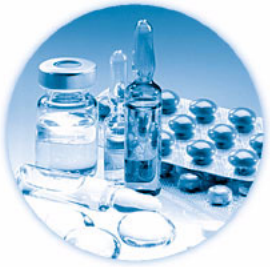

Agilent Cerity Networked Data System for Pharmaceutical QA/QC

Getting Started Guide

Agilent Technologies

Notices

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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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CAUTION

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Contents

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**.

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.

   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.

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 (5µM).

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

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.

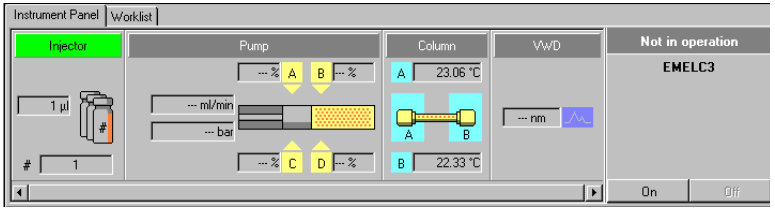
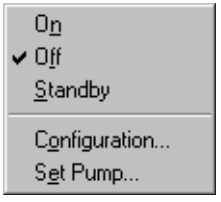
For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

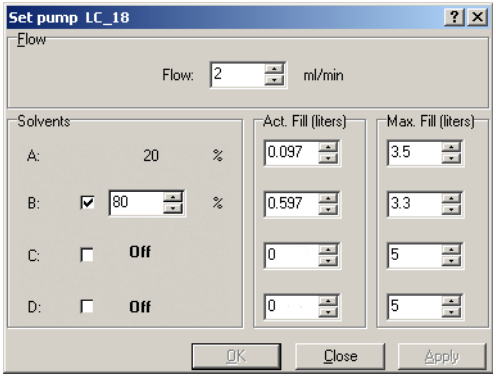
Make sure that the pump is on standby and the VWD lamp is off.

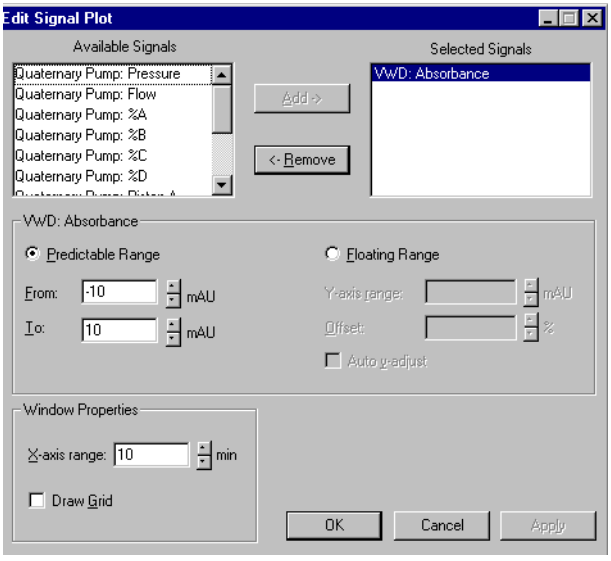
Make sure that the methods for this exercise have been set up or restored.

Task 1. Purge the pump from the Instrument Panel

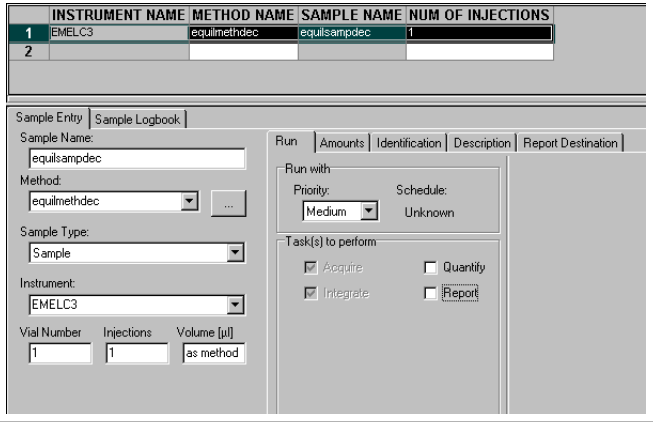
Steps	Detailed Instructions
1 Disengage pump and purge line B. Flow rate: 5ml/min %B = 100%	**a** Turn the black valve on the pump counterclockwise two full turns. **b** Select **Instrument** from the **Current View** list. **c** Select the instrument that you intend to equilibrate. The Instrument Panel appears, along with the Online Plot.  **d** Click the pump module on the Instrument Panel. A menu appears.  **e** Select **Set Pump**. **f** Enter a Flow of 5ml/min and %B=100, and click **OK**.
2 Purge line A and engage pump. %A = 100	**a** When there are no more bubbles in the line, repeat steps d and e from step 1. **b** Set %B = 0, and click **OK**. **c** When there are no more bubbles in the line, click the pump module, and select **Standby**. **d** Tighten the black valve.

Task 2. Equilibrate the instrument from the Instrument Panel

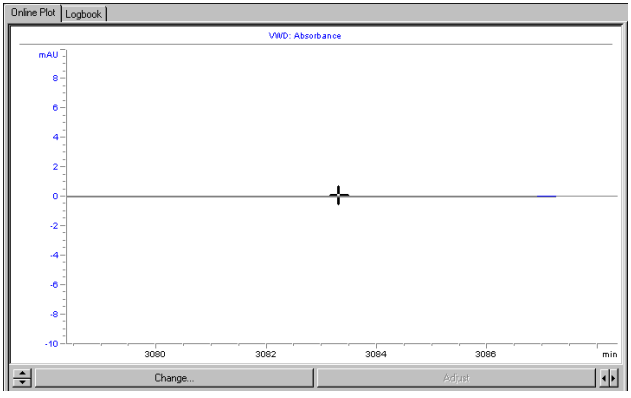
Steps	Detailed Instructions
1 Enter the pump parameters Methanol as Solvent B: • Flow rate: 2ml/min. • Solvent composition: 80%MeOH/20%H_2O Acetonitrile as Solvent B: • Flow rate: 1.5ml/min • Solvent composition: 65%ACN/35%H_2O	**a** Click the pump module on the Instrument Panel. **b** Select **Set Pump**. The Set Pump dialog box appears. **c** Enter the pump parameters as shown in the left column, and click **OK**.  **d** Click the pump module, and select **On**.
2 Turn the detector lamp on	**a** Click the detector module on the Instrument Panel. **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**.  **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
1 Enter the sample information Sample Name: equilsamp*iii*, where *iii* are your initials Method: defexer1 or equilmeth*iii* 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 equilsamp*iii*. **e** Select the **Method** as equilmeth*iii* 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**. 
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*iii*	**a** Select the sample, equilsamp*iii*, in the Sample Table. The Run button is now active. **b** Click the Run button  on the Actions toolbar.
2 Monitor the baseline until it is stable.	**a** Select the instrument that you want to equilibrate. 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**. 

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.

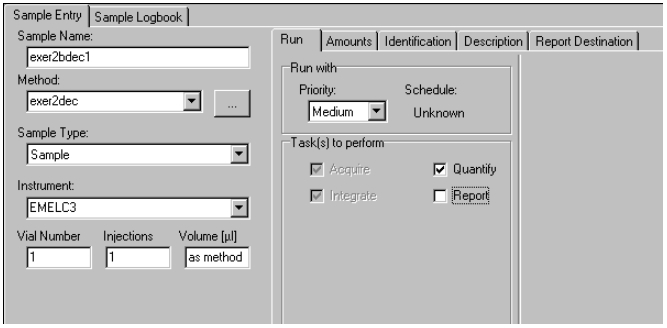
For the tasks on the next pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

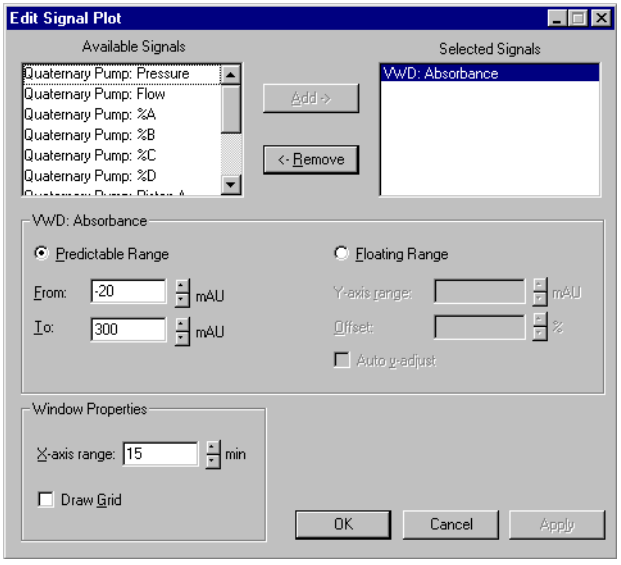
Read "Running Routine Samples" on page 9 for running routing samples.

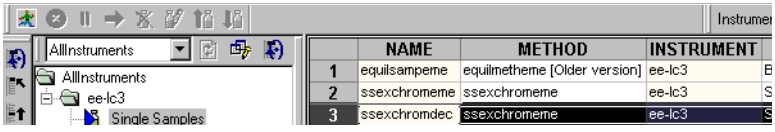
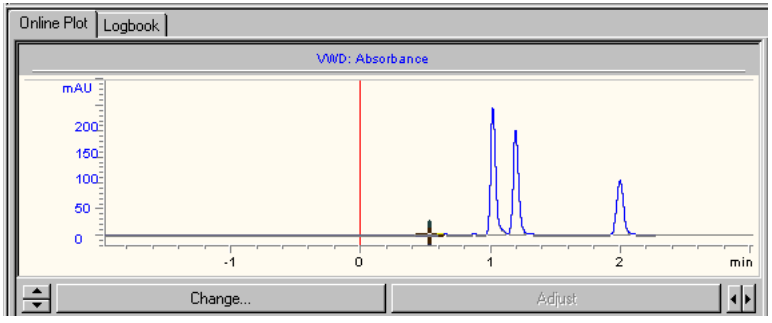
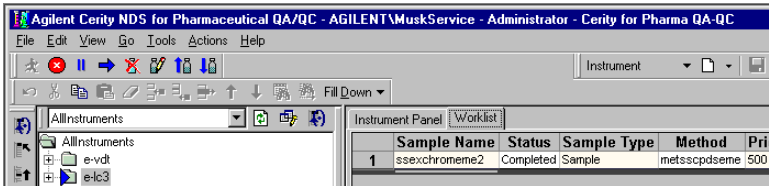
Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11. Make sure that the methods for this exercise have been set up or restored.

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 exchrom*iii*, where *iii* are your initials. • Select either defexer2, exer2*iii* (when first saved), equilmeth*iii* • Select the vial that contains the full-strength isocratic standard.	**a** Enter exchrom*iii* 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  . 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** Enter your electronic signature if required. **e** Click the **Save** button.

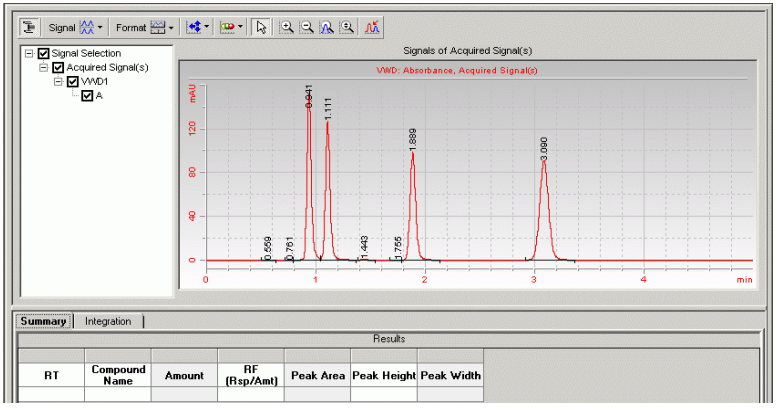
Task 2. Run the sample

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. 

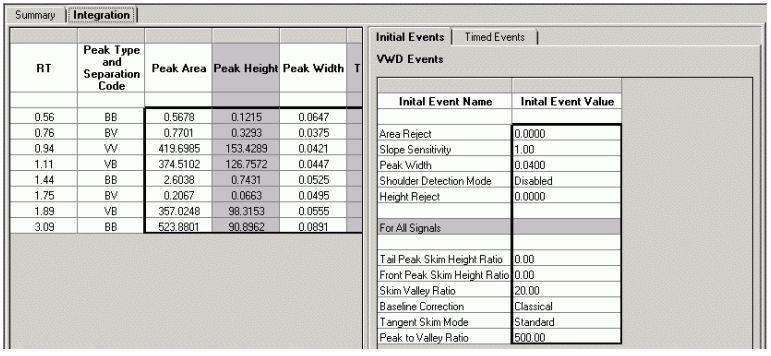
Steps	Detailed Instructions
2 Run the sample.	**a** On the selection tree, expand your instrument folder. **b** Select **Single Samples**. **c** Select the sample, exchrom*iii*. The Run button becomes available on the Tools toolbar.  **d** Click the **Run** button. You can also run the sample from the Sample View.
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.  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 exchrom*iii* #1 injection. **f** View the chromatogram and **Summary** results.



g Click the **Integration** tab to see the integration results.



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

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.

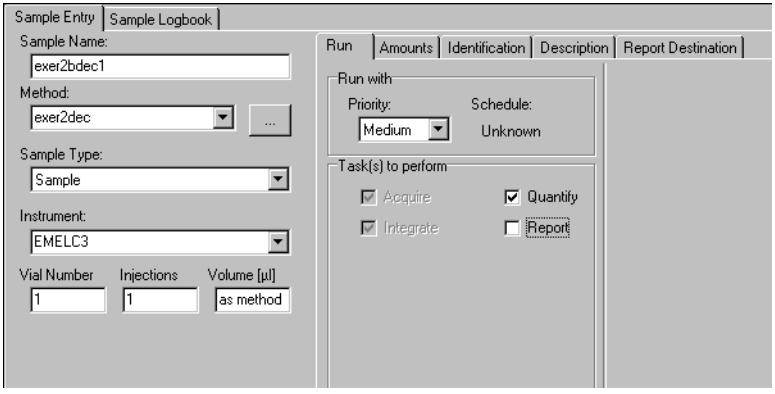
Before you start

Read "Running Routine Samples" on page 9.

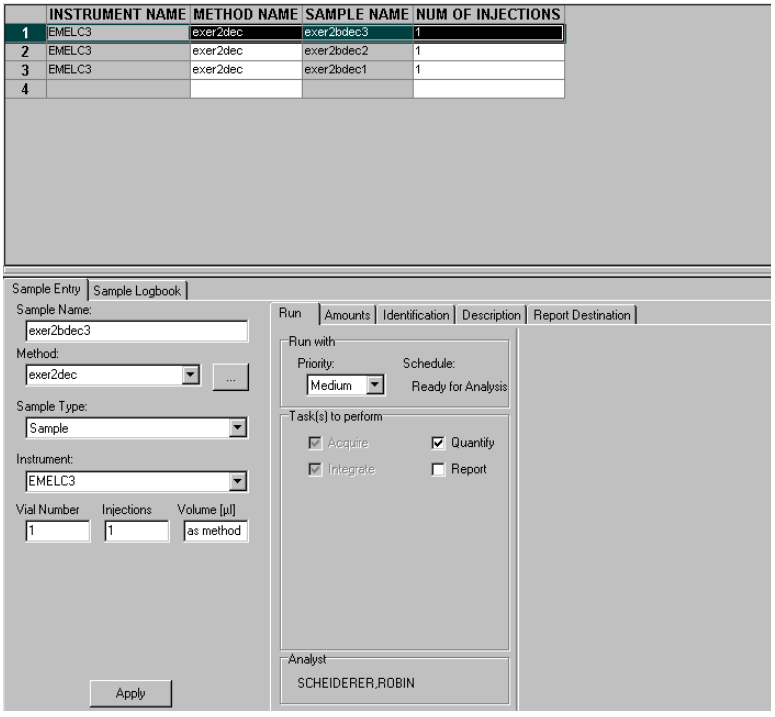
Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.

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

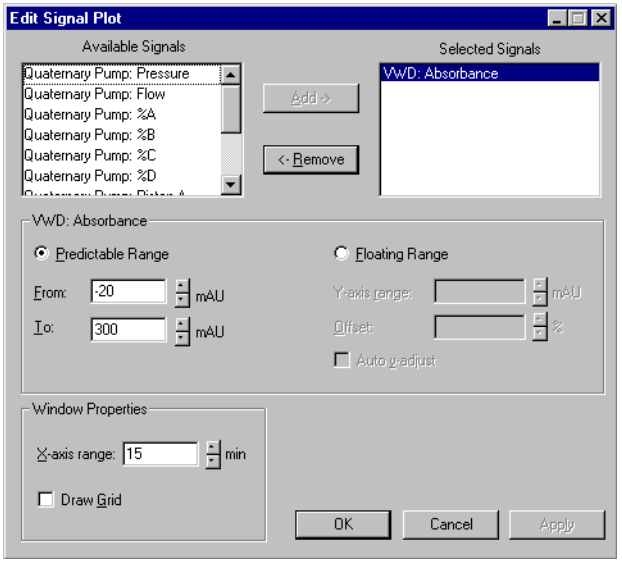
Task 1. Enter three single samples

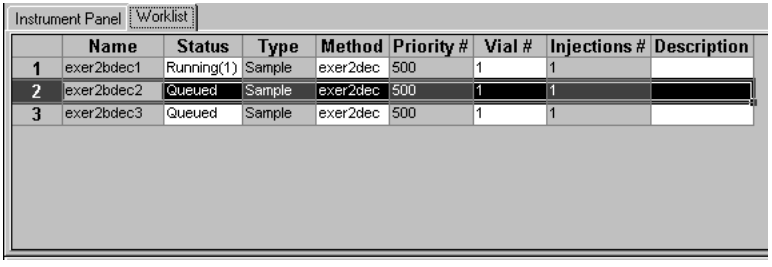
Steps	Detailed Instructions
1 Start the Instrument View and find the sample table for single samples.	**a** Select **Instrument** from the **Current View** list. **b** Expand your instrument folder **c** Select **Single Samples**. The sample table and **Sample Entry** tab sheet appear in the workspace.
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.	**a** Enter exer2b*iii*1 in the **Sample Name** box. **b** Select the exer2 method from the **Method** list (or copy of defexer2b). The instrument associated with the method appears in **Instrument** box. **c** Select **Sample** from the **Sample Type** list. **d** Enter the **Vial Number** that contains the standard. **e** Click **Apply** to put the sample information into the sample table. 
3 Enter the tasks that you want the system to do during the run	**a** Mark the **Quantify** check box, and clear the **Report** check box. 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.

Steps	Detailed Instructions
5 Repeat Steps 2 through 4 for the next two samples. Name these samples, exer2b*iii*2 and exer2b*iii*3.	**a** Select the empty row. **b** Start with Step 2a and finish with Step 4d for exer2b*iii*2. **c** Repeat steps a and b for exer2b*iii*3.



Task 2. Run the samples

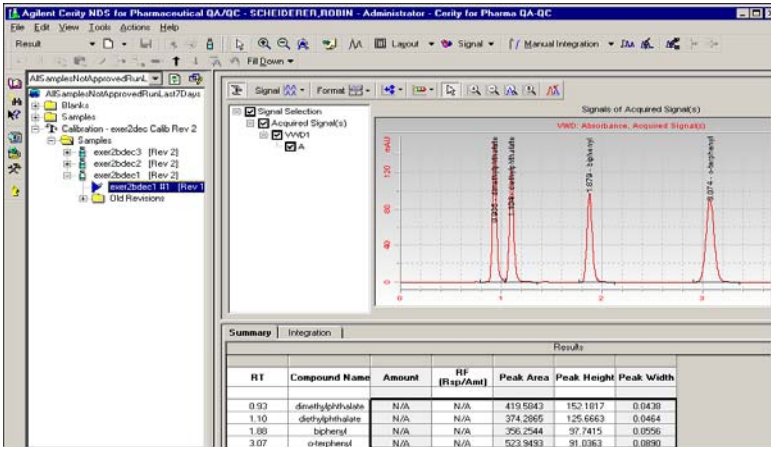
Steps	Detailed Instructions
1 Check that the instrument is ready.	**a** Select **Instrument** from the **Current View** list. **b** Click the **Online Plot** tab. **c** Click the **Change** button. The **Edit Signal Plot** dialog box appears. **d** Select the detector signal you need from the **Available 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. 

Steps	Detailed Instructions
2 Run the samples.	**a** Expand your instrument folder. **b** Select **Single Samples**. **c** Select the sample, exer2b*iii*1. **d** Click the **Run** button . **e** Select the sample, exer2b*iii*2. **f** Click the **Run** button. **g** Select the sample, exer2b*iii*3. **h** Click the **Run** button. The samples run in the order started, unless exer2b*iii*3 is of a higher priority than exer2b*iii*2. Then, exer2b*iii*3 runs before exer2b*iii*2. 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

	Name	Status	Type	Method	Priority #	Vial #	Injections #	Description
1	exer2bdec1	Running(1)	Sample	exer2dec	500	1	1	
2	exer2bdec2	Queued	Sample	exer2dec	500	1	1	
3	exer2bdec3	Queued	Sample	exer2dec	500	1	1	

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 - exer2*iii* 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 exer2b*iii*1 folder. **e** Select the exer2b*iii*1 #1 injection. **f** View the result. **g** Repeat steps d through f for the following samples: • exer2b*iii*2 • exer2b*iii*3. 

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.

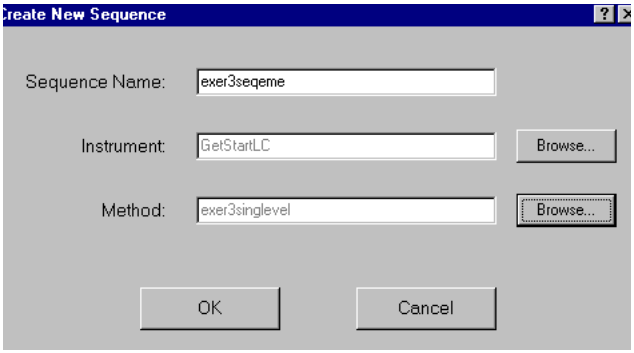
Before You Start

Read "Running Routine Samples" on page 9.

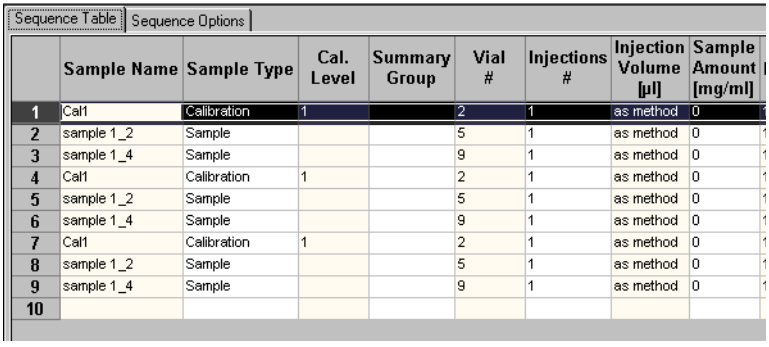
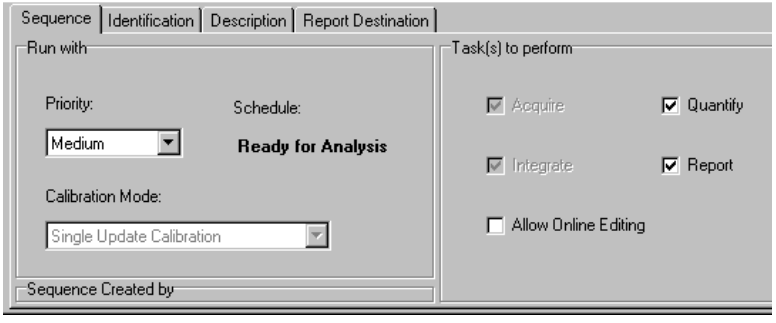
Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.

