

Automatic Resampling Using Chromeleon AutoDilution, Two Injection Loops, and the Dionex AS-DV Autosampler

Barbara Shao, Kris Bapten, John Madden, Terri Christison, Fei Pang, and Frank Hoefler

Introduction

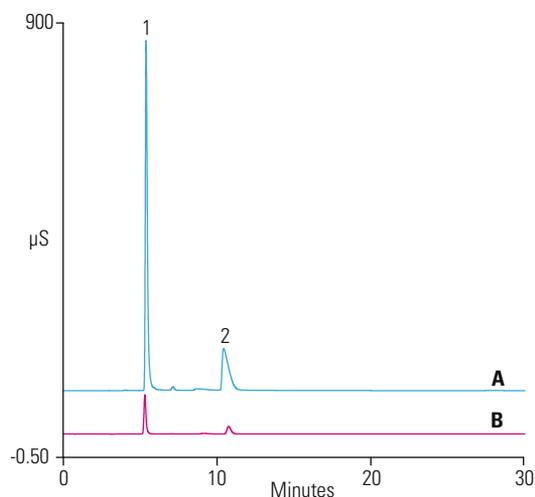
In environmental water analysis, it is often necessary to establish multiple calibration curves to accommodate samples varying in concentration or to dilute concentrated samples to fit the standard curve. AutoDilution, an important feature of Thermo Scientific Dionex Chromeleon software, provides a less time-consuming approach to analyzing out-of-calibration-range samples by utilizing a smaller sample loop compared to the one used for standards.

Operating AutoDilution using the Thermo Scientific Dionex AS Autosampler was previously demonstrated in Thermo Fisher Scientific, Inc. (formerly Dionex Corp.) Technical Notes (TN) 81, 83, and 84.¹⁻³ However, some customers prefer the Dionex AS-DV Autosampler which requires additional commands in the instrument program. Here, AutoDilution is demonstrated using the Dionex AS-DV. In AutoDilution, samples are first analyzed using a full-loop injection from a large loop while the Chromeleon™ software monitors the sample concentration. If the sample concentration is outside the specified range, AutoDilution will automatically reinject the sample using a smaller loop. All dilution runs can be stored in either the original sequence or a separate sequence for easy identification of reanalysis results.

The Chromeleon software's AutoDilution using the Dionex AS-DV Autosampler with two sample loops of different size offers a quick, easy, reliable, and economical method to perform sample analysis, thereby eliminating labor-intensive sample dilution. The Dionex AS-DV Autosampler in combination with a Thermo Scientific Dionex ICS-900, ICS-1100, ICS-1600, or ICS-2100 system is the cost-effective solution for environmental water and wastewater analysis. The use of AutoDilution requires the Chromeleon AutoDilution license (P/N 069725).

Column: Thermo Scientific Dionex IonPac AS25, 2 × 250 mm
 Eluent Source: Dionex EGC KOH Cartridge
 Eluent: 37 mM KOH
 Flow Rate: 0.25 mL/min
 Inj. Volume: A) 25 µL
 B) 2.5 µL
 Column Temp.: 30 °C
 Detection: Suppressed conductivity, Thermo Scientific Dionex ASRS 300 Suppressor, 2 mm, recycle, 23 mA
 Chromatograms: A) Original sample
 B) AutoDilution of Sample A

Peaks: 1. Chloride 97 mg/L
 2. Sulfate 50



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Figure 1: AutoDilution of a San Jose municipal water sample using two loops with the Dionex AS-DV Autosampler.

This study presents an example of automatic reinjection utilizing a smaller secondary loop fill and injection using an optional 10-port valve available on the Dionex AS-DV Autosampler and Dionex ICS-2100 system (Figure 1).

