TECHNICAL NOTE

Creating and fully utilizing the retention time standard processing method for a more automated IC workflow

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Keywords
Retention Time Standard Processing Method, Chromeleon 7, RTS, Integrion, Seven-Anion Standards, Six-Cation Standards, 7-Anion Standards, 6-Cation Standards

Goal

To describe how to create and use the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software Retention Time Standard Processing Method to automatically identify and properly label peaks.

Introduction

Retention time standard (RTS) processing methods help to fully automate the task of entering and updating component retention times in a processing method, thereby reducing the amount of time a user spends in front of a CDS and freeing that person to work on other projects or tasks. These methods also provide important system diagnostic and troubleshooting information by indicating whether peak response for any target analytes is outside of expected range.

The RTS processing methods provide predefined components with assigned peak area ratios. Each component is interpreted as a peak area ratio, rather than an absolute time or other time-based interpretation. The peak area ratios allow dynamic determination of expected retention times of each component. Using peak area ratios means that retention times in the component table do not need to be manually updated when they are inconsistent between methods or batches, due to variations such as changes in chromatographic run conditions.

The peak area ratios are determined using standards that have been specifically developed to have certain ratios of analytes, known as retention time standards. The peak area ratio for a component is a peak area percentage relative to the largest peak in the retention time standard.

For example, if the largest peak has an area of 0.09, and peak 5 has an area of 0.045, then the ratio of peak 5 to the reference (the largest peak) is 50%.

Software

- Dionex Chromeleon 7 Chromatography Data System (CDS) software, Version 7.2 SR3 or later

Reagents and Standards

- Thermo Scientific™ Dionex™ Seven Anion Retention Time Standard (P/N 088957) Thermo Scientific™ Dionex™ Seven Anion Retention Time Standard 100x Concentrate (P/N 302511)
- Thermo Scientific™ Dionex™ Six Cation Retention Time Standard (P/N 088964) Thermo Scientific™ Dionex™ Six Cation Retention Time Standard 100x Concentrate (P/N 088969)

Equipment

- A Thermo Scientific Dionex IC system, e.g., the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system

Software Setup

To design a run using an RTS processing method, setup is required both in the Console and the Studio.

Console Setup

In the Console, the retention time standards must be assigned and various injection columns can be revealed in the injection list to help identify and troubleshoot issues.

Once a new sequence has been created (see Editing Sequences and Injection Lists section in the Chromeleon 7 Help), right click on a column header in the injection list and select “Table Columns…” (Figure 1).

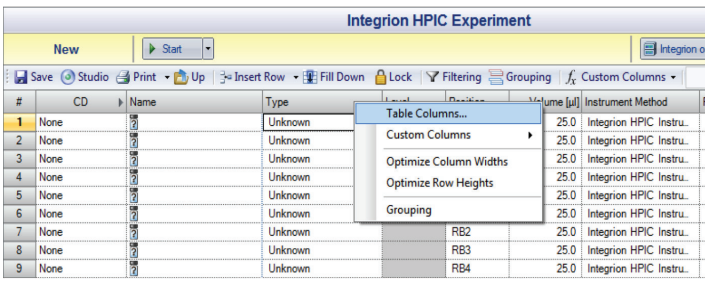


Figure 1: Access the Table Columns dialog by right-clicking one of the column headers in the injection list and selecting “Table Columns…”

This will reveal the Table Columns dialog: (Figure 2). On the left side of the dialog is a list of all available table columns as well as the order in which they are currently displayed. Those listed in a gray are not displayed. Select the Retention Time Standard listing and drag it up to its desired location among the visible columns.

