United States Pharmacopeia. Select EP, JP, USP, or All from the Suitability tab of the Processing Method window to obtain the results in Table 3-1. See the “Defining System Suitability Processing Method Parameters” topic in the Empower Help Find tab.

Table 3-1: System Suitability Results Based on Pharmacopeia

<table>
<thead>
<tr>
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<td>Results</td>
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Table 3-1 System Suitability Results Based on Pharmacopeia (Continued)

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<td>Asym@10</td>
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<td></td>
<td>—</td>
<td>—</td>
<td>Width @ Baseline</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>Width @ Tangent</td>
<td>Width @ Tangent</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>Width @ 4.4%</td>
<td>—</td>
</tr>
<tr>
<td>Width @ 5%</td>
<td>Width @ 5%</td>
<td>Width @ 5%</td>
<td>Width @ 5%</td>
<td>Width @ 5%</td>
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<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>Width @ 10%</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>Width @ 13.4%</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>Width @ 32.4%</td>
<td>—</td>
</tr>
<tr>
<td>Width @ 50%</td>
<td>Width @ 50%</td>
<td>Width @ 50%</td>
<td>Width @ 50%</td>
<td>Width @ 50%</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>Width @ 60.7%</td>
<td>—</td>
</tr>
<tr>
<td>f @ 5%</td>
<td>f @ 5%</td>
<td>f @ 5%</td>
<td>f @ 5%</td>
<td>—</td>
</tr>
</tbody>
</table>

1. EP and JP Relative Resolution are calculated using the formula for Resolution.
2. USP Relative Resolution is calculated using the formula for USP Resolution (HH).
3. EP, JP, and USP (All) Relative Resolution are calculated using the formula for USP Resolution.
3.2 Calculating Width at Percent of Peak Height

Empower System Suitability software uses the equation in Figure 3-1 to calculate width at percent of peak height used in several System Suitability equations referenced in this section.

System Suitability does not perform any calculation requiring a peak width if the peak width at the percentage of height cannot be calculated.

Where:

\[ y_2 = \frac{\%}{100}(y_{rt} - y_e) + y_e \]
\[ y_1 = \frac{\%}{100}(y_{rt} - y_s) + y_s \]

\[ x_1 = \text{Interpolated point on the chromatogram from } x_s \text{ to } x_{rt} \text{ where } y = y_1 \]
\[ x_2 = \text{Interpolated point on the chromatogram from } x_{rt} \text{ to } x_e \text{ where } y = y_2 \]

Figure 3-1 Calculation of Width at Percent of Peak Height
The peak width at percent height is calculated for baseline-resolved peaks (BB) but *not* for skinned peaks (such as GB or BG). It can be calculated for nonbaseline-resolved peaks (BS, SB, BR, and RB) depending on the height of the start and end points.

**Note:** The Peaks table (in the Review Main window and the Results window) displays a two-character label that describes the way a peak was integrated. See the "Integration Type Labels in the Peaks Table" topic in the Empower Help Find tab for a listing.

Peaks are identified as follows:

BB = Baseline to Baseline  
BV = Baseline to Valley  
VB = Valley to Baseline  
VV = Valley to Valley  
EE = Exponential to Exponential  
TT = Tangential to Tangential

If you manually draw or adjust a baseline, or move a drop line, manual integration is noted in the Int Type field by lowercase letters (b, v, t, and e). For example:

bV = Manual Baseline to Valley  
vV = Manual Valley to Valley  
bb = Manual Baseline to Manual Baseline

**If BV (or bv):**

If the $y$ value at the end time of the peak is greater than the $y$ value at the percent height at the start of the peak, then the peak width is not calculated.

**If VB (or vb):**

If the $y$ value at the start time of the peak is greater than the $y$ value at the percent height at the end of the peak, the peak width is not calculated.

**If VV (or vv):**

The peak width is not calculated if the $y$ value at either the start or end of the peak is greater than the percent height at the retention time of the peak, that is, $\text{pct/100}\cdot(\text{top } y - \text{bottom } y)$, where top $y = y$ value at retention time and bottom $y = y$ value of baseline at the retention time.

