Simplifying Complex Multi-Residue Pesticide Methodology in GC-MS/MS

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Overview

Easing Implementation of Multi-Residue Pesticide Methodology

The task of setting up a triple quadrupole GC/MS pesticide analysis can be daunting, regardless of your starting point. Perhaps you are brand new to GC/MS pesticide analysis, and you need all the help you can get. Maybe you analyze a small set of pesticides and want to expand your target list, or you analyze a large pesticide set in multiple runs on a single quadrupole and want to combine these into a single MRM analysis. Perhaps you already have a comprehensive MRM method, but want to move this to a Thermo Scientific™ TSQ™ 8000 triple quadrupole GC-MS/MS system to take advantage of its robustness, removable ion source under vacuum, and its ease in adding new target pesticides through AutoSRM. Whatever your starting point, when adopting new technology to address complex analytical challenges, you need tools that enable you to be productive, quickly.

With your needs and requirements in mind, the Thermo Scientific TSQ 8000 Pesticide Analyzer (Figure 1) has been developed. Provided within this comprehensive package are all the tools you need to set up a complex pesticide method, regardless of your starting point.

Everyone who is new to pesticide analysis on the TSQ 8000 GC-MS/MS system will appreciate the provided list of optimized pesticide transitions. Also, with an easy to follow step-by-step description of how to develop new transitions using AutoSRM, you'll find the ease of adding new pesticides to your MRM method is now a competitive advantage for your laboratory. And for those who need more assistance, the TSQ 8000 Pesticide Analyzer contains a complete instrument method developed on an included column with provided compound retention times and MRM parameters—eliminating days, if not weeks, of method development.

Figure 1. The TSQ 8000 Pesticide Analyzer. Details of its contents can be found in the *TSQ 8000 Pesticide Analyzer Brochure (BR10318)*.

In addition to simplified method startup, another advantage of using the analyzer is that it utilizes Timed-SRM methodology, allowing for easy-to-use, high-analyte-capacity methodology. The usability and scanning efficiency of Timed-SRM are complemented by the fast-scanning capability of the TSQ 8000 instrument, making the analysis of hundreds of pesticides, with a total of over one thousand transitions, not just possible, but easy.

Finally, the TSQ 8000 Pesticide Analyzer has the ability to analyze full scan data at the same time as your targeted MRM analysis. This allows you to harness the power of existing EI full scan libraries to, for example, find potential high-level contaminants you would otherwise miss in a targeted analysis, or monitor the matrix background for possible interference.

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Using the Startup Kit

Starting Point 1: Starting from Scratch

When creating your method within Thermo Scientific™ TraceFinder™ EFS software, the instrument control and data processing software included with the TSQ 8000 Pesticide Analyzer, the use of the TraceFinder Pesticide Compound Database (CDB) will greatly simplify the method development process. Multiple transitions for each compound in the database have been optimized on the TSQ 8000 instrument with AutoSRM to within ± 1 eV of the optimum collision energy.

Simply select the compounds of interest in the CDB (Figure 2). This will create not only the TraceFinder software processing method, but also the TSQ 8000 mass spectrometer acquisition list. Since the instrument employs Timed-SRM, SRM windows for data acquisition will be centered on your retention times, so that all peaks elute far from acquisition-window breaks. The complete step-by-step procedure, including software screen captures, is detailed in the *TSQ 8000 Pesticide Analyzer Installation Guide*, which is also included with the TSQ 8000 Pesticide Analyzer.

After selecting your compounds of interest, you are now ready to acquire samples in MRM with your TSQ 8000 instrument.

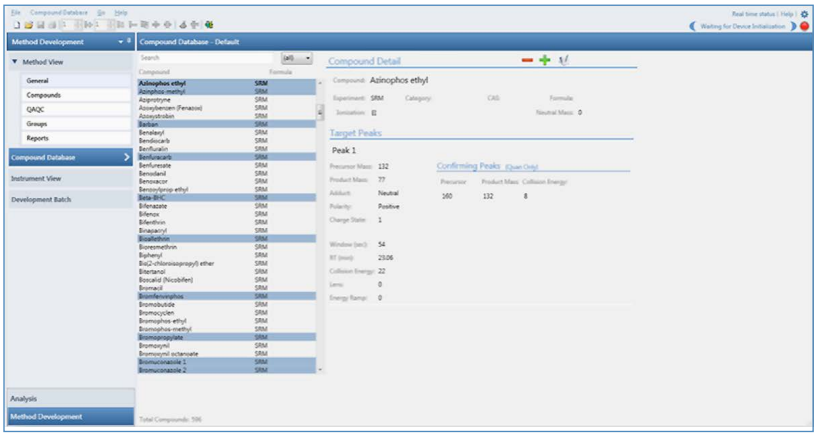

Figure 2. Selecting compounds from the TraceFinder EFS Compound Data Base. This will populate both your TraceFinder Processing Method and your acquisition list. For more information on creating TSQ 8000 methods with the TraceFinder CDB, see *AB52300: Thermo Scientific TSQ 8000 GC-MS/MS Method Sync.*

