



# **Dionex Column Selector Demo**

## **Version 0.93 for Windows<sup>®</sup>**

### **Getting Started Guide**

© 2001 - 2002 Dionex Corporation

# Introduction

Welcome to the Dionex Column Selector, the simulation software for Ion Chromatographic retention modeling and optimization. Dionex Column Selector is a powerful application that uses previously acquired (embedded) retention data to simulate all possible chromatograms in a defined search area. Dionex Column Selector determines the optimal eluent composition using a choice of two resolution criteria, Minimum Resolution or Normalized Resolution Product.

Dionex Column Selector is designed with the needs of a range of users in mind. It is intended for practical method development, educational and training purposes, refinement of existing methods and exploratory investigations including information gathering and experimental design overview.

Dionex Column Selector uses the most advanced retention prediction models available today to achieve accurate prediction of chromatograms. Embedded data, which have been acquired using a correct experimental design, are used to solve these models.

Dionex Column Selector uses these retention prediction algorithms to predict retention data across the entire search area. Dionex Column Selector ranks these virtual chromatograms according to two criteria, the Minimum Resolution or Normalized Resolution Product. A value of zero indicates that at least one peak pair is co-eluting. For the Minimum Resolution criterion, a value of 1.5 is generally regarded as baseline separation ( $< 0.2\%$  peak area overlap), although a value of 1.2 is often considered acceptable resolution for most applications ( $< 2\%$  peak area overlap). For the Normalized Resolution Product, a value of one indicates that all peaks are evenly resolved across the chromatogram.

Detailed information about the Retention Models and Resolution Algorithms can be found in Appendix A.

Dionex Column Selector has two options for finding the optimum eluent conditions, separation or time. A global optimum is defined as the chromatogram with a maximum value for the selected resolution criterion. Dionex Column Selector can also find the fastest chromatogram for which the value of the resolution criterion does

not fall below a specified value. This capability is designed to optimize the speed of a separation, rather than the resolution of peaks.

Dionex Column Selector allows the user to customize a simulation or optimization to match their own system through the adjustment of several key parameters including column, eluent and analyte selection, peak areas, peak shapes (asymmetry), void time and column efficiency (number of theoretical plates). Virtually any system can be simulated through the adjustment of these parameters.

Remember that no two columns are identical and the results obtained using the embedded databases in Dionex Column Selector are only considered typical of a particular column type. Results obtained may not necessarily match your exact column, but every effort has been made to attain as close a match as possible.

### **Getting Started contains the following parts:**

- Chapter 1, “Installing Dionex Column Selector,” contains information on installation procedures, system requirements, starting, uninstalling and upgrading Dionex Column Selector.
- Chapter 2, “Learning Dionex Column Selector. Tutorial 1 – The Wizard,” introduces the Dionex Column Selector wizard.
- Chapter 3, “Learning Dionex Column Selector. Tutorial 2 – Dual Species Eluent Optimization,” introduces the main interface for Dionex Column Selector and guides the user through the features of Dionex Column Selector that apply to dual species eluent systems, in particular, carbonate eluents.
- Chapter 4, “Learning Dionex Column Selector. Tutorial 3 – Single Species Eluent Optimization,” introduces the main interface for Dionex Column Selector and guides the user through the features of Dionex Column Selector that apply to single species eluent system, in particular, hydroxide eluents.
- Chapter 5, “Glossary,” explains some of the terms used in this manual.
- Appendix A, “Retention Models and Resolution Algorithms,” gives detailed information about the models and algorithms used in Dionex Column Selector.

# Chapter 1

## Installing Dionex Column Selector

### 1.1. Introduction

This chapter contains information on the following:

- system and software requirements.
- installation instructions.
- starting Dionex Column Selector.
- uninstallation instructions.

### 1.2. System Recommendations

#### Operating System Software

Dionex Column Selector is a 32-bit Windows<sup>#</sup> application. To run Dionex Column Selector you must have a 32-bit version of Windows such as Windows 95, 98, Me, NT 4.0, 2000 or XP. Dionex Column Selector will not run on earlier versions of Windows such as Windows 3.1, 3.11, NT 3.5 or NT 3.51.

#### Computer Hardware

Dionex Column Selector is compatible with Intel\* Pentium® class processors or higher, including Pentium Pro, Pentium II, Pentium III, Pentium 4, AMD† K6, Athlon®, Duron® and compatible processors.

#### Math Coprocessor

Dionex Column Selector is very floating point intensive and is therefore not compatible with processors that do not have a math coprocessor such as 386 and 486 SX processors.

---

<sup>#</sup> Windows is a registered trademark of the Microsoft Corporation

<sup>\*</sup> Intel is a registered trademark of the Intel Corporation

<sup>†</sup> AMD is a registered trademark of Advanced Micro Devices

## **Memory**

Dionex Column Selector is compatible with any memory size that runs your version of Windows. However for increased performance we recommend at least 32 MB of RAM for Windows 95 or Windows 98 and 64 MB of RAM for other Windows versions. For large numbers of analytes more memory may be required for acceptable performance.

## **Hard Drive Storage**

Dionex Column Selector consumes less than 10 MB of hard drive space.

## **Display**

Dionex Column Selector requires a minimum resolution of 800 x 600 and 256-color depth. However we strongly recommend a display capable of 1024 x 768 or higher resolution and at least 16-bit color.

## **1.2. Installing Dionex Column Selector**

The Dionex Column Selector installation program will decompress files and install them to the correct directories on your computer hard drive. The Dionex Column Selector installation program will also set up shortcuts in your start menu.

If Dionex Column Selector came on the Dionex Manuals and Literature CD-ROM click on the Virtual Column link and choose 'Run this program from its current location'. If you downloaded Dionex Column Selector as a self-extracting zip file, then it will be necessary to run the Dionex Column Selector Install.exe file. Click yes on the security warning and choose a directory to decompress the files to. The installer will automatically run the setup.exe file and install the application to your hard drive.

## **1.3. Starting Dionex Column Selector**

The following is only applicable if Dionex Column Selector has been installed correctly on your system.

- Position the mouse over the Start button of your task bar and left click on "Start".
- Position the mouse cursor over "Programs" and either left click or wait for the programs menu to open.

- Position the mouse over “Dionex Column Selector” and either left click or wait for the Dionex Column Selector menu to open.
- Left click on “Dionex Column Selector” to start the application.

#### **1.4. Uninstalling Dionex Column Selector**

The following is only applicable if Dionex Column Selector has been installed correctly on your system. Some minor variations may be noted with different versions of Windows. Please contact your computer support staff if the following does not apply to your system.

- Open the Control Panels by either navigating to the “Settings” menu of the Start menu, or by opening the “My Computer” window and opening Control Panels.
- Open the “Add/Remove Programs” control panel to show a list of all applications currently installed on your computer. Highlight “Dionex Column Selector” and click on “Change/Remove”.
- Follow the on screen instructions to remove Dionex Column Selector from your system. It is usually safe to remove all files from your system, but if in doubt, contact your local administrator or IS support staff.

## **Chapter 2**

# **Learning Dionex Column Selector. Tutorial 1 – The Wizard**

### **2.1. Introduction**

In order to run, Dionex Column Selector requires detailed information about the system it is trying to model. The Dionex Column Selector Data Editor is the part of the application designed to gather this information in as simple a manner as possible. This chapter will be divided into three main sections.

“Getting Started” will outline the procedure of starting Dionex Column Selector, the layout of the Data Editor and the files used by the Dionex Column Selector for storing column information (the Column Database) and user selection files.

“Using the Data Editor” takes you through the procedure of using the wizard interface and setting up a simulation or optimization. This tutorial will prepare you for Tutorial 2.

“Using the Data Editor selection files” introduces you to the concept of selection files, which speed up the process of using the Data Editor.

### **2.2. Getting Started**

Dionex Column Selector can be started using by using the Windows’ Start menu. You can navigate to the Dionex Column Selector short cut and start the application by clicking on “Start”, “Programs”, “Dionex Column Selector” and then left clicking the Dionex Column Selector application short cut.

When the program starts a blank main window and the Dionex Column Selector Date Editor windows are created, as shown in Figure 1.