There are two additional custom table columns that are useful when running the retention time standards:

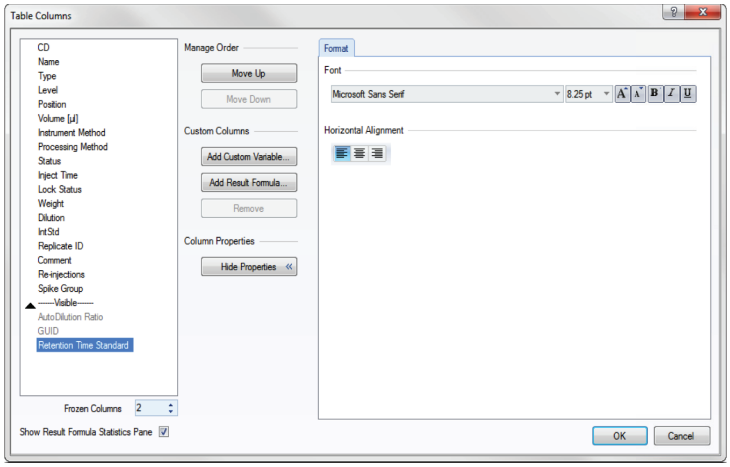


Figure 2: In the Table Columns dialog, select the Retention Time Standard column and drag it up to an appropriate position on the list.

- **The Retention Time Standard Error column** – Displays the associated descriptive error for a given invalid standard.
- **The Retention Time Standard Status column** – Displays whether or not a given standard is valid or invalid, based on a set of five criteria:

1. The injection is designated as a retention time standard in the injection list.
2. The injection status is either finished or interrupted.
3. The injection is assigned a processing method that has at least one RTS-enabled component.
4. For each RTS-enabled component in the processing method, there must be exactly one peak in the injection with an area ratio that matches the area ratio assigned to the component.
5. If a component is assigned to all channels, it will be evaluated against all channels of that injection.

The status is *valid* if all of the above are true.

The status is *invalid* if the injection meets the first three criteria above for a valid retention time standard, but does not meet the last two.

The status is *n.a.* if any of the following are true:

- The injection is not designated as a retention time standard in the injection list.
- The injection status is idle.
- The processing method assigned to the injection does not have at least one RTS-enabled component.

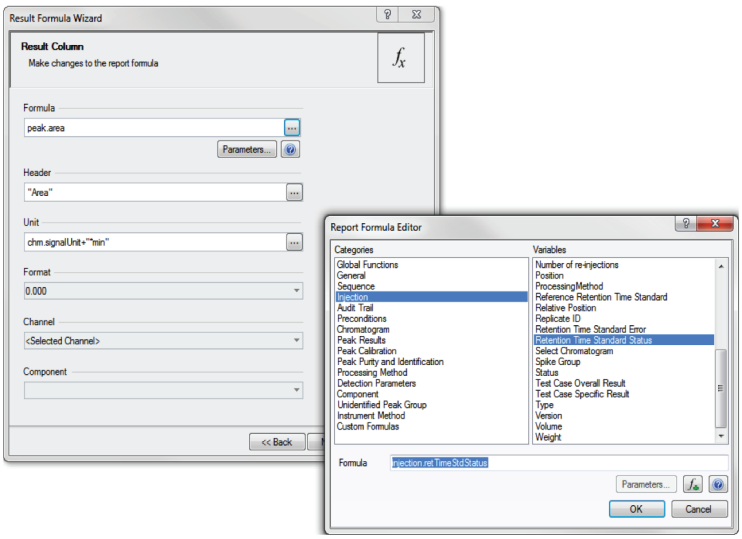


Figure 3: To create the Retention Time Standard Status and Retention Time Standard Error columns, click the Add Result Formula button in the middle of the Table Columns dialog to open the Result Formula Wizard, then click the ellipsis ("…") button at the end of the Formula field. In the Report Formula Editor, select the Injection category on the left, then find one of the two aforementioned variables on the right. **Repeat the process for the second variable.**

Using the Report Formula Wizard (Figure 3) and selecting the two variables, "Retention Time Standard Status" and "Retention Time Standard Error", will add the two custom table columns to both the table column and the injection lists (Figure 4).

Note: The wizard must be run twice to add both columns.

Once visible on the table columns list, simply drag them to the most appropriate position on the list.

