

Automatic Vial-to-Vial Dilutions Using Chromeleon AutoDilution and the ICS-3000 AS Autosampler with the Sample Prep Option

INTRODUCTION

Environmental and wastewater samples often contain compound concentrations that exceed the range defined by a standard curve. In the past, operators would need to identify these outliers, dilute the samples, and often rerun the standards along with the diluted samples. These time consuming steps have now been eliminated with a software enhancement called AutoDilution.

AutoDilution enables the Chromeleon® Chromatography Data System software to automatically determine when compound concentrations exceed a set concentration, then dilutes the samples, and rerun them along with standards if needed.

The Chromeleon AutoDilution license is a powerful tool for adding automatic dilutions, using a decision structure, to any Dionex Ion Chromatography systems with the AS autosampler. If the concentration of a specified compound (target compound) is over a predefined limit (threshold), Chromeleon will automatically create a new sequence to adjust the sample concentration and re-run the analysis. Up to 10 target compounds can be selected, each with their own separate threshold level.

This technical note presents an example of automatic dilutions using the Dilute function of the AS to make a dilution in an empty vial and run the diluted sample. Other injection volumes can be selected, as well as multiple conditionals. All dilution runs can be stored in a separate sequence, for easy identification of reanalysis results. This dilution sequence is run automatically after the original sequence.

Chromeleon AutoDilution offers a quick easy and reliable method to perform automatic dilutions of samples. Chromeleon AutoDilution requires the Chromeleon AutoDilution License P/N 069725.

PROCESS OVERVIEW

Chromeleon automatically determines if any of the target compounds are above the specified threshold level, any samples above this level will be added to a new dilution sequence. Chromeleon will then assign specific sequence parameters and methods using conditionals based on the concentration level or the target compounds or any of many Chromeleon variables. (See Fig. 1).

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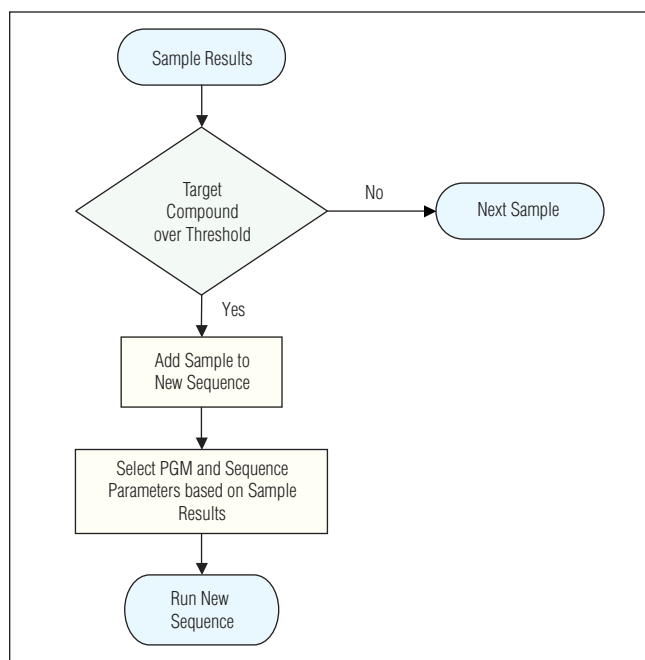


Figure 1. Flow chart.

The dilution of the samples is performed by calling a PGM file which contains a dilution step (vial-to-vial dilution). For this method, an AS with the sample prep option is required.

EQUIPMENT

Any Chromeleon connected Dionex ICS series Ion Chromatography System
 Chromeleon 6.8 Chromatography Data System software
 AutoDilution License P/N 069725
 AS Autosampler with Sample Preparation Option (for vial-to-vial dilutions) P/N 063103

PREPARATION

Vial-to-Vial Dilutions

In this method, actual dilutions of the original samples are made into empty vial using the Dilute function of an AS Autosampler with the Sample Preparation option. Only samples that have a target compound that is over the threshold level will be added to the dilution sequence and diluted. A conditional program will then select a PGM file to run the sample (containing an appropriate dilution command) depending on the concentration range of the target compounds. Dilution are made with any of up to four solution bottles that can be connected to the AS sample prep tower of the AS, or from another vial.

