Virtual Column Online

Getting Started Guide

Software Version 1.0 • November 2016

thermoscientific

Document History

Revision: 1.0. This manual is the original manual.

Table of Contents

1 Introduction

1.1 About this Document

Welcome to Virtual Column Online. This guide provides a quick reference to help you get started.

1.2 Virtual Column Online

Virtual Column Online is a web-based tool for simulating ion chromatography analyses. Based on known retention data acquired by Thermo Fisher Scientific as well as IC-specific retention algorithms, Virtual Column Online enables users to model the expected behavior of isocratic and linear gradient separations (see Section 9) for a variety of parameter and equipment combinations. This allows analysts to answer questions such as those listed below, and to determine the most appropriate settings for their intended application:

- What is the best column to use for a particular analysis?
- What eluent should I use for optimum separation of analytes?
- What eluent should I use for the fastest separation of analytes?
- How will changing the temperature of an analysis affect separation?

Note: Virtual Column Online calculates typical results for the selected parameter and equipment combination. Because no two columns or systems are identical, results under actual operating conditions may differ somewhat from those obtained using Virtual Column Online.

1.3 Compatibility

Virtual Column Online has been tested and is compatible with the following Internet browsers:

1. Microsoft™ Internet Explorer™ (version 11) or higher
2. Google™ Chrome™ (version 48) or higher
3. Mozilla™ Firefox™ (version 45) or higher

2 Start Virtual Column Online

2.1 Sign In

Registered users of the AppsLab Library of Analytical Applications can access Virtual Column Online free of charge.

To do so, simply sign in to Virtual Column Online using your existing AppsLab credentials:

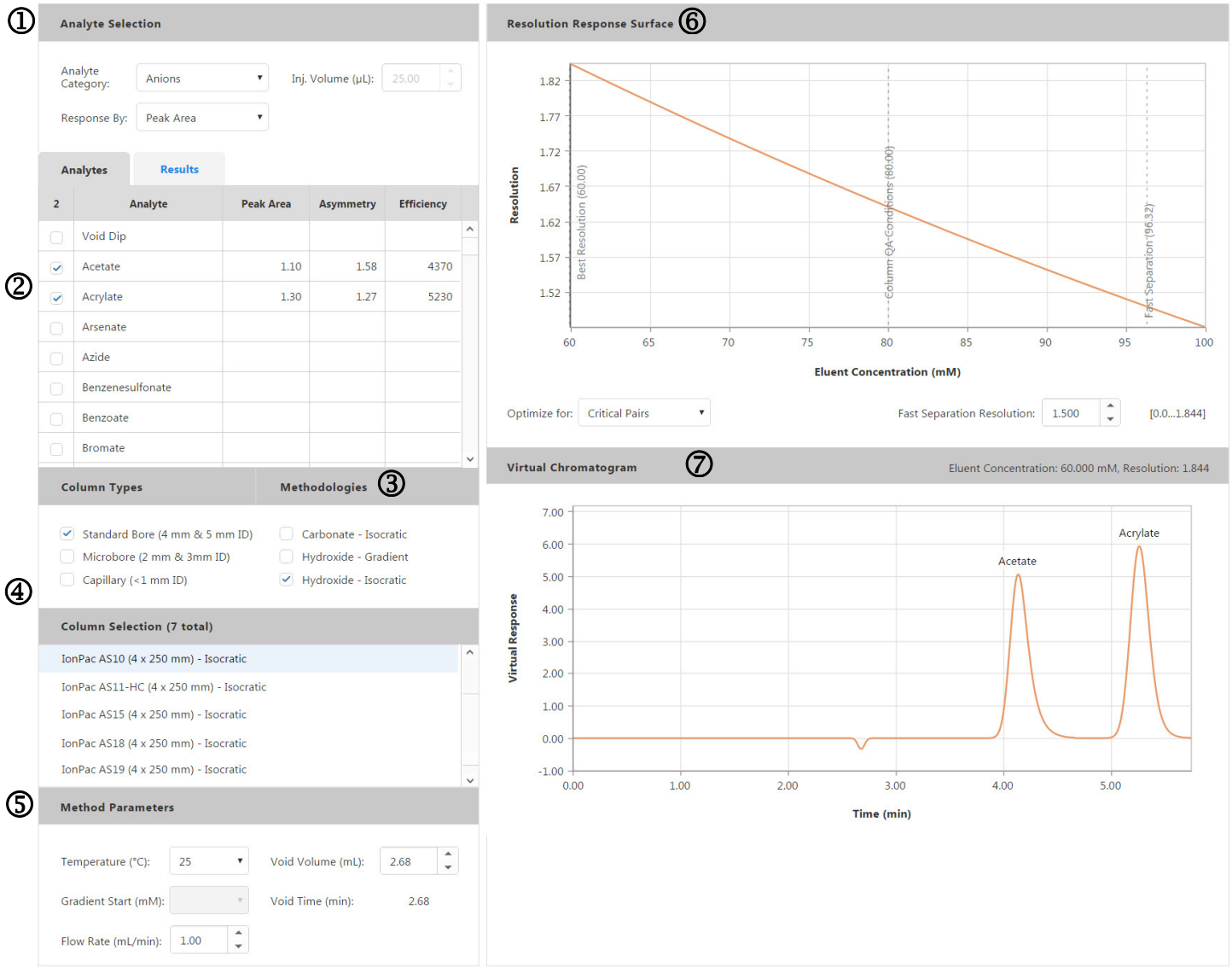
1. Open your Internet browser (see Section 1.3 for supported browsers).
2. Go to https://appslab.thermofisher.com/VirtualColumnOnline to open the welcome page.
3. Enter your **Email** or **Username** and your **Password** on the **Sign In** page.
4. Click **Sign In**.

You can also sign in to Virtual Column Online from within AppsLab:

1. Open your Internet browser (see Section 1.3 for supported browsers).
2. Go to http://www.thermofisher.com/appslab to open the welcome page.
3. Click **Sign In** in the upper right corner of the AppsLab page.
4. Enter your **Email** or **Username** and your **Password** on the **Sign In** page.
5. Click **Sign In**.
6. Click **Virtual Column Online** at the top right of the AppsLab page.

Tip: You can run several simultaneous sessions of Virtual Column Online in your Internet browser.

2.2 General Overview



No.	Explanation
①	Define analyte settings
②	Select two or more analytes
③	Select a methodology (optional)
④	Select a column
⑤	Define method parameters
⑥	View the Resolution Response Surface
⑦	View the Virtual Chromatogram

Tip: You can reset your selections at any time using the **Reset All** icon at the top of the screen (see Section 11.1).

Tip: Virtual Column Online has an inactivity timeout of 60 minutes. If no changes are made during this interval, all settings, such as compounds, column, etc. will be lost after this time.

Figure 1: General Overview

Once the appropriate settings have been made for the required analysis, Virtual Column Online calculates the corresponding retention data.

- The lowest resolution values for each eluent condition are reported on the **Resolution Response Surface** plot (see Section 9).
- Details of the analysis are also shown on the **Virtual Chromatogram** (see Section 10).

3 Define Analyte Settings

3.1 Overview

The **Analyte Selection** section at the top of the screen allows you to select the analyte category, response factor and injection volume.

Figure 2: Analyte Selection

3.2 Analyte Category

The **Analyte Category** drop-down list allows you to choose between the following types of analyte:

- Anions
- Cations
- Carbohydrates

3.3 Response Factor

Use the **Response By** drop-down list to select the response factor to be applied when calculating analyte retention and resolution.

- The default response factor is **Peak Area**.
- You can change this to **Concentration (mg/L)** or **Concentration (mM)** as appropriate.

Note: To determine the optimum eluent condition for peak resolution, you can select a specific resolution criterion using the **Optimize for** drop-down list (see Section 8).

3.4 Injection Volume

You can modify the default injection volume by entering a different value in the **Inj. Volume (µL)** field. This option is available if **Concentration (mg/L)** or **Concentration (mM)** is selected in the **Response By** drop-down list (see Section 3.3).

Note: Under actual operating conditions, peak shapes are affected by changes in the injection volume. However, if you change the injection volume in Virtual Column Online, the peak areas change accordingly, but the peak shapes on the **Virtual Chromatogram** do not. This may affect the actual observed resolution.