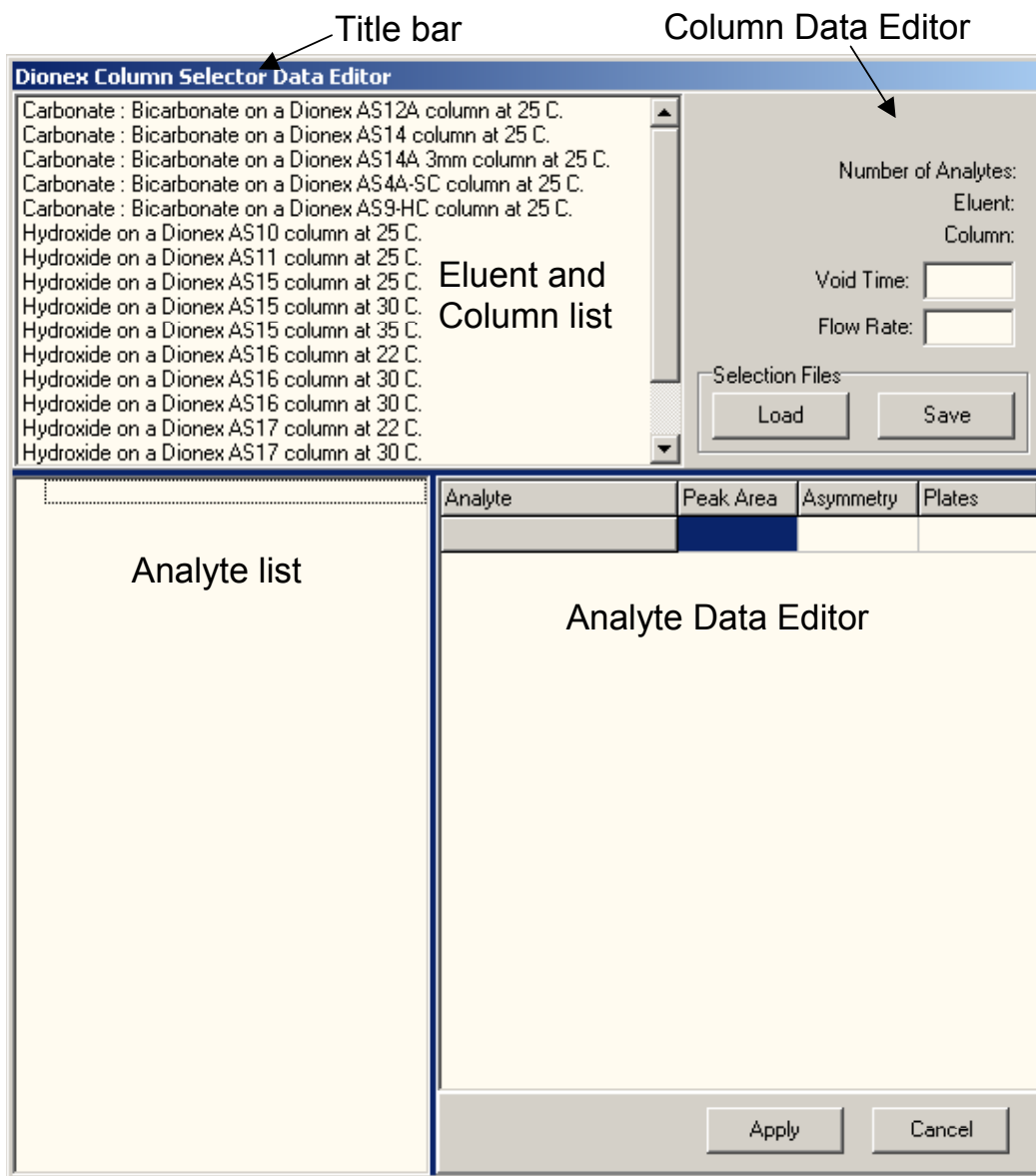


Figure 1. Dionex Column Selector Data Editor window. The list of eluent / column combinations may vary.

### Parts of the Wizard Window:

**Title Bar** – Use the title bar to move the window by placing the mouse cursor over the bar, click and hold the left mouse button then move the window to the desired position and release the mouse button when finished.

**Eluent / Column List** – The eluent / column list shows all available databases currently installed. Clicking on a particular eluent / column will load that database into memory, overwriting any previously selected database.

**Load and Save Selection Buttons** – The load and save selection buttons allow a selection to be loaded once a database is selected.

**Column Data Editor** – This section displays information about the column database that has been loaded. This includes the number of analytes in the database, the eluent and column names, the void time and the flow rate. The void time and flow rate can be edited to match a particular system.

**Analyte List** – When a database is loaded the list of analytes within the database will be presented here.

**Analyte Data Editor** –Analytes that are selected in the analyte list by checking the check box will appear in the analyte data editor with their default peak area, asymmetry and plates values. These values can be adjusted by entering new values.

### **2.3. Using the Data Editor**

There are four main areas in the Dionex Column Selector Data Editor.

#### **Choosing an Eluent / Column from the list.**

After the program has been started, the Data Editor should display a list of eluent / column entries just like Figure 1.

- Left click once on “Carbonate : Bicarbonate on a Dionex AS14A 3mm column” so that the selection is highlighted.

Each available eluent / column database in Dionex Column Selector is given an entry in this list. The selection of the database will directly affect how Dionex Column Selector calculates retention data. In this case Dionex Column Selector will simulate a Dionex AS14A 3mm column with a carbonate : bicarbonate dual species eluent.

#### **Choosing a selection of analytes.**

After loading the column database Dionex Column Selector will list all analytes available in the loaded database (Figure 2). The available analytes will vary from database to database.

- Left click once in the square check box to select each of the following analytes.

- Void Dip, Bromide, Chlorate, Chloride, Fluoride, Iodide, Molybdate, Nitrate, Nitrite, Phosphate, Phthalate, Sulfate and Thiocyanate.
- If necessary use the scroll bar on the right to access analytes below the bottom of the wizard window.

Number of Analytes: 13 / 27  
 Eluent: Carbonate : Bicarbonate  
 Column: Dionex AS14A 3mm  
 Void Time: 1.82  
 Flow Rate: 1.00

Selection Files  
 Load Save

| Analyte     | Peak Area | Asymmetry | Plates |
|-------------|-----------|-----------|--------|
| Void Dip    | 0.1       | 2         | 7000   |
| Bromide     | 1.9       | 1.4       | 7000   |
| Chlorate    | 2.4       | 1.8       | 7000   |
| Chloride    | 1.4       | 1.0       | 7000   |
| Fluoride    | 1         | 1.0       | 7000   |
| Iodide      | 4.8       | 2.2       | 7000   |
| Molybdate   | 5.4       | 1.9       | 7000   |
| Nitrate     | 2.1       | 1.5       | 7000   |
| Nitrite     | 1.6       | 1.8       | 7000   |
| Phosphate   | 2.6       | 1.3       | 7000   |
| Phthalate   | 11.7      | 1.8       | 7000   |
| Sulfate     | 3.3       | 1.2       | 7000   |
| Thiocyanate | 10.7      | 3.4       | 7000   |

Apply Cancel

**Figure 2. Dionex Column Selector Data Editor with the Carbonate : Bicarbonate on a Dionex AS14A 3mm column at 25 C database loaded.**

The void dip is always listed first; all other analytes are listed in alphabetical order. The number of analytes will display the number of chosen / number available.

## **Editing the analyte data.**

For each selected analyte, Dionex Column Selector will list the default peak area, asymmetry value and plate count. Default values for these are included in the column database to produce a typical separation and virtual chromatogram. These values can be changed to reflect the actual peak areas, asymmetries and plate counts of your exact system and column. For now we'll leave the values as they are, except the void peak, which we will set to have a peak area of 0.1 and asymmetry value of 2.

- Left click once on the Peak Area cell for "Void Dip". The value should become highlighted.
- Press the delete button to remove the existing value.
- Enter 0.1 and press the enter key.
- Left click once on the Asymmetry cell for "Void Dip". The value should become highlighted.
- Press the delete button to remove the existing value.
- Enter 2 and press the enter key.

If you do not select the void dip in step two, you will not be able to adjust its area in step three, nor will Dionex Column Selector calculate the resolution of the first peak from the void dip. However it will always be shown in the virtual chromatogram.

The default value for the number of theoretical plates is based on a new column. Changes in the number of theoretical plates due to column aging and degradation have not been taken into consideration. If the number of theoretical plates of your column differs from the default value, this step offers the opportunity to correct for any variations. Decreasing the number of theoretical plates will broaden the peaks and therefore decrease the resolution of a column.

## **Editing the column data.**

The embedded value for the void time is based on a typical system configured with that column and guard. Because flow rates and configurations vary, void times also vary from system to system. By matching the void time of your system Dionex Column Selector can model your system more accurately. Changing the Flow Rate variable immediately changes the Void Time in an inversely proportional manner the

higher the flow rate, the shorter the void time and consequently the shorter the overall chromatogram.

- Double left click on the Void Time text box. The value should become highlighted.
- Press the delete button to remove the existing value.
- Enter 1.25.
- Click on the apply button at the bottom right of the Data Editor window. The Data Editor window should close and the Dionex Column Selector main window should become active.

This step concludes the “Using the Wizard” tutorial. The “Using the Data Editor selection files” tutorial will teach you how selection files can save you time when repeating similar optimizations and simulations. Tutorial 2 will teach you how to use the main interface of Dionex Column Selector and assumes you have just completed this tutorial.

## **2.4. Using the Data Editor selection files**

The Data Editor selection files speed up the process of running the wizard when similar simulations and optimization have to be repeated. Data Editor selection files can be saved at any time after a database is loaded. The selection file stores the analytes that are selected as well as the peak areas, asymmetry values, plate counts and the void time. The chosen database is not stored in the selection file, so a single selection file can be applied to any database. As databases vary on which analytes are available, any analytes chosen in a selection file that are unavailable in a database will be ignored. A warning will be issued if this circumstance occurs.