Tip: Additional ranges can be added to the conditional program with higher dilution levels for higher ranges of target compound concentrations. However, with the wide dynamic range of the Dionex conductivity detectors, a single 1:10 dilution will generally be more than sufficient.

1. Create a copy of you PGM file. One copy of your PGM file will contain the Post-Acquisition step you will be creating, and will be used with your original run of undiluted samples. The other file (without the Post-Acquisition step) will be used for the dilution run. Name each file appropriately.
2. Add the Post-Acquisition step using the Create Purification Samples Wizard, to the one of the copies of the PGM file you created. This file will be used for you original runs (undiluted).

3. Add a dilution command to the file without the Post-Acquisition step. This file will be used prepare and run diluted samples. The dilution command can be entered by using the PGM Wizard. An example of the command is below:

```

Dilute ConcentrateVolume = 100.0, SourceVial =
CurrentVial+9, DiluentVolume = 900.0,
SourceReservoir = Reservoir_A, DestinationVial =
CurrentVial
  
```

This command makes a 1:10 dilution of the original sample (total of 1 mL) into an empty vial using the solution from reservoir A.

4. Add a User Defined column (named Vial_Position) to your original sequence. This column will contain the locations of the empty vials for each on your original samples. Due to the structure of the Dilute command, (SourceVial = CurrentVial +N) the empty vials must be placed before the vials for the original runs. Place your samples in locations 10 – 19 and empty vials in location 1 – 9. Place the first sample in location 10 on the Autosampler and an empty vial in location 1. In the sequence, enter the sample position at 10 in the POS column and enter 1 in the Vial_Position column for the first sample. Continue this sequence for the first nine samples. If additional samples are needed, begin again at location 31-39 for the samples and 21-30 for the empty vials.

Tip: For best results using the vial-to-vial dilution method, ensure that all connections to the sample preparation tower on the AS Autosampler are tight and not leaking. Be sure to prime and rinse the syringe before starting the sequence. Check all liquid fitting before beginning.

CREATE PURIFICATION SAMPLES WIZARD

The AutoDilution process is added to a current PGM file by adding a Post-Acquisition step. The Create Purification Samples post-acquisition step is available only if the timebase is connected to the related server, if this server is running, and if an AutoDilution license is installed.

The program for AutoDilution can be created using a built-in Wizard that guides users through the main steps required to create an AutoDilution program. The Wizard automatically shows dialogs needed to create the program. From the PGM, click on the Post-Acquisition steps icon to add a Post-Acquisition step. Choosing Click on This Line to Add a New Step and selecting Create Purification Samples will start the wizard program.

The dialogs used in the Wizard are shown below:

1. Number of Target Compounds
2. Target Compound X
3. Target Compound X Tests
4. Special Options
5. Conditional Program Selection
6. Sequence Properties
7. Sample Properties
8. Extended Sample Properties

Number of Target Compounds

The first dialog allows you to enter the number of target compounds are searched in the AutoDilution process. A maximum of 10 target compounds can be selected (Fig. 2), each with their own separate threshold level.

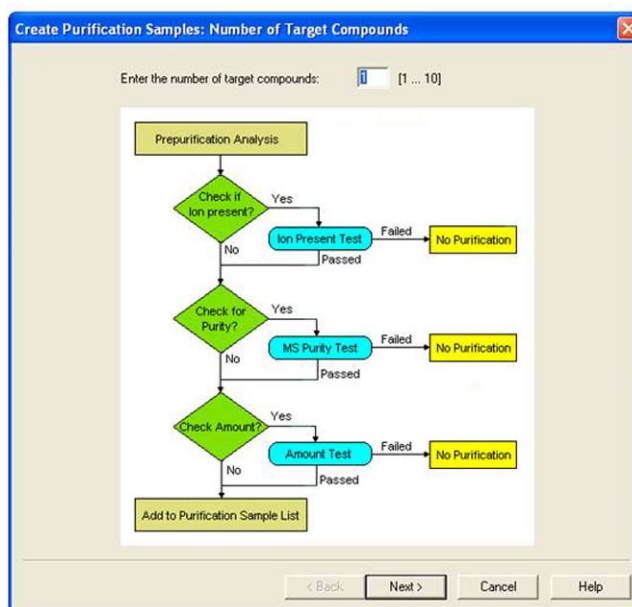


Figure 2. Number of target compounds dialog box.