### 3.3 Calculating Peak Width at Tangent

Empower System Suitability software calculates peak width at tangent to determine the width ($W$) when calculating the plate count using the tangent method (Figure 3-2). Peak width at tangent is calculated only if the peak widths at both the tangent percent +5 and the tangent percent −5 can be calculated. To complete the peak width at tangent
calculation, System Suitability software requires an intersection of the tangent lines with the baseline.

\[ W = x_B - x_F \]

Where:

- \( x_B \) = The \( x \) value of the point where the baseline intersects a line drawn between the points \( B_{hi} \) and \( B_{lo} \)

- \( x_F \) = The \( x \) value of the point where the baseline intersects a line drawn between the points \( F_{hi} \) and \( F_{lo} \)

- \( B_{hi} \) = The interpolated point on the back side of a peak at \((\% + 5) \times \) the height of the peak

- \( B_{lo} \) = The interpolated point on the back side of a peak at \((\% - 5) \times \) the height of the peak

- \( F_{hi} \) = The interpolated point on the front side of a peak at \((\% + 5) \times \) the height of the peak

- \( F_{lo} \) = The interpolated point on the front side of a peak at \((\% - 5) \times \) the height of the peak

- \( \% \) = The percentage at which you are calculating the tangent (for example, 50 or 61%)

Figure 3-2 Calculation of Peak Width at Tangent
3.4 Plate Count Equations

Plate count calculations determine column efficiency. This section includes figures that describe the equations used for the following plate count methods:

- EP plate count (Figure 3-3)\(^1\)
- JP plate count (Figure 3-4)
- USP plate count (Figure 3-5)
- 5-sigma (Figure 3-6)
- 4-sigma (Figure 3-7)
- 3-sigma (Figure 3-8)
- 2-sigma (Figure 3-9)
- Asymmetry-based (Figure 3-10)

\(^1\) Was half-height in previous versions

\[ N = 5.54 \left( \frac{R_t}{W} \right)^2 \]

- \( N \) = Plate count (the number of theoretical plates in a chromatographic column)
- \( R_t \) = Retention time
- \( W \) = Peak width at 50% of peak height

Figure 3-3 EP (European) Plate Count Equation
Plate count equations

**Figure 3-4 JP (Japanese) Plate Count Equation**

\[ N = 5.55 \left( \frac{R_t}{W} \right)^2 \]

- \( N \): Plate count (the number of theoretical plates in a chromatographic column)
- \( R_t \): Retention time
- \( W \): Peak width at 50% of peak height

**Figure 3-5 USP Plate Count Equation**

\[ N = 16 \left( \frac{R_t}{W} \right)^2 \]

- \( N \): Plate count (the number of theoretical plates in a chromatographic column)
- \( R_t \): Retention time
- \( W \): Peak width at baseline determined by tangents drawn to % of peak height

- % = 61% if Pharmacopeia choice is USP or the Tangent Percent entered by the user if the Pharmacopeia choice is All
**Figure 3-6** 5-Sigma Equation

\[ N = 25 \left( \frac{Rt}{W} \right)^2 \]

- \( N \) = Plate count (the number of theoretical plates in a chromatographic column)
- \( Rt \) = Retention time
- \( W \) = Peak width at 4.4% of peak height

**Figure 3-7** 4-Sigma Equation

\[ N = 16 \left( \frac{Rt}{W} \right)^2 \]

- \( N \) = Plate count (the number of theoretical plates in a chromatographic column)
- \( Rt \) = Retention time
- \( W \) = Peak width at 13.4% of peak height
Plate Count Equations

Figure 3-8  3-Sigma Equation

Figure 3-9  2-Sigma (Inflection) Equation

\[ N = 9 \left( \frac{Rt}{W} \right)^2 \]

\[ N = 4 \left( \frac{Rt}{W} \right)^2 \]

\( N \) = Plate count (the number of theoretical plates in a chromatographic column)

\( Rt \) = Retention time

\( W \) = Peak width at 32.4% of peak height

\( W \) = Peak width at 60.7% of peak height
3.5 Tailing or Symmetry Factor Equations

This section contains figures that describe the equations used to calculate the asymmetry of a peak. These figures illustrate the following tailing equations:

- USP tailing factor (Figure 3-11)
- Asymmetry\(^2\) (10%) tailing (Figure 3-12)
- Asymmetry (10%) tailing (Figure 3-13)
- Asymmetry\(^2\) (4.4%) tailing (Figure 3-14)
- Asymmetry (4.4%) tailing (Figure 3-15)

**Note:** The USP tailing factor is known as the symmetry factor in the European Pharmacopeia and the Japanese Pharmacopeia.

---

Tailing factor establishes the maximum permissible asymmetry of the peak. For pharmaceutical purposes, the tailing factor, $T$, is defined as the distance between the leading edge and tailing edge of the peak at a width of 5% of the peak height divided by twice the distance, $F$, between the peak maximum and the leading edge of the peak at 5% of peak height.

