Intelligent Use of Retention Time during Multiple Reaction Monitoring for Faster and Extended Compound Screening with Higher Sensitivity and Better Reproducibility

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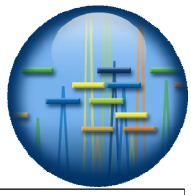
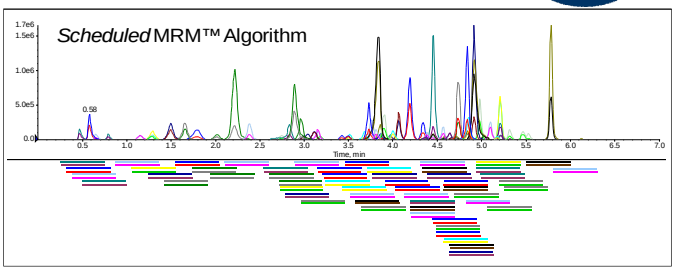
Key Features of *Scheduled* MRM™ Algorithm

- Intelligent use of retention times to maximize dwell times and optimize cycle time of MRM methods
- Increased number of monitored MRM transitions to screen and quantify more analytes per analysis
- Better Signal-to-Noise due to higher dwell times
- Greatly improved reproducibility and accuracy by detecting more data points across chromatographic peaks
- Faster sample analysis by applying UHPLC without compromising data quality





Introduction

LC-MS/MS instruments operating in Multiple Reaction Monitoring (MRM) are widely used for targeted quantitation and screening on triple quadrupole and hybrid triple quadrupole linear ion trap (QTRAP®) systems because of their well known selectivity and sensitivity. Extensive panels with a few hundred MRM transitions are used routinely in many laboratories, for example to screen for food contaminants and environmental pollutants or to identify drugs in intoxication cases in forensic laboratories.

However, the current limit of a few hundred transitions per chromatographic run limits the number of analytes that can be monitored per injection. This is further complicated by the demand for faster analysis through Ultra High Pressure Liquid Chromatography (UHPLC) without reducing the number of monitored analytes and without compromising reproducibility and accuracy.

With the new *Scheduled* MRM™ Algorithm offered in the Analyst® software version 1.5, MRM transitions of the targeted analytes are monitored only around the expected retention time. Thus, automated MRM scheduling decreases the number of concurrent MRM transitions, allowing both the cycle time and the dwell time to be optimized for highest sensitivity, accuracy, and reproducibility. In addition *Scheduled* MRM™ allows the monitoring of many more MRM transitions in a single acquisition or to speed up the analysis by the use of UHPLC or to combine both concepts without compromising data quality.

Key Principles of MRM and *Scheduled* MRM™ Algorithm

Dwell time is the time spent acquiring the targeted MRM transition during each cycle. While very short dwell times can be used (5-10 ms) for extended compound screening, higher dwell times are desirable for better Signal-to-Noise (S/N).

Duty cycle is effectively the amount of time spent monitoring an analyte, therefore the higher the duty cycle the better the data quality. Duty cycle is inversely proportional to the number of, concurrent MRM transitions monitored.

Therefore, an increase in multiplexing resulting in more concurrent MRM transitions can decrease the analytical reproducibility.

The ideal cycle time for an MRM method is a chromatographic consideration. A cycle time which provides 10-15 data points across the LC peak is optimal for accurate quantitation and reproducibility, especially for low abundant analytes. The relationship between number of MRM transitions, dwell time, duty cycle, and cycle time is illustrated in Figure 1.