- Environmental
- Water
- Wastewater
- Automation
- Dilution
- Sample Preparation

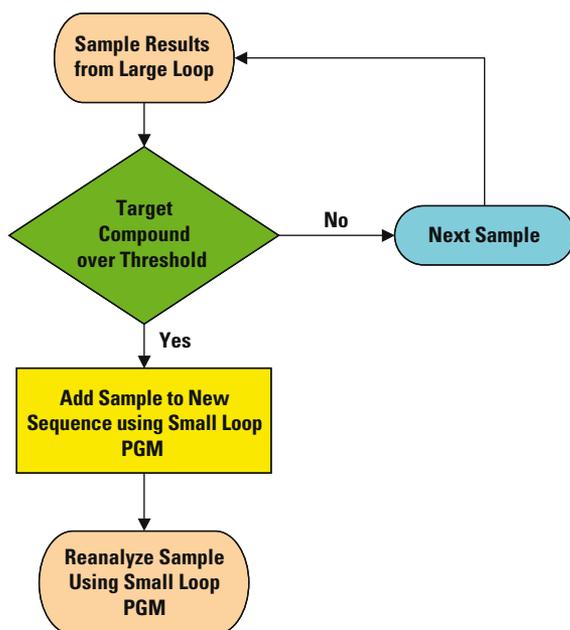


Figure 2: Flow chart of the AutoDilution process.

Process Overview

Figure 2 shows the flow chart of the AutoDilution process. First the sample is injected using the large loop. The Chromeleon software automatically determines if any of the target compounds are above the specified threshold level, then automatically reinjects the sample using the small loop at the end of the sequence. The diluted injections can be stored in either the original sequence or a separate sequence, for easy identification of reanalysis results. The Chromeleon software automatically specifies the sequence parameters programmed by the conditionals in the Create Purification Samples wizard.

Equipment

Dionex ICS-2100 system (or any Dionex ICS series system)
 Dionex AS-DV Autosampler equipped with a 10-port valve (P/N 068921)
 Chromeleon 6.8 AutoDilution License (P/N 069725)

Dionex AS-DV Autosampler Operation

Specific procedures to operate the Dionex AS-DV Autosampler with AutoDilution are described here. For detailed operations, refer to the Thermo Fisher Scientific, Inc. (formerly Dionex Corp.) AS-DV Autosampler Operator's Manual.⁴

Sampling

The Dionex AS-DV carousel holds 50 vials from which the Dionex AS-DV Autosampler samples in any order, with single or multiple injections from each vial. Before loading the carousel, always press Carousel Release button to reset the vial memory. After loading the vials in the carousel, press the Carousel Release button again to engage the carousel.

Specifying a Rinse

The Rinse command delivers rinse liquid from a vial to the 10-port valve through the open valve position and then to waste. In AutoDilution, the Rinse function is used to rinse any residual sample from the small loop when injecting through the large loop, or vice versa.

To perform a rinse cycle, the Dionex AS-DV Autosampler needs the position of the vial containing the rinse liquid, the rinse volume, and the time specified to start the rinse. In the Chromeleon Server Configuration program, first specify the position of the vials containing water for rinsing, such as Vials 45 to 50. In the Program Wizard for the Dionex AS-DV Autosampler, rinsing is programmed through the Basic Edit or Advanced Edit modes.

To use the Basic Edit mode, first select the mode, then select the Rinse check box, and enter the Volume. Using Basic Edit mode, the rinse is always delivered from the vials in the range specified in the Chromeleon Server Configuration program. Always enter the rinse commands at the end of the program so that both loops will be rinsed prior to the next injection, as shown in the Creating Program (PGM) Files section. If the first vial is empty, the rinse solution will be withdrawn from the next vial specified in the range, until all rinse vials are empty. To use the Advanced Edit mode, manually add the InjectPosition and DeliverRinse commands to the Command/Parameters table. Use this mode to define the volume and position where the rinse comes from, e.g., DeliverRinse Volume = 100, Position = 10. Enter these rinse commands at the end of the program, as shown.

Estimating the Delivery Volume

The Dionex AS-DV Autosampler calculates the delivery volume using the formula shown in the Total Volume Delivered Estimator:

$$\text{Total Volume} = (\text{Loop volume } 25 \mu\text{L} + \text{Delay Volume } 125 \mu\text{L}) \times \text{Flush Factor } 8 = 1200 \mu\text{L}$$

The large loop (25 μL) volume is used to estimate the delivery volume in both cases.

Sample Loops

Dilutions Using Both Large and Small Injection Loops and a 10-Port Valve

In this method, two sample loops on a 10-port valve are used for AutoDilution (Table 1). The original sample is loaded through Valve 2 in State B and injected through the large loop (25 μL) at State A using the Large Loop Program (PGM). The smaller injection volumes are achieved using a small loop (2.5 μL injection) in reverse, loading in State A and injecting in State B. The Chromeleon software monitors the sample concentration while injecting the sample using Large Loop PGM, and reinjects the samples using the Small Loop PGM (2.5 μL loop) if the concentration is over the specified threshold limit.

