

Timed SRM: Improved Capabilities for Multi-target Compound Analysis

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This technical document describes the use of unique timed SRM experiments on the Thermo Scientific TSQ Quantum XLS to achieve

- Higher sensitivity
- Higher number of target compounds
- Higher selectivity in real life matrix samples

Introduction

Multiple Reaction Monitoring (MRM) using the selected reaction monitoring technique (SRM) is the key operating mode for target compound quantitation with a triple quadrupole mass spectrometer. The TSQ Quantum XLS™ now offers improved analytical capabilities for higher sensitivity with multi-component trace target compound analyses. The new TSQ Quantum XLS features are of particular advantage for the low-level analysis of a large number of pesticides or drugs as target analytes in real-world matrix samples. The total number of compounds that can be included in the analysis has further been increased. Considerably more than 1000 compounds can be monitored with two SRM transitions each from different precursor ions, making the TSQ Quantum XLS the most powerful and most sensitive triple quadrupole GC-MS system today.

While *selected ion monitoring* (SIM) is a typical expression known from single quadrupole (SQ) mass spectrometers, the acronym SRM stands for the typical operation mode of *selected reaction monitoring* on a triple stage quadrupole mass spectrometer (TSQ) describing one single transition from a parent to a product ion.

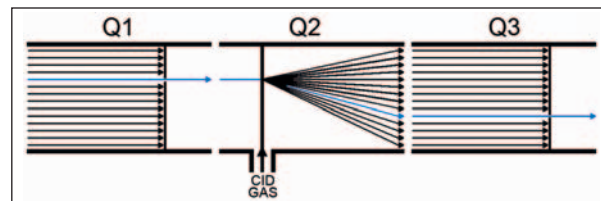


Figure 1: SRM transition process in a TSQ mass spectrometer: Q1 selects the analyte precursor ion, Q2 induces fragmentation, and Q3 measures an analyte specific structure fragment. Several consecutive SRM transitions form the MRM method.

While SIM and SRM are IUPAC terminology recommendations, the term MRM is commonly used describing the *multiple reaction monitoring* acquisition setup for a typical multi-component run using as many as several hundred consecutive SRM transitions. Both modes – SIM and SRM/MRM – are used for target compound quantitation in trace analysis and provide the potential for a highly sensitive detection.

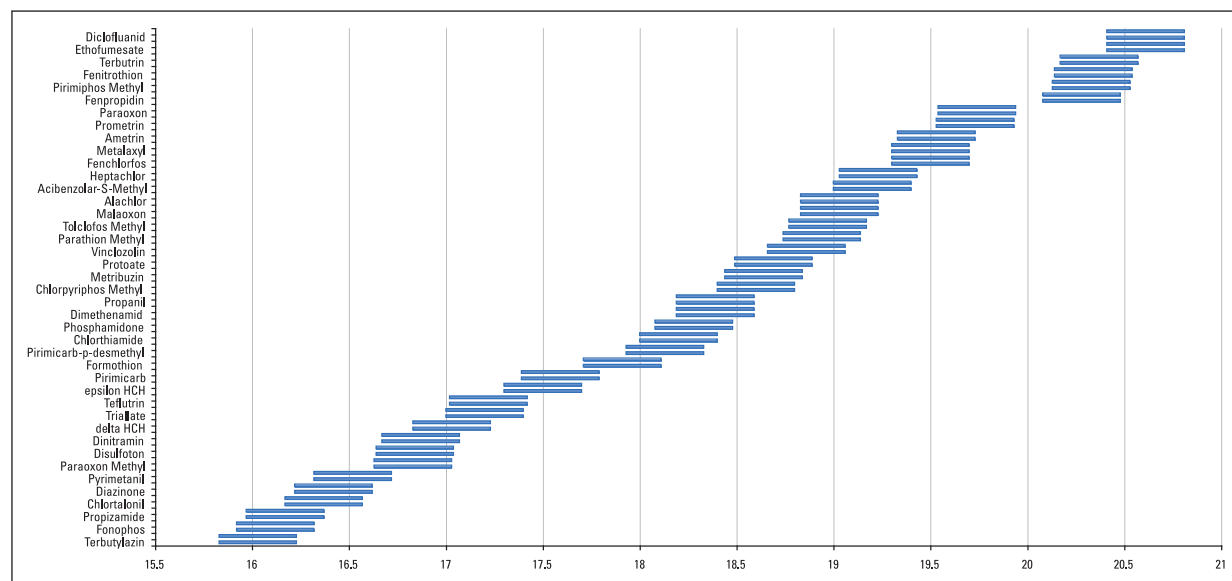


Figure 2: t-SRM setup on the TSQ Quantum XLS with 41 pesticides in a 5 min retention time window (same compounds and retention time window as in Figure 4) with two SRM transitions each. The number of SRM transitions that are acquired in parallel is significantly reduced, which shows the potential to speed up analysis times.