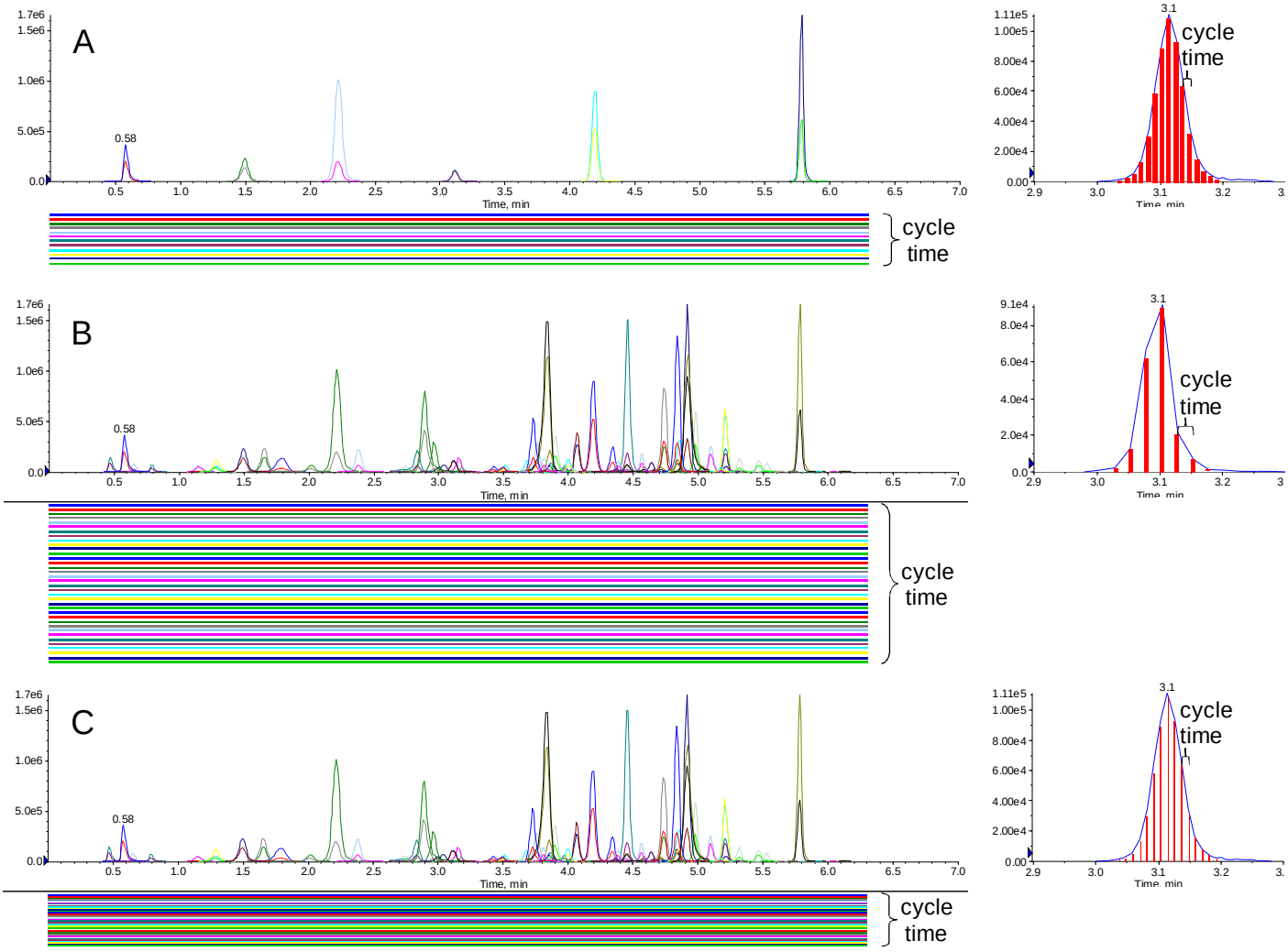


Figure 1. Considerations for Multiple Reaction Monitoring

(A) Traditionally, few MRM transitions are detected to quantify targeted analytes with high dwell times for best S/N and cycle times to collect enough data points across the LC peak for accurate and reproducible data (the width of the bars indicate the dwell time and the space between bars indicate the cycle time).

(B) Increasing the number of MRM transitions by maintaining the dwell time extends the cycle time resulting in very poor quantitative results because of an insufficient number of data points across the LC peak.

(C) Increasing the number of MRM transitions by decreasing the dwell time results in lower duty cycle and, thus, in lower S/N and higher limits of detection.

The *Scheduled* MRM™ Algorithm is illustrated in Figure 2. Prior knowledge of the retention of each analyte allows the MRM transition to be monitored only in a short time window. At any one point in time, the number of concurrent MRM transitions are significantly reduced resulting in much higher duty cycles for each analyte. The software computes maximum dwell times for the co-eluting compounds while still maintaining the desired cycle time.