PGM	Position A Function Valve_2 StateA	Position B Function Valve_2 StateB
Large Loop	Injects Large Loop contents to column	Loads Large Loop
Small Loop	Loads Small Loop	Injects Small Loop contents to column

Table 1: Functions of the 10-Port Valve Positions

Creating Program (PGM) Files

Examples of Large Loop and Small Loop PGM commands (Figures 4 and 5) show the program commands used in this application. The following examples use a 10-port valve on the Dionex ICS-2100 system. To create the program file:

- Rename the current PGM to Large Loop. This PGM will contain the postacquisition step and will be used on all initial runs using the large loop (25 μ L injection volume).
- Create a copy of this file named Small Loop (see the Chromeleon User's Manual for instructions on how to create a copy of a file). This PGM will reinject any samples that are above the threshold limit using a small sample loop (2.5 μ L injection volume).
- On the first line in the Large Loop PGM (Figure 4), add the command `Valve_2.StateB`. This command will rotate the 10-port injection valve to the LOAD position for the large loop.
- At time 0.000, after the `AcqOn` statement, enter the command `Valve_2.StateA`. This command will flip the 10-port injection valve to the INJECT position and transfer the contents of the large loop onto the column.
- To the first line in the Small Loop PGM (Figure 5), add the command `Valve_2.StateA`. This is the opposite of the Large Loop PGM command and will rotate the 10-port injection valve to the LOAD position for the small loop.
- At time 0.000, after the `AcqOn` statement, enter the command `Valve_2.StateB`. This again is the opposite of the Large Loop PGM and will flip the 10-port injection valve to the INJECT position for the small loop and transfer the contents of the small loop onto the column.

Figure 3A: Valve position B: load Large Loop or inject Small Loop.

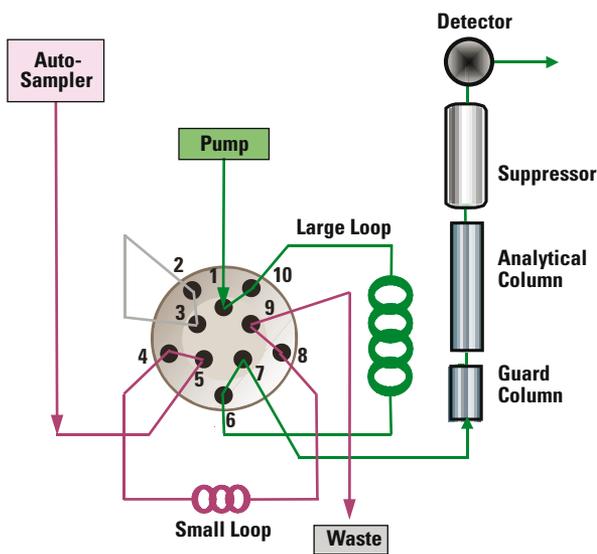


Figure 3B: Valve position A: load Small Loop or inject Large Loop.

Plumbing the 10-Port High-Pressure Valve

Figures 3A and 3B show the detailed plumbing connections for the 10-port valve

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

```

Pressure.LowerLimit = 200 [psi]
Pressure.UpperLimit = 3000 [psi]
%A.Equate = "%A"
CR_TC = On
DeliverSpeed = 4.0 [ml/min]
DelayVolume = 125 [μl]
FlushFactor = 10
DeliverSample
EndSamplePrep

Valve_2.State B
Data_Collection_Rate = 5.0 [Hz]
CellTemperature.Nominal = 35.0 [°C]
ColumnTemperature.Nominal = 30.0 [°C]
Suppressor_Type = ASRS_2mm

Suppressor_Current = 23 [mA]
ECD_Total.Step = 0.20 [s]
ECD_Total.Average = Off
Channel_Pressure.Step = 0.20 [s]
Channel_Pressure.Average = Off
Concentration = 37.00 [mM]
Curve = 5
Flow = 0.25 [ml/min]

0.000 Wait CycleTimeState
Autozero

Valve_2.State A

ECD_1.AcqOn
ECD_Total.AcqOn
Channel_Pressure.AcqOn

30.000 ECD_1.AcqOff
DeliverRinse Volume=1000
Valve_2.State B
ECD_Total.AcqOff
Channel_Pressure.AcqOff
End
    
```

