TECHNICAL NOTE

AutoDilution using Chromeleon 7 CDS software and two injection loops

Daniel Khor, Jay Lorch, and Jeff Rohrer, Thermo Fisher Scientific, Sunnyvale, CA, USA

Keywords: IRC, SST, Intelligent Run Control, Conditionals, Dionex Integrion HPIC System, Dionex ICS-5000+ HPIC System, AS-DV, AS-AP, Chromeleon, AutoDilution

Goal

To provide instructions to perform AutoDilution using Thermo Scientific™ Chromeleon™ 7 Chromatography Data System (CDS) software and two injection loops

Introduction

Challenging samples, such as wastewater, often contain compound concentrations that exceed the range defined by a calibration curve. Operators need to identify these outliers, dilute the samples, and rerun the standards along with the diluted samples. These time-consuming steps have been eliminated by the AutoDilution option, which is now an integral part of the Intelligent Run Control (IRC) feature. IRC enables the Chromeleon CDS software to automatically determine when compound concentrations exceed a set concentration, then dilute the samples by injecting a smaller volume, and rerun them along with standards, if needed (Figure 1).

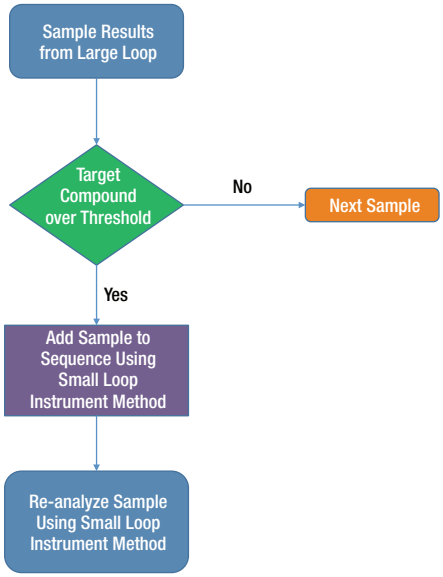


Figure 1. Flow chart of the Chromeleon 7 CDS software AutoDilution process.

ThermoFisher SCIENTIFIC

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

- Secondary loop fill (large and small loop) and injection using an optional 10-port valve, similar to the valves available for the Thermo Scientific™ Dionex™ AS-DV autosampler, Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, and in the Automation Manager (AM) for the Thermo Scientific™ Dionex™ ICS-5000+ Detector/Chromatography Compartment (DC).
  – If using Chromeleon 7 CDS software, refer to this note.
  – If using Chromeleon 7 CDS software, refer to TN 84.
- Smaller injection volume in the existing loop using partial loop injections with the Thermo Scientific™ Dionex™ AS Autosampler. (Not discussed in this technical note.)
  – If using Chromeleon 7 CDS software, refer to TN 183.
  – If using Chromeleon 6 CDS software, refer to TN 81.
- Vial-to-vial dilution performed with the Dionex AS Autosampler using the Sample Prep module. (Not discussed in this technical note.)
  – If using Chromeleon 7 CDS software, refer to TN 182.
  – If using Chromeleon 6 CDS software, refer to TN 83.

This technical note presents an example of AutoDilution via a secondary loop fill and injection using an optional 10-port valve available on the Dionex AS-DV autosampler, Dionex Integrion HPIC system, or in the Automation Manager for the Dionex ICS-5000+ DC. In this note, samples are first analyzed using a full-loop injection from a large loop (e.g., 100 µL loop). The Chromeleon CDS software monitors the sample concentration and reinjects the sample using the small loop (e.g., 10 µL loop) if the concentration is outside the specified range. This dilution sequence is run automatically after the original injection. The AutoDilution option offers a quick, easy, and reliable method to perform automatic reinjection of a smaller sample volume.