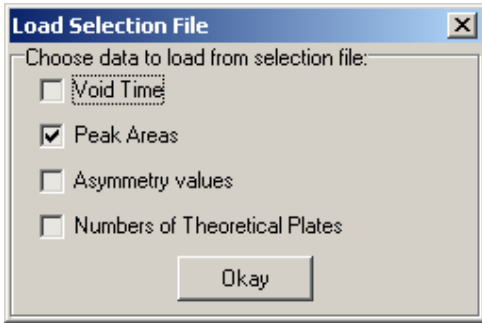
- If the Data Editor window is closed, open it by clicking on the View menu and select Data Editor. Click once on the “Carbonate : Bicarbonate on a Dionex AS14A 3mm column at 25 C database” and change the peak area, asymmetry and plate count of the Void Dip analyte to 0.1, 2 and 6000 respectively.
- Change the value of the Void Time to 1.25.
- Left click the “Save” button in the “Selection Files” group box to bring up the Save As dialog box.

- Enter a File Name for the selection file, taking note of the directory to which it is being saved by using the ‘Save in’ drop-down list box.
- Left click the Save button to save the selection file to the hard drive. This should return you to the Data Editor.
- Left click on Apply to close the Data Editor. The Dionex Column Selector main window should become the active window.

This procedure has saved the selection file to the hard drive. Rather than repeating steps two through five of the previous tutorial, it is now possible to open the selection file and skip steps two through five and obtain identical results.

### **Opening your selection file.**

- Open the data editor by clicking on the View menu and select Data Editor.
- Left click once on “Carbonate : Bicarbonate on a Dionex AS14A 3mm column” so that the selection is highlighted and the existing selections are overwritten. The default values should be restored.
- Left click once on the Load button in the Selection Files group box. This should open the Open dialog box. If the directory is different to the one used to save the file, navigate to the correct directory by using the ‘Look in’ drop-down list box. Left click once on the File Name that you chose to save your selection file as to highlight it. It should have a .vcx file extension.
- Left click once on the “Open” button. The open dialog box should close and a ‘Load Selection File’ confirmation box should appear, see Figure 3. Check all four values to overwrite the default values for the void time, peak areas, asymmetry values and numbers of theoretical plates.
- The Data Editor should now be restored to the state it was in when the selection file was saved. By not checking various values in the Load Selection File confirmation box default values can be used instead of being overwritten by the Load Selection function. This is helpful when comparing how different columns behave with the same analytes, but with their default column values.
- Left click once on the “Apply” button. This should close the Data Editor and the Dionex Column Selector main window should become active.



**Figure 3. Load Selection File confirmation box.**

This procedure will produce the same results as the tutorial in Section 2.3. Tutorial 2 will teach you how to use the main interface of Dionex Column Selector and assumes you have just completed this or the previous tutorial.

## Chapter 3

# Learning Dionex Column Selector. Tutorial 2 – The Main Window (dual species eluents)

### 3.1. Introduction

In order to run, Dionex Column Selector requires detailed information about the system it is trying to model. The Dionex Column Selector Data Editor is the part of the application designed to gather this information in as simple a manner as possible. This chapter assumes that the tutorial in Chapter 2 has been recently been completed. If not, restart the application and follow the steps in Chapter 2, section 2.3 “Using the Wizard”.

### 3.2. Getting Started

After completing the Wizard in the previous chapter, the application should look something like Figure 4.

#### **Parts of the Main Window:**

**Control menu button** – The control menu contains the commands to resize, move, maximize and close the Dionex Column Selector main window.

**Title bar** - The title bar contains information about the name of the application window and can be used to move the window.

**Minimize button** – The minimize button is used to minimize the application window to the task bar. The program will still be running but will take no space on the desktop.

**Maximize / restore button** – The maximize button is used to maximize the application windows to use the entire desktop area. When the window is maximized the restore button is used to restore the window to its original size.

**Exit button** – The exit button is used to close the application.

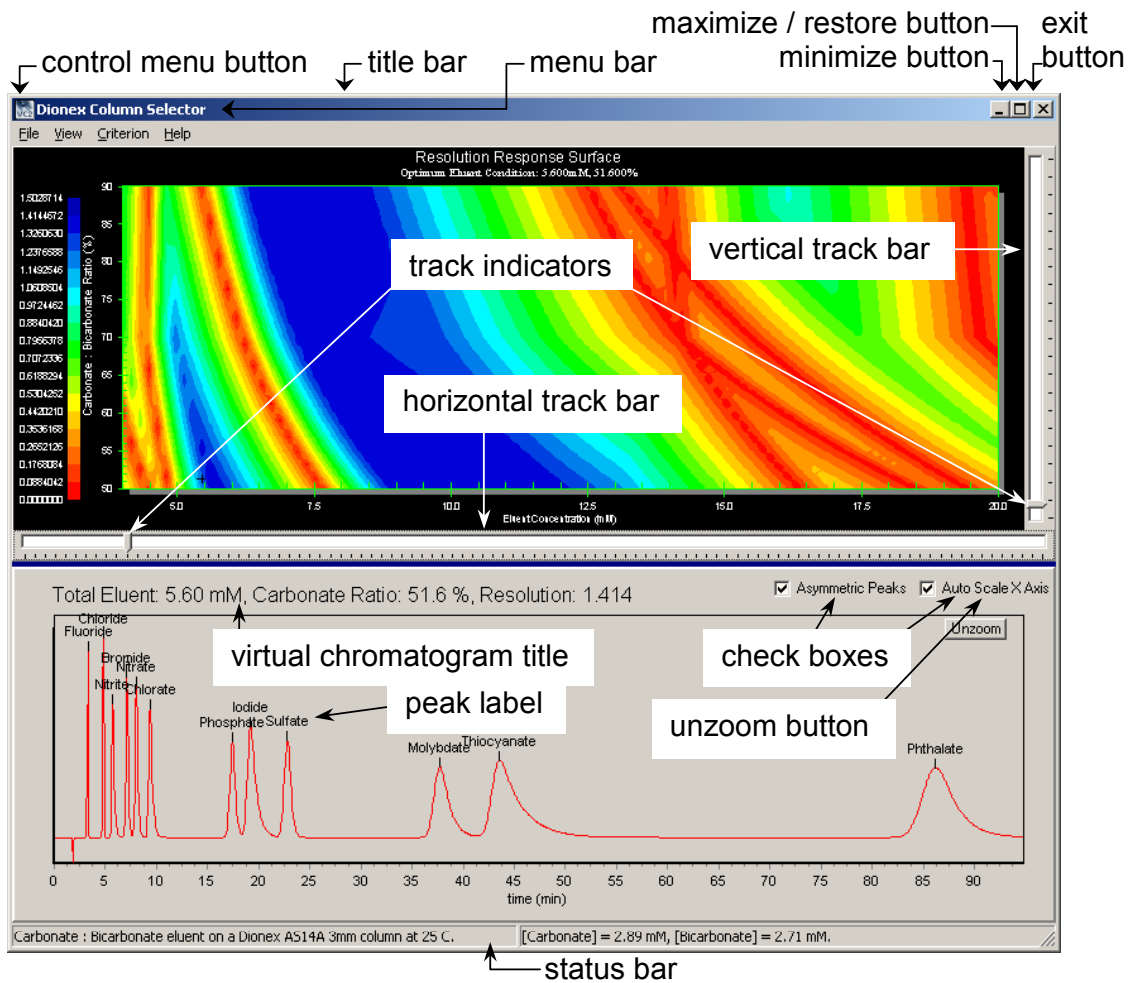


Figure 4. Dionex Column Selector main window for dual species eluents.

**Horizontal and vertical track bars** – These bars are used to adjust the eluent concentration and ratio respectively. They can be adjusted using the arrows keys to move one increment/decrement at a time, or by scrolling the track indicator.

**Virtual chromatogram title** – The title of the virtual chromatogram includes the total eluent concentration (sum of the carbonate and bicarbonate eluents), carbonate ratio (percentage of the eluent composed of carbonate) and resolution criterion value.

**Unzoom button** – This button is used to restore the chromatogram after zooming.

**Asymmetric Peaks check box** – This check box is used to turn the asymmetric peaks function on and off.

**Auto Scale X-Axis check box** – This check box is used to turn the auto scale x-axis function on and off.

**Peak labels** – These labels are used to identify the analyte for each peak.

### **3.3. Using Dionex Column Selector**

The Dionex Column Selector main window is split into two sections, the Resolution Response Surface and the Virtual Chromatogram. The Resolution Response Surface is a contour map of the resolution criteria across the entire search area. Areas of blue are maxima (high resolution) and areas of red are minima (low resolution).

The Virtual Chromatogram is a representation of the chromatogram that would be expected with the currently selected eluent conditions. The horizontal and vertical scroll bars can be used to select any eluent condition within the search area. The program starts by locating the global maximum and setting the initial eluent conditions to that value.

#### **Maximizing the Dionex Column Selector Window**

- Left click once on the maximize / restore button. The window should enlarge to fill the entire screen. Dionex Column Selector may take a few seconds to recalculate and redisplay the contour plot.

It is usually easier to work with a maximized window.

