Scout triggered MRM algorithm: The evolution of the MRM workflow

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Multiple reaction monitoring (MRM) as a mode of operation for mass spectrometry in LC-MS/MS workflows has long been considered the gold standard for both selectivity and sensitivity in targeted, quantitative analyses. As targeted panels grow in number of analytes of interest, for example, with increased regulation of residues or with increased interest in metabolite profiles, it becomes necessary to add retention time (RT) scheduling to the MRM method to capture the full panel without compromising data quality. Improvements in sensitivity, selectivity and number of analytes targeted in a single run can be realized by assigning retention times to analytes and allowing the MS to scan for the target MRM transition masses only during a narrow window of time in which the analyte is expected to elute chromatographically. The Scheduled MRM (sMRM) algorithm uses this strategy to allow for the analysis of hundreds-to-thousands more transitions in a single method, while maintaining reasonable cycle and dwell times for quantitative data quality.[1]

As retention time scheduling strategies have become the *de facto* standard for LC-MS/MS quantitative assays, optimization of the method has become more refined. For example, RT windows have become narrower to preserve data quality and methods have been optimized for the continuous and consistent running of many samples over long periods of time. As a result of small changes to the LC system over time or slight differences in the mobile phases, gradients and columns used, some expected peaks have shifted out of their scheduled RT windows. A peak that is not acquired due to a missed RT window must be reacquired with another injection and manual adjustments to the assigned retention times in the MRM method might be necessary. These re-injections and manual adjustments cost routine laboratories thousands of dollars in consumables cost, instrument time, precious sample volume and operator labor each year.

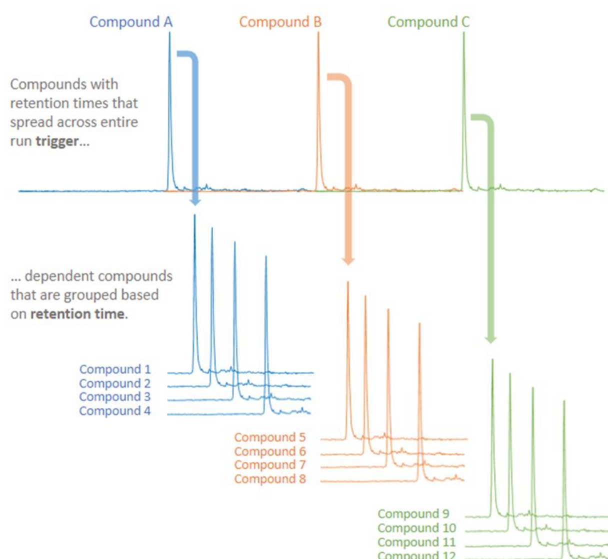

Figure 1. Principles of Scout triggered MRM analysis. Scout triggered MRM uses a marker transition to trigger MS analysis for a group of dependent target analytes. The marker transitions are typically staggered across the chromatographic run and dependent analytes are associated based on their retention times. The outcome is a targeted assay that is robust to retention time shifts.

Scout triggered MRM (stMRM) alleviates this pain point by removing the need to maintain RT windows for each transition and instead relies on marker transitions to trigger the acquisition of dependent MRMs. This new mode of targeted acquisition has been successfully demonstrated in both pesticides and proteomics screening and quantitation assays.[2-5]

Key Features of the Scout triggered MRM workflow

- Scout triggered MRM RT mode removes the need to maintain accurate retention times for all analytes in MRM acquisition methods, alleviating the need for reinjection due to missed peaks from unanticipated retention time shifts
- The ability to refine and adjust existing methods is simplified and does not require many exact retention time assignments
- Cross-experiment triggering of dependent transitions is newly introduced in Scout triggered MRM
- Data quality is preserved for analyte peaks collected in Scout triggered MRM vs. sMRM modes

Method Development

New MRM mode: Scout triggered MRM RT mode

In the updated Method Editor user interface (UI), there are new options for selecting types of MRM acquisition: MRM, Scheduled MRM and now 2 new Scout triggered MRM modes. One of the new modes is called Scout triggered MRM RT and is ideal for method development and method robustness, as it removes reliance on RT windows (Figure 2).