Process overview

The Chromeleon CDS software uses an associated calibration series to automatically determine if any of the target compound concentrations exceed that of the maximum calibration standard. Any samples with concentrations measuring above these levels are inserted as the next injection in the sequence and are reinjected according to the method described below.

Equipment

Option 1

a) Any Dionex Integrion or Thermo Scientific™ Dionex™ Aquion™ system

b) Dionex AS-AP or Dionex AS-DV autosampler equipped with the optional 10-port valve

Option 2

a) Dionex Integrion HPIC system with an optional 10-port valve or Dionex ICS-5000+ DC with Automation Manager

b) Dionex AS-AP or Dionex AS-DV autosampler

Preparation

Autosampler settings

In this method, lower sample injection volumes are achieved using a smaller loop. The original sample runs use a large loop (100 µL). The Chromeleon CDS software monitors the sample concentration and reinjects the samples using a small loop (10 µL) with a different instrument method if the concentration is over the specified threshold limit. See Table 1 for the valve functions. The autosampler should be set for the large loop (100 µL) in both cases.

Table 1. 10-port valve position and corresponding functions.

Instrument Method	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

Plumbing the 10-port high-pressure valve

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

1. Connect the tubing from the pump to Port 1
2. Connect the small loop to Ports 4 and 8
3. Connect Ports 2 and 3 together with approximately 3.2 in (8 cm) of tubing
   a. For microbore (2 mm column) plumbing: Use 0.005 in (0.125 mm) i.d. PEEK tubing
   b. For standard bore (4 mm column) plumbing: Use 0.010 in (0.25 mm) i.d. PEEK tubing
4. Connect the large loop to Ports 6 and 10
5. Connect the sample transfer line from the autosampler to Port 5
6. Connect the column to Port 7
7. Connect the 0.030 in. (0.75 mm) PEEK tubing waste line to Port 9

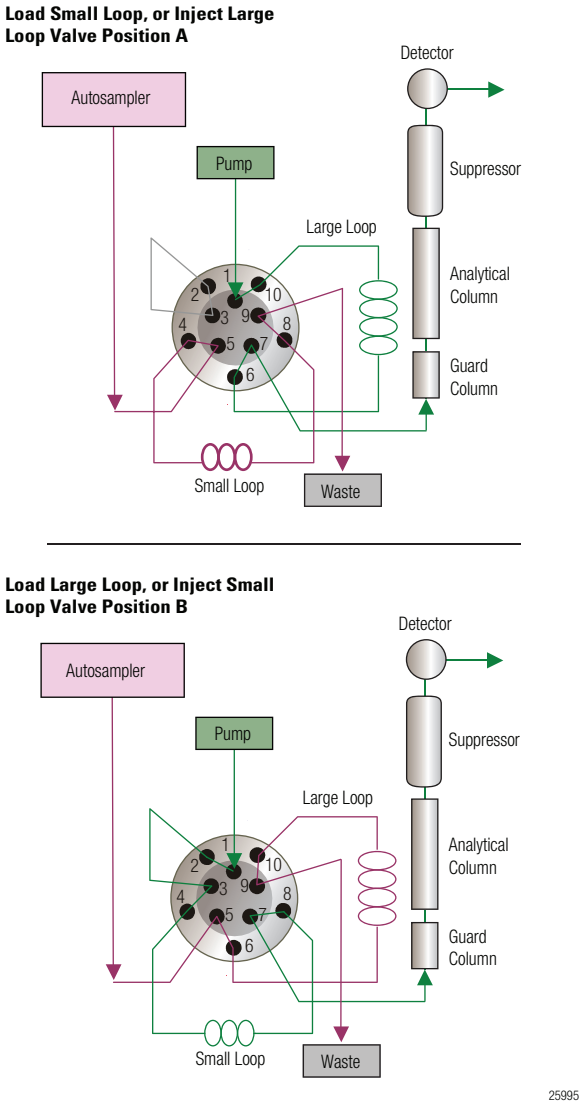


Figure 2. Valve plumbing.

