Using scout triggered MRM to improve allergen screening

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INTRODUCTION

Developing and maintaining large MRM assays can be challenging. Scheduling MRM transitions with retention time windows is necessary to achieve good assay performance as the numbers of MRM transitions per method increases. For methods with large numbers of MRMs, or that use fast gradients, the retention time windows must be as small as possible to achieve the desired dwell and cycle times. If retention times shift slightly, the peak might shift outside of the detection window and be missed or cut off by the edge of the acquisition window. Scout triggered MRM acquisition alleviates this issue by removing the need to maintain retention time windows.

Figure 1. Scheduled and scout triggered MRM acquisition methods. MRM transitions that require monitoring for scheduled MRM (A) and scout triggered MRM (B) are highlighted.

METHODS

A research version of SCIEX OS software was modified to enable scout triggered MRM acquisition. In this mode, when a scout MRM was detected, it triggered the acquisition of later eluting dependent MRMs. An existing allergen LC-MS/MS method was converted to a scout triggered MRM method by selecting the earliest eluting peptide as a scout for all the peptides indicative of each allergen of interest. All methods were run in SCIEX OS software on a SCIEX Triple Quad 7500 system. The data were processed in either Analytics or Explorer modules.

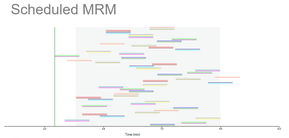

Figure 2A.
Screenshot from method editor showing allergen **scheduled** MRM method, with 95 MRM (45 peptides covering 12 allergens). All **95 MRM** require **retention time windows** to be applied and **maintained**.

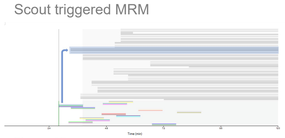

Figure 2B.
Scout triggered MRM **Group** method for the same 95 MRM (45 peptides covering 12 allergens). 12 peptides were used as scouts to trigger dependent confirmation peptide MRM. Thus, **only 24 MRM** required **retention time windows**.

24 scout triggered MRMs are easier to maintain than all 95 scheduled MRMs in the original method.

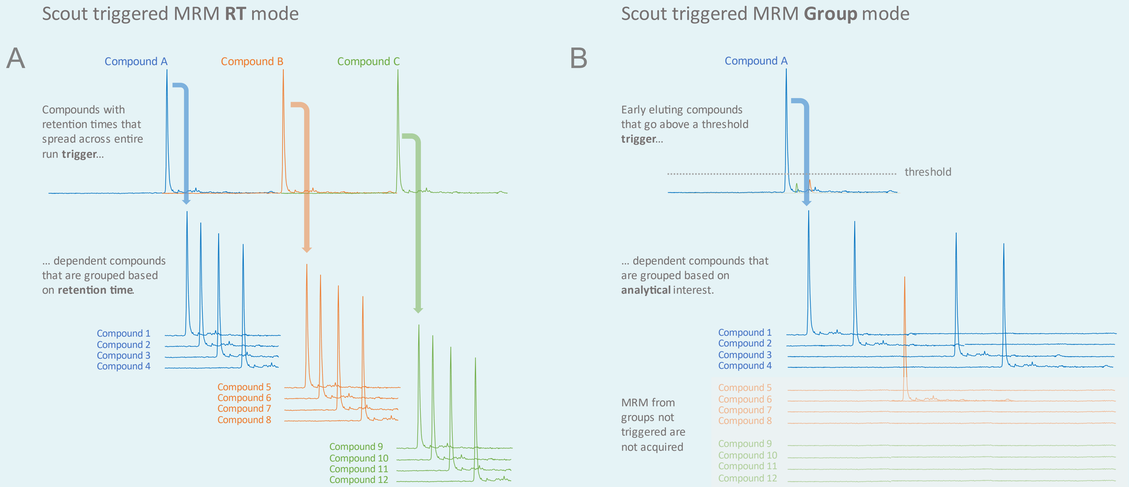

Figure 3. Scout triggered MRM acquisition modes. Panel A showing compounds grouped by retention time (RT) and triggered by detection of an RT marker compound. Panel B showing compounds grouped by analytical interest and triggered by detection of the first eluting compound in a group.

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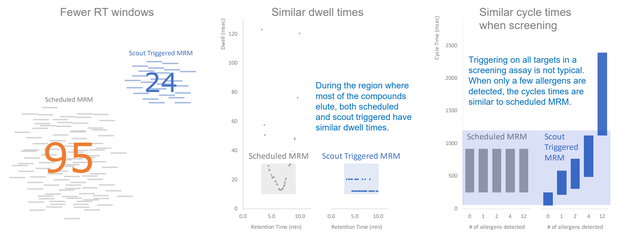

Figure 4. Comparison between scheduled and scout triggered MRM methods: number of retention time windows to maintain, dwell times and cycle times.

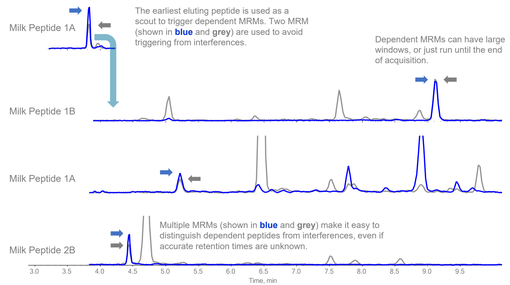

Figure 5. Example of a milk peptide (1A) used as a scout to trigger confirmation MRM that detected 3 additional milk peptides (1B, 2A, and 2B).

Adding Additional Allergens

Scout triggered MRM made it simpler to add new allergens to the existing method. For example, proteins from sesame seeds are of increasing interest. To detect sesame in this method, we predicted several MRM transitions for known sesame tryptic peptides. These transitions were tested with a sample containing sesame, and the most robust and early eluting peptide was selected as a scout to trigger dependent peptide MRMs. The dependent peptide MRMs were given large windows, as shown above for the other allergens (figure 5).