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Automatic Dilutions Using Chromeleon AutoDilution and Two Injection Loops

INTRODUCTION

Challenging samples, such as environmental and wastewater, often contain compound concentrations that exceed the range defined by a standard curve. Operators 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 to automatically determine when compound concentrations exceed a set concentration, then dilutes the samples, and reruns them along with standards if needed (see figure 1).

Depending on the hardware setup, the following options are available:

- Smaller injection volume in the existing loop using Partial loop injections with the AS Autosampler. Refer to Technical Note (TN) 81.
- Vial-to-vial dilution performed with the AS Autosampler using the Sample Prep module. Refer to Technical Note 83.
- Secondary loop fill (large and small loop) and injection using an optional 10-port valve, similar to the ones available for the AS-DV, ICS-1100/1600/2100, and in the Automation Manager for the ICS-3000 DC Detector/Chromatography Compartment.

This technical note presents an example of automatic dilution utilizing a secondary loop fill and injection using an optional 10-port valve available on the AS-DV, ICS-1100/1600/2100, and in the

Automation Manager for the ICS-3000 DC Detector/Chromatography Compartment. In this application, samples are first analyzed using a full-loop injection from a large loop, (e.g., 100 μ L loop). The data system monitors the sample concentration and re-injects the sample using the small loop (e.g., 10 μ L loop) if the concentration is outside the specified range. This dilution sequence is run automatically after the original sequence. A maximum of 10 target compounds can be selected, each with their own separate threshold level. All dilution runs can be stored in a separate sequence, for easy identification of reanalysis results.

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

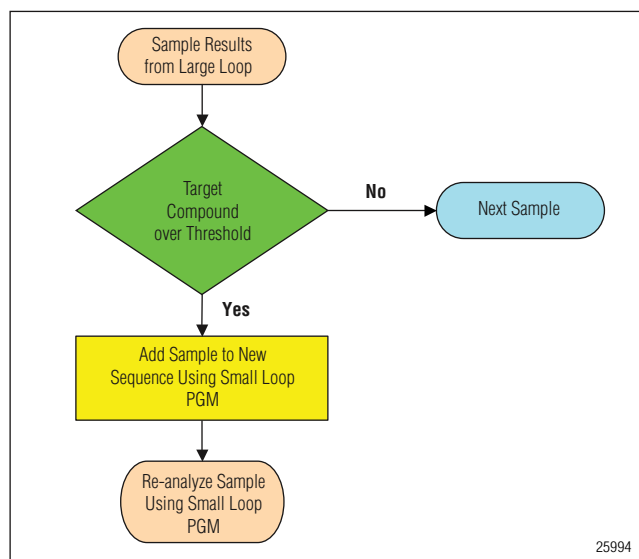


Figure 1. Flow chart of the AutoDilution process.

PROCESS OVERVIEW

Chromeleon software 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.

The dilution of the samples can be from partial loop injections (modified in the sequence properties Wizard dialog), or by calling a different PGM file, which contains a dilution step (using an AS with the sample prep option), or switches injection loops, as in the example below.

EQUIPMENT

- A. Any Dionex ICS System:
Dionex AS-DV autosampler equipped with the optional 10-port valve P/N 068921
AutoDilution License P/N 069725
- B. ICS-1100/1600/2100 system with an optional 10-port valve or ICS-3000 DC with AM (automation manager)
AS or AS-DV autosampler
AutoDilution License P/N 069725

PREPARATION

Dilutions Using a Large and Small Injection Loop and a 10-Port Valve

In this method, the lower injection volumes of samples are achieved with a small loop (10 μ L injection). The original runs of the samples are made using the Large Loop PGM (uses a 100 μ L large loop). Chromeleon software will monitor the sample concentration and re-inject the samples using the Small Loop PGM (10 μ L loop) if the concentration is over the specified threshold limit. See Table 1. The Autosampler should be set for the large loop (100 μ L) in both cases.

TABLE 1. 10-PORT VALVE POSITION FUNCTIONS

| PGM | Position A Function Valve_2.State A | Position B Function Valve_2.State B |
|------------|--|--|
| Large Loop | Injects Large Loop contents to column | Loads Large Loop |
| Small Loop | Loads Small Loop | Injects Small Loop contents to column |

Plumb the 10-port High-Pressure Valve

Figure 2 shows the detailed plumbing connections for the 10-port valve.