Starting Point 2: Starting from an Established GC Method

If you already have a preferred GC method, and know the retention times of your target compounds, you can update the pesticides in the CDB with the known retention times. Next, simply select the compounds you are interested in analyzing from the updated CDB, as shown in Figure 2. Again, this will create both the TraceFinder EFS processing method and the TSQ 8000 system Timed-SRM acquisition list, with acquisition windows centered on the retention times of the target peaks.

If you do not know exact retention times, you can easily widen acquisition windows while in TraceFinder EFS software for all compounds (Figure 3) to ensure your peaks fall within their acquisition window. Now update your TraceFinder EFS software method with the new retention times as you would in a normal data review, and your acquisition windows will be centered on each compound. After updating the retention times, follow the same step to reduce acquisition windows back to defaults in order to maximize dwell time for the analysis.

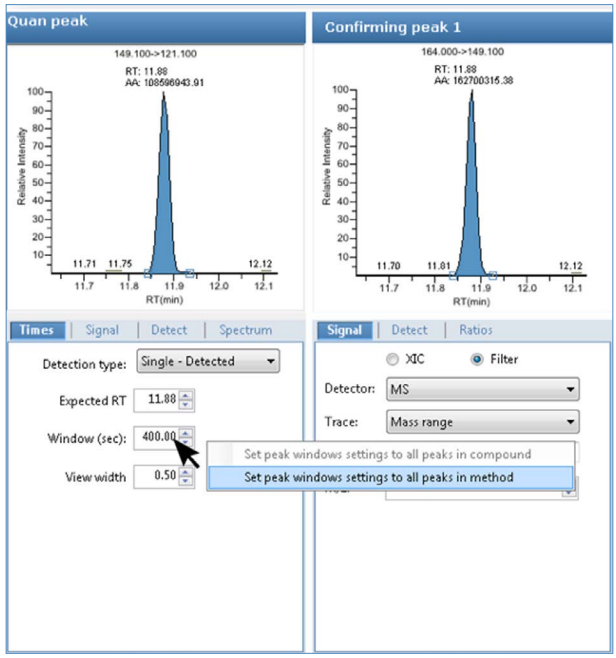

Figure 3. Widening acquisition windows in TraceFinder EFS software to find peaks with unknown retention times.

Tools to Get You Productive

The software features of the TSQ 8000 system have been designed with complex pesticide analysis in mind. These features include AutoSRM, a tool that makes the instrument the easiest for developing and adding new compounds to an existing pesticide method. Another useful feature is Timed-SRM, which enables accurate pesticide identification and quantitation, even for very dense pesticide methodologies. Finally, the ability of the TSQ 8000 instrument to perform simultaneous full scan/MRM provides the capability to identify general unknowns in conjunction with your target pesticides, filling a classic gap in targeted MRM analysis.

Addition of New Compounds

For those compounds provided in the TSQ 8000 Pesticide Analyzer CDB, the addition of new compounds to your methodology is extremely simple. If you are using the method and GC column provided with the TSQ 8000 Pesticide Analyzer, simply select additional compounds to your method from the CDB. The instrument software now adds the selected compounds to both the method acquisition list and the TraceFinder EFS software processing list with the correct retention times.

For those pesticides not yet in the TSQ 8000 Pesticide Analyzer CDB, AutoSRM can be used to quickly develop these new transitions (Figure 4). Once fully developed, the new compounds are easily imported into the CDB and added to your TraceFinder software method. A step-by-step walkthrough of this is described in detail in the *TSQ 8000 Pesticide Analyzer Installation Guide*, which is provided as part of the TSQ 8000 Pesticide Analyzer package. For more details on how AutoSRM works, see *AB52298: Introducing AutoSRM*.

