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New model to optimize triple quadrupole mass spectrometer performance for fast SRM methods

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Abstract

Purpose: Improve efficiency and speed of high throughput triple quadrupole MS operation for complex assays.

Methods: Interscan delays are calculated based on expected settling times for voltages on instrument optical elements and time-of-flight of ions through the system. Real-life assays are used to evaluate practical benefits of SRM speed increase on instrument performance.

Results: Experiments with pesticide and peptide assays showed potential for a sizeable increase in effective duty cycle when adopting the new model for settling time. Opportunity for increasing dwell times by a factor of 2 to 4, and maximum SRM speed above 800 s^{-1} were demonstrated.

Introduction

We had previously reported improvements to timing control of voltage changes in fast Triple Quadrupole SRM sequences. Recently we have implemented a comprehensive time-of-flight and settling time formula that allows maximizing throughput of SRM analysis and is achieving record high speeds while maintaining data integrity. Final parameters for the model were selected as a combination of predicted ion dynamics as well as empirical relationships based on the observation of the actual ion signal settling.

The validity of the new calculation approach has been verified using both test methods with constant dwell time transitions as well as a real-life pesticide panel with timed acquisition windows. We established that timing settings could be further optimized for different collision cell conditions.

Materials and methods

For a practical assay example, food samples were extracted using a modified Quick Easy Cheap Effective Rugged and Safe (QuEChERS) preparation protocol and without further clean-up. A large panel of pesticides was spiked into the extracts at a level corresponding to 10 µg/kg in the sample. The extracts were analyzed by LC-MS/MS using a validated method based on a Thermo Scientific™ Vanquish™ Flex UHPLC System connected to a Thermo Scientific™ TSQ Altis™ Plus mass spectrometer. A total of 595 compounds (526 in ESI+ and 69 in ESI-) were monitored in 22 minutes. The MS method contained 1186 SRMs acquired in timed SRM mode (SRM acquisition in a short retention time window) with dwell time prioritization and polarity switching.

Results

Calculation of settling time

In a multiple SRM method, instrument setting parameters are adjusted each time the next SRM transition is analyzed. Two major cases include switching between two different precursor ions (A) and two different product ions (B). The instrument has to wait for the amount time that it takes for the established flow of the next product ion to reach the detector. Figure 1 shows different contributions to the overall settling time.

Following is a set of simplified formulas to estimate it for cases A and B.

$$Case\ A\text{:}\ T_{SRMQ1} = \max[T_{TofQ1}^{precursor} + T_{setQ1}^{precursor} + T_{TofQ2}^{product}, T_{setQ3}^{product}] + T_{TofQ3}^{product}$$

$$Case\ B\text{:}\ T_{SRMQ3} = \max\left[T_{setQ2}^{product} + T_{TofQ2}^{product}, T_{setQ3}^{product}\right] + T_{TofQ3}^{product}$$

These are simplified for the case where the change in m/z between adjacent precursors in the SRM list is not very large, so the settings of SRIG and Q0/Q00 voltages have little effect on intensity. Those would need to be taken into account in a more general case.

Results

Specific dependency of the factors in the above equations varies depending on the type of the parameter. For example, the time-of-flight through devices can be reasonably well described as, for example,

$$T_{TofQ1}^{parent} = \alpha_{TofQ1}\sqrt{m/z}$$

Coefficients α_i depend on such factors as ion energy going through the multipole and pressure in the enclosure (in case of collision cell) and can be measured using various diagnostics routines.

For the voltage settling times certain empirical relationships can be established, and appropriate proportionality coefficients can be measured as well.

Figure 2 shows a typical signal stabilization curve vs. time that allows to estimate settling time for the SRM signal. Based on the acquisition of multitude of such curves in different conditions, plots like those in Figure 3 can be compiled. They show dependence of signal settling on both m/z of the ion as well as the amount of "mass jump" between two adjacent transitions. One can see that the dependence of time on the mass jump can be reasonably well approximated by a logarithmic function, particularly towards higher values.

Therefore, we used a dependence such as one below to approximate voltage settling timing.

$$T_{setQ1}^{parent} = \alpha_{setQ1}\log(m2 - m1)$$

Figure 1. Contribution of different factors to settling time

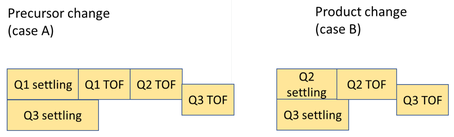
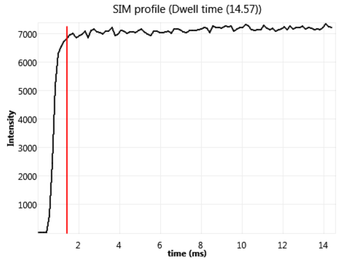
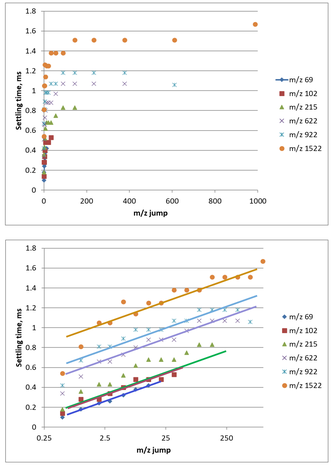


Figure 2. SRM transition settling curve (example)



Results

Figure 3. Signal settling time vs. target mass and "mass jump" between transitions. On the top, a linear scale is used for the jump amount, and on the bottom, it is presented on a logarithmic scale.



Initial values for all parameters were picked by fitting curves to plots such as one in Figure 3, and then refined to obtain fastest performance without compromising detected ion intensity. We had to pick a certain criteria for the latter, such as conservation of 80% of the signal level for the shortest dwell time (0.25ms) compared to the full intensity, which can be measured either by using a method with longer dwell time, or by inserting a "dummy" transition ahead of the test one which uses essentially the same m/z for both precursor and product.