Target Compound X

On the second wizard page, Target Compound X, defines how the target compounds are identified. Any connected detector can be selected from the drop-down list. Peaks may be selected by name (if you have already defined and entered the QNT method in the sample list, the names entered in the QNT method are listed for selection), retention time, greatest height or greatest area. The Mass Spectrometry Settings are not needed with conductivity detection (Fig. 3). This process is repeated for each target compound.

Figure 3. Target Compound X dialog box.

Target Compound X Tests

The Target Compound X Tests dialog defines the test used to determine if the sample is added to the new sequence and re-run (at a dilution). Custom tests can also be created using many of the standard Chromeleon variables, but are not covered by this Technical Note. For AutoDilution, the Amount Check should be selected. This test checks whether the amount of the target compound is above the threshold amount. Double-clicking on the test name will open the Amount Check Test dialog box. This dialog box allows you to select the channel, from which you want to check the amount of the target compound from a drop-down list. The threshold is the concentration value limit for the target compound. Any samples which have a target compound reported result (report units are ignored) above this concentration will be added to the dilution sequence. An amount check test with a specific threshold is defined for each target compound.

If chloride is selected as a target compound and selected “10” as the threshold, any samples that have a reported results above 10 (ppm or any concentration units from your QNT file) will fulfill this test and be added to the dilution sequence (Fig. 4).

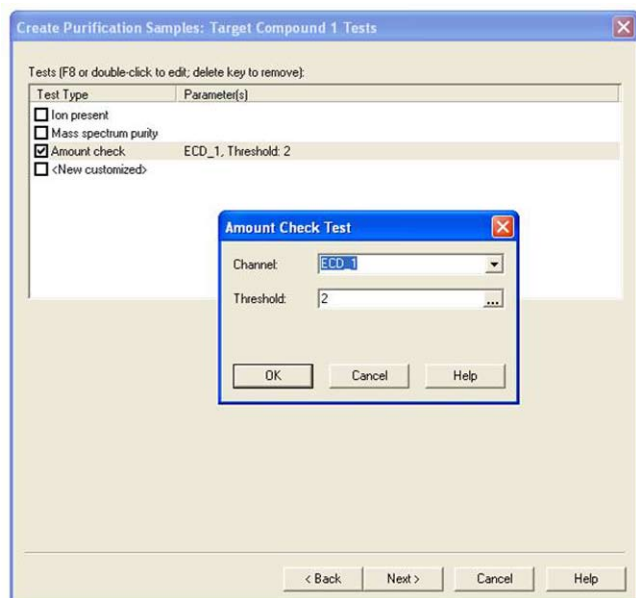


Figure 4. Target Compound X Test dialog box.

Special Options

The use of a conditional program is optional if multiple dilution levels will be used (each in a separate PGM). They can be specified in the conditional program selective dialog. Select Use Conditional Program from the Special Options Dialog box. Checking this box will open the Conditional Program wizard page on which you can define which PGM files will be used with the sample in the dilution sequence (Fig. 5).

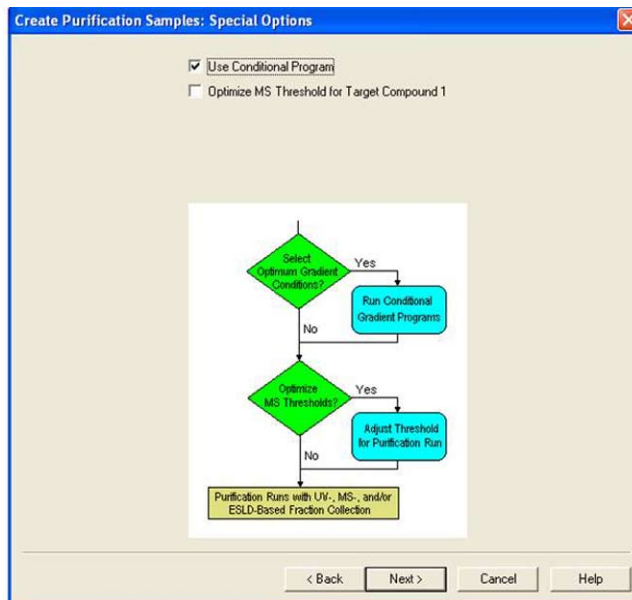


Figure 5. Special Options dialog box.