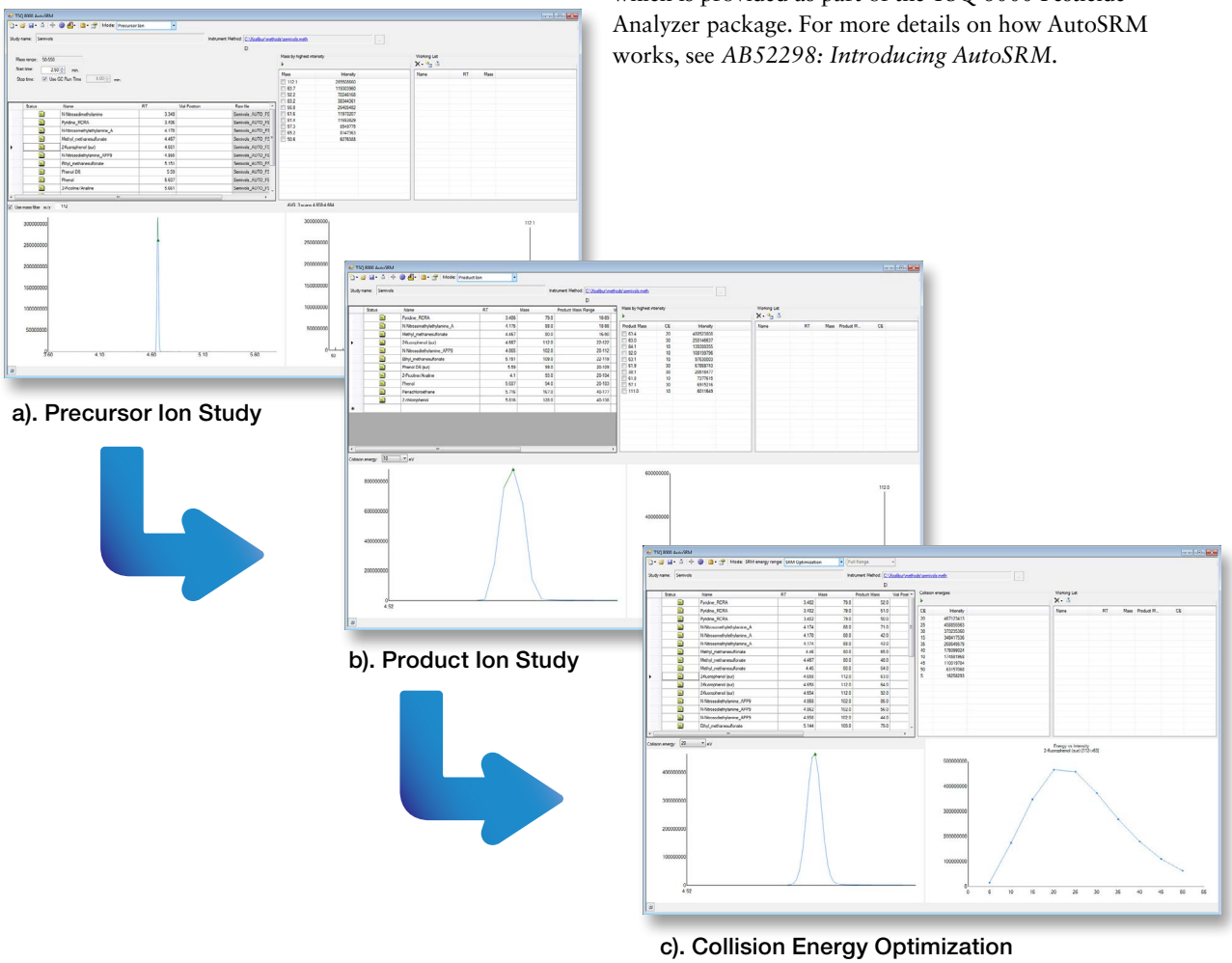

Figure 4. Screen shots showing the three-step process of AutoSRM. a.) In the first step, AutoSRM acquires full scan data for selecting precursor ions. b.) In the second step, product ions are selected from product ion scan data. c.) In the final step, collision energies are varied for each of the selected SRM's to determine the optimal collision energy.

High Compound Capacity Methods

One of the primary challenges of modern pesticide analysis is the sheer number of pesticides that need monitoring in order to meet international standards. This is particularly true in food analysis where products are transported across country borders, requiring exporters to meet the regulatory demands of many countries. Triple quadrupole instruments help meet this demand due to the high selectivity of MRM analysis, which allows for spectral separation of coeluting peaks due to unique reactions in the collision cell. This enables monitoring of more compounds in a single chromatographic run without prohibitive interference. However, due to the targeted nature of the MRM process, individual scan events must be created for each pesticide to be monitored, placing a strain on the amount of time devoted to the monitoring of each compound, and thus the sensitivity of the analysis of each compound.