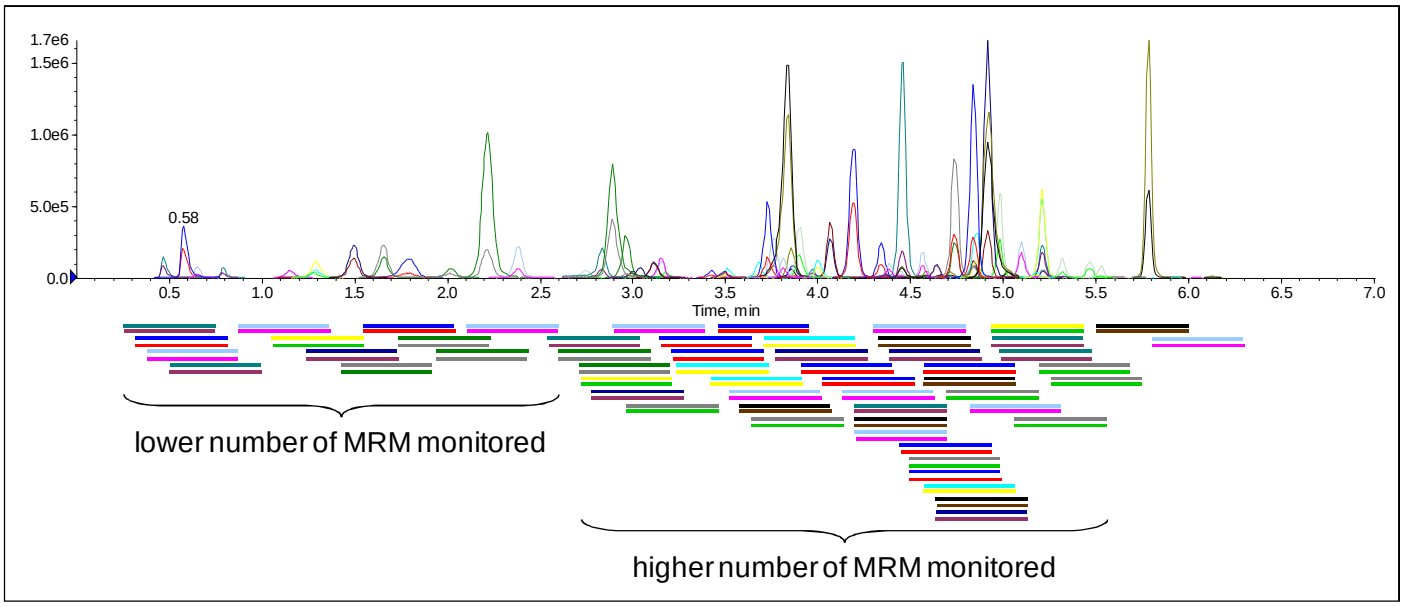


Figure 2. The *Scheduled* MRM™ Algorithm uses the knowledge of the elution of each analyte to monitor MRM transitions only during a short retention time window. This allows many more MRM transitions to be monitored in a single LC run, while maintaining maximized dwell times and optimized cycle time.

Good Chromatography is the Key for the Best LC-MS/MS Data

The key to the highest order multiplexing and optimal MS/MS performance is high quality and highly reproducible LC separation.

One of the user inputs to the software to automatically create the *Scheduled* MRM™ methods is the MRM Detection Window. This is an estimate of the LC peak width and chromatographic reproducibility expected, and should therefore reflect the time window around the supplied retention time which will contain the entire LC peak plus any shifts in chromatography. The narrower the peak widths and the more reproducible the elution, the tighter this MRM detection window can be and, thus, less concurrent MRM transitions are monitored. Reduced concurrency also means that higher dwell times will be used for each MRM, improving the data quality.