3.5 Analytes Table

3.5.1 Analytes for Analysis

In the **Analytes** table, you can select the individual analytes to be considered in the analysis.

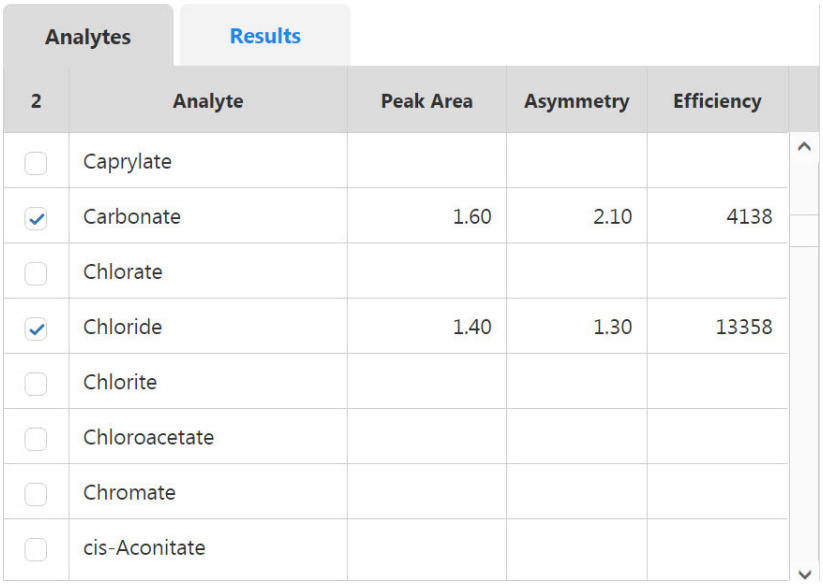


Figure 3: Analytes Table

Note: To display methodologies and columns that match your requirements, you must select two or more analytes.

3.5.2 Retention Data

After you select the required analytes and choose the relevant column and column type (see Section 4), the **Analytes** table displays the following information for each selected analyte:

- **Peak Area**: Available if **Peak Area** is selected in the **Response By** drop-down list (see Section 3.3).
- Concentration in **mg/L** or **mM**: Available if the corresponding concentration option is selected in the **Response By** drop-down list.
- **Asymmetry:** The peak asymmetry to be expected using the current parameters is shown here.
- **Efficiency:** The estimated peak efficiency based on the current parameters is shown here.

The values shown are default values that are derived from retention data acquired by Thermo Fisher Scientific. If you have data specific to your system, you can edit the corresponding values in the table. Virtual Column Online will then use these values in retention and resolution calculations.

Note: You can also calculate retention data for the **Void Dip** by selecting the corresponding check box in the **Analytes** table. If the **Void Dip** check box is not selected, the void dip is displayed on the **Virtual Chromatogram** (see Section 10), but retention and resolution data are not calculated for it.

4 Methodologies

Depending on the selected analyte category (see Section 3.2), you have the option of selecting one or more methodologies from the **Methodologies** list:

Analyte	Methodologies				
	Carbonate – Isocratic	Hydroxide – Isocratic	Hydroxide – Gradient	MSA – Isocratic	MSA – Gradient
Anion	✔	✔	✔	--	--
Cation	--	--	--	✔	✔
Carbohydrate	--	✔	✔	--	--

Table 1: Analyte Types and Methodologies

5 Columns

5.1 Column Types

In the **Column Types** list, you have the option of choosing one or more column types with an appropriate inner diameter for your application as follows:

- Standard bore
- Microbore
- Capillary

5.2 Column Selection

You must select an appropriate column from the **Column Selection** list.

Note: Columns for gradient applications are only listed if a gradient methodology (see Section 4) is selected.

Virtual Column Online now calculates retention and resolution data based on the parameters selected so far, and displays one or more corresponding **Resolution Response Surface** plots (see Section 9), as well as a **Virtual Chromatogram** (see Section 10).

6 Method Parameters

The **Method Parameters** section at the bottom of the screen allows you to select the relevant parameters for your analysis:

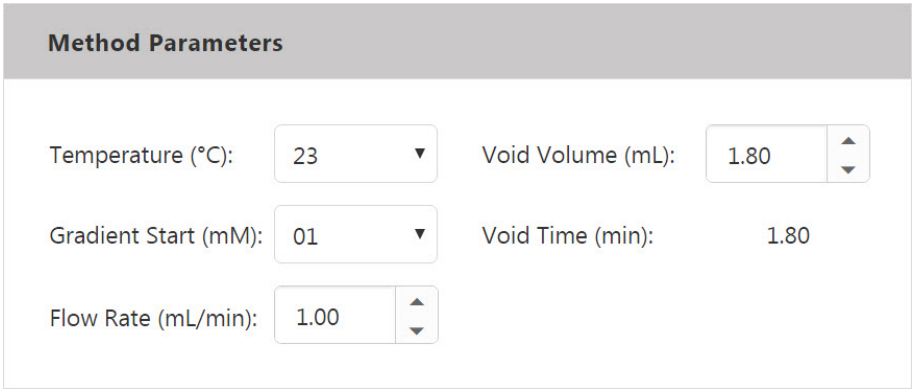


Figure 4: Method Parameters

The available parameters are:

- Temperature
- Gradient start (if applicable)
- Flow rate
- Void volume

6.1 Temperature

You can change the temperature for supported columns in the **Temperature** drop-down list. The results at different temperatures can be seen in the **Results** table (see Section 7), on the **Resolution Response Surface** (see Section 9), and on the **Virtual Chromatogram** (see Section 10).

Tip: The default value shown is the lowest applicable temperature for the selected column.

6.2 Gradient Start

When you select a column for a gradient methodology, a default value for the eluent concentration at the beginning of the gradient is displayed. You can change this setting in the **Gradient Start** drop-down list and view the results at different concentrations on the **Resolution Response Surface**. The **Virtual Chromatogram** and the **Results** table are also updated accordingly. For more information about gradient analyses, see Section 9.3.

Tip: This option is only applicable for gradient methodologies.

6.3 Flow Rate, Void Volume, and Void Time

Once you have selected the required analytes and chosen a column, Virtual Column Online calculates a default flow rate, void volume (or dead volume), and void time (or dead time) for the selected column.

- You can change the flow rate and/or void volume values in the fields provided so that your specific system is more accurately modeled on the **Resolution Response Surface**, on the **Virtual Chromatogram**, and in the **Results** table.
- The void time is calculated automatically based on the void volume and flow rate.

Note: Under actual operating conditions, peak shapes are affected by changes in the flow rate. However, if you change the flow rate in Virtual Column Online, the peak shapes on the **Virtual Chromatogram** are not changed. This may affect the actual observed resolution.

7 Results Table

Once you have selected the required analytes and chosen a column, the calculated results for each analyte are shown in the **Results** table.

Analytes | Results

Analyte	Ret. Time	Ret. Factor	Resolution
Fluoride	3.09	0.19	4.45
Chloride	5.17	0.99	3.18
Nitrite	6.65	1.56	2.73
Carbonate	8.52	2.28	**1.59***
Bromide	10.26	2.95	1.59
Sulfate	11.25	3.33	1.94
Nitrate	12.58	3.84	

Figure 5: Results Table

Results are calculated based on the analyte data (peak area or concentration, asymmetry, and efficiency) and on the selected column, temperature, void time, and eluent conditions. The analytes are listed in order of increasing retention time.

- **Ret. Time**: The time (in minutes) since injection.
- **Ret. Factor**: Also known as capacity factor, this is the ratio of the net retention time to the void time (also called dead time).
- **Resolution**: The degree of separation between the current peak and the next peak in the **Virtual Chromatogram**.
- **Peak Area**: The expected peak area for the respective concentration entered in the **Analytes** table (see Section 3.3) is shown if concentration in **mg/L** or **mM** is selected in the **Response By** drop-down list.

Note: Critical pairs (see Section 8.1.2) are indicated in the **Results** table by an asterisk and bold formatting. The asterisk is always assigned to the first analyte in the critical pair.