Figure 4: Example of Large Loop PGM commands.

```

Pressure.LowerLimit = 200 [psi]
Pressure.UpperLimit = 3000 [psi]
%A.Equate = "%A"
CR_TC = On
DeliverSpeed = 4.0 [ml/min]
DelayVolume = 125 [µl]
FlushFactor = 10

Valve_2.state A
DeliverSample
EndSamplePrep
Data_Collection_Rate = 5.0 [Hz]
CellTemperature.Nominal = 35.0 [°C]
ColumnTemperature.Nominal = 30.0 [°C]
Suppressor_Type = ASRS_2mm
Suppressor_Current = 23 [mA]
ECD_Total.Step = 0.20 [s]
ECD_Total.Average = Off
Channel_Pressure.Step = 0.20 [s]
Channel_Pressure.Average = Off
Concentration = 37.00 [mM]
Curve = 5
Flow = 0.25 [ml/min]

0.000 Wait CycleTimeState
Autozero

Valve_2.state B

ECD_1.AcqOn
ECD_Total.AcqOn
Channel_Pressure.AcqOn

30.000 ECD_1.AcqOff
DeliverRinse Volume=1000
Valve_2.state A
ECD_Total.AcqOff
Channel_Pressure.AcqOff

End

```

Figure 5: Example of Small Loop PGM commands.

- When using a Dionex AS-DV Autosampler, use a rinse step to remove the residual sample from both sample loops. Without the rinse, sample carryover will occur in the sample run. Add the command DeliverRinse Volume = 1000 to the end of the run, which does not increase the overall run time.
- The AutoDilution commands and conditionals are created in the Large Loop PGM using postacquisition steps and the Create Purification Samples wizard, which is described in detail in the following section.
- Create the sequence as the original run using the Large Loop PGM for all the samples. Reminder: Any reruns that are needed will be run from the same vial as the original run using the Small Loop PGM.

Using the Create Purification Samples Wizard

The AutoDilution process is added to the Large Loop PGM file by adding a postacquisition step. The Create Purification Samples postacquisition step is available only if the timebase is connected to the related server, the server is running, and 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. To create the conditionals for the AutoDilution program, open the Large Loop PGM and click on the postacquisition steps icon to add a postacquisition step. Click on *Click on this line to add a new step* and select Create Purification Samples to start the wizard program.

The dialogs used in the wizard are shown below:

- Number of Target Compounds
- Target Compound X
- Target Compound X Tests
- Special Options
- Conditional Program Selection
- Sequence Properties
- Sample Properties
- Extended Sample Properties

Number of Target Compounds

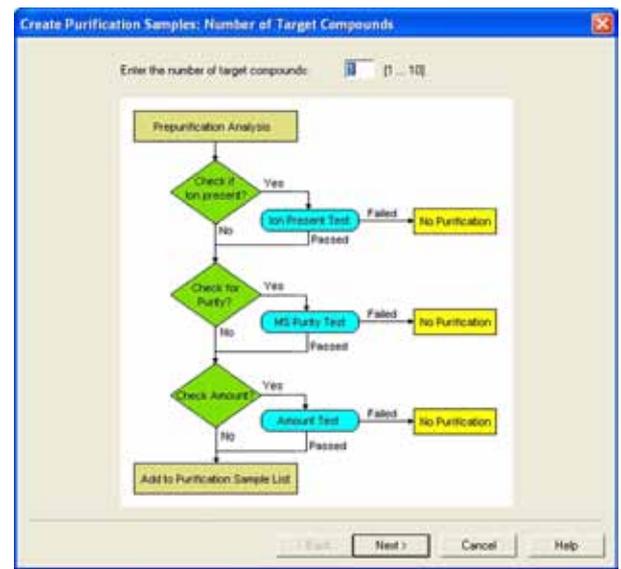


Figure 6: Number of target compounds dialog box.

In the first dialog, enter the number of target compounds that are searched in the AutoDilution process, up to a maximum of 10 target compounds (Figure 6).

Target Compound X

On the second wizard page, Target Compound X (shown in Figure 7) 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 operators 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 used for this application.

