

Agilent Cerity Networked Data System for Pharmaceutical QA/QC



Getting Started Guide



Agilent Technologies

Notices

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Software Revision

This guide is valid for A.02.xx revisions of the Agilent Cerity Networked Data System for Pharmaceutical QA/QC software, where xx refers to minor revisions of the software larger than or equal to 02 that do not affect the technical accuracy of this guide.

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Before you start

The Getting Started Exercises provide a quick way to learn the Cerity Pharmaceutical QA/QC application. Use the *Cerity Concepts Guide* to help you do the tasks in these exercises.

Setting Up Methods

If you develop methods for your laboratory, you should go through these exercises. You can use these methods to run samples and sequences with the Running Routine Samples exercises.

Running Routine Samples

If you run samples but do not develop methods, you can do these exercises with the default methods that come with the Cerity Networked Data System, or you can use the methods set up with the Setting Up Methods exercises.

Before you start

Make sure that you or your administrator transfer the default methods and example chromatogram from the Cerity CD-ROM to the database. For details to transfer the methods and make them usable for your system, turn to the next page.



Step 1. Restore the default methods

The default methods for the Basic and Advanced exercises are located on Cerity software CD in **\GettingStarted\DefaultMethods**.

1 Restore the default methods.

The default methods for the Basic and Advanced exercises are located on Cerity software CD in **\GettingStarted\ DefaultMethods**.

- 2 Select Start > Programs > Agilent Cerity > Administration and Maintenance > Archive and Restore.
- 3 Enter logon information and click **OK**.
- 4 Select Restore, and click Next.
- **5** Click the ... button.
- **6** Select **GettingStarted****DefaultMethods****Basic** (or **Advanced**) on the CD-Drive.
- 7 Click **OK**, click **Next**, and click **Yes** to the messages.
- 8 Click the >> button to move the default methods to the Restore Objects list.
- 9 Click Next, click Start, and click OK for each message that appears.

The following message appears: "These tables contain duplicates".

Step 2. Resolve database duplicates

- 1 Click Next.
- **2** Make sure that the **Select instruments to enable** check box is clear.
- **3** Click **Next** and select the second Administrator role.
- 4 Click **Rename**, enter the new role name Admin and click **OK**.
- 5 Click Next, click Start, and click OK.
- 6 Click **OK** and any **Close** buttons.

Step 3. Restore the example chromatogram

The example chromatogram is on Cerity-CD-1in **GettingStarted**\ **DefaultResults**. Make sure that the default example chromatogram has been restored.

- 1 Repeat step 1 through step 4 in "Step 1. Restore the default methods" on page 6.
- 2 Select \GettingStarted\DefaultResults on the CD-ROM drive, click OK, and click Next.
- **3** Select **defexchrom2a**, click **>**, and click **Next**.
- 4 Click **Start**, click **OK** to the messages that appear, and click **Close**.
- 5 Select Start > Programs > Agilent Cerity > Cerity Pharmaceutical QA/QC.
- 6 Enter logon information and click **OK**.
- 7 Select **Result** from the Current View list.
- 8 Select AllResultsRestored from the Query list.

Step 4. Copy the default method to use with your instrument

Refer to "Basic Exercise #2 Set up a method for single samples to identify compounds" on page 81 if you need to.

- **1** Select **Method** from the **Current View** list.
- 2 Select AllMethodsRestored from the Query list.
- **3** For each default method:
 - a Select File > New > Method.
 - **b** Click **Browse**, select **defaultmethodN** for Basic exercises, or **AdvdefaultmethodN** for Advanced exercises, and click **OK**.
 - c Name the new method defexerN, and click Next.
 - d Select the instrument where the method will be used, and click **Next**.
 - e Click *Next* until you reach the New Method Review panel.
 - f Click **Finish**, and click **Save** when the Save to the database message appears.
- **4** Select **AllMasterMethods** from the **Query** list.
- **5** Expand **defexerN**.
- **6** Expand **Instrument Setup**, and adapt the settings.
- 7 Adapt the instrument settings for the non-matching LC modules.

You can use the default methods ONLY on instruments with an Agilent VWD detector. Your other LC modules do NOT have to match the modules on which the default methods were set up (autosampler, quaternary pump, thermostatted column compartment).

If you have no instrument available with a VWD detector to use with these exercises, then the administrator or advanced user should set up the methods using the Setting Up Methods sections in this guide.

NOTE

The first time that you copy and rename **Advdefaultmethod4**, name it **defexer4a**. The first user will alter this method in Exercise 4b. You must then copy **Avdefaultmethod4** and rename it **defexer4b** for the second user to use the method.



Running Routine Samples

These exercises help you learn how to run routine samples. You can use the default methods for the "a" exercises or set up methods in the Setting Up Methods exercises. You must have results from the "a" exercises to do the "b" exercises. The set of basic and advanced exercises includes the topics below:

Basic Exercise 1 – Equilibrate the instrument Learn how to equilibrate the instrument with the instrument panel or with a method.

Exercise 2a – Run a single sample to produce an example chromatogram Learn how to produce an example chromatogram that you can use to set up integration and identification in a method.

Exercise 2b – **Run a group of single samples to identify compounds** Learn how to enter and run a group of single samples with a method to identify the compounds in the sample.

Exercise 3a – Run a sequence to quantify compounds with single-level calibration Learn how to run a sequence with single-level, single-update calibration, ESTD quantitation, and fixed amounts.

Exercise 3b – **Reintegrate and reprocess the results** Learn how to manually reintegrate the sequence results, and reprocess the results with the original method revision. For more information on running routine samples, see the *Concepts Guide*, "Sample Analysis".



Advanced Exercise 4a – Run a sequence to quantify compounds with multi-level calibration Learn how to run a sequence set up for multi-level, overall calibration, variable compound amounts and sample variables.

Exercise 4b – Change sample variables in the method and reprocess Learn how to reprocess the results with the most current version of the method and a version with new sample variables.

Exercise 5a – Run a sequence to quantify impurities Learn how to create and run a sequence set up for ISTD quantitation, custom calculations, limits, bracketed calibration and system suitability.

Exercise 5b – **Use a different method to reprocess** Learn how to reprocess with a new method.

Before you start Read "Before you start" on page 5.

If you plan to use default methods in these exercises, make sure that these methods are in your database. From the Query list, select AllMethodsRestored to view defexer1-5 or AllResultsRestored to view defexchrom2a.

Your system administrator must have configured an Agilent 1100 LC series liquid chromatograph for your system.

If you choose to do the Running Routine Samples exercises with the default methods, you must use an instrument with a VWD detector. If you use the methods created in the Setting Up Methods exercises, you need only an autosampler, pump (quaternary or binary) and UV-Vis detector (VWD, MWD, DAD).

Solvent A is water. Solvent B is methanol or acetonitrile.

Use Agilent Technologies column Eclipse XDB-C8 (or C-18), 4.6MM X 15 CM (5 μ M).

Prepare the following three vials of the isocratic standard, Agilent Part # 01080-68704: undiluted, diluted by factor 2, and diluted by factor 4.



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Basic Exercise #1a Equilibrate the instrument

This exercise contains a series of tasks to help you learn how to:

- Equilibrate the instrument with the instrument panel in the Cerity Pharmaceutical QA/QC application
- Enter and run an equilibration sample (blank run) with a method created to equilibrate the instrument

You can use a copy of the default method that comes with the system to equilibrate the instrument, or you can use the method created in the "Basic Exercise #1 Set up an equilibration method" on page 73.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Make sure that the pump is on standby and the VWD lamp is off.

Make sure that the methods for this exercise have been set up or restored.



Task 1. Purge the pump from the Instrument Panel

Steps

Detailed Instructions

- 1 Disengage pump and purge line B. Flow rate: 5ml/min
 - %B = 100%

- a Turn the black valve on the pump counterclockwise two full turns.
- **b** Select **Instrument** from the **Current View** list.
- c Select the instrument that you intend to equilibrate.

The Instrument Panel appears, along with the Online Plot.



d Click the pump module on the Instrument Panel.

A menu appears.



Task 2. Equilibrate the instrument from the Instrument Panel

Steps

.

•

•

Enter the pump parameters

Methanol as Solvent B:

Flow rate: 2ml/min. Solvent composition:

80%MeOH/20%H₂O

Flow rate: 1.5ml/min

Solvent composition:

65%ACN/35%H₂0

1

Detailed Instructions

a Click the pump module on the Instrument Panel.

b Select Set Pump.

The Set Pump dialog box appears.

c Enter the pump parameters as shown in the left column, and click **OK**.



b Select Lamp On.

Wait until baseline has stabilized.

Steps

Detailed Instructions

3 Monitor the baseline until it appears stable.

After this step, you are ready to do the remaining exercises, or you can move on to the next task to learn to equilibrate the instrument with a method.

a Click **Change** at the bottom of the Online Plot.

The Edit Signal Plot dialog box appears.

- b Select the detector signal you need from the Available Signals list, and click the Add button to put the signal in the Selected Signals list. (You can also select the pump pressure).
- c Set the Predictable Range (Y-axis) as -10 to +10.
- d Set the X-Axis range as 10 min.
- e Click OK.

| Edit Signal Plot | |
|-----------------------------|--------------------------|
| Available Signals | Selected Signals |
| Quaternary Pump: Pressure | Add > |
| VWD: Absorbance | |
| • <u>P</u> redictable Range | C <u>F</u> loating Range |
| Erom: -10 * mAU | Y-axis range: mAU |
| Io: 10 🔭 mAU | Offset: |
| | Auto g-adjust |
| Window Properties | |
| ⊻-axis range: 10 × min | |
| 🗖 Draw <u>G</u> rid | OK Cancel Apply |

- f Click the detector module after the lamp has been on for a few minutes.
- g Select Balance.

When the baseline stays at zero for a few minutes after the balance, the baseline is considered stable.

Task 3. Equilibrate the instrument with a method—Enter an equilibration sample

| Steps | Detailed Instructions | | | | |
|---|---|--|--|--|--|
| Enter the sample information Sample Name: equilsampiii, where i are your initials Method: defexer1 or equilmethiii See "Before you start" on page 5 for instructions on how to restore and copy the default methods. | a Select Instrument from the Current View list. b Expand the Sample Entry folder for the instrument that you need to equilibrate. c Select Single Samples. d Enter the Sample Name as equilsampiii. e Select the Method as equilmethii or defexer1. f Select the Sample Type as Blank Run. g Click Apply. You can also enter the sample in the Sample View when you need to enter samples and sequences during a run. | | | | |
| 2 Enter the tasks that the system will do during the analysis. | a Clear the Quantify and Report check boxes. b Click Apply. INSTRUMENT NAME METHOD NAME SAMPLE NAME NUM OF INJECTIONS IMELC3 CRUINCIASS CAUSING CONSTRUCTIONS Sample Entry Sample Logbook Sample Entry Sample Logbook Sample Name: cquisampdec Gquisampdec Gquisampdec Gquisampdec Gequimethdec Image: Sample Type: Sample Type: | | | | |
| 3 Save the sample to the database | a On the Standard toolbar, click . b Review the list of changes c Under Reason for changes, enter a reason or select a reason from the list. d Enter your electronic signature if required. e Click the Save button. | | | | |

Task 4. Equilibrate the instrument with a method—Run the equilibration sample

| Steps | Detailed Instructions |
|------------------------------------|---|
| 1 Run equilsamp <i>iii</i> | a Select the sample, equilsampiii, in the Sample Table. |
| | The Run button is now active. |
| | b Click the Run button and the Actions toolbar. |
| 2 Monitor the baseline until it is | a Select the instrument that you want to equilibrate. |
| stable. | The Instrument Panel appears, along with the Online Plot. |
| | b Click Change at the bottom of the Online Plot. |
| | The Edit Signal Plot dialog box appears. (See the figure on page 14.) |
| | c Select the detector signal you need from the Available Signals list, and click the Add button to put the signal in the Selected Signals list. d Set the Predictable Range as -10 to +10. e Set the X-Axis range as 10 min. f Click OK. |
| | Online Flot Logbook |
| | WWD: Absorbance |



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Basic Exercise #2a Run a single sample to produce an example chromatogram

This exercise contains a series of tasks to learn how to:

- Enter a sample to produce an example chromatogram
- Run the sample
- Review the results

An example chromatogram can be any chromatogram that you produce. Use the example chromatogram to test new integration parameters and identify peaks as compounds.

You can use one of the following methods for this exercise:

- A copy of the default method provided with the Cerity Networked Data System.
- The method saved in "Task 3. Save and audit method changes" on page 86 in the Setting Up Methods section.
- An equilibration method that you created in "Basic Exercise #1 Set up an equilibration method" on page 73.

For the tasks on the next pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Running Routine Samples" on page 9 for running routing samples.

Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11. Make sure that the methods for this exercise have been set up or restored.



Task 1. Enter a single sample

| Steps | | Detailed Instructions | | | | |
|-------|--|---|--|--|--|--|
| 1 | Start the Instrument View to find the sample table for single samples. | a Select Instrument from the Current View list. b Expand the folder for the instrument that will produce the example chromatogram. c Select Single Samples. The sample table and sample entry panel appear in the workspace. | | | | |
| 2 | Enter a sample with the following information: Name the sample exchromiii, where iii are your initials. Select either defexer2, exer2iii (when first saved), equilmethiii Select the vial that contains the full-strength isocratic standard. | a Enter exchrom<i>iii</i> in the Sample Name box. b Select a method from the Method list. The instrument associated with the method appears in the Instrument box. c Select Sample from the Sample Type list. d Enter the vial number for the sample in the Vial Number box. e Click Apply to put the sample information in the sample table. Use the default values for all other parameters | | | | |
| 3 | Enter the tasks to perform during the run. | a Clear the Quantify and Report check boxes. | | | | |
| 4 | Save the sample. | a On the Standard toolbar, click | | | | |

Task 2. Run the sample

| Steps | Detailed Instructions | | | | | |
|---|---|--|--|--|--|--|
| 1 Check that the instrument is ready for use. | a On the selection tree, select your instrument. b Click the Online Plot tab. c Click the Change button. | | | | | |
| | The Edit Signal Plot dialog box appears. | | | | | |
| | d Select the detector signal you need from the Available Signals list. e Click the Add button to put the signal in the Selected Signals list. f Select the Predictable Range option and set the predictable range from -20mAU to 300mAU. g Under Window Properties, enter 5 min in the X-Axis range box. h Click the OK button. | | | | | |
| | Edit Signal Plot | | | | | |
| | Available Signals Selected Signals | | | | | |
| | Quaternary Pump: Pressure ▲ Quaternary Pump: %A Quaternary Pump: %A Quaternary Pump: %C Quaternary Pump: %C Quaternary Pump: %C | | | | | |
| | - VWD: Absorbance | | | | | |
| | Predictable Range C Eloating Range | | | | | |
| | Erom: -20 + mAU Y-axis range: F mAU | | | | | |
| | I o: 300 mAU Offset: | | | | | |
| | 🗖 Auto gradjust | | | | | |
| | Window Properties X-axis range: 15 T Draw Grid | | | | | |
| | OK Cancel Apply | | | | | |

Basic Exercise #2a Run a single sample to produce an example chromatogram

| Steps | Detailed Instructions |
|---|---|
| 2 Run the sample. | a On the selection tree, expand your instrument folder. b Select Single Samples. c Select the sample, exchrom<i>iii</i>. The Run button <i>becomes available on the Tools toolbar.</i> |
| | Image: Second |
| 3 Monitor the signal, and track the status of the sample. | a On the selection tree, select your instrument. b Click the Online Plot tab to view the signal. Change the axes if necessary. |
| | |
| | C Click the Worklist tab to track the status of the sample. |
| | File Edit View Go Tools actions Help Image: Section Sections Help |

After you click the **Worklist** tab, the **Abort**, **Pause** and **Resume** buttons become available.

Task 3. Review the chromatogram

Steps

Detailed Instructions

- 1 Review the sample result and make sure all four peaks are integrated.
- a Select **Result** from the **Current View** list.
- b Select MySamplesRunLast24h from the Query list.
- c Expand the **Samples** folder.
- d Expand the exchrom*iii* folder.
- e Select the exchromiii #1 injection.
- f View the chromatogram and Summary results.



g Click the Integration tab to see the integration results.

| | | | | | | Initial Events Timed Eve | nts | |
|------|--|-----------|-------------|------------|---|------------------------------|--------------------|--|
| RT | Peak Type and Separation Code | Peak Area | Peak Height | Peak Width | т | VWD Events | | |
| | | | | | | Inital Event Name | Inital Event Value | |
| 0.56 | BB | 0.5678 | 0.1215 | 0.0647 | | | | |
| 0.76 | BV | 0.7701 | 0.3293 | 0.0375 | | Area Reject | 0.0000 | |
| 0.94 | W | 419.6985 | 153.4289 | 0.0421 | | Slope Sensitivity | 1.00 | |
| 1.11 | VB | 374.5102 | 126.7572 | 0.0447 | | Peak Width | 0.0400 | |
| 1.44 | BB | 2.6038 | 0.7431 | 0.0525 | | Shoulder Detection Mode | Disabled | |
| 1.75 | BV | 0.2067 | 0.0663 | 0.0495 | | Height Reject | 0.0000 | |
| 1.89 | VB | 357.0248 | 98.3153 | 0.0555 | | | | |
| 3.09 | BB | 523.8801 | 90.8962 | 0.0891 | | For All Signals | | |
| | | | | | | | | |
| | | | | | | Tail Peak Skim Height Ratio | 0.00 | |
| | | | | | | Front Peak Skim Height Ratio | 0.00 | |
| | | | | | | Skim Valley Ratio | 20.00 | |
| | | | | | | Baseline Correction | Classical | |
| | | | | | | Tangent Skim Mode | Standard | |
| | | | | | | Peak to Valley Ratio | 500.00 | |

Basic Exercise #2a Run a single sample to produce an example chromatogram



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Basic Exercise #2b Run a group of single samples to identify compounds

This exercise contains a series of tasks to learn how to:

- Enter a sample
- Run and track groups of single samples
- Review the results to check compound identification

You can use one of the following methods for this exercise:

- A copy of the default method provided with the Cerity Networked Data System (NDS).
- The method completed in "Basic Exercise #2 Set up a method for single samples to identify compounds" on page 81.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Running Routine Samples" on page 9.

Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.

Make sure that the methods for this exercise have been set up or restored.



Task 1. Enter three single samples

| Ste | ps |
|-----|------------|
| | - - |

Detailed Instructions

a Select Instrument from the Current View list.

- 1 Start the Instrument View and find the sample table for single samples.
- 2 Enter a sample with the following information:
 - Name the sample exer2b*iii*1, where *iii* are your initials.
 - Select the method for the sample: defexer2 or exer2*iii*
 - Select the Vial # that contains the the full-strength isocratic standard.

| b Expand your instrument folder c Select Single Samples. The sample table and Sample I | Entry tab sheet appear in the workspace. |
|---|---|
| a Enter exer2biii1 in the Sample b b Select the exer2 method from t The instrument associated with c Select Sample from the Sample d Enter the Vial Number that corre e Click Apply to put the sample in | Name box. he Method list (or copy of defexer2b). h the method appears in Instrument box. e Type list. htains the standard. hformation into the sample table. |
| Sample Entry Sample Logbook Sample Name: exer2bdec1 Method: exer2dec Sample Type: Sample Instrument: EMELC3 Vial Number Injections Volume [µ] 1 1 | Run Amounts Identification Description Report Destination Run with Priority: Schedule: Medium Unknown Task(s) to perform Acquire Quantify Integrate Report |

| 3 | Enter the tasks that you want the | a Mark the Quantify check box, and clear the Report check box. |
|---|-----------------------------------|---|
| | system to do during the run | You must mark the Quantify check box to identify the compounds, even though Calibration and Quantitation are not set up in the method. |
| | | b Click Apply. |
| 4 | Save the sample | a On the Standard toolbar, click 🔚 |
| | | The Save Changes To The Database dialog box appears. |
| | | b Review the List of changes. |
| | | c Under Reason for changes , enter a reason or select a reason from the list. |
| | | d Click the Save button. |

Basic Exercise #2b Run a group of single samples to identify compounds

| S | teps | Detailed Instructions | | | | | |
|----------|--|---|--|--|---|------------------------|--|
| <u>5</u> | teps Repeat Steps 2 through 4 for the next two samples. Name these samples, exer2biii2 and exer2biii3. | Detailed Instructions a Select the empty row. b Start with Step 2a and finish with Step 4d for exer2biii2. c Repeat steps a and b for exer2biii3. INSTRUMENT NAME METHOD NAME SAMPLE NAME NUM OF INJECTIONS 2 EMELC3 exer2dec exer2dec exer2dec a Select the empty row. | | | | | |
| | | Sample Entry Sample Logbook Sample Name: [exer2bdec3 Method: [exer2dec Sample Type: Sample Instrument: [EMELC3 Vial Number Injections Vol 1 1 1 a | Rur Rur Tr Murme (µ1) s method | Amounts Ident un with Priority: S Medium Acquire Acquire Integrate | ification Description chedule: Ready for Analysis I Quantify Report | n Report Destination | |

| Task 2. | Run | the | samp | les |
|---------|-----|-----|------|-----|
|---------|-----|-----|------|-----|

| Steps | Detailed Instructions |
|---------------------------------------|--|
| 1 Check that the instrument is ready. | a Select Instrument from the Current View list. b Click the Online Plot tab. c Click the Change button. |
| | The Edit Signal Plot dialog box appears. |
| | d Select the detector signal you need from the Available Signals list. e Click the Add button to put the signal in the Selected Signals list. f Select the Predictable Range option and set the range from -20mAU to 300mAU. g Under Window Properties, enter 15 min in the X-Axis range box. h Click the OK button. |
| | |
| | Edit Signal Plot |
| | Quaternary Pump: Pressure Add Quaternary Pump: %A Add Quaternary Pump: %B Quaternary Pump: %C Quaternary Pump: %D |
| | WWD: Absorbance |
| | <u>P</u> redictable Range <u>C</u> Eloating Range |
| | Erom: -20 🔭 mAU Y-axis range: 🔽 mAU |
| | I a 300 🛓 mAU Offset: |
| | Auto gradjust |
| | Window Properties <u>X</u> -axis range: 15 |
| | |

| Steps | Detailed Instructions | | |
|--|---|--|--|
| 2 Run the samples. | a Expand your instrument folder. b Select Single Samples. c Select the sample, exer2biii1. d Click the Run button . e Select the sample, exer2biii2. f Click the Run button. g Select the sample, exer2biii3. h Click the Run button. The samples run in the order started, unless exer2biii3 is of a higher priority than exer2biii2. Then, exer2biii3 runs before exer2biii2. The first sample started will always run first even if the sample is a lower priority than the other samples | | |
| 3 Monitor the signal, and track the status of the samples. | a Click the Online Plot tab to view the signal. Change the axes if necessary. b Click the Worklist tab, and track the status of the three samples Instrument Panel Worklist Instrument Panel Worklist Instrument Panel Worklist Instrument Panel Worklist Instrument Panel Worklist Instrument Pane | | |

Task 3. Review the chromatogram

| Steps | | Detailed Instructions |
|-------|--|---|
| 1 | Review the sample results and make sure all the compounds are identified in each sample. | a Select Result from the Current View list. b Expand the Calibration - exer2iii folder or defexer2 folder. Even though calibration was not set up in the method, the result appears in a Calibration folder. c Expand the Samples folder. d Expand the exer2biii1 folder. e Select the exer2biii1 #1 injection. f View the result. g Repeat steps d through f for the following samples: e exer2biii2 e exer2biii3 |
| | | Active JUS for Phonesenders QA/QC - 5CHEDERED, 100H4 - Administrator - Centry for Phones QA-QC Active Just determined by Active Just determine |



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration

This exercise contains a series of tasks to help you learn to:

- Create a sequence with a method set up for single-level, single-update calibration, ESTD quantification and fixed compound amounts
- Select report types and set up a directory for reports
- Run and track the sequence
- Review the results to make sure the compounds have been identified and quantified correctly
- Review the reports

You can choose between two methods to use with this exercise:

- a copy of the default method provided with the system.
- method that you created in "Basic Exercise #3 Set up a single-level calibrated method for a sequence" on page 91.

For the Basic exercises, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before You Start

Read "Running Routine Samples" on page 9.

Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.

Place all the vials of prepared samples into the ALS tray. Make sure that the methods for the exercise have been set up or restored.