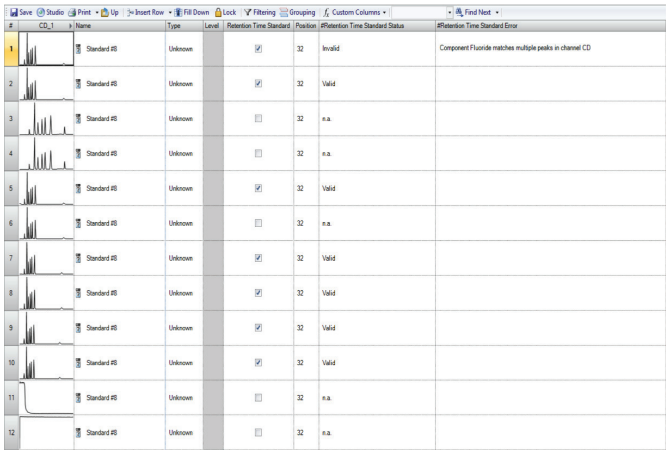


Figure 4: Example of an invalid Seven-Anion standard and its associated error.

Once the columns are set up, the retention time standards can be assigned by selecting the checkboxes found in the Retention Time Standard column (Figure 5). Chromeleon 7 CDS software will now identify these as the retention time standards against which all other standards and samples will be compared.

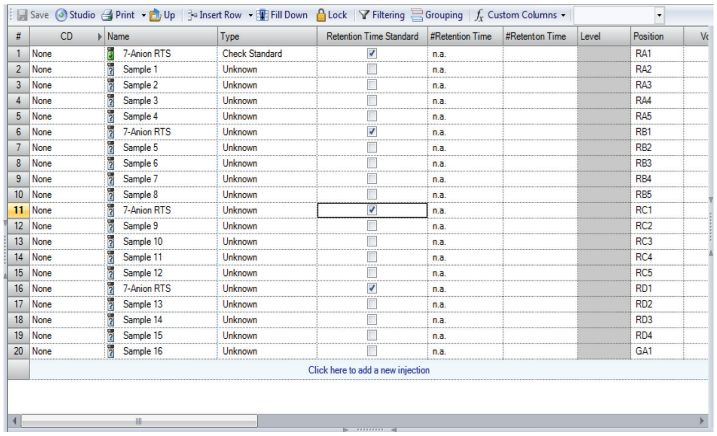


Figure 5: Enable the retention time standards by selecting the corresponding checkboxes in the Retention Time Standard column.

After adding the columns and identifying the standards, a processing method must be generated. To do this, click the Create button in the Menu Bar and select "Processing Method…". In the resulting Create Processing Method dialog, there are two default processing method templates: one for the 7-Anion Retention Time Standards and one for the 6-Cation Retention Time Standards (Figure 6). Select the appropriate method template and follow the wizard.

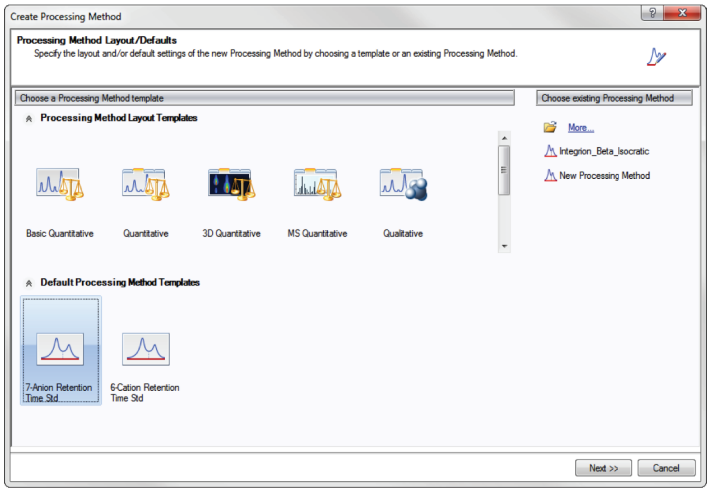


Figure 6: Select the appropriate default processing method template.