Key Words

- Dwell Time
- GC-MS/MS
- MRM Methods
- Quantitation
- Target Compound Analysis
- Timed SRM
- Triple Quadrupole

Segmented SRM Mode

As known from selected ion monitoring (SIM) experiments on a single quad GC-MS, masses to be recorded during a GC run are programmed by retention time. Typically, retention time windows are used. A similar analytical setup is used with triple quadrupole mass spectrometers using SRM transitions. The SRM time windows are programmed for data acquisition using the retention time windows in which the target analyte is expected to elute from the column. A sequence of retention time windows is used to cover the complete chromatogram in segments. Each window typically covers about 20 or more analytes, e.g. the target pesticide components. This data acquisition mode is commonly called “segmented SRM” mode (Figure 4).

The segmented mode offers a quick and straightforward solution for the setup of target compound methods on the TSQ Quantum XLS for a limited number of compounds with good peak separation on the chromatographic time scale. The chromatogram is segmented into several retention time segments that can be quickly filled up with the required SRM transitions of compounds eluting in this segment. Note that when using this method, it is preferable to switch segments in retention areas that are free of eluting analytes.

In some cases, overlapping windows may be required (Figure 4). With an increased number of components and increased coelution of compounds, it becomes more difficult to determine the window limits. Many compounds in a segment, additional transitions, and overlapping masses limit available dwell times for targets. The final number of segments to be used depends on the number of compounds to be detected. In segments with many analytes, there are a limited number of compounds in the segmented SRM mode that can be detected in parallel due to short dwell times for each compound. Typically the data acquisition for pesticides is using two SRM transition per compound. Also, the minimum number of data points defining the GC peak can limit dwell times for areas of multiple coelutions. This situation may require longer GC runs and longer retention times for better compound separation.

NEW Timed SRM with EZ Method Editor

The new “timed SRM” acquisition mode for multi-target component analysis significantly widens the scope of application of the TSQ Quantum XLS for a practically unlimited number of compounds. A new *EZ Method editor* for the setup of the data acquisition is provided for the use with timed SRM (t-SRM). For the first time the compound name, retention time and mass acquisition details come together in an MS method setup. Another important feature for flexibility and ease-of-use is provided with the EZ Method editor. t-SRM methods can be designed and maintained in an offline environment using standard spreadsheet programs (Microsoft® Excel®, OpenOffice™, etc.) and copied easily via *.csv format files to the TSQ Quantum XLS data acquisition in EZ Method.

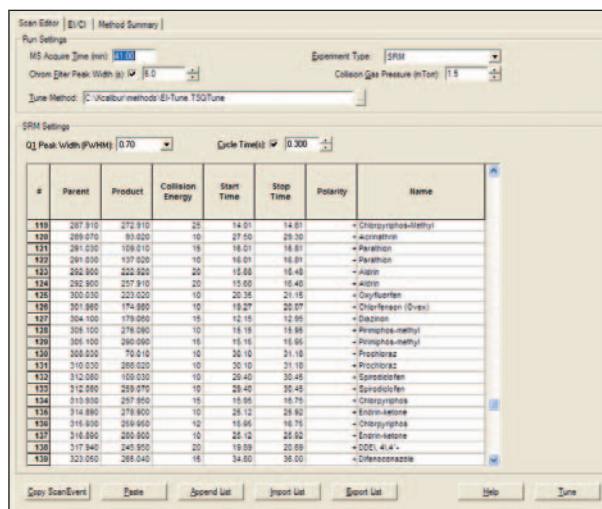
Timed SRM uses a completely different analytical strategy than the segmented setup. The SRM data acquisition window in t-SRM uses the compound retention time. The data acquisition for a particular target compound is done only in a short retention window around the known compound retention time, and not in a wide retention time segment. The compound acquisition window can also be individually set to cover close elution isomers. Figure 2 shows the t-SRM settings for the same compounds and retention time window that were demonstrated in Figure 4 with the former segmented SRM mode.