#### **Changing the eluent concentration and ratio**

Dionex Column Selector indicates the currently selected eluent conditions using a cross hair on the resolution response surface. If the selected eluent condition lies at the edge of the response surface, the cross hair may not be visible.

- Press and hold the right arrow key. The eluent concentration should increment. Hold the right arrow key down until the eluent concentration is 10.08 mM, and then release the key.
- Press the left or right arrow key once to move the eluent concentration one increment at a time.
- At 10.08 mM, the minimum resolution criterion should be 1.246 (your actual value might vary slightly).

- Press and hold the up arrow key. The eluent ratio should increment. Hold the up arrow key down until the eluent ratio is 62.0 % and then release the key.
- Press the up or down arrow key one to move the eluent ratio one increment at a time.
- The minimum resolution criterion should be 1.200 (your actual value might vary slightly).
- Position the cursor over the track indicator of the horizontal track bar. Press and hold the left mouse button. Move the cursor in a straight line to the left. The eluent concentration should decrease as the track indicator moves. When the eluent concentration reaches 5.60 mM release the left mouse button.
- Position the cursor over the track indicator of the vertical track bar. Press and hold the left mouse button. Move the cursor in a straight line downwards. The eluent ratio should decrease as the track indicator moves. When the eluent ratio reaches 51.6 % release the left mouse button.

The eluent concentration can also be modified by left clicking directly on the resolution response surface.

- Position the cursor over the resolution response surface approximately at the 7.5 mM and 80 % eluent conditions. Left click the mouse button once. The eluent conditions should change to approximately these values.
- Position the cursor over the 'Criterion' menu on the menu bar, left click once. A menu should appear. Position the cursor over the 'Find Global Optimum' menu, left click once.
- You should now be back at the global optimum eluent composition (5.60 mM, 51.6%) and the minimum resolution criterion should be 1.316 (your actual value might vary slightly).

When you change the eluent conditions using the track bars you may notice a delay of a few seconds before the cross hair updates.

The right pane of the status bar will show the composition of the selected eluent in terms of carbonate and bicarbonate concentrations. The sum of these two values will always match the total eluent value.

## Zooming and unzooming a part of the virtual chromatogram

- Position the cursor so that it is to the left of the void dip but above the height of the chloride peak. Press and hold the left mouse button. Move the cursor downwards and to the right, a rectangular box should be created from where you pressed the left mouse button to wherever you move the cursor. Move the cursor to just below the base of the 'Phosphate' peak and release the left mouse button.

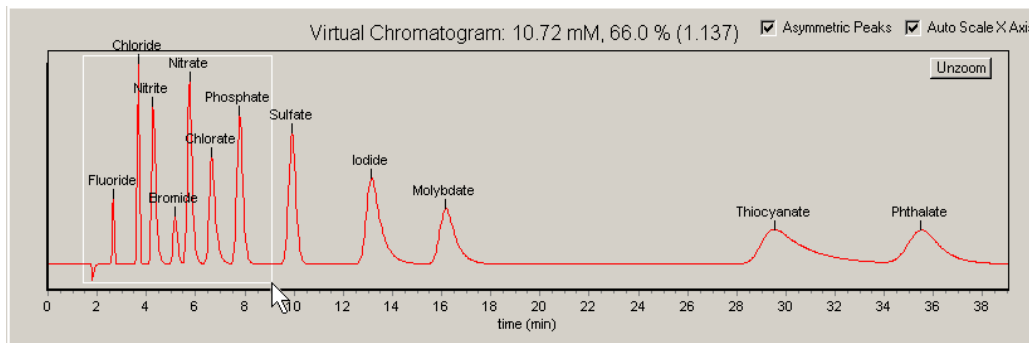


Figure 5. A Virtual Chromatogram displaying the rectangular zoom box.

- The seven peaks of fluoride, chloride, nitrite, bromide, nitrate, chlorate and phosphate should now occupy the entire virtual chromatogram.
- Left click once on the unzoom button to return to the original virtual chromatogram.

Zooming is always achieved by dragging a rectangle from top left to bottom right. Unzooming is achieved by dragging a rectangle from the bottom right to the top left (opposite to zooming).

## Auto scaling the X-Axis

- Left click once on the 'Auto Scale X-Axis' check box. The tick in the box should disappear and the virtual chromatogram should redraw itself with 96.2 minutes as the maximum on the time axis (your value might vary). The virtual chromatogram should no longer run to the end of the x-axis.
- Press and hold the left arrow key. The eluent concentration should decrement until the minimum of 4.0 mM is reached, then release the key.

- As the eluent concentration decremented, the virtual chromatogram should have been redrawn taking up more and more of the x-axis until all but the last few minutes of the x-axis is used.
- Press and hold the right arrow key. The eluent concentration should increment. Release the mouse button when the eluent concentration reaches 12.48 mM. The virtual chromatogram should now only take up about one third of the x-axis.
- Left click once on the Auto Scale X-Axis check box. A tick should appear and the virtual chromatogram should redraw itself so that the chromatogram uses the entire x-axis. This is the same chromatogram, just with a longer x-axis.

When Auto Scale is turned off the total time of the chromatogram is defined by the longest possible chromatogram for the entire search area.

### Finding the optimal and fastest eluent conditions

- Position the cursor over the 'Criterion' menu on the menu bar, left click once. A menu should appear (Figure 6). Position the cursor over the 'Find Fastest Chromatogram...' menu, left click once. A 'Fastest Chromatogram Finder' dialog box should appear (Figure 7).

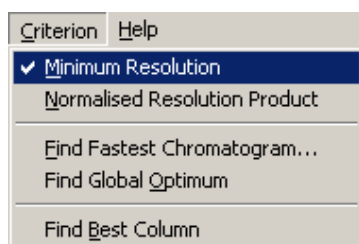


Figure 6. Criterion menu.

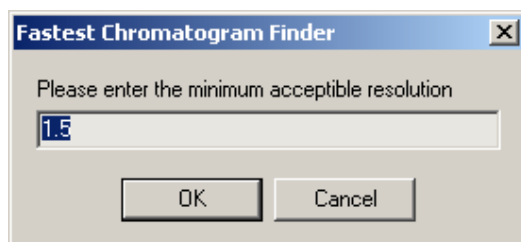


Figure 7. Fastest Chromatogram Finder dialog box.

- Enter a minimum acceptable resolution of 1.25 into the dialog box. Click OK.

- The eluent conditions should change to 7.04 mM, 90.0 % with a minimum resolution criterion of 1.251. The time axis of the Virtual Chromatogram should be about 34 minutes (your results may vary slightly).
- Left click on the 'Criterion' menu, then left click on 'Find Global Optimum'.
- Dionex Column Selector will return to the global optimum with the time axis of the Virtual Chromatogram about 65 minutes.

If you enter a minimum acceptable resolution greater than the global optimum, Dionex Column Selector will tell you that it couldn't find a chromatogram to match that criterion.

- Left click on the Criterion menu and select "Find Fastest Chromatogram...".
- Enter 1.5 into the Fastest Chromatogram Finder dialog box. Dionex Column Selector should inform you that there are no chromatograms that match that criterion. Click OK to continue.

### **Changing the Resolution Criterion**

- Left click on the 'Criterion' menu. There should be a tick next to 'Minimum Resolution', as in Figure 6. Left click on 'Normalized Resolution Product'. After completing the calculations, the Resolution Response Surface contour plot should change to a new response surface. The optimum eluent conditions should now be 7.84 mM, 73.6 % with a resolution criterion of 0.473 (your results may vary slightly).
- Left click on the 'Criterion' menu, and then click on 'Minimum Resolution' to change the resolution criterion back.

For difficult separations the minimum resolution criterion is helpful, as it will try to maximize the resolution of any difficult to separate peak pairs. For easy separations the normalized resolution criterion is helpful, as it will try to maximize the resolution of all peak pairs.

### **Turning Asymmetric peaks on and off**

When 'Asymmetric Peaks' is turned on, the resolution is calculated based on the Exponentially Modified Gaussian peak separation. Thus the asymmetry values can

impact the overall resolution significantly. Turning asymmetric peaks on and off should produce an easily identifiable change in the resolution response surface.

- Left click on the 'Asymmetric Peaks' check box. The tick in the box should disappear and the response surface should redraw itself, this time based on symmetrical (Gaussian) peaks. The optimum eluent conditions should now be 5.76 mM, 50.4 % with a resolution criterion of 1.965 (your results may vary slightly).
- Left click on the 'Asymmetric Peaks' check box to restore the asymmetry (Exponentially Modified Gaussian) calculations.

### Viewing the Resolution Response Surface in true 3D

- Left click on the 'View' menu (Figure 8) and click on '3D Plot'. After completing some calculations the contour plot should disappear and be replaced with a wire frame 3D plot over a contour plot. Two scroll bars should appear inside the existing track bars (Figure 9).

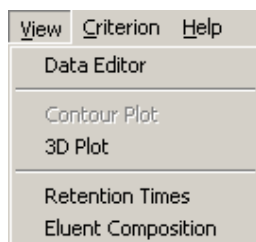


Figure 8. View menu.