Task 1. Create a new sequence

| Steps | Detailed Instructions | | | |
|--|---|--|--|--|
| Create a new sequence. Name the sequence exer3seqiii, where iii are your initials. Use one of the two methods: • defexer3 • exer3iii (created with Exercise 3 of Setting Up Methods) | a Click the New button, in the Standard toolbar, and select Sequence. The Create New Sequence dialog box appears. b Enter the Sequence Name as exer3seqiii. c Select the Instrument that will run the sequence. d Select the Method for the sequence. e Click OK. | | | |
| | Sequence Name: exer3seqeme | | | |
| | Instrument: GetStartLC Browse | | | |
| | Method: exer3singlevel Browse | | | |
| | OK Cancel f If the Save Changes to the Database dialog box appears, select the Reason for changes, if present, and click Save. | | | |

Task 2. Enter sample and sequence information

Steps

Detailed Instructions

1 Review the Sequence Table

a Select Instrument from the Current View list.

Note how the sequence table created. matches the sequence template setup in the method.

- b Expand the instrument you are using, and select the sequence you just
- c Review the table.

| | Sample Name | Sample Type | Cal. Level | Summary Group | Vial # | Injections # | Injection Volume [µl] | Sample Amount [mg/ml] |
|----|-------------|-------------|---------------|------------------|-----------|-----------------|-----------------------------|-----------------------------|
| 1 | Cal1 | Calibration | 1 | | 2 | 1 | as method | 0 |
| 2 | sample 1_2 | Sample | | | 5 | 1 | as method | 0 |
| 3 | sample 1_4 | Sample | | | 9 | 1 | as method | 0 |
| 4 | Cal1 | Calibration | 1 | | 2 | 1 | as method | 0 |
| 5 | sample 1_2 | Sample | | | 5 | 1 | as method | 0 |
| 6 | sample 1_4 | Sample | | | 9 | 1 | as method | 0 |
| 7 | Cal1 | Calibration | 1 | | 2 | 1 | as method | 0 |
| 8 | sample 1_2 | Sample | | | 5 | 1 | as method | 0 |
| 9 | sample 1_4 | Sample | | | 9 | 1 | as method | 0 |
| 10 | | | | | | | | |

during the run:

a Click the Sequence Options tab.

b Make sure that the Quantify and Report check boxes are marked for the Task(s) to perform.

| Sequence Identification | n Description Report Destination | ו | |
|-------------------------|----------------------------------|-----------------------|------------|
| -Run with | | Task(s) to perform | |
| Priority: | Schedule: | 🔽 Acquire | 🔽 Quantify |
| Medium | Ready for Analysis | 🔽 Integrate | 🔽 Report |
| Calibration Mode: | | | |
| Single Update Calibr | ation | 🔲 Allow Online Editin | ng |
| Sequence Created by | | | |

2 Enter the tasks to be performed

Quantify, Report.

Acquire and Integrate are always marked.

Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration

| Steps | | Detailed Instructions | | | | |
|-------|--|---|---|---|--|--|
| 3 | Enter the destination path for, but do not print, the reports: Enter Exercise3 <i>iii</i> , where " <i>iii</i> " are your initials. | a Click the Report Destination tab. b Clear the Printer check box, if necessary. c Mark the Path check box, and enter the directory, Exercise3<i>iii</i>. The system automatically creates this directory if it does not exist and places the generated reports into the directory Agilent\Cerity\Reports\Pharmaqc\Reports | | | | |
| | | Sequi | rence Identification Description Report Destination ort(s) to print Printer: Path: Exercise3def | Select | | |
| 4 | Select the following reports to be | a M | lark the Print check box to the left of | f the Report Types noted on the left | | |
| 4 | Select the following reports to be generated: | a M m | lark the Print check box to the left of largin. | f the Report Types noted on the left | | |
| 4 | Select the following reports to be generated: Single Injection | a M m b Cl | lark the Print check box to the left of aargin. lear all the Print check boxes that are | f the Report Types noted on the left e not those noted on the left margin. | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection | a M m b Cl | lark the Print check box to the left of largin. lear all the Print check boxes that are | f the Report Types noted on the left e not those noted on the left margin. | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | lark the Print check box to the left of nargin. lear all the Print check boxes that are Print Report Types | f the Report Types noted on the left e not those noted on the left margin. | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | lark the Print check box to the left of largin. lear all the Print check boxes that are Print Report Types Sample single injection | f the Report Types noted on the left e not those noted on the left margin. Report Template | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | lark the Print check box to the left of largin. lear all the Print check boxes that are <u>Print</u> Report Types <u>I</u> Sample single injection <u>Standard single injection</u> | f the Report Types noted on the left e not those noted on the left margin. Report Template | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | Print the Print check box to the left of largin. lear all the Print check boxes that are Print Report Types Image: Standard single injection Image: Standard single injection Image: Multi-Injection Summary Group | f the Report Types noted on the left e not those noted on the left margin. Report Template | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | Print the Print check box to the left of largin. lear all the Print check boxes that are Print Report Types Image: Sample single injection Image: Standard single injection | f the Report Types noted on the left e not those noted on the left margin. Report Template | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | Print the Print check box to the left of largin. lear all the Print check boxes that are Print Report Types Image: Standard single injection Image: Standard single Group Image: Standard Standards Group | the Report Types noted on the left e not those noted on the left margin. | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | Print the Print check box to the left of largin. lear all the Print check boxes that are Print Report Types Image: Sample single injection Image: Standard single Group Image: Sample Group Image: Sample Group | f the Report Types noted on the left e not those noted on the left margin. | | |
| 4 | Select the following reports to be generated: Single Injection Standard Injection Sequence | a M m b Cl | Print the Print check box to the left of leargin. lear all the Print check boxes that are Print Report Types Image: Sample single injection Image: Sample Group Image: Sample Group Image: Sample Group Image: Sample Group Image: Sample Group | f the Report Types noted on the left e not those noted on the left margin. | | |

Task 3. Run and track the sequence

Steps

Detailed Instructions

1 Make sure that the instrument is ready.

а Select the instrument for the sequence from the selection tree. b Make sure the instrument and column are equilibrated, and the conditions

Use the same conditions as set in the method.

Online Plot settings:

- Y-Axis range: -20 to 300
- X-Axis range: 15 minutes

are the same as those set in the method for the sequence.

Instrument Panel Worklist Colum Injector EMELC3 --- % A B --- % A 23.06 °C 1 μΙ --- ml/min --- nm **[**]# B 22.33 °C # On

Click **Change** at the bottom of the Online Plot. С

The Edit Signal Plot dialog box appears.

- **d** Select the detector signal you need from the Available Signals list, and click Add to place this signal on the right.
- e Set the Predictable Range as -20 to 300.
- Set the X-Axis range as 15 min. f
- Click OK.

| Selected Signals |
|------------------|
| : Absorbance |
| |
| ange |
| mAU |
| F % |
| |
| |
| |
| Cancel Apply |
| |

Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration



Note that the Abort, Pause and Resume buttons appear when you enter the Worklist.

Task 4. Review the results and reports

| Steps Detailed Instructions | | | | |
|--|--|---|--|--|
| Review the calibration table and curve for each revision of the calibration. | a Select Result from the Current View. b Select AllSeqNotApprovedRunLast7Days from the Query list. c Expand the exer3seq<i>iii</i> folder. d Select the Calibration - exer3seq<i>iii</i> Calib Rev 2 folder. | | | |
| | The calibration table and | Curve appear in the workspace. | | |
| | Articol Time 31 Coloradore Coloradore | Compound Name Weighed Assound Comment Signal Sheet Description RF (Rap/Ant) dmsthylphnulae 10.0000 V/V01A 43.302 bphreyd 15.0000 V/v01A 24.6555 | | |
| | | Sample Name Calibration Weighed Amount RF (Ptp/As o Weighed Amount Weighed Amount | | |
| | e Select the Calibration - e | SCHEDERERROOM (MAAMMANDO EMELCO (037/8/2002 (11/2259) sconeDireter/DayNeterco (Prev 7/Cit) xer3seq <i>iii</i> Calib Rev 3 folder. | | |
| | Review the calibration table and curve for each revision of the calibration. | eps Detailed Instructions Review the calibration table and curve for each revision of the calibration. a Select Result from the Curb Select AllSeqNotApprove calibration. a Select Result from the Curb Select AllSeqNotApprove c Expand the exer3seqiii for d Select the Calibration - e The calibration table and The calibration table and Image: the calibration of the | | |

Steps

2 Review the results for each calibration standard in each revision.

Note the different response factors used to quantify the samples.

Detailed Instructions

- a Expand the Calibration exer3seqiii Calib Rev 2 folder.
- b Expand the Calibrations folder.
- c Expand the Cal1 folder.
- d Select Cal1 #1.
- e Observe the response factor in the workspace.



- f Expand the Calibration exer3seqiii Calib Rev 3 folder.
- g Repeat steps b-c.
- h Select the second Cal1 standard.
- i Observe the response factor.
- j Expand the Calibration exer3seqiii Calib Rev 4 folder.
- k Repeat steps b-c.
- Select the third Cal1 standard.
- m Observe the response factor.
| occpa |
|-------|
|-------|

Detailed Instructions

3 Review the sample results for each revision.

Note the response factor used for the quantitation.

- a Expand the Calibration exer3seqiii Calib Rev 2 folder.
- **b** Expand the **Samples** folder.
- c Expand the Sample1 2 folder.
- d Select Sample1 2 #1.
- e Observe the response factor in the workspace.
- f Repeat steps c-e for Sample1 4.

| | E Signal 👯 | • Format 🔜 • 🚾 • 🕞 • 🔉 🔉 🕰 🛝 | | | | | | |
|---|-----------------|--|------------|----------------------------|-------------------|-------------|------------|----------------|
| | E 🗹 Signal Sele | Signal Selection Signals of Acquired Signal(s) | | | | | | |
| | 🗄 🗹 Acquire | Acquired Signal(s) VWD: Absorbance, Acquired Signal(s) | | | | | | |
| | | | | 1, 104 - diretterybrihadet | - 1.870 · bişheny | | | |
| | | | | | 2 | 3 | 4 | mi |
| | Summary Inte | egration | | Resu | llts | | | |
| | BT | Compound Name | Amount | RF (Rsp/Amt) | Peak Area | Peak Height | Peak Width | |
| | 0.93 | dimethylphthalate | 2.4563 | 43.5878 | 107.0642 | 38.4529 | 0.0438 | |
| | 1.10 | diethylphthalate | 1.7347 | 43.5878 | 94.5155 | 31.6319 | 0.0464 | |
| | 1.88 | biphenyl | 3.4917 | 24.6553 | 86.0889 | 23.8970 | 0.0556 | |
| | 3.07 | | 4.6752 | 27.3947 | 128.0765 | 22.6674 | 0.0890 | |
| | g Expand | I the Calibra | ation - ex | er3seq <i>iii</i> C | alib Rev 3 | folder. | | |
| | h Repeat | steps b-t. | | | | | | |
| | i Expand | l the Calibra | ation - ex | er3seq <i>iii</i> C | alib Rev 4 | folder. | | |
| | j Repeat | steps b-f. | | | | | | |
| Review the reports. | a Select | Start > Pro | grams > | Agilent Ce | rity > Rep | oort Viewe | r. | |
| · | h Select | File > Onen | - | - | • | | | |
| HINT : Use the Report Viewer to open | | | | | | | S. F | a ::: |
| the reports. | C Upen C | erity > Agi | ient > Ke | eports > Ph | armauC | > Keports | > Exercise | 3 <i>111</i> . |

d Open and view each report.

Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Basic Exercise #3b Reintegrate and reprocess the results

This exercise contains a series of tasks to help you learn to:

- Manually reintegrate the calibration standard results
- Change sample variable values
- Reprocess the sequence with the original method revision

You use the data produced in Exercise #3a.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Running Routine Samples" on page 9.



Task 1. Make changes to the results and sample information

Steps

Detailed Instructions

- Find the single injection result for the third quantitation of sample1_4 in the sequence exer3segiii.
- a Select **Result** from the Current View.
- **b** From the Query List, select **MySeqNotApprovedRunLast7days**.
- c Expand the exer3seqiii folder.
- d Expand the Calibration exer3iii Calib Rev 4 folder.
- e Expand the Samples folder.
- f Expand the sample 1 4 folder.
- g Select sample 1 4#1.
- h Click the Integration tab.



Basic Exercise #3b Reintegrate and reprocess the results

Steps

Detailed Instructions

2 Manually reintegrate the dimethylphthalate peak.

> Draw the baseline from the bottom left corner of the peak to the inflection point on the bottom right of the peak.

Note that the Amount and RF values disappear.

- a On the Integration toolbar, click A. A mouse pointer in the shape of a bell curve appears on the chromatogram.
- **b** Place the pointer at the bottom left of the peak at the intersection between the baseline and peak, and click once.
- c Hold the mouse button down, and move the pointer to the inflection point at the bottom right of the peak.
- d Release the mouse button.

The new baseline appears, but the bell curve pointer remains.

e On the Integration toolbar, click 💦 to change the pointer from a bell curve pointer to a normal pointer.



Detailed Instructions

- **3** Change sample variable values.
 - Dilution = 5
 - Purity = .9

a Select the sequence, exer3seqiii.

The sequence table and Sample Entry panel appear in the workspace.

- **b** Select the first sample 1 4 in the sequence.
- c Click the Amounts tab, and enter a default value for the Dilution factor of 5.
- d Enter a default value for the **Purity** of .9, and click **Apply**.
- e Repeat steps c and d for every sample 1_4 in the sequence.

| | Sample Nam | e Sample Type | Cal. Level | Custom Sample Group | Vial # | Injed |
|---------------------------------------|--|---------------|---------------|---|------------|----------|
| 1 | cal1 | Calibration | 1 | | 2 | 1 |
| 2 | sample 1_2 | Sample | | | 5 | 1 |
| 3 | sample 1_4 | Sample | | | 9 | 1 |
| 4 | cal1 | Calibration | 1 | | 2 | 1 |
| 5 | sample 1_2 | Sample | | | 5 | 1 |
| 6 | sample 1_4 | Sample | | | 9 | 1 |
| 7 | cal1 | Calibration | 1 | | 2 | 1 |
| 8 | sample 1_2 | Sample | | | 5 | 1 |
| 9 | sample 1_4 | Sample | | | 0 | 1 |
| Sampl Samp | e Entry Sequence | Logbook | Bup | Amounts I Identifica | tion Dec | cription |
| Sampl Samp Samp Samp Samp | e Entry Sequence le Name: nple 1_4 le Type: mple | Logbook | Run Sam | Amounts Identifica ple variables mple Amount: 0 | tion Des | cription |

Task 2. Reprocess the sequence results

| Steps | | Detailed Instructions | | | | | | |
|---|---|--|--|-----------------------|--|--|--|--|
| 1 | Open the Reprocess window. See Chapter 3, "Sample Analysis", in the <i>Concepts Guide</i> for a chart that helps you select the correct reprocessing option. | a Select the sequence, exer3seqiii. The Save Reasons for Changes dialog box appears. b Enter any information requested, and click Save. c Select Actions > Reprocess in the top menu bar. | | | | | | |
| 2 | Select the reprocessing option that uses all other method settings of the original method, except for the integration settings and default sample variable values. | a Select Use the method revision now attached to the b Click OK. The Cerity system uses the settings of the method origin sequence, the new manual integration setting and the values to process the sequence. | ne result . Jinally used t new sample | o run the variable | | | | |
| In the Cerity system all sample, sequence, method and instrument information is attached to the result. | | Sequence defseq3 - Reprocessed | | X | | | | |
| | | Reprocess Options | Revision | 11 | | | | |
| | | Print Reports | ОК | Cancel | | | | |

| Steps | Detailed Instructions | | | | | |
|--------------------------------------|---|--|--|--|--|--|
| 3 Track reprocessing until complete. | a Select the sequence, exer3seqiii.b Click the Sequence Options tab. | | | | | |
| | Sequence Table Sequence Options | | | | | |
| | Sequence Name: | Sequence Identification Description Report Destination | | | | |
| | defseq3 - Reprocessed | Driecher | | | | |
| | Instrument: | Medium Schedule: | | | | |
| | EMELC3 | | | | | |
| | Sequence Template | Single Update Calibration | | | | |
| | Apply | - Sequence Created by | | | | |
| | When the system has completed rep | rocessing, the message "Completed | | | | |
| | Reprocessing" appears on the Seque | nce Options panel | | | | |
| | Sequence Table Sequence Options | | | | | |
| | Sequence Name: | Sequence Identification Description Report Destination | | | | |
| | defseq3 - Reprocessed | | | | | |
| | Instrument: | Priority: Schedule: | | | | |
| | EMELC3 | | | | | |
| | Sequence Template | Calibration Mode: | | | | |
| | Apply | | | | | |
| | | Sequence Created by | | | | |



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Advanced Exercise #4a Run a sequence to quantify compounds with multi-level calibration

This exercise contains a series of tasks to help you learn how to:

- Create a sequence with a method set up for multi-level, overall calibration, ESTD quantitation and variable compound amounts
- Enter new information for an individual sample or standard
- Edit a sequence during a run
- Review the results to view the multi-level, overall calibration process.
- View the early quantitation single injection reports and the sequence report

You can choose between two methods to use with this exercise:

- A copy of defexer4*iii*, the instrument method copied from the default method provided with the system.
- Exer4*iii*, the method that you created in "Advanced Exercise #5 Set up a multi-level calibrated method for a sequence" on page 121.

For tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Running Routine Samples" on page 9.

Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.



Task 1. Create a new sequence, and enter sample and sequence information

| Steps | Detailed Instructions | | | | |
|---|---|--|--|--|--|
| Create a new sequence. Name the sequence exer4seqiii, where iii are your initials. Use one of the two methods: defexer4iii exer4iii (created with Exercise 4 of Setting Up Methods) | For detailed instructions, see "Task 1. Create a new sequence" on page 30. After you create a new sequence, the revision number is set to 1. | | | | |
| 2 Enter values for sample amounts and variables. For the first sample 1_2, enter: Sample Amount - 2.5 mg Dilution Factor - 2 Purity93 | a Select Instrument from the Current View list. b Expand the instrument folder. c Select exer4seqiii. d Select the first sample 1_2 in the Sequence Table. e Click the Amounts tab. f Enter 2.5 for the Sample Amount. g Change the Dilution Factor value to 2. h Change the Purity value to .93. | | | | |
| Enter compound amounts. To quantify a compound in a sample, you must select to use the compound amount for the standard. For the second set of calibration standards for dimethyl phthalate, enter compound amounts: Cal1 - 10.17 µg Cal2 - 37.62 µg | a Click the Sequence Table tab, and select Call from the second set of standards. b Enter 10.17 for the Compound amount. c Select Cal2 from the second set of standards. d Enter 37.62 for the Compound amount. Sequence Table Sequence Options via sample 1.4 Sample Via Injections injection a sample 1.4 Sample 1.4 Sample 1.4 Sample Amount 1 Sample Amount 1< | | | | |

Advanced Exercise #4a Run a sequence to quantify compounds with multi-level calibration

| St | teps | Detailed Instructions | | | | | | | |
|----|--|--|--|--|--|--|--|--|--|
| 4 | Enter the tasks to be performed during the run: Quantify, Report, Allow Online Editing | a Select the sequence that you just created. b Click the Sequence Options tab. c Make sure that the Quantify and Report check boxes are marked for the Task(s) to perform. d Mark the Allow Online Editing check box. Sequence Table Sequence Options Sequence Table Seque | | | | | | | |
| 5 | Enter the destination path for, but do not print, the reports: Enter Exercise4 <i>iii</i> , where <i>iii</i> are your initials. | • For the detailed instructions, see step 3 on page 32. | | | | | | | |
| 6 | Save the sequence | • On the Standard toolbar, click [] , and enter reasons for changes and your password, if necessary. | | | | | | | |
| | | After you save the sequence, the revision increments by one. Here, the revision number is set to 2. | | | | | | | |

Task 2. Edit the sequence during the run

| St | eps | Detailed Instructions | | | | | |
|----|---|--|--|--|--|--|--|
| 1 | Run the sequence after the instrument is ready. | For detailed instructions, see "Task 3. Run and track the sequence" on page 33, Steps 1 and 2. | | | | | |
| | | Note that the sequence disappears from beneath the instrument folder. | | | | | |
| | | After you run the sequence, the revision number increments by one. Here, the revision number is set to 3. | | | | | |
| 2 | Edit the sequence during the run: | a On the selection tree, select the instrument. | | | | | |
| | After the last peak comes off during | b On the instrument workspace, click the Worklist tab. | | | | | |
| | the run of the first standard, select to immediately quantify the first sample 1 4 in the sequence. | c Select the sequence. d After the last peak comes off during the run of the first standard, click ?? in the toolbar. | | | | | |
| | | The sequence in the worklist now says "Preparing to edit". When the sample run is complete, the sequence is stopped and the status says "Editable". | | | | | |
| | | Name Status Type Method Priority # Vial # Injections # Description 1 exer4seqdec Preparing to edit(1/1) Sequence exer4dec 500 N/A N/A Instrument Panel Worklist Worklist Mame Status Type Method Priority # Vial # Injections # Description 1 exer4seqdec Editable Sequence exer4dec 500 N/A N/A | | | | | |
| | | e Expand the instrument folder. (Note that the sequence has reappeared.) If you do not see the sequence, click the Redo Query button or F5. f Select the sequence, and select the first sample 1_4 in the Sequence Table. g Double-click the Immediate Quantitation cell. h Double-click Yes. i Save and run the sequence. The revision number increments to 4 after you save the sequence. The revision number increments to 5 after you run the sequence. j Select the instrument and click the Worklist tab. (The sequence starts with the second standard.) | | | | | |
| | | Instrument Panel Worklist Name Status Type Method Priority # Vial # Injections # Description | | | | | |

Task 3. Review the calibration results

Steps

Detailed Instructions

 Review the calibration table and curve.

If you ran the sample more than 7 days ago, you must modify the query to retrieve older results from the database. See the online *How To* help, "Define a query."

Note that when you first view the sequence result in the Result View, the revision number equals the number of saves that you made plus the number of run executions. In this exercise, the revision number for the sequence result is 5.

See Chapter 5, "Sample Analysis", in the *Concepts Guide* for information on sequence and calibration revisioning.

a Select **Result** from the Current View.

b Select AllSeqNotApprovedRunLast7Days from the Query list.

c Expand the exer4seqiii folder.

One folder appears that contains the calibration and single injection results.

d Select any one of the **Calibration** - **exer4seq***iii* **Calib Rev 5** folder. The calibration table and curve appear in the workspace.

| Calibration Table | | | | | | | | | |
|-------------------|--------------------|--------------|-------------------------------|----------------------|-------------------------------|-------------------------------|--|--|--|
| Compound Name | Weighed Amount | Comment | Signal Short Description | RF (Rs | p/Amt) | | | | |
| dimethylphthalate | 10.0000 40.0000 | | VWD1 A | 10.5 | 703 812 | | | | |
| biphenyl | 15.0000 60.0000 | | VWD1 A | 5.8 | 263 | | | | |
| | | | | | | | | | |
| | | | Compound Summary | | | | | | |
| dimethylphthalate | | | | | 3 | | | | |
| e ak Area | | 2 | Sample Name | Valid Calibration | Weighed Amount | RF (Rsp/Amt) | | | |
| 8 - 1 | | | cal1 #1 cal1 #1 | Yes Yes | 10.0000 10.1700 | 10.7547 10.5703 | | | |
| • | | | cal1 #1 cal2 #1 cal2 #1 | Yes Yes Yes | 10.0000 40.0000 39.7500 | 10.7154 10.9703 11.0560 | | | |
| | 20 We | igned Amount | cal2 #1 | Yes | 40.0000 | 10.9812 | | | |
| ak Are | 20 We | ighed Amount | | | | | | | |

e View how the system uses the standards in overall calibration to quantify the samples compared to single-level calibration in Exercise 3a.

Advanced Exercise #4a Run a sequence to quantify compounds with multi-level calibration

Steps

Detailed Instructions

2 Review the single injection results a for both sample 1 2 injections.

Note that the Amount is different for the first sample 1_2. Why?

The Amount is the compound amount in the sample. The value for this exercise represents the compound amount in the injection times the values of the dilution factor and purity. When you entered this sample, you changed these values.

- a Expand any one of the **Calibration** folders.
- **b** Expand the **Samples** folder.
- c Expand the first **sample 1_2** folder.
- d Select the single injection.
- e Note the value in the **Amount** column.



- f Expand the second sample 1 2 folder.
- g Select the single injection.
- h Compare the Amounts from the first and second sample1_2's.



