Using Field Strength Gradients to Enhance PATsmart™ ZipChip® System Analysis

Application Note

Repligen Corporation now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Overview

One of the great things about the PATsmart™ ZipChip® System is the ability to perform rapid, high resolution separations. The fast run times of the ZipChip System assays cut down the time needed for data collection and are amenable to higher throughput assays. However, sometimes it is difficult for some mass spectrometers to adequately sample the sharp, narrow peaks when the assays require complex MS acquisition strategies like MS/MS fragmentation.

It is easy to slow down the ZipChip System separation and widen the peaks by lowering the field strength, but this can increase the run time more than is desired. A solution to this problem and a new way to perform the ZipChip System analysis is to use a field strength gradient.

Field strength gradients are a new feature of the ZipChip System available with Software Version 1.3.0. This gives users the ability to alter the separation field strength during the analysis by ramping the field strength over a given period of time or stepping the field strength up or down at a specific time. Changing the field strength in these ways allows the user to fine tune the peak widths from the ZipChip System separation to facilitate in depth MS characterization of analytes for top-down and bottom- up characterization assays or with other sophisticated MS acquisition parameters.

Field Strength Gradients

Field strength gradients can be particularly useful for complex samples containing many analytes that elute over a wide time range, such as protein digests. The example in Figure 1 shows a protein digest analyzed at a constant field strength (top) and using a field strength gradient (bottom). The top separation was

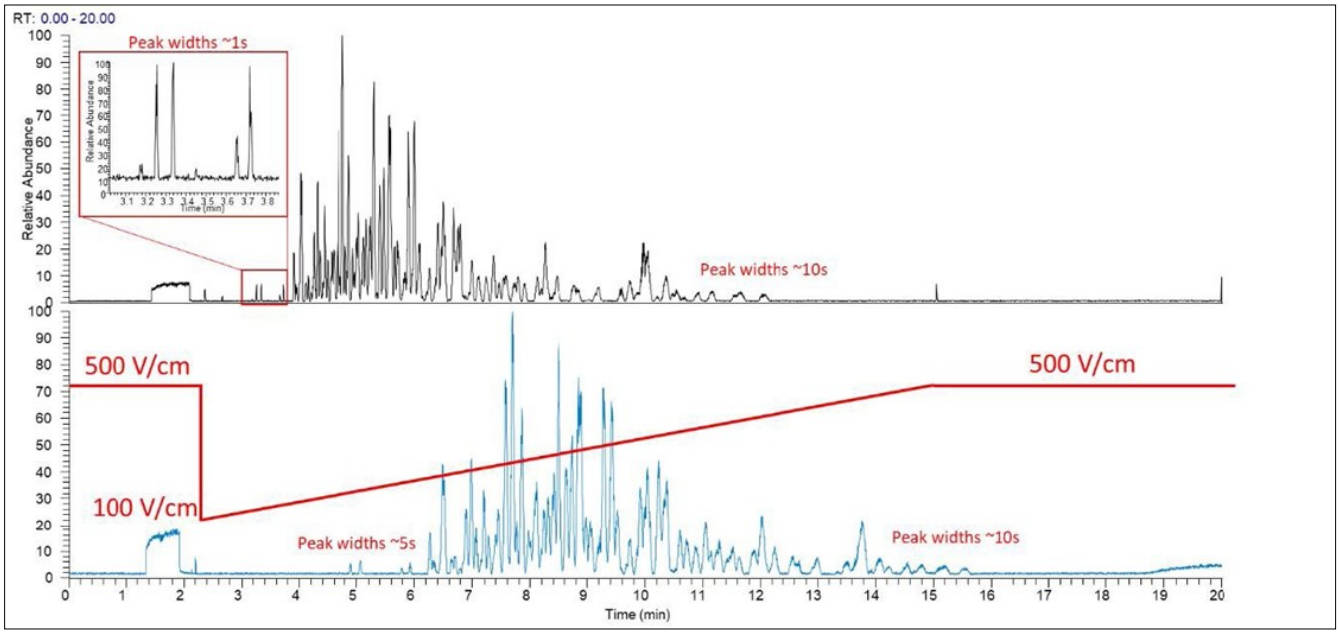

Figure 1. ZipChip System separation of a protein digest at constant 500 V/cm (top) and using a field strength gradient (bottom). The profile of the field strength gradient used is overlaid on the trace.

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run at a constant field strength of 500 V/cm. The earliest peptide peaks at ~3.5 min are only about 1 second wide. It is challenging to characterize peaks this narrow when performing MS/ MS fragmentation, but the peak width of the later eluting peaks (~10s) is sufficient.