8 Resolution Criteria

8.1 Optimize For

8.1.1 Overview

To determine the optimum eluent condition for peak resolution, select an appropriate resolution criterion from the **Optimize for** drop-down list beneath the interactive **Resolution Response Surface**.

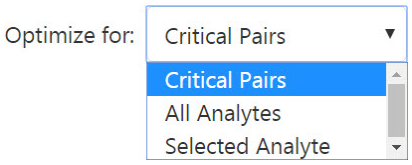


Figure 6: Resolution Criteria

The following resolution criteria are available: **Critical Pairs**, **All Analytes**, **Selected Analyte**.

Virtual Column Online adjusts the information provided in the **Results** table (see Section 10), on the **Resolution Response Surface** (see Section 9), and on the **Virtual Chromatogram** (see Section 10) in accordance with the selected resolution criterion.

Note: In the case of isocratic modeling, the **Column QA Conditions** (see Section 9.1.4) are also automatically calculated based on the selected resolution criterion and displayed on the **Resolution Response Surface** plot.

8.1.2 Critical Pairs

Critical Pairs is the default resolution criterion. When this option is selected, Virtual Column Online detects the least resolved peak pair for the selected eluent condition, thus optimizing the resolution of peak pairs that are hard to separate. This is useful when performing difficult separations.

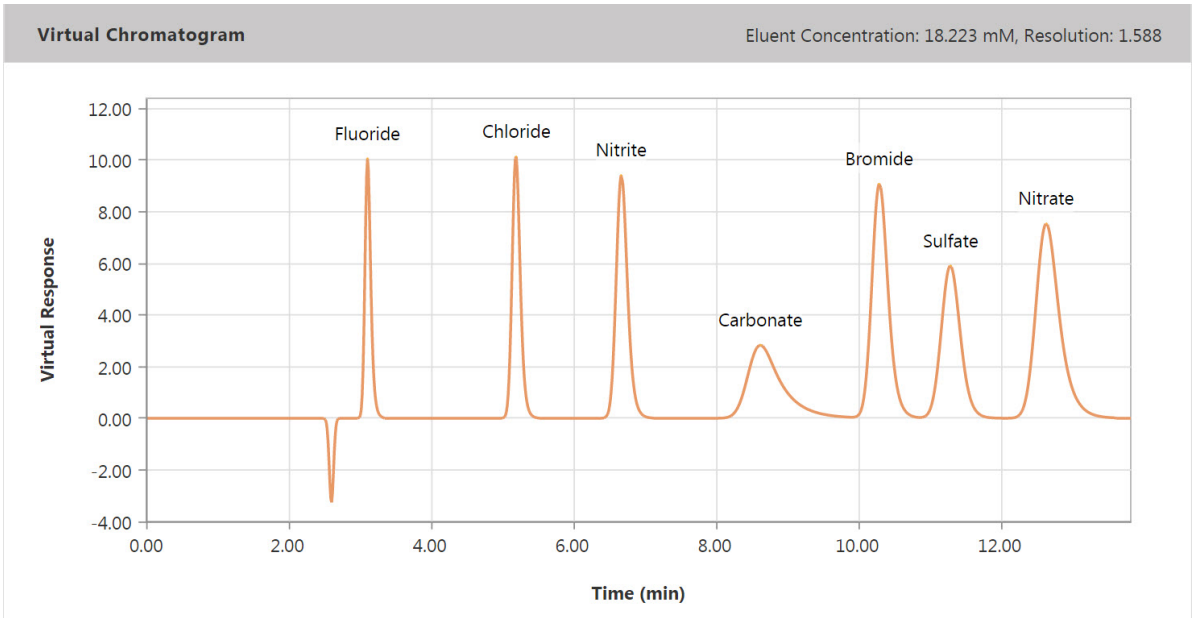


Figure 7: Critical Pairs (Resolution of 1.588)

In general, a resolution value of at least 1.5 (peak areas overlap by less than 0.2%) is regarded as a good baseline separation. For many applications, a value of 1.2 (peak areas overlap by less than 2%) is considered an acceptable separation. A value of 0 indicates that at least two peaks are eluting at the same retention time.

Analytes | Results

Analyte	Ret. Time	Ret. Factor	Resolution
Fluoride	3.09	0.19	4.45
Chloride	5.17	0.99	3.18
Nitrite	6.65	1.56	2.73
Carbonate	8.52	2.28	**1.59***
Bromide	10.26	2.95	1.59
Sulfate	11.25	3.33	1.94
Nitrate	12.58	3.84	

Figure 8: Results Table (Critical Pairs Shown in Bold)

Note: Critical pairs are indicated in the **Results** table by an asterisk and bold formatting. The asterisk is always assigned to the first analyte in the critical pair.

8.1.3 All Analytes

When **All Analytes** is selected, Virtual Column Online calculates the eluent condition that provides the most evenly resolved peaks across the entire **Virtual Chromatogram**.

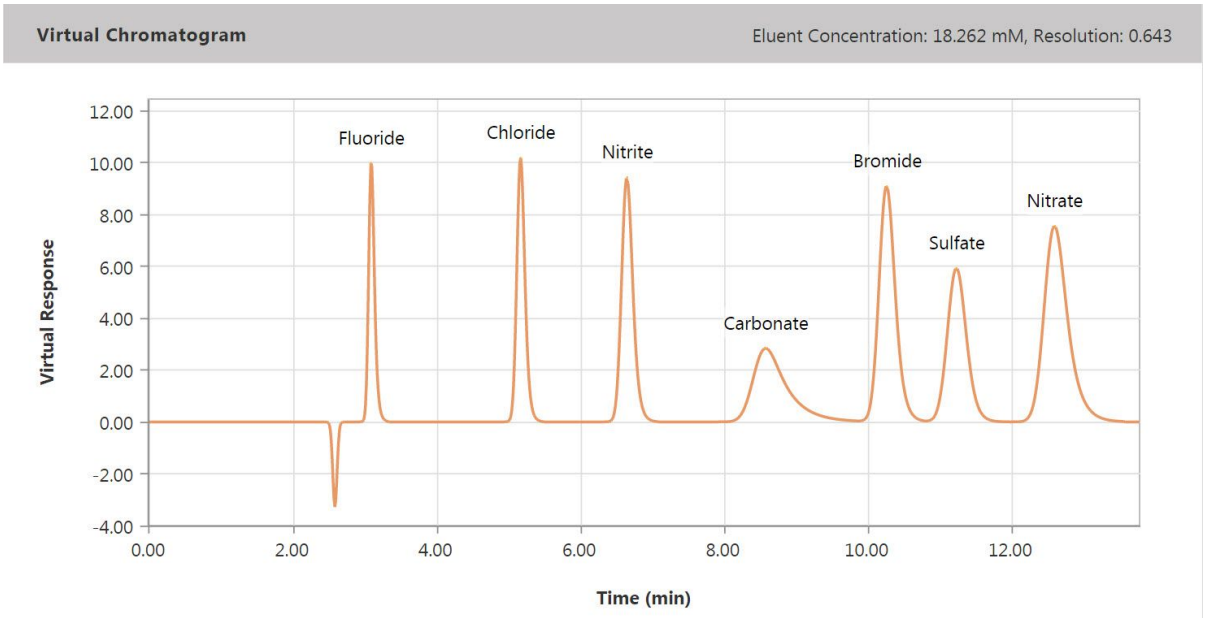


Figure 9: All Analytes (Normalized Resolution for All Peaks)

A normalized resolution product value of 1 indicates that all peaks are evenly resolved across the **Virtual Chromatogram**. A value of 0 indicates that at least one peak pair is co-eluting.

The normalized resolution product (r) is defined by the following equation:

$$r = \prod_{i=1}^{n-1} \left(\frac{R_{s_{i,i+1}}}{\frac{1}{n-1} \sum_{i=1}^{n-1} R_{s_{i,i+1}}} \right)$$

Figure 10: Normalized Resolution Equation

Here, n is the number of peaks and $R_{s_{i,i+1}}$ is the resolution of peaks *i* and *i*+1.