Table 1. Except from a modified pesticide (left) and peptide (right) methods

	Compound	Precursor (m/z)	Product (m/z)
328	Metconazole	320.35	70.104
329	Metconazole	320.35	125.087
330	Iprovalicarb	321.2	91.241
331	Iprovalicarb	321.2	119.24
332	**EMRS 322**	322	322
333	**EMRS 322-1**	322	322.05
334	Pyriproxyfen	322.35	96.126
335	Pyriproxyfen	322.35	129.2
336	Pyriproxyfen	322.35	185.153

	Compound	Precursor (m/z)	Product (m/z)
291	GISNEGQNASIK (	613.316	426.28
292	GISNEGQNASIK (	613.316	540.323
293	GISNEGQNASIK (	613.316	668.381
294	GISNEGQNASIK (	613.316	725.403
295	GISNEGQNASIK (	613.316	854.445
296	GISNEGQNASIK (	613.316	968.488
297	GISNEGQNASIK (	613.316	1055.52
298	GISNEGQNASIK (	613.316	1168.604
299	**EMRS 622**	622	622
300	**EMRS 622-1**	622	622.05

Results

Simplified pesticide and peptide assays test results

To evaluate the optimized settings two multi-SRM methods were used. They were based on model pesticide and peptide assays supplied by the Thermo Scientific™ Xcalibur™ Method Editor software. Both consisted of about 500 transitions. Table 1 shows excerpts from the transition lists. No timed windows were used, and some transitions were replaced with those reflecting m/z of ions from a standard Extended Mass Range calibrant solution (EMRS).

After parameters' optimization using rather conservative higher maximum SRM rates were achieved for different dwell times. In addition to better SRM rates, and in fact more importantly, longer dwell times can be used for the same cycle time with the new model which directly translates into sensitivity improvements.

The peptide assay is different from the pesticide one due to more numerous product ions for each precursor. Since it generally takes shorter time to switch between different product ions, that assay could allow speeds in excess of 800 SRM/s. The Table 2 bellow summarizes the improvements for both assays.

Table 2. SRM rates per second for the regular and new settling time modes for two assays and different dwell times. Gains in duty cycle are also given.

Assay	Pesticides			Peptides		
Dwell time	0.25 ms	0.5 ms	1 ms	0.25 ms	0.5 ms	1 ms
Standard model	467	416	375	625	540	424
Non-linear model	716	605	456	830	688	509
Duty cycle gain (same # transitions)	4	2.5	1.5	2.6	1.8	1.4

One important aspect that needs to be taken into account is variability of instrument settings between units. The highest possible performance can be achieved if tuning of optical elements is minimized. Alternatively, either fixed or certain minimum voltage gradients are established to assure sufficient speed of ions through the devices. We have been working worked on implementing such tuning approach for the regular instrument control software.

The other critical aspect is to adjust the expected time-of-flight through the collision cell if certain assays benefit from higher gas pressure. This requires including pressure factor in the above formulas. This feature's implementation is ongoing.

Real-world pesticide analysis in food samples

To confirm that the gains observed with test assays are realized in real-world applications, food samples extracted using a modified QuEChERS protocol were analyzed by a method containing 1186 transitions acquired in timed SRM mode with polarity switching.

We focused our attention on the portion of the chromatogram with the most simultaneous transitions per second (about 250, see Figure 4). In order to make the experiment more challenging we increased the number of points per chromatographic peak which pushed minimum dwell times to under 0.4ms. Then the data was acquired using both standard and non-linear settling time model.

As shown for a few selected compounds in Table 3, the reduction of cycle time from about 0.90 s to 0.59 s caused deterioration of RSDs for multiple injection series. This was due to estimated reduction of dwell time by 4x and duty cycle by 2.5x, which thus could have negative effect on the limit of quantitation. With the new model in place the dwell time was recovered, and the RSDs were improved.

Results

Moreover, even with a further reduced cycle time (0.36s), the overall duty cycle was better with the non-linear model, and the observed RDSs were comparable to the case of long cycle time (0.90s) with the standard model.

Reported peak intensities for non-linear model at the shortest dwell times was on average 85% of that for the standard model which was a good match for the original criteria.

In conclusion, shorter cycle times (or the higher number of points per chromatographic peak) could be achieved without performance degradation and sensitivity reduction. This also opens the door for further increase of the number of analytes in complex assays.

Figure 4. Timed SRM distribution plot showing the highest concentration of transitions around 14 min of retention time.

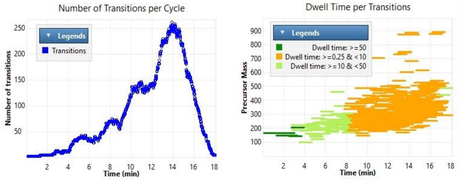
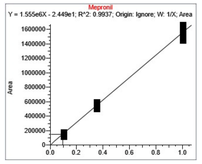


Table 3. Comparison of %RSD of injection series for a few pesticides, different models and cycle times at 0.1 ppb loading. Typical dilution curve is shown on the right. Peak areas around 10^5 may correspond to about 100 ions in a peak.

Model	Standard		Non-linear	
Cycle time, s	0.9	0.59	0.59	0.36
Coumaphos	17	22	8	17
Mepronil	11	20	12	15
Tebufenozide	16	29	13	11
Flufenacet	6	23	11	13



Conclusions

Application of the new non-linear model for predicting signal settling times in multiple SRM methods improved the instrument duty cycle which led to better sensitivity for complex assays. Maximum practical SRM speeds were shown to exceed 800 s^{-1} for certain assays.

Acknowledgements

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