Create Chromeleon CDS instrument methods

1. Rename the current instrument method **Large Loop**. This instrument method will be used for all initial runs and use the large injection loop (100 µL).

2. Create a copy of this file and name it **Small Loop**. This instrument method will be used to reinject any samples that are over the threshold limit and will use a small injection loop (10 µL).

3. To the first line in the **Large Loop** instrument method, add the command that will actuate the 10-port injection valve to the LOAD position for the large loop. The example below is for the 10-port valve on the Dionex Integrion HPIC system. If the optional 10-port valve is used on the Dionex AS-DV autosampler, the command would be *AuxValve B*.

   Note: If the autosampler is set to perform a sample loop rinse 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 outside the recorded run time and prevent the occurrence of a second water dip (a result of the loop containing rinse water). Alternatively, this command could be added to the end of the run.

4. To the first line in the **Small Loop** instrument method add the command that 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.

5. When using a Dionex AS-DV autosampler, an autosampler rinse step is recommended. 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 thereby limit the possibility of carryover. This time should be as short as possible, but not less than the time required to flush the loop volume five times at the given flow rate. An example of this command is 0.500 *Pump_ECD.HP_Valve.B* for the **Large Loop** instrument method or 0.050 *Pump_ECD.HP_Valve.A* for the **Small Loop** instrument method.

6. Create the sequence using the **Large Loop** instrument method. The **Small Loop** method must also be included in the sequence as an additional method but not initially assigned to any injections. This can be achieved via a copy/paste or drag-and-drop operation. Any samples that need to be rerun with a smaller volume will be done from the same vial as the original run, but will instead use the **Small Loop** instrument method file (as outlined below in IRC section).

Large Loop	ASAP Autosampler	AS-DV Autosampler
Load (State B)	Pump_ECD.HP_Valve_2.B	*AuxValve.B*
Inject (State A)	Pump_ECD.HP_Valve_2.A	*AuxValve.A*
Small Loop		
Load (State A)	Pump_ECD.HP_Valve_2.A	*AuxValve.A*
Inject (State B)	Pump_ECD.HP_Valve_2.B	*AuxValve.B*

Example of large loop instrument method commands

Time	Command	Value
{Initial Time}	Instrument Setup	
	Pump_ECD.HP_Valve.B	
	Sampler.FlushFactor	10
	Sampler.DelayVolume	125 [µl]
	Sampler.DeliverSpeed	4.0 [ml/min]
	Pump_ECD.Pressure.UpperLimit	3000.00 [psi]
	Pump_ECD.%A.Equate	"4.5 mM CO3/ 1.4 mM HCO3"
	Pump_ECD.Pressure.LowerLimit	200.00 [psi]
	Pump_ECD.Compartment_TC.Mode	On
	Pump_ECD.Compartment_TC.TemperatureSet	20.0 [°C]
	Pump_ECD.Column_TC.Mode	On
	Pump_ECD.Column_TC.TemperatureSet	35.0 [°C]
	Electrolytics.Suppressor.CurrentSet	41 [mA]
	Electrolytics.Suppressor.Type	AERS_4mm
	CDet.Rise_Time	0.5 [s]
	CDet.Data_Collection_Rate	5.0 [Hz]
	CDet.CellHeater.Mode	On
	CDet.CellHeater.TemperatureSet	35.0 [°C]
	CDet.Temperature_Compensation	1.7 [%]
	Pump_ECD.Flow.Nominal	1.2 [ml/min]
	Sampler.LoadPosition	
	Sampler.DeliverSample	
	Sampler.EndSamplePrep	
	Wait	Pump_ECD.Ready
	Wait	Compartment_TC.TemperatureState
	Wait	Column_TC.TemperatureState
	Click here to append a new command	
0.000	Inject	
	Wait	Sampler.CycleTimeState, Run=Hold, Timeout=Infinite
	Sampler.Inject	
	Click here to append a new command	
0.000	Start Run	
	Pump_1.Pump_1_Pressure.AcqOn	
	CDet.CD.AcqOn	
	CDet.CD_Total.AcqOn	
	CDet.Autozero	
	Pump_ECD.HP_Valve.A	
	Click here to append a new command	