Note: All Analytes is useful for easy separations, as it optimizes the resolution of all peak pairs (see Figure 9). However, for more difficult separations, such as those involving peaks that are not well resolved or in the case of large numbers of peaks, **All Analytes** may generate a **Virtual Chromatogram** with evenly resolved peaks, but not all of these peaks will necessarily be resolved.

8.1.4 Selected Analyte

With **Selected Analyte**, Virtual Column Online calculates the eluent condition that optimizes the resolution for the selected analyte. The resolution of the other peaks is not taken into consideration. **Selected Analyte** is useful if resolving a particular analyte peak is more critical than resolving all other peak pairs.

Note: Choosing the **Selected Analyte** option displays another drop-down list, from which you can select the analyte of interest.

In the example below, chloride is the selected analyte (also indicated by the blue peak label).

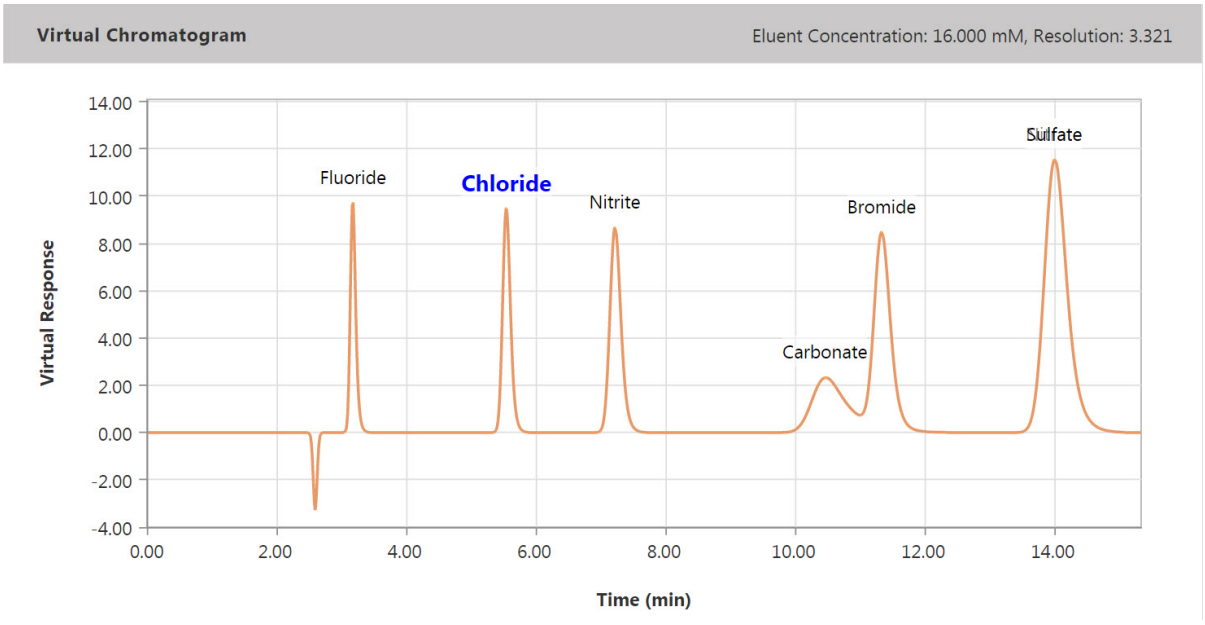


Figure 11: Selected Analyte

Notice that with this option, the carbonate/bromide and nitrate/sulfate peaks are not resolved.

9 Resolution Response Surface

9.1 General Features

9.1.1 Overview

The **Resolution Response Surface** is available for isocratic and gradient separations. It is comprised of up to two dynamic plots showing the lowest resolution values found at each possible eluent condition. Specific features of the plot may vary depending on which resolution criterion is selected (see Section 8), whether isocratic or gradient separation is chosen, and on whether the eluent is single- or dual-species.

9.1.2 Best Resolution

Regardless of which resolution criterion you select (see Section 8), Virtual Column Online automatically calculates the **Best Resolution** for your settings and indicates this on the **Resolution Response Surface** plot using a corresponding label.

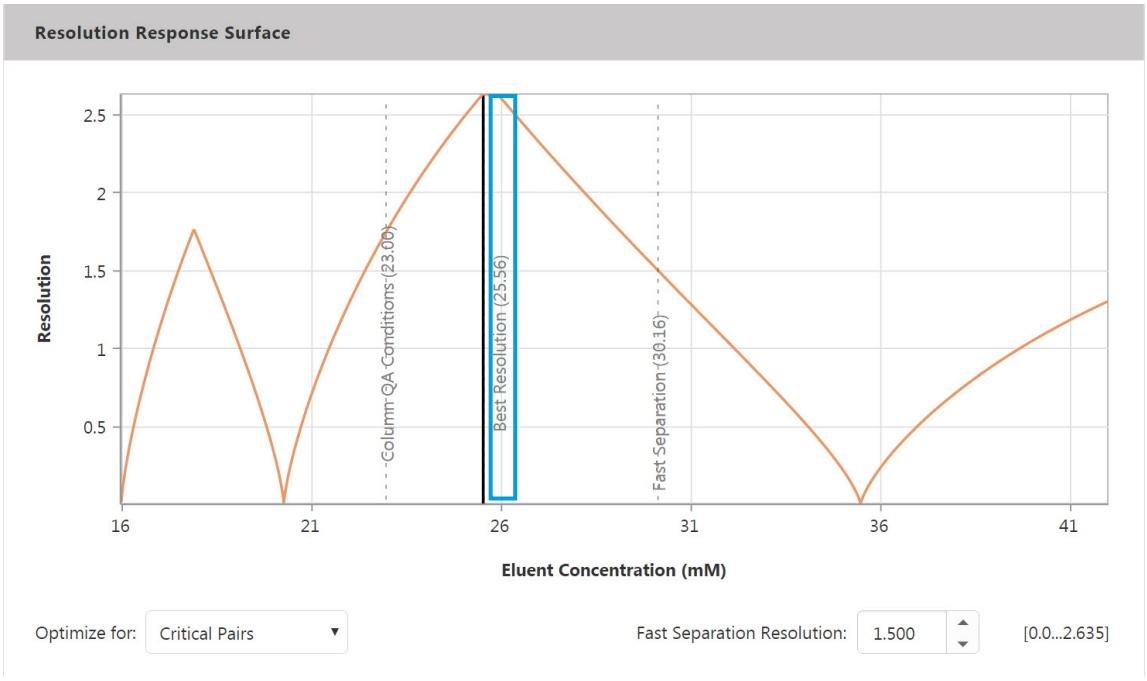


Figure 12: Isocratic Separation (Best Resolution)

The best resolution label indicates the eluent condition that offers the best resolution regardless of separation speed. The example above shows the result when an AS18 column is selected for an isocratic separation.

9.1.3 Fast Separation Resolution

Virtual Column Online also automatically calculates the **Fast Separation Resolution** and indicates this on the **Resolution Response Surface** plot using a dotted vertical line as well as a corresponding label.