Timed SRM for Pesticide Analysis

Using timed SRM significantly reduces the number of SRM transitions that are monitored in parallel at a certain retention time. At a constant acquisition rate (cycle time) a significantly longer measurement time (dwell time) is available for each transition resulting in higher sensitivity and lower quantitation limits, improved RSDs and the potential for even more data points per chromatographic peak.

Figure 5a clearly demonstrates the difference of the both acquisitions modes when monitoring the same number of pesticide compounds, with the increased signal to noise ratio (S/N) on the t-SRM traces. The same peaks are displayed in Figure 5b in the bar graph mode for easy display of the increased number of data points over the chromatographic peak. Data acquisition rate details can be seen in the zoomed view. An ideal peak profile for optimum peak area integration is achieved. With the given setup, increased sensitivity using longer dwell times per compound or additional capacity for monitoring more coeluting compounds can be achieved, depending on the analytical requirements.

The use of t-SRM is especially recommended for the setup of multi-component analyses, e.g. for the “500 Pesticides Method”, monitoring of prescribed drugs and drugs of abuse, POPs including dioxins and dl-PCBs and similar large target compound lists.¹



#	Parent	Product	Collision Energy	Start Time	Stop Time	Polarity	Name
118	287.910	272.910	25	14.01	14.01	-	Chlorzoxipros-methyl
120	289.070	18.020	10	27.50	29.50	-	Acromethin
121	291.230	109.010	15	18.01	18.01	-	Permethin
122	291.030	137.020	10	18.01	18.01	-	Permethin
123	292.000	222.020	20	18.01	18.40	-	Azin
124	292.000	237.910	20	18.01	18.40	-	Azin
125	300.030	223.020	10	20.38	21.18	-	Oxyfluorfen
126	301.980	174.000	10	19.27	20.07	-	Chlorfenzapir (Diast)
127	304.000	179.000	10	12.16	12.66	-	Imidacloprid
128	308.100	176.090	10	18.18	18.98	-	Permethin-methyl
129	308.100	280.080	15	18.18	18.98	-	Permethin-methyl
130	308.020	70.010	10	30.10	31.10	-	Prochloraz
131	310.020	200.020	10	30.10	31.10	-	Prochloraz
132	312.080	109.030	10	20.40	20.45	-	Sprindicifen
133	312.080	259.070	10	20.40	20.45	-	Sprindicifen
134	313.000	207.000	15	18.00	18.75	-	Chlorzoxipros
135	314.880	178.800	10	25.12	26.62	-	Enzin-ketone
136	316.000	209.000	12	19.00	19.75	-	Chlorzoxipros
137	318.000	200.000	10	25.12	26.62	-	Enzin-ketone
138	317.840	245.800	20	19.00	20.00	-	ODEL, et al.
139	323.040	269.040	18	14.00	16.00	-	Chlorocresazone

Figure 3: New EZ Method t-SRM acquisition page. The t-SRM table includes compound name, retention time, precursor, product ion, polarity and collision energy.