Example of small loop instrument method commands

Time	Command	Value
{Initial Time}	Instrument Setup	
	Pump_ECD.HP_Valve.A	
	Sampler.FlushFactor	10
	Sampler.DelayVolume	125 [µl]
	Sampler.DeliverSpeed	4.0 [ml/min]
	Pump_ECD.Pressure.UpperLimit	3000.00 [psi]
	Pump_ECD.%A.Equate	"4.5 mM CO3/ 1.4 mM HCO3"
	Pump_ECD.Pressure.LowerLimit	200.00 [psi]
	Pump_ECD.Compartment_TC.Mode	On
	Pump_ECD.Compartment_TC.TemperatureSet	20.0 [°C]
	Pump_ECD.Column_TC.Mode	On
	Pump_ECD.Column_TC.TemperatureSet	35.0 [°C]
	Electrolytics.Suppressor.CurrentSet	41 [mA]
	Electrolytics.Suppressor.Type	AERS_4mm
	CDet.Rise_Time	0.5 [s]
	CDet.Data_Collection_Rate	5.0 [Hz]
	CDet.CellHeater.Mode	On
	CDet.CellHeater.TemperatureSet	35.0 [°C]
	CDet.Temperature_Compensation	1.7 [%]
	Pump_ECD.Flow.Nominal	1.2 [ml/min]
	Sampler.LoadPosition	
	Sampler.DeliverSample	
	Sampler.EndSamplePrep	
	Wait	Pump_ECD.Ready
	Wait	Compartment_TC.TemperatureState
	Wait	Column_TC.TemperatureState
	Click here to append a new command	
0.000	Inject	
	Wait	Sampler.CycleTimeState, Run=Hold, Timeout=Infinite
	Sampler.Inject	
	Click here to append a new command	
0.000	Start Run	
	Pump_1.Pump_1_Pressure.AcqOn	
	CDet.CD.AcqOn	
	CDet.CD_Total.AcqOn	
	CDet.Autozero	
	Pump_ECD.HP_Valve.B	
	Click here to append a new command	

Using Chromeleon CDS software intelligent run control to enable AutoDilution

When a new System Suitability Test (SST) case is added to the processing method associated with a given injection, the AutoDilution process is enabled. The New Test Case Wizard guides users through the steps required:

1. Case Type
2. General
3. Injection condition
4. Evaluation
5. Peak/channel
6. Pass actions
7. Fail actions

Note: Test case templates can be adjusted to suit application requirements.

New test case

From the SST/IRC tab of the processing method, click on **Click here to add a new test case** to start the wizard (Figure 3).