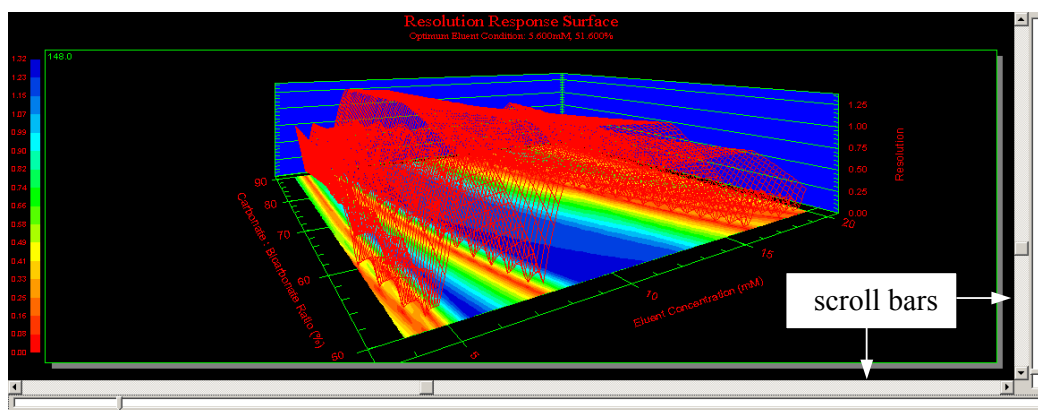
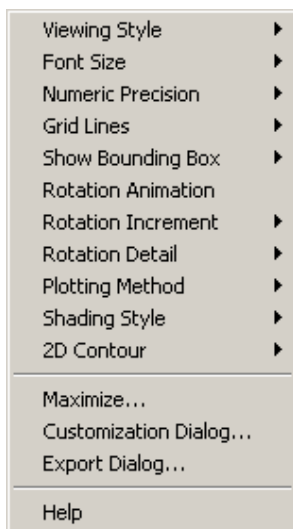


Figure 9. 3D Resolution Response Surface showing scroll bars.

- Use the horizontal scroll bar to rotate the wire frame around the z-axis, and the vertical inner scroll bar to rotate the wire frame around the x-axis.

- Right click on the 3D plot to activate the 3D plot context sensitive menu (Figure 10). 3D plotting controls are accessed through this menu.



**Figure 10. Response Surface context sensitive menu.**

- Left click on the ‘View’ menu and click on ‘Contour Plot’ to return to the default contour map.

Clicking on the 3D image of the response surface will not change the eluent conditions. Only the contour plot can be used in this way.

### **Viewing the Retention Times of the analytes**

- Left click on the ‘View’ menu (Figure 8) and click on ‘Retention Times’. A ‘Dionex Column Selector Retention Time Results’ table should be displayed (Figure 11).

| Analyte     | Ret. Time | Ret. Factor | Resolution |
|-------------|-----------|-------------|------------|
| Void Dip    | 1.25      | 0.00        | 2.508      |
| Fluoride    | 2.23      | 0.79        | 5.916      |
| Chloride    | 3.30      | 1.64        | 2.894      |
| Nitrite     | 3.94      | 2.15        | 1.734      |
| Bromide     | 4.88      | 2.90        | 1.414      |
| Nitrate     | 5.50      | 3.40        | 1.652      |
| Chlorate    | 6.44      | 4.15        | 3.777      |
| Phosphate   | 11.97     | 8.57        | 1.391*     |
| Iodide      | 13.20     | 9.56        | 1.400      |
| Sulfate     | 15.66     | 11.53       | 5.136      |
| Molybdate   | 25.93     | 19.75       | 1.428      |
| Thiocyanate | 29.98     | 22.98       | 2.759      |

\* Minimum Resolution

Close Print

**Figure 11. Dionex Column Selector Retention Time Results Table.**

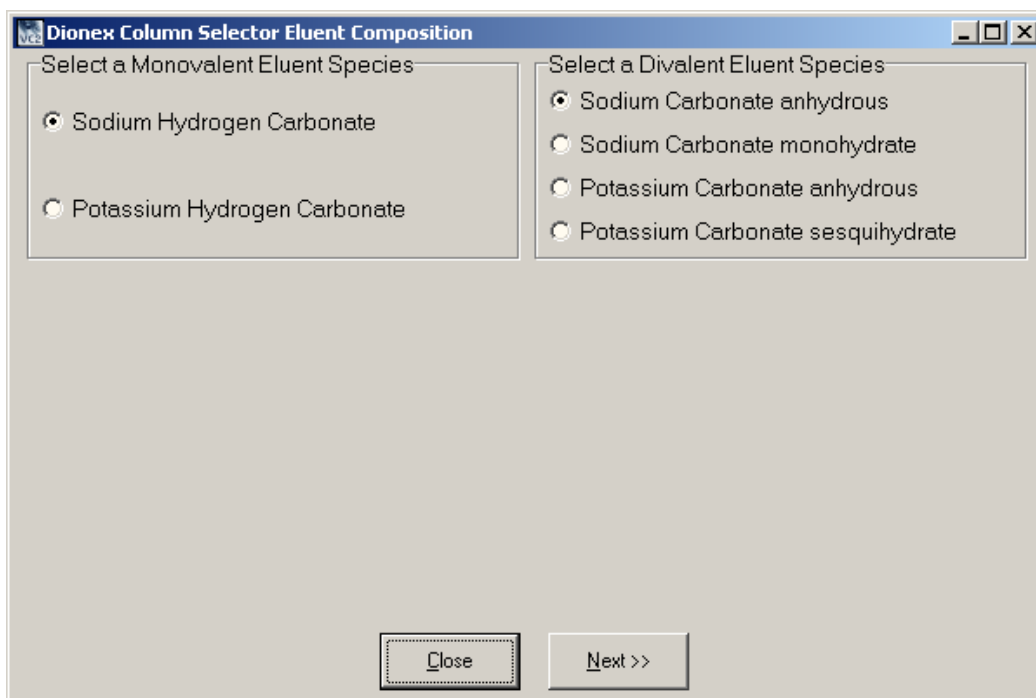
- Left click on the Print button to print a copy of the retention times.
- Left click on the Close button to close the window.

The column on the right gives the resolution between the analyte in that row and the next. For example in Figure 11, the resolution between Fluoride and Chloride is 5.478.

## Calculating the Eluent Composition

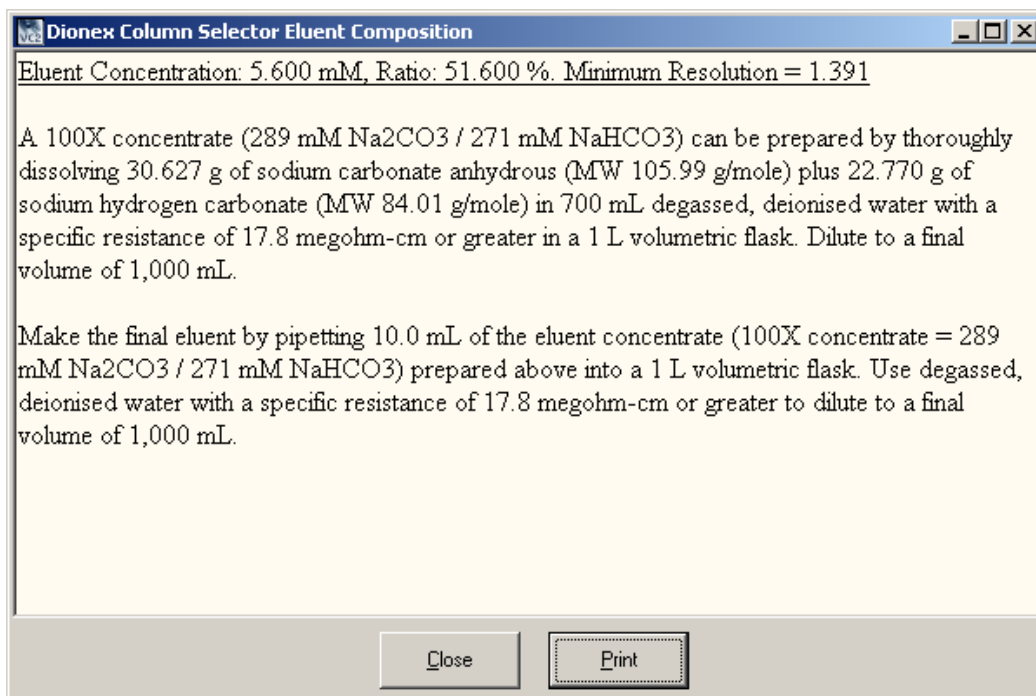
The Eluent Composition Calculator is a useful tool for making your own eluents manually. It is also possible to use a gradient or multi-valve pump to mix together carbonate and bicarbonate eluents in the correct ratio.

- Left click on the 'View' menu (Figure 8) and click on 'Eluent Composition'. A 'Dionex Column Selector Eluent Composition' window should appear (Figure 12).



**Figure 12. Dionex Column Selector Eluent Composition selection window.**

- Left click on ‘Sodium Hydrogen Carbonate’ radio button in the ‘Select a Monovalent Eluent Species’ group box, and ‘Sodium Carbonate Monohydrate’ radio button in the ‘Select a Divalent Eluent Species’ group box.