The separation could be run at 100 V/cm to increase the width of the early eluting peaks, but the overall run time would increase by a factor of 5 and the late eluting peaks would be excessively broadened. To increase the width of the early eluting peaks, achieve good peak shape of the later eluting peaks, and maintain a fast run time, a field strength gradient was used. In the bottom pane, the same sample is analyzed using a field strength gradient from 100 V/cm to 500 V/cm starting at 2.2 minutes and continuing until 15 minutes. As indicated in the figure, the width of the early eluting peptide peaks increases to approximately 5 seconds, which is wide enough to be adequately characterized by the mass spectrometer. The width and Gaussian shape of the later eluting peaks is maintained and the overall run time only increases by a few minutes.

Field Strength Steps

Another way to utilize this feature is to implement a single step down in field strength during a separation. This approach can be useful for samples containing fewer analytes that elute over a narrower time range, such as intact proteins or protein subunits. Figure 2 is an example of using a field strength step for antibody fragments generated from IdeS digestion and reduction. With a constant field strength of 500 V/cm the analysis is complete in approximately 5 minutes and results in peaks that are about 3 seconds wide. By dropping the field strength from 500 V/cm to 100 V/cm right before the first antibody fragment peak elutes, the peak widths are increased approximately 5x. This provides more time to characterize the peaks using MS parameters that result in a lower acquisition rate, such as high-resolution settings or top-down fragmentation techniques.

Building A Method With Field Strength Gradients

A field strength gradient can be added to a method by activating the Advanced Method options in the method editor of the ZipChip System App. The starting field strength is set by the Initial Field Strength at the top of the method editor. For a Field Strength Gradient, input the time the gradient should start in the Gradient Start field and enter the time the gradient should complete in the Gradient End Time field. This can be the at the end of the run or earlier in the acquisition. In the Gradient Field Strength Start, input the value at which the ramp should start and in the Gradient Field Strength End field input the field strength the system

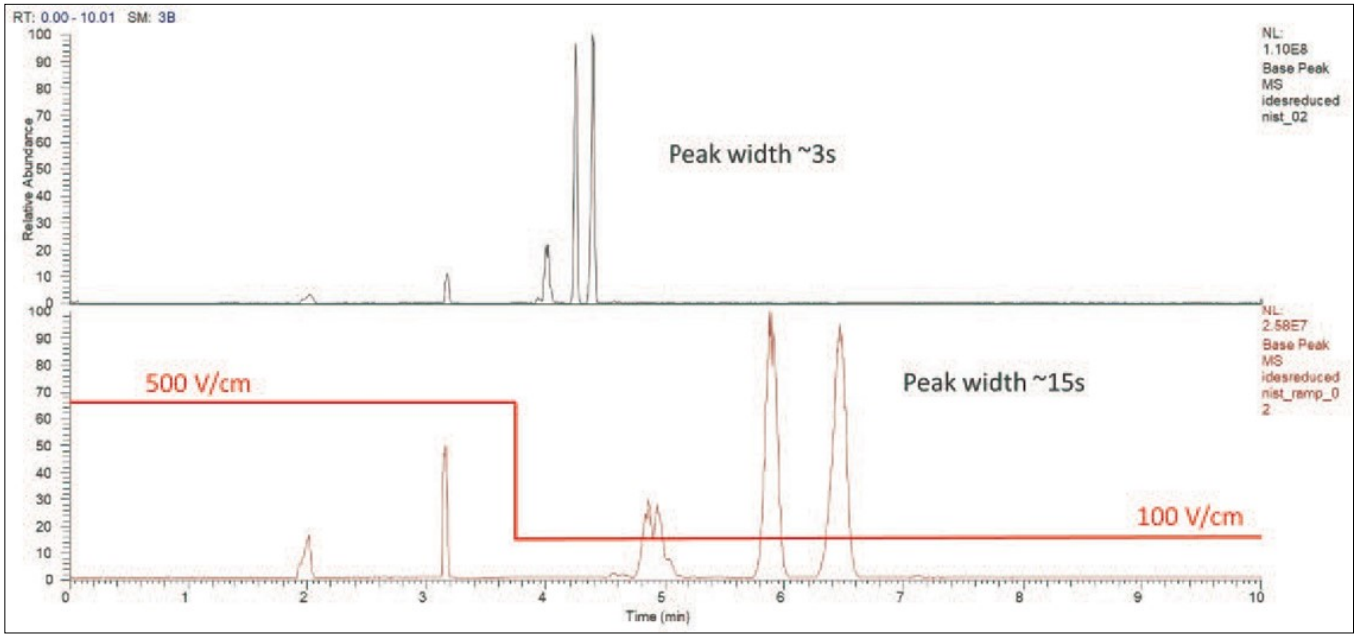

Figure 2. ZipChip System separation of antibody subunits using a constant field strength (top) and a field strength step (bottom). Utilizing the field strength step increases the width of the subunit peaks, making the analysis more amendable to the timescale of top-down MS/MS fragmentation experiments.