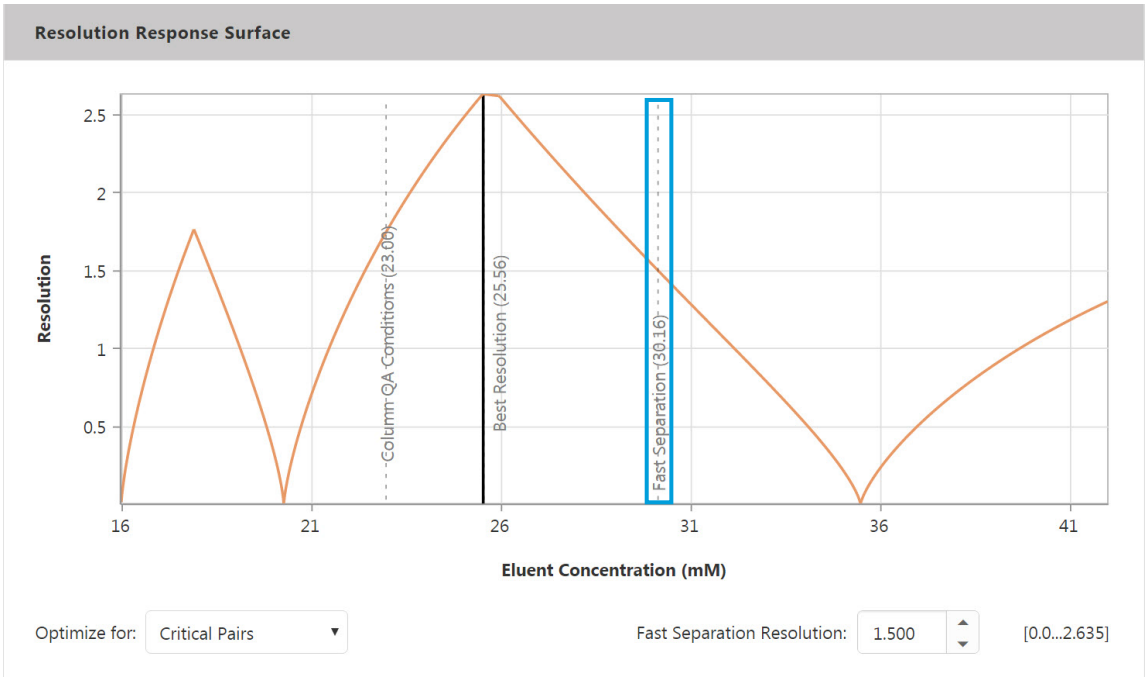


Figure 13: Isocratic Separation (Fastest Separation)

Fast Separation Resolution is optimized for separation speed rather than for peak resolution. In other words, Virtual Column Online calculates the eluent condition that offers the fastest separation at the minimum acceptable resolution.

In the example in Figure 13, resolution is calculated using the **Critical Pairs** criterion with a minimum acceptable resolution of 1.5. To change the minimum acceptable resolution, enter a new value in the **Fast Separation Resolution** field.

9.1.4 Column QA Conditions

When isocratic modeling is selected, Virtual Column Online automatically calculates the relevant column QA conditions based on the eluent composition specified during production control testing of the selected Thermo Scientific column. These are indicated on the **Resolution Response Surface** plot by a dotted vertical line as well as a corresponding label.

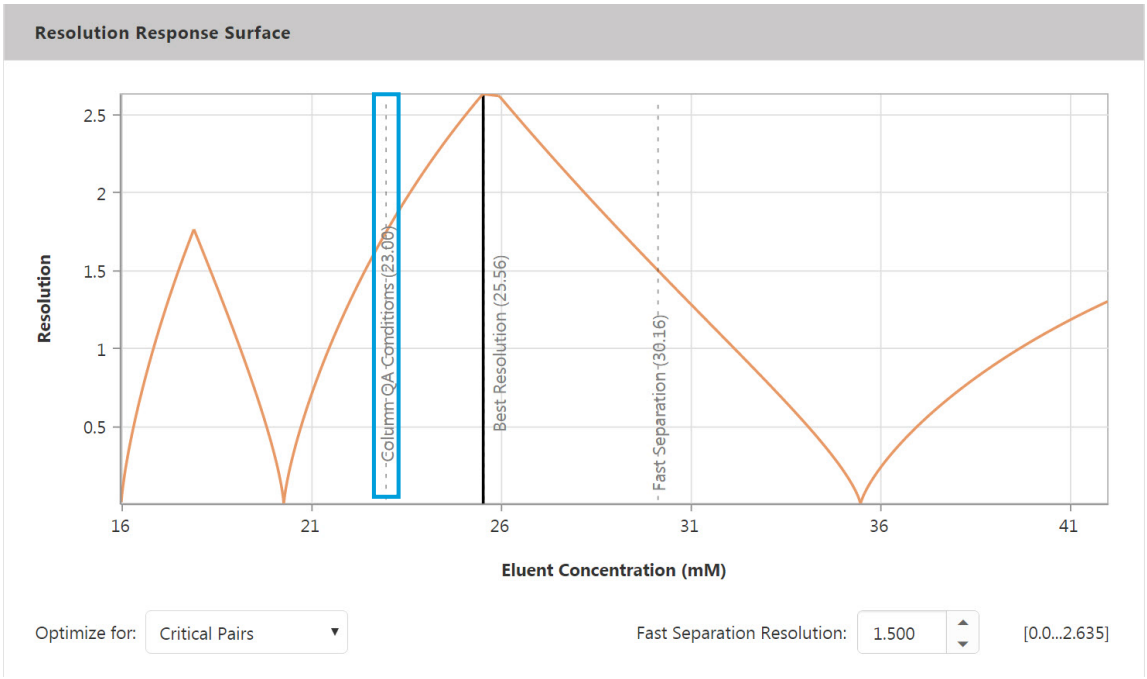


Figure 14: Isocratic Separation (Column QA Conditions)

The **Column QA Conditions** indicate the optimum eluent concentration, based on the current analyte, column, void time, temperature, and resolution criterion.

Note: The column QA conditions do not have any impact on analyte selection. In other words, even if the relevant column QA conditions include analytes that the user has not selected, these analytes are not added to the **Virtual Chromatogram**.

For information on production control tests, refer to the relevant column manual.

9.2 Isocratic Separation

9.2.1 Single-Species Eluents

When a single-species eluent is used, one **Resolution Response Surface** plot is shown where the resolution is plotted over eluent concentration. In the example below, the selected resolution criterion is **Critical Pairs**.

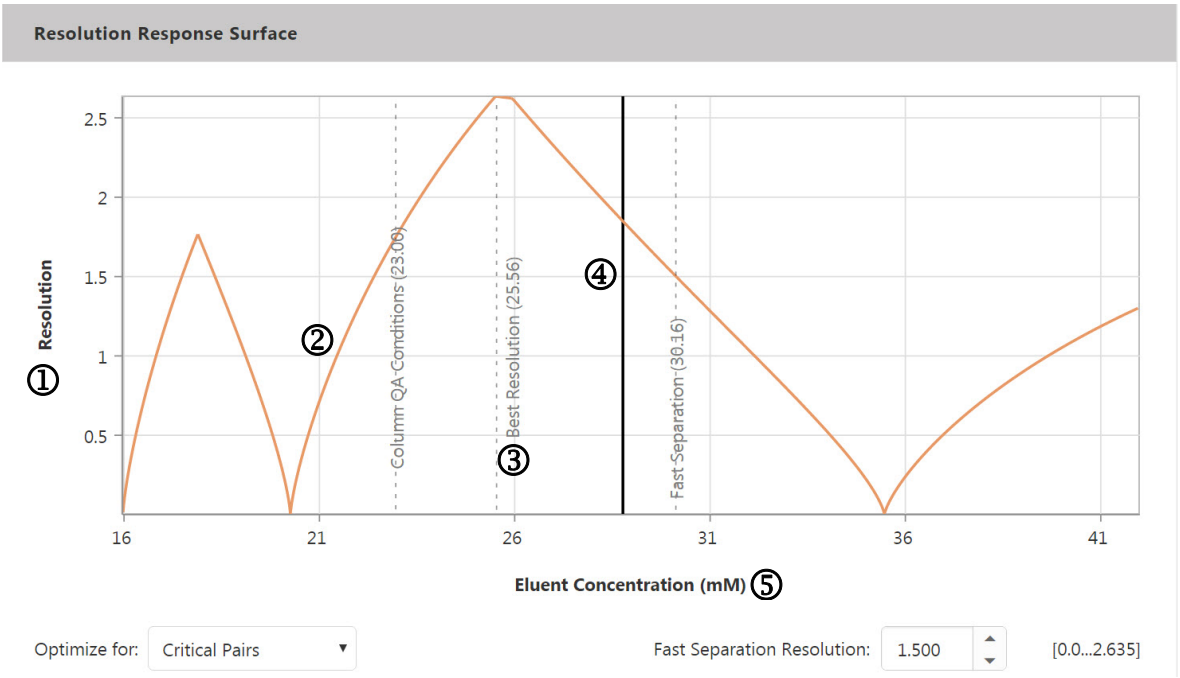


Figure 15: Eluent Concentration

No.	Explanation
①	Resolution range of the least resolved peak pairs on the **Virtual Chromatogram**
②	Plot of the lowest resolution value found at each eluent concentration
③	Optimum eluent concentration, based on the current analyte, column, void time, temperature, and resolution criterion selection
④	Currently selected eluent concentration (the concentration at which the **Virtual Chromatogram** is modeled)
⑤	Concentration of the eluent used across the **Resolution Response Surface**

9.2.2 Dual-Species Eluents

When a dual-species eluent is used, two **Resolution Response Surface** plots are displayed. The plot on the left shows the resolution plotted over the total eluent concentration, while the plot on the right shows the resolution plotted over the percentage of carbonate in the eluent. In the example below, the selected resolution criterion is **Critical Pairs**.

