Dynamic MRM Method Optimization for UHPLC-QQQ Multi-Residue Analytical Applications

ASMS 2009

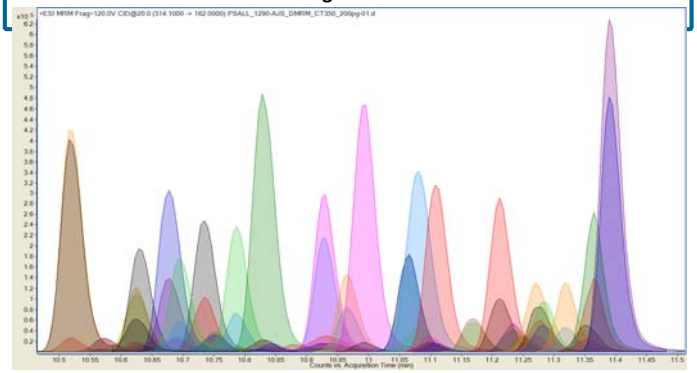
Harry Bunting, Jun Wang, Michael Flanagan, Agilent Technologies, Santa Clara, CA

WPY 604

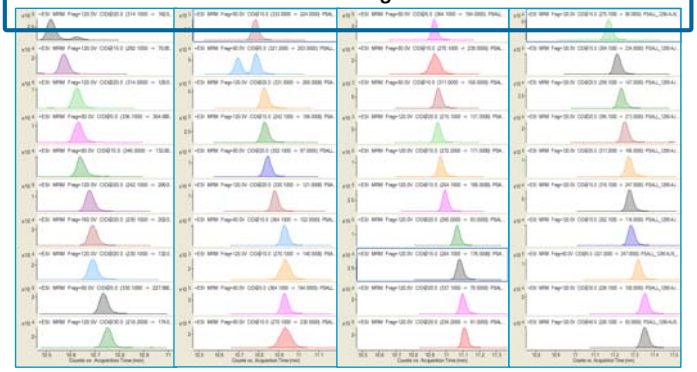
Introduction

Ultra High Performance Liquid Chromatography (UHPLC) is enabling efficient, high peak capacity separations which are finding increased relevance in complex, multi-residue analytical applications. As the need and challenge to rapidly analyze large numbers of analytes has grown, MRM methods with multiple time segments have been developed to reduce the scan cycle time and number of concurrent MRMs. These methods, however, have several critical limitations when applied to complex samples (e.g. several hundred pesticides). Dynamic MRM acquisition provides triple quad users with a new and rapid means to develop well-optimized multi-analyte LC/MS/MS methods. Utilizing analyte retention times, detection windows (Delta RT), and a constant scan cycle time, DMRM software automatically constructs DMRM timetables for the precise detection of multiple analytes as they chromatographically elute.

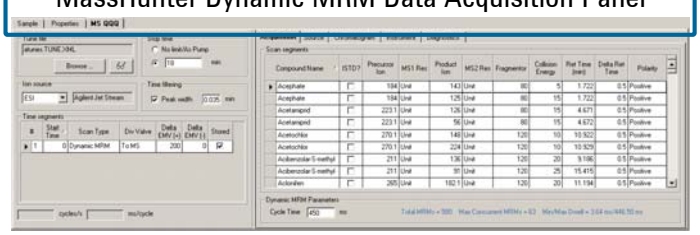
40 Pesticides Eluting in a 1 min RT Window



EICs of 40 Pesticides Eluting in a 1 min RT Window



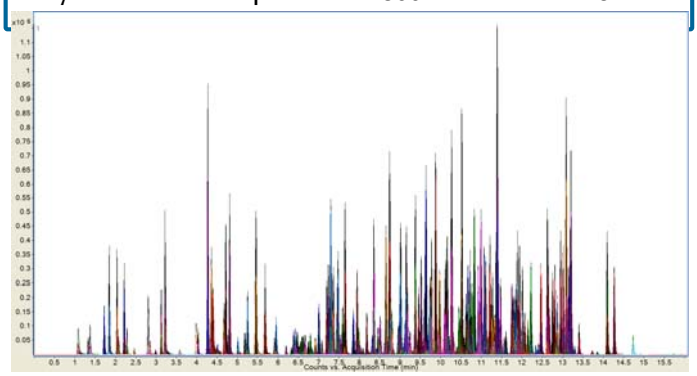
MassHunter Dynamic MRM Data Acquisition Panel