With a traditional style analysis, this issue can be partially resolved by slicing up the acquisition list into discreet time segments, so that all transitions are not being monitored at the same time. However, this can quickly lead to problems when analyzing more than 50 pesticides in one run. This is because, due to the density of the peaks in the heart of the method, it is difficult to find a time for a segment break when no target peaks are eluting.

This then forces a compromise between adding many compounds per segment, reducing individual SRM dwell times and sensitivity, and adding segment breaks between closely eluting peaks, which causes the risk of false negatives due to shifts in peak retention times outside of acquisition windows because, for example, a large bit of matrix coelutes with a peak.

The TSQ 8000 system takes an approach called Timed-SRM that eliminates this compromise. Timed-SRM removes the limitations of segmented SRM by centering acquisition windows on the retention time of each peak and allowing for acquisition window overlap, so that acquisition windows for all nearby eluting compounds are not forced to start and stop at the same time (Figure 5). The user simply needs to enter the retention time of each compound, and the instrument method takes care of the rest, eliminating the need for creating segments.

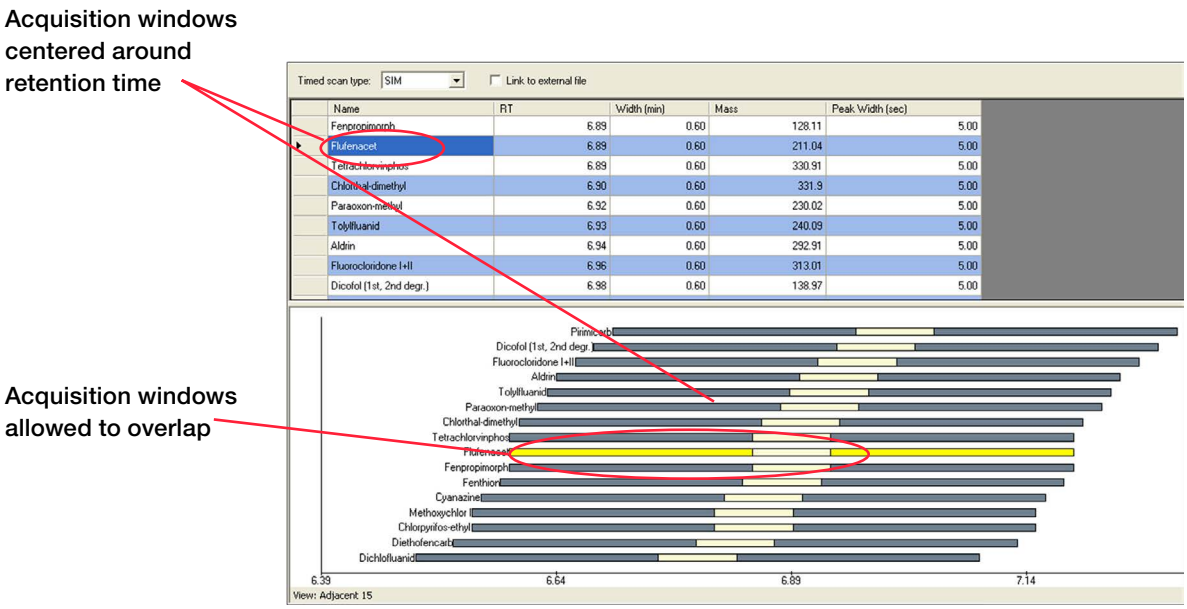

Figure 5. The TSQ 8000 system Timed-SRM Acquisition list, showing SRM acquisition windows centered on retention times and overlapping nearby transitions.

