

AutoDilution using Chromeleon 7 CDS software and vial-to-vial dilutions

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Keywords: IRC, SST, Intelligent Run Control, Conditionals, Integrion, ICS-5000+, AS-AP, Dilution, Vial, Chromeleon, AutoDilution

Goal

To provide instructions to perform AutoDilution using Thermo Scientific™ Chromeleon™ 7 Chromatography Data System (CDS) software and vial-to-vial dilutions using a Thermo Scientific™ Dionex™ AS-AP autosampler

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 now been eliminated with AutoDilution, which is an integral part of the Intelligent Run Control (IRC) feature. IRC enables the software to automatically determine when compound concentrations exceed a set concentration, then dilute the samples by injecting a smaller amount, and re-run them along with standards, if needed (Figure 1).

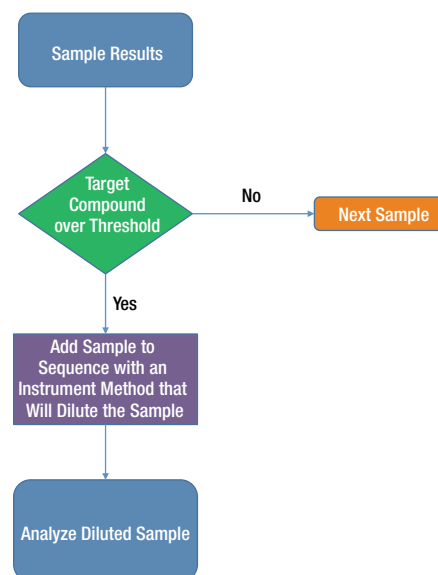


Figure 1. Flow chart of the Chromeleon 7 CDS AutoDilution process.

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+ IC system Detector/Chromatography Compartment (DC). (Not discussed in this technical note.)
 - If using Chromeleon 7 CDS software, refer to Thermo Scientific Technical Note 181 (TN 181).
 - If using Chromeleon 6 CDS software, refer to TN 84.
- Smaller injection volume in the existing loop using partial loop injections with the Dionex AS-AP Autosampler. (Not discussed in this technical note.)
 - If using Chromeleon 7 CDS software, refer to TN 183.
 - If using Chromeleon 6 CDS software and Thermo Scientific™ Dionex™ AS Autosampler, refer to TN 81.
- Vial-to-vial dilution performed with the Dionex AS-AP Autosampler.
 - If using Chromeleon 7 CDS software, refer to this note.
 - If using Chromeleon 6 CDS software and Dionex AS Autosampler, refer to TN 83.

This technical note presents an example of automatic dilutions using the Dilute function of the Dionex AS-AP autosampler to make a dilution in an empty vial and then run the diluted sample. Other injection volumes can be selected, as well as multiple conditionals. In this note, samples are first analyzed as is, without any dilutions made. The Chromeleon 7 CDS software monitors the sample concentration, dilutes, and re-injects the diluted sample using the same injection volume when 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 injection of a diluted sample.

Process overview

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

Equipment

- IC System
 - a) Dionex ICS-5000+ HPIC system, or
 - b) Dionex Integrion HPIC system, or
 - c) Thermo Scientific™ Dionex™ Aquion™ system
- Dionex AS-AP autosampler

Preparation

Autosampler settings

In this method, actual dilutions of the original samples are made into an empty vial using the Dilute function of the Dionex AS-AP autosampler. The Chromeleon CDS software will monitor the sample concentration, dilute and re-inject the samples with a different instrument method, if the concentration is over the specified threshold limit.

The typical configuration of the Dionex AS-AP autosampler is as follows:

- 250 µL syringe
- 1200 µL buffer loop

The recommended settings of the Dionex AS-AP autosampler are as follows:

- Vial size: 1.5 mL
- Sample dispense speed: 5 µL/s
- Sample draw speed: 10 µL/s
- Dilution mix properties:
 - Draw speed: 50 µL/s
 - Dispense speed: 50 µL/s
 - Iteration: 2
 - Volume: 50 µL

Create Chromeleon CDS instrument methods

1. Rename the current instrument method to **Undiluted**. This instrument method will be used for all initial runs with the original undiluted sample.
2. Create a copy of this file and name it **Diluted**. This instrument method will be used to dilute and re-inject any samples that are over the threshold limit.
3. To the **Diluted** instrument method, add the dilution commands. Refer to **Add dilution commands to the CDS instrument methods** section below.
4. Create the sequence using the **Undiluted** instrument method. The **Diluted** instrument 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. Samples requiring dilution will be diluted in the vial after the initial run, but will use the **Diluted** instrument method file (as outlined in **Using Chromeleon CDS Intelligent Run Control to enable AutoDilution** section below).