1. Connect the line from the pump to port 1.
2. Connect the Small Loop to ports 4 and 8.
3. Connect ports 2 and 3 together with a short piece of tubing.
4. Connect the Large Loop to ports 6 and 10.
5. Connect the sample line from the Autosampler to port 5.
6. Connect the column line to port 7.
7. Connect the green PEEK™ tubing waste line to port 9.

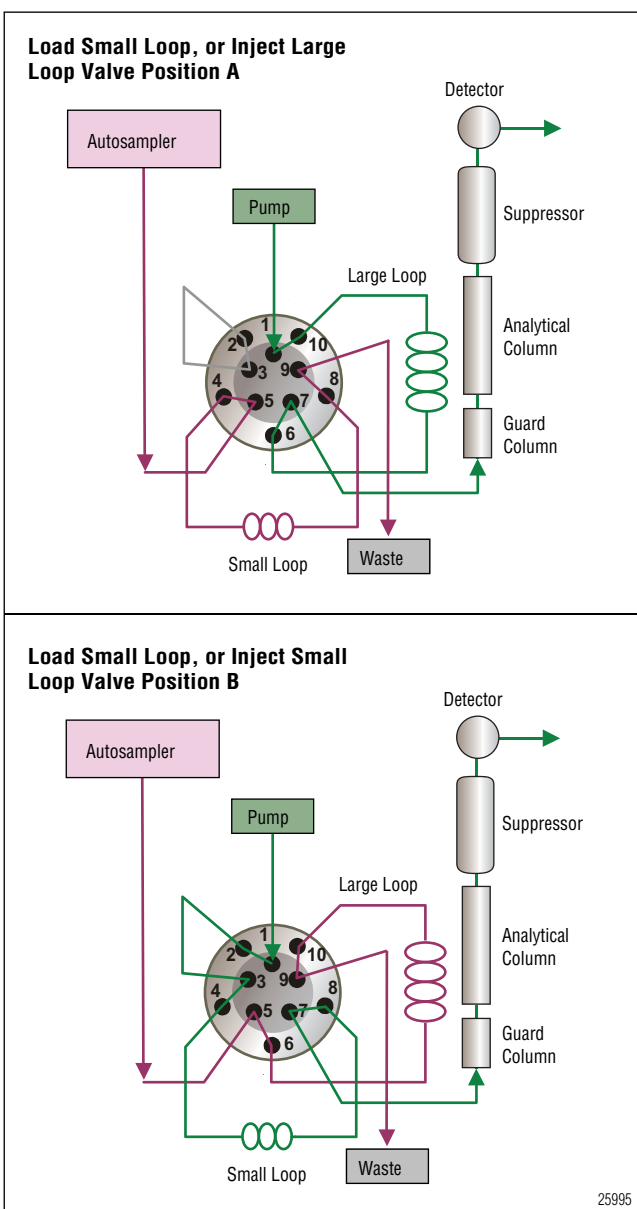


Figure 2. Valve plumbing.

CREATE PGM FILES AND POST-ACQUISITION STEP

1. Rename your current PGM **Large Loop**. This PGM will contain the Post-Acquisition Step and will be used on all initial runs using the large injection loop (100 µL injection volume).
2. Create a copy of this file named **Small Loop**. This PGM will be used to re-inject any samples that are over the threshold limit using a small injection loop (10 µL injection volume).
3. To the first line in the Large Loop PGM, add the command: `Valve_2.State <space> B`. This command will rotate the 10-port injection valve to the LOAD position for the large loop. The example below is for the 10-port valve on the ICS-1100/1600/2100 system. If using the optional 10-port valve on the AS-DV, the command would be `AuxValve<space>B`. Note: if the autosampler is set to perform a rinse through the sample loop, prior to or after an injection, the command to switch the valve to the Load position must be moved to a time of -2 minutes. This will place the valve switch outside the recorded run time and prevent the occurrence of a second water dip (since the loop would contain rinse water). Alternatively, this command could be added at the end of the run (in addition to time 0.00) which would not increase the overall run time.
4. At time 0.000, after the `AcqOn` statements, enter the command: `Valve_2.State <space> A`. This command will flip the 10-port valve to the INJECT position and transfer the contents of the large loop onto the column.
5. To the first line in the Small Loop PGM, add the command: `Valve_2.State <space> A`. This is the opposite from the Large Loop PGM command and will rotate the 10-port injection valve to the LOAD position for the small loop. If a rinse step is used, refer to the note in step 3 above.
6. At time 0.000, after the `AcqOn` statements, enter the command: `Valve_2.State <space> B`. This again is the opposite from the Large Loop PGM and will flip the 10-port valve to the INJECT position for the small loop and transfer the contents of the small loop onto the column.
7. When using an AS-DV, an autosampler rinse step is suggested. Alternatively, if no rinse is used, a second valve command can be entered to switch the inject valve back to the load state. This command will limit the time the valve is in the inject state and limit the possibility of carryover. This time should be as short as possible, but in any case, no less than the time required to flush the loop volume 5 times at the given flow rate. An example of this command is 0.500 `Valve_2.State B` for the Large Loop PGM or 0.050 `Valve_2.State A` for the Small Loop PGM
8. Add the Post-Acquisition step using the Create Purification Samples Wizard, to the Large Loop PGM file you created.
9. Create your sequence as normal using the Large Loop PGM for all the samples. Any re-runs that are needed will be run from the same vial as the original run using the Small Loop PGM.