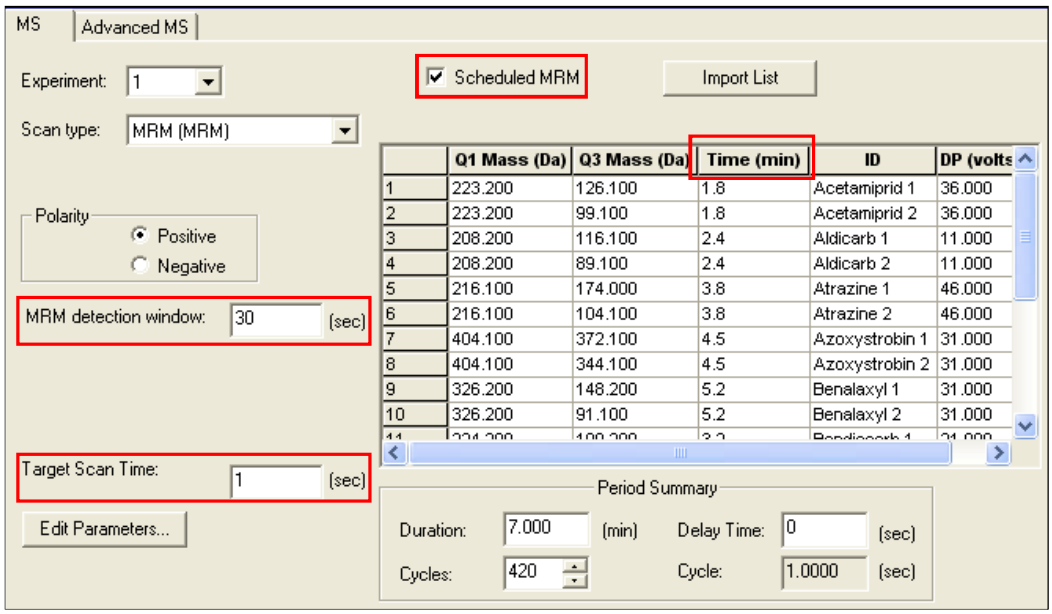


Figure 3. Acquisition method interface for *Scheduled* MRM™, in addition to traditional MRM parameters, the user provides retention times of all analytes, an MRM detection window, and a Target scan time. The software then automatically designs and optimizes the Scheduled MRM™ acquisition method.

Easy Method Creation

Another key advantage in *Scheduled* MRM™ is the ease at which powerful quantitative MRM acquisition methods can be created. The user is required to specify a few key parameters (Figure 3):[1]

- MRM transition: (Q1, Q3) and any compound dependent parameters
- Expected retention time for each MRM transition
- MRM detection window must be wide enough to allow the MRM peak to stay entirely within the window across all injections – consider the width of the LC peak at the base and the retention time stability
- Target scan time is effectively the cycle time – how often the chromatographic peak should be sampled. This is determined from the peak width at the base. The best accuracy and reproducibility is between 10-15 points across the peak
- Additionally, MRM ID, like compound name, for easier data processing and reporting

The software algorithm then automatically builds an acquisition method that schedules the appropriate MRM transitions to be screened over the chromatographic analysis at the appropriate times. Instead of monitoring all transitions all of the time, it will only look for those transitions within the targeted time window.

Results of Using the *Scheduled* MRM™ Algorithm

Increased Number of MRM Transitions

The number of MRM transitions which can be monitored in a single analysis depends on chromatographic peak width and required S/N (dwell time). Several publications show that AB SCIEX systems equipped with Linear Accelerator® collision cell can be used to detect several hundred transitions using traditional LC configurations.[2-4]

The automated MRM scheduling decreases the number of concurrent MRM transitions. Thus *Scheduled* MRM™ allows the monitoring of many more MRM transitions per cycle without the need to sacrifice data quality.

The example in Figure 4 shows an injection of more than 750 compounds typically analyzed in forensic laboratories to screen for toxic substances, such as drugs of abuse, pharmaceuticals and their metabolites.

Such screening methods are used frequently to screen for a large number of targeted compounds. The Scheduled MRM™ survey was used to automatically acquire Enhanced Product Ion (EPI) spectra on a 3200 QTRAP® LC/MS/MS system. The characteristic and high sensitivity spectra can be searched against a mass spectral library for compound identification.

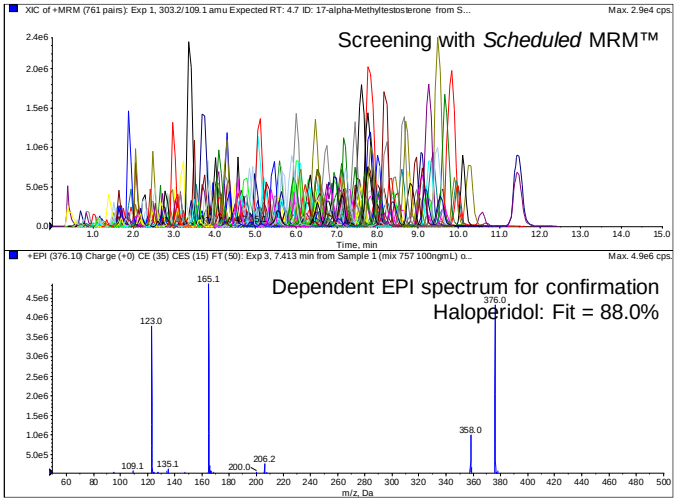


Figure 4. Using *Scheduled* MRM™ to increase the number of monitored MRM transitions for screening applications. The example shows an injection of more than 750 compounds relevant in forensic toxicology. The *Scheduled* MRM™ survey was used to automatically acquire EPI spectra for identification by library searching.