Experimental

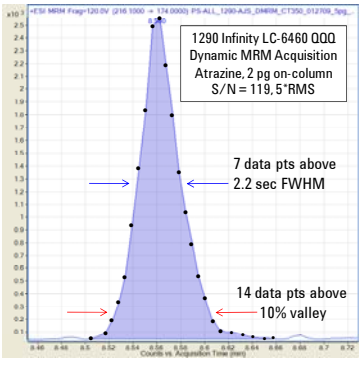
300 pesticides (600 MRM transitions) were analyzed in 15 min in dynamic MRM mode using a 1290 Infinity LC and 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology. The LC reversed phase column and flow rate were selected to maximize separation, MS sensitivity and analysis time. Calibration standards were analyzed in triplicate at 7 concentration levels ranging from 0.5 ppb to 500 ppb in solvent and in an apple extract. Data was also obtained in conventional MRM mode in four time-segments at 2 ppb to 100 ppb for statistical comparisons. Several pesticide DMRM methods were developed for RRLC and UHPLC with different mobile phases and gradient conditions. The methods include compound-specific MRM transitions/MS parameters information, RTs, and detection windows (Delta RT). The methods are stored in a compound database allowing users to select and import MRM compound information or build new acquisition methods.

Dynamic MRM acquisition of 300 Pesticides in 15 min



Dynamic MRM methods contrast with conventional MRM methods which rely on user-defined MRM time segments. With DMRM, specific analyte detection may take place over successive MRM timetables depending upon the number of concurrent MRMs and their respective RT detection windows. Within each DMRM timetable, all ion transitions will have the same dwell time, however, dwell times will vary for each timetable to ensure that all analytes are quantitatively sampled (i.e. provide a sufficient number of data points across all detected peaks). DMRM utilizes a constant cycle time for the DMRM timetables to ensure proper integration of peaks.

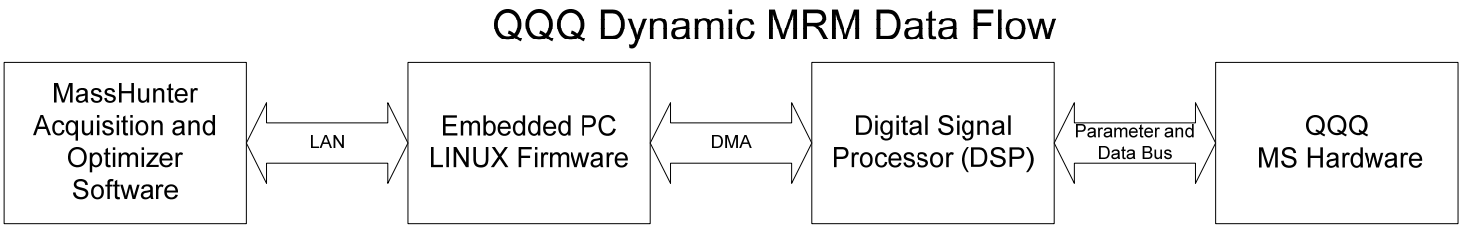
Dynamic MRM maxim:
Enhanced peak sampling, more data points across the peak!



Results and Discussion

Dynamic MRM Key Concepts

A flow diagram was developed to illustrate and explain the key SW/FW/HW communication channels and data flows associated with building and executing dynamic MRM methods.



- Compound-specific MRM transitions/MS parameters are imported from the Optimizer Database, spreadsheets or entered manually by the user.
- MassHunter creates a lookup recipe based on analyte RT detection windows (Delta RT) and cycle time, that groups ion transitions into small tables (up to 1000+) for DSP processing. These tables are similar to conventional MRM "Time Segments" but contain fewer ion transitions. MassHunter software allows up to 4000+ ion transitions (200 max/table) in a dynamic MRM method.
- MassHunter sends the list of ion transitions/MS parameters (up to 4000+) and lookup recipes to the embedded PC.
- Embedded PC sends the list of MRM transition parameters and lookup recipes to the DSP memory.