Example of Large Loop PGM Commands

Valve_2.State B

```
Sampler.AcquireExclusiveAccess
Flush                               Volume = 250
Pressure.LowerLimit =               200 [psi]
Pressure.UpperLimit =               3000 [psi]
%A.Equate =                         "%A"
CR_TC =                             On
Wait                                FlushState
Data_Collection_Rate =              5.0 [Hz]
CellTemperature.Nominal =            35.0 [°C]
ColumnTemperature.Nominal =          30.0 [°C]
Suppressor_Type =                   ASRS_4mm
Suppressor_Current =                 82 [mA]
ECD_Total.Step =                     0.20 [s]
ECD_Total.Average =                  Off
Channel_Pressure.Step =               0.20 [s]
Channel_Pressure.Average =            Off
Concentration =                      33.00 [mM]
Curve =                              5
Flow =                               1.00 [ml/min]
NeedleHeight =                       2 [mm]
CutSegmentVolume =                   10 [µl]
SyringeSpeed =                       4
CycleTime =                          0 [min]
WaitForTemperature =                 False
Wait                                 SampleReady
```

```
0.000  Autozero
        Load
        Wait                                CycleTimeState
        Inject
        Wait                                InjectState
        ECD_1.AcqOn
        ECD_Total.AcqOn
        Channel_Pressure.AcqOn
Valve_2.State A
        Sampler.ReleaseExclusiveAccess

12.000  ECD_1.AcqOff
        ECD_Total.AcqOff
        Channel_Pressure.AcqOff

End
```

Example of Small Loop PGM Commands

Valve_2.State A

```
Sampler.AcquireExclusiveAccess
Flush                               Volume = 250
Pressure.LowerLimit =               200 [psi]
Pressure.UpperLimit =               3000 [psi]
%A.Equate =                         "%A"
CR_TC =                             On
Wait                                FlushState
Data_Collection_Rate =              5.0 [Hz]
CellTemperature.Nominal =            35.0 [°C]
ColumnTemperature.Nominal =          30.0 [°C]
Suppressor_Type =                   ASRS_4mm
Suppressor_Current =                 82 [mA]
ECD_Total.Step =                     0.20 [s]
ECD_Total.Average =                 Off
Channel_Pressure.Step =              0.20 [s]
Channel_Pressure.Average =           Off
Concentration =                      33.00 [mM]
Curve =                              5
Flow =                               1.00 [ml/min]
NeedleHeight =                       2 [mm]
CutSegmentVolume =                   10 [µl]
SyringeSpeed =                       4
CycleTime =                           0 [min]
WaitForTemperature =                 False
Wait                                SampleReady
```

```
0.000  Autozero
        Load
        Wait                                CycleTimeState
        Inject
        Wait                                InjectState
        ECD_1.AcqOn
        ECD_Total.AcqOn
        Channel_Pressure.AcqOn
Valve_2.State B
        Sampler.ReleaseExclusiveAccess

12.000  ECD_1.AcqOff
        ECD_Total.AcqOff
        Channel_Pressure.AcqOff

        End
```

CREATE PURIFICATION SAMPLES WIZARD

The AutoDilution process is added to the Large Loop 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 is 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**. Clicking on **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. Sequence Properties
6. Sample Properties
7. Extended Sample Properties

Number of Target Compounds

The first dialog allows you to enter the number of target compounds that are searched in the AutoDilution process. A maximum of 10 target compounds can be selected. (See Fig. 3).