Task 4. Review the reports

| Steps | | Detailed Instructions | | | | | | | | |
|---|---|--|---|---|--|---------------------------------------|---|--------------------------------------|--|--|
| Review the two single injection reports for the first sample 1_2 and sample 1_4. Note that there is only one folder for each of the second set of samples because they were not marked for Immediate Quantitation. | | a Se b Se c Ex d Ex Sa e Do No f Ex 01 g Do No h Re | lect Start > P lect File > Op pand the Exer pand the 003 I mple single in uble-click defi- te the compor- pand the 003 I Sample singl uble-click defi- te the compor- peat steps d-g | rograms > en, or click cise4 <i>iii</i> fol Multi-Injec ijection fol ault.htm. und amoun Multi-Injec e injection ault.htm. und amoun for the 00 | Agilent Cerity > Rep the Open button. der. stion Summary Group lder. tts. stion Summary Group folder. tts. | ort Viewe folder, an 0001 folde | r. d expand er, and e oup folde | d the 01 xpand the ers. | | |
| 2 | View the sample amount for the first sample 1_2 in the Sequence Report. | a Cli b Ex | ck the Open b pand the Sequ | utton, and I ence folde | expand the Exercise4 er, and double-click def | <i>iii</i> folder. fault.htm. | | | | |
| | | - Sequ | Name | Position | Modified ini. volume | Amount | Unit | Cal. level | | |
| | | 1 | cal1 | 9 | (As Method) | 0.0000 | ma/ml | 1 | | |
| | | 2 | cal2 | 2 | (As Method) | 0.0000 | ma/ml | 2 | | |
| | | 3 | sample 1 2 | 5 | (As Method) | 2.5000 | mg/ml | 1 | | |
| | | 4 | sample 1 4 | 9 | (As Method) | 0.0000 | ma/ml | 1 | | |
| | | 5 | cal1 | 9 | (As Method) | 0.0000 | ma/ml | 1 | | |
| | | 6 | cal2 | 2 | (As Method) | 0.0000 | mg/ml | 2 | | |
| | | 7 | sample 1_2 | 5 | (As Method) | 0.0000 | mg/ml | 1 | | |
| | | 8 | sample 1_4 | 9 | (As Method) | 0.0000 | mg/ml | 1 | | |
| | | 9 | cal1 | 9 | (As Method) | 0.0000 | mg/ml | 1 | | |
| | | 10 | cal2 | 2 | (As Method) | 0.0000 | ma/ml | 2 | | |

Advanced Exercise #4a Run a sequence to quantify compounds with multi-level calibration



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Advanced Exercise #4b Change sample variables in the method and reprocess

This exercise contains a series of tasks to help you learn to:

- Change an integration setting in the method.
- Remove a calibration point.
- Change the sequence so that no sample is immediately quantified after processing
- Reprocess the sequence with the most current method revision.
- Add a new sample variable to the method.
- Reprocess the sequence after you add the new variable
- Regenerate the reports

You use the data produced in Exercise #4a.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Running Routine Samples" on page 9.



Task 1. Update the method and result

| St | teps | Detailed Instructions | | | | | | | |
|----|---|---|--------------------------------|---|--|--|--|--|--|
| 1 | Change the integration setting in the method. Set the Height Reject to 0. If you are using a copy of the defexer4 <i>iii</i> method, make sure that no one else has modified it. Check the old revisions. If it has been modified, make another copy of the default method. See "Before you start" on page 5. | a Select Method from the Current View. b Expand the exer4<i>iii</i> folder. c Expand the Data Analysis folder. d Select Integration. e Click the Height Reject cell, and enter 0. f Save the method. | | | | | | | |
| 2 | Remove the second Cal2 calibration point for dimethylphthalate. | a Select Result from the Current View. b Expand the exer4seqiii folder. c Select the Calibration - Exer4iii folder. d Click the Calibration cell for the second Cal2 calibration. e Click the button, and double-click the cell to change Yes | s to No . | | | | | | |
| | | Compound Summary | - | | | | | | |
| | | | | | | | | | |
| | | Sample Name | Valid Calibration | Weighed Amo | | | | | |
| | | 0 call #1 0 call #1 call #1 call #1 | Yes Yes Yes Yes No | 10.0000 10.1700 10.0000 40.0000 37.6200 | | | | | |
| | | cal2 #1 | Yes | 40.0000 | | | | | |

20

Weighed Amount

■| |

e Area ⊕

| St | eps | Detailed Instructions | | | | | | | | |
|----|---|--|--|--|--|--|------------------------|-----------|-----------------|--|
| 3 | Change the sequence so that no sample is immediately quantified during processing | a Se b In firs c Do d Re e Sa Note | lect the exer4 the sequence st Sample1_2. suble-click No peat steps b a ve the change that the revisi | seqiii seque table, doubl and c for the ed result. ion is increm | nce. e-click t first Sa ented b | the Immedia mple1_4. y 1. | nte Quantitatio | n cell f | or the | |
| | | Seque | nce Table Sequen | ce Options | | | | | | |
| | | | Sample Name | Sample Type | Cal. Level | Immediate Quantitation | Custom Sample Group | Vial # | Injections # | |
| | | 1 | cal1 | Calibration | 1 | NO | | 2 | 1 | |
| | | 2 | cal2 | Calibration | 2 | NO | | 3 | 1 | |
| | | 3 | sample 1_2 | Sample | | NO | | 5 | 1 | |
| | | 4 | sample 1_4 | Sample | | NO | | 9 | 1 | |
| | | 5 | cal1 | Calibration | 1 | NO | | 2 | 1 | |

Task 2. Reprocess and review the result

Steps

Detailed Instructions

- 1 Reprocess the sequence with the most current revision of the method.
 - Use the integration settings in the method.
 - Set up to print (regenerate) reports
- a Select the exer4seqiii sequence.
- **b** Select Actions > Reprocess.
- c Select Use the most current revision of the method that is attached to the result.
- d Mark the Use integration settings in the method check box.
- e Mark the **Print Reports** check box.
- f Click OK.
- g To follow reprocessing, click the Sequence Options tab.

| Reprocessed | | | |
|-------------------------|----------------------------|------------|--|
| | R | evision 13 | |
| | | | |
| now attached to the res | lt | | |
| | at is attached to the resu | ilt | |
| ion of the method th | | | |
| the method to | | | |
| ion of the method th | | | |

2 Make sure the integration change appears in the reprocessed result.

If you cannot see the calibration standard chromatogram because of the example chromatogram, click the Layout button and clear the Display Example Chromatogram check box.

- a Expand the second Calibration Exer4iii folder.
- **b** Expand the **Calibrations** folder and the **Cal1** folder.
- c Select Cal1 #1.

Note that one or more peaks are now integrated and appear in the Results Table.



Task 3. Add a new sample variable to the method and reprocess

| S | teps | Detailed Instructions | | | | |
|---|---|--|--|--|--|--|
| 1 | Add a new variable to the method. Add a divisor called "attenuation factor" with a default value of 3. | a Select Method from the Current View list. b Expand the current revision of exer4<i>iii</i>. c Select Sample Variables. d Type "attenuation factor" into a Divider cell of the System Sample Variables table. e Enter a Default Value of 3. f Save the method. | | | | |
| 2 | Reprocess the sequence with the revised method. Enter a new value for the Attenuation Factor of 7 for the first Sample 1_2. Set up to print (regenerate) reports. | a Select Result from the Current View list. b Select exer4seqiii. c Select Actions > Set up reprocessing for new sample entry fields. Set up reprocessing for new sample entry fields in the latest method revision Sequence exer4seqivs2 - Reprocessed Revision After you click DK: 1. A new revision of the result appears in the Selection Tree. 2. The most current revision of the method is attached to this result. 3. The new sample fields added to the current method revision appear in the sample entry panel. DK | | | | |
| | | d Click OK . The new Sample Entry panel appears. | | | | |
| | | e Click the Amounts tab, and enter 7 for the "Attenuation Factor". f Select Actions > Reprocess. g Select Use the method revision now attached to the result. h Mark the Print Reports check box. i Click OK. | | | | |

Steps

Detailed Instructions

- a Select Start > Programs > Agilent Cerity > Report Viewer.
- **b** Select **File > Open**.
- c Expand Exercise4*iii*-0002.
- d Expand the 003Multi-Injection Summary folder.
- e Expand the 01Sample Single Injection folder.
- f Double-click Default.htm.

The report appears with the new amount for Sample1_2.



| | Sample single injection compounds | | | | | | | | |
|------|-----------------------------------|-----------|--------|------|----------|------------|--|--|--|
| RT | Compound | Peak area | Amount | Unit | Resp. f. | Tailing f. | | | |
| 0.76 | N/A | 0.8370 | 0.4500 | N/A | 0.2471 | N/A | | | |
| 0.94 | dimethylphthalate | 124.1833 | 2.4779 | ug | 6.6582 | N/A | | | |
| 1.11 | diethylphthalate | 109.6416 | 1.7791 | N/A | 6.5501 | N/A | | | |
| 1.89 | biphenyl | 106.8904 | 3.7001 | ug | 3.8380 | N/A | | | |
| 3.11 | N/A | 153.0533 | 4.5837 | N/A | 4.4362 | N/A | | | |

3 Find the report for the first Sample1 2.

Note that the quantitation value is different after reprocessing. The software used the "Attenuation Factor" in the calculation. Advanced Exercise #4b Change sample variables in the method and reprocess



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Advanced Exercise #5a Run a sequence to quantify impurities

This exercise contains a series of tasks to help you learn to review results and reports of a sequence run with a method set up for multi-level, bracketed calibration, ISTD quantitation and variable compound amounts. You learn how to:

- Recognize the results of an overall calibration
- Find the system suitability calculations that were selected for the review layout in the method
- Find the custom calculations that were set up in the method
- Review the reports for the calculations that were set up in the report template

You can choose between two methods to use with this exercise:

- instrument method copied from the default method provided with the system, defexer5.
- method that was created in "Advanced Exercise #6 Set up a method for a sequence to quantify impurities" on page 133.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Running Routine Samples" on page 9.

Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.



Task 1. Set up and run the sequence

| St | eps | Detailed Instructions | | | |
|----|---|-----------------------|--|--|--|
| 1 | Create a new sequence. | • | For detailed instructions, see "Task 1. Create a new sequence" on page 30. | | |
| | Name the sequence exer5seqiii, where iii are your initials. | | | | |
| | Use one of the two methods: | | | | |
| | defexer5 exer5<i>iii</i> (created with Exercise 5 of Setting Up Methods) | | | | |
| 2 | Make sure that Quantify and Report are selected. | • | For detailed instructions, see "Task 2. Enter sample and sequence information" on page 31, Step 2. | | |
| 3 | Enter the destination path for, but do not print, the reports, and save the sequence. | • | For detailed instructions, see "Task 2. Enter sample and sequence information" on page 31, Step 3. | | |
| | Enter Exercise5 <i>iii</i> , where " <i>iii</i> " are your initials. | | | | |
| 4 | Run and track the sequence. | • | For detailed instructions, see "Task 3. Run and track the sequence" on page 33. | | |

Task 2. Review the results and reports

Steps

Detailed Instructions

 Compare the response factors for dimethylphthalate for the first set of bracketed samples with the second set.

Hint: If you can't see the RFs, click the bottom of the Compound Summary panel to make the scroll bar appear.

Note that the RFs for the second Cal1 and Cal2 for the first set of bracketed samples are the same as for the first Cal1 and Cal2 for the second set of bracketed samples.

- a Select **Result** from the Current View.
- b Select AllSeqNotApprovedRunLast7Days from the Query list.
- c Expand the exer3seqiii folder.
- d Select the second **Calibration exer3seq***iii* folder. The first calibration folder contains the blank run.
- e Scroll to see the RFs if not visible.
- f Select the third **Calibration exer5seq***iii* folder.
- g Scroll to see the RFs if not visible.
- h Compare the RFs.



2 Review the system suitability calculations for Cal1 #1 under the second calibration folder.

Note the values for the Average Percent Specified Impurity and the Average Percent Unspecified Impurity calculations that were set up as custom calculations in the method.

- a Expand the second Calibration exer3seqiii Calib folder.
- b Expand the Calibrations folder.
- c Expand the Cal1 folder.
- d Select Cal1 #1.
- e Review the Results Table for the system suitability calculations.

You may have to click the bottom of the Results table to see the scroll bar.

| | Results | | | | | | | |
|-----------------------------------|-------------------|------------|---------------|---------------|------------------------|--|--|--|
| RT | Compound Name | Peak Width | TailingFactor | SignalToNoise | Peak resolution USP | | | |
| 0.94 | dimethylphthalate | 0.0424 | 1.144 | 97.300 | N/A | | | |
| 1.11 | diethylphthalate | 0.0443 | 1.050 | 79.413 | 2.303 | | | |
| 1.89 | biphenyl | 0.0560 | 0.887 | 1041.299 | 9.108 | | | |
| 3.10 | | 0.0905 | 0.666 | 607.791 | 9.690 | | | |
| | | | | | | | | |
| | | Summary | y Results | | | | | |
| Percent Specified Impurity : | 13.42 | | | | | | | |
| Percent Unspecified Impurity : | 37.91 | | | | | | | |
| | | | | | | | | |

| 0.0000 |
|--------|
|--------|

Detailed Instructions

3 Review the percent impurity results for the first Sample1_2 and for the sample group. a Expand the second Calibration - exer3seqiii folder.
b Expand the Samples folder.

c Select the Sample1 2 folder.

Note that the percent impurity values N exceeded their limits.

Note that the average percent specified and unspecified impurities for both injections appear here.

| | | Results Table |
|------------------------------|------------|-----------------|
| | | |
| Compound Name | Injection# | |
| dimethylphthalate | | |
| | 1 | |
| | 2 | |
| diethylphthalate | | |
| | 1 | |
| | 2 | |
| biphenyl | | |
| | 1 | |
| | 2 | |
| Not Identified Peaks | | 1 |
| | 1 | |
| | 2 | |
| | ۷ | J |
| | | |
| | | |
| | | Summary Hesults |
| Avg Percent Specified | 13.65 | |
| | 13.03 | |
| Avg Percent Unspecified : | 37.80 | |

- d Expand the Group Results folder.
- e Select Samples.

The results for the average of the percent impurities over all samples appear here, as do the results of the limit checks for these impurities.

| | | Summary Results |
|--------------------------------------|------------|-----------------|
| Avg % S All Samples : | 13.73 | |
| Avg % S All Samples Limit Check : | Not Passed | |
| Avg % U All Samples : | 37.72 | |
| Avg % U All Samples Limit Check : | Not Passed | |
| | | |

| Stehs | Detailed Instructions | | | | | | | |
|--|--|---|--|---|-----------------------------------|--|---|--|
| 4 Review the sample single injection report for the first Sample1_2 and the report for the sample group. | a Select S b Select Fi c Expand I d Expand (e Expand (Note the the methesistic of the set of the s | tart > Programs ile > Open. Exercise5 <i>iii</i> . DO3Multi-Injectic D1Sample Single e system suitabil nod. | s > Agile onSumm Injectio ity calcu | ent Cerity > ary. n, and doul lation value | > Repor ble-click es in the | t Viewer . < default.ht e table tha | m. t was set up in | |
| | Retention | Compound | Amount | Response | Tailing | Peak | | |
| | Time | Name | Amount | Factor | Factor | USP | SignalToNoise | |
| | Time 0.93 | Name dimethylphthalate | 24.8892 | 0.1169 | 1.178 | USP N/A | SignalToNoise 237.192 | |
| | 7ime 0.93 1.10 | Name dimethylphthalate diethylphthalate | 24.8892 17.5561 | C.1169 | 1.178 | N/A | SignalToNoise 237.192 194.383 | |
| | 7ime 0.93 1.10 1.89 | Aimethylphthalate diethylphthalate biphenyl | 24.8892 17.5561 37.5000 | C.1169 0.1169 0.1169 0.0667 | 1.178 1.135 1.090 | N/A 2.308 9.129 | SignalToNoise 237.192 194.383 2554.088 | |

Note the percent impurity calculations and limits that were set up in custom calculations and the report template in the method.

| Avg % S All Samples: | 13.73 |
|----------------------|-------|
| Avg % U All Samples: | 37.72 |

| | | Sample group |) limit results | |
|---|------------------------------|-------------------|------------------|----------------|
| # | Sample name | Compound | Limit (Compound) | Limit (Sample) |
| 1 | sample 1_2 | dimethylphthalate | XXXXXXXXXXX | XXXXXXXXXXX |
| 2 | sample 1_4 dimethylphthalate | | XXXXXXXXXXX | XXXXXXXXXXXX |
| 3 | sample 1_2 | dimethylphthalate | XXXXXXXXXXX | XXXXXXXXXXXX |
| 4 | sample 1_4 | dimethylphthalate | XXXXXXXXXXX | XXXXXXXXXXXX |
| 1 | sample 1_2 diethylphthalate | | XXXXXXXXXXX | XXXXXXXXXXXX |
| 2 | sample 1_4 | diethylphthalate | XXXXXXXXXXX | XXXXXXXXXXXX |
| 3 | sample 1_2 | diethylphthalate | XXXXXXXXXXX | XXXXXXXXXXXX |
| 4 | sample 1_4 | diethylphthalate | XXXXXXXXXXX | XXXXXXXXXXXX |
| 1 | sample 1_2 | biphenyl | XXXXXXXXXXX | XXXXXXXXXXXX |
| 2 | sample 1_4 | biphenyl | XXXXXXXXXXX | XXXXXXXXXXXX |
| 3 | sample 1_2 | biphenyl | XXXXXXXXXXX | XXXXXXXXXXXX |
| 4 | sample 1_4 | biphenyl | XXXXXXXXXXX | XXXXXXXXXXX |

Avg % S All Samples Limit Check: Not Passed

Avg % U All Samples Limit Check: Not Passed

Advanced Exercise #5a Run a sequence to quantify impurities



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Advanced Exercise #5b Use a different method to reprocess

This exercise contains a series of tasks to help you learn to:

- set up a different method with a new calibrated compound
- set up reprocessing for a different method
- reprocess the sequence with the different method

You use the data produced in Exercise #5a.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before You Start

Read "Running Routine Samples" on page 9.



Task 1. Set up a different method

| Steps | Detailed Instructions | |
|--|--|--|
| Copy exer5<i>iii</i> and rename it exer5<i>iii</i>2. Or, copy defexer5. Or, use defexer5<i>iii</i>2 to reprocess. | a Select File > New > Method. b Click the Browse button in the Method Wizard. c Select exer5<i>iii</i>. d Enter a New Method Name of exer5<i>iii</i>2, and click Next. e Click Next until you reach the New Method Review panel. f Click Finish and click Save. | |
| 2 Add diethylphthalate as a calibrated compound. Cal Level 1 - 8 μg Cal Level 2 - 32 μg Set biphenyl as the ISTD for this compound. | a Expand the exer5<i>iii</i>2 folder. b Expand the Data Analysis folder. c Select Calibration. d Right-click the calibration table, and select Insert Compound. e Select diethylphthalate, click > and click OK. f In the calibration table, select diethylphthalate. g Click on the Level 1 Use Default Amount cell, and click the button. h Select the + sign, and enter 8 μg into the Weighed Amount and Unit cells. i Repeat steps g and h for Level 2 and 32 μg. j Select Quantitation. k Select diethylphthalate. I Mark the Use ISTD Compound check box, and select biphenyl. m Save the method. | |

Task 2. Reprocess the sequence result

Steps

method.

1

Detailed Instructions

Set up reprocessing for a different a Select Res

Select exer5iii2 or defexer5iii2.

See Chapter 3, "Sample Analysis", in the *Concepts Guide* for a chart that helps you select the correct reprocessing options.

- Select **Result** from the Current View.
- **b** From the Query List, select **MySeqNotApprovedRunLast7days**.
- c Select the exer5seqiii folder.
- d Select Actions > Set up reprocessing for a different method.

| 📴 Set up repro | cessing for a different method | | × |
|--|---|----------|--------|
| Sequence | exer5seqjws - Reprocessed | | 4 |
| | | Revision | 8 |
| Select Method | | | |
| exer5jws2 | | 4 | Browse |
| When you click (1. A copy of the r 2. The method th 3. The sample er | DK: esult appears in the Selection Tree after you click Redo Query, at you selected is attached to the copy of the result. itry fields appear in the sample entry panel. | | |
| | | ок | Cancel |

- e Click Browse, select exer5iii2 and click OK.
- f Click **OK**, and click **Save**.

A copy of the sequence appears in the selection tree, ready for reprocessing. This copy is now attached to the new method but has no folders underneath until it is reprocessed.

AllSeqNotApprovedRunLast7Days
AllSeqNotApprovedRunLast7Days
AllSeqNotApprovedRunLast7Days
Copy Df exer5seqiws - Reprocessed (4/12/02 6:51:27 AM) [Rev 1]
Copy Df exer5seqiws - Reprocessed (4/12/02 2:45:06 AM) [Rev 4]
Copy Df exer5seqiws - Reprocessed (4/12/02 1:35:31 AM) [Rev 5]
Copy Df exer5seqiws - Reprocessed (4/12/02 1:35:31 AM) [Rev 3]
Copy Df exer5seqiws - Reprocessed (4/12/02 1:50:23 AM) [Rev 3]
Copy Df exer5seqiws - Reprocessed (4/12/02 1:50:23 AM) [Rev 3]
Copy Df exer5seqiws - Reprocessed (4/12/02 1:50:23 AM) [Rev 3]
Copy Df exer5seqiws - Reprocessed (4/12/02 1:50:23 AM) [Rev 3]
Copy Df exer5seqiws - Reprocessed [Rev 18]
Copy Df exer5seqiws - Reprocessed

2 Enter amounts for the new calibrated compound, diethylphthalate, for every calibration standard.

Level 1-8

Level 2 - 32

a Select this copy (note the date and time after it).

- **b** Click the **Amount** tab on the Sample Entry panel in the sequence workspace.
- c For each Level 1 standard, mark the **Use** checkbox for diethylphthalate, and enter 8.
- d For each Level 2 standard, mark the **Use** checkbox for diethylphthalate, and enter 32.
- e Save the result.

| Steps | Detailed Instructions | | | |
|-----------------------|--|--|--|--|
| 3 Reprocess the copy. | a Select Actions > Reprocess b Make sure that Use the method revision now attached to the result is marked. c Click OK. d Monitor the reprocessing from the Sequence Options panel. e Click the Redo Query button. f Expand the copy. g Select a calibration folder. h Make sure that diethylphthalate is now included as a calibrated compound. | | | |
| | L Appleof Cerby NOS for Pharmascontical QA/QC - SCIIEDECRETINDUM - Administrator - Cerby for Pharma QA-QC En Edit year Tools Software Beb Read + D + I = I D D | | | |
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| | Bit | | | |
| | SCHEIDERER ROBIN (merfanz) [MELC3 (M4722002 (024025) MEM/Metersmethink and David Core ID exercises at 1 NAM | | | |



Setting Up Methods

These exercises help you learn how to set up methods for your laboratory. See Chapter 4, "Method Setup", in the *Concepts Guide* for background information that can help you use these exercises. The set of basic and advanced exercises includes the following topics:

Basic Exercise 1 – Set up an equilibration method Learn how to set up a method template and enter instrument parameters to equilibrate the instrument.

Exercise 2 – Set up a method for single samples to identify compounds Learn how to use an example chromatogram to set up integration and compound identification for single samples.

Exercise 3 – Set up a single-level calibrated method for a sequence Learn how to set up single-level, single-update calibration, ESTD quantitation, and fixed compound amounts.

Advanced Exercise 4 – Set up a multi-level calibrated method for a sequence Learn how to set up multi-level, overall calibration, ESTD quantitation, variable compound amounts, and sample variables.

> **Exercise 5 – Set up a method for a sequence to quantify impurities** Learn how to setup ISTD quantitation, custom calculations, limits, bracketed calibration, and system suitability.

After you set up the methods in Exercises 1-5, you can use them to run the samples and sequences in Exercises 1-5 of the section—"Running Routine Samples".



Before you start Read "Before you start" on page 5! Your system administrator must have configured an Agilent 1100 LC series liquid chromatograph for your system. If you plan to copy a default method to create a new method as in Exercises 3 and 5, make sure that the default methods are in your database. From the Query list, select AllMethodsRestored to view defexer1-5. If they do not appear, see the instructions in "Before you start" to transfer these methods from the CD-ROM

to your database.


Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Basic Exercise #1 Set up an equilibration method

This exercise provides a series of tasks to learn how to:

- Create a method template to set up instrument parameters
- Set up instrument parameters
- Save and audit method changes
- View the history of method changes

A *method template* is a framework to let you enter only those conditions and parameters that you need to acquire and process data. A *method* is a method template that contains entered parameter values.