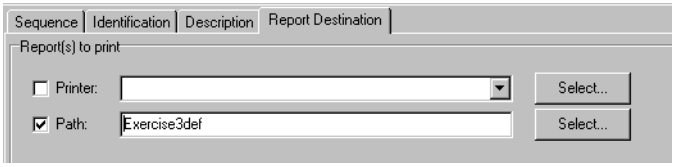
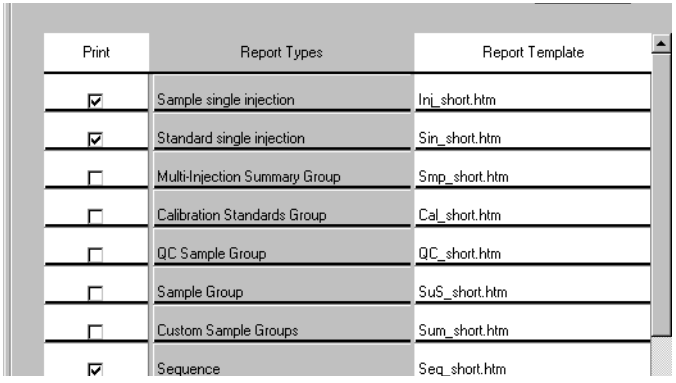
Place all the vials of prepared samples into the ALS tray. Make sure that the methods for the exercise have been set up or restored.

Task 1. Create a new sequence

Steps	Detailed Instructions
Create a new sequence. Name the sequence exer3seq*iii*, where *iii* are your initials. Use one of the two methods: • defexer3 • exer3*iii* (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 exer3seq*iii*. **c** Select the **Instrument** that will run the sequence. **d** Select the **Method** for the sequence. **e** Click **OK**.  **f** If the Save Changes to the Database dialog box appears, select the **Reason for changes**, if present, and click **Save**.

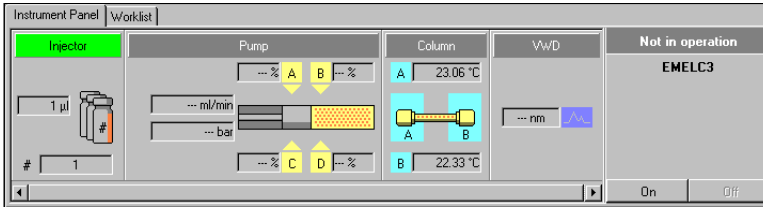
Task 2. Enter sample and sequence information

Steps	Detailed Instructions
1 Review the Sequence Table Note how the sequence table matches the sequence template setup in the method.	**a** Select Instrument from the Current View list. **b** Expand the instrument you are using, and select the sequence you just created. **c** Review the table. 
2 Enter the tasks to be performed during the run: Quantify, Report. Acquire and Integrate are always marked.	**a** Click the **Sequence Options** tab. **b** Make sure that the **Quantify** and **Report** check boxes are marked for the Task(s) to perform. 

Steps	Detailed Instructions
3 Enter the destination path for, but do not print, the reports: Enter Exercise3*iii*, where "*iii*" 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*iii*. The system automatically creates this directory if it does not exist and places the generated reports into the directory Agilent\Cerity\Reports\Pharmaqc\Reports 
4 Select the following reports to be generated: Single Injection Standard Injection Sequence	**a** Mark the **Print** check box to the left of the **Report Types** noted on the left margin. **b** Clear all the **Print** check boxes that are not those noted on the left margin. 
5 Save the sequence	**a** Click  , and enter reasons for changes and your password, if necessary.

Task 3. Run and track the sequence

Steps	Detailed Instructions
1 Make sure that the instrument is ready. Use the same conditions as set in the method. Online Plot settings: • Y-Axis range: -20 to 300 • X-Axis range: 15 minutes	**a** Select the instrument for the sequence from the selection tree. **b** Make sure the instrument and column are equilibrated, and the conditions are the same as those set in the method for the sequence.



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

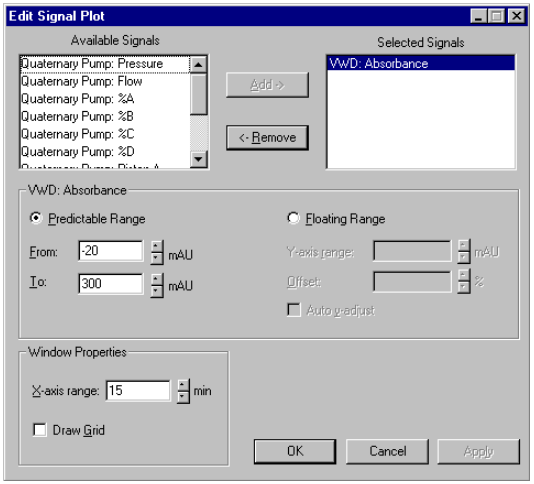
The Edit Signal Plot dialog box appears.

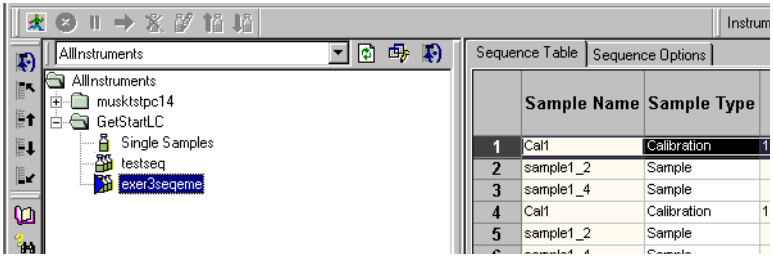
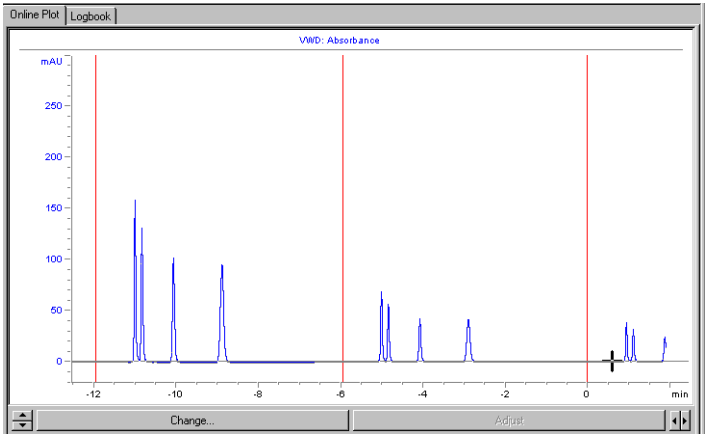
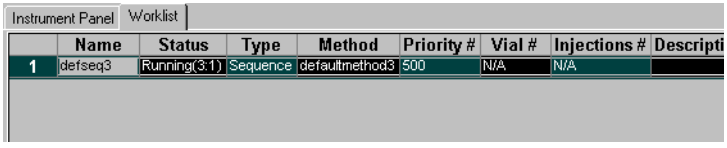
d Select the detector signal you need from the Available Signals list, and click **Add** to place this signal on the right.

e Set the **Predictable Range** as -20 to 300.

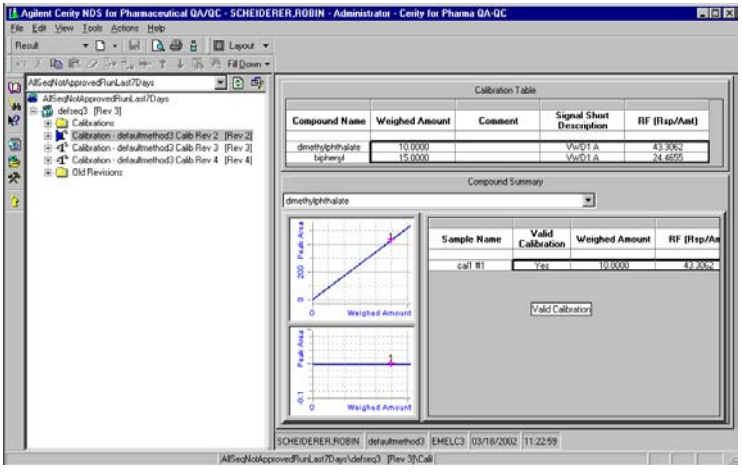
f Set the **X-Axis range** as 15 min.

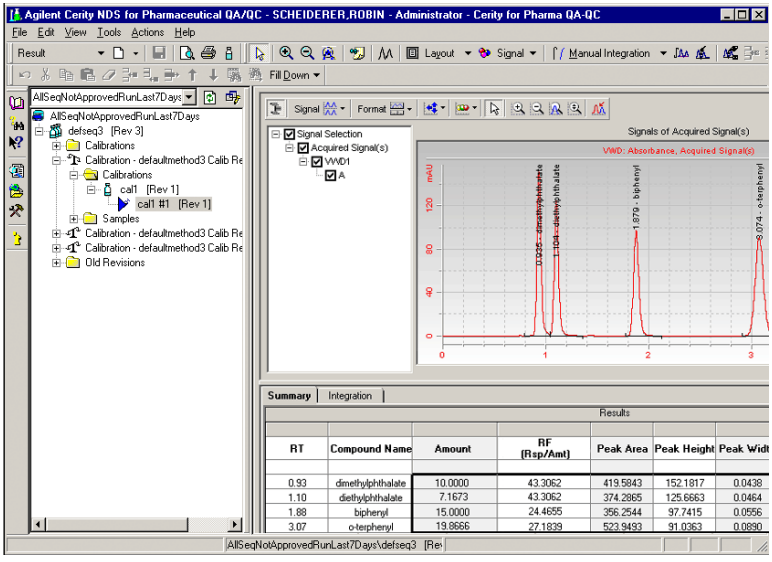
g Click **OK**.

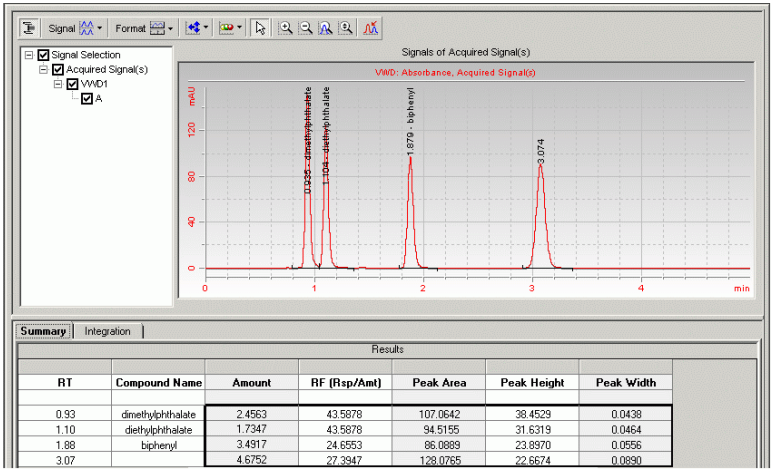


Steps	Detailed Instructions
2 Run the sequence.	**a** Expand the instrument folder. **b** Select the sequence that you just set up. The Run button,  , appears. **c** Click the **Run** button. 
3 Monitor the signal, and track the status of the sequence.	**a** Select the instrument. **b** Observe the signal in the **Online Plot** tab, and change the axes if you need to.  **c** Click the **Worklist** tab, and observe the status of the sequence.  Note that the Abort, Pause and Resume buttons appear when you enter the Worklist.

Task 4. Review the results and reports

Steps	Detailed Instructions
1 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*iii*** folder. **d** Select the **Calibration - exer3seq*iii* Calib Rev 2** folder. The calibration table and curve appear in the workspace.  **e** Select the **Calibration - exer3seq*iii* Calib Rev 3** folder. **f** Select the **Calibration - exer3seq*iii* Calib Rev 4** folder.

Steps	Detailed Instructions
2 Review the results for each calibration standard in each revision. Note the different response factors used to quantify the samples.	**a** Expand the **Calibration - exer3seq*iii* 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 - exer3seq*iii* Calib Rev 3** folder. **g** Repeat steps b-c. **h** Select the second Cal1 standard. **i** Observe the response factor. **j** Expand the **Calibration - exer3seq*iii* Calib Rev 4** folder. **k** Repeat steps b-c. **l** Select the third Cal1 standard. **m** Observe the response factor.

Steps	Detailed Instructions
3 Review the sample results for each revision. Note the response factor used for the quantitation.	**a** Expand the **Calibration - exer3seq*iii* 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.  **g** Expand the **Calibration - exer3seq*iii* Calib Rev 3** folder. **h** Repeat steps b-f. **i** Expand the **Calibration - exer3seq*iii* Calib Rev 4** folder. **j** Repeat steps b-f.
4 Review the reports. **Hint**: Use the Report Viewer to open the reports.	**a** Select **Start > Programs > Agilent Cerity > Report Viewer**. **b** Select **File > Open**. **c** Open **Cerity > Agilent > Reports > PharmaQC > Reports > Exercise3*iii***. **d** Open and view each report.

RT	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

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.

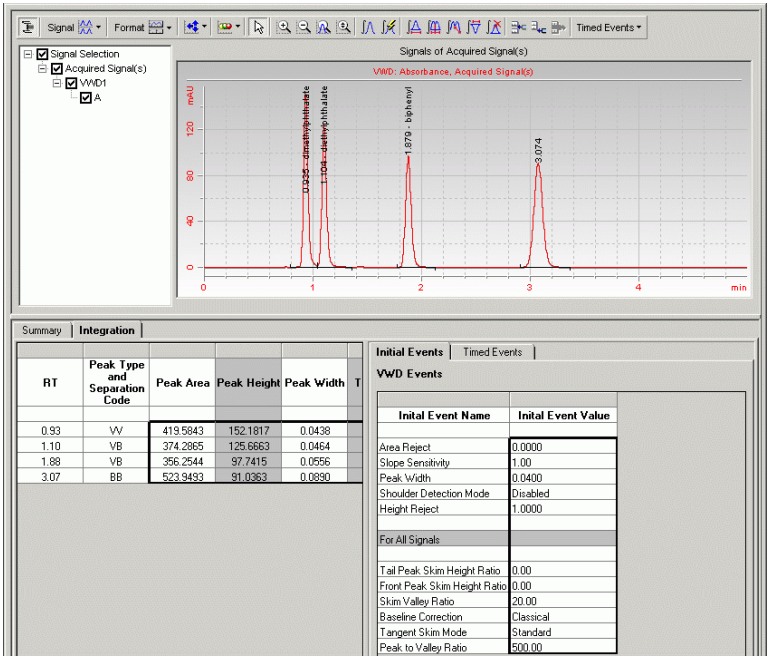
For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

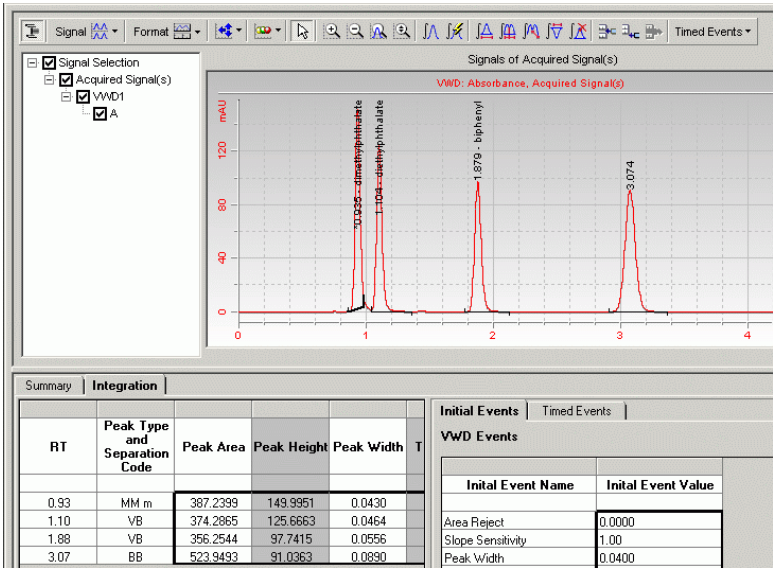
Read “Running Routine Samples” on page 9.

Task 1. Make changes to the results and sample information

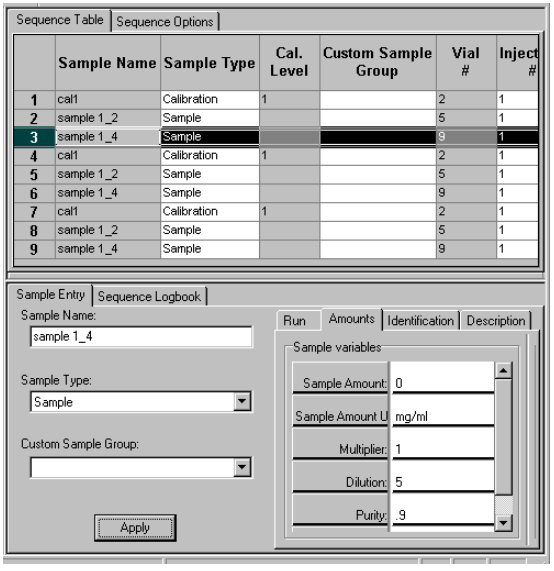
Steps	Detailed Instructions
1 Find the single injection result for the third quantitation of sample1_4 in the sequence exer3seq*iii*.	**a** Select **Result** from the Current View. **b** From the Query List, select **MySeqNotApprovedRunLast7days**. **c** Expand the **exer3seq*iii*** folder. **d** Expand the **Calibration - exer3*iii* 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.



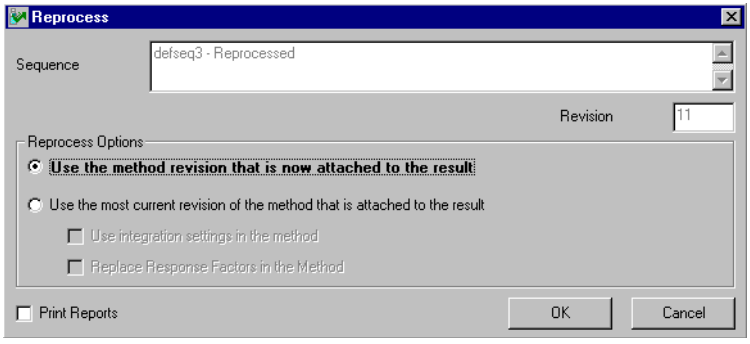
Steps	Detailed Instructions
2 Manually reintegrate the dimethylphthalate peak. Draw the baseline from the bottom left corner of the peak to the inflection point on the bottom right of the peak. Note that the Amount and RF values disappear.	**a** On the Integration toolbar, click  . A mouse pointer in 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.

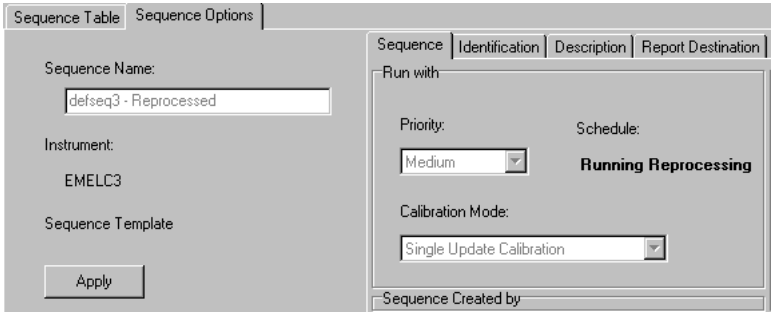
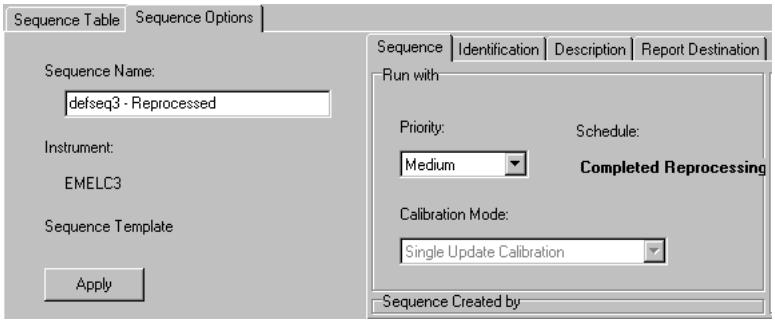


Steps	Detailed Instructions
3 Change sample variable values. • Dilution = 5 • Purity = .9	**a** Select the sequence, exer3seq*iii*. The sequence table and Sample Entry panel appear in the workspace. **b** Select the first sample 1_4 in the sequence. **c** Click the **Amounts** tab, and enter a default 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.



Task 2. Reprocess the sequence results

Steps	Detailed Instructions
1 Open the Reprocess window. See Chapter 3, "Sample Analysis", in the *Concepts Guide* for a chart that helps you select the correct reprocessing option.	**a** Select the sequence, exer3seq*iii*. 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. In the Cerity system all sample, sequence, method and instrument information is attached to the result.	**a** Select **Use the method revision now attached to the result**. **b** Click **OK**. The Cerity system uses the settings of the method originally used to run the sequence, the new manual integration setting and the new sample variable values to process the sequence. 

Steps	Detailed Instructions
3 Track reprocessing until complete.	**a** Select the sequence, exer3seq*iii*. **b** Click the **Sequence Options** tab.  When the system has completed reprocessing, the message "Completed Reprocessing" appears on the Sequence Options panel 

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.

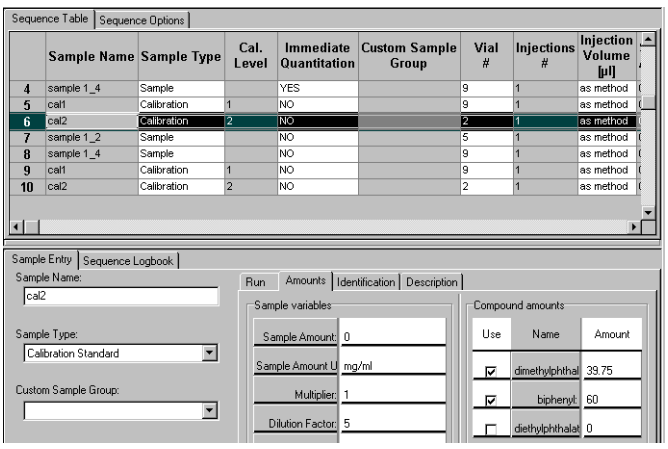
For tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

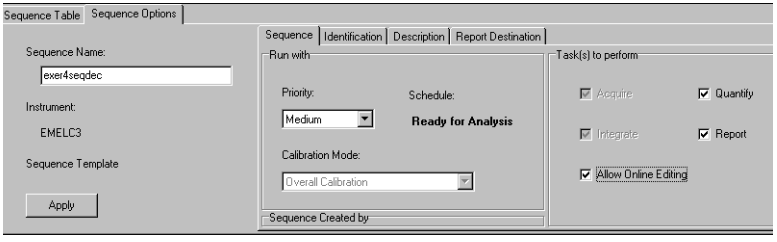
Before you start

Read "Running Routine Samples" on page 9.

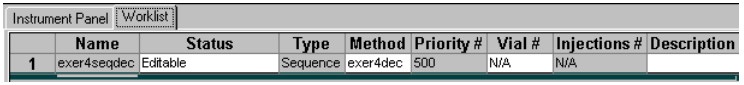
Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.

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

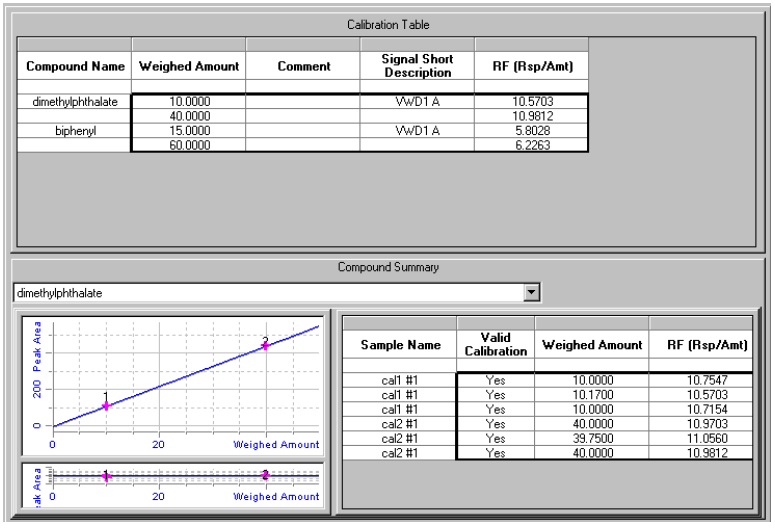
Steps	Detailed Instructions
1 Create a new sequence. Name the sequence exer4seq*iii*, where *iii* are your initials. Use one of the two methods: • defexer4*iii* • exer4*iii* (created with Exercise 4 of Setting Up Methods)	• For detailed instructions, see "Task 1. Create a new sequence" on page 30. After 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 • Purity - .93	a Select **Instrument** from the Current View list. b Expand the instrument folder. c Select exer4seq*iii*. 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.
3 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 Cal1 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. 