Add dilution commands to the CDS instrument methods

There are two different dilution approaches for the Dionex AS-AP autosampler: fixed and variable dilution ratios. Regardless of the concentration of the original sample, the former approach will always dilute the sample by a defined ratio. With AutoDilution enabled, the Chromeleon CDS software automatically examines the calibration data and calculates the raw AutoDilution ratio for each sample. By using this ratio, the component concentration will be brought into the middle of the calibration range.

1. Fixed dilution ratio

- a. Open the **Diluted** instrument method, click the Autosampler tab on the left, and the Sample Preparation tab above to access the sample preparation capabilities of the Dionex AS-AP autosampler
- b. Add the Dilute command. For a 10-times dilution,
 - i. SpSourceVial: CurrentVial+1
 - ii. SpSourceVol: 50.0
 - iii. SpDestVial: CurrentVial
 - iv. SpDiluent: G1
 - v. SpReagentVolume: 450.0

CurrentVial+1 is where the original sample is placed, and CurrentVial is where the empty vial will be placed in the autosampler. For example, if position of CurrentVial+1 is RA2, then the position of CurrentVial will be RA1. Using this example, the Dionex AS-AP autosampler will draw 50 µL of sample from the original sample vial (position RA2), dispense the aliquot into the defined empty vial (position RA1), and then dilute it with 450 µL of diluent from vial G1. Depending on the application, the diluent contained in vial G1 is typically deionized water.

Note: The positions of the vials (original and empty) must be aligned to the position identified in the Fail Actions windows of Chromeleon CDS IRC.

- c. Add the Mix_DrawDispense
 - i. SpSourceVial: CurrentVial
 - ii. Volume: 50.0 µL

Using the example above, the mixing is achieved by drawing and dispensing 50 µL from the diluted vial (position RA1).

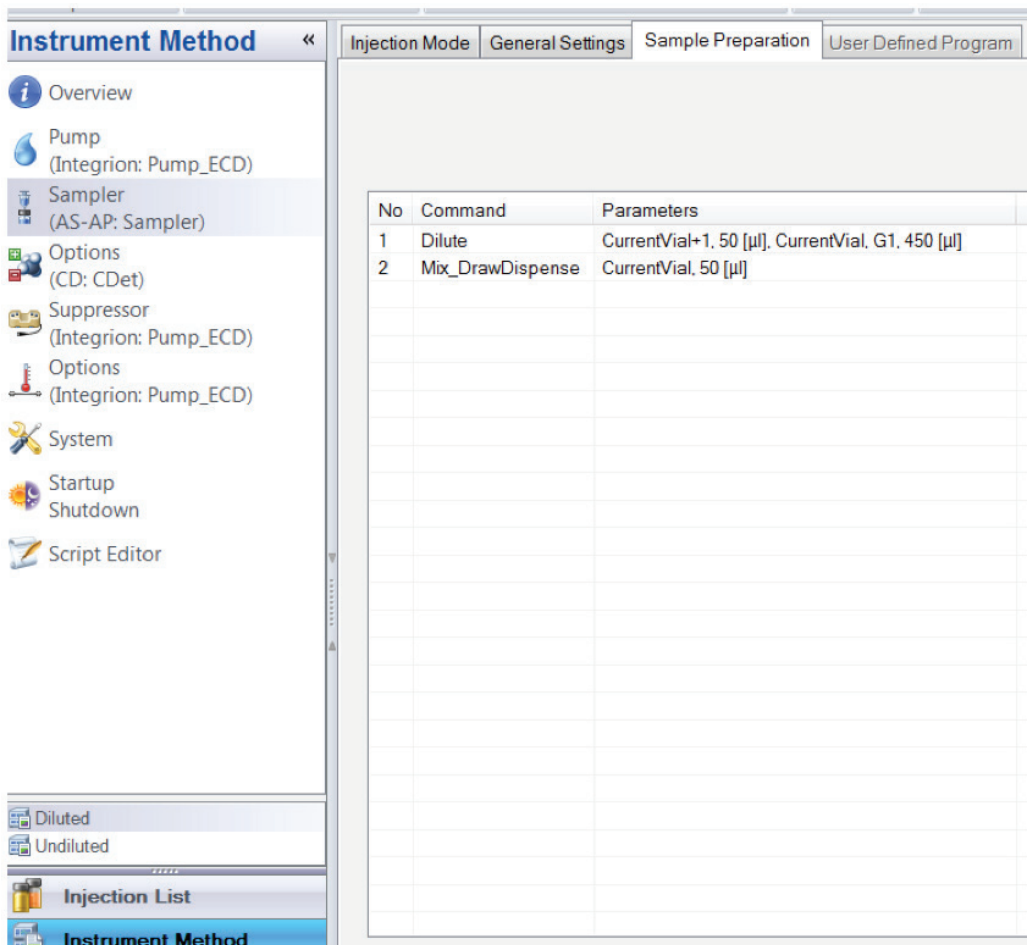


Figure 2. 10-fold dilution with the AS-AP autosampler.