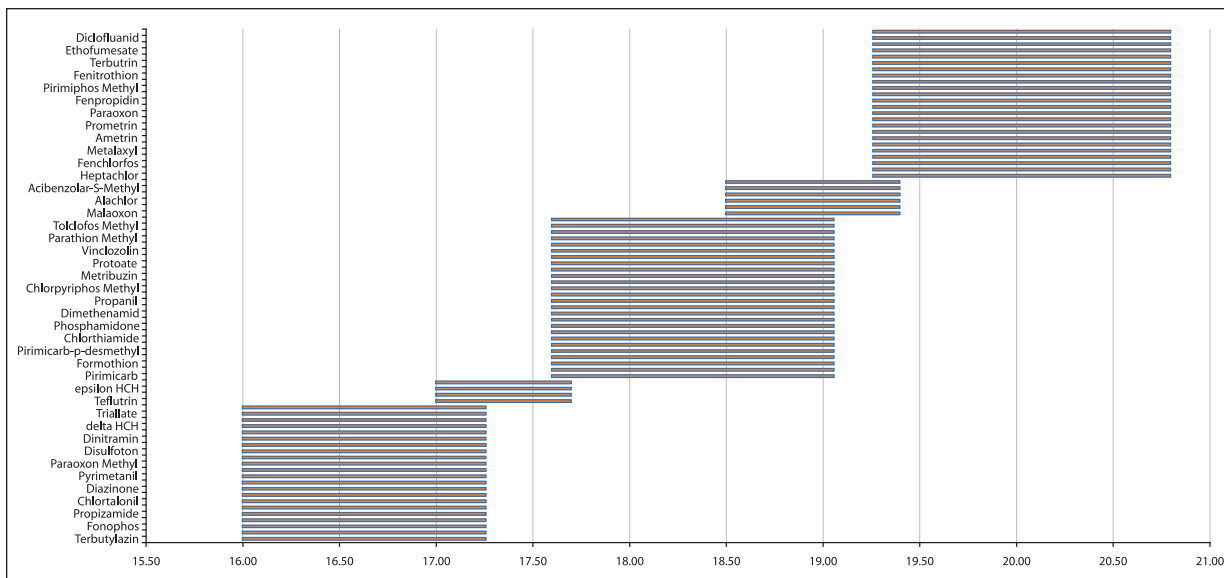


Figure 4: Sequential SRM data acquisition – The retention time segment of 16:00 to 21:00 min is shown with the sequential acquisition setup for 41 pesticide compounds eluting in this window. Time segments have been defined that cover up to 13 compounds for parallel detection.

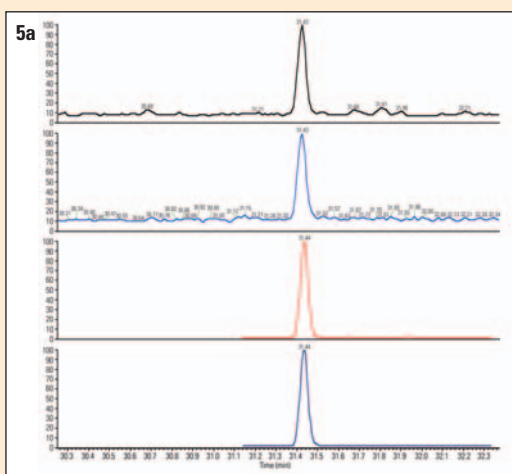


Figure 5a: Compare segmented SRM with timed SRM for sensitivity in pesticide analysis: The top two traces (black and light blue) show the use of segmented SRM mode with 29 parallel transitions. The bottom two traces (red and dark blue) show timed SRM mode, with improved signal-to-noise ratios.

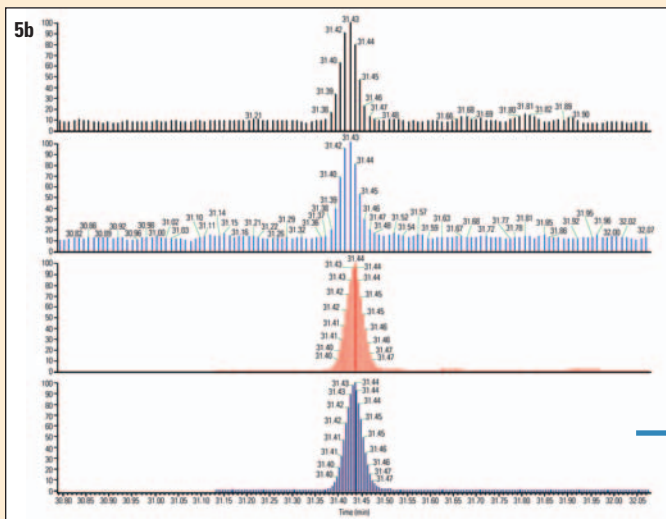


Figure 5b: Compare segmented SRM with timed SRM by acquisition rate: The top two traces (black and light blue) show segmented SRM mode with 29 parallel transitions, 10 ms dwell time each, 10 data points/peak. The bottom two traces show timed SRM using 150 ms/transition with 300 ms cycle time, 28 data points/peak (see peak detail to the right). Using t-SRM allows for a large number of points across the chromatographic peak.