Building a Scout triggered MRM RT method requires acquiring standards data for the target analytes using MRM mode to identify retention times and elution order. Assessment of the chromatogram allows for the assignment of marker transitions to different groups of target analytes or acquisition windows segmented across the LC run time. Once these windows and the elution order have been defined, the operator can assign intensity thresholds to each marker transition for triggering MS scanning for dependent target transitions. The defined RTs are not used during acquisition, but rather as inputs that define the elution order of the analytes. All transitions are acquired from the moment they are triggered by their assigned marker transitions within their designated acquisition window until the next marker transition exceeds its intensity threshold, through the duration of the run (Figure 3).

RT overlap

RT Overlap is a new parameter in the Method Editor UI. A dependent transition that elutes close to the boundary of its acquisition window might risk missing its trigger if it elutes outside of the defined window. The RT overlap parameter allows the user to define a tolerance around each marker transition so that any adjacent transitions eluting within this tolerance will be triggered by that marker. By setting this tolerance, a dependent transition, for example, could be triggered by its own user-assigned marker or by the next or preceding marker.

Cross-experiment triggering for the first time in SCIEX software

Scout triggered MRM introduces a new ability for cross-experiment triggering, by allowing marker transitions used in an experiment to be used to trigger acquisition of dependent transitions in another experiment. For example, in a polarity-switching method with 2 experiments using the Scout triggered MRM RT mode, marker transitions used in the positive ion mode can trigger acquisition in negative ion mode, and vice versa.

This cross-experiment triggering ability adds flexibility for the user to use markers in either polarity as appropriate, based on the internal or surrogate standards available.

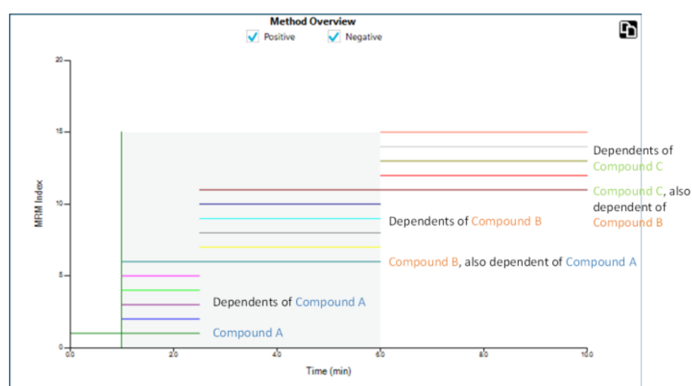

Figure 3. MRM transitions are triggered in acquisition windows across the chromatographic timescale. User-programmable trigger thresholds for the marker transitions define when a window begins. Elution order is critical and exact retention times are no longer necessary.

Experiment: MRM
Polarity: Positive | Spray voltage: 2500 V | Dynamic background subtraction
Mass Table: Import | MRM Mode: Scout triggered MRM RT (MRM / Scheduled MRM / Scout triggered MRM RT / Scout triggered MRM Group) | RT overlap: 0.2 min

	Group ID	Compound ID		ger shold (cps)	Retention time (min)	Q1 mass (Da)	Q3 mass (Da)	Edit dwell time	Dwell time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)	Triggered by: Compound ID
1	6-MAM-d3	6-MAM-d3 IS			1.66	331.100	165.000		6.568	100.0	10.0	49.0	8.0	Methamphetamine-d5 IS||Morphine-d6 IS
2	Amphetamine-d5	Amphetamine			1.44	141.100	93.000		7.838	30.0	10.0	21.0	8.0	Methamphetamine-d5 IS||Morphine-d6 IS
3	Benzoylecgonine-d3	Benzoylecgonine-d3 IS			1.93	293.100	171.200		5.021	80.0	10.0	25.0	12.0	MDPV-d8 IS||Methamphetamine-d5 IS
4	Buprenorphine-d4	Buprenorphine-d4 IS			2.62	472.300	400.200		3.615	120.0	10.0	52.0	18.0	MDPV-d8 IS
5	Carisoprodol-d7	Carisoprodol-d7 IS			2.40	268.100	183.100		3.615	35.0	10.0	11.0	12.0	MDPV-d8 IS
6	Codeine-d6	Codeine-d6 IS			1.55	306.200	152.200		7.838	100.0	10.0	83.0	10.0	Methamphetamine-d5 IS||Morphine-d6 IS
7	Fentanyl-d5	Fentanyl-d5 IS			2.43	342.300	105.100		3.615	90.0	10.0	50.0	8.0	MDPV-d8 IS
8	Hydrocodone-d6	Hydrocodone-d6 IS			1.76	306.200	202.100		6.568	100.0	10.0	39.0	14.0	Methamphetamine-d5 IS||Morphine-d6 IS
9	Hydromorphone-d6	Hydromorphone-d6 IS			1.23	292.100	185.100		40.624	100.0	10.0	39.0	12.0	Morphine-d6 IS||[start of run]
10	JWH 018 4-OH pentyl-d5 IS	JWH 018 4-OH pentyl-d5 IS			4.05	363.100	155.100		15.984	100.0	10.0	35.0	14.0	Nordiazepam-d5 IS
11	JWH 019 6-OH hexyl-d5 IS	JWH 019 6-OH hexyl-d5 IS			3.98	377.200	155.100		15.984	90.0	10.0	27.0	12.0	Nordiazepam-d5 IS
12	MDPV-d8	MDPV-d8 IS	✓	10000	2.12	284.100	134.100		4.116	70.0	10.0	27.0	15.0	MDPV-d8 IS||Methamphetamine-d5 IS
13	Meperidine-d4	Meperidine-d4 IS			2.06	252.200	224.100		5.021	86.0	10.0	29.0	16.0	MDPV-d8 IS||Methamphetamine-d5 IS
14	Mephedrone-d3	Mephedrone-d3 IS			1.84	181.100	148.100		5.021	46.0	10.0	26.0	6.0	Methamphetamine-d5 IS