For a symmetrical peak, the tailing factor, $T$, is 1.0, and the value of $T$ increases as tailing becomes more pronounced.

**Note:** The value of $F$ is reported in the f @ 5% field.

\[
T = \frac{W}{2F}
\]

- $T$ = Tailing factor
- $W$ = Peak width at 5% of peak height
- $Rt$ = Retention time
- $F$ = Time from width start point at 5% of peak height to $Rt$
Figure 3-12 Asymmetry\(^2\) (10\%) Tailing Equation

\[
(A_{10})^2 = \left(\frac{A}{B}\right)^2
\]

\(A_{10}\) = Asymmetry (10\%)

\(Rt\) = Retention time

\(A\) = Time from \(Rt\) to width end point at 10\% of peak height

\(B\) = Time from width start point at 10\% of peak height to \(Rt\)

---

Figure 3-13 Asymmetry (10\%) Tailing Equation

\[A_{10} = \frac{A}{B}\]

\(A_{10}\) = Asymmetry (10\%)

\(Rt\) = Retention time

\(A\) = Time from \(Rt\) to width end point at 10\% of peak height

\(B\) = Time from width start point at 10\% of peak height to \(Rt\)
Tailing or Symmetry Factor Equations

Figure 3-14  Asymmetry\(^2\) (4.4%) Tailing Equation

\[(A_{4.4})^2 = \left(\frac{B}{A}\right)^2\]

- \(A_{4.4}\) = Asymmetry (4.4%)
- \(R_t\) = Retention time
- \(A\) = Time from \(R_t\) to width end point at 4.4% of peak height
- \(B\) = Time from width start point at 4.4% of peak height to \(R_t\)

Figure 3-15  Asymmetry (4.4%) Tailing Equation

\[A_{4.4} = \frac{A}{B}\]

- \(A_{4.4}\) = Asymmetry (4.4%)
- \(R_t\) = Retention time
- \(A\) = Time from \(R_t\) to width end point at 4.4% of peak height
- \(B\) = Time from width start point at 4.4% of peak height to \(R_t\)
3.6 Resolution Equation

Resolution between peaks is measured to ensure that the system has the resolving power to separate closely eluting components of a mixture.

System Suitability software measures resolution between a peak and the preceding integrated peak. Relative resolution is measured between named peaks in the calibration table and their referenced peaks when a relative resolution reference peak is specified in the processing method.

Resolution is calculated as 2.0 times the retention time between two adjacent peaks divided by the sum of the width of the peaks. Theoretically, the peak width used in the Resolution formula should be the width of the peak at baseline. However, this peak width cannot be calculated for overlapping peaks. The peak width can be approximated by either the tangent width at 50% or by the peak width at 50% multiplied by a constant value of 1.7.

The different resolution equations implemented in Empower System Suitability software use the approximate peak widths specified by the United States Pharmacopeia (USP), European Pharmacopeia (EP), and Japanese Pharmacopeia (JP), as follows:

- The USP Resolution equation uses the baseline peak width calculated using lines tangent to the peak at 50% height (Figure 3-16).
- The USP Resolution (HH) equation uses the peak widths at half-height multiplied by a constant value of 1.7 (Figure 3-17).
- The European and Japanese Pharmacopeia Resolution equation uses peak widths at 50% of peak height multiplied by a constant value of 1.7, but replaces the 2.0 constant value in the numerator and the 1.7 constant value in the denominator by a single constant value of 1.18 in the numerator (Figure 3-18).

**Note:** When the 2.0 constant is divided by the 1.7 constant (and rounded to two decimal places) the result is a constant value of 1.18.

Resolution is calculated for both named and unnamed peaks, where the appropriate peak width can be calculated. Resolution is not calculated for skimmed peaks.

**Note:** Resolution is never measured for the first peak in a chromatogram because there is no preceding peak to use in the calculation.
Resolution Equation 35

\[ R = \frac{2.0(Rt_2 - Rt_1)}{(W_2 + W_1)} \]

**R** = Resolution

**Rt** = Retention time

**W_1 + W_2** = Sum of peak widths at baseline between tangent lines drawn at 50% peak height

Figure 3-16 USP Resolution Equation
System Suitability Equations

\[ R = \frac{2.0(Rt_2 - Rt_1)}{1.7(W_2 + W_1)} \]

- **R** = Resolution
- **Rt** = Retention time
- **W_1 + W_2** = Sum of peak widths at 50% peak height

Figure 3-17  USP Resolution (HH) Equation
3.7 Capacity Factor (k’) Equation

Capacity factor (k’) is a measurement of the retention time of a sample molecule, relative to the column dead volume. Figure 3-19 describes the capacity factor (k’) equation.