2. Variable dilution ratio

- a. Follow the steps as described in the fixed dilution ratio
- b. Click the Script Editor on the left, locate the sampler dilute commands (Line 37 in Figure 3), and manually edit the script as follows:
 - i. Update 50 [µL] to $500 * \text{system.injection.autodilutionratio}$
 - ii. Update 450 [µL] to $500 * (1 - \text{system.injection.autodilutionratio})$

Using these commands, the Dionex AS-AP autosampler will prepare 500 µL total volume of diluted sample in a 1.5 mL vial. So, if the AutoDilutionRatio is 0.1, the Dionex AS-AP autosampler will take 50 µL of sample and 450 µL of diluent.

Note: For this approach, the automatic dilution mode box must be checked. Otherwise, the AutoDilutionRatio will not be calculated. This is defined under Fail Actions window of the Chromeleon CDS IRC.

Instrument Method	Time	Command	Value
Overview	24	Sampler.WashVolume	250.0 [µl]
Pump (Integriion: Pump_ECD)	25	Sampler.SampleHeight	2.000 [mm]
Sampler (AS-AP: Sampler)	26	Sampler.WasteSpeed	20.0 [µl/s]
Options (CD: CDet)	27	Sampler.DispenseDelay	2.0 [s]
Suppressor (Integriion: Pump_ECD)	28	Sampler.DispSpeed	5.0 [µl/s]
Options (Integriion: Pump_ECD)	29	Sampler.DrawSpeed	10.0 [µl/s]
System	30	Sampler.DrawDelay	2.0 [s]
Startup Shutdown	31	Sampler.DilutionMixDispenseSpeed	50.0 [µl/s]
Script Editor	32	Sampler.DilutionMixIterations	2
	33	Sampler.DilutionMixSpeed	50.0 [µl/s]
	34	Pump_ECD.Flow.Nominal	1.2 [ml/min]
	35	Wait	Pump_ECD.Ready
	36	Sampler.PunctureOffset	3 [mm]
	37	Sampler.Dilute	CurrentVial+1, $500 * \text{system.injection.autodilutionratio}$, CurrentVial, G1, $500 * (1 - \text{system.injection.autodilutionratio})$
	38	Sampler.Mix_DrawDispense	CurrentVial, 50 [µl]

Figure 3. Editing the Chromeleon script to enable variable dilution.

Using Chromeleon CDS software intelligent run control to enable AutoDilution

The AutoDilution process is enabled by adding a new System Suitability Test (SST) case to the processing method associated with a given injection. 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: The test case templates can be adjusted to suit application requirements.

New test case

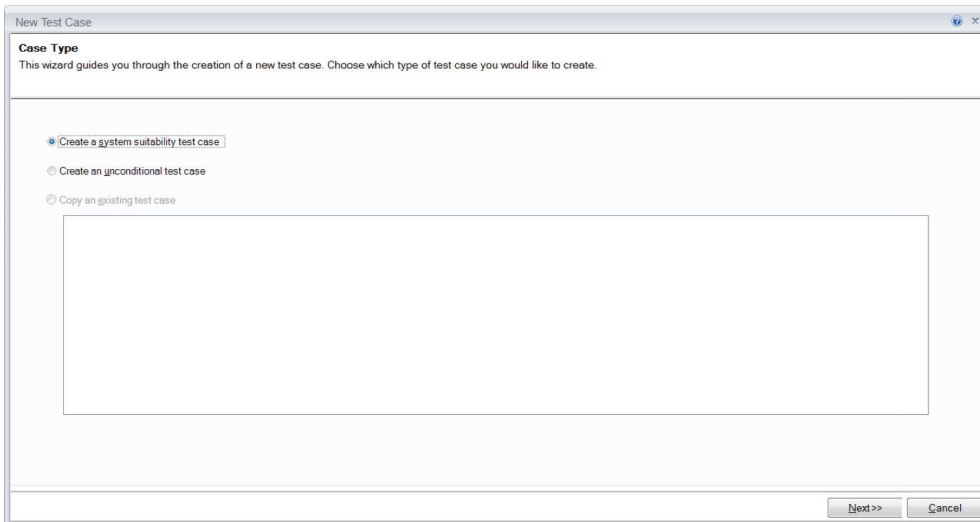
From the SST/IRC tab of the processing method, clicking on **Click here to add a new test case** starts the wizard (Figure 4).