Figure 2. The updated UI includes new MRM mode options, such as Scout triggered MRM RT.

Experimental Design

Varying the LC system and LC components

To challenge and verify the ability of the Scout triggered MRM workflow, adjustments to a targeted MRM method were made to simulate common changes a laboratory might make to an existing method or system. Each of these has the potential to impact the exact retention time of an eluting analyte and thus cause Scheduled MRM algorithm methods to require manual adjustments or re-injections due to the shifted RTs. A common pesticide mix was used as a representative analyte panel.

Experimental changes to the system included:

1. Changing analytical columns to different stationary phases
2. Swapping the entire LC system (switch from LC system from Lab 1 to LC system from Lab 2, and vice versa)
3. Varying the column configuration by adding a delay column between the solvent mixer and the analytical column to chromatographically separate background contamination
4. Changing the LC method parameters such as gradient, flow rate and runtime

In most of these experiments, all analytes were detected and captured within their designated acquisition windows, despite the perturbances to the LC environment (Figure 4). Some exceptions led to missed triggering because the change in LC condition was dramatic enough to change the elution order of the marker compounds relative to the dependents.

Improved Scout triggered MRM performance with more markers

In Scout triggered MRM RT mode, higher MRM concurrency, especially in regions where transitions overlap between different acquisition windows, might decrease dwell times and potentially increase the cycle time. Impacts to cycle time risk compromising signal quality but this can be easily mitigated by introducing more marker compounds to narrow the concurrency into more defined acquisition windows. With Scout triggered MRM RT, the user only needs to manage a set of acquisition windows with associated markers, rather than all the individual RT windows when using sMRM. This makes method building and maintenance easier for the user while still providing the advantage of minimizing peak cut-offs from RT shifts.

Comparing data quality to Scheduled MRM algorithm

An example pesticide, omethoate, was used to demonstrate comparison of quality between data acquired by sMRM and Scout triggered MRM. Similar data performance between the 2 modes was observed based on the peak area, ion ratio and number of data points acquired across half peak height. For omethoate, however, it can be assumed that further RT shift in sMRM mode would risk cutting off the peak and the sample would need to be reacquired with a modified RT window. In contrast, in Scout triggered MRM acquisition, omethoate is less sensitive to RT shifts within its acquisition window (Figure 5).