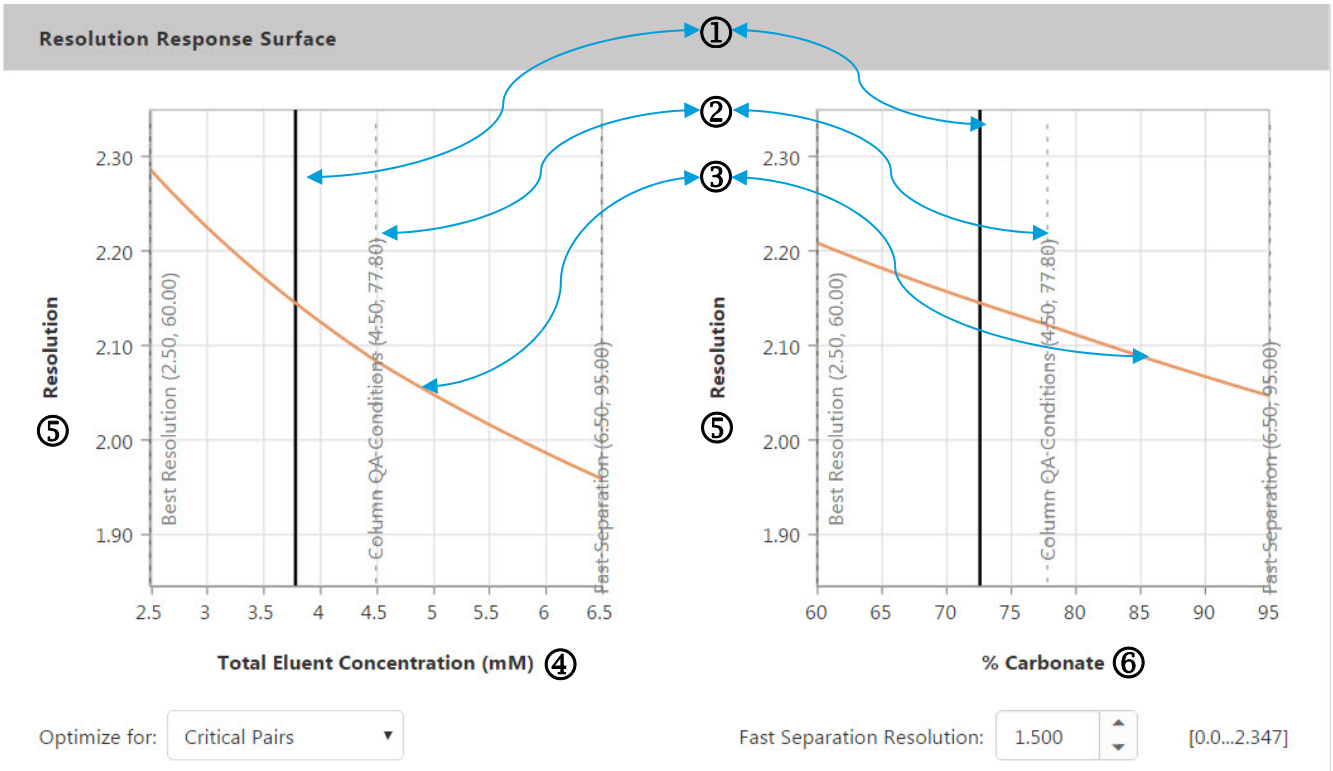


Figure 16: Dual-Species Eluents

No.	Explanation
①	Currently selected eluent concentration (the concentration at which the **Virtual Chromatogram** is modeled)
②	Optimum eluent concentration, based on the current analyte, column, void time, temperature, and resolution criterion selection
③	Plot of the lowest resolution value found at each eluent concentration
④	Concentration of the eluent used across the **Resolution Response Surface**
⑤	Resolution range of the least resolved peak pairs on the **Virtual Chromatogram**
⑥	Percentage of carbonate in the eluent

Where two **Resolution Response Surface** plots are shown, selecting x in the left chart (total eluent concentration) will influence the right chart (% Carbonate) and vice versa. In other words, resolution and the Virtual Chromatogram are determined by the selection of x in both charts.

9.2.3 Eluent Concentration

Adjusting Eluent Concentration

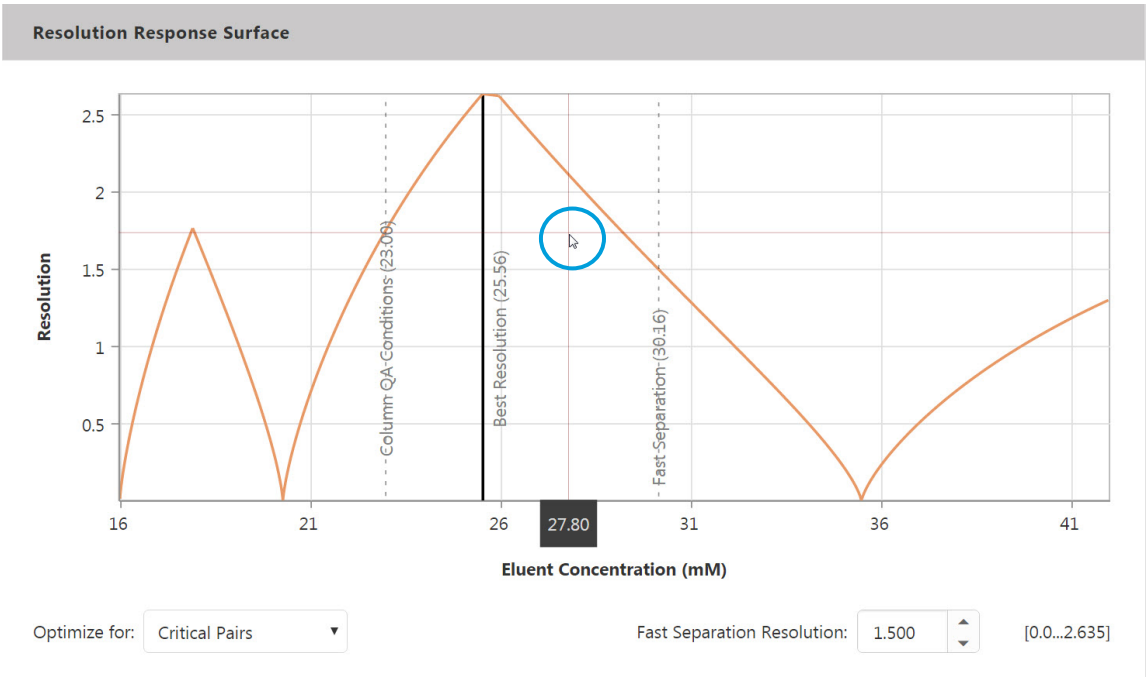


Figure 17: Adjusting Eluent Concentration

To manually select a desired eluent concentration for an isocratic separation:

- Move the mouse pointer over the **Resolution Response Surface**. A crosshair is displayed indicating the current location of the mouse pointer on the plot.
- Click on the **Resolution Response Surface** at the desired eluent concentration.

  The black vertical bar indicating the selected eluent concentration is repositioned accordingly and the **Virtual Chromatogram** is updated to reflect the new concentration.

Note: If a gradient methodology is selected, moving the vertical bar adjusts the gradient slope rather than the eluent concentration, see Section 9.3.

Effect on Separation Speed

In the examples for isocratic separation provided below, the run time of the **Virtual Chromatogram** is reduced from 10.67 to 9.75 minutes if the eluent concentration is adjusted from 25.555 to 28.990 mM.