Use this method to equilibrate the instrument as outlined in the chapter "Basic Exercise #1a Equilibrate the instrument" on page 11.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Setting Up Methods" on page 71 for setting up methods.



Task 1. Create a method template to enter instrument parameters

| Steps | Detailed Instructions |
|---|---|
| Create a new method template for a single sample. Name the method template, equilmethiii, where iii are your initials. | a Select File > New > Method or click and select Method. The Method Wizard appears. b On the New Method panel, enter the Method Name, equilmethiii. c Select Single Sample. |
| | Method Wizard |
| | New Method New Method name : |
| | Image: |
| | < <u>B</u> ack. <u>Next</u> > ⊟nith <u>C</u> ancel |
| | d Click Next to scroll to the Instrument panel. |

| Steps | Detailed Instructions | | |
|---|--|--|--|
| 2 Select the instrument to equilibrate. | a On the Instrument panel, select the instrument you need to equilibrate. The instruments that appear in the Available Instruments list depends or your configuration of the Cerity Networked Data System. | | |
| | Method Wizard | | |
| | Instrument Select the Instrument for your Method. | | |
| | Adiable Instruments: Adiable Instruments: | | |

- **b** Click **Next** to scroll to the Data Analysis panel.
- 3 Clear all data analysis selections. a On the Data Analysis panel, clear the Compound Identification check box.



| Steps | Detailed Instructions | | |
|--|--|--|--|
| 4 Review and save the method template. | a On the New Method Review panel, review the setting in the Method Wizard Settings section. b Add the words "Test Comment" in the Comment section. c Click Finish. | | |
| | Method Wizard | | |
| | New Method Review Comment to the Method setup: | | |
| | Settings made on the "Instrument" Panel This instrument was selected: "EMELC3" Settings made on the "Data Analysis" Panel: "Compound Identification" was not checked "Calibration and Quantification" was not checked "Calibration and Quantification" was not checked "Include System Suitability Calculations" was not checked "Includ | | |
| | G Click Save if the Save changes to the Database dialog box appears. | | |
| 5 View the Method Wizard settings in the method. | After you save the method template, the Method View appears. a Select the method you just created - equilmethiii. b View the Method Description in the workspace. Notice that the Method Description corresponds to the Comment section of the New Method Review panel in the Method Wizard. | | |
| | Anilent Cerity NDS for Pharmaceutical 0A/0C - SCHEIDEBER R0BIN - Administrator - Cerity for Pharma 0A-0C | | |
| | Ele Edt View Iools Actions Help Method Mainterimethods AllMasterMethods AllMasterMethods Copy of exerChang Exercise EquilinetImag Exercise | | |

Task 2. Enter the instrument conditions for the equilibration

Steps

•

•

•

1 Set the pump parameters:

Methanol as Solvent B:

Flow rate: 2ml/min.

Solvent composition:

Stoptime: 10 min.

80%MeOH/20%H₂0

Acetonitrile as Solvent B:

Flow rate: 1.5ml/min

 Solvent composition: 65%ACN/35%H₂O Stoptime: 10 min.

Detailed Instructions

- a On the selection tree, expand the equilmethiii method folder.
- **b** Expand the **Instrument Setup** folder and select **Quaternary Pump** or **Binary** Pump.
- c Enter the Flow as 2 ml/min.
- d Under **Solvents**, mark the **B** check box and enter 80 in the % box. The percent of solvent A is automatically set to 20 %.
- e Under **Stoptime**, select the **min** option and enter 10.
- Under Posttime and Pressure Limits, accept the default values. f

| Setup Timetable Auxiliary & Data Curves | |
|---|-----------------------------|
| Flow Flow: 2 Im ml/min | Stoptime: |
| Solvents A: 20 % | C 10 min |
| B: 🔽 80 🛋 % | • Off |
| C: 🗖 Off | Pressure Limits |
| D: D Off | Min: 0 📩 bar Max: 400 🛫 bar |

2 Set the autosampler (ALS) injection volume to zero

- a Select the ALS folder.
- **b** Click the **Setup** tab.
- c Under Injection, select Standard Injection,
- d Set the Injection Volume to zero.

| Standard Injection | Injection Volume: 🔍 🛒 µl |
|--|--------------------------|
| C Injection with Needle Wash | Wash Vial: 1 |
| C Use Injector Program | |

| St | eps | Detailed Instructions | |
|--|----------|---|--|
| 3 Set the same stoptime for all | | a Select the ALS folder, | |
| | modules. | b Click the Auxiliary & Time tab. | |
| Stoptime: 10 min. c Under Stoptime d Select the DAD configuration. | | c Under Stoptime, select the as Pump option. | |
| | | d Select the DAD, MWD, or VWD folder that appears in your detector configuration. | |
| | | e Under Stoptime, select the as Pump/Injector option. | |
| | | f Select the TCC folder. | |
| | | g Under Stoptime , select the as Pump/Injector option. | |
| | | h Accept default values for all other module parameters | |

Task 3. Save and audit method changes

Steps

Detailed Instructions

Save Changes To The Database

1 Save the method.

The Cerity administrator must set up auditing for the Save Changes To The Database dialog box to appear. The Cerity administrator can provide a list of reasons and require you to enter your electronic signature to complete the dialog box. These requirements can only appear when a Cerity GMP license is installed and Auditing is set up by the Cerity administrator.

a On the Standard toolbar, click 🔲

The Save Changes To The Database dialog box appears.

List of changes

۸ Change the 'Stoptime' from 'no Limit' to 'as Pump/Injector' for the TCC Setpoint. Change the 'Stoptime' from 'no Limit' to 'as Pump/Injector' for the VWD Setpoint. Change the 'Flow' from '0' to '2' for the Quaternary Pump Setpoint. Change the 'Stoptime' from 'no Limit' to '5' for the Quaternary Pump Setpoint. Change the 'Solvent D Ratio' from '0' to 'off' for the Quaternary Pump Setpoint. Change the 'Solvent C Ratio' from '0' to 'off' for the Quaternary Pump Setpoint. Change the 'Solvent B Ratio' from '0' to '80' for the Quaternary Pump Setpoint. Change the 'Solvent A Ratio' from '100' to '20' for the Quaternary Pump Setpoint. Change the 'Injection Volume' from '5' to '0' for the ALS Setpoint Change the 'Stoptime' from '10' to 'as Pump' for the ALS Setpoint. 1 Reason for changes ٩ <u>S</u>ave Discard Cancel

- b Review the List of changes.
- Under Reason for changes, enter a reason or select a reason from the list. С
- d Click the Save button.

2 View the history of changes to the method.

If you need to use this method before you set up other methods, use the method with Running Routine Samples, Basic Exercise #1, Equilibrate the instrument.

- a On the selection tree, select the method, equilmethiii.
- b View the list of changes to the method.

| Description | ltem | Comment | E-Sig | Timestamp |
|----------------------------|--------------------------|---------------|-------|----------------------|
| Change the 'Stoptime' from | | | | |
| 'no Limit' to 'as | | | | |
| Pump/Injector' for the TCC | | Initial | | |
| Setpoint. | TCC Setpoint | configuration | None | 03/17/2002, 16:31:51 |
| Change the 'Stoptime' from | | | | |
| 'no Limit' to 'as | | | | |
| Pump/Injector' for the VWD | | Initial | | |
| Setpoint. | VWD Setpoint | configuration | None | 03/17/2002, 16:31:51 |
| Change the 'Flow' from 'D' | | | | |
| to '2' for the Quaternary | | Initial | | |
| Pump Setpoint. | Quaternary Pump Setpoint | configuration | None | 03/17/2002, 16:31:51 |
| Change the 'Stoptime' from | | | | |
| 'no Limit' to '5' for the | | Initial | | |
| Quaternary Pump Setpoint. | Quaternary Pump Setpoint | configuration | None | 03/17/2002, 16:31:51 |

Individual setpoint changes can only appear in the history of changes when a Cerity GMP license is installed and Auditing is set up by the Cerity administrator.

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Basic Exercise #1 Set up an equilibration method



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Basic Exercise #2 Set up a method for single samples to identify compounds

This exercise contains a series of tasks to learn how to:

- Create a method template for single samples to include only compound identification in the method
- Set up and save the method to produce an example chromatogram
- Use an example chromatogram to set up integration
- Set up compound identification

A *method template* is a framework to let you enter only conditions and parameters that you need to acquire and process data.

Use the method created in the first part of this exercise to enter and run a single sample to produce an example chromatogram. You can use the completed method to enter and run a group of samples to identify compounds. See "Basic Exercise #2a Run a single sample to produce an example chromatogram" on page 17 and "Basic Exercise #3b Reintegrate and reprocess the results" on page 39.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Setting Up Methods" on page 71 for setting up methods.



Task 1. Create a method template to identify compounds only

| St | teps | Detailed Instructions |
|----|--|--|
| 1 | Create a new method template for a single sample. • Name the method template, exer2 <i>iii</i> , where <i>iii</i> are your initials. | c Select File > New > Method or click and select Method. The Method Wizard appears. d Enter exer2<i>iii</i> in the Method Name box. e Select Single Sample. Method Wizard P * * * * * * * * * * * * * * * * * * |
| 2 | Select an instrument for the method. | f Click Next to scroll to the Method Wizard Instrument panel. a On the Instrument panel, select the instrument that will run the sample. a On the Instrument panel, select the instrument that will run the sample. Kethod Wizard Instrument Select the Instrument for your Method. Available Instruments: SetVDTI B: 0000E Analog to Digital Convetter SetVDTI B: 0000E Analog to Digital Convetter SetVDTI B: 0000E Analog to Digital Convetter B: 0000 |

🔽 UV Spectra

Agilent 1100 Series Diode Array Detector
 Agilent 1100 Series Diode Array Detector [Thermostatted I
 Agilent 1100 Series Standard Autosampler
 Agilent 1100 Series Thermostatted Autosampler

≟- 35900E Analog to Digital Converter

Selected Instrument: <None>
Do you want to acquire UV Spectra?

msklc3

DigVDT

Ĩ

b Click **Next** to scroll to the Data Analysis panel.

| Steps | Detailed Instructions | | | | |
|---|--|--|--|--|--|
| 3 Mark only Compound Identification. | a On the Data Analysis panel, Clear the Calibration and Quantification, Includ Noise Calculations and Include System Suitability Calculations check boxes. | | | | |
| | Method Wizard | | | | |
| | Data Analysis | Do you want to include Compound Identification? | Compound Identification | | |
| | 9 | Do you want to include UV Spectral Compound Purity? | LV/ Purity | | |
| | - | Do you want to include UV Spectral Compound Confirmation? | UV Confirmation | | |
| | | Do you want to include Calibration and Quantitation? | Calibration and Quantitation | | |
| | | Do you want to use Custom Calculations? | Custom Calculations | | |
| | | Do you want to include System Suitability Calculations? | Include System Suitability Calculations | | |
| | < <u>B</u> e | ick <u>N</u> ext > | <u>Finish</u> | | |
| | b Click Next to scroll to t | he Identification panel. | , | | |
| 4 Complete the setup of the method template. | a Click Next, and click th b Click Save if the Save (| e Finish button. Changes to the Database | e dialog box appears. | | |
| Do not mark any check box on the Method Wizard Identification panel. | | - | | | |

Task 2. Enter the instrument conditions for the equilibrium

Steps

Detailed Instructions

- 1 Enter the pump parameters:
 - Methanol as Solvent B:
 - Flow rate: 2ml/min.Solvent composition:
 - 80%MeOH/20%H₂0
 - Stoptime: 5 min.
 - Acetonitrile as Solvent B:
 - Flow rate: 1.5ml/min
 - Solvent composition: 65%ACN/35%H₂0
 - Stoptime: 6 min.

- a On the selection tree, expand the exer2*iii* method folder.
- b Expand the Instrument Setup folder and select the Quaternary Pump or Binary Pump.
- c Enter the **Flow** as 2 ml/min.
- d Under **Solvents**, mark the **B** check box and enter 80 in the % box. The percent of solvent A is automatically set to 20 %.
- e Under Stoptime, select the min option and enter 5.

| Setup Timetable Auxiliary & Data Curves | |
|---|-----------------------------|
| Flow Flow: 2 * ml/min | Stoptime: O no Limit |
| A: 20 % | © 5 in min |
| B: 🔽 80 💼 % | |
| C: 🗖 Off | Pressure Limits |
| D: D Off | Min: 0 📼 bar Max: 400 📼 bar |

2 Enter the injection volume and stop time for the autosampler.

- Injection Volume: 1µl
- Stop Time: same as pump

| а | On the | selection | tree, select | the | ALS | folder. |
|---|--------|-----------|--------------|-----|-----|---------|
|---|--------|-----------|--------------|-----|-----|---------|

- **b** Click the **Auxiliary & Time** tab.
- c Under Stoptime, select the as Pump option.
- d Click the Setup tab and select Standard Injection.
- e Enter 1µl for the Injection Volume.

| Setup Auxiliary & Time | |
|------------------------------|--------------------------|
| Injection | |
| C Standard Injection | Injection Volume: 1 👘 μί |
| C Injection with Needle Wash | Wash Viel: |
| O Use Injector Program | |
| | |

| Steps | Detailed Instructions | |
|--|--|---|
| 3 Make sure the stop time is the same for all instrument modules. Stop Time: same as pump | a On the selection tree, select the b Under Stoptime select as Pump c On the selection tree, select the d Under Stoptime, select as Pump Signal & Time Timetable Options Special Setpo Signal Wavelength: 254 = nm Peakwidth (Responsetime) >0.10 min (2.0 s) V | VWD folder. //njector. TCC folder. p/Injector. ints Stoptime: |

Task 3. Save and audit method changes

| Steps | Detailed Instructions |
|---|---|
| 1 Save the method. After you save the method here, you can use the method to produce a | a On the Standard toolbar, click . you The Save Changes To The Database dialog box appears. n |
| example chromatogram. | Save Changes To The Database ? 🗙 |
| See "Basic Exercise #2a Run a si sample to produce an example chromatogram" on page 17. Continue with Task 4 after you produce an example chromatogra | Ingle List of changes Sequence template updated due to changes in compound calibration Method. Change the Compound Name' from New Compound' to 'o-terphenyl' for the 'Compound' in the Calibration. Change the 'Compound Name' from New Compound' to 'o-terphenyl' for the 'Compound' in the Calibration. Change the 'Compound Name' from New Compound' to 'dethylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from New Compound' to 'dethylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from New Compound' to 'dethylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from New Compound' to 'dethylphthalate' for the 'Compound' in the Calibration. Added Compound New Compound with Expected Time 1.07397056425605. High Time Limit 1.32625482839 Added Compound New Compound' with Expected Time 1.0343977305102. High Time Limit 1.320087423 Added Compound New Compound' with Expected Time 0.934924245150261, High Time Limit 0.958297351: Reason for changes |
| | h Review the List of changes |
| | c Under Reason for changes, enter a reason or select a reason from the list. d Click the Save button. |
| | The Cerity administrator must set up auditing for the Save Changes to the Database dialog box to appear. The Cerity administrator can provide a list of reasons and require you to enter your electronic signature to complete the |

dialog box.

Task 4. Select an example chromatogram and set up integration

Steps

1 Select an example chromatogram.

to produce the example

page 17.

recommended.

If no chromatogram of the isocratic

sample exists, you must run a sample

chromatogram. See "Basic Exercise

#2a Run a single sample to produce

chromatogram to set up integration

an example chromatogram" on

You do not need the example

and identification, but it is

Detailed Instructions

- a On the selection tree, expand the exer2iii method folder, if necessary.
- b Expand the Data Analysis folder.
- c Select Example Chromatogram.
- d On the Tools toolbar, click AA.

Select example chromatogram ? • [AllSamplesNotApprovedRunLast7Days SAIISamplesNotApprovedRunLast7Days 🗄 🧰 Blanks 🚊 🔂 Samples 🖻 🔓 defexchrom2a [Rev 2] 🗡 defexchrom2a #1 [Rev 1] 🗄 🦲 Old Revisions 🗄 🖕 🔓 exer2dec [Rev 2] ⊕ -1[°] Calibration - exer2dec Calib Rev 2 [Rev 2] Expand the Samples folder. е f Expand the exchromiii or defexchrom2a folder. a Select the sample name with the injection number.

h Click the **Select** button.

The example chromatogram appears in the workspace.



| Steps | Detailed Instructions | | |
|---|---|--|--|
| 2 Change the initial event values so that there are only four integrated peaks. | a On the selection tree, select Integration under Data Analysis. The example chromatogram appears with the integration events tables. b Change the Height Reject event value to 1 (or the lowest value that will sti integrate the four main peaks). c Click I f on the Actions toolbar | | |
| | Example Chromatogram | | |
| | MARK ALCOST | | |



Task 5. Set up compound identification

Steps **Detailed Instructions** 1 Set up the compound table for the а On the selection tree, select the **Identification** item for Data Analysis. following compounds: b On the Tools toolbar, click +++-RT=.9 to 1.1, dimethylphthalate The peaks appear with the names New CompoundN in the compound table, where N = 1 - 4. RT=1.1 to 1.2, diethylphthalate c Under **Compound Name**, select the first cell and enter dimethylphthalate. RT=1.8 to 2.1, biphenyl After you select the cell, enter the name. The previous entry is overwritten. RT=3 to 3.2, o-terphenyl **d** Under **Compound Name**, select the second cell and enter diethylphthalate. e Under Compound Name, select the third cell and enter biphenyl. f Under **Compound Name**, select the fourth cell and enter o-terphenyl. Compound Options ¥. 8 8 min Identification Confirmation Compound Resolution Reference Use Default Time Window Time Reference Peak Compound Name Expected Time Peak Signal Lo DAD1 A DAD1 A DAD1 A DAD1 A dimethyl phthalate 1.1668 1.9700 3.1861 diethyl phthalate biphenyl N/A N/A

Steps

Detailed Instructions

2 Save the method.

If you need to run this method to identify compounds before you set up the other methods in these exercises, use the method with "Basic Exercise #2b Run a group of single samples to identify compounds" on page 23.

| | - | | |
|----------------|--------------|---------------|----------------|
| The Save Chang | ges To The D | atabase dialo | g box appears. |

a On the Standard toolbar, click 🛄

| Save Changes To The Database | ? × |
|---|-------------------------------------|
| List of changes Focuses togethe undeted due to observe is concerned estimation Method | |
| Change the 'Compound Name' from 'New Compound' to 'oterphenyl' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'oterphenyl' for the 'Compound' in the Calibration. | - |
| Change the 'Compound Name' from 'New Compound2' to 'diethylpithalate' for the 'Compound' in the Calibrat Change the 'Compound Name' from 'New Compound1' to 'dimethylpithalate' for the 'Compound' in the Calibrat Added Compound New Compound4 with Expected Time 3.07391365386018, High Time Limit 3.15076150 Added Compound New Compound3 with Expected Time 1.8739705425805, High Time Limit 1.92635483 Added Compound New Compound2 with Expected Time 1.10439877305102, High Time Limit 1.13200874 Added Compound New Compound1 with Expected Time 0.34324245150261, High Time Limit 0.9582973 | ion. 152! 1831 233 151; |
| | ₹ F |
| Reason for changes | |
| Updated | - |
| Save Discard Cancel | |

- **b** Review the **List of changes**.
- c Under Reason for changes, enter a reason or select a reason from the list.
- d Click the Save button.

The Cerity administrator must set up auditing for the **Save Changes to the Database** dialog box to appear. The Cerity administrator can provide a list of reasons and require you to enter your electronic signature to complete the dialog box.



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Basic Exercise #3 Set up a single-level calibrated method for a sequence

This exercise contains a series of tasks to learn how to:

- Create a method template for a sequence to include single-level, single-update calibration and ESTD quantitation
- Set up calibration and quantitation with fixed compound amounts
- Set up a sequence template

A *sequence template* is a sequence table containing the order of calibration standards and samples that you need to run with this method. A sequence template is useful if the order, sample names and characteristics are similar every time you run a sequence with this method.

You can use this method with "Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration" and "Basic Exercise #3b Reintegrate and reprocess the results".

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Setting Up Methods" on page 71 for setting up methods.



Task 1. Copy a method to create a method template for a sequence

Steps

- 1 Create a new method template from an existing method.
 - Name the method template, exer3iii, where iii are your initials.
 - Use exer2iii or defexer2 as the template for the new method template.
 - Make sure that only Compound Identification and Calibration and Quantitation are marked.

You copy a method when you want to keep the instrument and data analysis settings from the old method. You do not have to take the time to enter the settings into the new method.

Detailed Instructions

- a Select File > New > Method or click and select Method.
 The Method Wizard New Method panel appears.
- b Click the Browse button and select exer2*iii* or defexer2 from the Method Template Selection dialog box.
- c Enter exer3*iii* in the **New Method Name** box.
- d Select Sequence.



- e Click Next until you reach the Data Analysis panel.
- f Mark the Calibration and Quantitation check box.



Steps

Detailed Instructions

- 2 Make selections to keep the compound table and set up new calibration.
 - Make selections to set up: single-level calibration fixed compound amounts single-update calibration sequence-specific calibration
- a Click Next to scroll to the Compound Table panel.
- b Select the Keep Compound Calibration from Method template option. This option lets you keep the compound table from the previous method (even though no calibration was set up).



- c Click Next to scroll to the Identification panel.
- d Do not mark any check boxes on the **Identification** panel.
- e Click Next to scroll to the Calibration panel.
- f Select Fixed Amount and use the default options.

| Method Wizard | | × |
|---------------|--|---|
| Calibration | Do the standards in your method always contain Fixed Amounts or Variable Amounts? Does this method use more than one concentration level of the calibrated compound(s)? What kind of Calibration do you need? | Variable Amount Fixed Amount Multi Level 2 Uverall Calibration G Single Update Calibration Bracketing |
| | What kind of Calibration Procedure do you need? | Instrument Specific Celibration Sequence Specific Celibration |

| Steps | Detailed Instructions | | |
|--|--|--|--|
| 3 Set up Quantitation and review your new method. | a Click Next to scroll to the Quantitation panel. b Make sure that the Limit checks check box is clear and the ESTD option is selected. | | |
| | Method Wizard ? × Quantitation Do you want to include limit checks on the calculated results ? Limit checks | | |
| | Which Calibration Mode do you C ESTD Want to use in your Method ? C ISTD | | |
| | " Sack INext> Drish Cancel | | |
| | c Click Next to scroll to the New Method Review panel. | | |
| | d Keview the Method Wizard Settings. Click the Finish button to save your new method | | |

Task 2. Select an example chromatogram

Steps

Detailed Instructions

- **1** Select an example chromatogram. a On the selection tree, expand the exer3iii folder. • Use the example chromatogram you b Expand the Data Analysis folder. produced with Basic Exercise 2a or c Select the Example Chromatogram item. 2b of the "Basic Exercise #3a Run a d On the Tools toolbar, click AA. sequence to quantify compounds Select example ch ? × with single-level calibration" and • AlSamplesNotApprovedBunLast7Days "Basic Exercise #3b Reintegrate anplesNotApprovedRunLast7Day: AllSamplesNotApprovedRunLast7Day and reprocess the results". Blanks 🗟 Samples Or, use defexchrom2a. defexchrom2a [Rev 2] • You do not need to select an example chromatogram. However, it is easier to modify compound identification if you do.
 - AllSamplesNotApprovedRunLast7Days/Samples/defexchrom2a [Rev 2]/defexchrom2a H1 [Rev 1]
 - e Select the sample name with the injection number to produce the example chromatogram.
 - f Click the Select button.

The example chromatogram appears in the workspace.



After you have selected the example chromatogram, you can see the integration and identification settings that belong to the original method.

Task 3. Modify compound identification

Steps

Detailed Instructions

- 1 Remove a compound from the compound table.
- a On the selection tree, select **Identification** under the Data Analysis folder.
- **b** Select the **o-terphenyl** cell.
- Remove the o-terphenyl compound.
- c Right-click the o-terphenyl cell and select Remove Compound.



