Real-Time Dynamic Background Subtraction: Improving the Automated Ion Selection Process

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Overview

Information Dependent Acquisition (IDA) is a software tool that helps select the best ion(s) to collect further information on during an LC analysis. Its main purpose is to collect as much information as possible in every injection. This corresponds to MS, MS/MS and even MS^3 for the selected ion(s), all on an LC time scale.

Dynamic Background Subtraction (DBS) [patent-pending] makes this process much easier, while at the same time providing the user with better, more relevant data.

Introduction

Automated LC/MS/MS workflows using Information Dependent Acquisition (IDA) provide the framework for deriving maximum information from every LC experiment. IDA lets you focus on specific ions of interest for increased productivity. The benefits of IDA are the simultaneous collection of single MS and MS/MS data in order to maximize the information in a single injection. The challenge in this process is the determination of the ion of interest that is not necessarily the most intense ion, all on an LC time scale.

Selection criteria tools available for IDA can help to select the correct ion of interest. The selection criterion can include: upper and lower mass cut-offs, isotope pattern matching, Inclusion and Exclusion lists, as well as Dynamic Exclusion. However, if the selection criteria are not set correctly, it can lead to missed ions and therefore re-injection. To help combat this scenario, a new ion selection tool was developed.

To develop this tool, the ideal selection conditions were considered, which include the use of single MS scans, the capability of detecting minor peaks in the presence of high background conditions and closely or even co-eluting peaks. Also, it is important not to make any assumptions or have any prior knowledge of the background ions or sample, and perform it in real-time.

This technical note describes the concept of Dynamic Background Subtraction and how the process leads to better, more relevant data.

Dynamic Background Subtraction: The Concept

The concept of Dynamic Background Subtraction is to subtract the previous survey scan(s) from the current one, before applying any other IDA selection criteria. This essentially eliminates ion response variability that one observes in typical background conditions. As an example, in a full scan MS spectrum there are many masses present, most of which are coming from something other than the injected sample. These ions, although variable to a small extent from scan to scan, are continuously eluting and are always present in the background. These ions can be eliminated on an LC time scale by averaging previous survey scans and subtracting those from the current survey scan being collected. The resulting spectrum after the subtraction has been applied will be dominated only by those ions that are truly changing significantly over time. It is these ions that are selected to do further experimentation on, MS/MS or even MS^3. The concept is depicted in Figure 1.