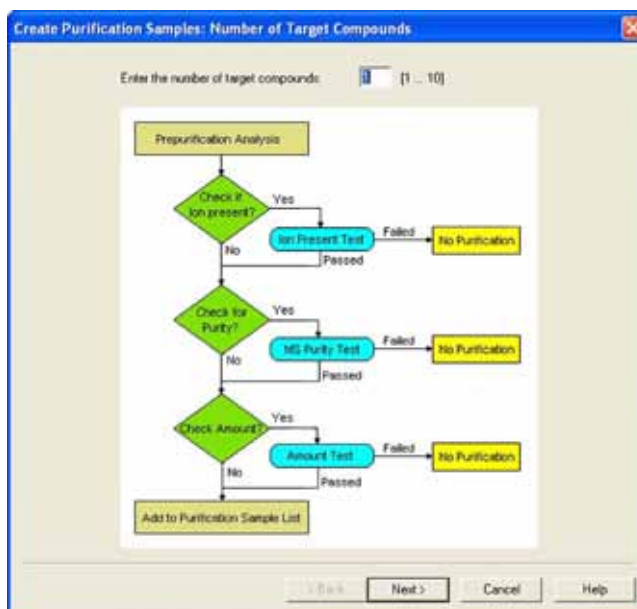


Figure 3. Number of target compounds dialog box.

Target Compound X

On the second page of the wizard, Target Compound X defines how the target compounds are identified (See Fig. 4). 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.

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 is 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. (See Fig. 5). 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.

If, for example, chloride is chosen as a target compound, and 2 is selected as the threshold, any samples that have a reported results above 2 (ppm or any concentration units from your QNT file) will fulfill this test and be added to the dilution sequence.

Figure 4. Target Compound X dialog box.

Figure 5. 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 dialog. Checking this box will open the Conditional Program wizard page where you can define which PGM files will be used with the sample in the dilution sequence. Since all re-runs will use only a single PGM file (Small Loop.pgm), a conditional program is not needed. Uncheck both boxes (See Fig. 6).

Sequence Properties

The Sequence Properties dialog box is where the name of the dilution sequence is entered. (See Fig. 7). Enter a name that will be readily recognized as containing the diluted samples from the original run. A new sequence can be created for the re-run samples, or they can be appended to the end of the current sequence. If you use the {seq.name} variable without an extension, for example, 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.

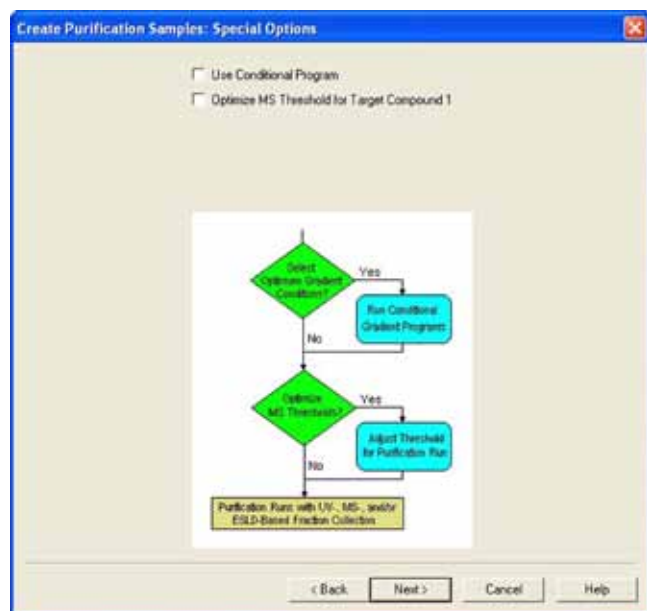


Figure 6. Special Options dialog.

Sequence Properties

Name: {seq.name}_1_10_new

Title: {seq.title} (used only for new seq)

☒ Add sequence to batch

Additional Samples

☐ Pre-run samples: Inserted before the first sample of type "Preparation".

Use template: []

☐ Post-run samples: Appended to the sequence.

Use template: []

Additional samples are copied to the sequence only once. If the sequence already contains a sample of type "Preparation", no pre-run or post-run samples are added.

< Back Next > Cancel Help

Figure 7. Sequence Properties dialog box.

Sample Properties

Program: PGM:\DIONE\AEB644C2_locat20090216_SEQ\Small Loop

Qnt. method: QNT:\DIONE\AEB644C2_locat2001_locatProtot2manual_SEQ\default

Type: Unknown Status: High

| Sample field | Value |
|-----------------|-------------------------|
| Name | {smp.name} (prep) |
| Int. Vol. | 10 |
| Weight | {smp.sample_weight,4} |
| STD Amount | {smp.amount,4} |
| Dil. Factor | {smp.dilution_factor,4} |
| Comment | {smp.comment} |
| Sample ID | {smp.id} |
| Replicate ID | {smp.replicate} |
| Std. Add. Group | |
| Ref. Amount Set | |

< Back Next > Cancel Help

Figure 8. Sample Properties dialog box.