**Figure 13. Dionex Column Selector Eluent Composition results window.**

- Left click on the Next button to advance to the results page (Figure 13). A description of how to make up the eluent from Sodium Hydrogen Carbonate and Sodium Carbonate Monohydrate should be displayed.
- Left click on the Print button to print the description.
- Left click on the Close button to close the window.

## Printing the Response Surface and Virtual Chromatogram

- Left click on the 'File' menu (Figure 14) and click on 'Print' to print both the response surface and the virtual chromatogram.

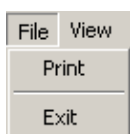


Figure 14. File menu.

- To print only the response surface, right click on the response surface to bring up the context sensitive menu (Figure 10) and click on 'Export Dialog' on the menu to open the Exporting Resolution Response Surface window (Figure 15).

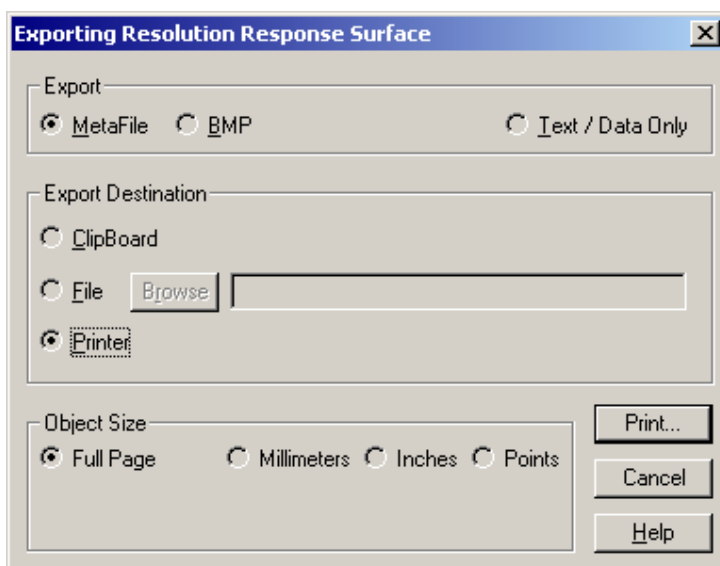


Figure 15. Exporting Resolution Response Surface window.

- In the 'Export Destination' box select 'Printer' by left clicking on the Printer radio button.

- Left click on the Print button.

On some systems the response surface does not print from the File / Print command. The Export Dialog method is a work around for this problem.

## **Comparing different Columns**

Dionex Column Selector can calculate the optimized resolution criterion for every available column in its databases using the existing analyte list. This is an easy way to find if there is a better mobile/stationary phase combination for a particular separation.

- Left click on the Criterion menu (Figure 6) and click on 'Find Best Column'.
- Dionex Column Selector will warn you that some analytes are not found in certain databases. Iodide, Thiocyanate, Molybdate and Phosphate will not be found in the AS10 database, and Iodide and Thiocyanate will not be found in the AS15 database. This is because these columns are unsuitable for these analytes.
- After a few moments of calculations a Best Column page will open displaying the best criterion value found for each column, see Figure 16.
- Notice the AS10 and AS15 databases have \* marks next to their criterion values, this is because these criterion values were calculated without the missing analytes being taken into consideration.
- A low value will tell you that a particular column is unsuitable for a separation. A high value tells you that a particular column might be suited to a certain separation.
- In this case it should be obvious that the AS14A is a suitable column for this particular set of analytes while the AS16 would be a poor choice.
- Click Okay to continue.

The highest criterion value may not necessarily be the best column for a particular separation. Further investigation might be necessary to determine other factors such as peak asymmetry and chromatogram length, both are important factors before choosing a column.

| Eluent and Column   | Best Criterion Value |
|---|----------------------|
| Carbonate : Bicarbonate on a Dionex AS12A column at 25 C.     | 1.003                |
| Carbonate : Bicarbonate on a Dionex AS14 column at 25 C.      | 1.435*               |
| Carbonate : Bicarbonate on a Dionex AS14A 3mm column at 25 C. | 1.414                |
| Carbonate : Bicarbonate on a Dionex AS4A-SC column at 25 C.   | 1.161                |
| Carbonate : Bicarbonate on a Dionex AS9-HC column at 25 C.    | 1.304                |
| Hydroxide on a Dionex AS10 column at 25 C.                    | 0.074*               |
| Hydroxide on a Dionex AS11 column at 25 C.                    | 0.717*               |
| Hydroxide on a Dionex AS15 column at 25 C.                    | 0.484*               |
| Hydroxide on a Dionex AS15 column at 30 C.                    | 2.559*               |
| Hydroxide on a Dionex AS15 column at 35 C.                    | 2.357*               |
| Hydroxide on a Dionex AS16 column at 22 C.                    | 0.141                |
| Hydroxide on a Dionex AS16 column at 30 C.                    | 0.185                |
| Hydroxide on a Dionex AS16 column at 30 C.                    | 0.233                |
| Hydroxide on a Dionex AS17 column at 22 C.                    | 0.347                |
| Hydroxide on a Dionex AS17 column at 30 C.                    | 0.381                |
| Hydroxide on a Dionex CarboPac PA20 column at 30 C.           | 0.000*               |
| Methanesulfonic Acid on a Dionex CS12A column at 25 C.        | 0.000                |
| Methanesulfonic Acid on a Dionex CS12A column at 40 C.        | 0.000                |

\* One or more analytes are not present in this database

Okay

Figure 16. Virtual Column Find Best Column results window.

## Restarting Dionex Column Selector

- Left click on the ‘View’ menu (Figure 8) and click on ‘Data Editor’. The Dionex Column Selector data editor should open.
- Left click once on “Carbonate : Bicarbonate on a Dionex AS14A 3mm column” so that the selection is highlighted.
- Left click once on the Load button in the Selection Files group box. This should open the Open dialog box. Left click once on the file name that you chose to save your selection file as to highlight it. It should have a .vex suffix.
- Left click once on the “Open” button and select all values for the confirmation box. The Data Editor should now have a void time value of 1.25.
- Left click once on the “Apply” button. This should close the data editor and the Dionex Column Selector main window should become active.

Once the View / Data Editor command has been issued, all data in memory is erased. Pressing Cancel on the Dionex Column Selector Data Editor will return you to a blank main window. However the last values from the Data Editor will still be selected so immediately clicking “Apply” will return you to the main window with the same parameters.

### **Closing Dionex Column Selector**

- Left click on the ‘File’ menu (Figure 14) and click on ‘Exit’.
- Alternatively you can use the exit button (Figure 4).

## Chapter 4

### Learning Dionex Column Selector. Tutorial 3 – The Main Window (single species eluents)

#### 4.1. Introduction

In order to run, Dionex Column Selector requires detailed information about the system it is trying to model. The Dionex Column Selector Data Editor is the part of the application designed to gather this information in as simple a manner as possible. This chapter will start with a brief guide to the Data Editor, which will open a single species eluent database. Familiarity with the Data Editor from Chapter 2 is assumed.

#### 4.2. The Data Editor

- Start the application by clicking on the shortcut in the Start Menu, Programs, Dionex Column Selector menu.
- Left click once on 'Hydroxide on a Dionex AS15 column' (Figure 17).
- Tick the following check boxes in the analyte list to select a series of analytes – Void Dip, Acetate, Bromide, Chlorate, Chloride, Chromate, Fluoride, Methanesulfonate, Nitrite, Oxalate, Phosphate, Succinate and Sulfate.
- Change the Void Dip peak area to 0.25.
- Left click the Apply button to complete the Data Editor.

**Dionex Column Selector Data Editor**

Carbonate : Bicarbonate on a Dionex AS12A column at 25 C.  
 Carbonate : Bicarbonate on a Dionex AS14 column at 25 C.  
 Carbonate : Bicarbonate on a Dionex AS14A 3mm column at 25 C.  
 Carbonate : Bicarbonate on a Dionex AS4A-5C column at 25 C.  
 Carbonate : Bicarbonate on a Dionex AS9-HC column at 25 C.  
 Hydroxide on a Dionex AS10 column at 25 C.  
 Hydroxide on a Dionex AS11 column at 25 C.  
**Hydroxide on a Dionex AS15 column at 25 C.**  
 Hydroxide on a Dionex AS15 column at 30 C.  
 Hydroxide on a Dionex AS15 column at 35 C.  
 Hydroxide on a Dionex AS16 column at 22 C.  
 Hydroxide on a Dionex AS16 column at 30 C.  
 Hydroxide on a Dionex AS16 column at 30 C.  
 Hydroxide on a Dionex AS17 column at 22 C.  
 Hydroxide on a Dionex AS17 column at 30 C.  
 Hydroxide on a Dionex CarboPac PA20 column at 30 C.  
 Methanesulfonic Acid on a Dionex CS12A column at 25 C.  
 Methanesulfonic Acid on a Dionex CS12A column at 40 C.