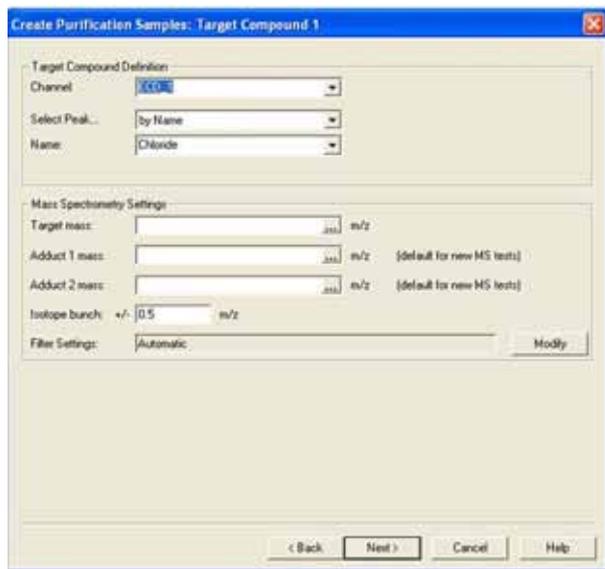


Figure 7: Target Compound X dialog box.

Target Compound X Tests

The Target Compound X Tests dialog in Figure 8 defines the test used to determine if the sample is rerun (at a dilution). Custom tests can also be created using many of the standard Chromeleon software variables, but are not covered in this document. 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 operators to select the channel, from which they can then 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 that 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 selected as a target compound and 10 is selected as the threshold, any samples that have reported results above 10 (ppm or any concentration units from the QNT file) will fulfill this test and be added to the dilution sequence.

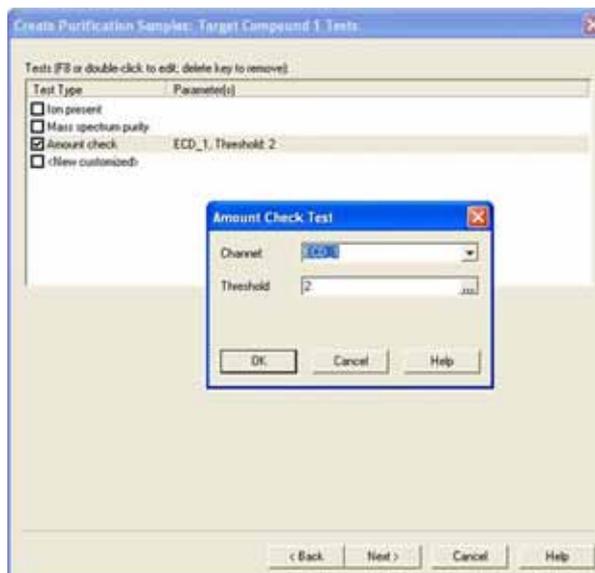


Figure 8: Target Compound X Test dialog box.

Special Options

Use of a conditional program (Figure 9) 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 on which operators can define which PGM files will be used with the sample in the dilution sequence. Because all reruns will use only a single PGM file (Small Loop.pgm), a conditional program is not needed. Uncheck both boxes.

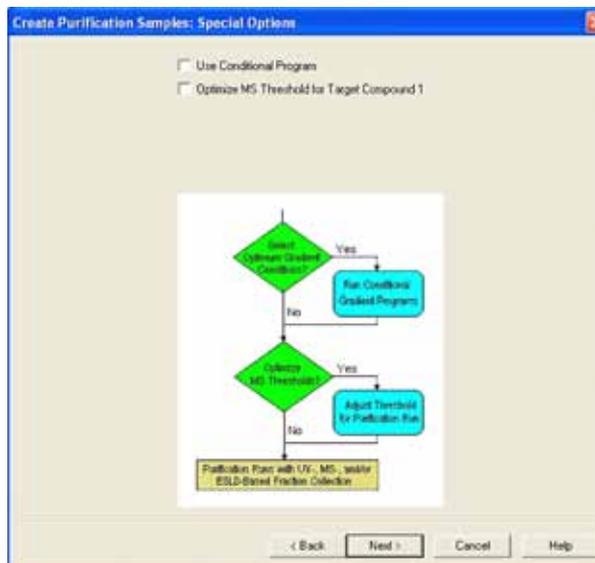


Figure 9: Special Options dialog box.