Steps	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. 
5 Enter the destination path for, but do not print, the reports: Enter Exercise4*iii*, where *iii* 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.

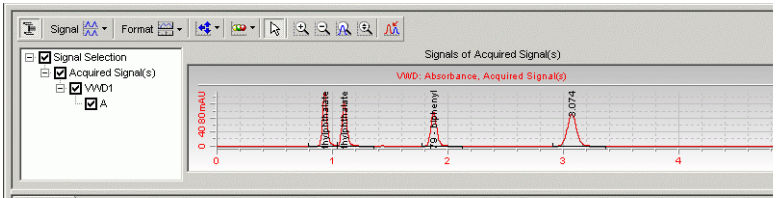
Task 2. Edit the sequence during the run

Steps	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: After the last peak comes off during the run of the first standard, select to immediately quantify the first sample 1_4 in the sequence.	**a** On the selection tree, select the instrument. **b** On the instrument workspace, click the **Worklist** tab. **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".   **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.) 

Task 3. Review the calibration results

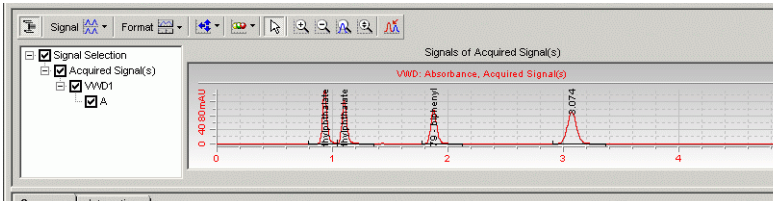
Steps	Detailed Instructions
1 Review the calibration table and curve. If you ran the sample more than 7 days ago, you must modify the query to retrieve older results from the database. See the online *How To* help, "Define a query." Note that when you first view the sequence result in the Result View, 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 **exer4seq*iii*** 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.  **e** View how the system uses the standards in overall calibration to quantify the samples compared to single-level calibration in Exercise 3a.

Steps	Detailed Instructions
2 Review the single injection results 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.



RT	Compound Name	Amount	RF (Rsp/Amt)	Peak Area	Peak Height	Peak Width
0.93	dimethylphthalate	16.6528	10.9313	195.7383	69.9187	0.0431
1.10	diethylphthalate	11.6001	10.9230	170.3073	57.6104	0.0455
1.88	biphenyl	23.2052	6.1513	153.4873	42.9007	0.0550
3.07		31.9479	6.8819	236.4102	42.5603	0.0855

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.



RT	Compound Name	Amount	RF (Rsp/Amt)	Peak Area	Peak Height	Peak Width
0.93	dimethylphthalate	44.7873	10.9132	195.5095	69.9118	0.0430
1.10	diethylphthalate	31.1959	10.8913	169.8817	57.5508	0.0454
1.88	biphenyl	62.4277	6.0983	152.2814	42.5782	0.0550
3.07		85.8281	6.8598	235.5047	42.0430	0.0862

Task 4. Review the reports

Steps	Detailed Instructions
1 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** Select **Start > Programs > Agilent Cerity > Report Viewer**. **b** Select **File > Open**, or click the **Open** button. **c** Expand the **Exercise4*iii*** folder. **d** Expand the **003 Multi-Injection Summary Group** folder, and expand the **01 Sample single injection** folder. **e** Double-click **default.htm**. Note the compound amounts. **f** Expand the **003 Multi-Injection Summary Group 0001** folder, and expand the **01 Sample single injection** folder. **g** Double-click **default.htm**. Note the compound amounts. **h** Repeat steps d-g for the **004 Multi-Injection Summary Group** folders.
2 View the sample amount for the first sample 1_2 in the Sequence Report.	**a** Click the **Open** button, and expand the **Exercise4*iii*** folder. **b** Expand the **Sequence** folder, and double-click default.htm.

Sequence samples

	Name	Position	Modified inj. volume	Amount	Unit	Cal. level
1	cal1	9	(As Method)	0.0000	mg/ml	1
2	cal2	2	(As Method)	0.0000	mg/ml	2
3	sample 1_2	5	(As Method)	2.5000	mg/ml	1
4	sample 1_4	9	(As Method)	0.0000	mg/ml	1
5	cal1	9	(As Method)	0.0000	mg/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	mg/ml	2

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

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

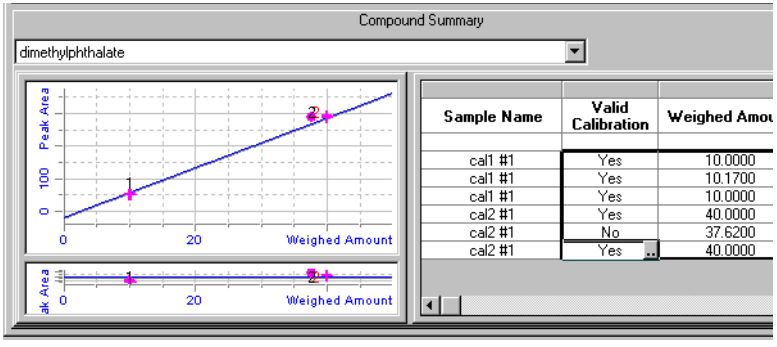
You use the data produced in Exercise #4a.

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

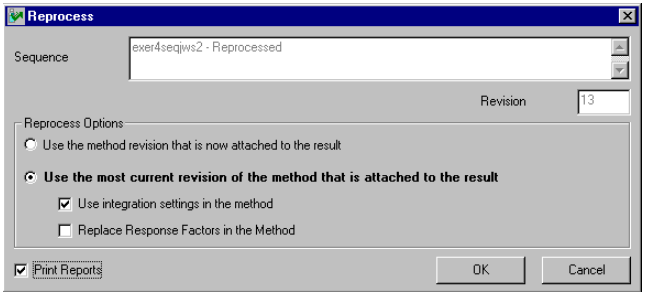
Steps	Detailed Instructions
1 Change the integration setting in the method. Set the Height Reject to 0. If you are using a copy of the defexer4*iii* method, make sure that no one else has modified it. Check the old revisions. If it has been modified, make another copy of the default method. See "Before you start" on page 5.	**a** Select **Method** from the Current View. **b** Expand the **exer4*iii*** 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 **exer4seq*iii*** folder. **c** Select the **Calibration - Exer4*iii*** folder. **d** Click the Calibration cell for the second Cal2 calibration. **e** Click the **..** button, and double-click the cell to change Yes to **No**. 

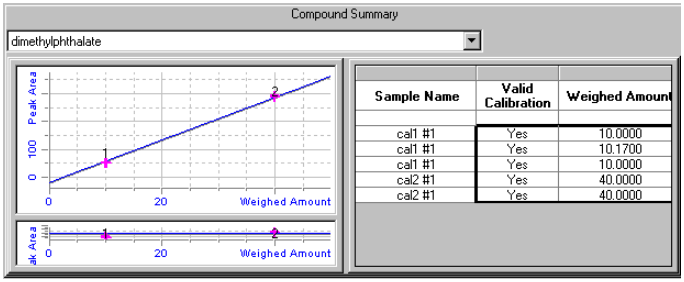
Steps	Detailed Instructions
3 Change the sequence so that no sample is immediately quantified during processing	**a** Select the exer4seq*iii* sequence. **b** In the sequence table, double-click the **Immediate Quantitation** cell for the first Sample1_2. **c** Double-click **No**. **d** Repeat steps b and c for the first Sample1_4. **e** Save the changed result. Note that the revision is incremented by 1.

Sequence Table | Sequence Options

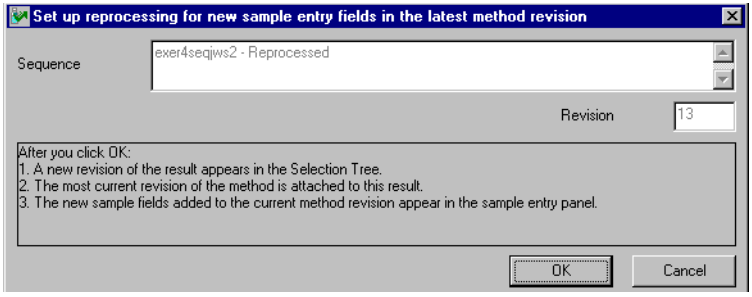
	Sample Name	Sample Type	Cal. Level	Immediate Quantitation	Custom Sample Group	Vial #	Injections #
1	cal1	Calibration	1	NO		2	1
2	cal2	Calibration	2	NO		3	1
3	sample 1_2	Sample		NO		5	1
4	sample 1_4	Sample		NO		9	1
5	cal1	Calibration	1	NO		2	1

Task 2. Reprocess and review the result

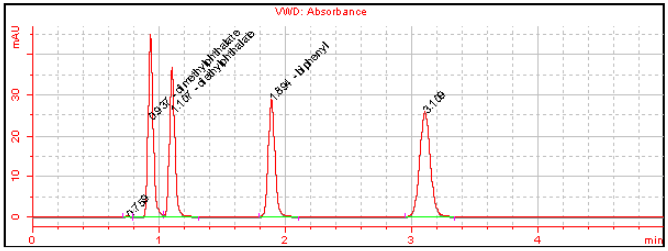
Steps	Detailed Instructions
1 Reprocess the sequence with the most current revision of the method. • Use the integration settings in the method. • Set up to print (regenerate) reports	**a** Select the exer4seq*iii* sequence. **b** Select **Actions > Reprocess**. **c** Select **Use the most current revision 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. 
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 - Exer4*iii*** 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.

Steps	Detailed Instructions
3 Review the calibration summary.	• Select the second **Calibration - Exer4*iii*** folder. Note that the calibration point that you removed before reprocessing is gone. 
4 Review the reports for the first set of samples to make sure that they were quantified with all the calibration standards.	**a** Select **Start > Programs > Agilent Cerity > Report Viewer**. **b** Select **File > Open**. **c** Expand **Exercise4*iii*-0001**. Note that only one report exists for all the samples. After initial processing two reports existed for the first Sample1_2 and Sample1_4.

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

Steps	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*iii*. **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 exer4seq*iii*. **c** Select **Actions > Set up reprocessing for new sample entry fields**.  **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
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.	**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

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

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.

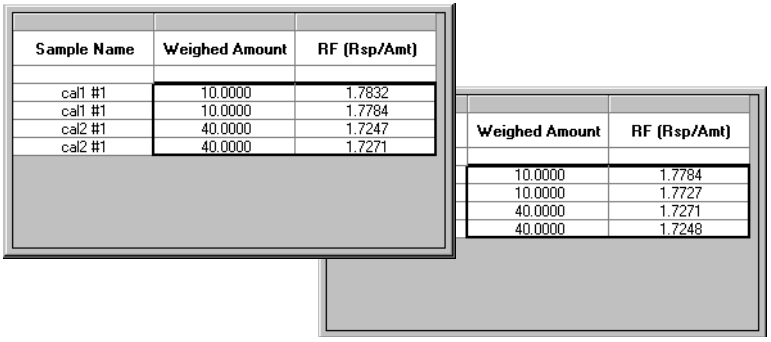
Equilibrate the instrument. See "Basic Exercise #1a Equilibrate the instrument" on page 11.

Task 1. Set up and run the sequence

Steps	Detailed Instructions
1 Create a new sequence. Name the sequence exer5seq*iii*, where *iii* are your initials. Use one of the two methods: • defexer5 • exer5*iii* (created with Exercise 5 of Setting Up Methods)	• For detailed instructions, see "Task 1. Create a new sequence" on page 30.
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. Enter Exercise5*iii*, where "*iii*" are your initials.	• For detailed instructions, see "Task 2. Enter sample and sequence information" on page 31, Step 3.
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

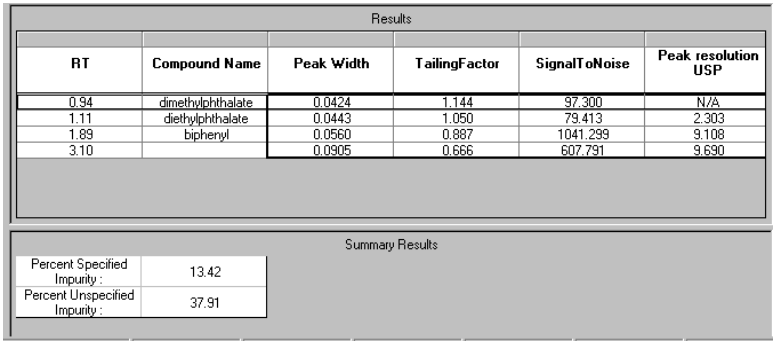
Steps	Detailed Instructions
1 Compare the response factors for dimethylphthalate for the first set of bracketed samples with the second set. **Hint**: If you can't see the RFs, click the bottom of the Compound Summary panel to make the scroll bar appear. Note that the RFs for the second Cal1 and Cal2 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 **exer3seq*iii*** 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.



Sample Name	Weighed Amount	RF (Rsp/Amt)
cal1 #1	10.0000	1.7832
cal1 #1	10.0000	1.7784
cal2 #1	40.0000	1.7247
cal2 #1	40.0000	1.7271

Weighed Amount	RF (Rsp/Amt)
10.0000	1.7784
10.0000	1.7727
40.0000	1.7271
40.0000	1.7248

Steps	Detailed Instructions
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 - exer3seq*iii* 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 Results

Percent Specified Impurity :	13.42
Percent Unspecified Impurity :	37.91

Steps	Detailed Instructions
3 Review the percent impurity results for the first Sample1_2 and for the sample group. Note that the percent impurity values exceeded their limits.	**a** Expand the second **Calibration - exer3seq*iii*** folder. **b** Expand the **Samples** folder. **c** Select the **Sample1_2** folder. 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
	2

Summary Results

Avg Percent Specified :	13.65
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

Steps	Detailed Instructions
4 Review the sample single injection report for the first Sample1_2 and the report for the sample group.	**a** Select **Start > Programs > Agilent Cerity > Report Viewer**. **b** Select **File > Open**. **c** Expand **Exercise5*iii***. **d** Expand 003Multi-InjectionSummary. **e** Expand 01Sample Single Injection, and double-click default.htm. Note the system suitability calculation values in the table that was set up in the method.

Retention Time	Compound Name	Amount	Response Factor	Tailing Factor	Peak resolution USP	SignalToNoise
0.93	dimethylphthalate	24.8892	0.1169	1.178	N/A	237.192
1.10	diethylphthalate	17.5561	0.1169	1.135	2.308	194.383
1.89	biphenyl	37.5000	0.0667	1.090	9.129	2554.088
3.11	N/A	48.6177	0.0741	1.043	9.713	1489.322

f Expand **Exercise5*iii***.
g Expand **Sample Group**, and click default.htm.

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	XXXXXXXXXX	XXXXXXXXXX
2	sample 1_4	dimethylphthalate	XXXXXXXXXX	XXXXXXXXXX
3	sample 1_2	dimethylphthalate	XXXXXXXXXX	XXXXXXXXXX
4	sample 1_4	dimethylphthalate	XXXXXXXXXX	XXXXXXXXXX
1	sample 1_2	diethylphthalate	XXXXXXXXXX	XXXXXXXXXX
2	sample 1_4	diethylphthalate	XXXXXXXXXX	XXXXXXXXXX
3	sample 1_2	diethylphthalate	XXXXXXXXXX	XXXXXXXXXX
4	sample 1_4	diethylphthalate	XXXXXXXXXX	XXXXXXXXXX
1	sample 1_2	biphenyl	XXXXXXXXXX	XXXXXXXXXX
2	sample 1_4	biphenyl	XXXXXXXXXX	XXXXXXXXXX
3	sample 1_2	biphenyl	XXXXXXXXXX	XXXXXXXXXX
4	sample 1_4	biphenyl	XXXXXXXXXX	XXXXXXXXXX

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

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.

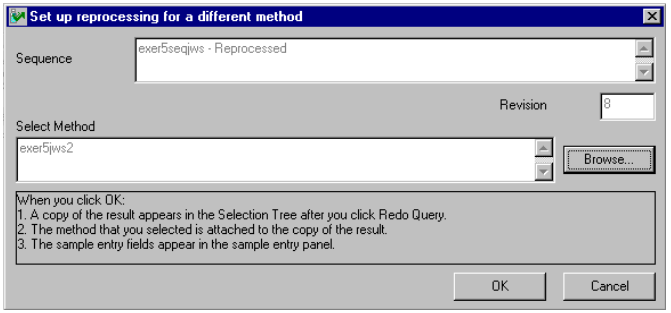
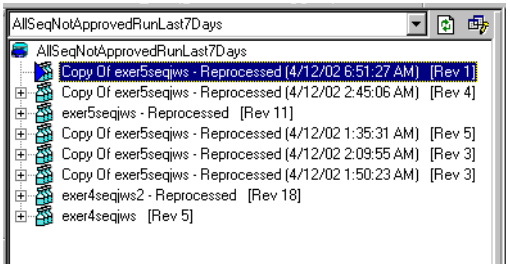
Before You Start

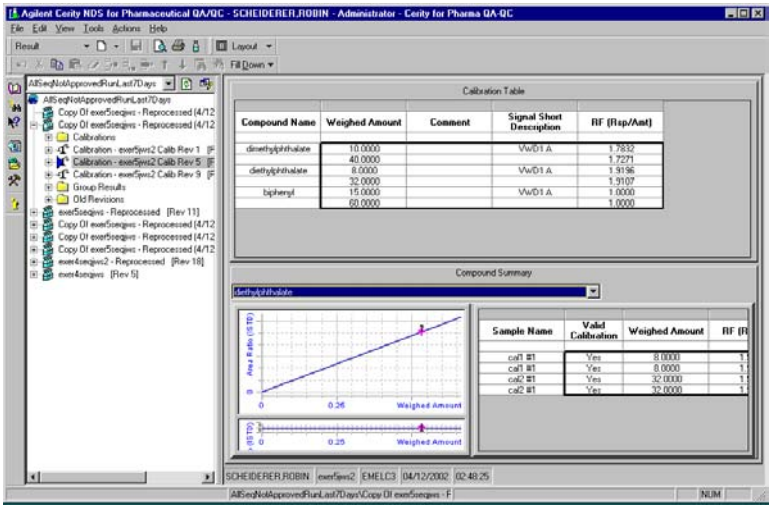
Read “Running Routine Samples” on page 9.

Task 1. Set up a different method

Steps	Detailed Instructions
1 Copy exer5*iii* and rename it exer5*iii*2. • Or, copy defexer5. • Or, use defexer5*iii*2 to reprocess.	**a** Select **File > New > Method**. **b** Click the **Browse** button in the Method Wizard. **c** Select **exer5*iii***. **d** Enter a **New Method Name** of exer5*iii*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*iii*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. **l** Mark the **Use ISTD Compound** check box, and select biphenyl. **m** Save the method.

Task 2. Reprocess the sequence result

Steps	Detailed Instructions
1 Set up reprocessing for a different method. Select exer5*iii*2 or defexer5*iii*2. See Chapter 3, "Sample Analysis", in the *Concepts Guide* for a chart that helps you select the correct reprocessing options.	**a** Select **Result** from the Current View. **b** From the Query List, select **MySeqNotApprovedRunLast7days**. **c** Select the **exer5seq*iii*** folder. **d** Select **Actions > Set up reprocessing for a different method**.  **e** Click **Browse**, select exer5*iii*2 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. 
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. 

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.

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.

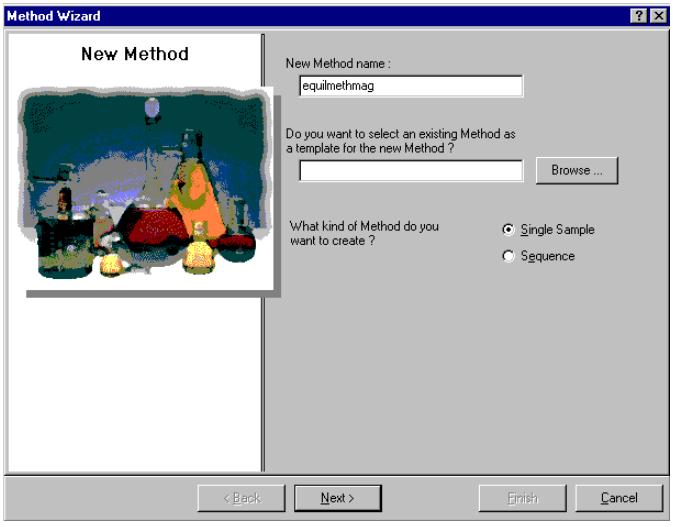
Use this method to equilibrate the instrument as outlined in the chapter "Basic Exercise #1a Equilibrate the instrument" on page 11.

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.

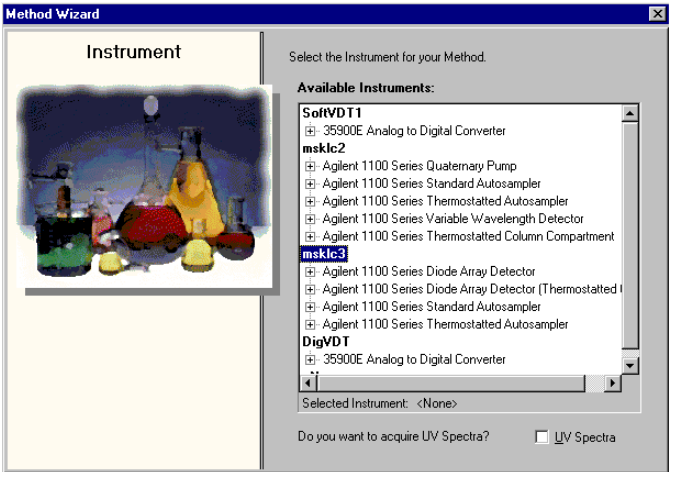
Before you start

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

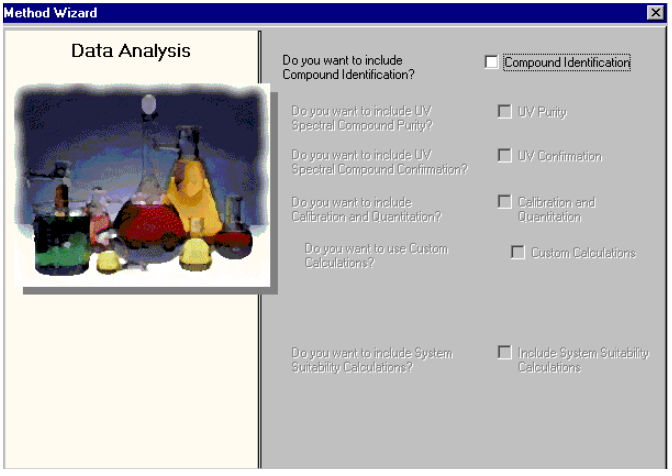
Task 1. Create a method template to enter instrument parameters

Steps	Detailed Instructions
1 Create a new method template for a single sample. • Name the method template, equilmeth*iii*, 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**, equilmeth*iii*. **c** Select **Single Sample**.  **d** Click **Next** to scroll to the Instrument panel.