Conditional Program Selection

This section is active only if the Use Conditional Program box is checked. The Conditional Program Selection dialog allows different PGM files (with a specific dilution command) to be used for each sample based on a defined range of the sample results. This allows you to enter multiple dilutions based on the concentration of the sample (peak.amount). To enter the selection criterion, click on the {..} button and select a variable from the Report Categories in the Edit/Select Result Formula dialog box. For most dilutions applications, the selection criterion should be Peak.Amount. The Compute For box indicates the target compound for which the criterion is used. If you have determined several target compounds, click the arrow and select a compound from the drop-down list. In the From and To cells, enter the lower and upper values (enter the threshold value from above in the To cell) for the selection criterion. In the first row, enter the range for your standard

calibration and in the program column, enter your standard program. In the Otherwise row, enter the PGM you created (without the Post-Acquisition step). To avoid the possibility of entering an endless loop, program files entered in the section should not contain a Post-Acquisition step. For vial-to-vial dilution, this PGM must contain the Dilute function. Additional rows can be entered with PGM files containing higher dilutions for higher peak.amount ranges (Fig. 6).

It should be noted that if you enter several programs (rows), the first program for which the conditions are fulfilled is used. In all cases in which no program condition is fulfilled or in which Chromeleon cannot evaluate the selected criterion in the defined context, the program defined on the bottom line is used. Therefore, be sure to define a program on the “Otherwise use” line.

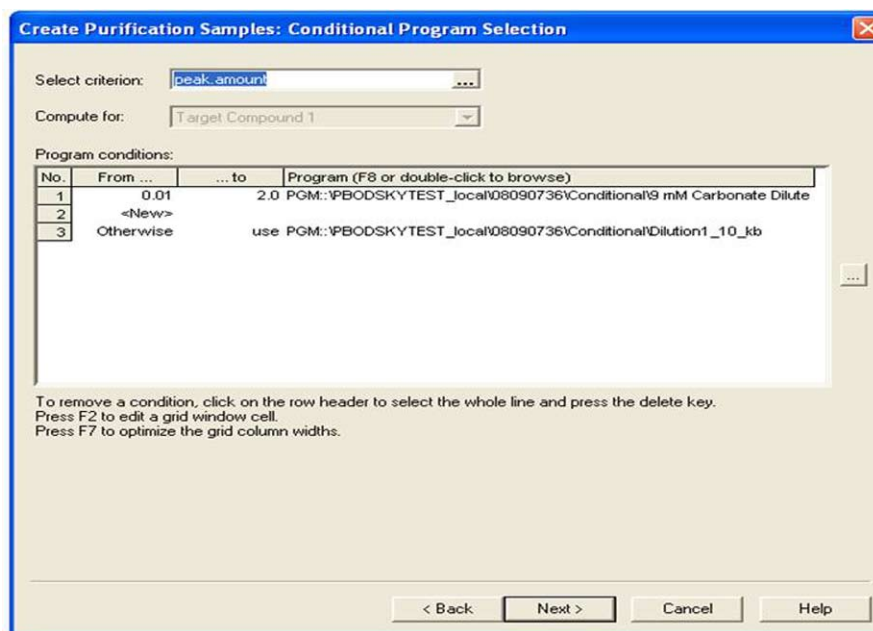


Figure 6. Conditional Program Selection dialog box.

Sequence Properties

The Sequence Properties dialog box is where the name of the new dilution sequence is entered. A new sequence can be created for the re-run samples, or they can be appended to the end of the current sequence. Enter a name that will be readily recognized as containing the diluted samples for your original run. If you use variable sequence name without an extension, Chromeleon does not create a new sequence. The newly created samples are appended to the existing samples in the current sequence instead.

The Sequence Properties dialog is also where you can specify any standards that are needed to run before your dilutions. In Pre-run samples, a previously created sequence containing the needed standards can be selected.

The sequence you select will automatically run before the dilution sequence. If desired, standards can be selected to run before or after the diluted samples (Fig. 7).