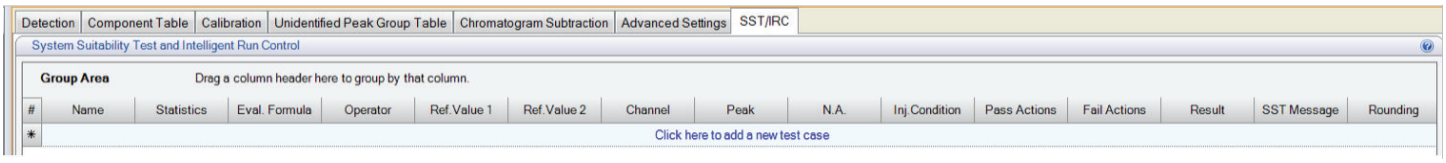
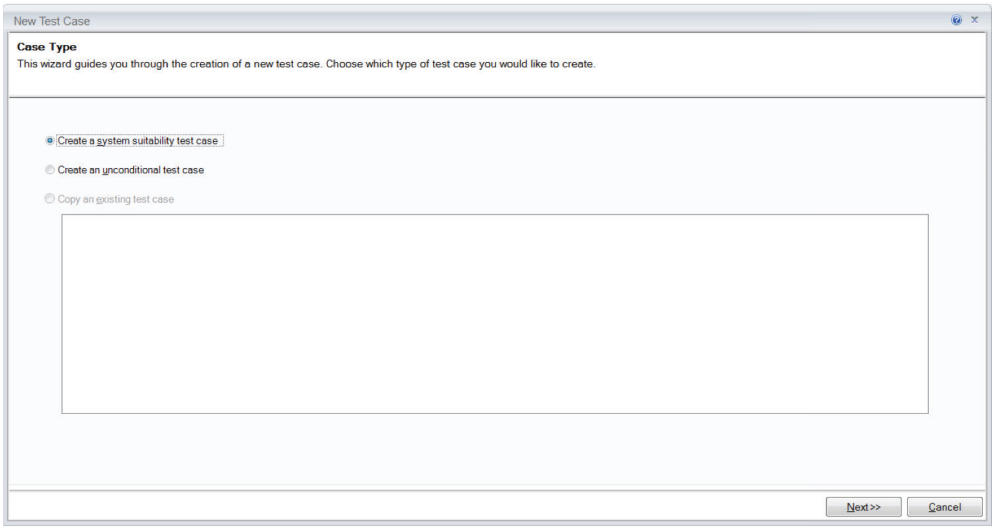


Figure 3. Creating a new test case from Chromeleon 7 CDS software Processing Method File.

Case type

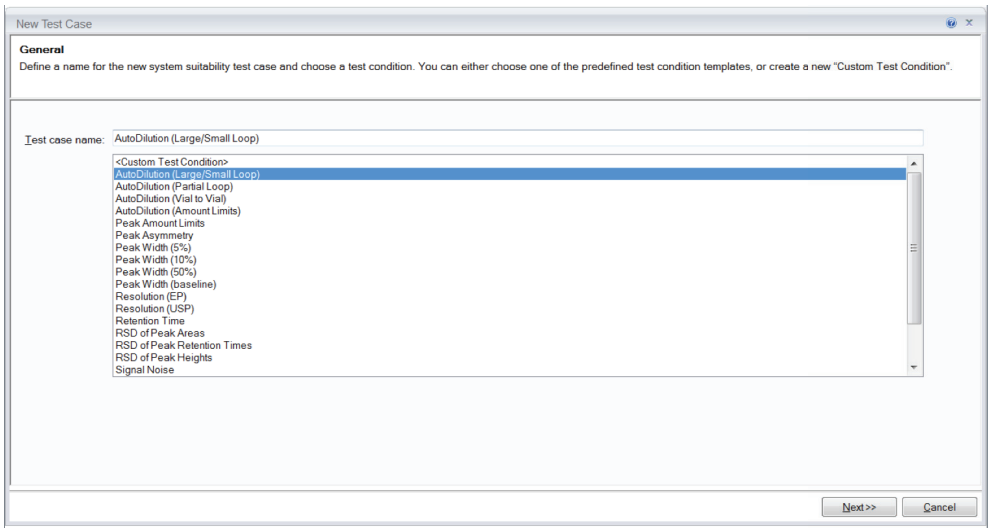
For AutoDilution, select **Create a system suitability test case**, then click **Next**. Depending on the requirements, multiple conditionals, also known as SSTs, may be created. The wizard provides guidance on the creation of a new test case.