Task 4. Set up calibration

Steps

1 Set up calibration for

dimethylphthalate.

dimethylphthalate - 10µg

Detailed Instructions

- a On the selection tree, select **Calibration** under the Data Analysis folder.
- **b** On the calibration table, select dimethylphthalate.
- c On the **Options** tab, enter 10 in the **Weighed Amount** box and μg in the **Amount Unit** box.

| dimethylphthalate diethylphthalate biphenyl | 0.9349 1.1044 1.8794 | 10.0000 0.0000 15.0000 | нд | area area area | 0.000 N/A 0.000 |
|---|----------------------------|------------------------------|----|----------------------|-----------------------|
| diethylphthalate biphenyl | <u>1.1044</u> 1.8794 | 0.0000 | рц | area area | N/A 0.000 |
| biphenyl L | 1.8794 | 15.0000 | pų | area | 0.000 |
| | | | | | |
| Dptions | : dimethylpht | halate | | | |
| Weighed Amount : | 10 | | | | |
| Amount Unit : | ua | | | | |
| | | | | | |
| Comment : | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Basic Exercise #3 Set up a single-level calibrated method for a sequence

Detailed Instructions

2 Set up calibration for biphenyl.

Biphenyl - 15µg

a On the calibration table, select biphenyl.

b On the **Options** tab, enter 15 in the **Weighed Amount** box and μ g in the **Amount Unit** box.

| Compounds | | | | | |
|-------------------|---------------|----------------|-------------|--------------------------|--------------|
| Compound Name | Expected Time | Weighed Amount | Amount Unit | Quantitation Based On | RF (Rsp/Amt) |
| dimethylphthalate | 0.9349 | 10.0000 | μg | area | 0.0000 |
| diethylphthalate | 1.1044 | 15.0000 | | area | N/A |
| | | | | | |
| Options | | | | | |
| Compound Nam | e: biphenyl | | | | |
| Weighed Amount : | 15 | | | | |
| Amount Unit : | μg | | | | |
| Comment : | | | | | |

| Steps | Detailed Instructions | Detailed Instructions | | |
|--|--|---|--|--|
| 3 Remove diethylphthalate from calibration table. | a On the calibration table, right-cliftrom the shortcut menu. The Select Compound(s) dialog b In the Calibration Table list, selection c Click the < button to put diethyled d Click the OK button. | a On the calibration table, right-click anywhere and select Remove Compour from the shortcut menu. The Select Compound(s) dialog box appears. b In the Calibration Table list, select diethylphthalate. c Click the < button to put diethylphthalate in the Available Compounds list d Click the OK button. | | |
| | 🖷 Select Compound(s) | × | | |
| | Available Compounds | Calibration Table | | |
| | diethylphthalate | <pre>dimethylphthalate biphenyl </pre> | | |
| | Compound Info : | OK Cancel | | |

Task 5. Set up quantitation for all four peaks

| S | teps | Detailed Instructions | |
|--|------|--|---|
| Base the quantitation of diethylphthalate on dimethylphthalate. Use an amount multiplier of .8. | | a On the selection tree, select Quantitation Setup under the Data Analysis folder. b Click the Uncalibrated Compounds tab. c Under Compound Calibration Type, select the Use Compound option. d Select dimethylphthalate from the Use Compound list. e Enter .8 in the Amount Multiplier (Compound) box. | |
| | | Calibrated Compounds Uncalibrated Compounds Unidentified Peaks | |
| | | Compound Name Expected Time Compound Calibration Type (Compound) RF (Rsp/Amt) Compound Group | , |
| | | diethylphthalate 1.1044 dimethylphthalat 1.0000 N/A | |
| | | Compound Name dethylphthalate Compound Calibration Type Compound Group © Use Compound dimethylphthalate Amount Multiplier (Compound) Amount Multiplier (Compound) C Manual Response Factor N/A Compound Info | |
| | | | |

Detailed Instructions

2 Base the quantification of the unidentified peak on biphenyl.

Use an amount multiplier of .9.

- a Click the Unidentified Peaks tab.
- b Under Use for Quantitation, select the Use Compound option.
- c Select biphenyl from the Use Compound list.
- d enter .9 in the Amount Multiplier (Unidentified Peak) box.

| Calibrated Compounds Ur | ncalibrated Compounds Uni | identified Peaks | |
|------------------------------|---|---------------------------|--|
| Use Quanti | For fication Amount Multiplier (Unidentified Peak) | RF (Unidentifed Peaks) | |
| Not Identified Peaks dimethy | phthalate 1.0000 | N/A | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| - Lles For Quantification | | | |
| Use Compound | hiskoud | | |
| | | _ | |
| Amount Multiplier (Uniden | uned Heak) [.9 | | |
| C Manual Response Factor | N/A | | |
| O No Quantification | | | |

Task 6. Set up the sequence template

| 1 E s | | |
|--------------------------|--|---|
| s | nter the following calibration standards and samples into the sequence template: | a On the selection tree, select Sequence Template for the method. b On the sample table, enter the calibration standard for row one. Enter Cal1 in the Sample Name box. |
| (5 0 0 0 | Cal1- full-strength isocratic standard Sample 1_2 - isocratic standard diluted 1/2 with methanol Sample 1_4 - isocratic standard diluted 1/4 with methanol | Select Calibration Standard from the Sample Type list. Enter the Vial# where this standard is located in the ALS. Click the Apply button to put the sample information into the sample table. Enter sample 1_2 for row two. Select Row 2 in the sample table. Enter sample 1_2 in the Sample Name box. Solect Sample from the Sample Time list. |
| You tem unt Dat | NOTE cannot set up a sequence aplate with calibration standards il you have set up calibration in ca Analysis. | Select Sample from the Sample Type list. Enter the Vial# where this sample is located in the ALS. Click the Apply button to put the sample information into the sample table. d Enter sample 1_4 for row three. Select Row 3 in the sample table. Enter sample 1_4 in the Sample Name box. Select Sample from the Sample Type list. Enter the Vial# where this sample is located in the ALS. Click the Apply button to put the sample is located in the ALS. Click the Apply button to put the sample information into the sample table. |

| | Sample Name | Sample Type | Cal. Level | Summary Group | Vial # | Injections # | Volume [µl] | Samp Amou [mg/n |
|---|-------------|-------------|---------------|------------------|-----------|-----------------|----------------|-----------------------|
| 1 | Cal1 | Calibration | 1 | | 2 | 1 | as method | 0 |
| 2 | sample 1_2 | Sample | | | 5 | 1 | as method | 0 |
| 3 | sample 1_4 | Sample | | | 9 | 1 | as method | 0 |
| - | | | | | - | | | - |

Steps

Detailed Instructions

2 Enter two more sets of Cal1, sample1_2 and sample 1_4 into the template.

Hint: Use the Fill Down Wizard and Copy command.

The standards and samples in the final template appear in the following order:

- calibration standard
- two samples,
- calibration standard
- two samples,
- calibration standard
- two samples

- a Click Fill Down on the Edit toolbar, and select Fill Down Wizard. The Fill Down Wizard appears.
- **b** Under **Range**, select **Append**, enter 6, and click **Next**.

- c On the **Sample Names** panel, enter callin the **Name** box, and click **Next**.
- d On the Vial Numbers panel, clear the Define Vial numbers? check box, and click Finish.
- e When the **Apply Sample Changes** dialog box appears, click **Yes**. See that the six new rows display copies of the first row of the template.
- f Select the two samples on lines 2 and 3, and click the **Copy** button on the Edit toolbar.
- g Select rows 5 and 6, and click the **Paste** button on the Edit toolbar.
- h Select rows 8 and 9, and click the Paste button on the Edit toolbar.

| | Sample Name | Sample Type | Cal. Level | Summary Group | Vial # | Injections # | Injection Volume [µl] | Samp Amou [mg/n |
|----|-------------|-------------|---------------|------------------|-----------|-----------------|-----------------------------|-----------------------|
| 1 | Cal1 | Calibration | 1 | | 2 | 1 | as method | 0 |
| 2 | sample 1_2 | Sample | | | 5 | 1 | as method | 0 |
| 3 | sample 1_4 | Sample | | | 9 | 1 | as method | 0 |
| 4 | Cal1 | Calibration | 1 | | 2 | 1 | as method | 0 |
| 5 | sample 1_2 | Sample | | | 5 | 1 | as method | 0 |
| 6 | sample 1_4 | Sample | | | 9 | 1 | as method | 0 |
| 7 | Cal1 | Calibration | 1 | | 2 | 1 | as method | 0 |
| 8 | sample 1_2 | Sample | | | 5 | 1 | as method | 0 |
| 9 | sample 1_4 | Sample | | | 9 | 1 | as method | 0 |
| 10 | | | | | | | | |

| St | eps | Detailed Instructions | | | | |
|----|--|--|---|--|--|--|
| 3 | Set how the calibration will be updated: First Cal1- Replace (both RF and RT) Second Cal1 - Average for RF and Floating average for RT (Weighted 60% after RT) Third Cal1 - Average for RF and Floating average for RT (Weighted 75% ofter RT) | a On the sequence table, select the first Cal1. b Click the Run tab. c Under Calibration, select Replace from the Response Factor Update list and select Replace from the Retention Time Update list. d Select the second Cal1 in the sequence table. e Select Average from the Response Factor Update list and Floating average from the Retention Time Update list. f Select 60%. g Repeat steps d and e for the third Cal1. | | | | |
| | | Sample Name Sample Type Cal. Level Custom Sample Group Vial # Injections # Injection bill 1 call Calibration 1 2 1 as method 0 3 sample 1_2 Sample 5 1 as method 0 4 call Calibration 2 1 as method 0 4 call Calibration 2 1 as method 0 5 sample 1_2 Sample 5 1 as method 0 5 sample 1_2 Sample 9 1 as method 0 6 sample 1_2 Sample 9 1 as method 0 7 call Calibration 1 2 1 as method 0 9 sample 1_2 Sample 9 1 as method 0 0 9 sample 1_4 Sample 9 1 as method 0 0 10 sample 1_4 Sample Sample 9 1 as method | nple ount Multipl 1 1 1 1 1 1 1 1 1 1 1 | | | |
| | | | | | | |

4 Save the method.

After you complete this method, you may use it to run a sequence. See "Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration" on page 29 and "Basic Exercise #3b Reintegrate and reprocess the results" on page 39.

a Click 📕, and enter your reasons for changes and electronic signature, if necessary.



Agilent Cerity Networked Data System for Pharmaceutical QA/QC Getting Started Guide

Advanced Exercise #4 Set up a method for single samples to acquire and use spectra

This exercise contains a series of tasks to learn how to:

- Create a method template for single samples and spectra, to include only compound identification in the method
- Set up and save the method to produce an example chromatogram
- Use an example chromatogram to set up integration
- Set up compound identification
- Set up UV compound confirmation
- Set up UV purity
- Set up spectra handling
- NOTE

You will need a detector capable of acquiring spectra (DAD or FLD) and a UV Spectral Acquisition license to complete this exercise.

Use the method created in the first part of this exercise to enter and run a single sample to produce an example chromatogram. You can use the completed method to enter and run a group of samples to identify compounds.

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Setting Up Methods" on page 71 for setting up methods.



Task 1. Create a method template to identify compounds only

| St | eps | Detailed Instructions |
|----|--|--|
| 1 | Create a new method template for a single sample. Name the method template, exer4 <i>iii</i> , where <i>iii</i> are your initials. | b Select File > New > Method or click and select Method. The Method Wizard appears. c Enter exer4 in the Method Name box. d Select Single Sample. |
| | | New Method New Method name : exer2jws Do you want to select an existing Method as a template for the new Method ? What kind of Method do you want to create ? What kind of Method do you want to create ? © Sequence |

- e Click Next to scroll to the Method Wizard Instrument panel.
- 2 Select an instrument for the method. Select an instrument with either a DAD or an FLD.
- a On the Instrument panel, select the instrument that will run the sample.b Mark the UV Spectra check box.

| Method Wizard | × |
|-----------------------------|--|
| Method Wizard Instrument | Select the Instrument for your Method. Available Instruments: SoftVDT1 |
| | Agilent 1100 Series Thermostatted Autosampler DigVD T S3500E Analog to Digital Converter |
| | Selected Instrument: «None» Do you want to acquire UV Spectra? 🔽 UV Spectra |
| | |

c Click Next to scroll to the Data Analysis panel.

| On the Data Analysis pa boxes, and clear the Ca Calculations and Inclusion | anel, mark the UV Purity libration and Quantifica de System Suitability Ca | and UV Confirmation cho ition, Include Noise alculations check boxes. |
|--|--|---|
| Method Wizard | 1 | <u>? ×</u> |
| Data Analysis | Do you want to include Compound Identification? | Compound Identification |
| | Do you want to include UV Spectral Compound Purity? | VV Purity |
| - | Do you want to include UV Spectral Compound Confirmation? | VV Confirmation |
| | Do you want to include Calibration and Quantitation? | Calibration and Quantitation |
| | Do you want to use Custom Calculations? | Custom Calculations |
| | Do you want to include System Suitability Calculations? | ☐ Include System Suitability Calculations |
| | A Off the Data Analysis per boxes, and clear the Ca Calculations and Includ Method Wizard Data Analysis Data Analysis | A off the bata Analysis paner, mark the over unity boxes, and clear the Calibration and Quantifica Calculations and Include System Suitability Ca Method Wizard Data Analysis Day want to include UV Spectral Compound Identification? Do you want to include UV Spectral Compound Purity? Do you want to include UV Spectral Compound Purity? Do you want to include UV Spectral Compound Purity? Do you want to include UV Spectral Compound Analysis Do you want to include System Calculations? |

- 4 Complete the setup of the method a Click Next,
 - a Click Next, and click the Finish button.
 - **b** Click **Save** if the Save Changes to the Database dialog box appears.

Do not mark any check box on the Method Wizard Identification panel.

template.

Task 2. Enter the instrument conditions for the equilibrium

Steps

Detailed Instructions

- 1 Enter the pump parameters:
 - Methanol as Solvent B:
 - Flow rate: 2ml/min.Solvent composition:
 - 80%MeOH/20%H₂O
 - Stoptime: 5 min.

Acetonitrile as Solvent B:

- Flow rate: 1.5ml/min
- Solvent composition: 65%ACN/35%H₂O
- Stoptime: 5 min.

- a On the selection tree, expand the exer4*iii* method folder.
- b Expand the Instrument Setup folder and select the Quaternary Pump or Binary Pump.
- c Enter the **Flow** as 2 ml/min.
- d Under **Solvents**, mark the **B** check box and enter 80 in the % box. The percent of solvent A is automatically set to 20 %.
- e Under Stoptime, select the min option and enter 5.

| Setup Timetable Auxiliary & Data Curves | |
|---|-----------------------------|
| Flow | Stoptime: |
| Flow: 2 📩 ml/min | 🔿 no Limit |
| Solvents | € 5 min |
| | Posttime: |
| B: 🔽 80 🛨 % | • Uff |
| C: Dff | C D min min |
| D: D Off | Min: 0 🔅 bar Max: 400 😇 bar |

2 Enter the injection volume and stop time for the autosampler.

- Injection Volume: 1µl
- Stop Time: As pump
- a On the selection tree, select the ALS folder.
- **b** Click the **Auxiliary & Time** tab.
- c Under Stoptime, select the As pump option.
- d Click the Setup tab and select Standard Injection.
- e Enter 1µl for the Injection Volume.

| Setup | Auxiliary & Time | |
|-------|------------------------------|--------------------------|
| Inje | ction | |
| c | Standard Injection | Injection Volume: 1 🚅 µl |
| c | C Injection with Needle Wash | WashViai: 1 |
| c | OUse Injector Program | |
| | | |
| Steps | | Detailed Instructions | | | | |
|--|---|---|---|--|--|--|
| 3 Make sure the stop time is the same for all instrument modules. Stop Time: As pump/injector | | a On the selection tree, select the DAD or FLD folder. b Under Stoptime, select As pump/injector. c On the selection tree, select the TCC folder. d Under Stoptime, select As pump/injector. | | | | |
| | | Signal & Time Timetable Options Signal Store Sample Bw Store Sample Bw A: I 250 mm 10 mm I 400 mm 100 mm nm B: I Not used C: I Not used D: I Not used E: I Not used Stoptime: I As pump / injectod C Mo imit I Mint | Spectrum Store: All Range: 190 to: 450 rm to: 450 rm rm Step: 2 rm rm Threshold: 10 Peakwidth (Responsetime) >0.10 min (2.0 s) | | | |
| 4 | Set up spectral acquisition parameters. Signal • A: 254 nm, Bw: 4 nm • Reference: 400 nm, Bw: 100 nm Spectrum • Store: All • Range: 190 nm • to: 450 nm • Step: 2 nm | a On the selection tree, select the DAD or FLD folder. b Under Signal, mark the Store check box for Signal A, wavelength to 254 nm and the Bw to 10 c Mark the On/Off check box and set the Reference wa Bw to 100. d Under Spectrum, select to store All spectra, and set the 450 nm with a Step size of 2. | , set the Sample avelength to 400 and the the Range from 190 to | | | |

Task 3. Save and audit method changes

| Steps | | Detailed Instructions | | | |
|-------|--|---|--|--|--|
| 1 | Save the method. After you save the method here, you can use the method to produce an example chromatogram | a On the Standard toolbar, click 🔚 . The Save Changes To The Database dialog box appears. | | | |
| | can use the method to produce an example chromatogram. See "Basic Exercise #2a Run a single sample to produce an example chromatogram" on page 17. Continue with Task 4 after you produce an example chromatogram. | List of changes Sequence template updated due to changes in compound calibration Method Change the 'Compound Name' from 'New Compound' to 'oterphenyl' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'biphenyl' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound' to 'dishtylphthalate' for the 'Compound' in the Calibration. Added Compound New Compound's with Expected Time 1.10439877305120. High Time Limit 1.1320874233 Added Compound New Compound' with Expected Time 0.934924245150261. High Time Limit 0.958297351: | | | |
| | | b Review the List of changes. c Under Reason for changes, enter a reason or select a reason from the list. d Click the Save button. | | | |
| | | The Cerity administrator must set up auditing for the Save Changes to the Database dialog box to appear. The Cerity administrator can provide a list of reasons and require you to enter your electronic signature to complete the | | | |

dialog box.

Task 4. Enter and run a single sample

| Steps | | Detailed Instructions | | | |
|-------|---|---|--|--|--|
| 1 | Start the Instrument View to find the sample table for single samples. | a Select Instrument from the Current View list. b Expand the folder for the instrument that will produce the example chromatogram. c Select Single Samples. The sample table and sample entry panel appear in the workspace. | | | |
| 2 | Enter a sample with the following information: Name the sample exchrom3Diii, where iii are your initials. Select either exer4iii Select the vial that contains the full-strength isocratic standard. | a Enter exchrom3D<i>iii</i> in the Sample Name box. b Select a method from the Method list. The instrument associated with the method appears in the Instrument box. c Select Sample from the Sample Type list. d Enter the vial number for the sample in the Vial Number box. e Click Apply to put the sample information in the sample table. Use the default values for all other parameters | | | |
| 3 | Enter the tasks to perform during the run. | a Clear the Quantify and Report check boxes. | | | |
| 4 | Save the sample. | a On the Standard toolbar, click | | | |

Advanced Exercise #4 Set up a method for single samples to acquire and use spectra

| Steps | | Detailed Instructions | | | | | |
|-------------------|---|---|--|--|--|--|--|
| 5 Run the sample. | | a On the selection tree, expand your instrument folder. b Select Single Samples. c Select the sample, exchromiii. The Run button becomes available on the Tools toolbar. | | | | | |
| | | Image: Sector of the sector | | | | | |
| 6 Mo sta | nitor the signal, and track the tus of the sample. | a On the selection tree, select your instrument. b Click the Online Plot tab to view the signal. Change the axes if necessary. See "Basic Exercise #2a Run a single sample to produce an example chromatogram" on page 17 for detailed instructions. | | | | | |
| 7 Rev sur | view the sample result and make e all four peaks are integrated. | a Select Result from the Current View list. b Select MySamplesRunLast24h from the Query list. c Expand the Samples folder. d Expand the exchrom3D<i>iii</i> folder. e Select the exchrom3D<i>iii</i> #1 injection. f View the chromatogram and results. | | | | | |

Task 5. Select an example chromatogram and set up integration

Steps

1 Select an example chromatogram.

chromatogram to set up integration

You do not need the example

and identification, but it is

recommended.

Detailed Instructions

- a On the selection tree, expand the exer4 method folder, if necessary.
- b Expand the Data Analysis folder.
- c Select Example Chromatogram.
- d On the Tools toolbar, click AA.

Select example chromatogram ? • [AllSamplesNotApprovedRunLast7Days SAIISamplesNotApprovedRunLast7Days 🗄 📄 Blanks 🚊 🔂 Samples 🖻 📮 defexchrom2a [Rev 2] 🗡 defexchrom2a #1 [Rev 1] 🗄 📋 Old Revisions 🗄 📲 🔓 exer2dec [Rev 2] Expand the Samples folder. е f Expand the exchrom3Diii folder. a Select the sample name with the injection number. h Click the Select button. The example chromatogram appears in the workspace.



| Steps | Detailed Instructions | | | | | |
|---|---|--|--|--|--|--|
| 2 Change the initial event values so that there are only four integrated peaks. | a On the selection tree, select Integration under Data Analysis. The example chromatogram appears with the integration events tables. b Change the Height Reject event value to 1 (or the lowest value that will still integrate the four main peaks). | | | | | |
| | c Click M on the Actions toolbar | | | | | |
| | c Click M on the Actions toolbar | | | | | |
| | C Click M on the Actions toolbar Example Chromatogram VWD: Absorbance | | | | | |

-3.07 8 8 0 min ά Initial Events I imed Events Results VWD • Select... Signal Short Description ВT Peak Are Initial Event Name Initial Event Value Initial Event Name Area Reject Slope Sensitivity Peak Width Shoulder Detection Mode Height Reject 0.0000 1.0000 0.0400 VWD1 A VWD1 A VWD1 A VWD1 A 0.9349 1.1044 1.8794 3.0739 419.5843 374.2865 356.2544 523.9493 Disabled 1.0000 For All Signals Tail Peak Skim Height Ratio Front Peak Skim Height Ratio Skim Valley Ratio Baseline Correction 0.0000 0.0000 0.0000 20.0000 Classical Tangent Skim Mode Peak to Valley Ratio Standard 500.0000

Task 6. Set up compound identification

Set up the compound identification

table for the following compounds:

RT=.9 to 1.1, dimethylphthalate

RT=1.1 to 1.2, diethylphthalate

RT=1.8 to 2.1, biphenyl

RT=3 to 3.2, o-terphenyl

Steps

1

Detailed Instructions

a On the selection tree, select the Identification item for Data Analysis.

b On the Tools toolbar, click +++

The peaks appear with the names New CompoundN in the compound table, where N = 1 - 4.

- **c** Under **Compound Name**, select the first cell and enter dimethylphthalate. After you select the cell, enter the name. The previous entry is overwritten.
- **d** Under **Compound Name**, select the second cell and enter diethylphthalate.
- e Under **Compound Name**, select the third cell and enter biphenyl.
- f Under **Compound Name**, select the fourth cell and enter o-terphenyl.



