RADAR

Understanding sample complexity
Improving quantitative data quality

Uncertainty in chromatographic method development and quantitative experiments often arises when co-eluting unknown interferences reduce performance. In particular, targeted multiple reaction monitoring (MRM) analysis of compounds in complex matrices is a difficult task and it is often impossible to separate all the interfering compounds present in matrix from the target analytes. It is likely that an analyst performing a routine MRM experiment would be unaware if interferences were present or if they were changing from sample to sample as subtle changes in analysis results are difficult to detect.

Some key issues for quantitative methods are:

- Calculating compound concentrations in addition to the detection of unexpected contaminants.
- Characterisation of the background matrix for every sample, increasing data quality.
- Detection of analytes that are not in a targeted MRM screening method.
- Improving method development by discovering more matrix components.

In order to address these challenges a tandem quadrupole instrument must be able to rapidly switch between MS qualitative scanning and MS/MS quantitative modes so as not to affect the duty cycle time of the instrument. This is a key requirement when performing UPLC®, UPC²® or GC separations which produce narrow peak widths offering less time to acquire data for the sample component peak as it arrives at the mass analyser. Within this narrow time window it is still important to maintain sensitivity and mass resolution as well as providing reproducible data from injection to injection.

To maintain high quality reproducible data, RADAR™ was introduced on the Xevo® TQ MS in 2008 and has since become a well-established technology available on the Xevo TQD and Xevo TQ-S instruments.

In RADAR mode, it is possible to monitor for matrix interferences, metabolites, impurities and degradants in a sample while accurately quantifying target compounds without losing sensitivity or performance. Understanding the matrix of individual samples and monitoring changes in matrix across different samples will lead to continuous improvement of quality of the services provided by the laboratory.

PRINCIPLE OF OPERATION

Innovative T-Wave™ collision cell design permits the use of RADAR which is operated by rapidly switching between MRM (MS/MS) mode and MS full scan acquisition. Switching between the two modes occurs in only 5 ms ensuring that the duty cycle time is kept to a minimum and maximizing data points across the peak. Setting up a RADAR experiment is a simple process as the analyst can add an MS scan function to any MRM method and use the automatic dwell time feature to create optimal MRM channel dwell times. Figure 1 shows the two modes of acquisition that traditionally would require two separate injections but can now operate together.

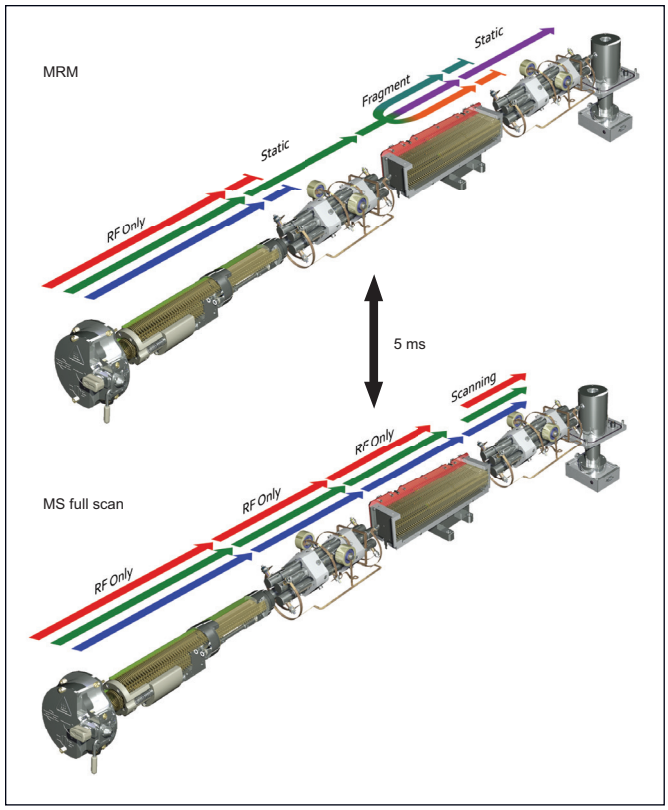

Figure 1. Xevo TQD ion path showing MRM and MS full scan mode acquisition.

Assessing background changes in matrix while performing low level MRM analysis[1]

In the food safety laboratory, a very useful function is the ability to detect and measure ultra-trace levels of hazardous pesticides in a variety of complex food matrices. This experiment combined monitoring a challenging number of pesticide analytes at low concentrations with simultaneously recording MS full scan data on the Xevo TQ-S instrument with ACQUITY UPLC® chromatographic separation.

To improve pesticide detection, a sample preparation technique was utilised with Waters® DisQuE™ Dispersive SPE (QuEChERS). However this sample prep technique is a generic method designed to allow for good recovery of a wide range of pesticide compounds with differing physiochemical properties. This lack of specificity is therefore an advantage for multi-analyte recovery but the penalty is that a large proportion of the background matrix is also recovered. The full scan RADAR data (as shown in Figure 2) aids in observation of the background matrix in every sample leading to improvements in sample clean-up as potential sources of ion suppression can be removed or the chromatography adjusted if they are known. This leads to robust and reliable method development and allows monitoring of the background matrix in samples over time.