System Suitability Test and Intelligent Run Control															
Group Area															
Drag a column header here to group by that column.															
#	Name	Statistics	Eval. Formula	Operator	Ref. Value 1	Ref. Value 2	Channel	Peak	N.A.	Inj. Condition	Pass Actions	Fail Actions	Result	SST Message	Rounding
*	Click here to add a new test case														

Figure 4. Creating a new test case from Chromeleon 7 CDS software Processing Method file.

Case type

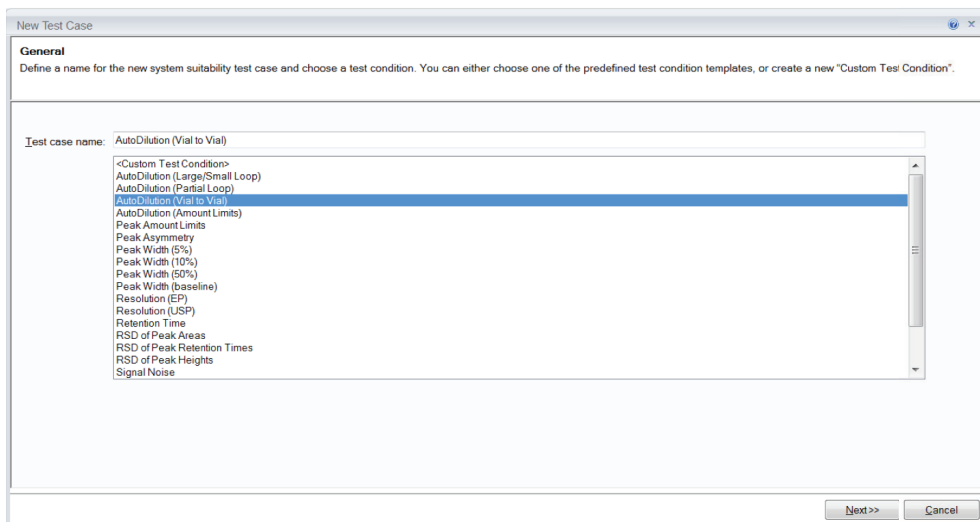
For AutoDilution, select **Create a system suitability test case** and click **Next**. Depending on the requirements, multiple conditionals (also known as SSTs) may be created. This wizard provides guidance on the creation of a new test case.



General

Select the predefined test case. For AutoDilution involving dilution of samples in a different vial, select **AutoDilution (Vial to Vial)** and click **Next**.

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



Injection condition

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

The screenshot shows the 'New Test Case' dialog box with the 'Injection Condition' tab selected. The title bar reads 'New Test Case'. Below the title bar, the text 'Injection Condition' is followed by the instruction 'Define the set of injections to which the test condition applies.' The main area contains three radio buttons: 'Apply to all injections' (unselected), 'Injection type:' (selected), and 'Custom condition:' (unselected). The 'Injection type:' dropdown is set to 'Unknown'. Below it, the 'Injection property:' dropdown is set to 'Injection Number', followed by an equals sign and another dropdown menu. A 'More...' button is located at the bottom right of the main area. At the bottom of the dialog box, there are three buttons: '<< Back', 'Next >>', and 'Cancel'.

Evaluation

Evaluation 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.

The screenshot shows the 'New Test Case' dialog box with the 'Evaluation' tab selected. The title bar reads 'New Test Case'. Below the title bar, the text 'Evaluation' is followed by the instruction 'Define the test condition to be evaluated for this test case.' The main area contains several fields: 'Statistics:' dropdown set to 'None'; 'Evaluation formula:' dropdown set to 'peak amount'; 'Operator:' dropdown set to 'between'; 'Reference values:' field with '0' and 'component.amount("max")' separated by a minus sign; a checkbox 'Round "Evaluation Result" and "Reference Value" to:' with '0' and 'decimal places'; 'Statistics condition' section with 'Include at least: 1' and 'and at most: 2' injections (incl. current injection); a checkbox 'Only include injections with:' followed by a dropdown set to 'Injection Number' and an equals sign; and 'Evaluation failure' section with radio buttons 'Treat as "passed"' (selected) and 'Treat as "failed"'. At the bottom of the dialog box, there are three buttons: '<< Back', 'Next >>', and 'Cancel'.