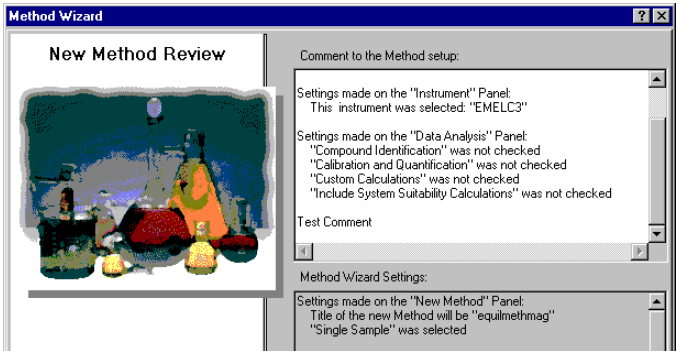
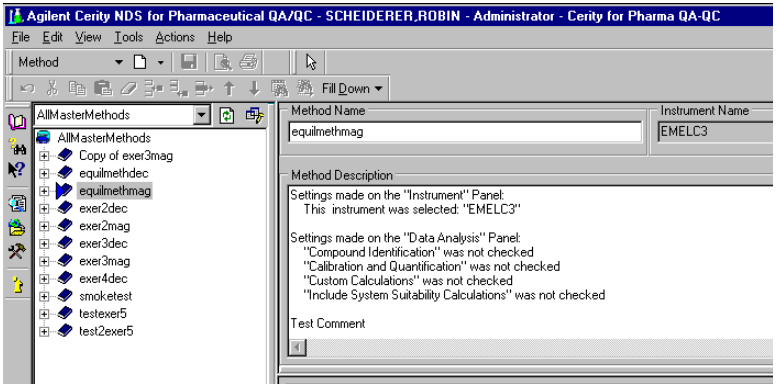
Steps	Detailed Instructions
2 Select the instrument to equilibrate.	**a** On the Instrument panel, select the instrument you need to equilibrate. The instruments that appear in the **Available Instruments** list depends on your configuration of the Cerity Networked Data System.



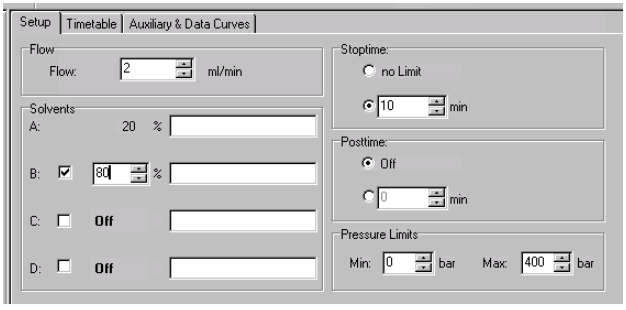
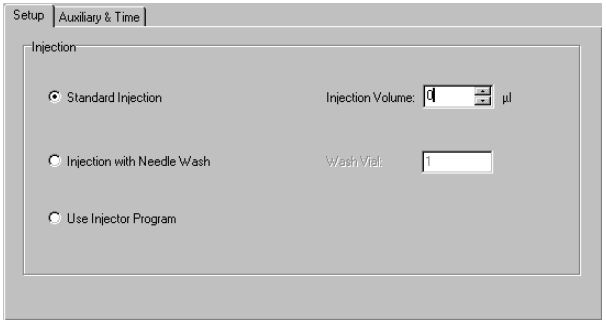
	b Click **Next** to scroll to the Data Analysis panel.
3 Clear all data analysis selections.	**a** On the Data Analysis panel, clear the **Compound Identification** check box.



	b Click **Next** to scroll to the New Method Review panel.

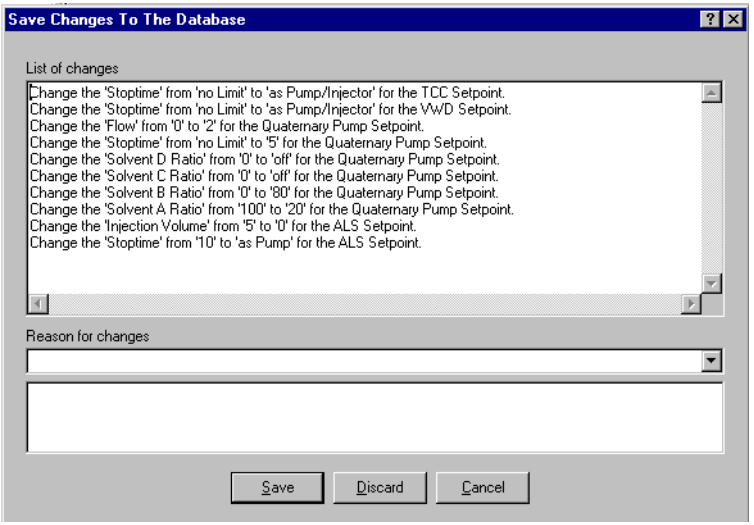
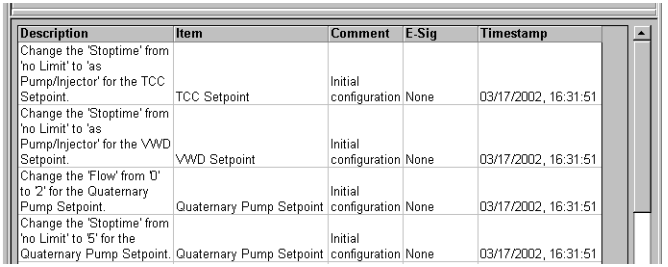
Steps	Detailed Instructions
4 Review and save the method template.	**a** On the New Method Review panel, review the setting in the **Method Wizard Settings** section. **b** Add the words "Test Comment" in the **Comment** section. **c** Click **Finish**.  **d** Click **Save** if the Save Changes to the 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 - equilmeth*iii*. **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. 

Task 2. Enter the instrument conditions for the equilibration

Steps	Detailed Instructions
1 Set the pump parameters: Methanol as Solvent B: • Flow rate: 2ml/min. • Solvent composition: 80%MeOH/20%H_2O • Stoptime: 10 min. Acetonitrile as Solvent B: • Flow rate: 1.5ml/min • Solvent composition: 65%ACN/35%H_2O • Stoptime: 10 min.	**a** On the selection tree, expand the equilmeth*iii* 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. **f** Under **Posttime** and **Pressure Limits**, accept the default values. 
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. 

Steps	Detailed Instructions
3 Set the same stoptime for all modules. Stoptime: 10 min.	**a** Select the **ALS** folder, **b** Click the **Auxiliary & Time** tab. **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
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.  **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.
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, equilmeth*iii*. **b** View the list of changes to the method.  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.

Description	Item	Comment	E-Sig	Timestamp
Change the 'Stoptime' from 'no Limit' to 'as Pump/Injector' for the TCC Setpoint.	TCC Setpoint	Initial configuration	None	03/17/2002, 16:31:51
Change the 'Stoptime' from 'no Limit' to 'as Pump/Injector' for the VWD Setpoint.	VWD Setpoint	Initial configuration	None	03/17/2002, 16:31:51
Change the 'Flow' from '0' to '2' for the Quaternary Pump Setpoint.	Quaternary Pump Setpoint	Initial configuration	None	03/17/2002, 16:31:51
Change the 'Stoptime' from 'no Limit' to '5' for the Quaternary Pump Setpoint.	Quaternary Pump Setpoint	Initial configuration	None	03/17/2002, 16:31:51

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.

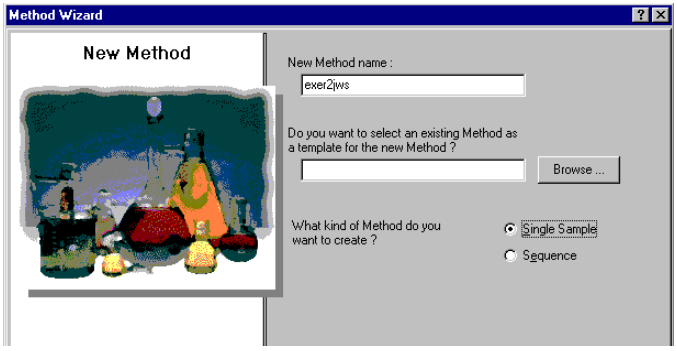
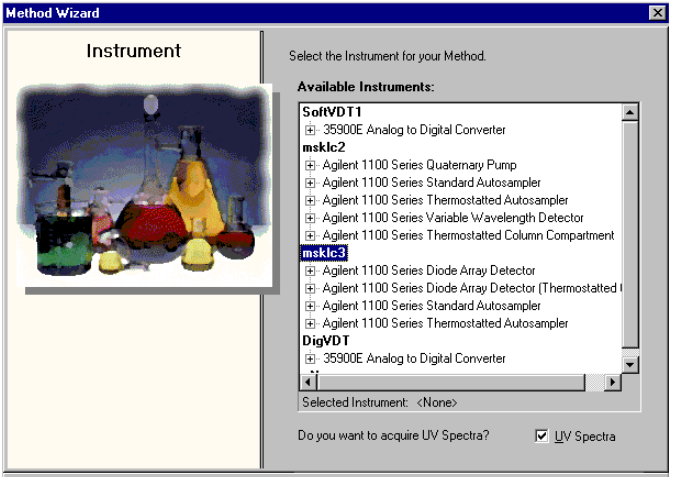
Use the method created in the first part of this exercise to enter and run a single sample to produce an example chromatogram. You can use the completed method to enter and run a group of samples to identify compounds. See "Basic Exercise #2a Run a single sample to produce an example chromatogram" on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

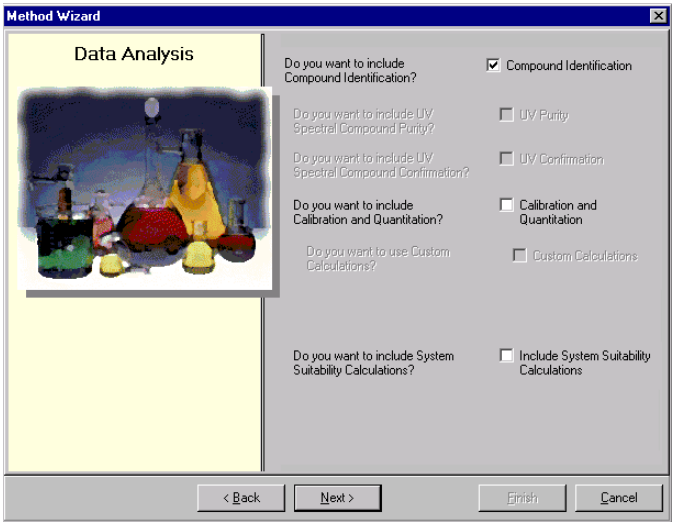
For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

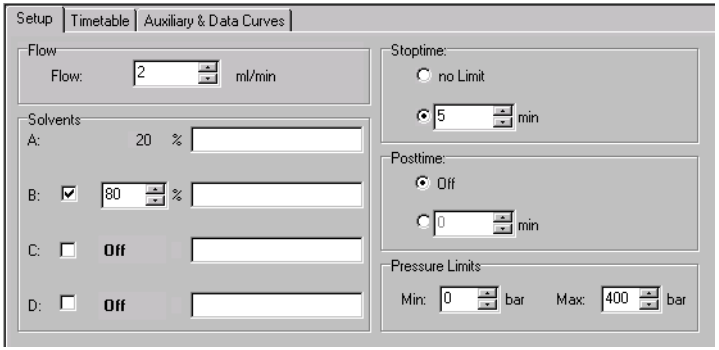
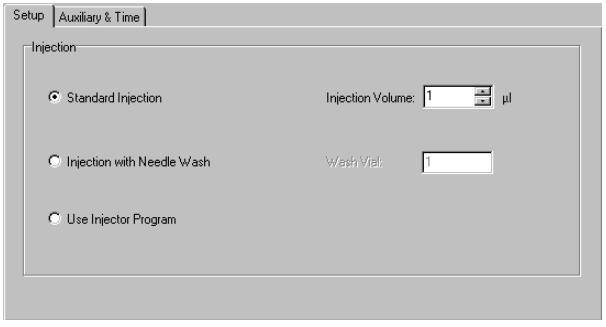
Read "Setting Up Methods" on page 71 for setting up methods.

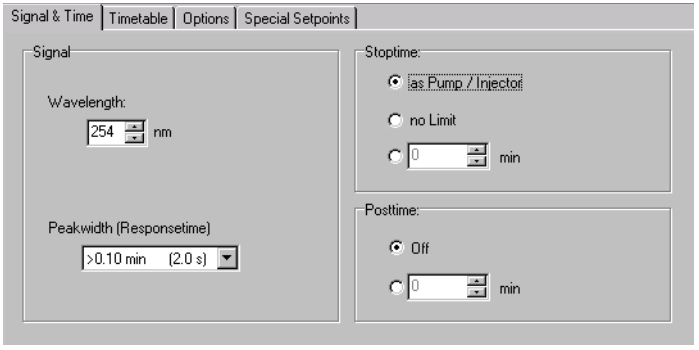
Task 1. Create a method template to identify compounds only

Steps	Detailed Instructions
1 Create a new method template for a single sample. • Name the method template, exer2*iii*, where *iii* are your initials.	**c** Select **File > New > Method** or click and select **Method**. The Method Wizard appears. **d** Enter exer2*iii* in the **Method Name** box. **e** Select **Single Sample**.  **f** Click **Next** to scroll to the Method Wizard Instrument panel.
2 Select an instrument for the method.	**a** On the Instrument panel, select the instrument that will run the sample.  **b** Click **Next** to scroll to the Data Analysis panel.

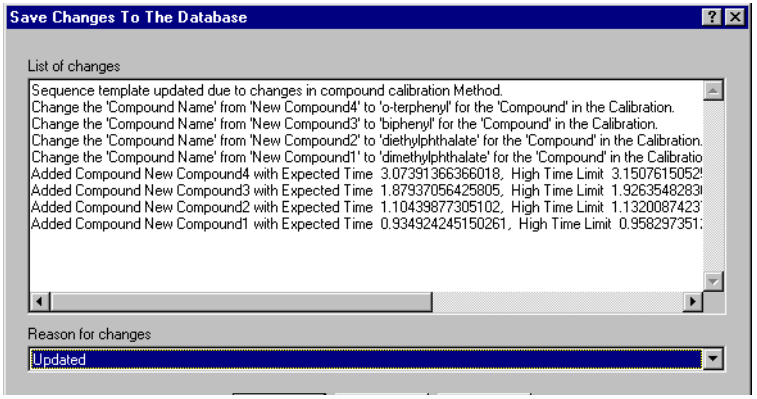
Steps	Detailed Instructions
3 Mark only Compound Identification.	**a** On the Data Analysis panel, Clear the **Calibration and Quantification, Include Noise Calculations** and **Include System Suitability Calculations** check boxes.  **b** Click **Next** to scroll to the Identification panel.
4 Complete the setup of the method template. Do not mark any check box on the Method Wizard Identification panel.	**a** Click **Next,** and click the **Finish** button. **b** Click **Save** if the **Save Changes to the Database** dialog box appears.

Task 2. Enter the instrument conditions for the equilibrium

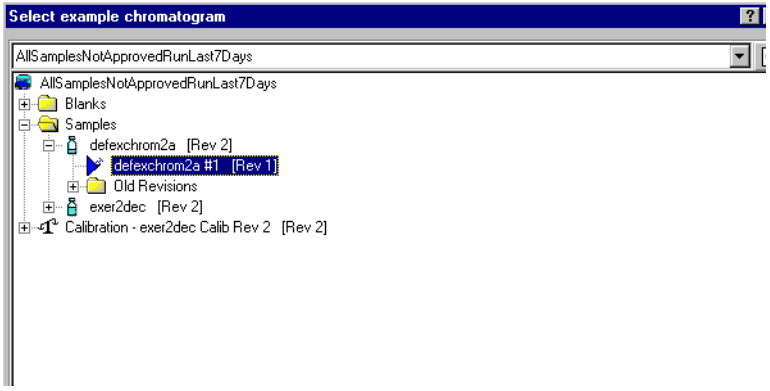
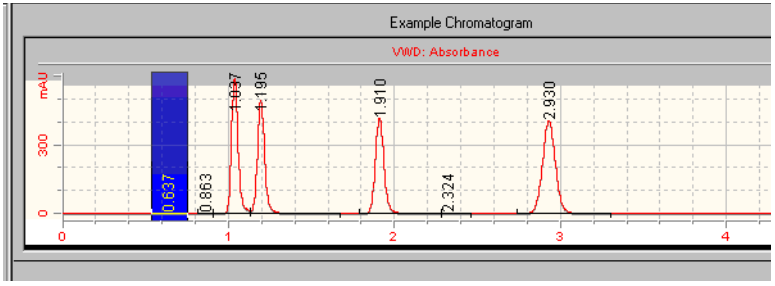
Steps	Detailed Instructions
1 Enter the pump parameters: Methanol as Solvent B: • Flow rate: 2ml/min. • Solvent composition: 80%MeOH/20%H_2O • Stoptime: 5 min. Acetonitrile as Solvent B: • Flow rate: 1.5ml/min • Solvent composition: 65%ACN/35%H_2O • Stoptime: 6 min.	**a** On the selection tree, expand 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. 
2 Enter the injection volume and stop time for the autosampler. Injection Volume: 1µl Stop Time: same 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**. 

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 **VWD** folder. **b** Under **Stoptime** select **as Pump/Injector**. **c** On the selection tree, select the **TCC** folder. **d** Under **Stoptime**, select **as Pump/Injector**. 

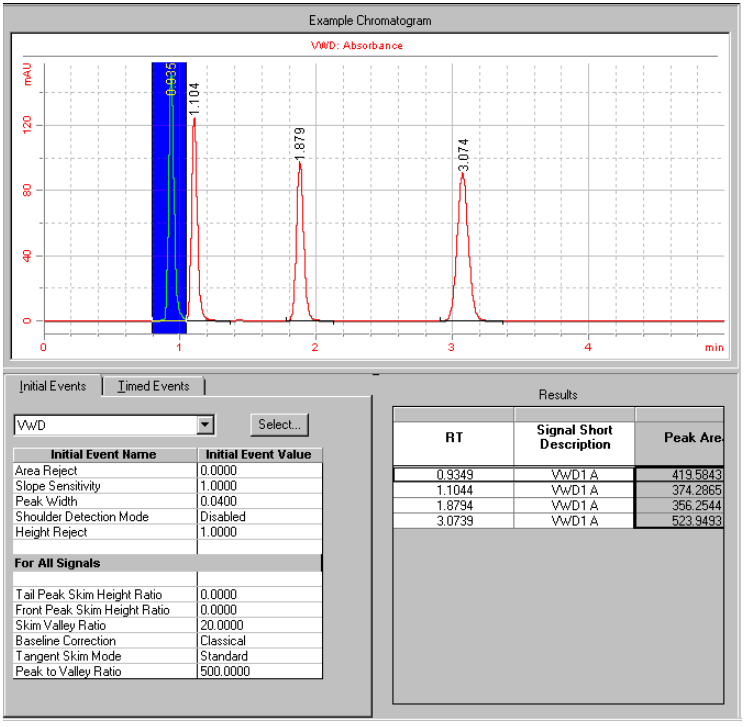
Task 3. Save and audit method changes

Steps	Detailed Instructions
1 Save the method. After you save the method here, you can use the method to produce an example chromatogram. 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.	**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. 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

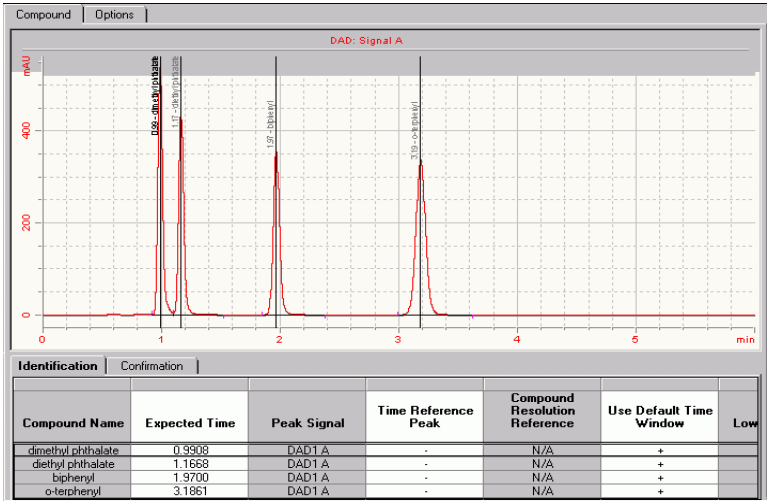
Steps	Detailed Instructions
1 Select an example chromatogram. If no chromatogram of the isocratic sample exists, you must run a sample to produce the example chromatogram. See "Basic Exercise #2a Run a single sample to produce an example chromatogram" on page 17. You do not need the example chromatogram to set up integration and identification, but it is recommended.	**a** On the selection tree, expand the exer2*iii* method folder, if necessary. **b** Expand the **Data Analysis** folder. **c** Select **Example Chromatogram**. **d** On the **Tools** toolbar, click  .  **e** Expand the Samples folder. **f** Expand the exchrom*iii* or defexchrom2a folder. **g** 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 on the Actions toolbar

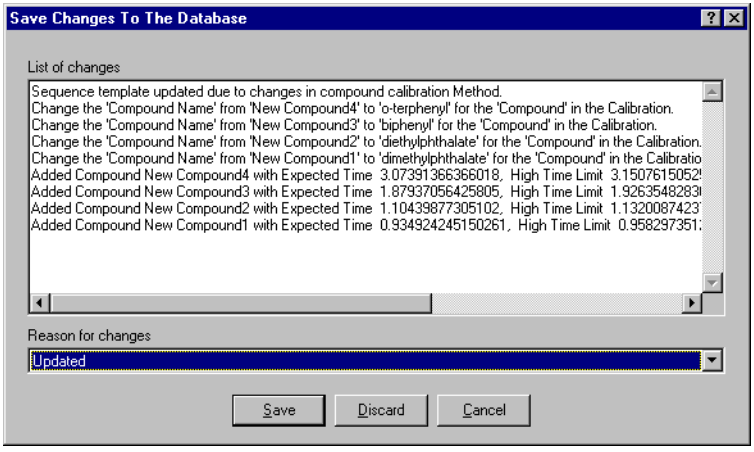


Task 5. Set up compound identification

Steps	Detailed Instructions
1 Set up the compound 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	**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.



Compound Name	Expected Time	Peak Signal	Time Reference Peak	Compound Resolution Reference	Use Default Time Window	Low
dimethyl phthalate	0.9908	DAD1 A	-	N/A	+	
diethyl phthalate	1.1668	DAD1 A	-	N/A	+	
biphenyl	1.9700	DAD1 A	-	N/A	+	
o-terphenyl	3.1861	DAD1 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.	**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. 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.

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.

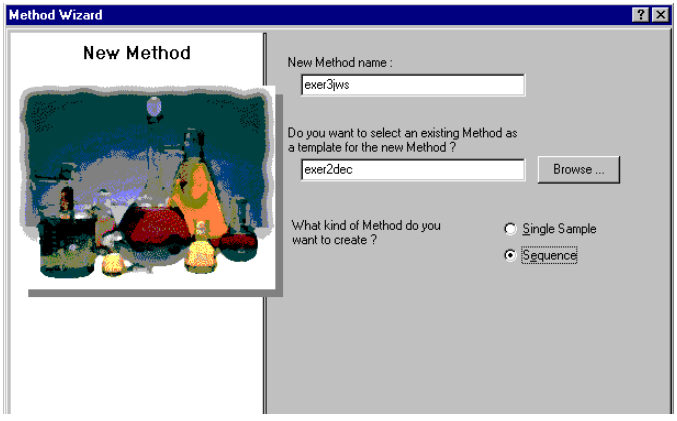
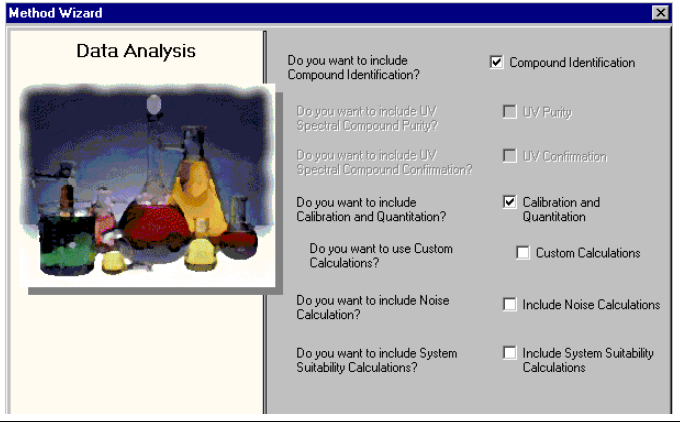
You can use this method with "Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration" and "Basic Exercise #3b Reintegrate and reprocess the results".