Task 7. Set up UV spectral compound confirmation

| Steps | | Detailed Instructions | | | | | | | |
|---|---|---------------------------------|---------------------|-------------|---|--------------|--------------------------|-----------------|--|
| 1 Set up UV spectral compound confirmation for all compounds | a On the Identification workspace, click the Confirmation tab. b In the Confirmation table, select the first line. c Mark the Use UV spectral compound confirmation check box in the panel below the table. d Mark the Use default options check box in the panel below the table. e Select the other lines in the Confirmation table, and repeat (c) and (d) for each compound. Note that when the checkboxes are marked, a plus sign is entered into the Use UV spectral compound confirmation and Use Defaults columns of the Confirmation table. | | | | | | | | |
| | | Identification Confirmation | | | | | | | |
| | | Compound Name | Expected Time | Peak Signal | Use UV Spectral Compound Confirmation | Use Defaults | Background correction | Use M Backgi | |
| | | dimethyl phthalate | 0.9908 | DAD1 A | + | + | Automatic | | |
| | | diethyl phthalate | 1.1668 | DAD1 A | + | + | Automatic | - | |
| | | biphenyl | 1.9700 | DAD1 A | • | + | Automatic | | |
| | | o-terpnenyl | 3.1861 | DADTA | + | + | Automatic | | |
| | | Use UV spectra | al compound confirm | nation | | For | mat 🔛 🖌 📐 | વ્ ગ્ | |

| Steps | Detailed Instructions | | | | | |
|--|---|--|--|--|--|--|
| 2 Set up the default UV spectral compound confirmation options | a In the panel below the Confirmation table, click the button to the right of the Use default options check box. The Spectral Confirmation Defaults dialog box appears. | | | | | |
| | Spectral Confirmation Defaults | | | | | |
| | DAD | | | | | |
| | Background Correction Calculations | | | | | |
| | C None Solice threshold [mAU]: 5 | | | | | |
| | Automatic | | | | | |
| | C Manual | | | | | |
| | Background freint | | | | | |
| | | | | | | |
| | -1 Reject : 950 | | | | | |
| | | | | | | |
| | OKCancel | | | | | |
| | b In the Background Correction group, select the Automatic option. c In the Calculations group, set the Noise threshold to 5 mAU. d Leave the Levels at their default values. | | | | | |
| 3 Select a reference spectrum for confirmation | a Un the Standard tooldar, click | | | | | |
| commation | The Compound Reference Spectrum Selection dialog box for the selected compound appears. | | | | | |
| | b On the selection tree, expand the <i>exchrom3Diii</i> folder. | | | | | |
| | c Select the sample name with the injection number. | | | | | |
| | d On the dialog box toolbar, click 🔼 | | | | | |
| | e Un the example chromatogram, select the peak for the selected compound. | | | | | |
| | The apex spectrum of the selected compound is displayed in the spectrum window. | | | | | |
| | f From the Compound drop-down list, select the next compound. | | | | | |
| | g Select the peak for this compound. | | | | | |
| | h Repeat (f) and (g) for all remaining compounds. | | | | | |
| | i Close the Compound Reference Spectrum Selection dialog box. | | | | | |

Task 8. Set up UV purity

| Steps | Detailed Instructions | | | | | |
|---|--|--|---|------------------|--|--|
| Set up Spectra Handling parameters Set up the Wavelength Range Set up Background Correction | a On the selection tree, select the UV Purity item for Data Analysis. The UV purity options panel is displayed in the workspace. | | | | | |
| Set up Peak Spectra Set up Calculations Set up Levels | DAD Wavelength Range | 220 | Peak Spectra Number of spectra: Minimum response range [mAU] | 5 💌 | | |
| | Background Correction | 0 | Calculations Noise threshold [mAU]: | 990 | | |
| | b In the Wavelength Rang the adjacent field. c In the Background Corr d In the Peak Spectra ground Minimum response range e In the Calculations ground f Leave the Levels at their | ge group, mar rection group, up, set the Nu uge at its defau up, set the Noi ir default value | k the Low check box and ent select the Automatic option i mber of spectra to 7 . Leave t ult value. se threshold to 5 mAU. es. | er 220 in the | | |

Task 9. Set up spectra handling

Steps

Detailed Instructions

- 1 Set up UV Purity parameters
 - Set up the Wavelength Range
 - Set up Background Correction
 - Set up Peak Spectra
- a On the selection tree, select the **Spectra Handling** item for Data Analysis. The spectra handling options panel is displayed in the workspace.

| DAD | | | |
|---|-----|---|-------|
| Wavelength Range Low (nm): High (nm): | 210 | Peak Spectra Number of spectra: Minimum response range [mAU]; | All 🔽 |
| -Background Correction- | | | |
| C None | | | |
| Automatic | | | |
| C Manual | | | |
| 🗚 🗖 Background 1 [min]: | 0 | | |
| Background 2 [min]: | 0 | | |

b In the **Wavelength Range** group, clear both check boxes.

This ensures that the complete wavelength range is displayed.

- c In the **Background Correction** group, select the **Automatic** option.
- d In the Peak Spectra group, set the **Number of spectra** to **All**. Leave the **Minimum response range** at its default value.

Advanced Exercise #4 Set up a method for single samples to acquire and use spectra

| Steps | Detailed Instructions |
|--------------------|--|
| 2 Save the method. | a On the Standard toolbar, click 🔚 . |
| | The Save Changes To The Database dialog box appears. |
| | Save Changes To The Database |
| | List of changes |
| | Sequence template updated due to changes in compound calitration Method. Change the 'Compound Name' from 'New Compound'to 'o-terpheny' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound'to 'o 'bipheny' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound'to 'o 'dientylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound'to 'o 'dientylphthalate' for the 'Compound' in the Calibration. Change the 'Compound Name' from 'New Compound'to 'o 'dientylphthalate' for the 'Compound' in the Calibration. Change the 'Compound New Compound' with Expected Time 3.07391366356018. High Time Limit 1.92635482831 Added Compound New Compound's with Expected Time 1.10439877305102, High Time Limit 1.92635482831 Added Compound New Compound' with Expected Time 0.934924245150261, High Time Limit 1.958297351: Added Compound New Compound' with Expected Time 0.934924245150261, High Time Limit 1.958297351: |
| | Reson for change |
| | |
| | b Review the List of changes. |
| | c Under Reason for changes , enter a reason or select a reason from the list. |
| | d Click the Save button. |
| | The Cerity administrator must set up auditing for the Save Changes to the Database dialog box to appear. The Cerity administrator can provide a list of reasons and require you to enter your electronic signature to complete the dialog box. |



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Advanced Exercise #5 Set up a multi-level calibrated method for a sequence

This exercise contains a series of tasks to learn how to:

- Use an existing method to create a new method template for a sequence
- Include multi-level, overall calibration and ESTD quantitation in the method
- Set up calibration and quantitation with variable compound amounts for a calibration table with two levels
- Set up system sample variables
- Set up a sequence template for overall calibration
- Select a new report template for a single standard injection report

See "Basic Exercise #3 Set up a single-level calibrated method for a sequence" on page 91 to learn what a sequence template is.

You can use this method with "Advanced Exercise #4a Run a sequence to quantify compounds with multi-level calibration" on page 45 and "Advanced Exercise #4b Change sample variables in the method and reprocess" on page 53.

For the tasks in this exercise, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Setting Up Methods" on page 71 to set up methods.



Task 1. Copy a method to create a new method template for a sequence

Steps

1 Copy the method to create a new template.

- Copy either exer3iii or defexer3.
- Name the method template,
- exer4iii, where *iii* are your initials. • Change nothing until you reach the

Compound Table panel. Note that the Method Wizard panels

contain the method selections from Exercise 3.

Detailed Instructions

- a Select File > New > Method or click 🗋 and select Method. The Method Wizard appears.
- **b** On the New Method panel, click the **Browse** button, and select exer3*iii* or defexer3.
- c Enter exer4*iii* in the **New Method Name** box.

| New Method New M | ethod name : 4eme |
|--------------------------------------|--|
| Do you a templ exert What H | want to select an existing Method as ate for the new Method ? 3singlevel Browse wind of Method do you C Single Sample to create ? C Sequence |

d Click **Next** until you reach the Compound Table panel.

2 Set up the Compound Table panel. a On the Compour

Because you will set up a multi-level calibration, you set up a new calibration table.

a On the Compound Table panel, select **Set up a new Compound Calibration**.



b Click **Next** until you reach the **Calibration** panel.

Steps

Detailed Instructions

3 Set up the calibration panel.

Choose to set up:

- multi-level calibration (2 levels)
- · variable compound amounts
- overall calibration
- sequence-specific calibration

a Select Variable Amount.

- b Mark the Multi Level check box, and enter 2 levels.
- c Select Overall Calibration.

Method Wizard ?) Calibration Do the standards in your Variable Amount method always contain Fixed Amounts or Variable Amounts? O Fixed Amount Does this method use more than one concentration level of the Multi Level 2 calibrated compound(s)? What kind of Calibration do Overall Calibration you need? C Single Update Calibration C Bracketing What kind of Calibration C Instrument Specific Procedure do you need? Calibration Sequence Specific Calibration < <u>B</u>ack Next > Cancel d Click Next until you reach the New Method Review panel. On the New Method Review panel, review the Method Wizard Settings. a

- 4 Review your new method template.
- **b** Click the **Finish** button to save your new method.
- c Save all changes to the database, with a reason if necessary.

Task 2. Set up example chromatogram and compound identification

Steps

results".

Select an example chromatogram.

you produced with "Basic Exercise #3a Run a sequence to quantify

compounds with single-level calibration" and "Basic Exercise #3b

Reintegrate and reprocess the

Or, use defexchr2a. (To use this

with a VWD detector.)

restored result.

chromatogram, use an instrument

If you cannot see the sample whose chromatogram that you want to select, select another query. **Hint**: The result, defexchr2a, is a

Use the example chromatogram that

1

Detailed Instructions

- a On the selection tree, expand the new method template, exer4iii.
- **b** Expand the **Data Analysis** folder and select **Example Chromatogram**.
- c On the Tools toolbar, click AA

The Select example chromatogram dialog box appears.



- d Select the injection from the analysis that contains the example chromatogram for the new method. If you do not see the defexchrom2a under the Samples folder, select the query, AllResultsRestored.
- e Click the Select button.

The example chromatogram appears in the workspace.



The integration parameters are retained from the Exercise 3 method. You do not have to set up integration.

| Steps | | Detailed Instructions | | |
|-------|--|-----------------------|---|--|
| 2 | Set up the compound table for these compounds: | a b | On the selection tree, select Identification under the Data Analysis folder. On the Tools toolbar, click —. | |
| | RT=0.9-1.1 min, dimethylphthalate RT=1.1-1.3 min, diethylphthalate | | The peaks appear with the names New Compound one through four in the compound table. | |
| | RT=1.8-2.0 min, biphenyl Do not identify the fourth peak. In another exercise, you will set up the fourth peak as an unspecified impurity that is not identified based on retention time. | c d e f g | Under Compound Name , select the first cell and enter dimethylphthalate. After you select the cell, enter the name. The previous entry is overwritten. Under Compound Name , select the second cell and enter diethylphthalate. Under Compound Name , select the third cell and enter biphenyl. Under Compound Name , right-click the fourth cell. Select Remove Compound . On the Identification workspace, view the three identified peaks and one unidentified peak. | |
| | | C | ompound Options | |
| | | | VWD: Absorbance | |

dimethy/phthalate

0-0349

Expected Time

0.9349 1.1044 1.8794 Peak Signal

VWD1A VWD1A VWD1A

8

Identification

Compound Name

dimethylphthalate diethylphthalate biphenyl 4

High Time Limit

t

Low Time Limit

1.0768

Use Default Time Window

Time Reference Peak

Task 3. Set up calibration and quantitation

Steps

Detailed Instructions

- a On the selection tree, select **Calibration** under the Data Analysis folder.
- b On the Compounds table, select dimethylphthalate.
- c On the **Options** sheet, click the **Use Default Amount** cell and select +. When you make this selection, the amount that you enter in the Weighed Amount cell for each level appears in the Amounts sheet in Sample Entry.
- d For level 1, enter 10 in the Weighed Amount box and μg in the Amount Unit box.
- e For level 2, enter 40 in the Weighed Amount box.
- f Repeat steps c-e for biphenyl.

| Compound Name | Level Id | Weighed Amount | Use Default Amount | Amount Unit | Quantitation Based On | |
|------------------------|--------------------------|---------------------------|-----------------------|----------------------------|--------------------------|--|
| dimethylphthalate | 1 | 10.0000 | + | ug | area | |
| | 2 | 40.0000 | | | | |
| diethylphthalate | 1 | 0.0000 | • | | area | |
| | 2 | 0.0000 | | | | |
| Dipnenyi | | 15.0000 | + | uq | area | |
| Compound Name biphenyl | | | | | | |
| Compound Nam | biphenyl | Use Default | | | - | |
| Compound Nam | biphenyl Weighed Amo | Use Default Amount | Amount Unit | Lo w Amount Lim | it Use Low | |
| Compound Nam | biphenyl Weighed Amor | unt Use Default Amount | Amount Unit | Low Amount Limi | it Use Low | |

1 Set up calibration for dimethylphthalate and biphenyl.

Default amounts for dimethylphthalate:

- Level 1 10µg
- Level 2 40µg

Default amounts for biphenyl:

- Level 1 15µg
- Level 2 60µg

When you set up a method with variable compound amounts, the application lets you enter the actual weight (concentration) of the standard compounds in sample entry.

| Steps |
|-------|
|-------|

Detailed Instructions

2 Remove diethylphthalate from the calibration table.

The system has automatically added all compounds from the compound identification table to the calibration table.

In this step, remove diethylphthalate to use it as an uncalibrated compound that is quantified based on the response factors of a different compound. a On the calibration table, right-click anywhere and select **Remove Compound** from the shortcut menu.

The Select Compounds dialog box appears.

- **b** In the **Calibration Table** list, select diethylphthalate.
- c Click the < button to put diethylphthalate in the Available Compounds list.
- d Click the **OK** button.

| 🛋 Select Compound(s) | | | × |
|----------------------|----|-------------------------------|----------|
| Available Compounds | | Calibrati | on Table |
| diethylphthalate | >> | dimethylphthalate biphenyl | |
| | > | | |
| | < | | |
| | | | |
| Compound Info : | | | |
| | | ОК | Cancel |

3 Set up quantitation as you did in Exercise 3.

See "Task 5. Set up quantitation for all four peaks" on page 100.

Task 4. Set up system sample variables

| St | teps | Detaile | ed Instructions | | | | |
|----|---|--|-----------------|-------------------|---------|--|--|
| 1 | Set up a multiplier called "dilution factor". Use a default value of 5. | a On the selection tree, select Sample Variables. b Double-click the Dilution cell, and add the word Factor. c Enter a default value of 5. | | | | | |
| 2 | Set up a divisor called "correction factor". Use a default value of 2. | correction a Click the Divisor cell once, and enter the name, Correction Fa b Enter a default value of 2. System Defined Sample Variables (Set by the user in Sample Entry and used in | | | | | |
| | | | | | Default | | |
| | | | Variable ID | Display Name | Value | | |
| | | 1 | Multiplier_1 | Multiplier | 1 | | |
| | | 2 | Multiplier_2 | Dilution Factor | 5 | | |
| | | 3 | Multiplier_3 | Purity | 1 | | |
| | | 4 | Multiplier_4 | | 1 | | |
| | | 5 | Multiplier_5 | | 1 | | |
| | | 6 | Divider_1 | Correction Factor | 2 | | |
| | | 7 | Divider_2 | | 1 | | |
| | | 8 | Divider_3 | | 1 | | |
| | | 9 | Divider_4 | | 1 | | |

Task 5. Edit the sequence template

| Steps Detailed Instructions | | | | | | |
|--|--|--|--|--|--|--|
| Edit the template to look like this: two calibration standards (Lev1,2) two samples, two calibration standards two samples, two calibration standards Wo calibration standards Wo calibration standards NOTE You cannot set up or edit a sequence template with | Note that the sequence template still contains the information for the method from Exercise 3 but no longer identifies calibration standards. a On the selection tree, select Sequence Template. b On the sample table, select the calibration standard for row one. c Select Calibration Standard from the Sample Type list. d Move to another row or click the Apply button. e Repeat steps b-d for the next two standards. f Select the standard in the first row. g Click the Insert button in the toolbar. h Change the Sample Name of the second standard to Cal2. i Set the Vial# to 3 and the Calibration Level to 2. | | | | | |
| up calibration in Data Analysis. | K Repeat steps g-j for the next two standards. I Select the last two sample rows, and click the Delete button. | | | | | |
| 2 Set up to quantify the first sample, Sample 1_2, immediately. When you make this selection, Sample 1_2 will be quantified using | a Double-click the cell for Sample 1_2 under the heading, Immediate Quantitation. b Double-click the Yes that appears. | | | | | |
| the first set of calibration standards. | Sampla Name Sample Ture Cal. Immediate Custom Sample Vial Injections | | | | | |

| | Sample Name | Sample Type | Cal. Level | Immediate Quantitation | Custom Sample Group | Vial # | Injections # | |
|----|-------------|-------------|---------------|---------------------------|------------------------|-----------|-----------------|---|
| 1 | cal1 | Calibration | 1 | NO | | 2 | 1 | ĺ |
| 2 | cal2 | Calibration | 2 | NO | | 3 | 1 | [|
| 3 | sample 1_2 | Sample | | YES | | 5 | 1 | [|
| 4 | sample 1_4 | Sample | | NO | | 9 | 1 | |
| 5 | cal1 | Calibration | 1 | NO | | 2 | 1 | ſ |
| 6 | cal2 | Calibration | 2 | NO | | 3 | 1 | [|
| 7 | sample 1_2 | Sample | | NO | | 5 | 1 | [|
| 8 | sample 1_4 | Sample | | NO | | 9 | 1 | [|
| 9 | cal1 | Calibration | 1 | NO | | 2 | 1 | ĺ |
| 10 | cal2 | Calibration | 2 | NO | | 3 | 1 | į |
| 11 | | | | | | | | ĺ |

Sample 1_2, along with the other samples, will also be quantified at a later time using the average of all the

calibration standards.

Detailed Instructions

| 3 | Use the default compound amounts for all standards. | a Click the Amounts tab on the Sample Entry panel b For each calibration standard: Select the standard in the sequence table. Under Compound amounts, mark the Use check boxes for dimethylphthalate and biphenyl. | | | | | | | | | |
|---|---|---|-----------------------------|-------------|---------------|---------------------------------|------------------------|-----------|-----------------|-----------------------------|---------------------------|
| | | | Sample Name | Sample Type | Cal. Level | Immediate Quantitation | Custom Sample Group | Vial # | Injections # | Injectior Volume [µl] | ¹ Samp Amou |
| | | 1 | cal1 | Calibration | 1 | NO | | 2 | 1 | as method | 0 |
| | | 2 | cal2 | Calibration | 2 | NO | | 3 | 1 | as method | 0 |
| | | 3 | sample 1_2 | Sample | | YES | | 5 | 1 | as method | 0 |
| | | 4 | sample 1_4 | Sample | 4 | NO | | 9 | 1 | as method | 0 |
| | | 5 | call2 | Calibration | 2 | NO | | 2 | 1 | as method | 0 |
| | | 7 | sample 1 2 | Sample | ~ | NO | | 5 | 1 | as method | 0 |
| | | 8 | sample 1_4 | Sample | | NO | | 9 | 1 | as method | 0 |
| | | 9 | cal1 | Calibration | 1 | NO | | 2 | 1 | as method | 0 |
| | | 10 | cal2 | Calibration | 2 | NO | | 3 | 1 | as method | 0 |
| | | 11 | | | | | | | | | |
| | | Sampl | e Name: | | Run -Sai | Amounts Ider | tification Description | Compo | und amounts | | |
| | | Sampl | e Type: bration Standard | • | s | ample Amount: 0 | | Use | Name | | Amount |
| | | - 1 · | | | Sa | mple Amount U | ı/ml | R | dimethylphthal | ate (u 40 | |
| | | Custo | n Sample Group: | ▼ New | | Multiplier: 1 | | | diethylphth | alate: 0 | |
| | | | | | _ | Dilution Factor: 5 | | | bipheny | l [ug]: 60 | _ |
| | | Vial N 3 | umber Injections | Volume [µl] | | Purity: 1 rrection Factor: 2 | | | | | |

Steps

Task 6. Select a new report template for a report

| St | eps | Detailed Instructions | | | | |
|----|--|---|--|--|--|--|
| 1 | Select a report template for a single standard injection report | a On the selection tree, select Reporting. b On the Reporting table, select the Standard single injection report type. c Click the Select Template button. The Select Report Template dialog box appears. d On the Select Report Template dialog box, select the template for the Standard Single Injection Detailed report. e Click OK. | | | | |
| | | Iemplates DK Iemplates DK Implates Cancel Implates Dk Implates Cancel Implates Dk Implates Cancel Implates Dk Implates Cancel Implates Devices.html (Instrument) Implates Implate single injection report) Implate Implate single single injection detailed report) Implate Implate single injection Condensed Report) Implate Implate single injection Detailed Report) Implate Implate single injection Condensed Report) Implate Implate single injection Detailed Report) Implate Implate single injection Condensed Report) | | | | |
| 2 | Select these report types to print: Sample single injection Standard single injection | a Double-click the Print cell for the Multi-Injection Summary Group report to change Yes to No. b Repeat step a for the Calibration Standards Group report to change Yes to No. | | | | |

| Print | Report Type | Report Template | | |
|-----------|-------------------------------|------------------|--|--|
| Yes | Sample single injection | exer5injdec.html | | |
| Yes | Standard single injection | sin_d.html | | |
| Yes | Multi-Injection Summary Group | Smp_short.htm | | |
| No | Calibration Standards Group | Cal_short.htm | | |
| No | QC Sample Group | QC_short.htm | | |
| Yes | Sample Group | exer5sgdec.html | | |
| No | Custom Sample Groups | Sum_short.htm | | |
| Yes | Sequence | Seq_short.htm | | |
| No | Customer Report 1 | Composite_1.xml | | |
| No | Customer Report 2 | Composite_2.xml | | |
| No | Customer Report 3 | Composite_3.xml | | |
| | | | | |
| Select Te | mplate Edit Template | | | |

3 Save the method.

a On the Standard toolbar, click 🔛 , and enter your reasons for changes and electronic signature, if required.

Advanced Exercise #5 Set up a multi-level calibrated method for a sequence



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Advanced Exercise #6 Set up a method for a sequence to quantify impurities

This exercise contains a series of tasks to learn how to:

- Include custom, noise and system suitability calculations in the method for a sequence
- Include bracketed calibration and ISTD quantitation in the method
- Set up a custom calculation to average the percent impurities of all the samples in the sequence over multiple injections
- Set limits for custom and system suitability calculations
- Set up a sequence template for bracketing, multiple injections and a blank run for a S/N calculation
- Set up the Result View layout to see the custom and system suitability calculations.
- Edit a report template for a sample group report to include the custom and system suitability calculations

You can use this method with "Advanced Exercise #5a Run a sequence to quantify impurities" on page 61 and "Advanced Exercise #5b Use a different method to reprocess" on page 67.

In the tasks in this exercise, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

Read "Setting Up Methods" on page 71 to set up methods.



Task 1. Copy a method to create a new method template for a sequence

Steps

Detailed Instructions

- 1 Copy the method to create a new template.
 - Copy either exer4*iii* or defexer4*iii*. You can use the original method from Exercise 4 or the modified method from Exercise 4b.
 - Name the method template, exer5iii, where iii are your initials.

Note that the Method Wizard panels contain the method selections in Exercise 4.

- a Select File>New>Method or click D and select Method. The Method Wizard appears.
- b Click the Browse button, and select exer4iii or defexer4iii.
- c Enter exer5iii in the New Method Name box.

| New Method name : exer5dec | |
|--|--|
| | |
| Do you want to select an existing M a template for the new Method ? exer4dec | ethod as Browse |
| What kind of Method do you want to create ? | ⊙ <u>S</u> ingle Sample ⊙ S <u>e</u> quence |
| | Do you want to select an existing M a template for the new Method ? exer4dec What kind of Method do you want to create ? |

| Steps | Detailed Instructions | Detailed Instructions | | | | | |
|--|---|--|--|--|--|--|--|
| 2 Include the capability to set up custom calculations and system suitability calculations | a On the Data Analysis panel, mark the Custom Calculations check box. b Mark the Include Noise Calculations check box. Note that when you mark the Include Noise Calculations check box, the Include System Suitability check box appears marked and dimmed. | | | | | | |
| | Method Wizard | | ? > | | | | |
| | Data Analysis | Do you want to include Compound Identification? | Compound Identification | | | | |
| | | Do you want to include UV Spectral Compound Purity? | 🔲 UV Purity | | | | |
| | | Do you want to include UV Spectral Compound Confirmation? | UV Confirmation | | | | |
| | | Do you want to include Calibration and Quantitation? | Calibration and Quantitation | | | | |
| | | Do you want to use Custom Calculations? | Custom Calculations | | | | |
| | | Do you want to include Noise Calculation? | Include Noise Calculations | | | | |
| | | Do you want to include System Suitability Calculations? | Include System Suitability Calculations | | | | |
| | Click Next to scroll to the Co | Impound Table panel. | | | | | |
| 3 Select a Compound Table option. | a On the Compound Table pan | el, Select Keep Compound C | alibration from | | | | |

Even though you are changing the mode of calibration to Bracketing, you can keep the calibration setup from Exercise 4.

Method template.



b Click **Next** until you reach the **Calibration** panel.

| St | teps | Detailed Instructions | | | | | | |
|----|--|---|---|--|--|--|--|--|
| 4 | Select Calibration options. | a On the Calibration panel, select Bracketing . | | | | | | |
| | Select Bracketing and keep all other options the same. | er Method Wizard | | | | | | |
| | | Calibration | Do the standards in your method always contain Fixed Amounts or Variable Amounts? | Variable Amount Fixed Amount | | | | |
| | | | Does this method use more than one concentration level of the calibrated compound(s)? | 🔽 Multi Level 🛛 🛛 🛛 | | | | |
| | | | What kind of Calibration do you need? | Overall Calibration Single Update Calibration Bracketing | | | | |
| | | | What kind of Calibration Procedure do you need? | Instrument Specific Calibration | | | | |
| | | | | Sequence Specific Calibration | | | | |
| | | b Click Next to scroll to the Quar | ntitation panel. | | | | | |
| 5 | Select Quantitation options | a On the Quantitation panel, manb Select ISTD. | rk the Limit checks check | box. | | | | |
| | | Method Wizard | | ? | | | | |
| | | Quantitation | Do you want to include limit checks on the calculated results ? | Limit checks | | | | |
| | | | Which Calibration Mode do you want to use in your Method ? | € ESTD © ISTD | | | | |
| | | Click Next to scroll to the New | Method Beview papel | | | | | |

6 Review your new method template.

The new method contains the same data analysis and sequence template information as in the method for Exercise 4.