Example A **Example B**

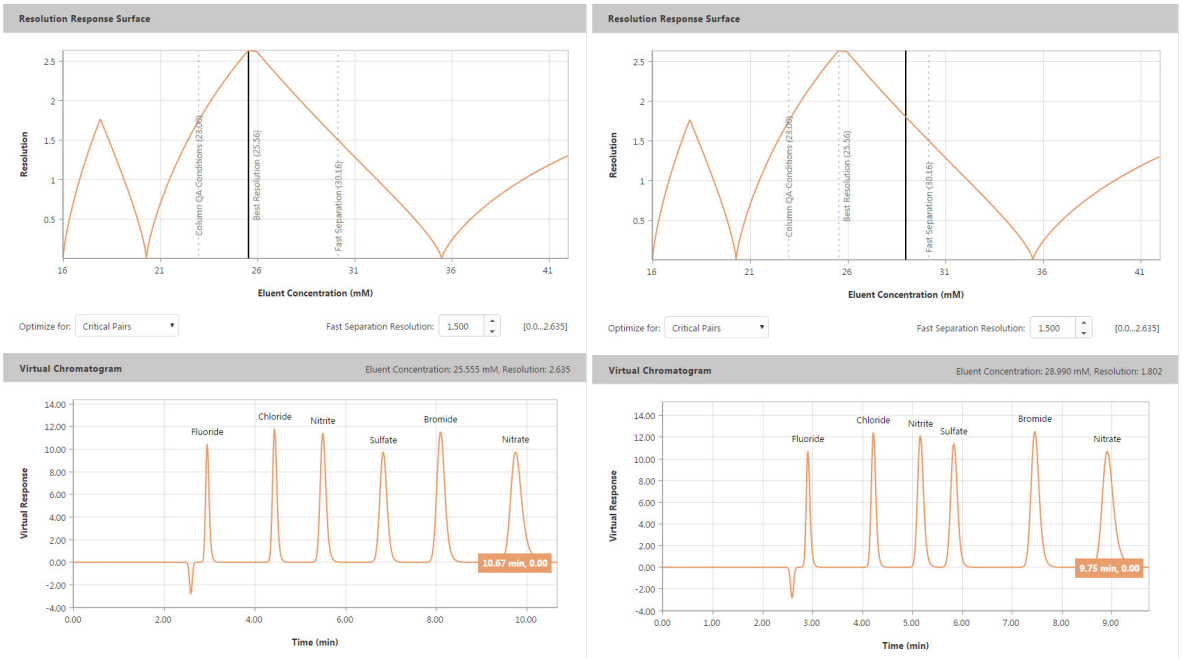


Figure 18: Resolution Response Surface (Comparison of Separation Speeds)

9.3 Gradient Separation

9.3.1 Overview

The gradient predictions provided by Virtual Column Online are based on gradient data acquired under conditions equivalent to zero delay time. Accordingly, the composition of the mobile phase remains constant until the sample enters the column, thus eliminating any effects of isocratic elution of the sample before the gradient ramp begins.

9.3.2 Resolution Response Surface

When a gradient separation is selected, one **Resolution Response Surface** plot is shown where the resolution is plotted over the gradient slope. In the example below, the selected resolution criterion is **Critical Pairs**.

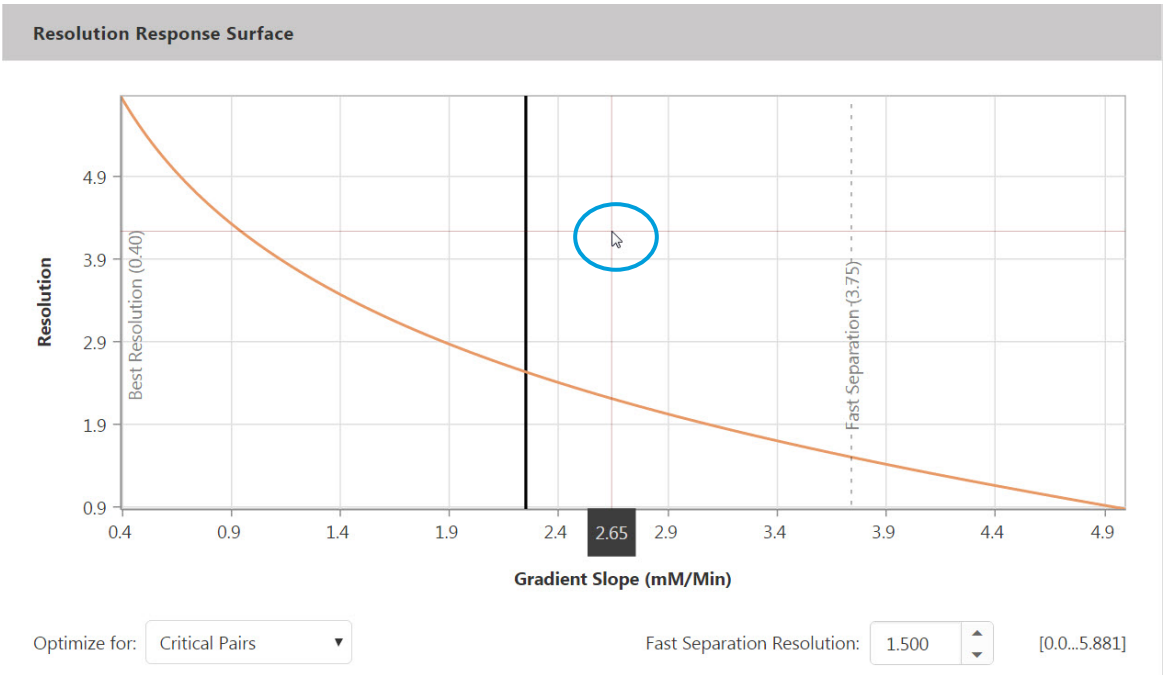


Figure 19: Gradient Separation (Critical Pairs)

To manually adjust the gradient slope for a gradient separation:

- Move the mouse pointer over the **Resolution Response Surface**. A crosshair is displayed indicating the current location of the mouse pointer on the plot.
- Click on the **Resolution Response Surface** at the desired gradient value.

  A black vertical bar indicating the selected gradient value is positioned accordingly and the **Virtual Chromatogram** is updated to reflect the new gradient slope.

Note: If an isocratic methodology is selected, moving the vertical bar adjusts the eluent concentration rather than the gradient slope, see Section 9.2.3.

Note: If you try to reproduce results obtained in Virtual Column Online for a gradient separation under actual operating conditions, remember that actual results may include a delay time that is not taken into consideration in Virtual Column Online. This is only likely to be of relevance where high starting concentrations are used in conjunction with low gradient slopes. In such cases, monovalent species with short run times may show a shift in retention time compared to other species.

10 Virtual Chromatogram

10.1 Overview

The **Virtual Chromatogram** simulates an actual analysis using the currently selected analytes, column, void time, temperature, resolution criterion, and eluent condition. The **Virtual Chromatogram** is updated whenever the selected analytes or other parameters are changed. In the example below, the **Critical Pairs** resolution criterion (see Section 8) is selected.