Number of Analytes: 13 / 39  
 Eluent: Hydroxide  
 Column: Dionex AS15  
 Void Time:   
 Flow Rate:

Selection Files:

| Analyte          | Peak Area | Asymmetry | Plates |
|------------------|-----------|-----------|--------|
| Void Dip         | 0.25      | 1         | 4279   |
| Acetate          | 1.2       | 1.0       | 3300   |
| Bromide          | 6.3       | 1.0       | 5800   |
| Chlorate         | 11.1      | 1.1       | 4700   |
| Chloride         | 2.3       | 1.0       | 6400   |
| Chromate         | 11.5      | 1.0       | 5000   |
| Fluoride         | 1         | 1.1       | 6000   |
| Methanesulfonate | 1.8       | 1.0       | 4400   |
| Nitrite          | 3         | 1.0       | 5800   |
| Oxalate          | 3.7       | 1.0       | 4700   |
| Phosphate        | 8.1       | 1.0       | 4100   |
| Succinate        | 2.7       | 1.0       | 3700   |
| Sulfate          | 3.3       | 1.0       | 5100   |

Void Dip  
 Acetate  
 Acrylate  
 Arsenate  
 Bromate  
 Bromide  
 Carbonate  
 Chlorate  
 Chloride  
 Chlorite  
 Chromate  
 Ethanesulfonate  
 Fluoride  
 Formate  
 Fumarate  
 Glycolate  
 Iodate  
 Lactate  
 Malate  
 Maleate  
 Methacrylate  
 Methanesulfonate  
 Molybdate  
 Monofluorophosphate  
 n-Butyrate  
 Nitrate  
 Nitrite  
 Oxalate  
 Phosphate  
 Phthalate  
 Propanesulfonate  
 Propionate  
 Selenate  
 Selenite  
 Succinate  
 Sulfate  
 Sulfite  
 Tartrate  
 Thiosulfate  
 Tungstate

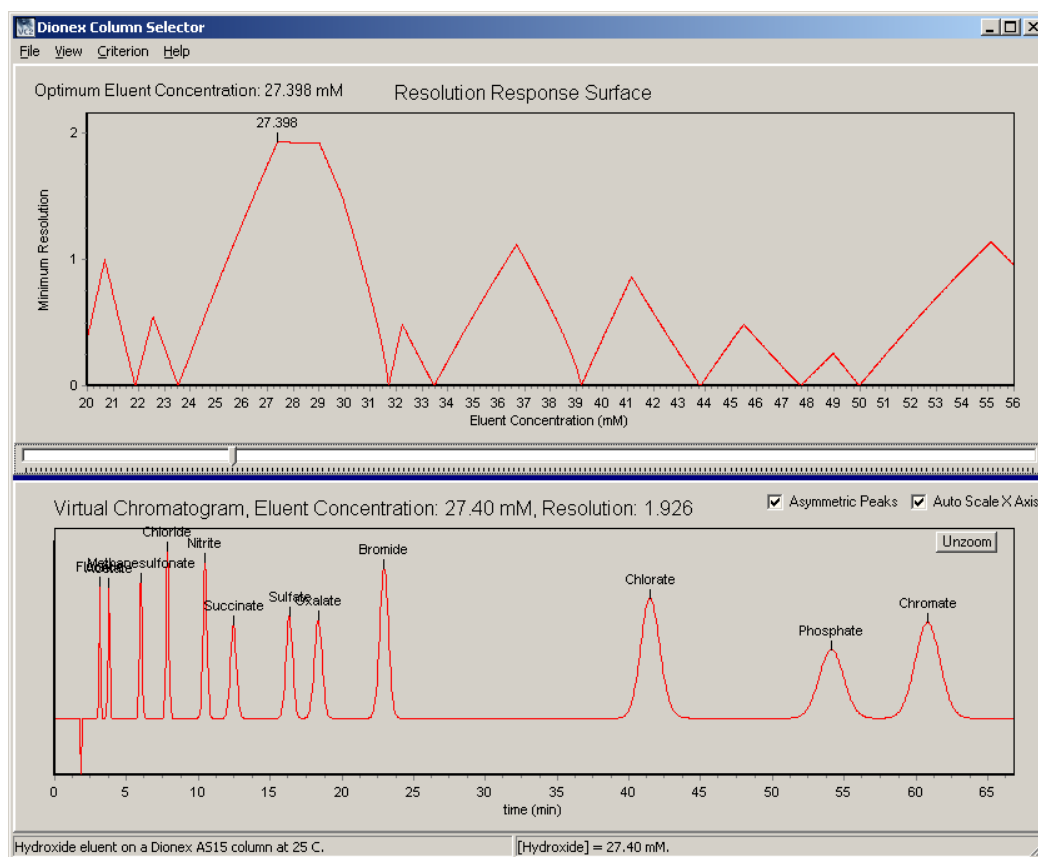
**Figure 17. Dionex Column Selector Data Editor displaying the contents of a Dionex AS15 database.**

This procedure will open a Hydroxide database, which is a single species eluent database. Dionex Column Selector will automatically detect this and switch to single

species eluent mode. This results in the resolution response surface being a line graph rather than a contour plot.

### 4.3. Getting Started

After completing the Wizard, the Dionex Column Selector main form should look something like Figure 18.



**Figure 18. Dionex Column Selector main window for single species eluents.**

The parts and functions of the windows are the same as for dual species eluents (Figure 4) with the exception that there is no vertical track bar.

### 4.4. Using Dionex Column Selector

The Dionex Column Selector main window is split into two sections, the Resolution Response Surface and the Virtual Chromatogram. The Resolution Response Surface is a line graph of the resolution criterion over the entire search area.

The Virtual Chromatogram is a representation of the chromatogram that would be expected at the currently selected eluent condition. The horizontal track bar can be

used to select any eluent condition within the search area. The program starts by locating the global maximum and setting the initial eluent conditions to that value.

In this tutorial, only functions that differ with the dual species eluent systems will be covered. Functions that are equivalent in both modes have been covered in the previous chapter.

### **Changing the eluent concentration**

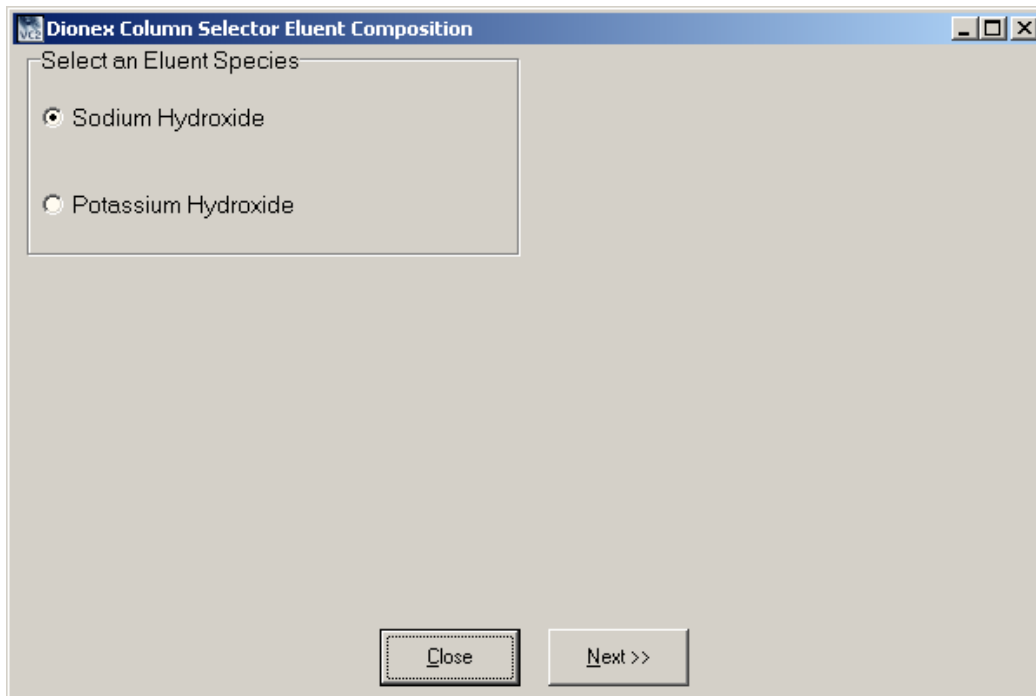
- Press and hold the right arrow key. The eluent concentration should increment. Hold the right arrow key down until the eluent concentration reaches 36.67 mM, and then release the key.
- Press the left or right arrow keys once to move one increment at a time.
- The minimum resolution criterion should be 1.113.
- Position the cursor over the track indicator of the horizontal track bar. Press and hold the left mouse button. Move the cursor in a straight line to the left or right. The eluent concentration should decrease or increase as the scroll indicator moves. When the eluent concentration reaches a desired value release the left mouse button.

The eluent concentration can also be modified by left clicking directly on the resolution response surface line.

- Position the cursor over the red line of the resolution response surface line graph at the peak about 29 mM. The cursor should change to a cross hair. Left click once while the cursor is in the form of a crosshair.

### **Other differences from dual species eluent mode**

- There is no 3D plotting capability for the line graph.
- There is only one class of eluent species available for eluent composition calculations, thus the Dionex Column Selector eluent composition window contains only one group box (Figure 19).