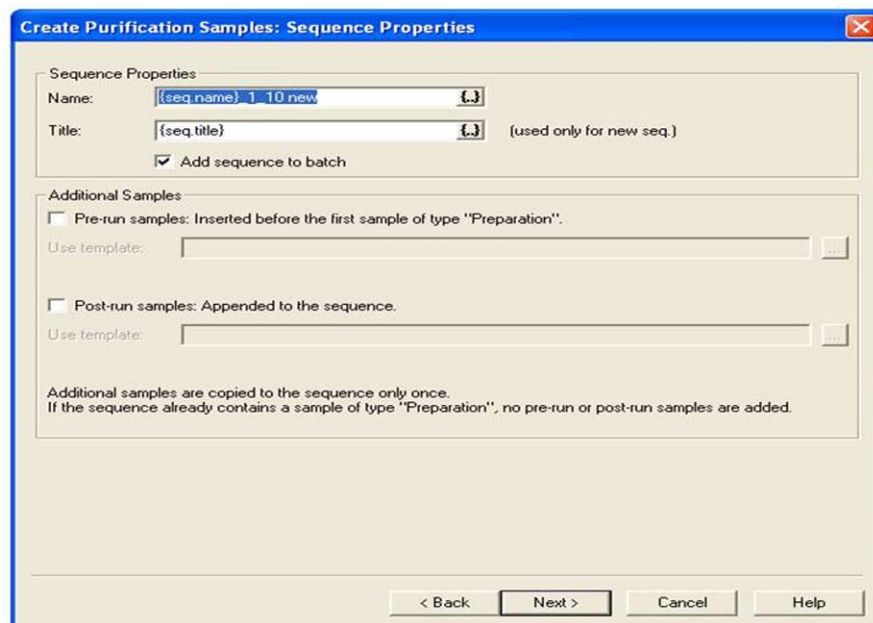


Figure 7. Sequence Properties dialog box.

Sample Properties

If you are using only one dilution level (one PGM for all re-runs), you can enter the program file (PGM) to be used with the dilution sequence in the program box. To add a program, open the Browse dialog box and select a program. If you have entered a Conditional Program (as is these examples), the entry <Conditional Programs> appears grayed out in this field. In this case, the field cannot be edited. The QNT method can also be entered. This is generally the same QNT method used in the original samples. The sample type and status cannot be edited (Fig. 8).

| Sample field | Value |
|------------------|-------------------------|
| Name | {smp.name} (prep) |
| Inj. Vol. | 25 |
| Weight | {smp.sample_weight;4} |
| ISTD Amount | {smp.amount;4} |
| Dil. Factor | {smp.dilution_factor;4} |
| Comment | {smp.comment} |
| Sample ID | {smp.ident} |
| Replicate ID | {smp.replicate} |
| Std. Add. Group | |
| Ref. Amount Set | |
| *Valve_Cycles | {smp.Valve_Cycles} |
| *Loop_Size | {smp.Loop_Size} |
| *Transfer_Volume | {smp.Transfer_Volume;3} |
| *Vial_Position | {smp.Vial_Position} |

Figure 8. Sample Properties dialog box.

Extended Sample Properties

The last Wizard dialog box is the Extended Sample Properties. In the Extended Sample Properties dialog box, you can select the tray and vial locations for the diluted samples.

For the vial-to-vial dilution method, since the samples are diluted into the previous empty vials, you should select Use Analytic Sample Tray and Get from User-Defined Column from your original sequence (Fig. 9).

Purification sample position

Tray: Use analytic sample tray
 Use fixed tray: []
 Get from user-defined column: []

Vial: Same as analytic sample vial (even if on a different tray)
 Get from user-defined column: {smp.Vial_Position}

Number of preparative injections

Fixed number: 1
 Compute number:

Inject volume: 25 µl
Sample volume: [] µl

Figure 9. Extended Sample Properties dialog box.

BENEFITS OF CHROMELEON AUTODILUTION

Chromeleon AutoDilution offers a quick, easy, and reliable method to perform automatic logical dilutions of samples, saving time and money, and increasing productivity.

1. Automated check for samples that exceed threshold.
2. Automated re-run of dilution samples if needed.
3. All dilution and dilution re-runs can be stored in a separate sequence for easy identification.

Chromeleon is a registered trademark of Dionex Corporation.

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Dionex Corporation

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America

U.S./Canada (847) 295-7500

South America

Brazil (55) 11 3731 5140

Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
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Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com



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