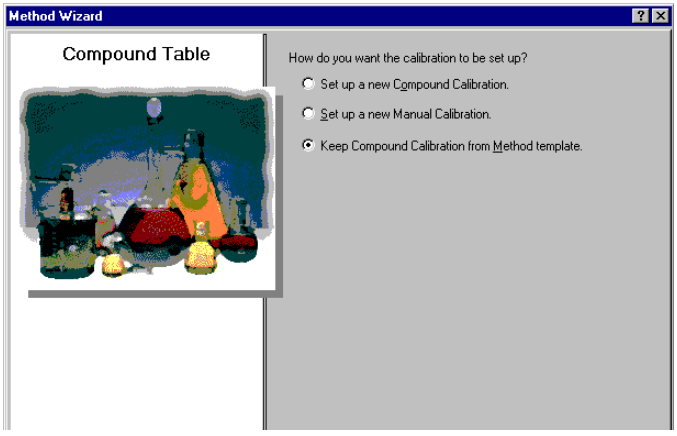
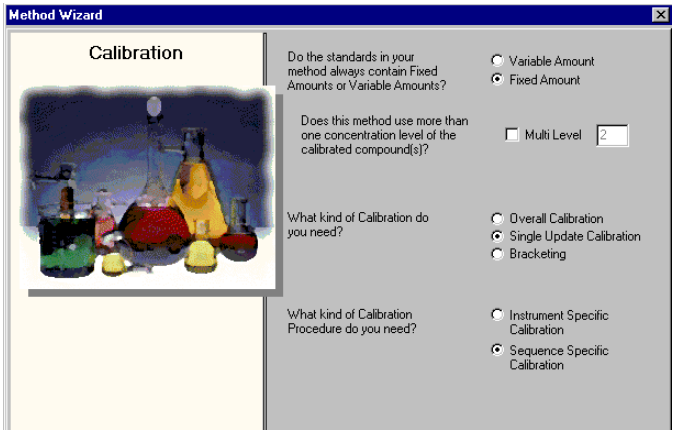
For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

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

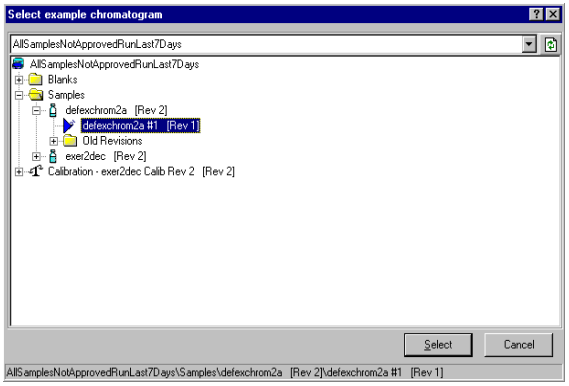
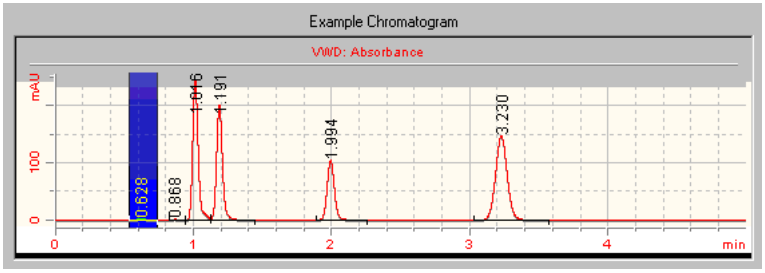
Task 1. Copy a method to create a method template for a sequence

Steps	Detailed Instructions
1 Create a new method template from an existing method. • Name the method template, exer3*iii*, where *iii* are your initials. • Use exer2*iii* 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.	**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. 

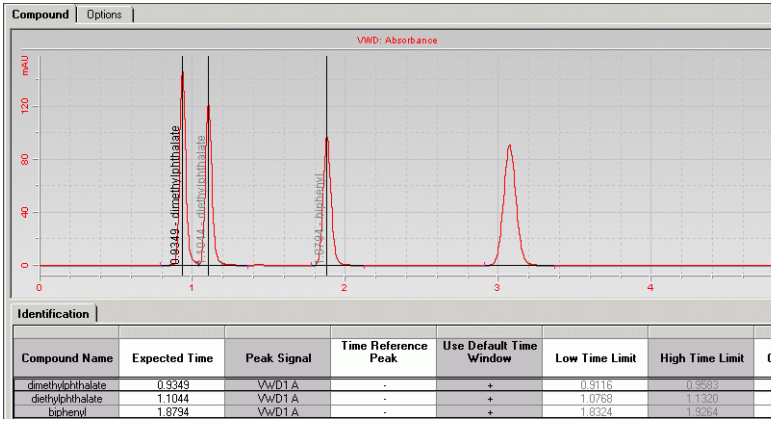
Steps	Detailed Instructions
3 Set up Quantitation and review your new method.	**a** Click **Next** to scroll to the **Quantitation** panel. **b** Make sure that the **Limit checks** check box is clear and the **ESTD** option is selected.  **c** Click **Next** to scroll to the **New Method Review** panel. **d** Review the **Method Wizard Settings**. **e** Click the **Finish** button to save your new method.

Task 2. Select an example chromatogram

Steps	Detailed Instructions
1 Select an example chromatogram. • Use the example chromatogram you produced with Basic Exercise 2a or 2b of the "Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration" and "Basic Exercise #3b Reintegrate and reprocess the results". • Or, use defexchrom2a. You do not need to select an example chromatogram. However, it is easier to modify compound identification if you do.	**a** On the selection tree, expand the exer3*iii* folder. **b** Expand the **Data Analysis** folder. **c** Select the **Example Chromatogram** item. **d** On the **Tools** toolbar, click  .  **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.

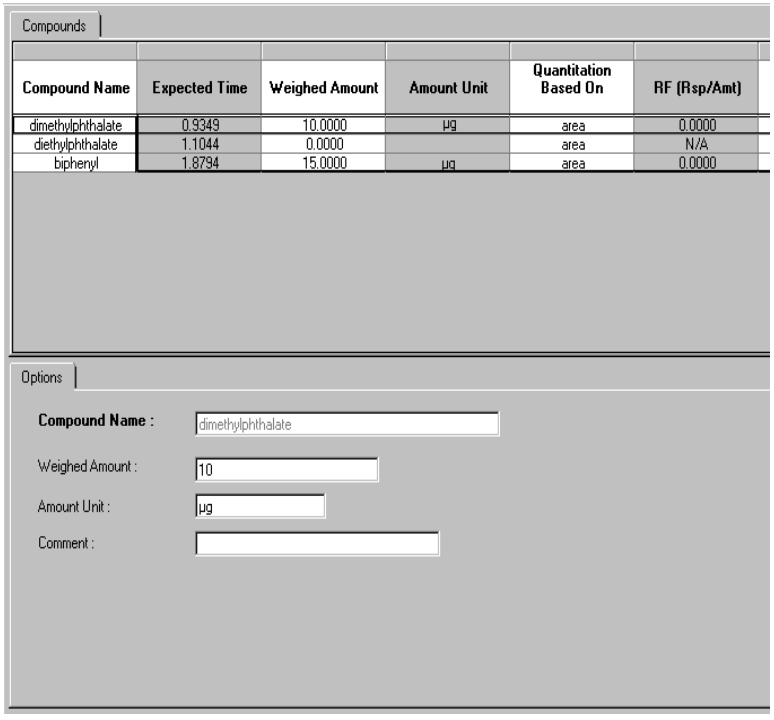
Task 3. Modify compound identification

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

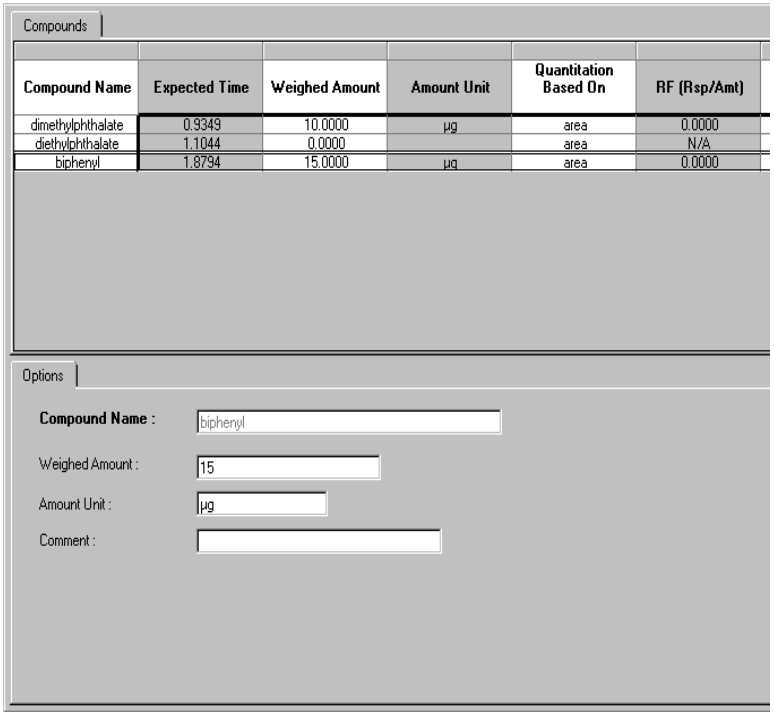


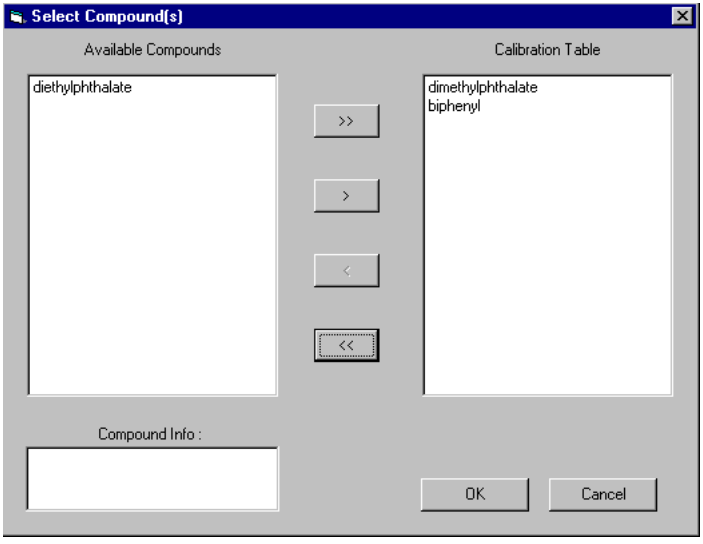
Task 4. Set up calibration

Steps	Detailed Instructions
1 Set up calibration for dimethylphthalate. dimethylphthalate - 10µg	**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.



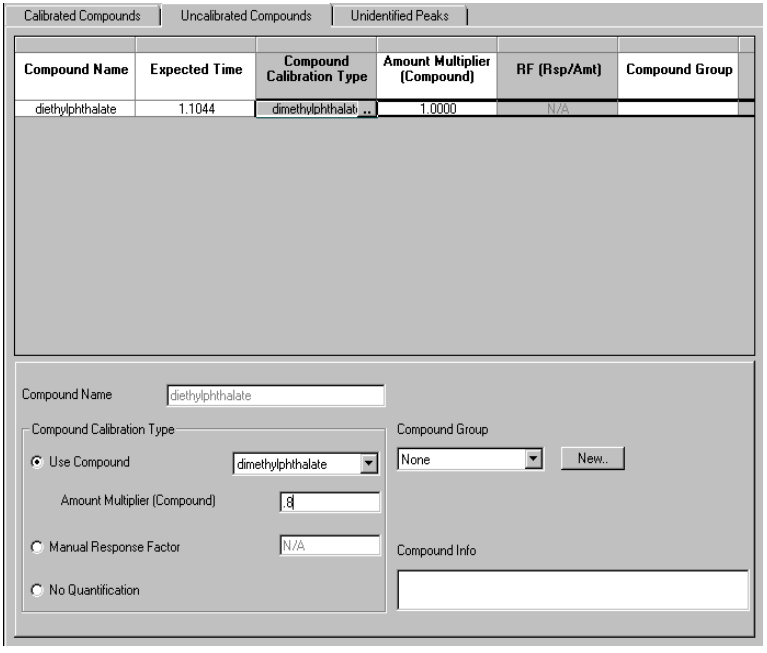
Steps	Detailed Instructions
2 Set up calibration for biphenyl. Biphenyl - 15µg	**a** On the calibration table, select biphenyl. **b** On the **Options** tab, enter 15 in the **Weighed Amount** box and µg in the **Amount Unit** box.



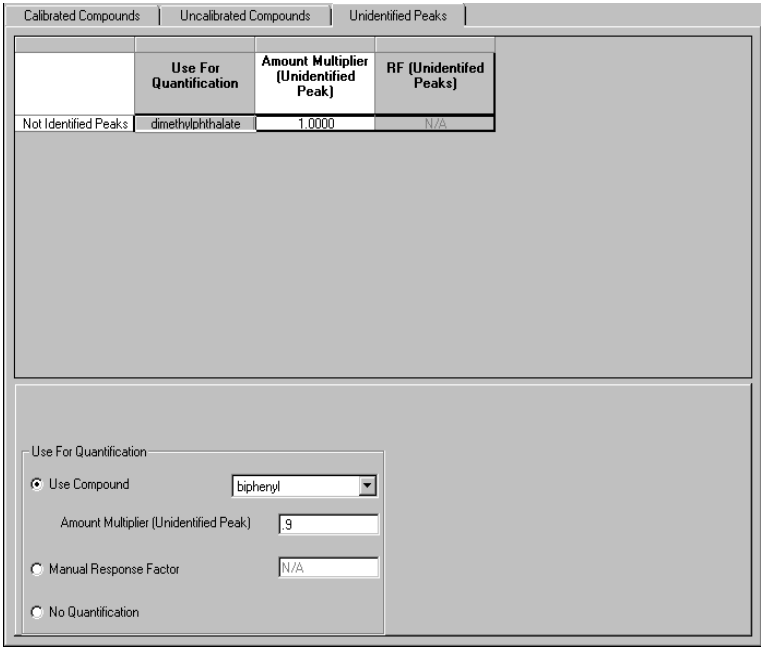
Steps	Detailed Instructions
3 Remove diethylphthalate from the calibration table.	**a** On the calibration table, right-click anywhere and select **Remove Compound** from the shortcut menu. The **Select Compound(s)** dialog box appears. **b** In the **Calibration Table** list, select diethylphthalate. **c** Click the **<** button to put diethylphthalate in the **Available Compounds** list. **d** Click the **OK** button. 

Task 5. Set up quantitation for all four peaks

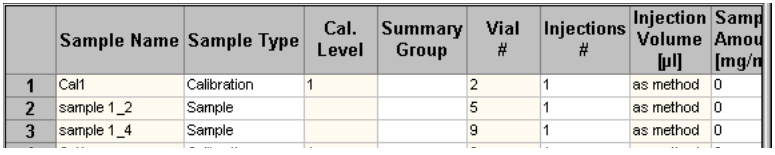
Steps	Detailed Instructions
1 Base the quantitation of diethylphthalate on dimethylphthalate. Use an amount multiplier of .8.	**a** On the selection tree, select **Quantitation Setup** under the Data Analysis folder. **b** Click the **Uncalibrated Compounds** tab. **c** Under **Compound Calibration Type**, select the **Use Compound** option. **d** Select dimethylphthalate from the **Use Compound** list. **e** Enter .8 in the **Amount Multiplier (Compound)** box.



Steps	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.

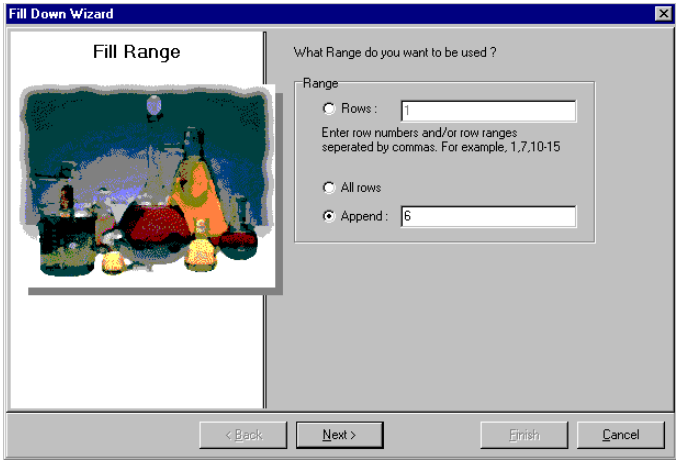


Task 6. Set up the sequence template

Steps	Detailed Instructions
1 Enter the following calibration standards and samples into the sequence template: 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 **NOTE** You cannot set up a sequence template with calibration standards until you have set up calibration in Data Analysis.	**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. • 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. **c** Enter sample 1_2 for row two. • Select Row 2 in the sample table. • Enter sample 1_2 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 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 information into the sample table. 

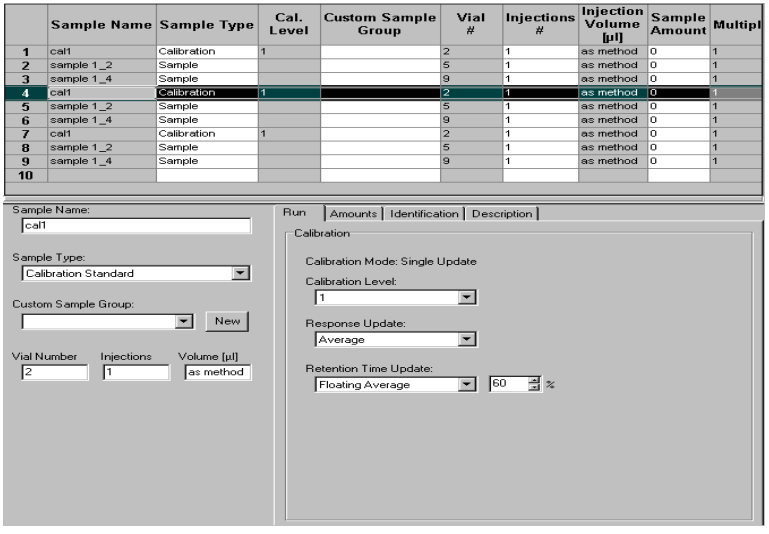
	Sample Name	Sample Type	Cal. Level	Summary Group	Vial #	Injections #	Injection Volume [µl]	Samp Amou [mg/n
1	Cal1	Calibration	1		2	1	as method	0
2	sample 1_2	Sample			5	1	as method	0
3	sample 1_4	Sample			9	1	as method	0

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 cal1in 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								

Steps	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% after 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. 
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.

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.

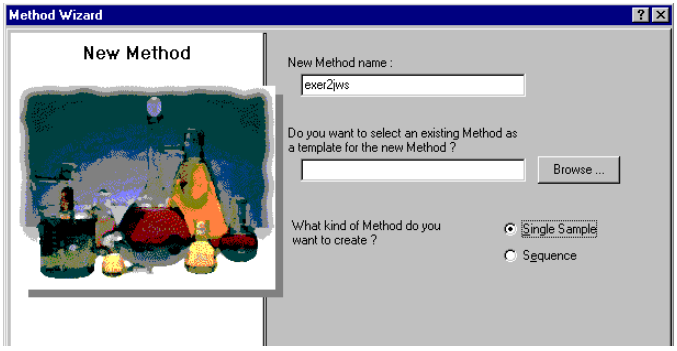
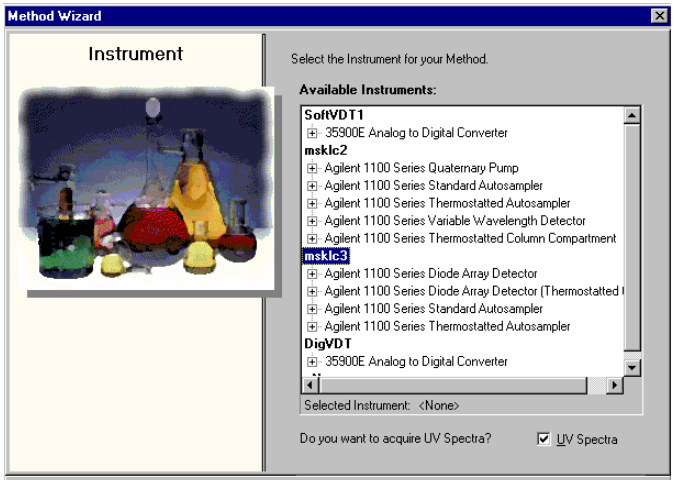
Use the method created in the first part of this exercise to enter and run a single sample to produce an example chromatogram. You can use the completed method to enter and run a group of samples to identify compounds.

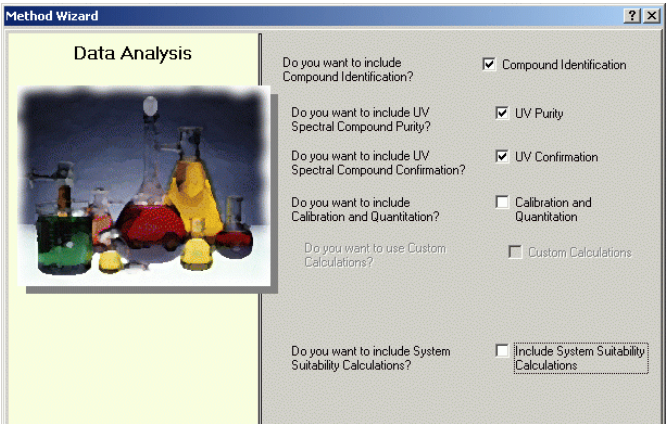
For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

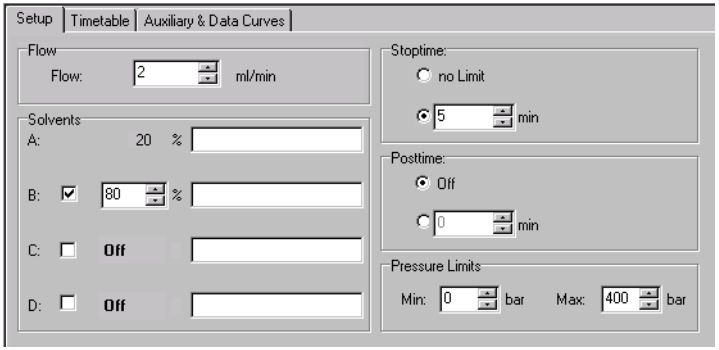
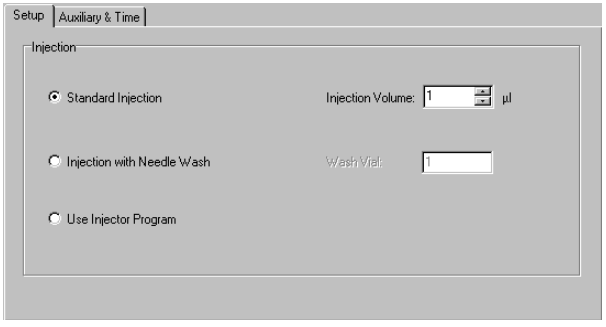
Read "Setting Up Methods" on page 71 for setting up methods.

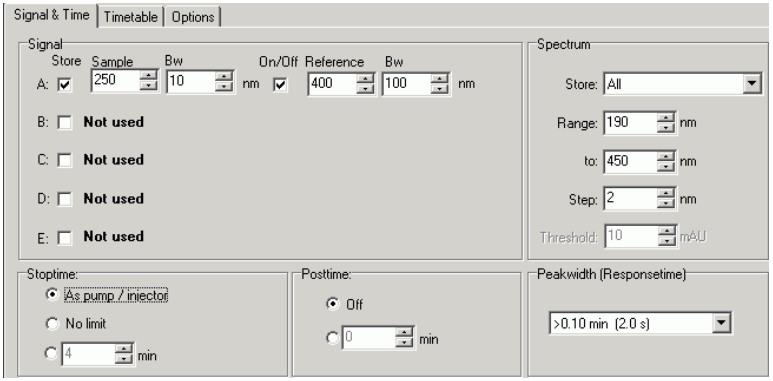
Task 1. Create a method template to identify compounds only

Steps	Detailed Instructions
1 Create a new method template for a single sample. Name the method template, exer4*iii*, where *iii* 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**.  **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.  **c** Click **Next** to scroll to the Data Analysis panel.