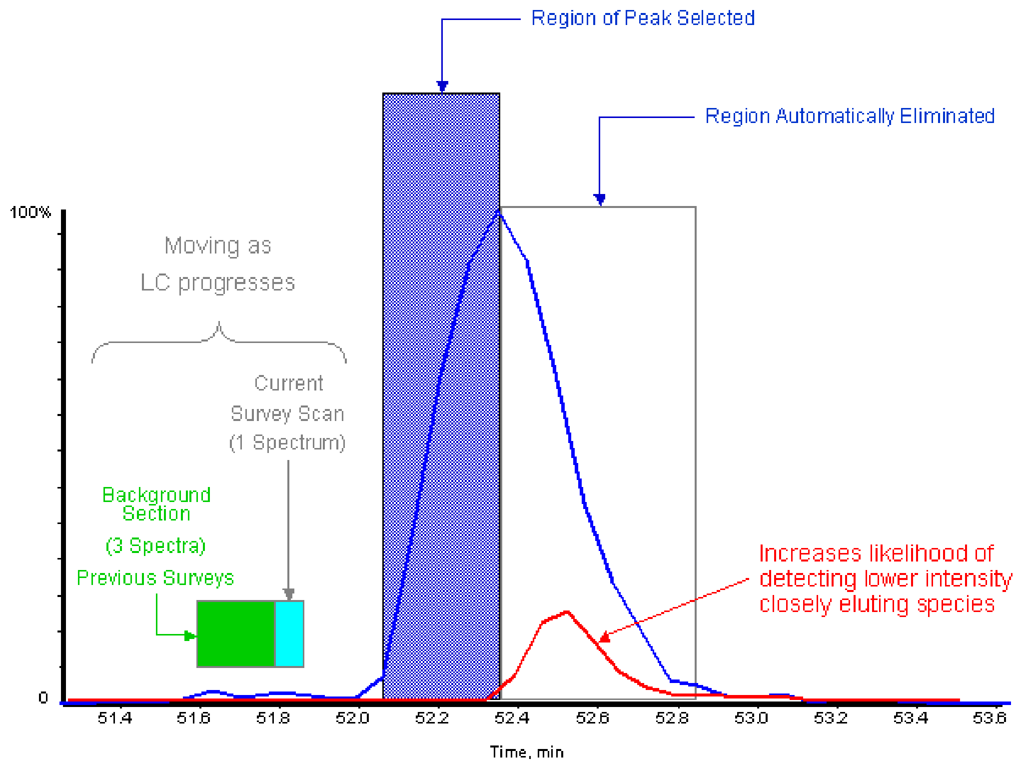

Figure 1. The Concept of Dynamic Background Subtraction. The previous survey scan(s) is subtracted from the current survey scan in IDA before any other IDA selection criteria are applied. This ensures that IDA will only select an ion on the rising edge of its signal. When the signal reaches its apex and begins to decline, it will be subtracted from itself and the ion will no longer be selected as the ion of interest. This increases the likelihood that a lower intensity species that may be closely or even co-eluting, is selected by IDA as the ion of interest.

Dynamic Background Subtraction: The Application

In an automated experiment (IDA) the goal is to obtain as much information as possible in one injection. Depending on the selection criteria chosen, this can lead to a large amount of data that may not be useful for the sample set being analyzed. As an example, without any prior knowledge of the sample or background conditions, MS/MS data may be collected on background or interfering ions. Dynamic Background Subtraction improves the ion selection process, which leads to better, more relevant data. Figure 2 shows the analysis of bromazepam using an IDA experiment with an MS survey scan over a 450 amu mass range to trigger the collection of MS/MS data. In this example, the most intense ion (m/z 315) happens to be an interfering ion that even skews the isotope ratio of the bromazepam analyte (expecting about a 1:1 isotope ratio distribution between the m/z 316 and m/z 318 ions).

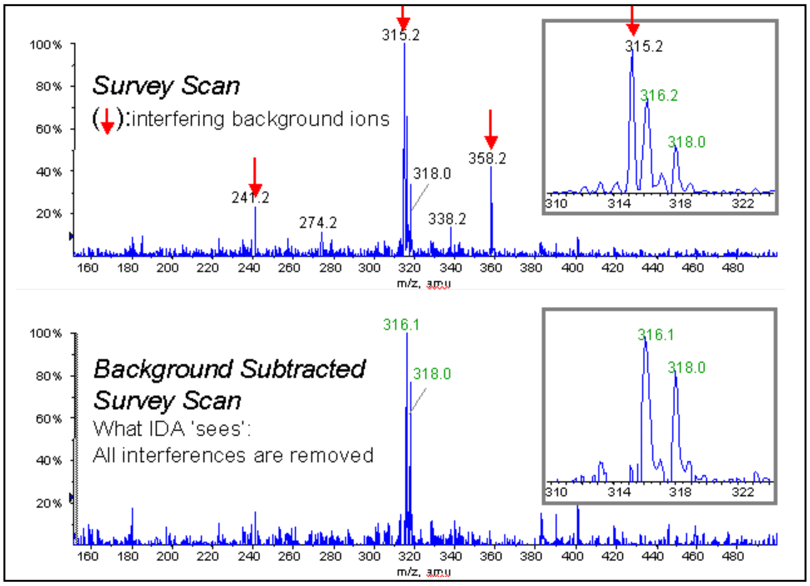

Figure 2. IDA experiment using Dynamic Background Subtraction (DBS). A) Q1 survey scan over 450 amu mass range with 3 other dominant ions other than bromazepam (highlighted with red arrow). B) The same experiment with DBS turned on. The ion selection process is better, because the background ions are subtracted out. IDA now selects only those ions that are truly changing over an LC timescale for further experiments.

Figure 3 shows an IDA experiment of a spiked matrix sample of tamoxifen. The Q TRAP® system was used with an EMS (Enhanced MS) survey scan over 320 amu mass range to trigger the collection of MS/MS data. The IDA selection criteria were very simple with DBS turned on and an intensity threshold of 1000 counts. No other IDA criteria were used (no mass cut-off or Dynamic Exclusion). In this example, the number and intensity of background ions is quite high. In a typical IDA experiment, before DBS, more of the criteria settings would be required in order to make intelligent ion selection. Without prior knowledge of the sample or background conditions, an automated experiment would step down through the ions based on intensity. In this case, the 'real' ion of interest would be missed on a LC timescale because of its intensity relative to the background.