General

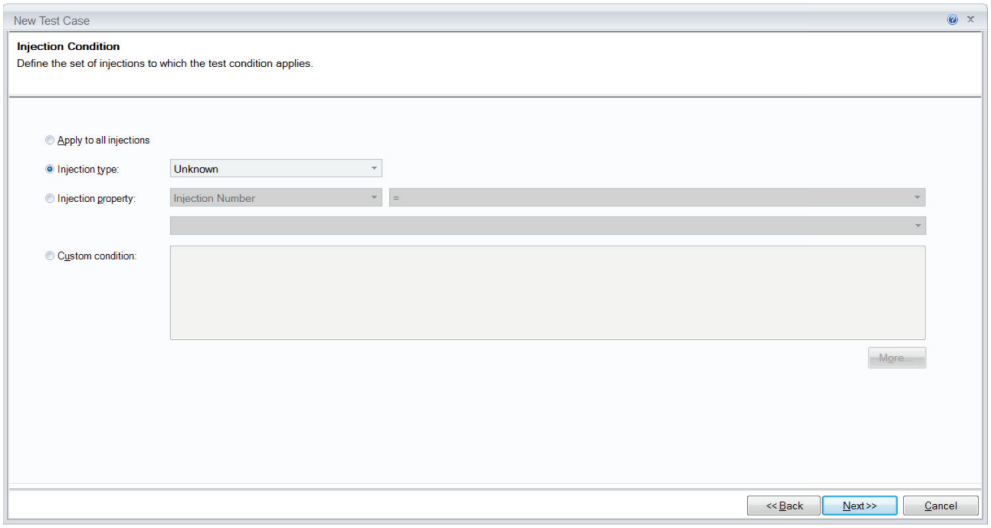
Select the predefined test case. For AutoDilution involving large and small injection loops, select **AutoDilution (Large/Small Loop)** and click **Next**.

Note that there are four AutoDilution test case templates and each is designed for a specific application.



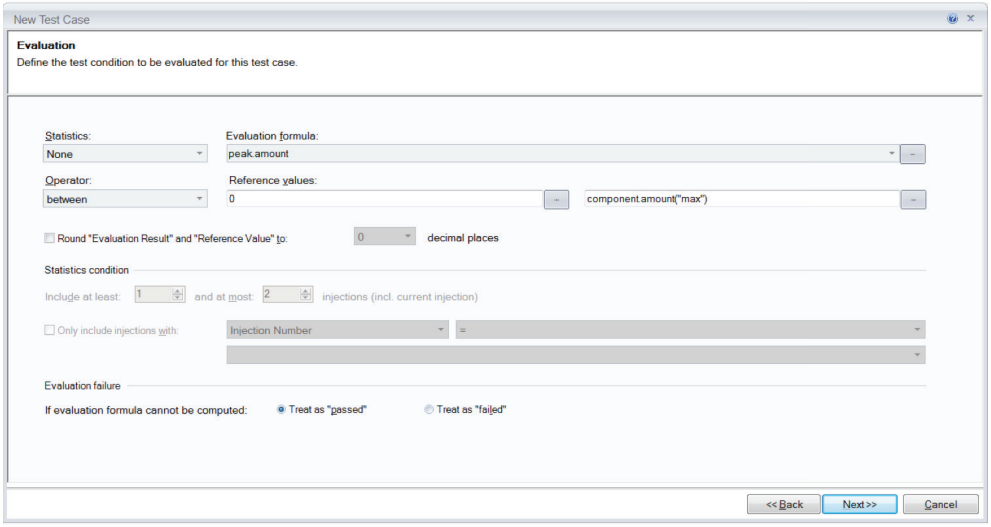
Injection condition

This window defines the set of injections where the test condition (AutoDilution) applies. Typically, the default setting is to apply the test condition to the injection type set as Unknown.