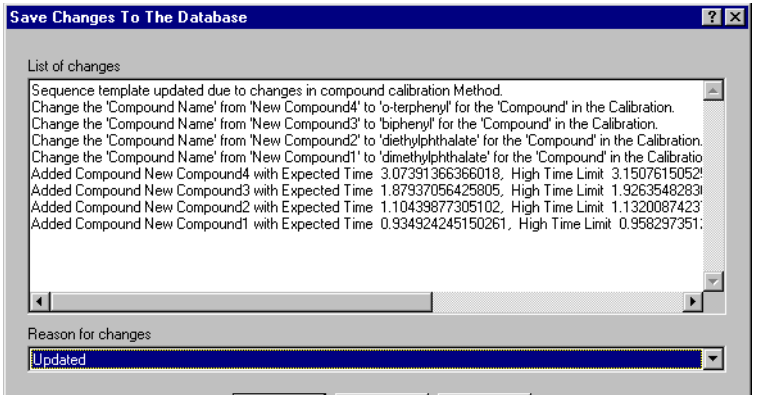
Steps	Detailed Instructions
3 Mark Compound Identification, UV Purity and UV Confirmation.	**a** On the Data Analysis panel, mark the **UV Purity** and **UV Confirmation** check boxes, and clear the **Calibration and Quantification, Include Noise Calculations** and **Include System Suitability Calculations** check boxes.  **b** Click **Next** to scroll to the Identification panel.
4 Complete the setup of the method template. Do not mark any check box on the Method Wizard Identification panel.	**a** Click **Next,** and click the **Finish** button. **b** Click **Save** if the Save Changes to the Database dialog box appears.

Task 2. Enter the instrument conditions for the equilibrium

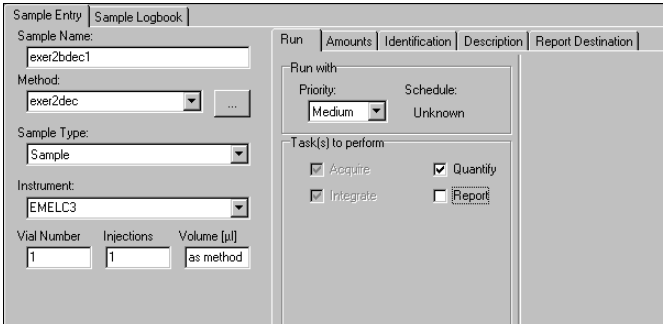
Steps	Detailed Instructions
1 Enter the pump parameters: Methanol as Solvent B: • Flow rate: 2ml/min. • Solvent composition: 80%MeOH/20%H_2O • Stoptime: 5 min. Acetonitrile as Solvent B: • Flow rate: 1.5ml/min • Solvent composition: 65%ACN/35%H_2O • Stoptime: 5 min.	a On the selection tree, expand the 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. 
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**. 

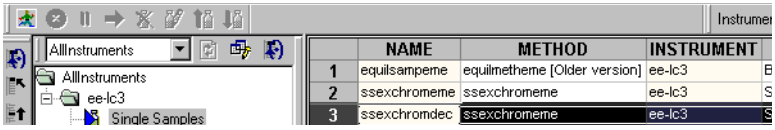
Steps	Detailed Instructions
3 Make sure the stop time is the same for all instrument modules. Stop Time: As pump/injector	**a** On the selection tree, select the **DAD** or **FLD** folder. **b** Under **Stoptime,** select **As pump/injector**. **c** On the selection tree, select the **TCC** folder. **d** Under **Stoptime**, select **As pump/injector**. 
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**, set the **Sample** wavelength to 254 nm and the **Bw** to 10 **c** Mark the **On/Off** check box and set the **Reference** wavelength to 400 and the **Bw** to 100. **d** Under **Spectrum**, select to store **All** spectra, and set the **Range** from 190 to 450 nm with a **Step** size of 2.

Task 3. Save and audit method changes

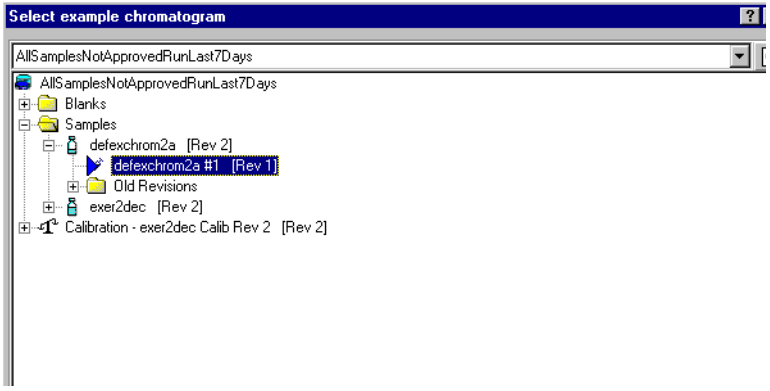
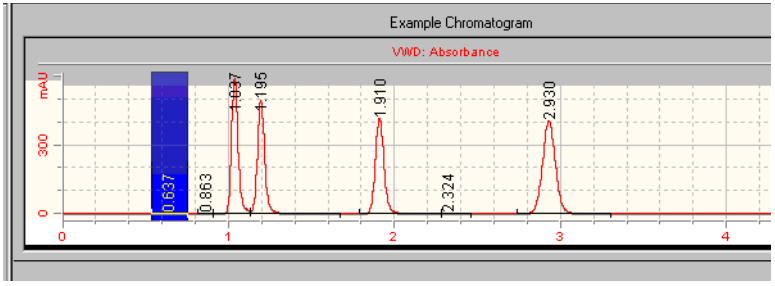
Steps	Detailed Instructions
1 Save the method. After you save the method here, you can use the method to produce an example chromatogram. See "Basic Exercise #2a Run a single sample to produce an example chromatogram" on page 17. Continue with Task 4 after you produce an example chromatogram.	**a** On 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. 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

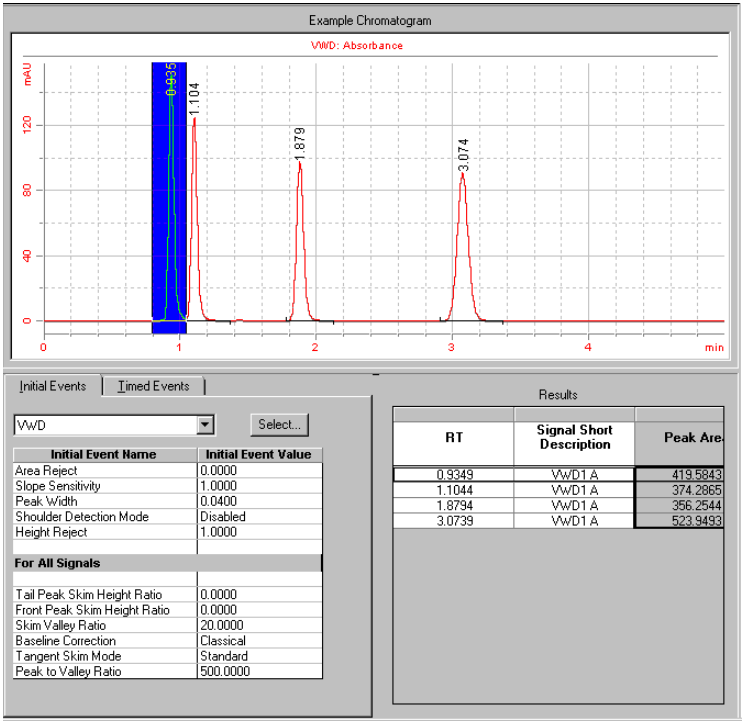
Steps	Detailed Instructions
1 Start the Instrument View to find the sample table for single samples.	**a** Select **Instrument** from the **Current View** list. **b** Expand the folder for the instrument that will produce the example chromatogram. **c** Select **Single Samples**. The sample table and sample entry panel appear in the workspace.
2 Enter a sample with the following information: • Name the sample exchrom3D*iii*, where *iii* are your initials. • Select either exer4*iii* • Select the vial that contains the full-strength isocratic standard.	**a** Enter exchrom3D*iii* 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  . 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** Enter your electronic signature if required. **e** Click the **Save** button.

Steps	Detailed Instructions
5 Run the sample.	**a** On the selection tree, expand your instrument folder. **b** Select **Single Samples**. **c** Select the sample, exchrom*iii*. The Run button becomes available on the Tools toolbar.  **d** Click the **Run** button. You can also run the sample from the Sample View.
6 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. See "Basic Exercise #2a Run a single sample to produce an example chromatogram" on page 17 for detailed instructions.
7 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 exchrom3D*iii* folder. **e** Select the exchrom3D*iii* #1 injection. **f** View the chromatogram and results.

Task 5. Select an example chromatogram and set up integration

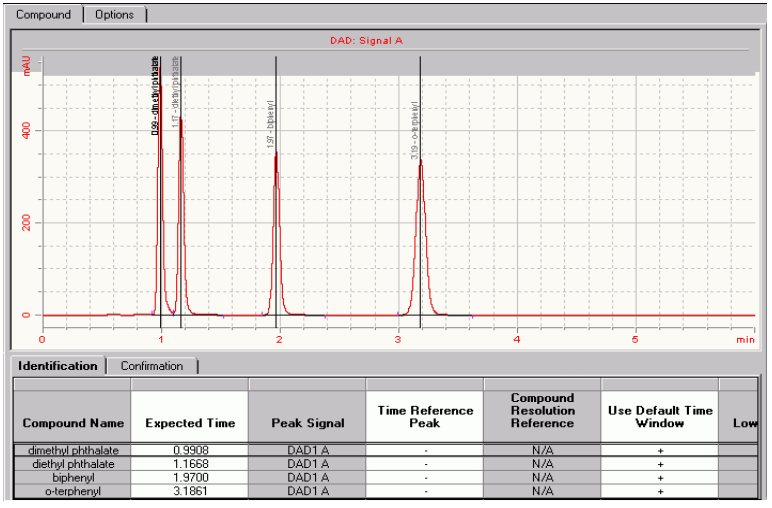
Steps	Detailed Instructions
1 Select an example chromatogram. You do not need the example chromatogram to set up integration and identification, but it is recommended.	**a** On the selection tree, expand the exer4 method folder, if necessary. **b** Expand the **Data Analysis** folder. **c** Select **Example Chromatogram**. **d** On the **Tools** toolbar, click  .  **e** Expand the Samples folder. **f** Expand the exchrom3D*iii* folder. **g** 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 on the Actions toolbar



Task 6. Set up compound identification

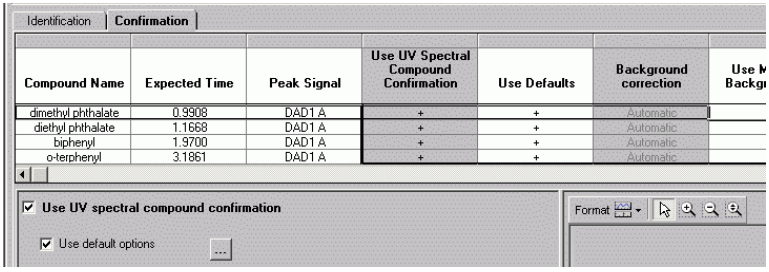
Steps	Detailed Instructions
1 Set up the compound identification table for the following compounds: RT=.9 to 1.1, dimethylphthalate RT=1.1 to 1.2, diethylphthalate RT=1.8 to 2.1, biphenyl RT=3 to 3.2, o-terphenyl	**a** On the selection tree, select the **Identification** item for Data Analysis. **b** 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.

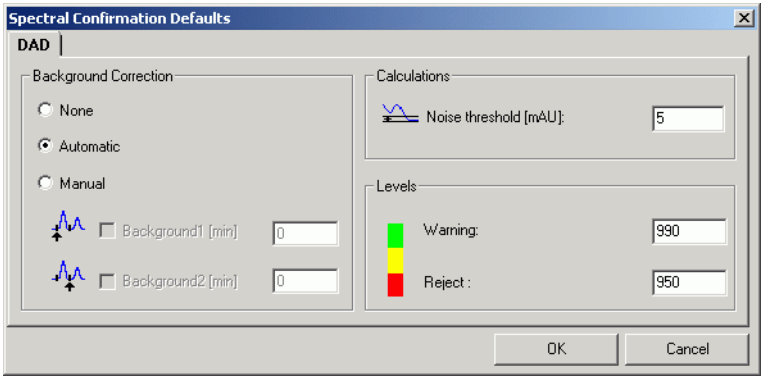


Compound Name	Expected Time	Peak Signal	Time Reference Peak	Compound Resolution Reference	Use Default Time Window	Low
dimethyl phthalate	0.9908	DAD1 A	-	N/A	+	
diethyl phthalate	1.1668	DAD1 A	-	N/A	+	
biphenyl	1.9700	DAD1 A	-	N/A	+	
o-terphenyl	3.1861	DAD1 A	-	N/A	+	

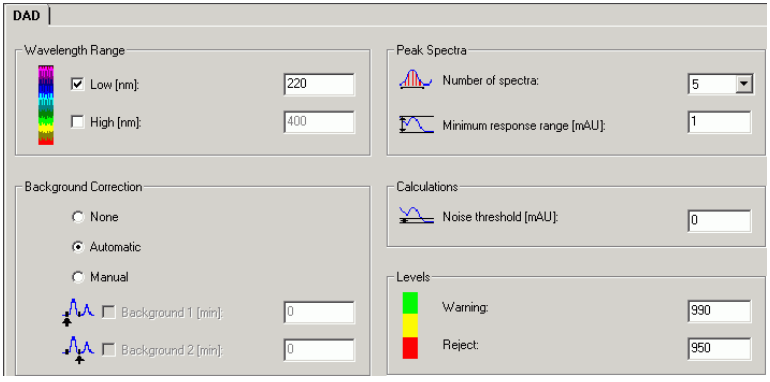
Task 7. Set up UV spectral compound confirmation

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

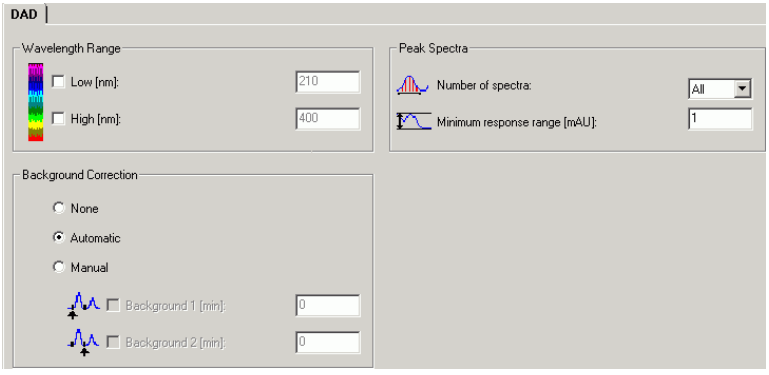


Steps	Detailed Instructions
2 Set up the default UV spectral compound confirmation options	**a** In the panel below the **Confirmation** table, click the **...** button to the right of the **Use default options** check box. The **Spectral Confirmation Defaults** dialog box appears.  **b** In the **Background 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** On the Standard toolbar, click  . The **Compound Reference Spectrum Selection** dialog box for the selected compound appears. **b** On the selection tree, expand the *exchrom3Diii* folder. **c** Select the sample name with the injection number. **d** On the dialog box toolbar, click  . **e** On 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.

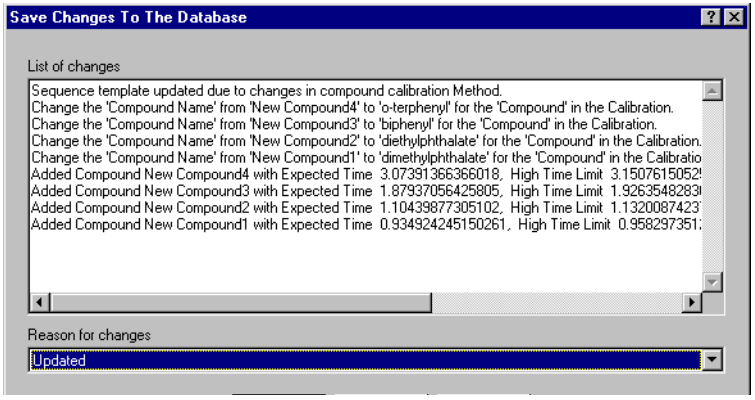
Task 8. Set up UV purity

Steps	Detailed Instructions
1 Set up Spectra Handling parameters • Set up the Wavelength Range • Set up Background Correction • Set up Peak Spectra • Set up Calculations • Set up Levels	**a** On the selection tree, select the **UV Purity** item for Data Analysis. The UV purity options panel is displayed in the workspace.  **b** In the **Wavelength Range** group, mark the **Low** check box and enter 220 in the adjacent field. **c** In the **Background Correction** group, select the **Automatic** option. **d** In the Peak Spectra group, set the **Number of spectra** to **7**. Leave the **Minimum response range** at its default value. **e** In the Calculations group, set the **Noise threshold** to **5** mAU. **f** Leave the **Levels** at their default values.

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.  **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.

Steps	Detailed Instructions
2 Save the method.	**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.

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.

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.

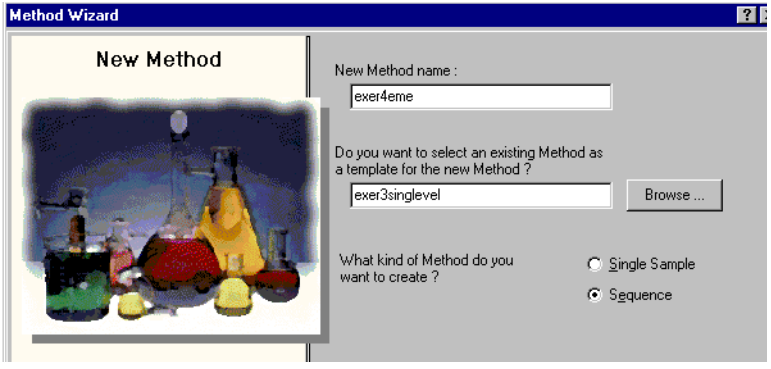
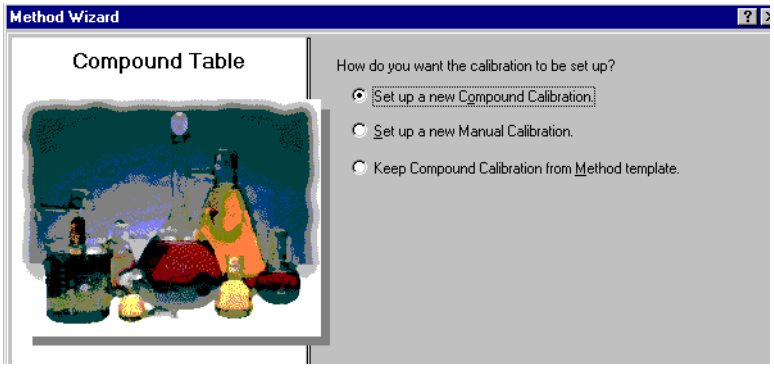
You can use this method with "Advanced Exercise #4a Run a sequence to quantify compounds with multi-level calibration" on page 45 and "Advanced Exercise #4b Change sample variables in the method and reprocess" on page 53.

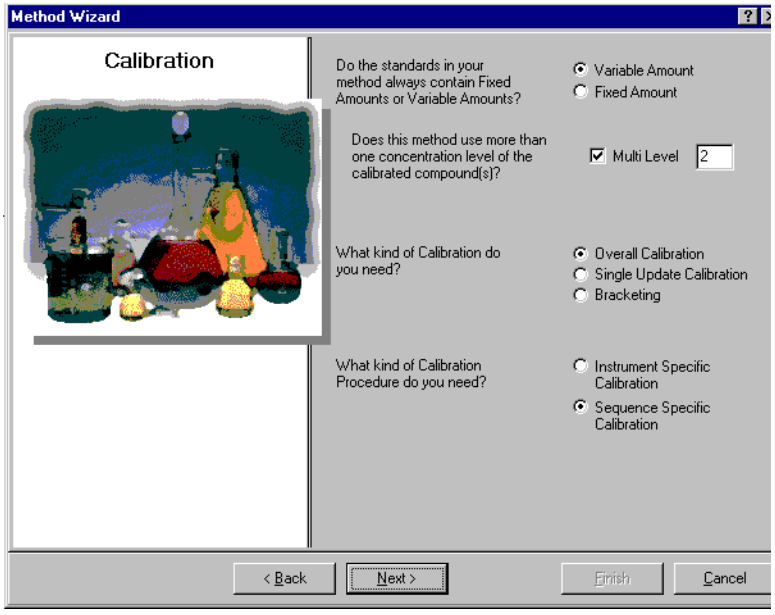
For the tasks in this exercise, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Before you start

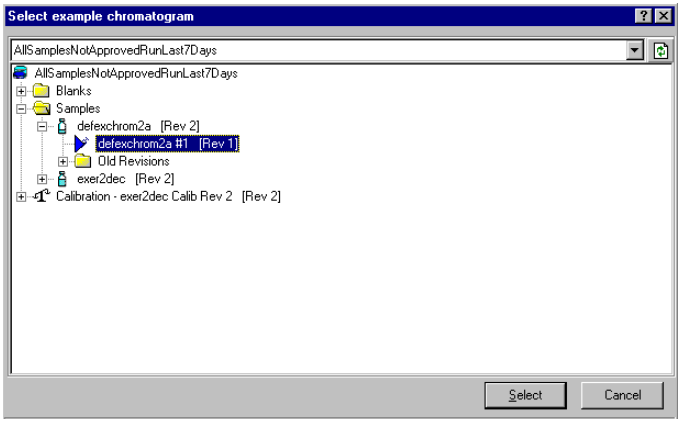
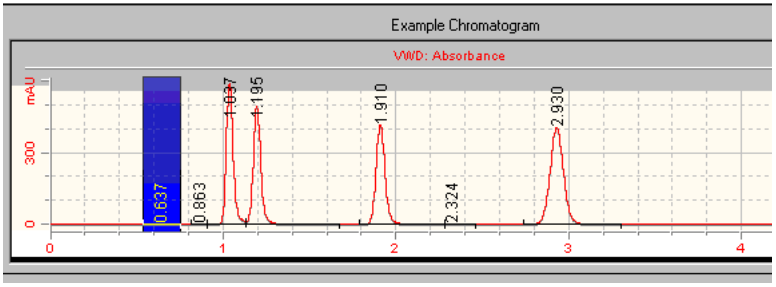
Read "Setting Up Methods" on page 71 to set up methods.

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

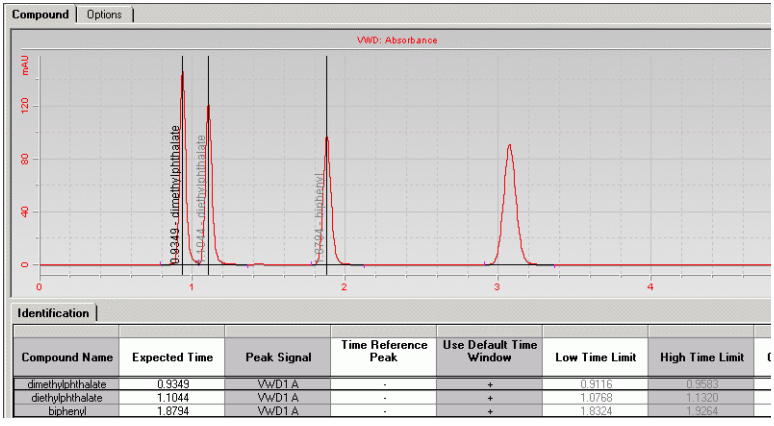
Steps	Detailed Instructions
1 Copy the method to create a new template. • Copy either exer3*iii* or defexer3. • Name the method template, exer4iii, where *iii* are your initials. • Change nothing until you reach the Compound Table panel. Note that the Method Wizard panels contain the method selections from Exercise 3.	**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.  **d** Click **Next** until you reach the Compound Table panel.
2 Set up the Compound Table panel. 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**.  **d** Click **Next** until you reach the **New Method Review** panel.
4 Review your new method template.	**a** On the **New Method Review** panel, review the **Method Wizard Settings**. **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	Detailed Instructions
1 Select an example chromatogram. Use the example chromatogram that you produced with "Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration" and "Basic Exercise #3b Reintegrate and reprocess the results". Or, use defexchr2a. (To use this chromatogram, use an instrument with a VWD detector.) If you cannot see the sample whose chromatogram that you want to select, select another query. **Hint**: The result, defexchr2a, is a restored result.	**a** On the selection tree, expand the new method template, exer4*iii*. **b** Expand the **Data Analysis** folder and select **Example Chromatogram**. **c** On the **Tools** toolbar, click  . 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.