- a On the New Method Review panel, review the Method Wizard Settings.
- **b** Click the **Finish** button to save your new method.
- c Save the changes to the database, with a reason, if necessary.

Task 2. Edit quantitation for an internal standard

Steps

Detailed Instructions

1 Set up the ISTD quantitation.

Set biphenyl as the internal standard and use it for the quantitation of dimethylphthalate.

- a Expand the method that you just created, and expand the Data Analysis folder.
- **b** On the selection tree, select **Quantitation Setup**.
- c Click the Calibrated Compounds tab.
- **d** On the calibration table, select biphenyl.
- e Under Internal Standard, mark Set this Compound as the ISTD.
- f Select dimethylphthalate.
- g Under Internal Standard, mark **Use ISTD compound**.
- h Click the down arrow, and select biphenyl from the list.

| Compound Name | Expected Time | Compound Group | ISTD | ISTD Name | |
|-------------------|------------------------------------|----------------|----------------|-----------|---|
| dimethylphthalate | 0.9349 | | | biphenyl | |
| biphenyl | 1.8902 | | ISTD | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Compound Name | dimethylphthalat | e | | | |
| Compound Name | dimethylphthalat | e | Compound Group | | |
| Compound Name | dimethylphthalat | e | Compound Group | New. | 1 |
| Compound Name | dimethylphthalat nd as the ISTD | e | Compound Group | New |] |

Task 3. Set up a custom calculation to average the percent impurities of all the samples in a sequence

| Steps | Detailed Instructions |
|--|--|
| Set up to calculate the percent of impurity in each single injection. The isocratic standard is a well-defined sample with known compounds. To help you learn how to set up a custom calculation, pretend that the composition of the isocratic standard is the following: Main compound - dimethylphthalate Specified impurity - diethylphthalate ISTD - biphenyl Unspecified impurity - unknown peak You can also point and drag the cell reference to specify the cells in the calculation. | a In the selection tree, select Custom Calculations under Data Analysis. b Click the Single Injection tab, if necessary. c Add a column that contains the Amount variable for all compounds/peaks. Right-click the table, and select Add Column. In the Existing Column sheet, expand Compounds and select Amount. Click Apply. d Add a column for the percent specified impurity calculation. Click the Add a New Custom Calculation Column tab. Enter the Variable ID for the specified impurity as anything you want, e.g. PercentSpecifiedImpurity (no spaces). Enter the Display Name, e.g., Percent Specified Impurity. Select the Level as Single Inj. Variables, then click Apply. e Add a column for the percent unspecified impurity calculation. Enter the Variable ID, the Display Name, and select the Level as Single Inj. Variables, and click OK. f Enter the formula for the percent specified impurity calculation into the Single Inj. Variables cell. Enter the syntax =D8 / SUM (D7 : D13) * 100, which represents the amount of diethylphthalate divided by the sum of the amounts of all the peaks x 100. You can use the f_x button to find the SUM function, or you can type SUM. g Enter the formula for the percent unspecified impurity calculation into the Single Inj. Variables cell. (Use same syntax as for the specified impurity.) |
| | 4 Single Injection 5 Single Inj. Variables |
| | 6 - Identified Compounds |

0.9993

1.9968 3.0126

4.0158

4.9725

6.0583

7

8

9

11

12 13

dimethylphthalate

diethylphthalate

biphenyl 10 - Not Identified Peaks

Unknown 1

Unknown n

| Steps | Detailed Instructions | | | | |
|---|---|---|--|--|--|
| 2 Set up to calculate the average percent of impurity for all injections of a sample. Do this for both the specified and unspecified impurity. | a On the Custom Calc b Add a column for pe Right-click the tat On the Existing Co Specified Impurit Click Apply. c Add a column for the Click Apply. d Add a column for the Click Apply. d Add a column for the Click the Add a Ni Enter the Variable Enter the Display Enter the Level as e Add a column for the injections of a samp Enter the Variable Click OK. f Enter the formula for Multiple Inj. Variable Enter the syntax = percent impurity or f_x button to acces | ulations worksp rcent specified ole, and select A olumn sheet, exp y . e percent unspense specified Impu e average of the e UD as anything Name as a varia is Multiple Inj. Va e average of the le. ID, Display Nar r the average of e cell. =AVERAGE(D6:E calculation for eas s the AVERAGE r the average of | ace, click t mpurity. dd Column band User cified impurity. percent sp culation Co you want, ant of the l riables, an percent u he and Lev the percer 8), which inch sample function, of the percer | the Multi 1. Defined, urity. Decified in blumn tal e.g., Avg D, e.g., A d click A nspecifie el as Mu nt specifie represent e or all inj pr you can t unspec | -injection tab. and select Percent mpurity for all injections. b. PercentSpecified. vg Percent Specified. pply . d impurity for all ltiple Inj. Variables. ed impurity into the ts the average of the ections. You can use the n type AVERAGE. |
| | | D | | 6 | |
| | | DE | r Now | G | |
| | 2 | Percent Percent Specified Unspecifi Impurity Impurity | Avg Percent d Specified | Avg Percent Unspecified | |
| | 3 - | | | | |
| | 4 Multi-Injection Summary | | | | |
| | 5 - Multiple Inj. Variable | | 2.00 | 2.00 | |
| | 6 Single Inj. #1 | 1.00 0.99 | | | |
| | 7 | 2.00 2.02 | | | |
| | 8 Single Inj. #n | 3.01 2.98 | | | |
| | 9 - dimethylphthalate | | | | |
| | 10 Single Inj. #1 | | | | |
| | 11 10 Single Ini #r | | | | |
| | ™ Single inj. #n | <u>Beesseen en een een een een een een een e</u> | | 40000000 | |

Advanced Exercise #6 Set up a method for a sequence to quantify impurities

| S | teps | De | etailed Instructions | | | | | |
|---|--|----------------------------|---|---|--|---|---|---|
| 3 | Set up to calculate the average percent of impurity for all samples. Do this for both the specified and unspecified impurity. | a b c d f g | Click the Sample Gro Add a column for ave Right-click the tab Expand User Defin Click Apply. Add a column for the On the Existing Co Unspecified. Click Apply. Add a column for the Click the Add a Ne Enter the Variable Enter the Display I Enter the Level as Add a column for the samples, e.g., AvgPe Enter the Variable Click OK. Enter the formula for Enter the syntax = percent impurity c access the AVERA | pup tab i erage pe le, and s ned, and e average lumn sh e average w Custo ID as ar Name as Sample e average rcentUA ID, Disp the ave AVERAC alculatic GE func the ave | n the Cus rcent spe elect Ad select Ad e percent eet, expa e of the p om Calcu hything yo s a varian Group Va e of the p IllSample lay Name rage of th GE(F6:F8), on for all s tion, or yy rage of th | stom Calc scified im d Column yg Percen unspecif nd User I ercent sp lation Co bu want, t of the II ariables, a ercent ur s. and Leve he percen which re samples. bu can ty he percen | eulations purity. It Specified in Defined, Defined, Lecified i lumn tal e.g., Avg D, e.g., A und click aspecifie el as San t specifi present You can pe AVER t unspec | workspace. fied. urity. and select Avg Percent mpurity for all samples. b. PercentSAIISamples. Apply. d impurity for all nple Group Variables. ed impurity. s the average of the use the f _x button to CAGE. cified impurity for all |
| | | _ | | | | | | - |
| | | | A B C | D | E | F | G | |
| | | 2 | | Avg Percent Specified | Avg Percent Unspecified | Avg % S All Samples | Avg % U All Samples | ī |
| | | 3 | - | | | | | |
| | | 4 | Samples | | | | | |
| | | 5 | - Sample Group Variable | | | 1.99 | =AVERAGE | - |
| | | 6 | Sample #1 | 0.99 | 1.01 | | (E6:E8) | - |
| | | 7 | | 2.01 | 1.98 | | | |
| | | 8 | Sample #n | 2.97 | 3.01 | | | - |
| | | 9 | - dimethyiphthalate | | | | | - |
| | | 10 | ampie #i | | | | | |
| | | 1.4.4 | | | and a second | | | |

Task 4. Set up limits for the custom and system suitability calculations

| Steps | Detailed Instructions |
|---|--|
| Set up limits for system suitability calculations If tailing factor > than 1.7, then say Not Passed - all samples and only dimethylphthalate If USP resolution < than 1.5, then say Not Passed - all samples and all compounds If signal to noise is less than 5, then say Not Passed. | a Select Limits under Data Analysis. b Make sure the Single Injection sheet appears. c Right-click the Limits table, and select Insert New Limit. d Expand the Peak folder, and select TailingFactor. e From the Condition list, select >, and for Value, enter 1.7. f From the Apply to list, select dimethylphthalate, and click OK. g Repeat steps c and d for Peak resolution USP. h From the Condition list, select < , and for Value, enter 1.5. i Click OK. j Repeat steps c and d for SignalToNoise. k From the Condition list, select <, and for Value, enter 5. |
| dimethylphthalate If USP resolution < than 1.5, then say Not Passed - all samples and all compounds If signal to noise is less than 5, then say Not Passed. | e From the Condition list, select >, and for Value, enter 1.7. f From the Apply to list, select dimethylphthalate, and click OK. g Repeat steps c and d for Peak resolution USP. h From the Condition list, select < , and for Value, enter 1.5. i Click OK. j Repeat steps c and d for SignalToNoise. k From the Condition list, select <, and for Value, enter 5. l Click OK. |

| Li | mit Options for: | | | | | | | |
|----|------------------|-----------------------------|-------------|-----------------------------|-----------|-------|---|-----|
| | Single Injection | Multi Injection | Summary G | roups | | | | |
| | | | | | | | | |
| | Variable ID | Н | eader | Units | Condition | Value | | |
| | | | | | | | | |
| | SignalToNoise | Signa | IToNoise | | < | 5 | | |
| IΓ | TailingFactor | TailingFactor TailingFactor | | TailingFactor TailingFactor | | | > | 1.7 |
| | USP_Resolution | Peak re | olution USP | | < | 1.5 | | |

2 Set up limits for both the average of the specified impurity and the average of the unspecified impurity for all samples.

- If specified impurity > 10%, not passed
- If unspecified impurity > 5%, not passed

Hint: The tab Summary Groups let you set limits for all the variables and calculations associated with sample-type groups, such as sample group, calibration standard group, custom sample group and QC group.

- a Click the Summary Groups tab.
- **b** Right-click the table, and select **Insert New Limit**.
- c In the Insert New Limit dialog box, expand the **Single Values** folder and select Avg % S All Samples.
- d From the **Data Set** list, select Sample.
- e From the Condition list, select >.
- f Enter a value of 10, and click **OK**.
- g Repeat steps b-f for the Avg % U All Samples and a value of 5.

| Variable ID | Header | Units | Data Set | Apply To |
|--------------------------|-------------------|-------|----------|----------------------|
| AvgPercentKAllSamples Av | g % K All Samples | | All | Selected Variable ID |
| AvgPercentUAlISamples Av | g % U All Samples | | All | Selected Variable ID |

Task 5. Edit the sequence template for bracketing and multiple injections

| St | eps | Detailed Instructions | | | | | | | | | | |
|----|---|-----------------------|---|--|---|--|--|---|--|--------------------------------------|---|------------------------------|
| 1 | Set up the brackets Quantify the first set of samples with the average RFs of the first and second sets of standards. Quantify the second set of samples with the average RFs of the second and third set of standards. | a b c d e | Select Seq Double-clia Double-clia Double-clia Double-clia | uence Te ck the Bra ck the Bra ck the Bra ck the Bra | mplat acketi acketi acketi acketi | e in the ng cell 1 ng cell 1 ng cell 1 ng cell 1 | selection to for Cal1 in r for Cal1 in r for Cal2 in r for Cal2 in r | ree. ow 1, ow 5, ow 6, ow 1 | and do and do and do), and d | uble-c uble-c uble-c ouble- | lick Op lick Op lick Cla click C | ien. ien. ose. lose |
| 2 | Enter a blank sample in the first row and enter two injections for each sample. | a b c d | Select row 1, and click the Insert button. (Use tooltip.) Enter NoiseBlank for the Sample Name , and select Blank Run for the Type . Enter a different Vial#, and click Apply . Enter 2 for the Injections # for each sample in the sequence. | | | | | | or the S | lamı | | |
| | | | Sample Name | Sample Type | Cal. Level | Bracketing | Custom Sample Group | Vial # | Injections # | Injection Volume [µl] | Sample Amount | |
| | | 1 | NoiseBlank | Blank Run | | | | 4 | 1 | as method | 0 | |
| | | 2 | cal1 | Calibration | 1 | Open | | 2 | 1 | as method | 0 | |
| | | 3 | calz | Calibratión | 2 | None | | 3 5 | 1 | as method | 0 | |
| | | 4 | sample 1_2 | Sample | | | | 9 | 2 | as method | 0 | |
| | | 6 | cal1 | Calibration | 1 | Open | | 2 | 1 | as method | 0 | |
| | | 7 | cal2 | Calibration | 2 | Close | | 3 | 1 | as method | 0 | |
| | | 8 | sample 1_2 | Sample | | | | 5 | 2 | as method | 0 | |
| | | 9 | sample 1_4 | Sample | | | | 9 | 2 | as method | 0 | |
| | | 10 | cal1 | Calibration | 1 | None | | 2 | 1 | as method | 0 | |
| | | | | | | | | | | | | |

Task 6. Set up the Result View layout to see custom and systemsuitability calculations

| St | eps | Detailed Instructions |
|----|--|--|
| 1 | Set up to view the percent specified impurity and the percent unspecified impurity. | a On the selection tree, expand the Data Review Layout folder. b Select Single Injection in the selection tree. c Select Summary Table in the workspace. d Select Percent Specified Impurity from the Available Items list, and click > to move it to the Display Items list. e Repeat step d for Percent Unspecified Impurity, and click Apply. |
| | | Single Injection Summary Results Table Summary Table Summary Table Unaritation Method (ESTD/S Quanitation Type (Area/Heigh Rel RT Reference Time Sample Amount Up Down |
| 2 | Set up to view the tailing factor, USP resolution and the S/N for each compound. | a Select the Results Table. b Select Tailing Factor from the Available Items list, and click > to move it to the Display Items list. c Repeat step b for Peak resolution USP and SignalToNoise, and click Apply. |
| | | Single Injection Summary Available Columns Display Columns Signal Short Description Signal Short Description Peak To Peak Noise Signal Short Description Peak resolution USP Signal Wander Signal Onite Symmetry Signal Noise Symmetry Signal Onite Display Columns Peak Noise Peak resolution USP Signal Onite Symmetry Signal Onice Up Down |
| 3 | Set up to view the average of the specified impurity and the average of the unspecified impurity for each sample. | a In the selection tree, select Multiple Injection. b Select the Summary Table in the workspace. c Select Avg Percent Specified from the Available Items list, and click > to move it to the Display Items list. d Repeat step b for Avg Percent Unspecified, and click Apply. |
| | | Multi-Injection Summary Results Table Summary Table Sample Amount Sample Name Sample Name |

| Steps | Detailed Instructions |
|--|--|
| 4 Set up to view the average of the percent specified and unspecified impurities in all the samples and their limit checks. | a Select Samples in the selection tree. b Select Summary Table in the workspace. c Select Avg % S All Samples from the Available Items list, and click > to move it to the Display Items list. d Repeat step c for Avg % U All Samples, Avg % S All Samples Limit Check and Avg % U All Samples Limit Check. e Click Apply. |
| | Sample Group Results Table Available Items Display Items Ava & S All Samples Imit Check I |
| | Apply |
Task 7. Edit a report template for the sample group

Steps

Detailed Instructions

- 1 Edit a report template for a sample single injection report.
 - Edit the inj.html report.
 - Add a column for USP resolution and Signal to Noise to the existing compounds table under the chromatogram.
 - Save the template as exer5injiii, where iii is your initials.
- a On the selection tree, select **Reporting**.
- **b** Select the Sample single injection report type, and click **Edit Template...**.
- c Double-click Individual Report Templates, and double-click inj.html.
- d Place the cursor in the last column of the compounds table located beneath the chromatogram.
- e Right-click the table, and select **Table Properties**. The Compound Table Properties dialog box appears.
- f In the Select Column Fields list, mark the Peak resolution USP and SignalToNoise check boxes, and click OK.



The compound table in the resulting template looks like this:

| Retention Time | Compound Name | Amount | Response Factor | Tailing Factor | Peak resolution USP | SignalToNoise |
|-------------------|------------------|--------|--------------------|-------------------|---------------------------|---------------|
| #####.## | × | ###.## | X.DDDD | #####.### | ##.### | ##_### |

g Select File > Save As, enter exer5injiii, and click OK.

| Steps | Detailed Instructions | | |
|--|--|--|--|
| 2 Edit the sample group detailed report template (sus_d.html). Insert an html table under the Sample group variables table. Enter the text for the Avg. % S Impurity All Samples and Avg% U Impurity All Samples. Enter the placeholder for the values for the % impurities. Under the Sample Group Limits table, enter the Limit check information for the sample group. Save the template as exer5sgiii, where iii are your initials. | a Exit the Report Template Editor. b Select the Sample Group report type, and click Edit Template c Double-click Individual Report Templates, and double-click sus_d.html. d Insert a line below the Sample group variables table, and click the Insert HTML table button. e In the Insert Table dialog box, select the Classic Table Style and click OK. f Click the Fields tab and expand the Sample Group folder. g Expand the Sample Group Variables Results folder. h Place the cursor into the first cell of the HMTL table, press the Alt key and double-click Avg % S All Samples. i Place the cursor into the second cell in the first row and double-click Avg % S All Samples. j Repeat steps h and i for Avg % U All Samples, using the second row. k Place the cursor below the Sample group limit results table. I Press the Ctrl key and double-click Avg % S All Samples Limit Check. m Do the same for Avg % U All Samples Limit Check. n Select File > Save As, enter exer5sgiii and click Save. | | |

After you finish, the template displays as the Sample group template

Sample group (detailed)

| Sequence name: | ****** |
|-----------------|--|
| Sequence Start: | sys_Date, sys_Time |
| Sequence End: | sys_Date, sys_Time |
| Method (rev): | XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX |

Number of unidentified peaks: ##

| | Sample grou | p variables | | |
|----|-------------|-------------|--|-----------|
| # | Sample name | Amount | Position | lnj. vol. |
| ## | ****** | ##.DDDD | $\times\!\!\!\times\!\!\!\times\!\!\times\!\!\times\!\!\times\!\!\times\!\!\times\!\!\times\!\!\times\!\!\times\!\!\times\!\!\times$ | ###.DD |

| Avg % S All Samples: | ##.DD |
|----------------------|-------|
| Avg % U All Samples: | ##.DD |

Sample group limit results

| # | Sample name | Compound | Limit (Compound) | Limit (Sample) |
|----|-------------|----------|---------------------|----------------|
| ## | ***** | ***** | ******* | ****** |

Avg % S All Samples Limit Check: XXXXXXXXXXX

Task 8. Select report templates and report types

| Steps | Detailed Instructions | | | |
|---|--|---|---|---|
| Select report templates for report types. Use exer5injiii for the Sample single injection report. Use exer5sgiii for the Sample group report. | a Exit t b Select c Select d Select e Select | he Cerity Report Template et the Sample single injecti et exer5injiii and click OK . et the Sample group report et exer5sgiii and click OK . | Editor. on report type and type and click Sel | d click Select Template ect Template |
| Select these report types to print. Sample single injection Standard single injection Multi-injection summary | a Doub chan b Repe | le-click the Print cell for th ge No to Yes . at instruction (a) for the Sa | e Multi-Injection s ample Group repor | Summary Group report t t to change Yes to No . |
| Sample group Sequence | Yes Yes No No Yes No Yes No No Select T | Sample single injection Standard single injection Multi-Injection Summary Group Calibration Standards Group QC Sample Group Sample Group Custom Sample Groups Sequence Customer Report 1 Customer Report 2 Customer Report 3 emplate | exer5injdec.html sin_d.html Smp_short.htm Cal_short.htm OC_short.htm <u>exer5sodec.html</u> Sum_short.htm Seq_short.htm Composite_1.xml Composite_2.xml Composite_3.xml | |
| 3 Save the method | On the S | Standard toolbar, click | and enter your rea | asons for changes and |

electronic signature, if necessary

Advanced Exercise #6 Set up a method for a sequence to quantify impurities



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Advanced Exercise #7 Calculate the mean area sum of the unidentified impurities per lot

This exercise contains a series of tasks to learn how to set up a custom calculation to calculate the mean of the area sum of the unidentified impurities per lot of samples:

- Set up to sum the peak areas of the unidentified peaks in a single injection
- Set up to average the area sums of the unidentified peaks for all injections of a sample
- Set up to calculate the mean area sum for the samples in the sample group

NOTE

You do not have to identify compounds to set up this calculation so that you can practice the instructions on an empty method.



Task 1. Set up to sum the peak areas of the unidentified peaks in a single injection

| Steps | | Detailed Instructions | | |
|-------|--|--|--|--|
| 1 | On the single injection worksheet, add a column to contain the existing integration result, peak area. | a On the selection tree, expand the folder for the relevant method. b Expand the Data Analysis folder. c Select Custom Calculations. d In the custom calculator workspace, click the Single Injection tab. e Right click in the worksheet and select Add Column from the context menu. | | |
| | | The Add Column dialog box appears. | | |
| | | f In the Existing Column tab, expand the Peaks section and select Peak Area. Click OK to close the dialog box. | | |
| | | ≫ Add Column 🗵 | | |

| Add a new Cu | stom Calculation Column | Existing Column |
|------------------|---|-----------------|
| Existing Items | Peaks Peaks Peaks Peaks Peaks Peaksen Peaksen Expected Rait Peak Height Peaksen Peak Height Peaksen P | Signal gnal |
| Information | | |
| Initial Value(s) | Start Value 1 Prec Increment Value 1 These values are used to fill the co calculation set up. | ision [%] 2 |
| <u>O</u> K | Cancel | Apply |

The workspace now contains a column with the peak areas for the not identified peaks.

| Steps | | Detailed Instructions | | |
|-------|---|--|--|--|
| 2 | Add a column to contain the new calculation for the area sum of the unidentified peaks. | a Right click in the worksheet and select Add Column from the context menu. The Add Column dialog box appears. b Click the Add a new Custom Calculation Column tab. c Enter Area Sum Not Ident Peaks in the Display Name field. d Click the Level down arrow and select Not Identified Peaks Summary. | | |
| | | Add a new Custom Calculation Column Existing Column Display Name Area Sum Not Ident Peaks Level Not Identified Peaks Summary Units: The workspace now contains a column for the new variable Area Sum Not Ident Peaks. | | |
| 3 | Enter the formula for the area sum of the unidentified peaks. | a In the Not Identified Peaks summary line of the new column, enter the formula to sum the areas of the unidentified peaks. Hint: Use the syntax, =SUM(D8:D10). | | |
| | | A B C D E 1 New New 2 Peak Area Sum 3 - 4 Single Injection 5 Single Injection 6 - Identified Compounds 6 - Identified Compounds 6 - Identified Peaks | | |

0.9993

1.9968 3.0126

Unknown 1

.. Unknown n

8 9

10

Task 2. Set up to average the area sums of the unidentified peaks for all injections of a sample

| S | teps | Detailed Instructions |
|---|--|--|
| 1 | On the multi-injection summary worksheet, add a column to contain the variable set up in the single injection worksheet, the area sum of the unidentified peaks. | a In the custom calculator workspace, click the Multi Injection tab. b Right click in the worksheet and select Add Column from the context menu. The Add Column dialog box appears. c In the Existing Column tab, expand the User Defined section and select Area Sum Not Ident Peaks. Click OK to close the dialog box. |
| | | Add Column X Add a new Custom Calculation Column Existing Column |

| Evisting Itoms | Realized Development | |
|----------------|--------------------------|--|
| E Aloung Homo | Multiple Inj. Hesuits | |
| | Method Settings | |
| | Compounds | |
| | Peaks | |
| | -User Defined | |
| | Area Sum Not Ident Pasko | |

The workspace now contains a column with the area sum for the unidentified peaks.