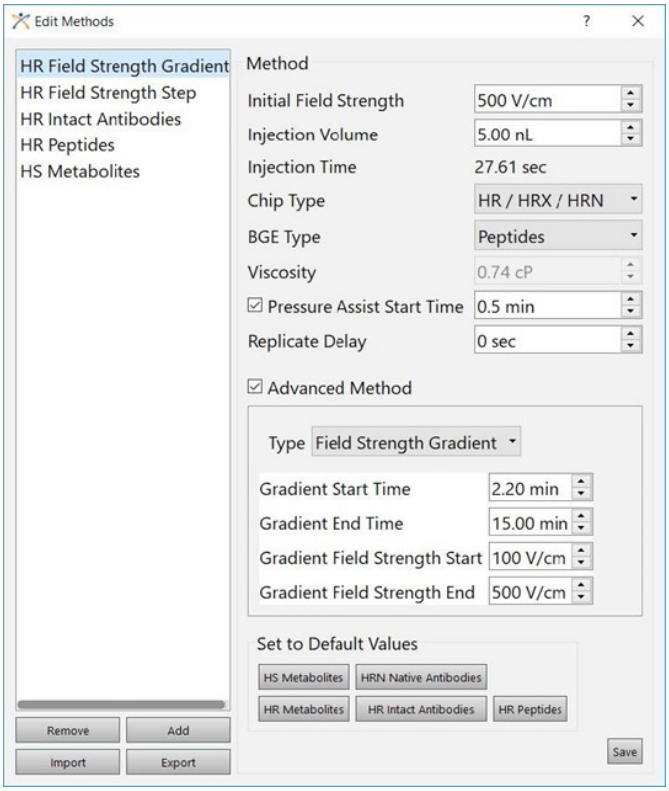

Figure 3. Example of the ZipChip System method using a Field Strength Gradient.

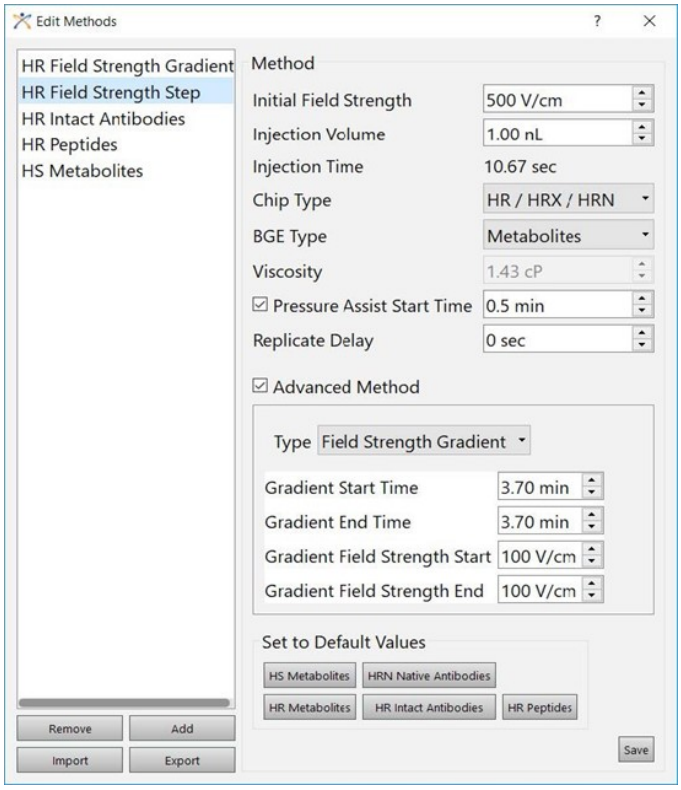

Figure 4. Example of the ZipChip System method using a Field Strength Step.

should ramp to during the analysis. The field strength will return to the Initial Field Strength after the Gradient End Time. The example in Figure 3 shows how the method used in Figure 1 was built. The analysis will start at a field strength of 500 V/cm and at 2.20 min the field strength drops to 100 V/cm. From 2.20 min to 15 min the field strength will gradually increase to 500 V/cm. The field strength will remain at 500 V/cm for the remaining duration of the analysis.

For a Field Strength Step, input the time the step should occur in the Gradient Start Time field. Set the Gradient End Time to be the same value. Set the Gradient Field Strength Start to the desired field strength to step to and set the Gradient Field Strength End to be the same value. The field strength step method used in Figure 2 is depicted in Figure 4. The analysis will start at a field strength of 500 V/cm and at 3.70 min the field strength will drop to 100 V/cm for the remaining duration of the run.

Summary

Field Strength Gradients are a useful tool when analyzing complex samples that require sophisticated MS acquisition parameters, such as top-down and bottom-up protein characterization. The feature of the ZipChip software provides the ability to either ramp or step the field strength during analysis to widen analyte peaks without a drastic increase in overall run time. It is easily implemented by activating the advanced method options in the method editor and can be run in both the Singles Pane and in a larger sequence. Whether its high speed analysis or in-depth MS/MS characterization, users can easily optimize their ZipChip separations to best suit their needs.

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