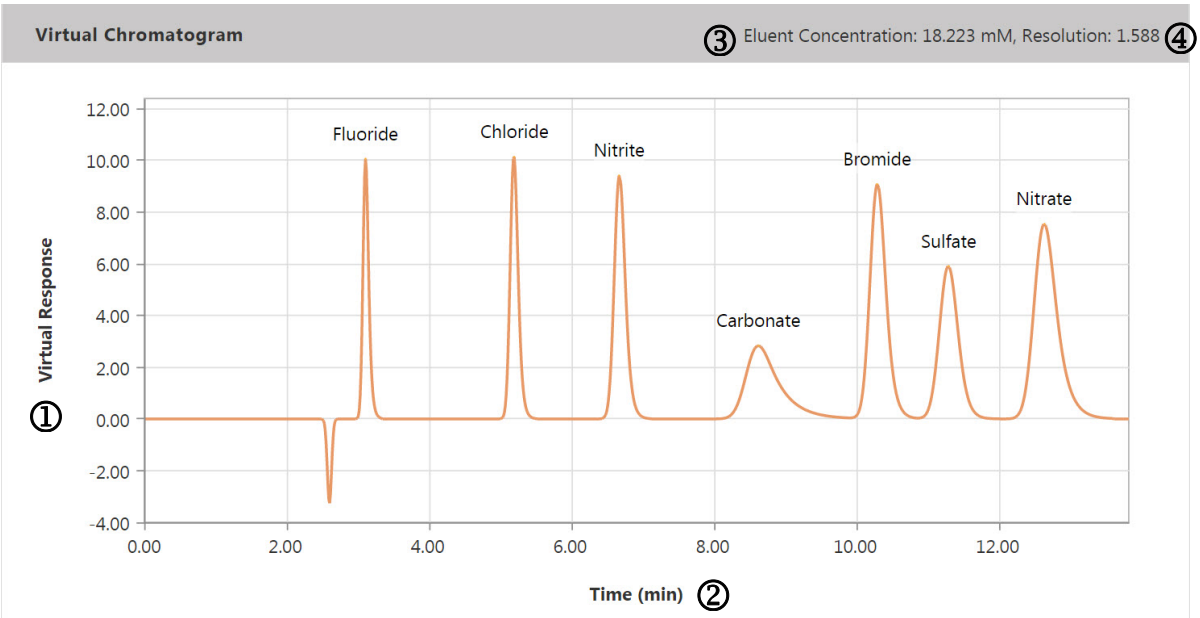


Figure 20: Virtual Chromatogram (Critical Pairs)

No.	Explanation
①	Range of response values on the current **Virtual Chromatogram**
②	Retention timescale of the current **Virtual Chromatogram**
③	Concentration of the currently selected eluent
④	Resolution of the least resolved peak pair on the current **Virtual Chromatogram**

10.2 Zooming/Unzooming

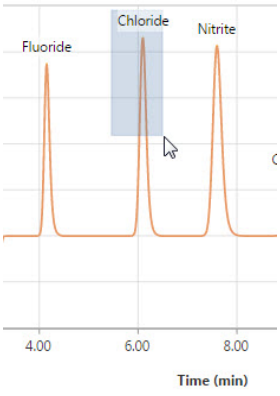


Figure 21: Virtual Chromatogram (Zooming)

- To zoom into an area of the **Virtual Chromatogram**, select the area of interest by forming a box around it using the mouse pointer.

Note: Zooming is also possible by moving the mouse inside the plot area and using the mouse wheel.

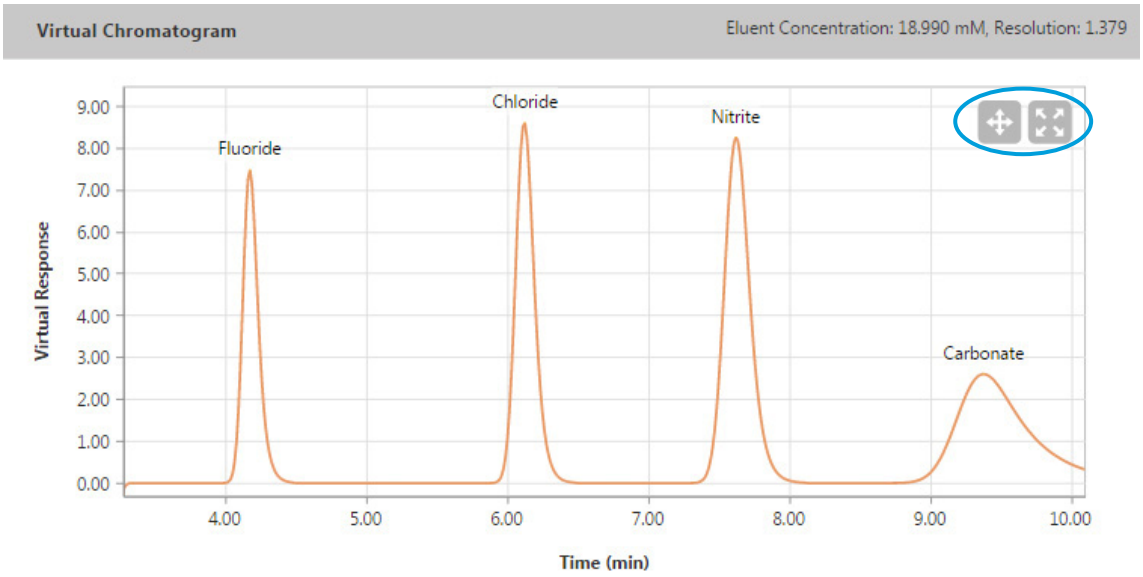


Figure 22: Virtual Chromatogram (Panning and Reset Icons)

- To pan across the area of interest, click the (**Pan**) button now shown and use the mouse pointer to drag the **Virtual Chromatogram** until the relevant area is shown.
- To display the full **Virtual Chromatogram** again, click the (**Reset**) button.

10.3 Gradient Profile

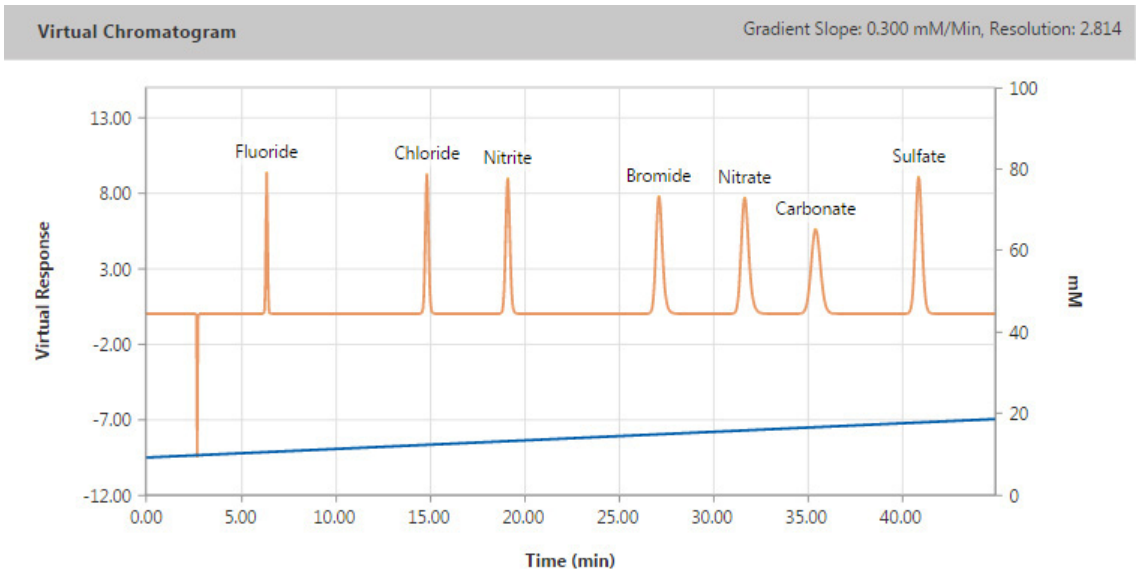


Figure 23: Gradient Profile

When gradient modeling is selected, a gradient profile (shown in blue) is automatically overlaid over the peaks (shown in red) on the **Virtual Chromatogram**.

11 Application Icons

Application icons are provided at the top left of the screen that offer additional functions.

11.1 Reset All

You can reset your selections at any time using the **Reset All** icon.

Figure 24: Reset All Icon

11.2 Eluent Preparation

When isocratic modeling is selected, Virtual Column Online can provide detailed instructions for preparing eluents in accordance with the currently selected conditions.

1. Click the **Eluent Preparation** icon.

Figure 25: Eluent Preparation Icon

2. On the **Eluent Preparation** page, click the required **Eluent Species** to display the appropriate eluent concentration as well as the best resolution in each case. Additional information may also be provided.
3. Click **Print** to print the contents of the **Eluent Preparation** page.
4. Click **Close** to exit the page.

11.3 Print PDF

Using the **Print PDF** icon, you can generate an export PDF that contains details of the analysis that you have simulated.

Figure 26: Print Icon

These details include:

- Analytes and analyte selection settings
- Method parameters
- Analysis results
- The **Resolution Response Surface**
- The **Virtual Chromatogram**

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