Dynamic MRM Maximum Cycle Time Calculation:

$$\text{Max Cycle Time (ms)} = \frac{\text{Delta RT (min)}}{\text{64 data pts}} \times \frac{\text{60,000 ms}}{\text{min}}$$

Dynamic MRM Attributes

✓Improved analyte RT accuracy, S/N, %RSDs (Figs. A, B)
✓Optimized dwell times and cycle times consistent with the total number of concurrent MRMs.
✓Truer representation of peaks (better symmetry, less flat tops, pointed apexes or step-like, irregular shapes (Fig. C)
✓Improved peak sampling (greater # of evenly spaced data points across the peak) ensures better quantitative results.
✓Reproducible RTs (Figs. A, C)
✓Greater ease of use – eliminates the requisite to group analytes into time segments according to their elution patterns. Users first analyze a multi-analyte calibration standard with chromatographic conditions they chose, then optimize their method by utilizing Quantitative Data Analysis and dynamic MRM method acquisition software.

- The DSP dynamically builds MRM transition tables throughout an LC/MS run based on analyte RT windows using the list of ion transitions and lookup recipes.
- To maintain a constant cycle time, individual dwell times are adjusted to maintain a constant sampling rate across all peaks ensuring accurate quantitation.
- For each ion transition, the DSP sends MS parameters to the QQQ in the form of address/data pairs. These are used to set the MS quadrupoles and other MS HW parameters correctly during acquisition.
- The MS takes an integrated abundance measurement at the selected ion and sends the unfiltered abundance data back to embedded PC firmware in the form of a structure containing header and abundance information.
- Peak abundance data returning from the DSP/MS is Burst/Time Filtered in embedded Linux firmware. Data is sent back to MassHunter for subsequent processing by Qualitative or Quantitative Data Analysis SW programs.

Conventional Time-Segmented MRM Drawbacks

•Inherent "dead time" data loss when monitoring analyte peaks eluting near or between time segment boundaries. Time segmented MRM methods may require duplicate monitoring of specific analytes which elute over adjoining time segments. (Fig. A)

•Peak shape anomalies (flat tops, irregular steps) due to insufficient peak sampling often observed at low analyte levels. Results in poor quantitation, reproducibility, and RT variability. (Fig. C)

•Significant number of false positives, split peaks or missed peaks at or near LOQ.

•Requires users to pre-define MRM time segments and group analytes/MRM transitions according to their TS elution patterns. Fixed time-segmented MRM methods may be adversely impacted if additional analytes are added or if analyte retention times shift significantly.

Results and Discussion

Two sets of experiments were run, one with dynamic MRM acquisition and the other by conventional MRM mode (2 ms fixed dwell time) with four distinct time segments (TS). The time segments were constructed based upon the number of MRMs/time segment and TS boundary-elution criteria. 20 min, RRLC analysis, 2.1x100 mm Eclipse-Plus C-18,1.8µm.

	Cycle Time (ms)	Start Time (min)	Total # MRMs	Overlapping TS MRMs
TS1	704	0	128	0
TS2	682	8.5	124	8
TS3	1050	11.1	191	12
TS4	990	13.7	144	8