Studio Setup

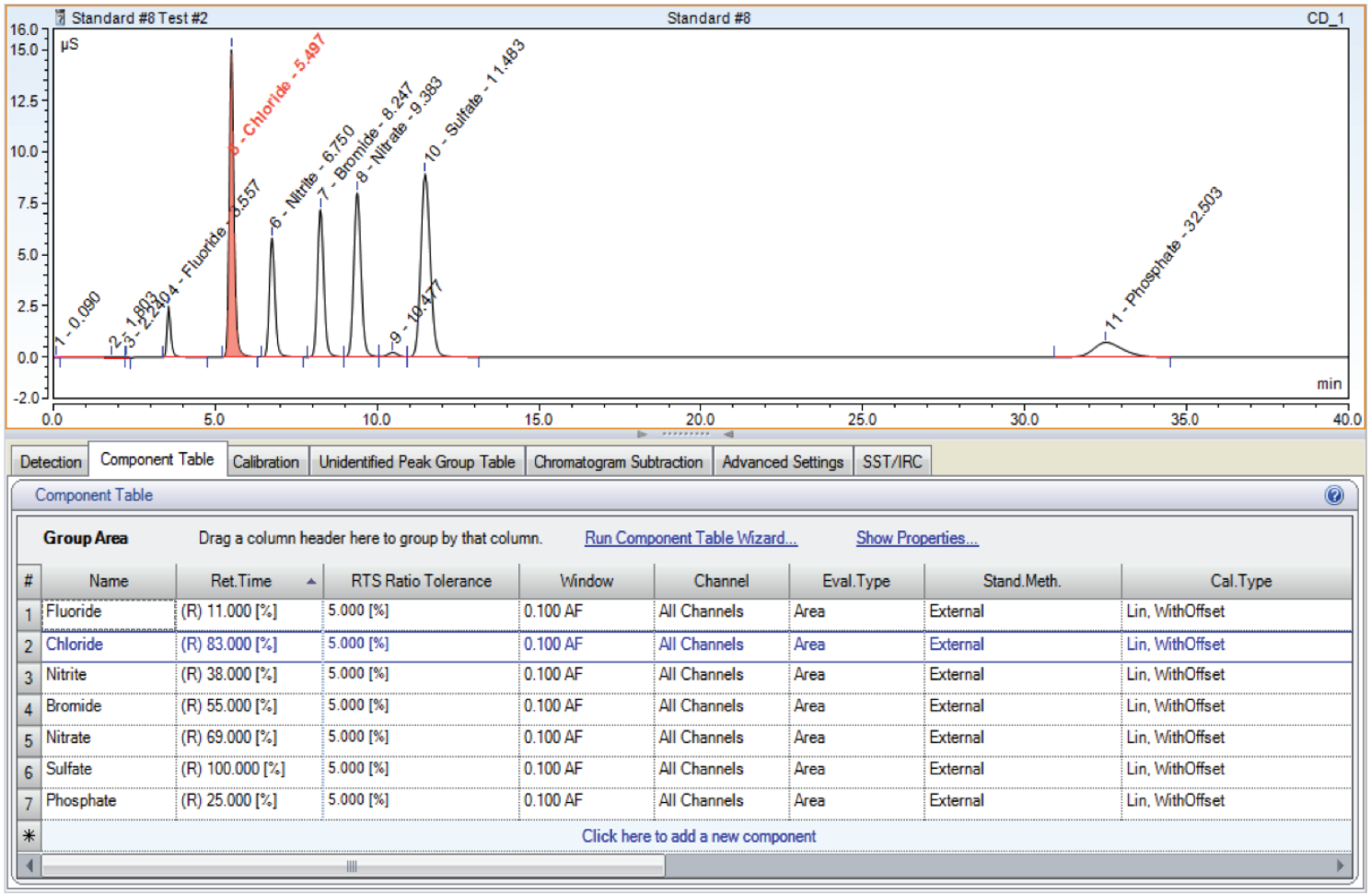
The created processing method will open in the Chromeleon 7 CDS software Studio window (Figure 7). Note that the retention time column, rather than showing actual retention times, instead shows the peak area ratios indicated by "(R) <peak area ratio value> [%]". The RTS Ratio Tolerances are also shown and are set to the percentage expected to give the best results. Each can be adjusted by the user, if necessary.

