

	<b>Standard Operating Procedure</b> <b>Creating Processing Method Files</b> <b>using Chromeleon Series 7.X Software</b>		<b>SOP Number</b> <b>D-812</b>	<b>Revision</b> <b>4</b>
			<b>Effective Date</b> 01/27/23	<b>Page</b> <b>Page 1 of 5</b>
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## 1.0 Purpose

The purpose of this procedure is to provide a general overview for creating HPLC Processing Method files for quantitation of analyte(s).

## 2.0 Scope

This procedure applies to processing method files created, stored and altered on lab computer(s) dedicated to Dionex HPLC system(s). This procedure applies specifically to the Chromeleon Series 7.X software. See Chromeleon Series 7.X electronic help for specific details and alternate methods for creating processing method files.

## 3.0 Responsibility

- 3.1 It is the responsibility of QC and Analytical Chemists to follow this procedure.
- 3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.
- 3.3 It is the responsibility of the QC Laboratory Management and/or Analytical Development to keep this procedure current with the latest Ion Labs practices.

## 4.0 Definitions

- 4.1 **QC** – Quality Control
- 4.2 **Processing Method** – A processing method includes all parameters that are used for the evaluation of a peak or the entire chromatogram and also any calibration information used to calculate sample amounts. The processing method consists of the following:

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- 4.2.1 The Cobra Peak Detection algorithm
- 4.2.2 All settings for automatic peak integration and assignment (identification)
- 4.2.3 The parameters (calibration constants) that are part of the area/concentration (amount) conversion formulas as well as the calibration points required for the calculation.

## **5.0 References**

- 5.1 Chromeleon Series 7.X Electronic Help
- 5.2 Thermo Scientific Chromeleon 7.2 Operator Training Level 1, pp.103-140

## **6.0 Procedure**

- 6.1 From within the Chromeleon console, bring up the sequence to be processed then double-click on one of the chromatogram thumbnails. Click Processing Method in the ribbon..
- 6.2 Select the Quantitative template.
- 6.3 Give the Processing Method a name. Use the ellipsis at the end of the Location field to designate where the new Processing Method should be saved. Provide a descriptive comment in the space provided that includes the analyte(s) analyzed. Assign the new Processing Method to the applicable injection(s). Select Finish.
- 6.4 The Processing Method opens in the Chromatography Studio and may be edited at this time. The processing method is best viewed by selecting the Calib & PM Preset View in the Navigation Section of the Data Processing Home tab Ribbon. The Detection tab of the Processing Method displays the detection parameters that are currently applied to the data. Use Cobra Wizard to set automatic integration parameters. (Optimizations made in Cobra Wizard are added to the Processing Method.)

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6.5 The Cobra Wizard is accessed from the Detection Parameters group of the Processing tab of the Chromatogram Tools contextual ribbon. That is, click on the chromatogram, select the Processing tab on the ribbon and select Cobra Wizard. The Cobra Wizard comprises five steps:

6.5.1 Step 1: Integration Area – The area of integration is defined by a rectangle drawn with the mouse cursor over the area of the chromatogram which contains the peaks of interest. Leave the Consider Void Peak checkbox unchecked.

6.5.2 Step 2: Baseline Noise Range – For each chromatogram Chromeleon determines a quiet portion of the baseline from which to calculate the noise. This calculation is then used to determine whether a peak should be considered a peak or part of the baseline noise. Autorange works well for most applications

6.5.3 Step 3: Cobra Smoothing Width – Use this wizard page to specify the width (time span) from which several successive data points are interpreted as peak or noise. Click the Smoothing width radio button..

6.5.4 Step 4: Minimum Area – Set a minimum area threshold below which peaks are not integrated. Minimum area may be left at the Auto setting, the user may draw a rectangle representing the value to be used, or the user may click on a peak that approximately represents the threshold value..

6.5.5 Step 5: Channel and Injection Type – If desired, the Cobra Wizard settings can be applied to selected Injection Types and/or Channels. Click Finish.

6.5.6 Once the wizard is finished, review the other chromatograms in the sequence to ensure the parameters set with the wizard are appropriate for all injections. Detection Parameters may be edited in the Parameter Table if the chromatogram requires further optimization after completing the Cobra Wizard..

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6.6 Component Table Creation – in general, peaks are identified based on their retention time under the conditions used. In order to assign these identities, a component table must first be created. Components are entered on the Component Table tab in the Processing Method. To build a Component Table the user may either click on the last line on the Component Table (Click here to add a new component), select Component Table Wizard, or select Add Component from the Processing tab of the Ribbon:

6.6.1 Click Here to Add a New Component: Use when only a few components must be added. User must manually enter the name, retention time and window into the table.

6.6.2 Run Component Table Wizard: Use when many peaks must be added at once, and the only peaks integrated are the peaks of interest. User is only required to enter the names; Chromeleon determines the retention time and window.

6.6.3 Add Component: Select this tool from the Component Table group of the Processing tab of the Chromatogram Tools contextual ribbon. The user may then drag the cursor along the chromatogram to indicate the retention time and width of the window for each peak.

6.6.4 Component names, retention times and windows may be viewed and edited anytime on the Component Table.

6.7 Calibration Curve Creation – Calibration Standard concentrations are entered on the Component Table. The association between Calibration Standards and their corresponding level in the Calibration Curve is created on the Injection List.

6.7.1 To associate the Calibration Standards on the Injection List, verify that the desired injections are assigned the Calibration Standard Type. In the Level column select the level of the Calibration Curve to which the standard corresponds.

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6.8 Save the Processing Method by clicking the Save button next to the Processing Method in the Navigation Pane, or by clicking the Save button on the Quick Access toolbar, or by using the Save section of the Application Menu (Charlie Button).

6.8.1 The filename is generated in the following format: D-XXX\_Analyte(s).

## 7.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	05/06/10	New	-	-
1	03/07/13	Updated format, changed title, added additional information	13-0128	B. Johns
2	02/19/15	Scheduled review: updated Format. Clarified SOP.	15-0173	B. Johns
3	01/02/19	Scheduled review: updated SOP to Chromeleon Series 7.	19-0012	J. Maignan
4	01/16/23	Scheduled review: updated responsibilities. Changed logo. Updated document format.	CC-23-0025	K. Burris