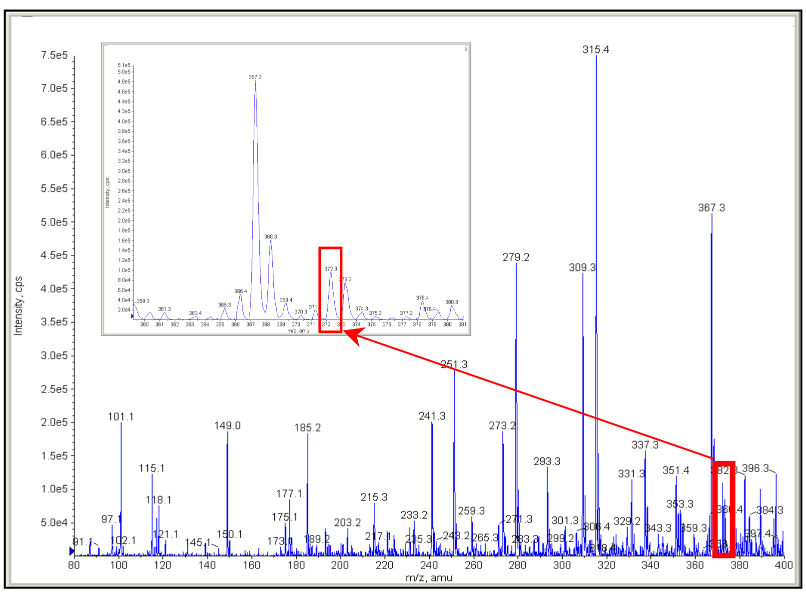

Figure 3. Tamoxifen MS/MS collected using DBS in the presence of high background conditions. The m/z 372 was chosen to perform MS/MS on with minimal IDA selection criteria and DBS turned on.

Dynamic Background Subtraction can be used with any survey scan. In multi-component screening methods, the best way to screen is to use MRM transitions, as hundreds of compounds can be looked for in each injection. Along with higher throughput analyses, this means that the chromatography will squeeze most if not all of the analytes into a much shorter period of time, resulting in closely or co-eluting species; which is fine when using the specificity of MRM transitions. However, some analytes being monitored may be in high enough concentration that will result in chromatographic tailing, thus 'masking' lower level species based on intensity alone.

Proper IDA selection criteria is then ever more important and the use of Dynamic Exclusion is a must (removing that ion from candidate selection for a set period of time). With DBS, after the chromatographic peak has reached its apex it will subtract itself out, thus leaving other ions available for selection. Also, some MRM transitions will inherently have a high background intensity level. DBS has the effect of 'normalizing' this signal. This is shown in Figure 4.

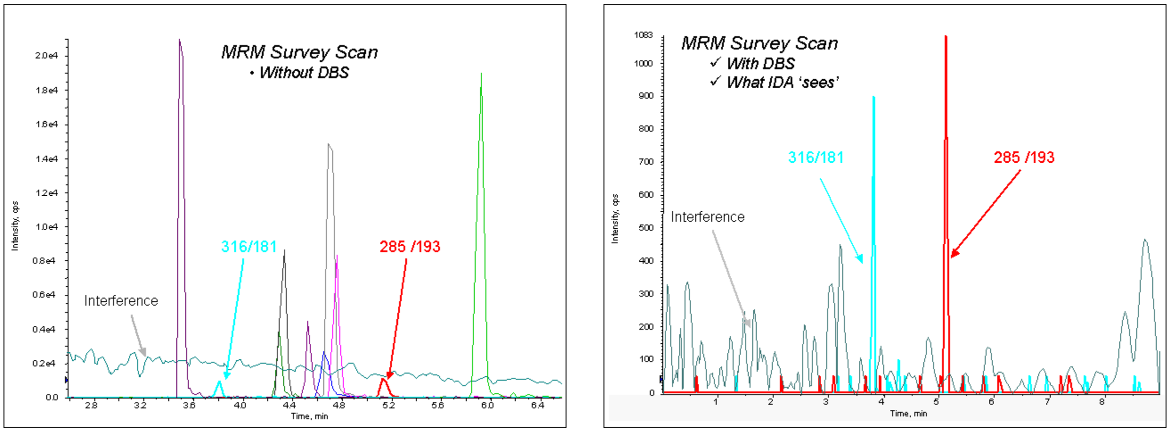

Figure 4. Multi-component analysis using MRM transitions and DBS. A) Without dynamic exclusion, the high background counts of one MRM transition (shown as Interference) eliminates the possibility of getting MS/MS information on the 316/181 or 285/193 analytes, because they never go above the intensity level of the interfering transition. B) DBS has the effect of normalizing this by subtracting it out. The 316/181 and 285/193 transitions are now selected to collect MS/MS data.

Summary

- Real-time Dynamic Background Subtraction simplifies the automated decision making process in IDA, leading to better quality, and more relevant MS/MS data.

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