\[ k' = \frac{R_t}{V_0} - 1.0 \]

\( k' \) = Capacity factor  
\( R_t \) = Retention time  
\( V_0 \) = Void volume time

Figure 3-19 Capacity Factor (k’) Equation
3.8 Selectivity ($\alpha$) Equation

Selectivity ($\alpha$) is the relative retention of two peaks in a chromatogram (the ratio of two $k'$ values). Figure 3-20 describes the equation used to compute selectivity.

\[
\alpha = \frac{Rt_2 - V_0}{Rt_1 - V_0}
\]

$\alpha$ = Selectivity (also called alpha)  
$Rt_1$ = Retention time of the first peak  
$Rt_2$ = Retention time of the second peak  
$V_0$ = Void volume time

Figure 3-20 Selectivity Equation for Peak at $Rt_2$

**Note:** Selectivity is never measured for the first peak in a chromatogram because there is no preceding peak to use in the calculation.
3.9 Baseline Noise and Drift Measurements

Empower software calculates noise and drift from a segment of the baseline between the Baseline Start time and Baseline End time. To obtain a valid noise calculation, the baseline interval must be free of component peaks.

Noise

The software calculates noise based on the maximum voltage change over a 30-second interval. The reported noise value is an average of a number of 30-second intervals as determined by the % Run Time Over Which to Average parameter.

To determine the 30-second intervals, the software begins counting from the Baseline Start time and the Baseline End time toward the middle of the chromatogram. Any points left over after the last full 30-second interval are not included in the calculation.

Table 3-2 identifies the specific conditions used to average the regions shown in the example in Figure 3-21.

Table 3-2 Conditions Used to Average Regions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Setting</th>
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<tr>
<td>Total Run Time</td>
<td>10 minutes</td>
</tr>
<tr>
<td>% of Run Time to Average</td>
<td>5%</td>
</tr>
<tr>
<td>Average Time</td>
<td>0.5 minute (5% of 10 minutes)</td>
</tr>
<tr>
<td>Baseline Start</td>
<td>1 minute</td>
</tr>
<tr>
<td>Baseline End</td>
<td>9 minutes</td>
</tr>
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Table 3-2

<table>
<thead>
<tr>
<th>Averaged region</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 1.5 minutes</td>
<td>1 minute (BL Start + Average Time)</td>
</tr>
<tr>
<td>8.5 to 9 minutes</td>
<td>8.5 minutes (BL End – Average Time)</td>
</tr>
</tbody>
</table>

Figure 3-21 Percent of Run Time to Average
A maximum change in millivolts (low to high) is calculated for each interval by subtracting the highest voltage in the interval from the lowest voltage, then the millivolt change values for all intervals are averaged and reported as the noise value.

If the averaged region contains fewer than 30 seconds, noise is reported as a blank.

**Drift**

Drift is the comparison of the millivolt (mV) readings at Baseline Start and Baseline End. To calculate drift, the software subtracts the millivolt value at the Baseline Start time from the millivolt value at the Baseline End time.

### 3.10 Statistical Quantity Equations

These figures describe equations used to calculate the following statistical quantities:

- $X_{\text{mean}}$ ([Figure 3-22](#))
- Standard deviation ([Figure 3-23](#))
- % RSD ([Figure 3-24](#))

#### Mean ($X_{\text{mean}}$)

$$X_{\text{mean}} = \frac{\sum_{i=1}^{n} (X_i)}{n}$$

- $X_{\text{mean}}$ = Arithmetic mean of all observations
- $X_i$ = One observation
- $n$ = Number of observations

*Figure 3-22 Mean ($X_{\text{mean}}$) Equation*

#### Standard Deviation

$$S = \sqrt{\frac{\sum_{i=1}^{n} (X_i - X_{\text{mean}})^2}{n - 1}}$$

- $S$ = Standard deviation
- $X_{\text{mean}}$ = Arithmetic mean of all observations
- $X_i$ = One observation
- $n$ = Number of observations

*Figure 3-23 Standard Deviation Equation*
Figure 3-24  % RSD Equation

\[ R = \frac{100}{X_{\text{mean}}} \times \sqrt{\frac{\sum_{i=1}^{n} (X_i - X_{\text{mean}})^2}{n - 1}} \quad \text{or} \quad R = 100 \times \frac{S}{X_{\text{mean}}} \]

\( R \) = Relative standard deviation in %
\( X_{\text{mean}} \) = Arithmetic mean of all observations
\( X_i \) = One observation
\( n \) = Number of observations
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