Sequence Properties

The Sequence Properties dialog box (Figure 10) is where the name of the dilution sequence is entered. Enter a name that will be readily recognized as containing the diluted samples for the original run. A new sequence can be created for the rerun samples, or they can be appended to the end of the current sequence. If operators use the variable without an extension, e.g. {seq.name}, the Chromeleon software will not create a new sequence, but append the newly created samples to the existing samples in the current sequence instead. The Sequence Properties dialog is also used to specify running a previously created sequence containing standards that need to run before the dilutions. The sequence an operator selects will automatically run before the dilution sequence. If desired, standards can be selected to run before or after the diluted samples.

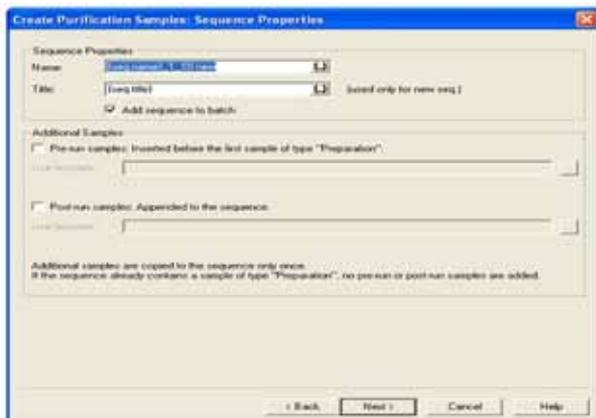


Figure 10: Sequence Properties dialog box.

Sample Properties

In the Program box on the Sample Properties dialog (Figure 11), enter the PGM file to be used for the rerun samples. Open the dialog box and select the *Small Loop* PGM file that was created earlier. Also enter the QNT method, which is generally the same QNT method used for the original samples. Enter the dilution factor in the sequence if the same QNT method is used to quantitate the dilution samples. Dilution factors are discussed in the Confirming the Loop Volumes section. If the operator has run a calibration for the Small Loop, select this QNT file here. 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*. The entry cannot be edited. If a dilution factor is used, it can be entered in the Sample field list by double-clicking the respective line.

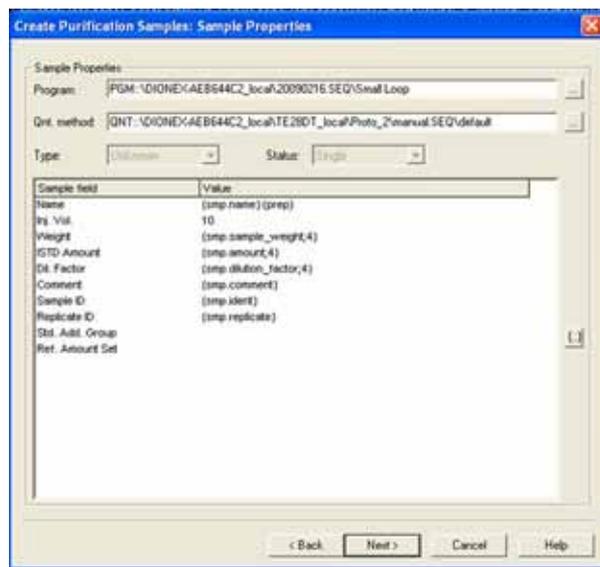


Figure 11: Sample Properties dialog box.

Extended Sample Properties

The last wizard dialog box is the Extended Sample Properties (Figure 12). In the Extended Sample Properties dialog, select the tray and vial locations for the diluted samples. For this method, the small loop injections are made from the same vial as the Large Loop undiluted run. Select *Use analytic sample tray* and *Same as analytic sample vial*. The number of preparative injections for both methods is one. The postacquisition step creation is now complete and ready to run.

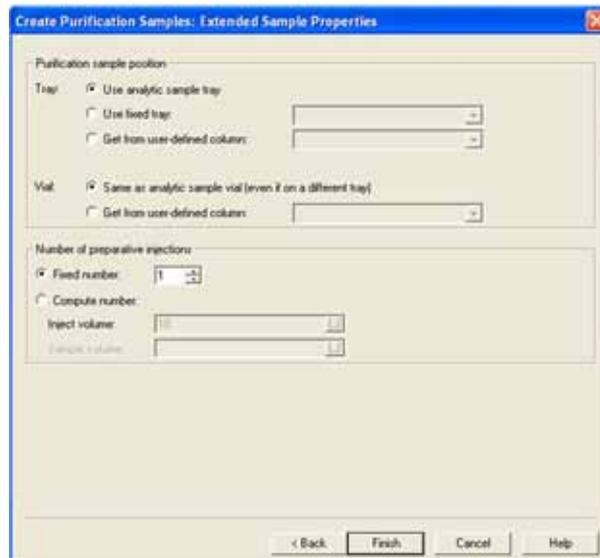


Figure 12: Extended Sample Properties dialog box.