Peak/Channel

Peak/Channel 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 that needs to be monitored. If only some of the components need to be monitored, a separate test case must be designed for each.

Note: The order of test cases defines the priority. This means that if there are five test cases where test case 1 passes and test case 2 does not, a dilution of that injection will be performed based on case 2 and the rest of the test cases ignored.

The screenshot shows the 'New Test Case' dialog box with the 'Peak / Channel' tab selected. The title bar reads 'New Test Case'. Below the title bar, the text 'Peak / Channel' is displayed, followed by the instruction 'Define the set of components and channels the test condition should be applied to.' The main area is divided into two sections: 'Component / peak selection' and 'Channel selection'. In the 'Component / peak selection' section, the 'All components' radio button is selected. Other options include 'Component name', 'Detected peak number' (set to 1), and 'Detected peak with' (set to Highest and Area). In the 'Channel selection' section, the 'Default channel' radio button is selected, and the 'Default Channel' dropdown menu is visible. At the bottom right, there are three buttons: '<< Back', 'Next >>', and 'Cancel'.

Pass actions

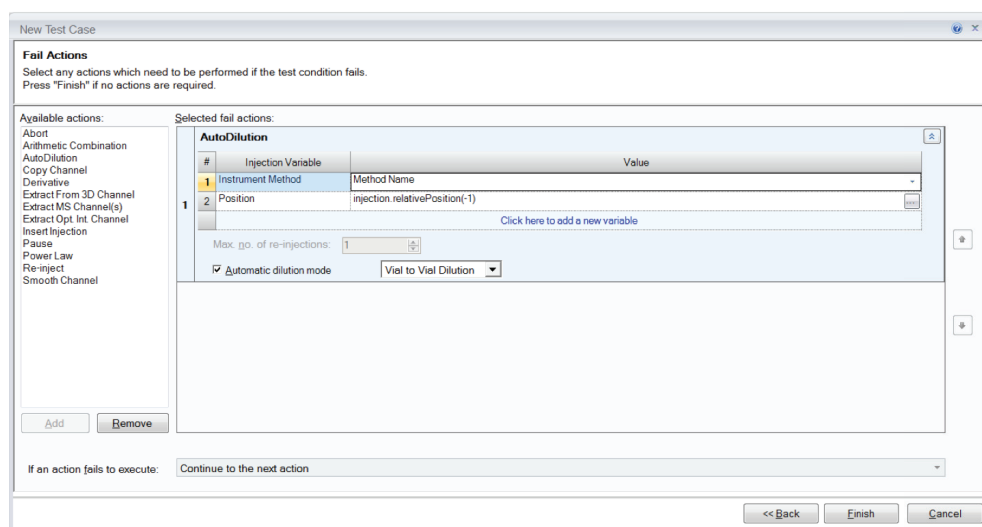
Pass Actions defines the actions to be performed when the test condition passes. For AutoDilution, typically, there are no actions required for this application.

The screenshot shows the 'New Test Case' dialog box with the 'Pass Actions' tab selected. The title bar reads 'New Test Case'. Below the title bar, the text 'Pass Actions' is displayed, followed by the instruction 'Select any actions which need to be performed when the test condition passes. Actions can be added more than once.' The main area is divided into two sections: 'Available actions:' and 'Selected pass actions:'. The 'Available actions:' list includes: Abort, Arithmetic Combination, AutoDilution, Copy Channel, Derivative, Extract From 3D Channel, Extract MS Channel(s), Extract Opt. Int. Channel, Insert Injection, Pause, Power Law, Re-inject, and Smooth Channel. The 'Selected pass actions:' area is currently empty and displays '<No items selected>'. There are 'Add' and 'Remove' buttons at the bottom left of the 'Selected pass actions:' area. At the bottom of the dialog, there is a dropdown menu for 'If an action fails to execute:' with the option 'Continue to the next action' selected. At the bottom right, there are three buttons: '<< Back', 'Next >>', and 'Cancel'.

Fail actions

Fail Actions defines the actions to be performed when the test condition fails. The **Instrument Method** and **Position** values must be updated.

- For **Instrument Method**, select the **Diluted** instrument method to be used when the test condition fails.
- For **Position**, select *injection.relativePosition(-1)*.
 - An empty vial will need to be placed one position before each sample. For example, if the position of the original sample is RA2, then the empty vial needs to be placed in position RA1.



Benefits of Chromeleon 7 CDS IRC with AutoDilution

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

1. Increases productivity, saving time and money.
2. Automatically checks for samples that exceed the calibration threshold.
3. Automatically re-runs diluted samples bringing them back into the calibration range.

Find out more at thermofisher.com/chromatography