Evaluation

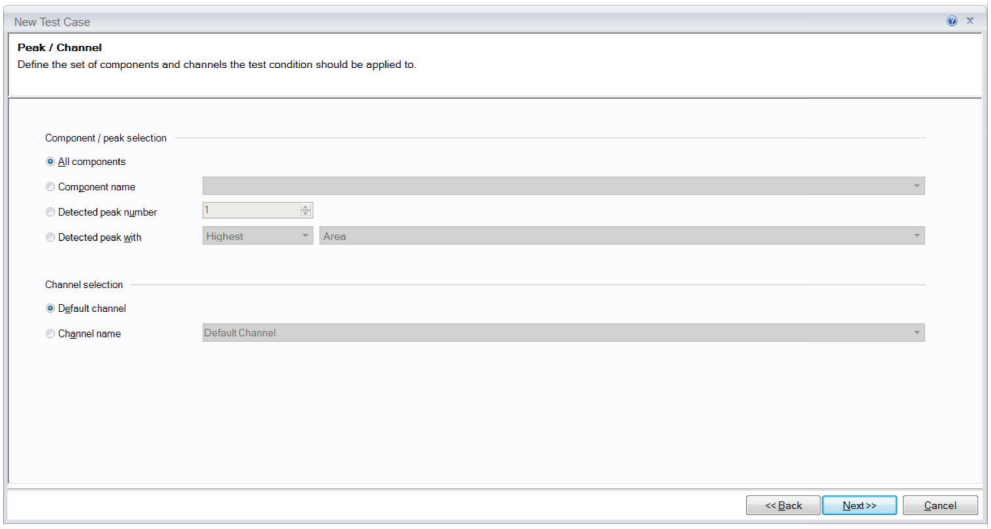
This window defines the test condition to be evaluated for this test. By default, it is set to enable AutoDilution when a sample exceeds the range of the calibration standards.



Peak/channel

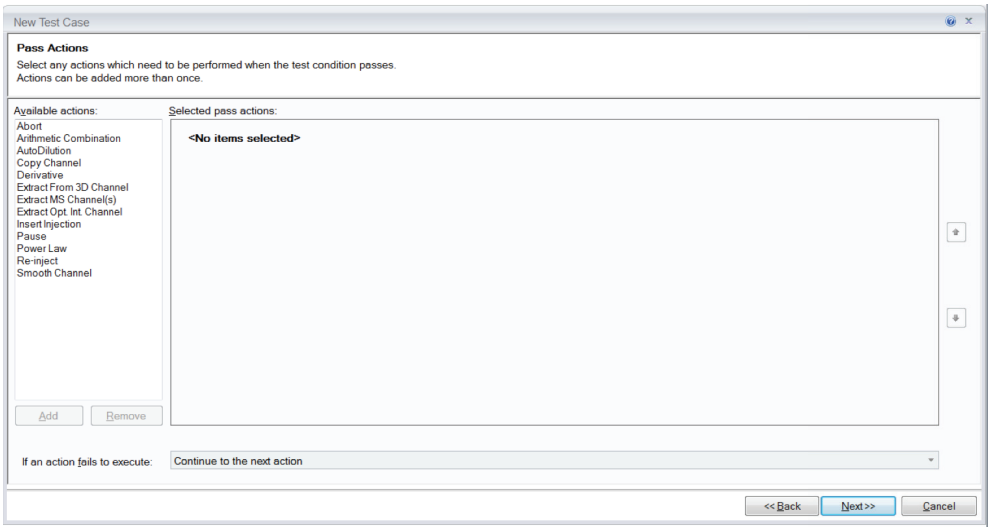
This window defines the set of components and channels to which the test condition should be applied. Select **All components** if the results of all identified analytes need to be monitored. Otherwise, select **Component name** and then the specific component to be monitored. If multiple components need to be monitored, a separate test case must be designed for each.

Note: In the processing method, the order of test cases defines the priority, meaning that if there are five test cases and test case 1 passes, but test case 2 does not, a dilution of that injection will be performed and the rest of the test cases ignored.