Sample Properties

In the Program box on the Sample Properties dialog, enter the PGM file to be used for the re-run samples. (See Fig. 8). Open the dialog box and select the Small Loop PGM file that you created earlier. The QNT method can also be entered. If you have run a calibration for the Small Loop, select this QNT file. The Type field indicates the Sample Type of the samples to be created. The sample type is always Unknown. The entry cannot be edited.

The Status field indicates the status of the samples to be created. The sample type is always Single and cannot be edited. If a dilution factor is used, it can be entered in the Sample field list by double clicking the respective line.

Extended Sample Properties

The last Wizard dialog box is the Extended Sample Properties. In the Extended Sample Properties dialog, you can select the tray and vial locations for the diluted samples. (See Fig. 9).

For this method, the small loop injections are made from the same vial as the Large Loop un-diluted run. Select **Use analytic sample tray** and **Same as analytic sample vial**. The number of preparative injections for both methods is one.

The post-acquisition step creation is now complete and ready to run.

LOOP CALIBRATION

It is recommended that a calibration curve be created for both loop sizes by running a calibration for each PGM (Large Loop and Small Loop). Two QNT files will need to be generated, one for the Large Loop (used in the original run), and one for the Small Loop (used with the re-run samples).

Optionally, a calibration can be run for the Large Loop and a dilution factor can be entered in the Sample Properties dialog for the Small Loop. The dilution factor is the ratio of the volume of the large loop to the volume of the small loop. If a dilution factor is used, the relative loop volumes should be confirmed using the procedure below.

CONFIRMING THE LOOP VOLUMES

1. Obtain a 1000 mg/L (ppm) bromide standard (for anion determinations) or a 1000 mg/L (ppm) lithium standard (for cation determinations).
2. Prepare a 1.0 mg/L (ppm) stock standard.
3. Create a sequence using the Large Loop PGM file and run 4 injections of the 1 mg/mL (ppm) standard.

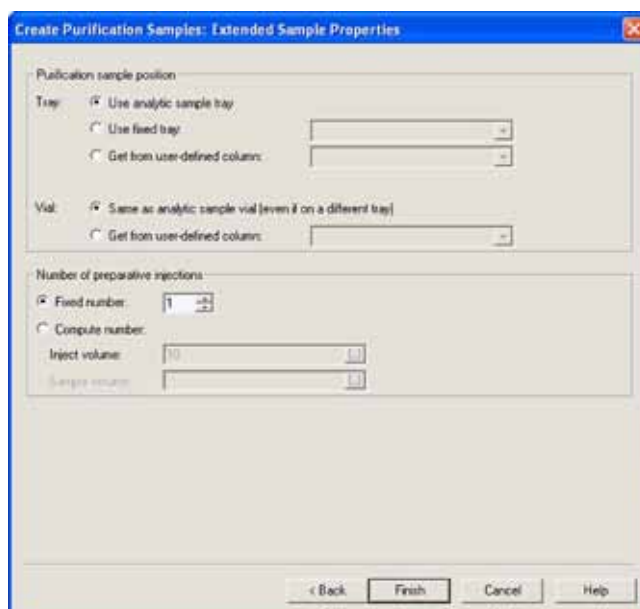


Figure 9. Extended Sample Properties dialog box.

4. Verify that the %RSD is 5% or lower.
5. Create a sequence using the Small Loop PGM file and run 4 injections of the 1.0 mg/L (ppm) standard.
6. Calculate the small loop relative volume and use this as the injection volume in the sequence in the Sample Properties dialog (Fig. 8).
7. Use the equation:

$$\text{Small Loop Volume} = \text{Large Loop Volume} \times \frac{\text{Average Area Small Loop}}{\text{Average Area Large Loop}}$$

BENEFITS OF CHROMELEON AUTODILUTION

Chromleon AutoDilution offers a quick, easy, and reliable method to perform automatic dilutions of samples.

1. Increases productivity, saving time and money.
2. Automated check for sample that exceed threshold.
3. Automated re-run of samples with a small injection loop.
4. All dilutions runs can be stored in a separate sequence for easy identification.

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