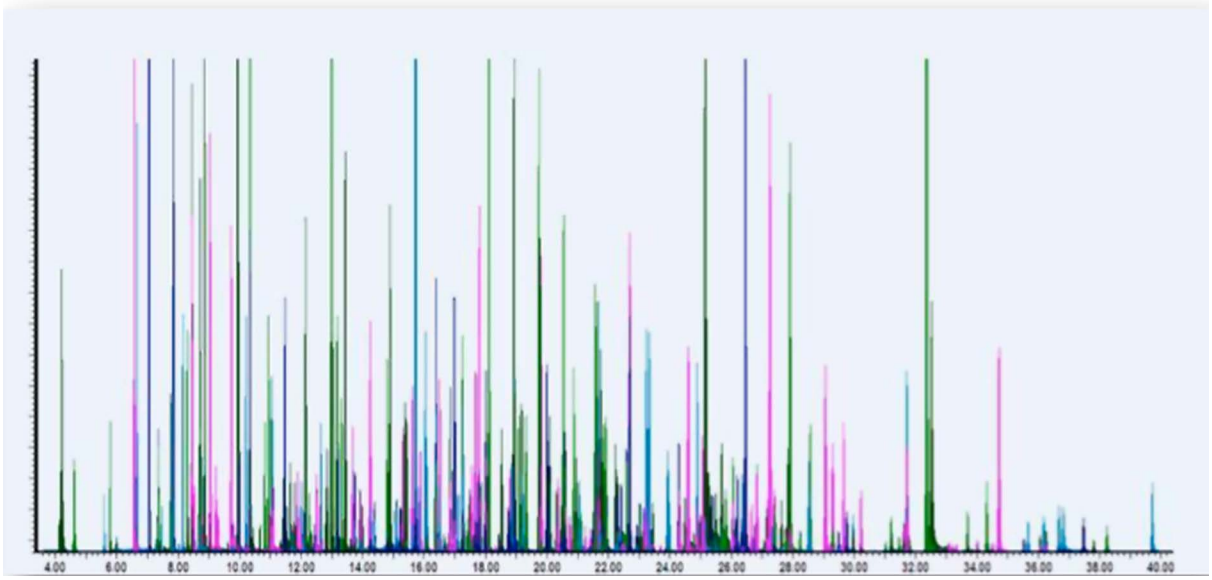

Figure 6. Real-world acquisition of over 300 pesticides in a single chromatographic run using Timed-SRM.

Figure 6 shows a real-world example of a pesticide analysis of over 300 compounds using Timed-SRM. As shown in the Table 1 comparison with Segmented-SRM, Timed-SRM increases both the sensitivity of the analysis by reducing the number of transitions being acquired simultaneously and the time between when target peaks elute and when their acquisition window begins or ends.

Table 1. Comparison of Segmented-SRM vs. Timed-SRM for method of over 300 pesticides. Timed-SRM can dramatically reduce the average number of transitions occurring simultaneously, while increasing the minimum time between an eluting peak and an acquisition window break.

	Segmented-SRM	Timed-SRM
Average number of simultaneous transtions during run	55 Transitions	15 Transitions
Shortest time interval between a compound retnetion time and an acquisiton window break	5 Seconds	15 Seconds

General Unknown Screening

Another limitation of the classic MRM approach to pesticide analysis is that, due to its targeted nature, if a compound is not part of your target list, you are not going to find it, even if it is present in large quantities in your sample. This limitation is removed with capability of the TSQ 8000 system to perform simultaneous full scan/MRM.

The TSQ 8000 system allows you to set up a full scan acquisition throughout the duration of your MRM analysis. Each acquisition will then have full scan data to identify non-target compounds, in addition to MRM data to confirm and quantitate the target list. This mode of analysis is facilitated with the TraceFinder EFS software qualitative processing view within its standard quantitative batch analysis, which automatically detects, identifies, and reports non-target compounds (Figure 7).

Figure 7. Qualitative view of TraceFinder EFS software for analyzing fruit juice with simultaneous full scan/Timed-SRM on the TSQ 8000 system. In addition to quantitating and confirming the 158 target compounds by MRM (top), TraceFinder EFS software has identified three high-level unknowns by full scan analysis (bottom): 2,4-bis(1,1-dimethylethyl)-phenol, triethyl citrate, and Vitamin E.

Conclusion

For the lab just starting up a complex pesticide analysis by triple quadrupole GC-MS, the TSQ 8000 Pesticide Analyzer offers the easiest and quickest path to success. The included methodology, consumables, and SRM transition list with accurate retention times enable the creation of your pesticide method to be as simple as selecting the compounds you want to analyze. With multiple SRM transitions per compound optimized to within ± 1 eV, the pesticide analyzer is useful for anyone who wants to take advantage of the unique features of the TSQ 8000 system designed to make complex pesticide analysis simple.

The TSQ 8000 Pesticide Analyzer fully takes advantage of these features, including the ability to do Timed-SRM, which significantly increases low-level sensitivity through a more efficient SRM scheduling. Also, the full scan/MRM capability of the TSQ 8000 mass spectrometer combines the elite quantitation capabilities of MRM analysis with classic general unknown identification through full scan quadrupole library searching. Finally, the ability to easily develop and add new pesticides to an existing pesticide method through AutoSRM makes the TSQ 8000 Pesticide Analyzer the most flexible system for expanding your pesticide target list to meet future regulatory or client demands.

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