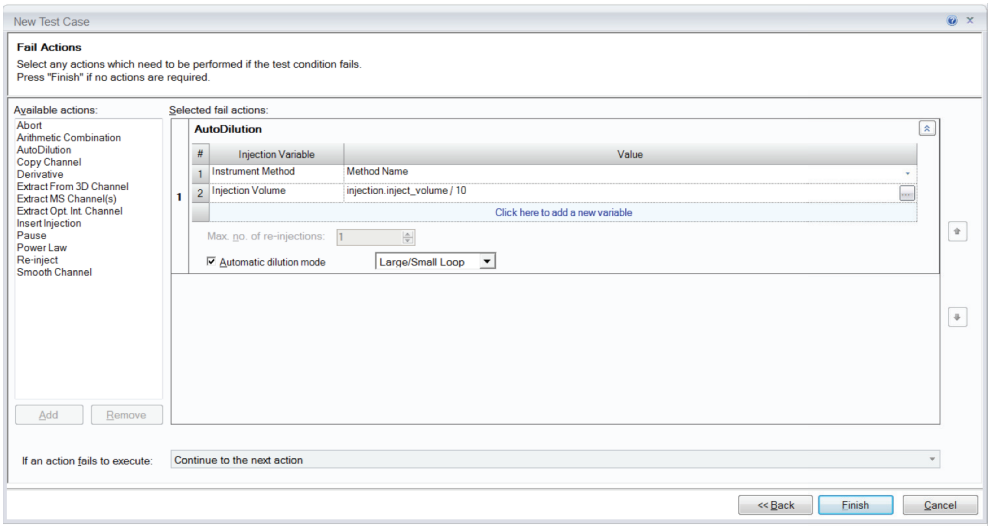
Pass actions

This window defines the actions that need to be performed when the test condition passes. For AutoDilution, typically, there is no action required for this application.



Fail actions

This window defines the actions to be performed when the test condition fails. **Instrument Method** and **Injection Volume** need to be updated. For **Instrument Method**, set **Small Loop** as the instrument method to be used when the test condition fails. Set **Injection Volume** to injection. *inject_volume/x*, where x is the ratio between the large loop and small loop. This ratio can be accurately determined by following the instructions in Step 7 of **Confirming the Loop Volumes**.



Loop calibration

Create a calibration curve 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.

Additionally, create a calibration curve for both loop sizes by running a calibration for each instrument method (**Large Loop** and **Small Loop**). Two processing method 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). For this application, the recommended large-to-small loop ratio is between 2 to 10. For example, if the large loop has a volume of 100 µL, then the volume of the small loop should be between 10 to 50 µL.

thermo scientific

Confirming the loop volumes

1. Obtain a 1000 mg/L (ppm) bromide standard (for anion determinations) or a 1000 mg/L lithium standard (for cation determinations).
2. Prepare a 1.0 mg/L (ppm) stock standard.
3. Create a sequence using the Large Loop instrument method and run 4 injections of the 1 mg/mL (ppm) standard.
4. Verify that the RSD is 5% or less.
5. Create a sequence using the Small Loop instrument method and run 4 injections of the 1.0 mg/L (ppm) standard.
6. Verify that the RSD is 5% or less.
7. Calculate the small loop relative volume and use this as the injection volume in the sequence in the Sample Properties dialog.
8. Use the equations:

$$\text{Small Loop Volume} = \text{Large Loop Volume} \times \text{Loop Ratio}$$

$$\text{Loop Ratio} = \frac{\text{Average Area of Small Loop}}{\text{Average Area of Large Loop}}$$

Benefits of the Chromeleon 7 CDS software IRC with AutoDilution

AutoDilution offers a quick, easy, and reliable method to perform automatic injections of lower sample volume. As a result, it:

- Increases productivity, saving time and money.
- Automatically checks for samples that exceed the calibration threshold.
- Automatically reruns samples with a smaller injection loop bringing them back into the calibration range.

Find out more at **thermofisher.com/chromatography**

TN17247-EN 0916S

ThermoFisher SCIENTIFIC