Loop Calibration

It is important to calibrate both loops or to run calibration standards on both loops to produce the most accurate dilution results. A calibration curve can 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 rerun samples). Optionally, a calibration can be run for the Large Loop and a dilution factor of 10 (operators need to confirm the loop volumes) can be entered for the dilution samples in the sequence. If a dilution factor is used, the loop volumes should be confirmed in the following procedure.

Confirming the Loop Volumes

- Obtain a 1000 mg/L (ppm) bromide standard (for anion determinations) or a 1000 mg/L (ppm) lithium standard (for cation determinations)
- Prepare a 10 mg/L (ppm) stock standard
- Create a sequence using the Large Loop PGM file and run four injections of the 10 mg/L (ppm) standard
- Verify that the %RSD is 5% or better
- Create a sequence using the Small Loop PGM file and run four injections of the 10 mg/L (ppm) standard
- Use this equation for the dilution factor:

$$\text{Dilution Factor} = \frac{\text{Average Area Large Loop}}{\text{Average Area Small Loop}}$$

- Use this equation to calculate the injection volume, and enter it in the sequence in the Sample Properties dialog:

$$\text{Volume of the Small Loop} = \frac{\text{Volume of the Large Loop}}{\text{Dilution Factor}}$$

Benefits of Chromeleon AutoDilution

The Chromeleon software's AutoDilution offers a quick, easy, and reliable method to deliver samples in the calibrated range of the instrument.

- Automated check for samples that exceed threshold
- Automated rerun of samples with a small injection loop
- All dilution runs can be stored in a separate sequence for easy identification
- Increased productivity, saved time, and reduced cost

For more information on methods to automate dilution using the Dionex AS Autosampler, refer to Dionex TN 81, TN 83, and TN 84.¹⁻³ In Dionex TN 84, AutoDilution by the Dionex AS Autosampler is discussed using two loops with the Dionex ICS-1100/1600/2100 systems and in the Automation Manager for the Dionex ICS-5000 and ICS-3000 DC Detector/Chromatography Compartment.³ In Dionex TN 81, AutoDilution by the Dionex AS Autosampler is achieved by injecting smaller amounts in the existing loop using partial-loop injections.¹ In Dionex TN 83, the Dionex AS Autosampler performs vial-to-vial dilutions using the Sample Prep module.²

References

1. *Automated Dilutions Using Chromeleon AutoDilution and the Partial Loop Injection Capability of the ICS-3000 AS Autosampler*. 2009, Thermo Fisher Scientific, Inc. (formerly Dionex Corp.) Technical Note 81, LPN 2205. www.dionex.com/en-us/webdocs/70792-TN81-Chromeleon-AutoDilution-06Mar09-LPN2205.pdf (accessed Nov 28, 2011).
2. *Automatic Vial-to-Vial Dilutions Using Chromeleon AutoDilution and the Partial Loop Injection Capability of the ICS-3000 AS Autosampler with the Sample Prep Option*. 2009, Thermo Fisher Scientific, Inc. (formerly Dionex Corp.) Technical Note 83, LPN 2245. www.dionex.com/en-us/webdocs/70900-TN83-Automatic-Dilutions-17Mar09-LPN2245.pdf (accessed Nov 28, 2011).
3. *Automated Dilutions Using Chromeleon AutoDilution and Two Injection Loops*. 2011, Thermo Fisher Scientific, Inc. (formerly Dionex Corp.) Technical Note 84, LPN 2255. www.dionex.com/en-us/webdocs/73254-TN84-ChromeleonAutodilution-05May2010-LPN2255-01.pdf (accessed Nov 28, 2011).
4. *AS-DV Operator's Manual*. 2009, Thermo Fisher Scientific, Inc. (formerly Dionex Corp.) Document No 065259. www.dionex.com/en-us/webdocs/73226-Man-IC-AS-DVoperators-May2010-DOC065259-03.pdf (accessed Nov 28, 2011).

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TN111_E 07/16SV LPN2961