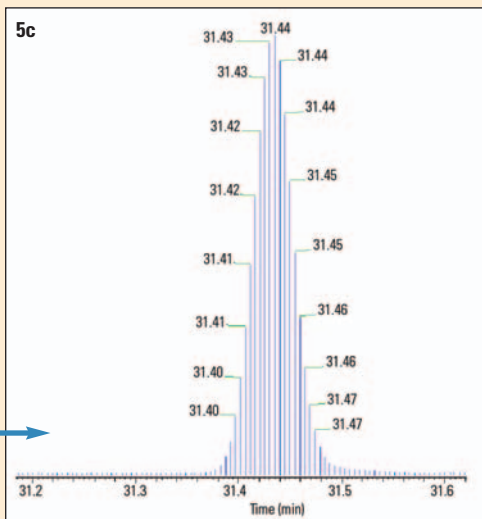


Figure 5c: Zoom view of the t-SRM peak of Figure 5b

Analytical Benefits of t-SRM

There are numerous analytical benefits for operation of the TSQ Quantum XLS using the EZ Method with t-SRM:

- Higher Sensitivity – Optimum dwell times for each component screened
- Constant Sensitivity – Independent of the total number of compounds in the t-SRM acquisition method
- More target compounds – A total of 3000 SRM transitions can be monitored in one run
- Easy method maintenance – The EZ Method editor covers compound name, retention time and acquisition details
- Flexible method preparation – Import of *.csv files for multi-compound data acquisition
- Rugged method design – Sufficient retention time window of 30 s or less covers minor peak shifts due to variable matrix conditions with no compromise on dwell times
- Individual acquisition windows – Isomers, e.g. HCHs, pyrethroids, etc., can be detected in one window
- Safe compound detection – Screening is not affected by slight matrix-induced retention time shifts
- Compliant with international directives – Two SRM transitions from individual precursors for each compound guaranteed
- Earn maximum number of identification points – Monitor two precursor ions, each with one product ion²

- Screening and compound confirmation – Earn maximum identification points for reliable target confirmation²
- More safety with varying matrices – More SRMs transitions per compound possible for changing matrix samples; use the Thermo Scientific QuanLab Forms local method editor to easily adjust as needed
- Maintain chromatographic resolution – Optimum number of data points for each chromatographic peak
- Highest selectivity in matrix samples – t-SRM runs with highly resolved SRM mode (H-SRM) for higher sensitivity with real-world samples
- Higher productivity – Prepared for fast GC on short columns with higher master acquisition rate

Both modes – *segmented SRM* and *timed SRM* – are available with the TSQ Quantum XLS for ease-of-use for both low and very high number of target compound analysis methods and for compatibility with already established and validated lab methods.

Further Reading

1. The Pesticides Analyzer Reference Manual, Thermo Fisher Scientific Inc., PN 120390, Revision A, May 2009.
2. COMMISSION DECISION of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities, 17.8.2002.

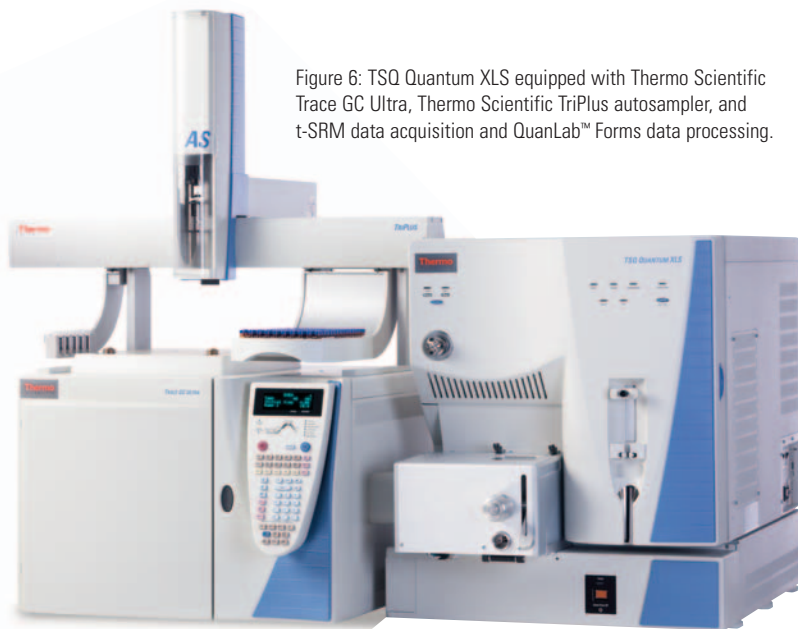


Figure 6: TSQ Quantum XLS equipped with Thermo Scientific Trace GC Ultra, Thermo Scientific TriPlus autosampler, and t-SRM data acquisition and QuanLab™ Forms data processing.

Original data acquired using the Thermo Scientific TSQ Quantum GC. Performance of the Thermo Scientific Quantum XLS typically meets or exceeds these results.

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