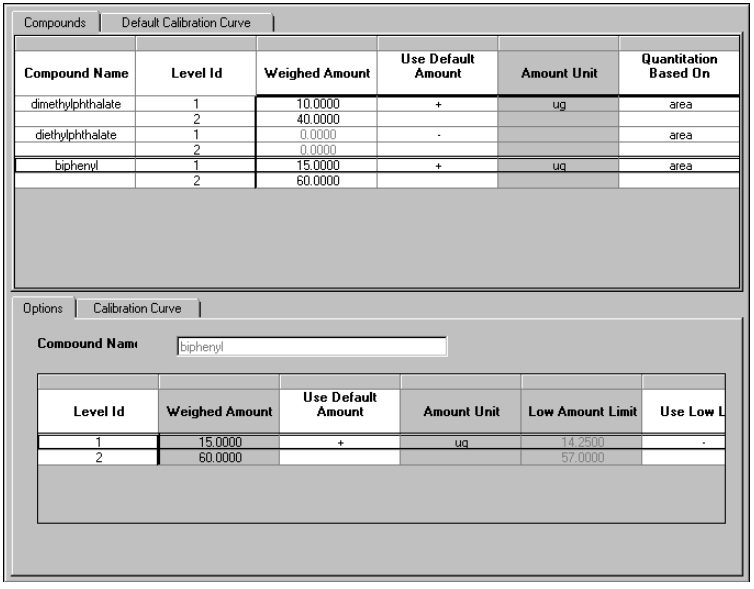
Steps	Detailed Instructions
2 Set up the compound table for these compounds: RT=0.9-1.1 min, dimethylphthalate RT=1.1-1.3 min, diethylphthalate RT=1.8-2.0 min, biphenyl Do not identify the fourth peak. In another exercise, you will set up the fourth peak as an unspecified impurity that is not identified based on retention time.	**a** On the selection tree, select **Identification** under the Data Analysis folder. **b** On the Tools toolbar, click  . The peaks appear with the names New Compound one through four in the compound table. **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,** right-click the fourth cell. **g** Select **Remove Compound**. On the Identification workspace, view the three identified peaks and one unidentified peak.

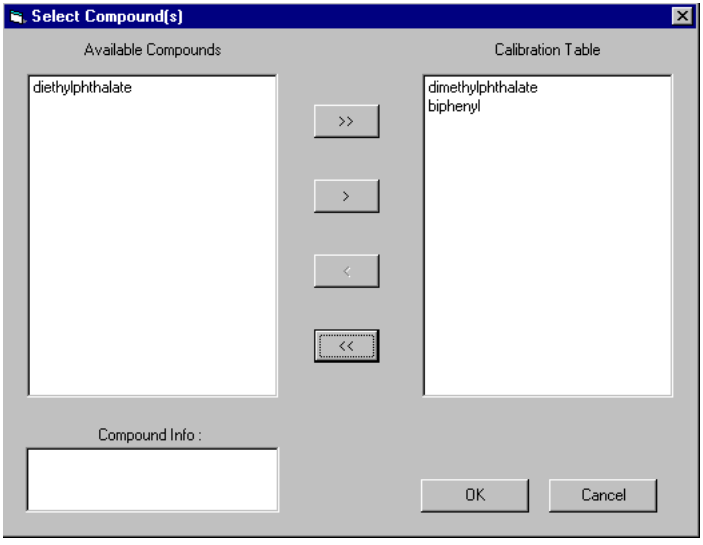


Compound Name	Expected Time	Peak Signal	Time Reference Peak	Use Default Time Window	Low Time Limit	High Time Limit
dimethylphthalate	0.9349	VWD1 A	-	+	0.9116	0.9583
diethylphthalate	1.1044	VWD1 A	-	+	1.0768	1.1320
biphenyl	1.8794	VWD1 A	-	+	1.8324	1.9264

Task 3. Set up calibration and quantitation

Steps	Detailed Instructions
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.	**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.



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. 
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

Steps	Detailed 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.	**a** Click the Divisor cell once, and enter the name, Correction Factor. **b** Enter a default value of 2.

System Defined Sample Variables (Set by the user in Sample Entry and used in quantification)

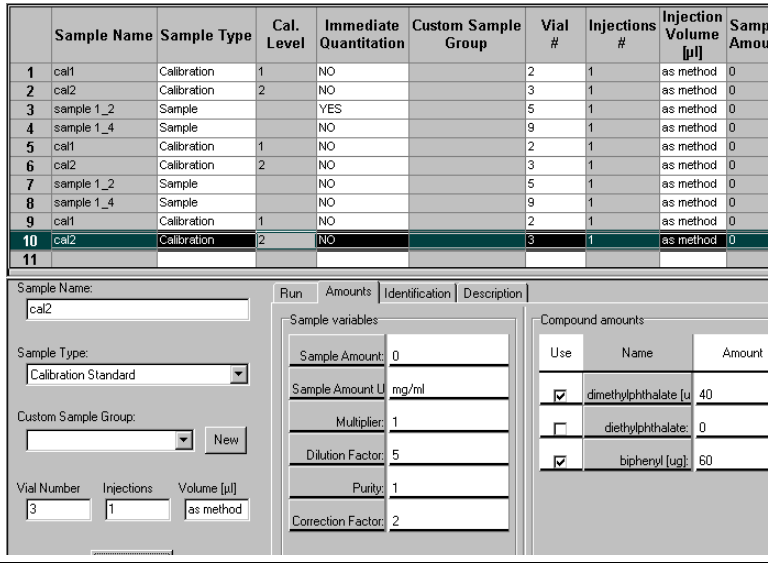
	Variable ID	Display Name	Default Value
1	Multiplier_1	Multiplier	1
2	Multiplier_2	Dilution Factor	5
3	Multiplier_3	Purity	1
4	Multiplier_4		1
5	Multiplier_5		1
6	Divider_1	Correction Factor	2
7	Divider_2		1
8	Divider_3		1
9	Divider_4		1

Task 5. Edit the sequence template

Steps	Detailed Instructions
1 Edit the template to look like this: • two calibration standards (Lev1,2) • two samples, • two calibration standards • two samples, • two calibration standards **NOTE** **You cannot set up or edit a sequence template with calibration standards until you set up calibration in Data Analysis.**	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. **j** Click **Apply**. **k** Repeat steps g-j for the next two standards. **l** 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 the first set of calibration standards. Sample 1_2, along with the other samples, will also be quantified at a later time using the average of all the calibration standards.	**a** Double-click the cell for Sample 1_2 under the heading, **Immediate Quantitation**. **b** Double-click the **Yes** that appears.

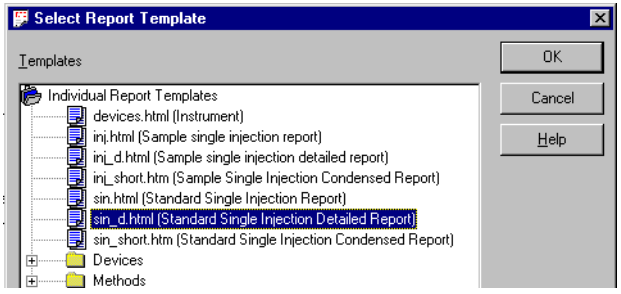
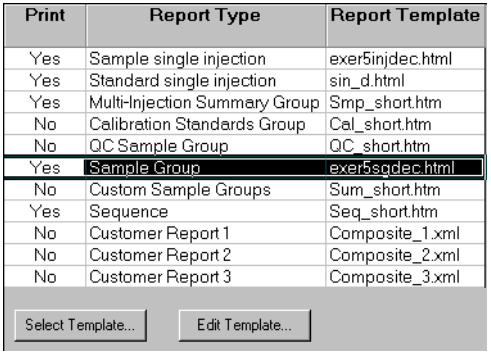
	Sample Name	Sample Type	Cal. Level	Immediate Quantitation	Custom Sample Group	Vial #	Injections #
1	cal1	Calibration	1	NO		2	1
2	cal2	Calibration	2	NO		3	1
3	sample 1_2	Sample		YES		5	1
4	sample 1_4	Sample		NO		9	1
5	cal1	Calibration	1	NO		2	1
6	cal2	Calibration	2	NO		3	1
7	sample 1_2	Sample		NO		5	1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

Steps	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 #	Injection Volume [µl]	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		NO		9	1	as method	0
5	cal1	Calibration	1	NO		2	1	as method	0
6	cal2	Calibration	2	NO		3	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									

Task 6. Select a new report template for a report

Steps	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**. 
2 Select these report types to print: • Sample single injection • Standard single injection • Sequence	**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**. 
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

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

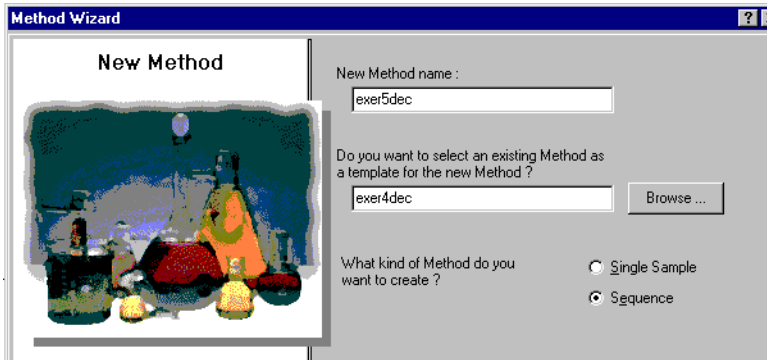
You can use this method with “Advanced Exercise #5a Run a sequence to quantify impurities” on page 61 and “Advanced Exercise #5b Use a different method to reprocess” on page 67.

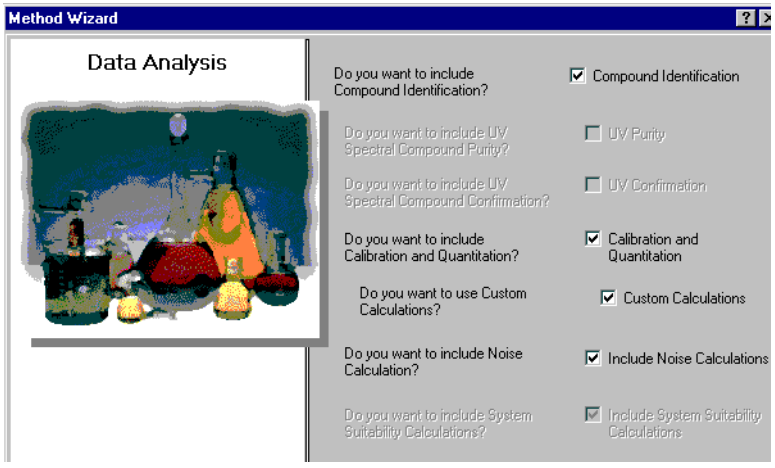
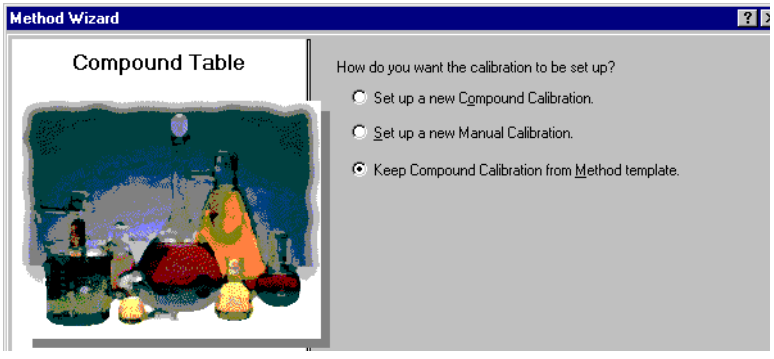
In the tasks in this exercise, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

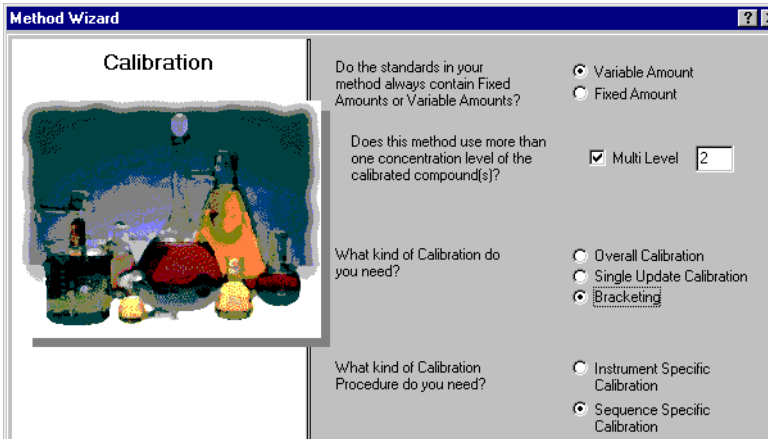
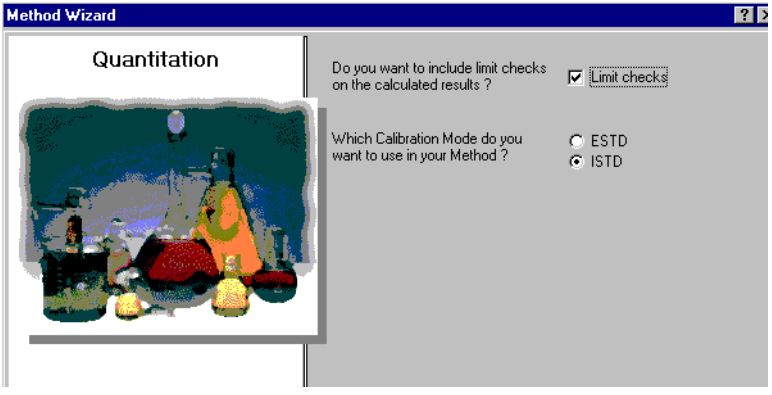
Before you start

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

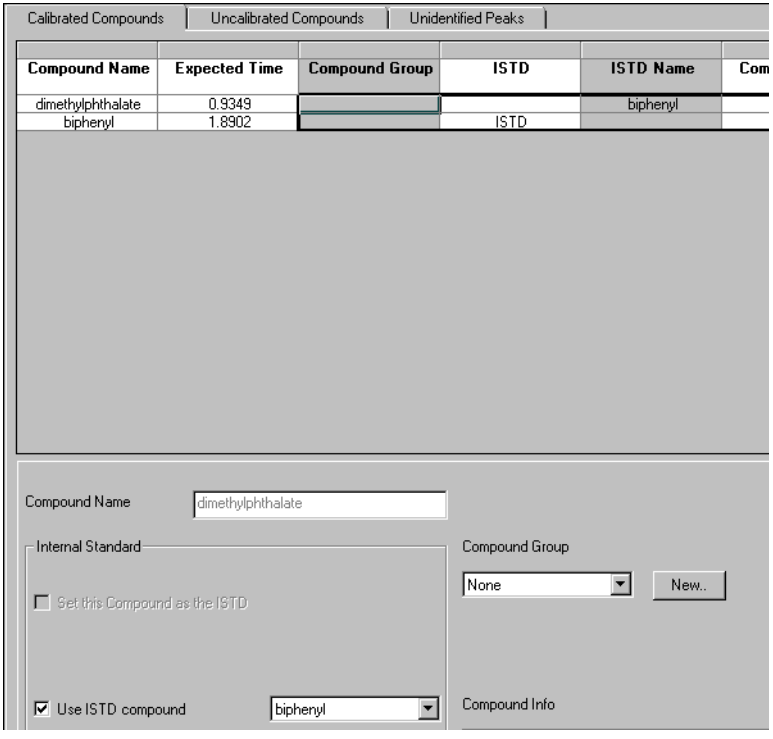
Steps	Detailed Instructions
1 Copy the method to create a new template. • Copy either exer4*iii* or defexer4*iii*. You can use the original method from Exercise 4 or the modified method from Exercise 4b. • Name the method template, exer5*iii*, where *iii* are your initials. Note that the Method Wizard panels contain the method selections in Exercise 4.	**a** Select **File>New>Method** or click and select **Method**. The Method Wizard appears. **b** Click the **Browse** button, and select exer4*iii* or defexer4*iii*. **c** Enter exer5*iii* in the **New Method Name** box.  **d** Click **Next** until you reach the Data Analysis panel.

Steps	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.  **c** Click **Next** to scroll to the Compound Table panel.
3 Select a Compound Table option. Even though you are changing the mode of calibration to Bracketing, you can keep the calibration setup from Exercise 4.	**a** On the Compound Table panel, Select **Keep Compound Calibration from Method template**.  **b** Click **Next** until you reach the **Calibration** panel.

Steps	Detailed Instructions
4 Select Calibration options. Select Bracketing and keep all other options the same.	**a** On the **Calibration** panel, select **Bracketing**.  **b** Click **Next** to scroll to the **Quantitation** panel.
5 Select Quantitation options	**a** On the **Quantitation** panel, mark the **Limit checks** check box. **b** Select **ISTD**.  **c** Click **Next** to scroll to the **New Method Review** panel.
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.



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

Steps	Detailed Instructions
1 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.)

		Amount	New Percent Specified Impurity	New Percent Unspecified Impurity
1				
2				
3	-			
4	Single Injection			
5	Single Inj. Variables		9.48	19.07
6	- Identified Compounds			
7	dimethylphthalate	0.9993		
8	diethylphthalate	1.9968		
9	biphenyl	3.0126		
10	- Not Identified Peaks			
11	Unknown 1	4.0158		
12	..	4.9725		
13	Unknown n	6.0583		

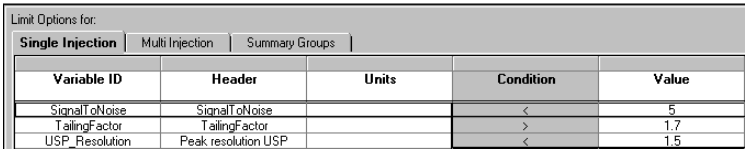
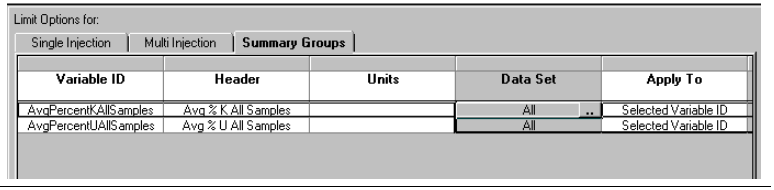
Steps	Detailed Instructions
2 Set up to calculate the average percent of impurity for all injections of a sample. Do this for both the specified and unspecified impurity.	**a** On the Custom Calculations workspace, click the **Multi-injection** tab. **b** Add a column for percent specified impurity. • Right-click the table, and select **Add Column**. • On the Existing Column sheet, expand User Defined, and select **Percent Specified Impurity**. • Click **Apply**. **c** Add a column for the percent unspecified impurity. • Select **Percent Unspecified Impurity**. • Click **Apply**. **d** Add a column for the average of the percent specified impurity for all injections. • Click the **Add a New Custom Calculation Column** tab. • Enter the **Variable ID** as anything you want, e.g., AvgPercentSpecified. • Enter the **Display Name** as a variant of the ID, e.g., Avg Percent Specified. • Enter the **Level** as Multiple Inj. Variables, and click **Apply**. **e** Add a column for the average of the percent unspecified impurity for all injections of a sample. • Enter the Variable ID, Display Name and Level as Multiple Inj. Variables. • Click **OK**. **f** Enter the formula for the average of the percent specified impurity into the Multiple Inj. Variable cell. • Enter the syntax =AVERAGE(D6:D8), which represents the average of the percent impurity calculation for each sample or all injections. You can use the f_x button to access the AVERAGE function, or you can type AVERAGE. **g** Enter the formula for the average of the percent unspecified impurity.

	A	B	C	D	E	F	G
1						New	New
2				Percent Specified Impurity	Percent Unspecified Impurity	Avg Percent 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			..				
12			Single Inj. #n				

Steps	Detailed Instructions
3 Set up to calculate the average percent of impurity for all samples. Do this for both the specified and unspecified impurity.	**a** Click the **Sample Group** tab in the Custom Calculations workspace. **b** Add a column for average percent specified impurity. • Right-click the table, and select **Add Column**. • Expand **User Defined**, and select **Avg Percent Specified**. • Click **Apply**. **c** Add a column for the average percent unspecified impurity. • On the Existing Column sheet, expand User Defined, and select **Avg Percent Unspecified**. • Click **Apply**. **d** Add a column for the average of the percent specified impurity for all samples. • Click the **Add a New Custom Calculation Column** tab. • Enter the **Variable ID** as anything you want, e.g., AvgPercentSAllSamples. • Enter the **Display Name** as a variant of the ID, e.g., Avg % S All Samples. • Enter the **Level** as Sample Group Variables, and click **Apply**. **e** Add a column for the average of the percent unspecified impurity for all samples, e.g., AvgPercentUAllSamples. • Enter the Variable ID, Display Name and Level as Sample Group Variables. • Click **OK**. **f** Enter the formula for the average of the percent specified impurity. • Enter the syntax =AVERAGE(F6:F8), which represents the average of the percent impurity calculation for all samples. You can use the f_x button to access the AVERAGE function, or you can type AVERAGE. **g** Enter the formula for the average of the percent unspecified impurity for all samples.

	A B C	D	E	F	G
1				New	New
2		Avg Percent Specified	Avg Percent Unspecified	Avg % S All Samples	Avg % U All Samples
3	-				
4	Samples				
5	- Sample Group Variable			1.99	=AVERAGE (E6:E8)
6	Sample #1	0.99	1.01		
7	..	2.01	1.98		
8	Sample #n	2.97	3.01		
9	- dimethylphthalate				
10	Sample #1				
11	..				

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

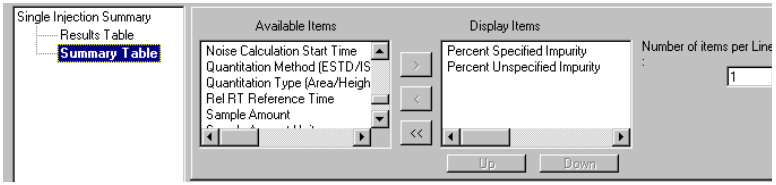
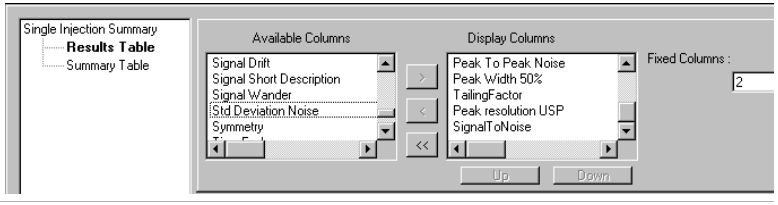
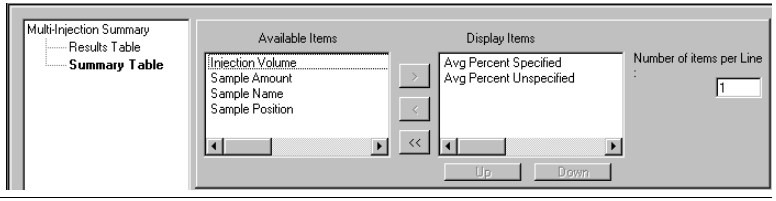
Steps	Detailed Instructions
1 Set up limits for system suitability calculations • If tailing factor > than 1.7, then say Not Passed - all samples and only dimethylphthalate • If USP resolution < than 1.5, then say Not Passed - all samples and all compounds • If signal to noise is less than 5, then say 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. **l** Click **OK**. 
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. 