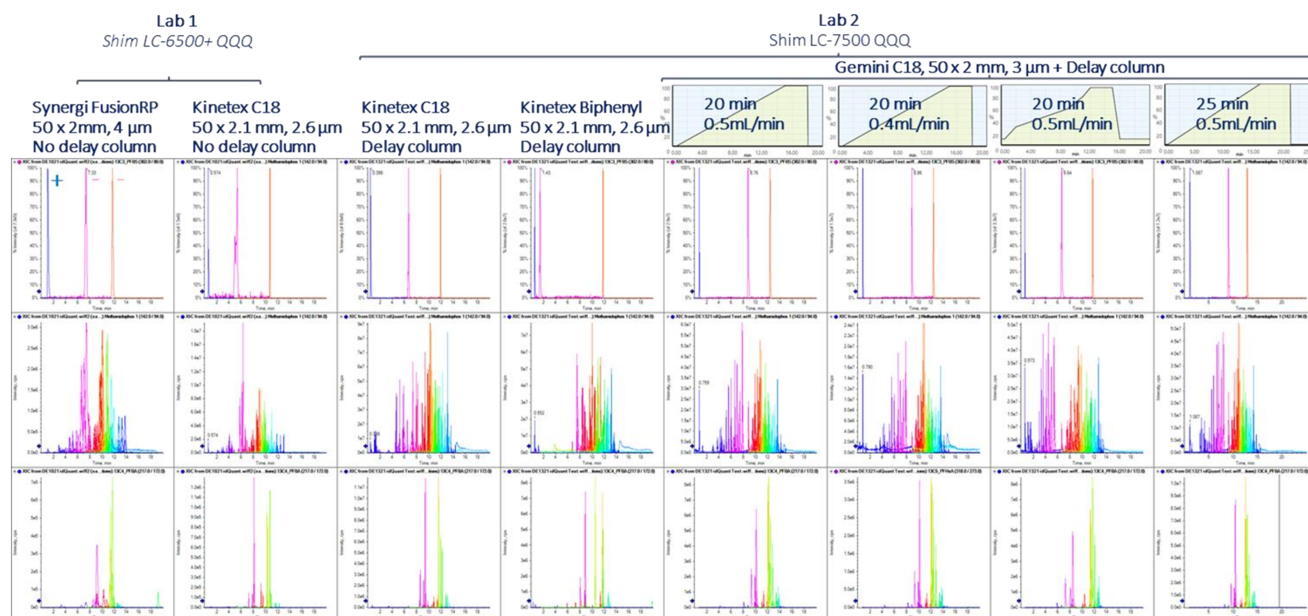

Figure 4. Testing robustness of the Scout triggered MRM approach to varied chromatographic challenges. Despite changes to the LC system, such as adding a delay column, changing laboratories and altering flowrate, the Scout triggered MRM workflow was able to trigger and detect the target analytes. The workflow is shown to overcome common method adjustments that affect RT without requiring major rebuilding of the acquisition method.

Conclusions

Developing and maintaining large MRM assays can be challenging. Time scheduling MRM acquisition with retention time windows is required to achieve good assay performance. However, if retention times shift, peaks might shift outside the detection window and be missed. Scout triggered MRM alleviates this issue by removing the need to maintain retention time windows for individual compounds.

Most common adjustments to methods that routine laboratories encounter, such as switching LC systems, adding a delay column or refining LC method parameters, can lead to retention time shifts. These scenarios were tested here to demonstrate the ability of the Scout triggered MRM method to acquire the target panel data, even when retention time shifts occur. Larger perturbations to elution order may still require acquisition method adjustment in some cases. Preserving data quality, such as maintaining appropriate cycle and dwell times for method sensitivity and precision, can be achieved by the addition of more marker transitions. This is analogous to narrowing RT windows in a sMRM method and is considered an advantageous tradeoff for a method with increased resilience against RT shifts and need for reinjection.

Scout triggered MRM workflow introduces, for the first time, an ability to trigger data acquisition between experiments, such as a marker being shared between positive and negative ion modes. This brings increased flexibility to the final assay and allows for refinement in the Scout triggered MRM method development.

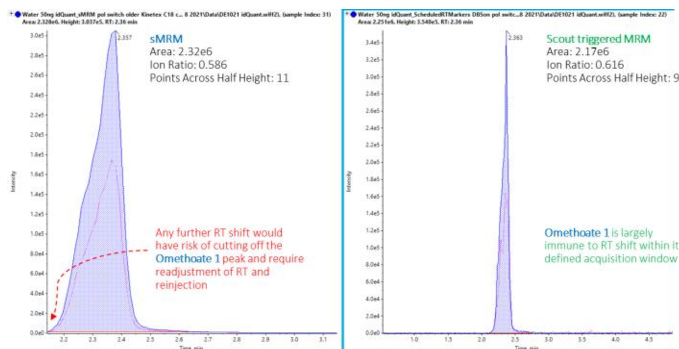

Figure 5. Omethoate in sMRM vs. Scout triggered MRM. Data quality is comparable between sMRM and Scout triggered MRM modes for parameters such as peak area, ion ratio and points across half height. A slight RT shift in the sMRM trace risks this peak becoming cut off or missed, rendering the sample unusable and requiring reinjection. In contrast, the Scout triggered MRM peak is less sensitive to this.

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