Fig. A

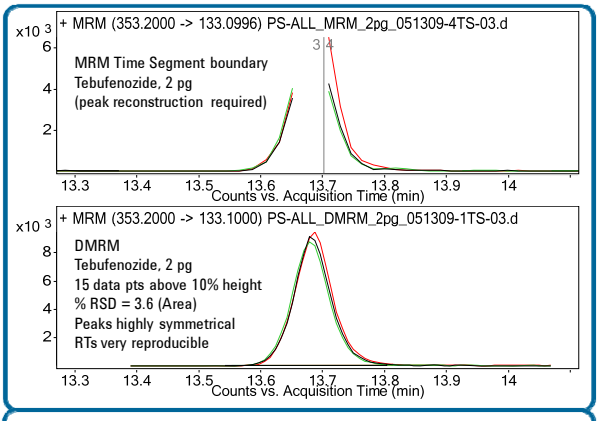


Fig. B

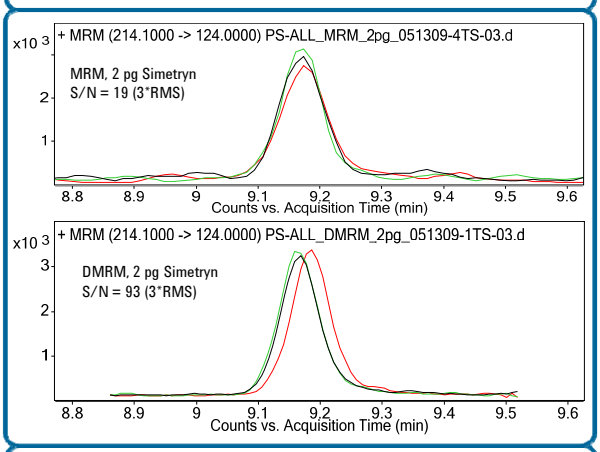
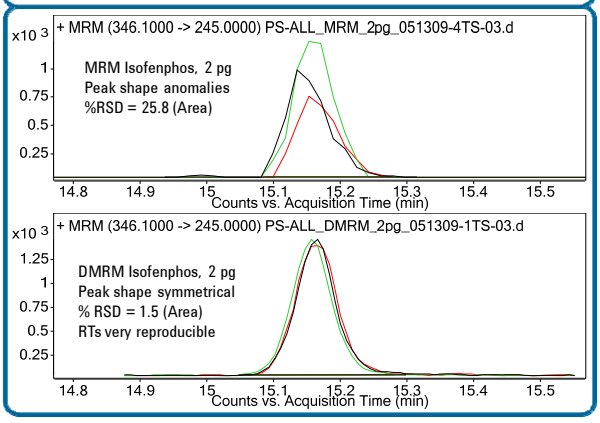


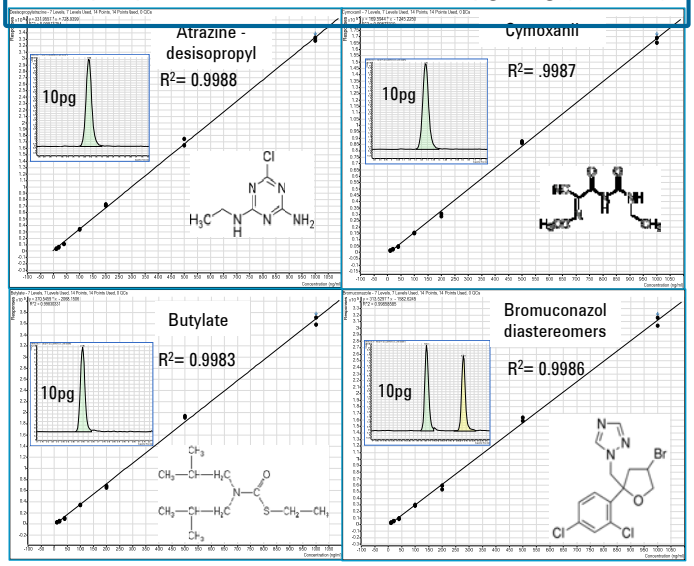
Fig. C



300 pesticides were analyzed in replicates of 3 at the 2 pg, 10 pg and 100 pg levels. For TS-MRM, mean %RSDs (area) were 13.4, 7.4 and 2.8 respectively. For DMRM, the results were 4.9, 2.3, and 1.3 respectively.

In separate experiments, the same pesticide standard mix was analyzed in replicates (N=9) at the 250 pg level by 1290 Infinity LC-G6460 QQQ with Agilent Jet Stream Technology. The mean %RSD was 3.9. An FDA pesticide standard mix consisting of ~250 pesticides, and several commercially available standard mixes yielded similar results and linear calibration curves.

Exemplary DMRM Calibration Plots, 10pg–1ng (7 levels)



Acknowledgements: Pesticide analytical standards used for these studies were a generous gift of Dr. Imma Ferrer and Dr. Michael Thurman, University of Colorado, Boulder, CO, Center for Environmental Mass Spectrometry (CEMS).

Conclusions

- Dynamic MRM is a key enabling technology for fast, accurate LC/MS quantitation of complex samples
- Matches performance of Agilent 6400 Series Triple Quads with the separation power of the 1200 Series RRLC and 1290 Infinity UHPLC
- Offers users major improvements in ease of use and productivity, particularly when utilized with a compound database.