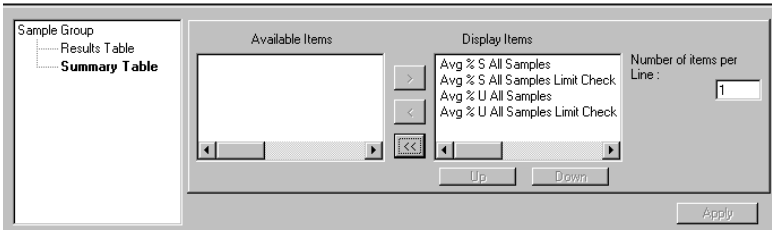
Task 5. Edit the sequence template for bracketing and multiple injections

Steps	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** Select **Sequence Template** in the selection tree. **b** Double-click the **Bracketing** cell for Cal1 in row 1, and double-click **Open**. **c** Double-click the **Bracketing** cell for Cal1 in row 5, and double-click **Open**. **d** Double-click the **Bracketing** cell for Cal2 in row 6, and double-click **Close**. **e** Double-click the **Bracketing** cell for Cal2 in row 10, and double-click **Close**.
2 Enter a blank sample in the first row and enter two injections for each sample.	**a** Select row 1, and click the **Insert** button. (Use tooltip.) **b** Enter NoiseBlank for the **Sample Name**, and select Blank Run for the **Sample Type**. **c** Enter a different Vial#, and click **Apply**. **d** Enter 2 for the Injections # for each sample in the sequence.

	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	cal2	Calibration	2	None		3	1	as method	0
4	sample 1_2	Sample				5	2	as method	0
5	sample 1_4	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
11	cal2	Calibration	2	Close		3	1	as method	0

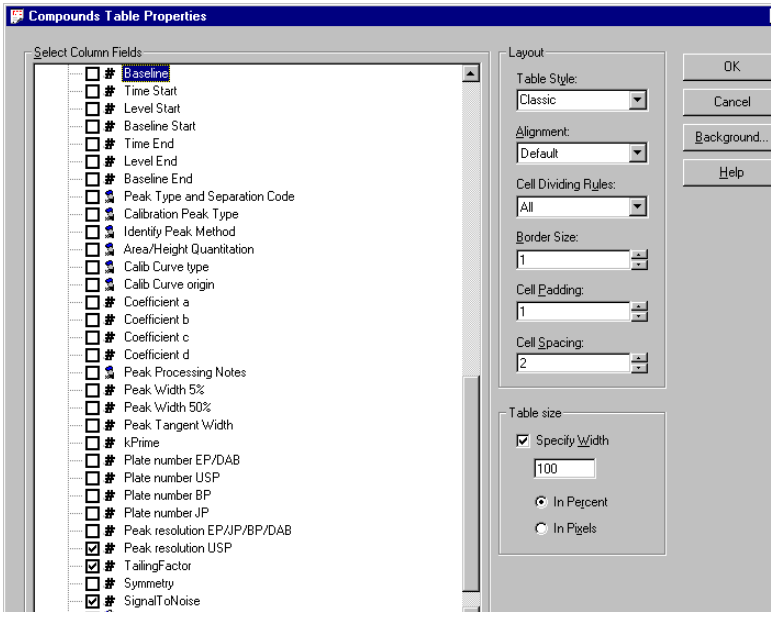
Task 6. Set up the Result View layout to see custom and system suitability calculations

Steps	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**. 
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**. 
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**. 

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**. 

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 exer5inj*iii*, 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	###.##	X.DDDD	#####.###	##.###	##.###

g Select **File > Save As**, enter exer5inj*iii*, 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 exer5sg*iii*, 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. **l** 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 exer5sg*iii* and click **Save**.

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

Sample group (detailed)

Sequence name:	XXXXXXXXXXXXXXXXXXXX
Sequence Start:	sys_Date, sys_Time
Sequence End:	sys_Date, sys_Time
Method (rev):	XXXXXXXXXXXXXXXXXX (###)

Number of unidentified peaks: ##

Sample group variables

#	Sample name	Amount	Position	Inj. vol.
##	XXXXXXXXXXXXXXXXXX	##.DDDD	XXXXXXXX	###.DD

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

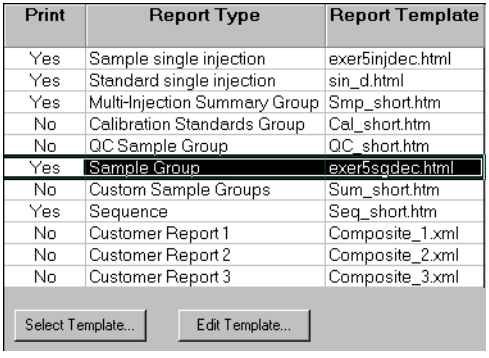
Sample group limit results

#	Sample name	Compound	Limit (Compound)	Limit (Sample)
##	XXXXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXXXX	XXXXXXXXX	XXXXXXXXX

Avg % S All Samples Limit Check: XXXXXXXXX

Avg % U All Samples Limit Check: XXXXXXXXX

Task 8. Select report templates and report types

Steps	Detailed Instructions
1 Select report templates for report types. • Use exer5inj*iii* for the Sample single injection report. • Use exer5sg*iii* for the Sample group report.	**a** Exit the Cerity Report Template Editor. **b** Select the Sample single injection report type and click **Select Template...**. **c** Select exer5inj*iii* and click **OK**. **d** Select the Sample group report type and click **Select Template...**. **e** Select exer5sg*iii* and click **OK**.
2 Select these report types to print. • Sample single injection • Standard single injection • Multi-injection summary • Sample group • Sequence	**a** Double-click the **Print** cell for the Multi-Injection Summary Group report to change **No** to **Yes**. **b** Repeat instruction (a) for the Sample Group report to change **Yes** to **No**. 
3 Save the method	On the Standard toolbar, click  , and enter your reasons for changes and electronic signature, if necessary

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

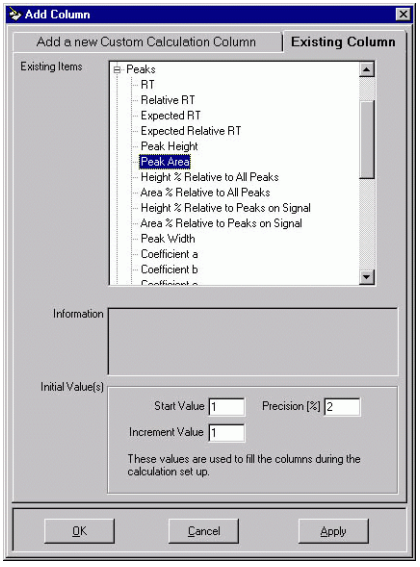
Advanced Exercise #7
Calculate the mean area sum of the unidentified impurities per lot

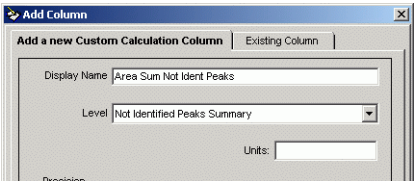
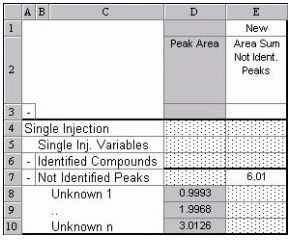
This exercise contains a series of tasks to learn how to set up a custom calculation to calculate the mean of the area sum of the unidentified impurities per lot of samples:

- 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.

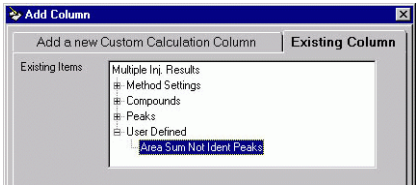
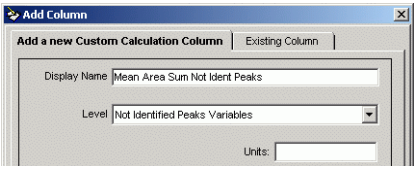
Task 1. Set up to sum the peak areas of the unidentified peaks in a single injection

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.  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**.  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
2				Peak Area	Area Sum Not Ident. Peaks
3		-			
4			Single Injection		
5			Single Inj. Variables		
6		-	Identified Compounds		
7		-	Not Identified Peaks		6.01
8			Unknown 1	0.9993	
9			..	1.9968	
10			Unknown n	3.0126	

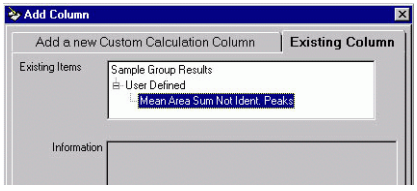
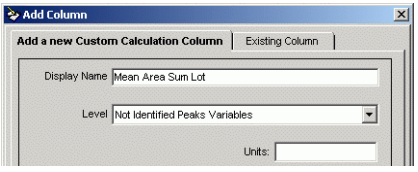
Task 2. Set up to average the area sums of the unidentified peaks for all injections of a sample

Steps	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.  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.	**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 Not Ident Peaks` in the **Display Name** field. **d** Click the **Level** down arrow and select **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					New
2				Area Sum Not Ident. Peaks	Mean Area Sum Not Ident. Peaks
3	-				
4	Multi-Injection Summary				
5	-	Multiple Inj. Variable			
6			Single Inj. #1		
7			..		
8			Single Inj. #n		
9	-	Not Identified Peaks			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

Steps	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**.  The workspace now contains a column for the new variable **Mean Area Sum Per Lot**.

Steps	Detailed Instructions
3 Enter the formula for the mean of the area sums for the lot.	**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					New
2				Mean Area Sum Not Ident. Peaks	Mean Area Sum per Lot
3	-				
4	Samples				
5	-	Sample Group Variable			
6			Sample #1		
7			..		
8			Sample #n		
9	-	Not Identified Peaks			2.01
10			Sample #1	1.00	
11			..	2.02	
12			Sample #n	3.01	

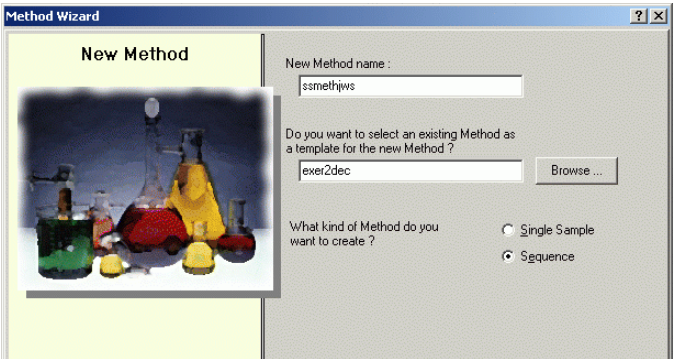
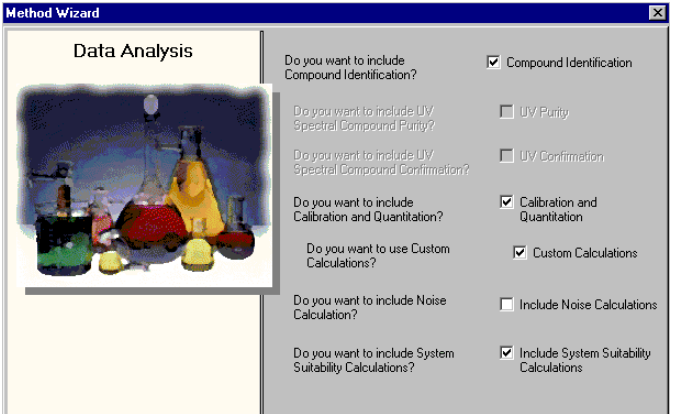
Advanced Exercise #7 Calculate the mean area sum of the unidentified impurities per lot

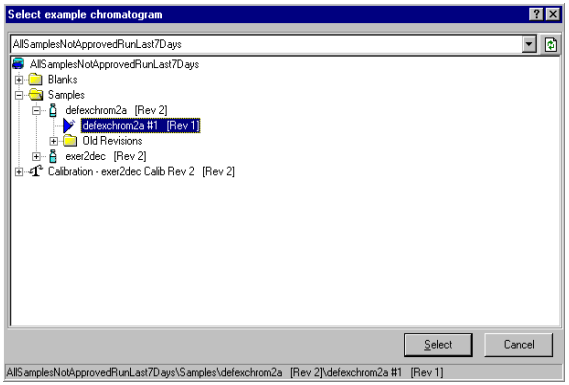
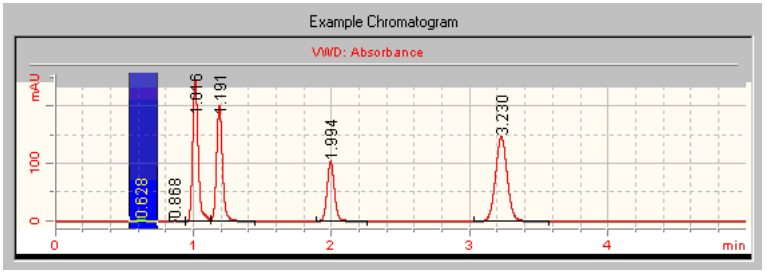
Advanced Exercise #8
Set up a Group Identifier with calculations for system suitability

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:

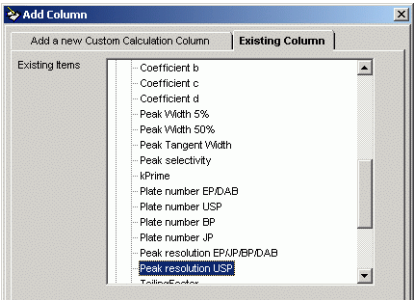
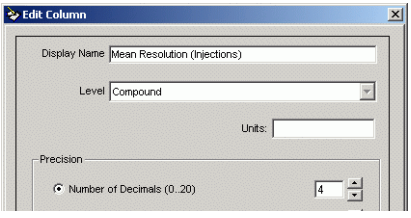
- Set up a method to include System Suitability Calculations
- Set up the custom calculations for the system suitability test
- Set up the limit conditions
- Identify the system suitability samples in the sequence table

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

Steps	Detailed Instructions
1 Create a new sequence method • Name the method ssmeth*iii,* 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 ssmeth*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**, **Custom Calculations** and **Include System Suitability** check boxes.  **g** Click through the remaining panels, making appropriate selections until you have completed the Method Wizard.

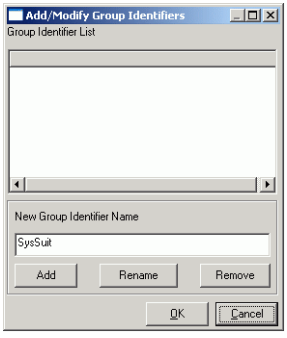
Steps	Detailed Instructions
2 Select an example chromatogram • Use the example chromatogram you produced with Basic Exercise 2a or 2b of the "Basic Exercise #3a Run a sequence to quantify compounds with single-level calibration" and "Basic Exercise #3b Reintegrate and reprocess the results". • Or, use defexchrom2a.	**a** On the selection tree, expand the exer3*iii* folder. **b** Expand the **Data Analysis** folder. **c** Select the **Example Chromatogram** item. **d** On the **Tools** toolbar, click  .  **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.  **g** After you have selected the example chromatogram, you can see the 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	Detailed Instructions
1 On the multi-injection summary worksheet, add a column to contain the resolution of each component.	**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.	**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 (Injections)` in the **Display Name** field. **d** Click the **Level** down arrow and select **Compound**. **e** Set the **Number of Decimals** to 4.  The workspace now contains a column for the new variable **Mean Resolution (Injections)**.

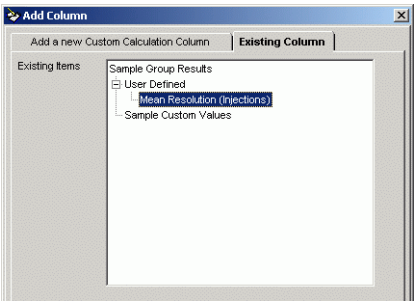
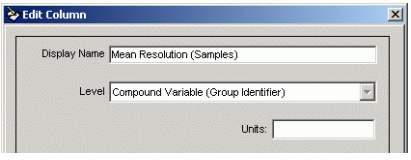
Steps	Detailed Instructions
3 Enter the formula for the mean of the resolutions for the replicate injections for each compound.	**a** In each of the compound variables lines of the new column, enter the formula to average the resolutions of the replicate injections. **Hint:** Use the syntax, =AVERAGE(D10:D12).
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**.

	A	B	C	D	E
1					New
2				Peak resolution USP	Mean Resolution (Injections)
3	-				
4	Multi-Injection Summary				
5	-	Multi Injection Variable			
6			Single Injection #1		
7			...		
8			Single Injection #n		
9	-	dimethylphthalate			2.0029
10			Single Injection #1	0.999	
11			...	1.997	
12			Single Injection #n	3.013	
13	-	diethylphthalate			5.0156
14			Single Injection #1	4.016	
15				4.973	

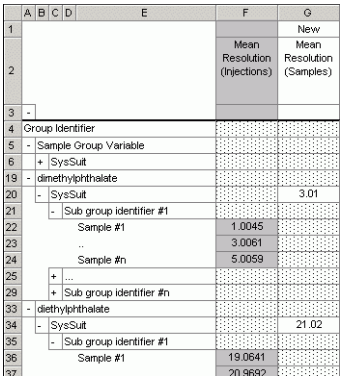


c Click OK to close the **Add/Modify Group Identifiers** dialog box.

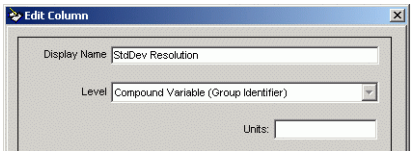
The workspace now contains new groups of lines under each section for the **Group Identifier SysSuit**.

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

Steps	Detailed Instructions
7 Enter the formula for the mean of the resolutions across samples for each compound.	**a** In the **SysSuit** line of the new column for dimethylphthalate, enter the formula to average the resolutions of the samples. **Hint:** Use the syntax, =AVERAGE(F22:F24). **b** Extend the selection to include the SysSuit lines for each compound in the **Mean Resolution (Samples)** column. **Hint:** Hold the left mouse button down while selecting the cells. **c** Right click in the worksheet and select **Fill Down** from the context menu. The formula is copied into each of the available cells.
8 Add a column to contain the new calculation for the standard deviation of the mean resolutions.	**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 `StdDev Resolution` in the **Display Name** field. **d** Click the **Level** down arrow and select **Compound Variable (Group Identifier**.

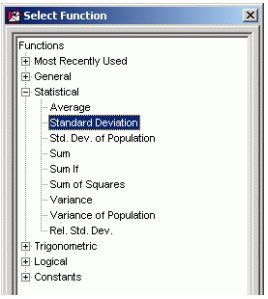


	A	B	C	D	E	F	G
1							New
2						Mean Resolution (Injections)	Mean Resolution (Samples)
3	-						
4	Group Identifier						
5	-	Sample Group Variable					
6		+	SysSuit				
19	-	dimethylphthalate					
20		-	SysSuit				3.01
21			-	Sub group identifier #1			
22					Sample #1	1.0045	
23					..	3.0061	
24					Sample #n	5.0059	
25			+	...			
29			+	Sub group identifier #n			
33	-	diethylphthalate					
34		-	SysSuit				21.02
35			-	Sub group identifier #1			
36					Sample #1	19.0641	
37						20.9692	



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

Steps	Detailed Instructions
9 Enter the formula for the standard deviation of the mean resolutions.	**a** Select the **SysSuit** line of the new column for dimethylphthalate, right click in the worksheet and select **Select Function** from the context menu. The **Select Function** dialog box appears. **b** Expand the **Statistical** section, select **Standard Deviation** and click **Select**.



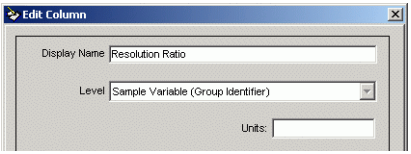
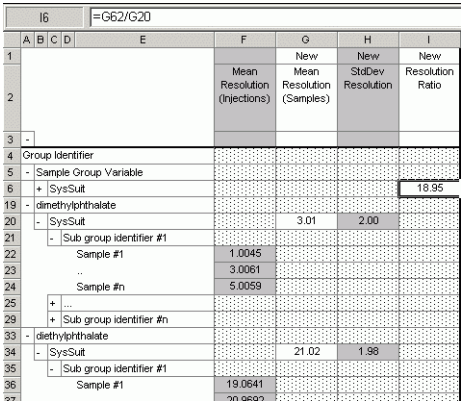
The **STDDEV** function is copied to the selected cell.

c Add the cell references for the standard deviation calculation:

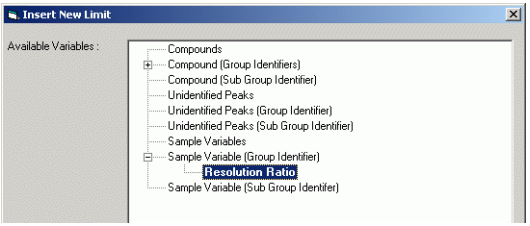
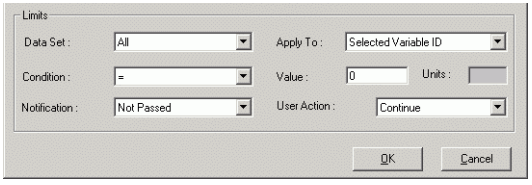
The syntax is =STDEV(F22:F24).

d Fill down the column with the new calculation.

	A B C D E	F	G	H
1			New	New
2		Mean Resolution (Injections)	Mean Resolution (Samples)	StdDev Resolution
3	-			
4	Group Identifier			
5	- Sample Group Variable			
6	+ SysSuit			
19	- dimethylphthalate			
20	- SysSuit		3.01	2.00
21	- Sub group identifier #1			
22	Sample #1	1.0045		
23	..	3.0061		
24	Sample #n	5.0059		
25	+ ...			
29	+ Sub group identifier #n			
33	- diethylphthalate			
34	- SysSuit		21.02	1.98
35	- Sub group identifier #1			
36	Sample #1	19.0641		

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**.  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 dimethylphthalate peak. **Hint:** Use the syntax, =G62/G20. 

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

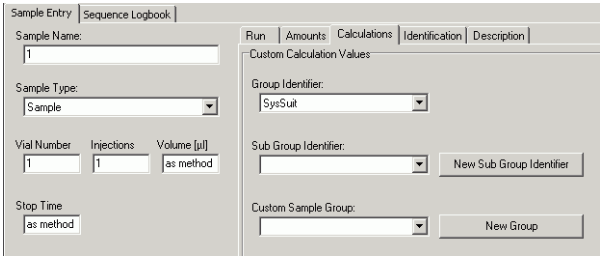
Steps	Detailed Instructions
2 Add two more system suitability limit checks: • If the ratio is greater than 0.8 (but less than 0.9), give a warning, but continue the run. • If the ratio is less than 0.8, the check is not passed; abort the run.	**a** For each limit check, display the **Insert new limit** dialog box, expand the **Sample Variable (Group Identifier)** section and select **Resolution Ratio**. **b** Set the parameters to: • **Data Set: SysSuit** • **Apply To: Selected Variable ID** • **Condition: >** • **Value: 0.8** • **Notification: Warning** • **User Action: Continue** and • **Data Set: SysSuit** • **Apply To: Selected Variable ID** • **Condition: <** • **Value: 0.8** • **Notification: Not Passed** • **User Action: Abort**

Limit Options for:

Single Injection | Multi Injection | Summary Groups | **Group Identifier**

Header	Units	Data Set	Apply To	Condition	Value	Notification	User Action
Resolution Ratio			Selected Variable ID	<	0.75	Not Passed	Abort
Resolution Ratio			Selected Variable ID	>	0.8	Warning	Continue
Resolution Ratio			Selected Variable ID	>	0.9	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.  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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