**Figure 19. Dionex Column Selector Eluent Composition window for single species eluents.**

- The line graph cannot be printed individually.

## Chapter 5

### Glossary

Terms in bold have their own entry.

**3D Plot:** A true three dimensionally rendered representation of a surface.

Analyte.

Asymmetry value.

**Auto-Scale:** A feature used to ensure that a virtual chromatogram stretches across the entire width of the x-axis.

**Check box:** A check box consists of a square box and text label that indicates a choice the user can make by selecting the box. Any number of the options in the set can be selected at a time.

Column (stationary phase).

**Column database file:** A file saved to the hard drive with a .vcl extension that contains retention data collected using a correct experimental design. Dionex Column Selector uses these databases to solve its internal retention models so that retention data can be predicted.

**Database file:** see **Column database file**.

**Dialog box:** A window displayed in the user interface to solicit input from the user and/or to display output to the user.

**Dionex Column Selector main window:** The main working part of Dionex Column Selector where the resolution response surface and virtual chromatogram is drawn.

**Dionex Column Selector Data Editor:** A set of simple steps in a window designed to gather detailed information about the system Dionex Column Selector is trying to model.

**Drop-down list box:** A list box that displays a current setting, but can be opened to display a list of choices.

**Dual Species Eluent.** An eluent mixture that contains two species such as bicarbonate and carbonate.

Eluent (mobile phase).

Eluent composition.

Embedded.

Empirical End Points Model.

Experimental design.

Global optimum.

**Group box:** A rectangle that surrounds a set of controls, such as **check boxes** or **radio buttons**, and contains a label. The sole purpose of a group box is to organize controls related by a common purpose (usually indicated by the label).

Linear Solvent Strength Model.

Local optimum.

**Maximize:** To display a window at its largest size.

**Minimize:** To minimize the size or appearance of a window; in some cases this means to hide the window.

Minimum resolution criterion.

Normalized resolution product criterion.

Optimization.

Peak area.

Peak pair.

**Radio button:** Radio buttons are used to select one of several options. A radio button contains a small circle with text next to it, when selected; the circle has a smaller, filled circle inside it. Selecting one button in a set deselects the previously selected button, so only one of the options in the set can be selected at a time.

Resolution.

Resolution criterion.

Response surface.

**Restore:** To return a window to its pre-**minimized** or pre-**maximized** size and position.

Retention factor.

Retention modeling.

Retention time.

Search area.

**Selection File:** A file saved to the hard drive with a .vcx extension that contains data normally gathered during the Dionex Column Selector Wizard.

Simulation.

Single Species Eluent.

Theoretical Plates.

**Unzoom:** Decreases the magnification of a part of a window to restore it to its original size.

**Virtual chromatogram:** A chromatogram drawn by Dionex Column Selector based on predicted retention times and embedded peak areas and asymmetry values.

Void Dip.

Void Time (Void Volume).

**Wizard Selection File:** See **Selection File**.

**Zoom:** Increases the magnification of a part of a window to aid in visualization.

## Appendix A

### Retention Models and Resolution Algorithms

Dionex Column Selector includes 2 built in models, one for single species eluents and the other for dual species eluents. Both models are based on the Linear Solvent Strength model<sup>1</sup>.

The single species eluent model is called the Empirical End Points approach to the Linear Solvent Strength Model<sup>2</sup> and is defined by the following equation, (1):

$$\log k' = C_1 + C_2 \log E_T \quad (1)$$

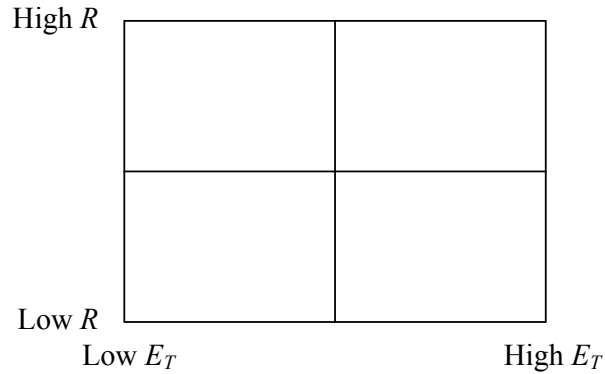
where  $k'$  is the retention factor,  $C_1$  and  $C_2$  are constants for a given analyte and column, and  $E_T$  is the eluent concentration of the eluting species.

Embedded data, which have been acquired according to a correct experimental design, are used to solve the two constants,  $C_1$  and  $C_2$ . For each analyte three retention times have been measured at known eluent concentrations, two at the end-points of the search area, and one extra point near the center of the search area. Values for  $C_1$  and  $C_2$  are solved for each consecutive pair, giving two equations, one applicable to one half of the search area, and the other to the remaining half of the search area<sup>3</sup>.

The dual species eluent model expands on the Empirical End Points approach to the Linear Solvent Strength Model, allowing prediction for both total eluent concentration and the ratio between two eluting species. The interface for Dionex Column Selector uses total eluent concentration and eluent ratio ( $R$ ), however the dual species eluent model uses total eluent concentration and the concentration of doubly charged eluent species ( $[E^{2-}]$ ) as parameters. The concentration of doubly charged eluent species can be calculated from total eluent concentration and eluent ratio using the following equation, (2):

$$[E^{2-}] = \frac{E_T \cdot R}{100} \quad (2)$$

Embedded data for dual species eluent systems has once again been acquired using a correct experimental design. For each analyte nine retention times have been measured at known eluent compositions according to Figure 23.



**Figure 20. Correct Experimental Design for Dual Eluent Species**

The experimental design can be thought of as consisting of four quadrants, where each quadrant is defined by the four retention times at the end points. The dual species eluent model is solved separately for each individual quadrant.

The dual species eluent model comprises three set equations. The first two are given by equations (3) and (4):

$$\log k' = C_{11} + C_{21} \log E_T \quad (\text{for low R}). \quad (3)$$

$$\log k' = C_{12} + C_{22} \log E_T \quad (\text{for high R}). \quad (4)$$

These equations are identical to the single species eluent equations and, once solved, can be used to calculate retention times on the top or bottom horizontal lines of the search area.

The third equation is given in equation (5):

$$\log k' = D_1 + D_2 \log [E^2] \quad (5)$$

To calculate a retention factor for a given  $E_T$  and  $[E^2]$ , equations (3) and (4) must be solved for each quadrant initially. Once these equations have been solved, retention factors can be calculated for a given  $E_T$  value at both low and high values of R using equations 3 and 4 respectively. These two retention factors are then used to solve for  $D_1$  and  $D_2$  in equation (5). Once  $D_1$  and  $D_2$  are known for a given  $E_T$  value, retention factors for any value of R can be calculated for that  $E_T$  value using equation (5).  $D_1$  and  $D_2$  must be solved separately for each value of  $E_T$ .

Dionex Column Selector calculates retention times for all selected analytes across the entire search area. If a single species eluent is used then 1000 retention times per analyte are calculated. If a dual species eluent is used then a grid of 100 x 100 retention times per analyte are calculated. From this data Dionex Column Selector constructs a ‘virtual chromatogram’ for any  $E_T$  and value of  $R$  the user chooses.

Dionex Column Selector ranks these virtual chromatograms by assigning them a value based on one of two resolution criteria, Minimum Resolution or Normalized Resolution Product<sup>1</sup>. For each peak pair a resolution value is calculated using equation (6):

$$R_s = \left( \frac{\alpha - 1}{\alpha + 1} \right) \left( \frac{k'}{1 + k'} \right) \frac{\sqrt{N}}{2} \quad (6)$$

where  $\alpha$  is the separation factor given by the ratio of retention factors for the peak pair,  $k'$  is the average retention factor for the peak pair and  $N$  is the number of theoretical plates.

For the minimum resolution criterion the chromatogram is assigned the minimum resolution of all the adjacent peak pairs in the chromatogram. The minimum resolution criterion gives a value of zero to a chromatogram that has one or more peak pairs co-eluting. A value of 1.5 is generally regarded as baseline separation, although a value of 1.2 is often considered acceptable resolution for most applications.

The normalized resolution product ( $r$ ) is defined by equation (7):

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

where  $n$  is the number of peaks and  $R_{S_{i,i+1}}$  is the resolution between peaks  $i$  and  $i+1$ .

The normalized resolution product criterion gives a value of zero to a chromatogram that has one or more peak pairs co-eluting, and a value of one for a chromatogram that has evenly spaced peaks.

---

<sup>1</sup> P.R. Haddad and P.E. Jackson, Ion Chromatography: Principles and Applications, Elsevier, Amsterdam, 1990.

<sup>2</sup> Critical Comparison of Retention Models for Optimisation of the Separation of Anions in Ion Chromatography. 1. Non-Suppressed Anion Chromatography. John E. Madden and Paul R. Haddad. J. Chromatogr. A, 829 (1999) 65 - 80.

<sup>3</sup> J.E. Madden, G.W. Dicoski, M.J. Shaw and P.R. Haddad, Unpublished Data.