AutoDilution using Chromeleon 7 CDS software and partial loop injection

Daniel Khor, Jay Lorch, Jeff Rohrer; Sunnyvale, CA USA

Key words
IRC, SST, Intelligent Run Control, conditionals, Integrion, ICS-5000+, AS-AP, partial loop, Chromeleon, AutoDilution

Goal
To provide instructions to perform AutoDilution using Thermo Scientific™ Chromeleon™ 7 Chromatography Data System (CDS) software and partial loop injections 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 the AutoDilution option, an integral part of the Intelligent Run Control (IRC) feature of Chromeleon 7 CDS software. IRC enables the software to automatically determine when compound concentrations exceed a set concentration, then reanalyze those the samples by injecting a smaller sample volume along with standards, if needed (Figure 1).

![Flow chart of the Chromeleon 7 CDS AutoDilution process.](image-url)

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
  - If using Chromeleon 7 CDS software, refer to this note.
  - 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: (Not discussed in this technical note.)
  - If using Chromeleon 7 CDS software, refer to TN 182.
  - If using Chromeleon 6 CDS software and Dionex AS Autosampler, refer to TN 83.

This technical note presents an example of AutoDilution using the partial loop injection function of the Dionex AS-AP autosampler. Other injection volumes can be selected, as well as multiple conditionals. In this note, samples are first analyzed by performing a full loop injection. The Chromeleon CDS software monitors the sample concentration, and re-injects the sample using a smaller injection volume if the concentration is over the specified threshold. 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 above these levels will be inserted as the next injection in the sequence and will be reinjected according to the method described below.

**Equipment**
- **IC System**
  - Dionex ICS-5000+ HPIC system, or
  - Dionex Integrion HPIC system, or
  - Thermo Scientific™ Dionex™ Aquion™ system
- Dionex AS-AP autosampler

**Preparation**

**Autosampler settings**
In this method, lower sample injection volumes are achieved using partial loop injections. The initial sample run uses a full loop injection (e.g., 100 µL). The Chromeleon CDS software monitors the sample concentration and reinjects the samples with a smaller injection volume (e.g., 10 µL), 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
Create Chromeleon CDS software instrument methods

1. Rename the current instrument method to Full Loop. This instrument method will be used for all initial runs.

2. Create a copy of this file and name it Partial Loop. This instrument method will be used to reinject any samples that are over the threshold limit with a smaller injection volume.


4. Create the sequence using the Full Loop instrument method. The Partial Loop 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 that need to be reinjected will be injected after the initial run, but will use the Partial Loop instrument method file (as outlined in Using Chromeleon CDS Intelligent Run Control to enable AutoDilution section, below).

Dionex AS-AP partial loop settings in Chromeleon CDS instrument method

When the Dionex AS-AP autosampler is configured for partial loop injections, the injection volume entered in the sequence determines the volume injected. A different sample loop is not required to perform partial loop injections (Figure 2).

1. Open the Partial Loop instrument method, click the Autosampler tab on the left, and select the Injection Mode tab above to access the injection settings for the Dionex AS-AP autosampler.

2. Change the settings from PushFull to PushPartial.

3. Accept the recommended values.

4. Save the instrument method.

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Figure 2. Configuring the Partial Injection mode of the Dionex AS-AP autosampler.
Using Chromeleon CDS 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, click on **Click here to add a new test case** to start the wizard (Figure 3).

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 smaller injection volumes by partial loop injection, select **AutoDilution (Partial Loop)** 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 **Unknown**.
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.

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.
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.

Fail Actions
Fail Actions defines the actions to be performed when the test condition fails. Add Instrument Method and Injection Volume by clicking **Click here to add a new variable.** Then, set Partial Loop as the instrument method to be used for the reinjection, and a smaller injection volume (e.g., 10 µL) as the injection volume.
Benefits of Chromeleon 7 CDS 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 re-runs samples with a smaller injection volume bringing them into the calibration range.