Better Sensitivity and Reproducibility

Figure 5 shows a comparison of using traditional MRM and *Scheduled* MRM™ detection for the screening of pesticides in fruit and vegetable samples. A 4000 QTRAP® LC/MS/MS system was used to detect 150 MRM transitions.

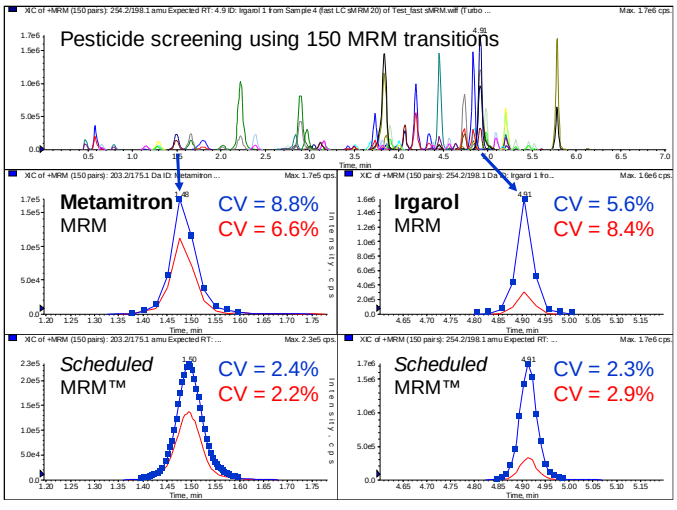


Figure 5. Using *Scheduled* MRM™ to optimize dwell times and number of data points across the LC peak in a pesticide screening method with 150 MRM transitions. The *Scheduled* MRM™ method shows significantly better sensitivity and reproducibility.

The *Scheduled* MRM™ Algorithm automatically optimizes dwell times enabling detection with higher sensitivity and better

reproducibility by collecting more data points across the LC peak. The improvement in sensitivity and reproducibility depends on the number of concurrent MRM transitions. Narrow LC peaks and highly stable retention times allow setting a smaller MRM detection window for best *Scheduled* MRM™ performance.

Faster analysis using UHPLC without compromising data quality

The use of small particle size columns and faster gradients results in narrower LC peaks. Traditional MRM would require decreasing the number of transitions or compromising quality to maintain the number of transitions.

The chromatograms in Figure 6 show examples of traditional, fast and ultra fast LC to monitor 150 MRM transitions. *Scheduled* MRM™ allows accelerated analysis without the need to compromise the number of monitored compounds and/or data quality. The data were acquired using a 4000 QTRAP® LC/MS/MS system. A Phenomenex Synergi 2.5u Fusion-RP 50x2 mm column with different gradients of water/methanol and 5 mM ammonium formate was used. The gradient conditions are shown in Table 1.

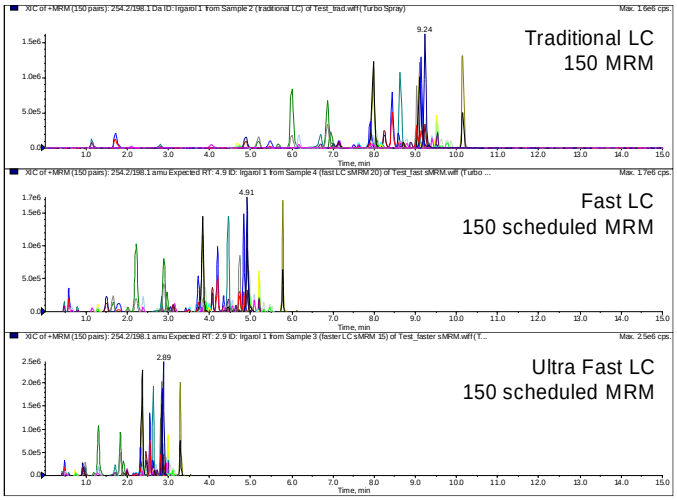


Figure 6. *Scheduled* MRM™ allows fast and ultra fast LC separation using small particle column while maintaining the number of monitored MRM transitions without compromising data quality.