- 2 Add a column to contain the new calculation for the mean of the area sums of the unidentified peaks for all the injections.
- Right click in the worksheet and select Add Column from the context menu.
 The Add Column dialog box appears.
- **b** Click the **Add a new Custom Calculation Column** tab.
- c Enter Mean Area Sum Not Ident Peaks in the Display Name field.
- d Click the Level down arrow and select Not Identified Peaks Variables.

| dd a new Custo | n Calculation Column Existing Column |
|----------------|--------------------------------------|
| Display Name | Mean Area Sum Not Ident Peaks |
| Level | Not Identified Peaks Variables |
| | _ |

The workspace now contains a column for the new variable **Mean Area Sum Not Ident Peaks**.

| Steps | Detailed Instructions |
|--|--|
| 3 Enter the formula for the mean of the area sums of the unidentified peaks for all the injections. | a In the Not Identified Peaks variables line of the new column, enter the formula to average the area sums of the unidentified peaks. Hint: Use the syntax, =AVERAGE(D8:D10). |
| | A B C D E 1 Area Sum Mean Area Not listri. New Sum Not listri. Sum Not listri. 2 Peaks Ident. Peaks |

| - | | | 140.44 |
|----|------------------------|---------------------------------|--------------------------------------|
| 2 | | Area Sum Not Ident. Peaks | Mean Area Sum Not Ident. Peaks |
| 3 | - | | |
| 4 | Multi-Injection Summ | harγ | |
| 5 | - Multiple Inj. Variab | le in in in it | |
| 6 | Single Inj. #1 | | |
| 7 | | | |
| 8 | Single Inj. #n | | |
| 9 | - Not Identified Peal | (S | 2.00 |
| 10 | Single Inj. #1 | 0.99 | |
| 11 | | 2.02 | |
| 12 | Single Inj. #n | 2.98 | |

Task 3. Set up to calculate the mean area sum for the samples in the sample group

| S | teps | Detailed Instructions |
|---|---|--|
| 1 | On the sample group worksheet, add a column to contain the variable set up in the multi- injection worksheet, the mean of the area sums for all injections. | a In the custom calculator workspace, click the Sample Group tab. b Right click in the worksheet and select Add Column from the context menu. The Add Column dialog box appears. c In the Existing Column tab, expand the User Defined section and select Mean Area Sum Not Ident Peaks. Click OK to close the dialog box. |
| | | The workspace now contains a column with the mean area sum for the not identified peaks. |
| 2 | Add a column to contain the new calculation for the mean of the area sums of the unidentified peaks for a sample lot. | a Right click in the worksheet and select Add Column from the context menu. The Add Column dialog box appears. b Click the Add a new Custom Calculation Column tab. c Enter Mean Area Sum Lot in the Display Name field. d Click the Level down arrow and select Not Identified Peaks Variables. |
| | | Add a new Custom Calculation Column Existing Column Display Name Mean Area Sum Lot Level Not Identified Peaks Variables Unite: The workspace now contains a column for the new variable Mean Area Sum Per Lot. |

| St | teps | D | etailed Inst | truct | tions | |
|----|--|--------------|---|------------------------------|---------------------------------------|--------------------------|
| 3 | Enter the formula for the mean of the area sums for the lot. | a | In the Not formula to Hint: Use | t Ide ave the s | ntified erage tl syntax, | Peaks ne area =AVE |
| | | 1 | A B C | | D | E |
| | | 2 | | | Mean Area Sum Not Ident. Peaks | Mean Area Sum per Lot |
| | | 3 | - Samples | | | |
| | | 5 6 7 | Sample Group ∨ Sample #1 | √ariable | 2 | |
| | | 8 9 10 | Sample #n - Not Identified Pe Sample #1 | eaks | 1.00 | 2.01 |
| | | 11 12 | Sample #n | | 2.02 3.01 | |

Advanced Exercise #7 Calculate the mean area sum of the unidentified impurities per lot



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Advanced Exercise #8 Set up a Group Identifier with calculations for system suitability

This exercise contains a series of tasks to learn how to set up a custom calculation to calculate the ratio of the resolutions of the first and last peaks, and stop the sequence if it falls outside a defined range:

- Set up a method to include System Suitability Calculations
- Set up the custom calculations for the system suitability test
- Set up the limit conditions
- Identify the system suitability samples in the sequence table



Task 1. Set up a method to include System Suitability Calculations

Steps

Detailed Instructions

- 1 Create a new sequence method
 - Name the method ssmethiii, where *iii* are your initials.
 - Use exer2iii or defexer2 as the template for the new method.
- a Select File > New > Method or click and select Method.
 The Method Wizard New Method panel appears.
- b Click the Browse button and select exer2*iii* or defexer2 from the Method Template Selection dialog box.
- c Enter ssmethiii in the New Method Name box.
- d Select Sequence.



- e Click Next until you reach the Data Analysis panel.
- f Mark the Calibration and Quantitation, Custom Calculations and Include System Suitability check boxes.



have completed the Method Wizard.

Steps

•

2 Select an example chromatogram

Run a sequence to quantify

Or. use defexchrom2a.

results".

compounds with single-level

calibration" and "Basic Exercise

#3b Reintegrate and reprocess the

• Use the example chromatogram

you produced with Basic Exercise

2a or 2b of the "Basic Exercise #3a

Detailed Instructions

- a On the selection tree, expand the exer3iii folder.
- b Expand the Data Analysis folder.
- c Select the Example Chromatogram item.
- d On the Tools toolbar, click AA.



AllSamplesNotApprovedRunLast7Days\Samples\defexchrom2a [Rev 2]\defexchrom2a #1 [Rev 1]

- e Select the sample name with the injection number to produce the example chromatogram.
- f Click the Select button.

The example chromatogram appears in the workspace.



After you have selected the example chromatogram, you can see the q integration and identification settings that belong to the original method. h Click Save if the Save Changes to the Database dialog box appears.

Task 2. Set up the custom calculations for the system suitability test

Steps

1 On the multi-injection summary

worksheet, add a column to contain

the resolution of each component.

Detailed Instructions

- a In the Data Analysis folder, select the Custom Calculator item.
- **b** In the custom calculator workspace, click the **Multi Injection** tab.
- c Right click in the worksheet and select Add Column from the context menu.

The Add Column dialog box appears.

d In the Existing Column tab, expand the Peaks section and select Peak resolution USP. Click OK to close the dialog box.



The workspace now contains a column with the peak resolutions for each injection for each component.

- 2 Add a column to contain the new calculation for the mean of the peak resolutions for the replicate injections.
- Right click in the worksheet and select Add Column from the context menu.
 The Add Column dialog box appears.
- **b** Click the **Add a new Custom Calculation Column** tab.
- c Enter Mean Resolution (Injections) in the Display Name field.
- d Click the Level down arrow and select Compound.
- e Set the Number of Decimals to 4.

| Display Name Mean Resolution (Injections) | |
|---|--------|
| | |
| Level Compound | Ŧ |
| | inito: |
| 0 | (ms. j |
| Precision | |
| • Number of Decimals (020) | 4 |

The workspace now contains a column for the new variable **Mean Resolution** (Injections).

| 3 Enter the formula for the mean of the resolutions for the replicate injections for each compound. a In each of the compound variables lines of the new column, ent to average the resolutions of the replicate injections. Hint: Use the syntax, =AVERAGE(D10:D12). A B C D E New Peak New Pea | Steps | De | etailed Instructio | ns | | | |
|--|---|----|---|---------------------------------|-------------------------------|--|------|
| A B C D E 1 Peak resolution USP New Peak resolution USP New Peak resolution USP 3 - - 4 Multi-hjection Summary - 5 - Multi-hjection Variable - 6 Single hjection #1 - 7 - - 8 Single hjection #1 0.999 10 Single hjection #1 0.999 11 1.997 12 Single hjection #1 0.919 13 - (destry-hydrhalate 5.0156 | 3 Enter the formula for the mean of the resolutions for the replicate injections for each compound. | а | In each of the co to average the r Hint: Use the sy | ompounc esolutio ntax, =A | l variat ns of th VERA(| es lines of the new column, e ereplicate injections. E(D10:D12). | ente |
| 1 New 2 Paid 3 resolution 4 Multi-Injection Summary 5 - [Multi Injection Variable 6 Strigle Injection #1 7 8 Single Injection #1 9 - [dimethr/spithalate 11 12 Single Injection #1 13 - [dimethr/spithalate 13 - [dimethr/spithalate | | | АВ С | D | E | | |
| 2 Peak Mean 2 resolution Resolution 3 - - 4 Multi-hjection Summary - 5 - [Multi-hjection Variable - 6 - [Multi-hjection Variable - 7 Single injection #1 - 8 Single injection #1 - 9 - dimethydythradate 2.0029 10 Single injection #1 0.999 11 - 1.997 12 Single injection #1 0.301 13 - [diettrioption #1 4.015 | | 1 | | | New | | |
| 2 resolution USP Hesolution (vijections) 3 - - 4 Multi-injection Summary 1 5 - Multi-injection Variable 1 6 Single Injection #1 1 7 | | | | Peak | Mean | | |
| 3 - 4 Multi-hjection Summary 5 - (Multi-hjection Variable 6 Sknje hjection #1 7 - 8 Sknje hjection #1 9 - (dimethydphthalate 10 Sknje hjection #1 11 | | 2 | | USP | (Injections) | | |
| 4 Multi-lijection Summary 1 5 - [Multi-lijection Variable 1 6 Single Injection #1 1 7 | | 3 | - | | | | |
| 5 - Multi Injection Variable 6 Single Injection #1 7 8 Single Injection #n 10 Single Injection #1 11 12 Single Injection #n 13 - diathylyphthalate 13 - diathylyphthalate 4 Single Injection #n | | 4 | Multi-Injection Summary | | | | |
| 6 Single Injection #1 7 8 Single Injection #n 9 - dimethylphthalate 10 Single Injection #1 11 12 Single Injection #n 13 - dimethylphthalate 13 - dimethylphthalate 5 Single Injection #1 4 Single Injection #1 | | 5 | Multi Injection Variable | | | | |
| 7 | | 6 | Single Injection #1 | | | | |
| 8 Single Injection #n 2 9 - dimethyphthate 2.0029 10 Single Injection #1 0.990 11 1.997 12 Single Injection #n 3.013 13 - distribution #1 4.016 | | 7 | | | | | |
| 10 Single injection #1 0.999 2.0023 11 1.997 1.997 12 Single injection #n 3.013 5.0156 13 - diethylphthalate 5.0156 1.916 | | 8 | Single Injection #n | | 2,0000 | | |
| 10 Single injection #n 3.013 11 1.997 12 Single injection #n 3.013 13 - distripriprintinate 5.0156 14 Single injection #1 4.018 | | 9 | jumetriviprimatate Single Injection #1 | 0.999 | 2.0028 | | |
| 12 Single Injection ≇n 3.013 13 - (attriy/pathtblate 5.0156 14 Single Injection ≇1 4.016 | | 11 | Single injection #1 | 1.997 | | | |
| 13 - diethylpithiaite 5.0156 14 Single injection #1 4.016 | | 12 | Single Injection #n | 3.013 | | | |
| 14 Single Injection #1 4,016 | | 13 | - diethylphthalate | | 5.0156 | | |
| | | 14 | Single Injection #1 | 4.016 | | | |

4 On the Group Identifier worksheet, add a new Group Identifier for the system suitability samples. a Right click in the worksheet and select Add/Modify Group Identifiers from the context menu.

The Add/Modify Group Identifiers dialog box appears.

b Enter SySSuit in the New Group Identifier Name field and click Add.

| Add/Modify Group Identifiers |
|------------------------------|
| |
| |
| |
| • |
| New Group Identifier Name |
| SysSuit |
| Add Rename Remove |
| <u>Q</u> K <u>Cancel</u> |

c Click OK to close the Add/Modify Group Identifiers dialog box. The workspace now contains new groups of lines under each section for the

Group Identifier SysSuit.

Advanced Exercise #8 Set up a Group Identifier with calculations for system suitability

| Steps | Detailed Instructions |
|---|--|
| 5 On the Group Identifier worksheet, add a column to contain the mean resolution of each component. | a Right click in the worksheet and select Add Column from the context menu. The Add Column dialog box appears. b In the Existing Column tab, expand the User Defined section and select Mean Resolution (Injections). Click OK to close the dialog box. |
| | Add a new Custom Calculation Column Existing Column Existing items Sample Croup Results Sample Custom Values Sample Custom Values Sample Custom Values The workspace now contains a column with the mean peak resolutions for each component. Note that the worksheet sets up sub-group identifiers for each component; this exercise does not use sub-group identifiers, so the only numbers of interest are under Sub group identifier #1 in each case. To simplify the worksheet, you can collapse the and Sub group identifier #2 rows for each compound. |
| 6 Add a column to contain the new calculation for the mean of the pea resolutions for different samples. | a Right click in the worksheet and select Add Column from the context menu. The Add Column dialog box appears. b Click the Add a new Custom Calculation Column tab. c Enter Mean Resolution (Samples) in the Display Name field. d Click the Level down arrow and select Compound Variable (Group Identifier). |
| | Edit Column Display Name [Mean Resolution (Samples)] Level Compound Variable (Group Identifier) Units: The workspace now contains a column for the new variable Mean Resolution (Samples). |

| 31 | eps | Detailed Instruction | S | | |
|----|---|--|-------------------------------------|--|-------------------------|
| 7 | Enter the formula for the mean of the resolutions across samples for each compound. | a In the SysSuit line formula to averag Hint: Use the synt | e of the r e the res :ax, =AV | w column for dimethy utions of the samples AGE(F22:F24). | phthalate, enter the |
| | | b Extend the select Mean Resolution | ion to ind (Sample | ide the SysSuit lines f) column. | or each compound in the |
| | | Hint: Hold the left | mouse | tton down while seled | ting the cells. |
| | | • Bight click in the | vorksha | and select Fill Down | from the context menu |
| | | A B C D E | F | G New Mean | |
| | | 2 | Resolution (Injections) | esolution amples) | |
| | | 3 - | | | |
| | | 3 - 4 Group Identifier | | | |
| | | 3 - 4 Group Identifier 5 - Sample Group Variable 6 + SvsSuit | | | |
| | | 3 - 4 Group Identifier 5 - 5 - 6 + 5 + 5 + 5 - 6 + 19 - 19 - | | | |
| | | 3 - 4 Group Identifier 5 - 5 - 6 + 7 SysSut 10 - 9 - 9 - 5 - 5 - | | 301 | |
| | | 3 - 4 Group Identifier 5 - Sample Group Variable 6 + SysSuit 19 - dimethylphthalate 20 - SysSuit 21 - Sub group Identifier #1 21 - Sub group Identifier #1 | | 301 | |
| | | 3 - 4 Group Identifier 5 - 6 + 9 - 19 - dimethylphthalate - 10 - 20 - 21 - 22 Sample #1 23 - | 1.0045 | 300 | |
| | | 3 - 4 Group Identifier 5 - Sample Group Variable 6 + SysSuit 19 - dimethylphthalate 20 - SysSuit 21 - Sungble group Identifier #1 22 Sample #1 23 24 Sample #n | 1.0045 3.0061 5.0059 | 3.01 | |
| | | 3 - 4 Group Identifier 5 - 5 - 6 + 10 oimethylohthalate 20 - 21 - 22 Sample 41 23 24 Sample #n 25 24 Sample #n | 1.0045 3.0061 5.0059 | 3.01 | |
| | | 3 - 4 Group Identifier 5 - 5 - 6 + 7 Group Variable 6 + 7 Group Variable 20 - 21 - 22 Sample #1 23 - 24 Sample #1 25 + 4 Sub group Identifier #1 28 - 4 Sub group Identifier #1 | 1.0045 3.0061 5.0059 | 301 | |
| | | 3 - 4 Group Identifier 5 - 6 + 19 - dimethylphthalate - 20 - - Sub group Identifier #1 21 - 23 - 24 Sample #1 25 4 Sample #1 5 24 23 24 24 26 + 24 + 27 28 + | 1.0045 3.0061 5.0059 | 301 | |
| | | 3 - 4 Group Identifier 5 - Sample Group Variable 6 + SyrsSuit 19 - dimethryghthalate 20 - SyrsSuit 21 - Sungle #1 23 - Sungle #1 24 Sample #1 25 + 24 Sample #1 25 + 26 - Sub group Identifier #1 27 - Sub group Identifier #1 33 - diethryghthalate 4 - Sysbuit | 1.0045 3.0061 5.0059 | 301 | |

- **b** Click the **Add a new Custom Calculation Column** tab.
- c Enter StdDev Resolution in the Display Name field.
- d Click the Level down arrow and select Compound Variable (Group Identifier.

| Display Na | me StdDev Resolution | |
|------------|--|---|
| | | |
| 1. | vel Compound Variable (Group Identifier) | - |

The workspace now contains a column for the new variable StdDev Resolution.

Advanced Exercise #8 Set up a Group Identifier with calculations for system suitability

| Steps | | Detailed Instructions | | | |
|---|------------------------------|--|---|---|--|
| Enter the formula for deviation of the mean | the standard resolutions. | Select the SysSuit line of the worksheet and select The Select Function dialo | the nev Select | v colum Functio | n for dimethylphthalate, right click in n from the context menu. |
| | | b Expand the Statistical se | ction, s | elect St | andard Deviation and click Select. |
| | | Sector Function Punctions Most Recently Used Ceneral Average Statustical Average Statustical Statustical Statustical Sum of Population Sum of Supares Variance Variance of Population Rel Stat Dev. Trigonometric Logical Constants | | | |
| | | The STDDEV function is of Add the cell references for The syntax is =STDEV(F2 d Fill down the column with | opied to or the st 2:F24). o the ne | o the se andard w calcu | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of Add the cell references for The syntax is =STDEV(F2 d Fill down the column with | opied to or the st 2:F24). In the ne | o the se andard w calcu | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of c Add the cell references for The syntax is =STDEV(F2 d Fill down the column with | opied to or the st 2:F24). In the ne | o the se andard w calcu H New | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of c Add the cell references for The syntax is =STDEV(F2 d Fill down the column with a b c b e f c Mean Resolution Resolution | G New Besolution (Samples) | b the se andard w calcu H New StaDev Resolution | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of c Add the cell references for The syntax is =STDEV(F2 d Fill down the column with A B C D E F Mean Resolution 3 - 4 Group Identifier | opied to or the st 2:F24). In the ne New Resolution (Samples) | o the se andard w calcu H New StdDev Resolution | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of c Add the cell references for The syntax is =STDEV(F2 d Fill down the column with ABCDEFF | opied to r the st 2:F24). n the ne New Mean Resolution (Samples) | o the se andard w calcu H New StdDev Resolution | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of c Add the cell references for The syntax is =STDEV(F2 d Fill down the column with ABCDEFF | opied to r the st 2:F24). n the ne | o the se andard w calcu H New Stopey Resolution | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of c Add the cell references for The syntax is =STDEV(F2 d Fill down the column with A B C D E F A B | opied to r the st 2:F24). n the ne New Mean Resolution (Samples) | o the se andard w calcu H New StdDev Resolution 2.00 | lected cell. deviation calculation: llation. |
| | | The STDDEV function is of c Add the cell references for The syntax is =STDEV(F2 d Fill down the column with A B C D E F A B | opied to r the st 2:F24). n the ne New Mean Resolution (Samples) | o the se andard w calcu H New StOev Resolution | lected cell. deviation calculation: llation. |

| Steps | Detailed Instructions | | | | | | |
|---|--|--|--|--|--|--|--|
| 10 Add a column to contain the new calculation to be used for system suitability. | a Right click in the worksheet and select Add Column from the context menu. The Add Column dialog box appears. b Click the Add a new Custom Calculation Column tab. c Enter Resolution Ratio in the Display Name field. d Click the Level down arrow and select Sample Variable (Group Identifier. | | | | | | |
| | ► Edit Column Usplay Name Resolution Ratio Level Sample Variable (Group Identifier) Units: The workspace now contains a column for the new variable Resolution Ratio. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylohthalate peak. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20 Iso the syntax, =G62/G20 | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20. | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20 Image: Second S | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20 Image: Source of the syntax is t | | | | | | |
| 11 Enter the formula for the resolution ratio. | a In the SysSuit line of the new column for the Sample Group Variable, enter the formula to ratio the resolution of the o-terphenyl peak with that of the dimethylphthalate peak. Hint: Use the syntax, =G62/G20 Image: Second Seco | | | | | | |

Task 3. Set up the limit conditions

Steps

Detailed Instructions

- 1 On the Group Identifier Limits panel, add a system suitability limit check:
 - If the ratio is greater than 0.9, the check is passed; continue the run.
- a In the Data Analysis folder, select the Limits item.
- **b** In the Limits panel, click the **Group Identifier** tab.
- c Right click in the table header and select **Insert new limit** from the context menu.

The Insert new limit dialog box appears.

d Expand the Sample Variable (Group Identifier) section and select Resolution Ratio.

| , Insert New Limit | | 2 |
|----------------------|---|---|
| vailable Variables : | Compounds | |
| | Compound (Group Identifiers) | |
| | Compound (Sub Group Identifier) | |
| | Unidentified Peaks | |
| | | |
| | Unidentified Peaks (Sub Group Identifier) | |
| | Sample Variables | |
| | Sample Variable (Group Identifier) | |
| | Resolution Ratio | |
| | Sample Variable (Sub Group Identifer) | |
| | | |

- e In the Limits group, set the following parameters:
 - Data Set: SysSuit
 - Apply To: Selected Variable ID
 - Condition: >
 - Value: 0.9
 - Notification: Passed
 - User Action: Continue

| Data Set : | All | • | Apply To : | Selected Va | riable ID | - |
|----------------|------------|---|---------------|-------------|-----------|---|
| Condition : | = | • | Value : | 0 | Units : | |
| Notification : | Not Passed | • | User Action : | Conti | nue | - |

f Click **OK** to add the new limit check to the table.

| S | teps | Detailed Instructions | | | | | | |
|---|--|---|--|--|-------------------------------|--------------------|---------------------------------|---------------------------|
| 2 | Add two more system suitability limit checks: If the ratio is greater than 0.8 (but less than 0.9), give a warning, but continue the run. If the ratio is less than 0.8, the check is not passed; abort the run. | a For each limit c Sample Variable b Set the parame Data Set: Sys Apply To: Sel Condition: > Value: 0.8 Notification: User Action: and Data Set: Sys Apply To: Sel Condition: < Yalue: 0.8 Notification: and Data Set: Sys Apply To: Sel Condition: Value: 0.8 Notification: Value: 0.8 Notification: User Action: | heck, dis le (Grou ters to: sSuit ected Va Warnin Continu sSuit ected Va Not Pas Abort | splay the p Identif ariable II g e ariable II ssed | e Insert n fier) sect D | ew limi ion and | it dialog b select Re | ox, expand solution Ra |
| | | Single Injection Multi Inject | ion) Summa | ry Groups) Gi | roup Identifier | | | |
| | | Header Units | Data Set | Apply To | Condition | Value | Notification | User Action |
| | | Resolution Ratio | | Selected Variable ID Selected | < | 0.75 | Not Passed | Abort |
| | | Resolution Ratio | | Variable ID Selected Variable ID | > | 0.8 | Warning Passed | Continue |

Task 3. Identify the system suitability samples in the sequence table

| Steps | | Detailed Instructions | | | | | | |
|-------|--|---|--|--|--|--|--|--|
| 1 | If necessary, prepare a sequence for the method. | a See "Task 1. Create a new sequence" on page 30. | | | | | | |
| 2 | Enter samples into the sequence table. | a See "Task 2. Enter sample and sequence information" on page 31. | | | | | | |
| 3 | Identify the system suitability test samples in the sequence table. | a Select the system suitability test sample line in the sequence table. b In the Sample Entry tab of the workspace, click the Calculations tab. c Click the Group Identifier down arrow and select SysSuit from the list. | | | | | | |
| | | Sample Type: Group Identifier: Sample Vial Number Injections Volume [µ] I Iss method Stop Time as method Custom Sample Group: New Group New Group | | | | | | |
| | | The group identifier name is added into the Group Identifier column of the sequence table. Samples identified with this name will be used in the custom calculator worksheet and in the limits checks. | | | | | | |

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In This Book

This Getting Started Guide is a collection of basic and advanced exercises that provide a quick way to learn the Cerity Pharmaceutical QA/QC application.

The exercises are grouped into two groups:

Running Routine Samples exercises help lab technicians learn how to run routine samples.

Setting up methods

exercises help Chemists learn how to set up methods for the laboratory.

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