Retention Time Standard Data Processing

Once the setup is completed and a sequence is run, the data is processed as follows:

- For each injection designated as a retention time standard, the Chromeleon 7 CDS software determines whether or not the retention time standard is valid.
- For each injection in the sequence that is assigned an RTS processing method, the Chromeleon 7 CDS software searches for the first valid retention time standard assigned the same processing method. This is the reference retention time standard for that injection. The search proceeds upward most recent (including the current injection) and then downward, if the upward search fails to find a valid retention time standard.

When the Chromeleon 7 CDS software finishes processing the data, each of the component names will be assigned to the appropriate peak.



#	Name	Ret.Time	RTS Ratio Tolerance	Window	Channel	Eval.Type	Stand.Meth.	Cal.Type
1	Fluoride	(R) 11.000 [%]	5.000 [%]	0.100 AF	All Channels	Area	External	Lin, WithOffset
2	Chloride	(R) 83.000 [%]	5.000 [%]	0.100 AF	All Channels	Area	External	Lin, WithOffset
3	Nitrite	(R) 38.000 [%]	5.000 [%]	0.100 AF	All Channels	Area	External	Lin, WithOffset
4	Bromide	(R) 55.000 [%]	5.000 [%]	0.100 AF	All Channels	Area	External	Lin, WithOffset
5	Nitrate	(R) 69.000 [%]	5.000 [%]	0.100 AF	All Channels	Area	External	Lin, WithOffset
6	Sulfate	(R) 100.000 [%]	5.000 [%]	0.100 AF	All Channels	Area	External	Lin, WithOffset
7	Phosphate	(R) 25.000 [%]	5.000 [%]	0.100 AF	All Channels	Area	External	Lin, WithOffset

Figure 7: The Seven-Anion Standard processing method, seen in the bottom half of the screen, contains the default peak area ratios in the Ret. Time column and the tolerance settings for those ratios in the RTS Ratio Tolerance column. Note the chromatogram with all peaks properly labeled.

Troubleshooting

Although setting up and running an RTS method is generally quite easy, several factors could potentially affect the final results of an injection. The table below describes these potential issues and their resolution.

Issue	Possible Resolution
No evaluated channel.	No data found for assigned channel. Ensure that data acquisition occurred.
If using a default RTS processing method and component x matches more than one peak for channel y, for example, "Component Fluoride matches multiple peaks in channel CD". This may occur if there are two peaks with similar area within the chromatogram.	Ensure that a freshly prepared RTS was used. There may be contamination or possibly degradation of the standard. Check that the number of peaks in the chromatogram match the number of components in the processing method. Run a blank using the same water used to prepare the RTS and check for contamination. Check background and eluent water quality. Review and ensure that the integration parameters are optimized.
If using a custom-created RTS processing method, component x matches more than one peak for channel y, for example, "Component Fluoride matches multiple peaks in channel CD". This may occur if there are two peaks with similar area within the chromatogram.	Check all the possible resolutions for the default method (see above). Ensure that the RTS Ratio Tolerance in the processing method is not set too high.
If using a default RTS processing method and component x does not match any peak for channel y, for example, "Component Bromide does not match any peak in channel CD".	Ensure that a freshly prepared RTS was used. Check that the number of peaks in the chromatogram matches the number of components in the processing method. Ensure that the run is long enough to elute all components. Run a blank using the same water used to prepare the RTS and check for contamination. Make sure that the method is suitable to resolve all peaks. Insufficient resolution can lead to large variations in area measurements. Check background and eluent water quality.
If using a custom-created RTS processing method and component x does not match any peak for channel y, for example, "Component Bromide does not match any peak in channel CD".	Check all the resolutions listed for the default method (see above). Ensure that the RTS Ratio Tolerance in the processing method is not set too low. Ensure that the Area Ratio set under the "Ret. Time" column in the component table is correct.

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