Table 1. Traditional, fast and ultra fast LC gradients to detect 150 MRM transitions of pesticides on a 4000 QTRAP® LC/MS/MS system

	Traditional LC (2150 psi)			Fast LC (4330 psi)			Ultra Fast LC (4570 psi)		
Step	**Time (min)**	**Flow (µL/min)**	**A%/B%**	**Time (min)**	**Flow (µL/min)**	**A%/B%**	**Time (min)**	**Flow (µL/min)**	**A%/B%**
0	0	250	80/20	0	500	70/30	0	500	60/40
1	8	250	10/90	5	500	10/90	2	500	10/90
2	14	250	10/90	6	500	10/90	4	500	10/90
3	15	250	80/20	7	500	70/30	5	500	60/40
4	20	250	80/20	10	500	70/30	8	500	60/40

Figure 7 shows results of the analysis of fruit extracts analyzed with a traditional LC and MRM method in comparison to a fast LC and *Scheduled* MRM™ method. The samples were extracted using a QuEChERS procedure before analysis.

Several pesticides were detected, quantified and identified using MRM ratio calculation, including Imazalil at 42 µg/kg and Thiabendazole at 3.4 µg/kg in grapefruit, Metazachlor at 8.9 µg/kg in apricot, and Methomyl at 4.7 µg/kg in grapes. The use of *Scheduled* MRM™ for this analysis allowed faster sample analysis with better sensitivity and reproducibility. In addition, data exploration was easier because of a more selective acquisition.

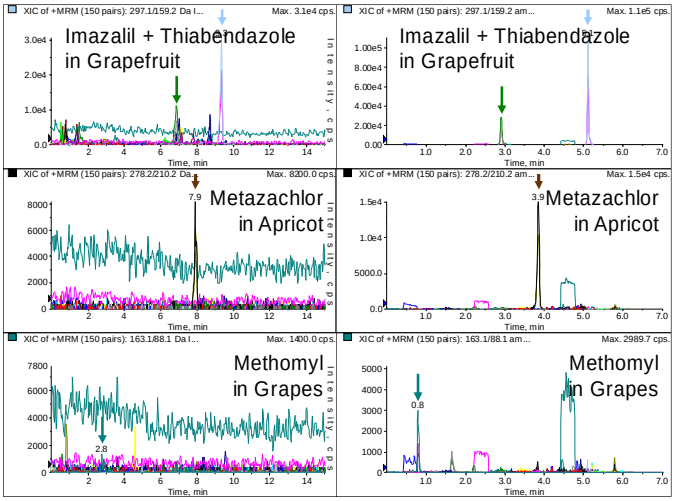


Figure 7. Comparison of traditional LC and MRM with fast LC and *Scheduled* MRM™ for the analysis of pesticides in fruit extracts, the new method allowed faster analysis with better sensitivity and reproducibility. Also cleaner data display made data exploration easier.

Summary

The new *Scheduled* MRM™ Algorithm offered in Analyst® software version 1.5 automatically monitors MRM transitions of the targeted analytes only around the expected retention time. The scheduling decreases the number of concurrent MRM transitions, allowing both the cycle time and the dwell time to be optimized for highest sensitivity, accuracy, and reproducibility. In addition, *Scheduled* MRM™ allows the monitoring of many more MRM transitions in a single acquisition and/or accelerating the analysis by the use of UHPLC maintaining highest data quality.

References

1 *Scheduled* MRM™ tutorial

2 C. A. Mueller et al.: 'Development of a Multi-Target Screening Analysis for 301 Drugs Using a QTRAP Liquid Chromatography/Tandem Mass Spectrometry System and Automated Library Searching' Rapid Commun. Mass Spectrom. 19 (2005) 1332-1338

3 A . Schreiber et al.: 'Simultaneous Quantitative Screening and Qualitative Confirmation of 300 Pesticides Using the New 3200 QTRAP® LC/MS/MS System' Application Note AB SCIEX (2005)

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Publication number: 1282310-01

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