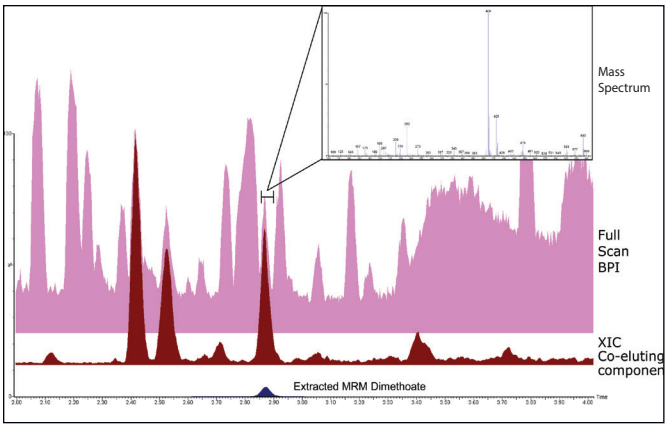

Figure 2. Extracted MRM chromatogram for dimethoate (0.01 mg/kg) in grape with overlaid simultaneous full scan and extracted mass chromatogram of co-eluting matrix component. Also shown (inset) is mass spectrum of co-eluting component.

Detecting contaminants in samples submitted for routine MRM targeted analysis[2]

Infant formulae and follow-on formulae milk are heavily regulated for both nutritional content and maximum allowable amount of contaminants. Unexpected adulterants in formula milk such as melamine (a toxic additive designed to mask dilution of milk with water) are extremely important to detect early so that the product is not released to the consumer. Therefore the ability to perform a background full scan analysis in addition to targeted MRM for vitamins in milk is critical to ensure the safety of the product and detect contaminants early in the production of formula milk. In this experiment prepared infant formula was spiked with melamine at 1 ppm and data was acquired using Xevo TQ MS in RADAR mode with an ACQUITY UPLC for chromatography. Water-soluble vitamins were measured using MRM transitions and melamine was detected using the MS full scan data with an identifiable mass spectrum for melamine shown in Figure 3. This method allows the analyst to perform quantitative MRM analysis and at the same time detect unexpected adulterants in a food sample. Similar approaches to the detection of unexpected contaminants, extra drugs and metabolites in samples submitted for MRM targeted analysis are useful in other applications.[3,4]

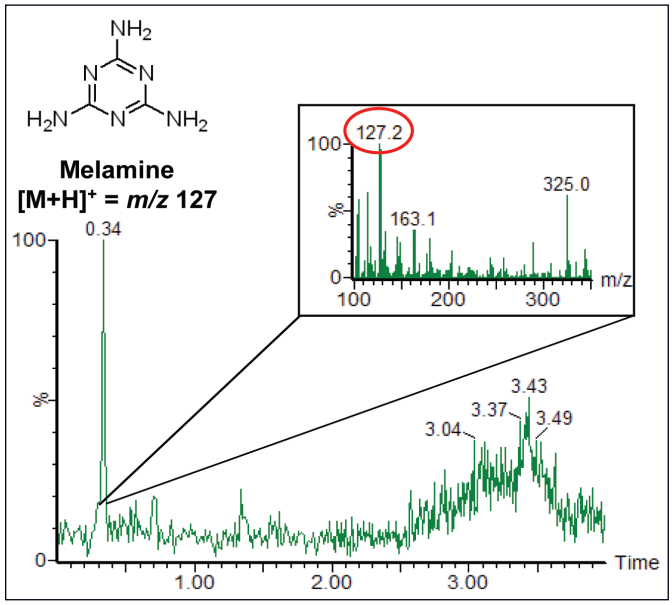

Figure 3. XIC for melamine from the full scan RADAR data. Inset shows the background subtracted spectrum acquired at 0.34 minutes.

Improving chromatography method development[5]

Bioanalysis methods must be transferable, reproducible and robust despite matrix variations. It is important to resolve analyte peaks from endogenous interferences to reduce matrix effects that may lead to poor assay reproducibility. A major source of interference in serum and plasma is the phospholipids (characterized by producing fragment ions with *m/z* 184) which may reduce sensitivity and robustness of methods. This experiment demonstrates that an assay for ranitidine (a common H2 receptor antagonist) could be rapidly developed, and potential interferences monitored, to ensure appropriate separation of the matrix from the analyte. Figure 4 shows the original chromatographic conditions (4A) with analyte/interference coelution and the final chromatography conditions (4B) with ranitidine well-resolved from the endogenous matrix compounds. This technique provided faster method development, easy troubleshooting, avoided the need for repeat injections and would allow the detection of *in vivo* metabolites during preclinical and clinical development. It is useful to recognise that RADAR can also aid sample preparation method development[6] when trying to resolve interferences in very complex matrices.

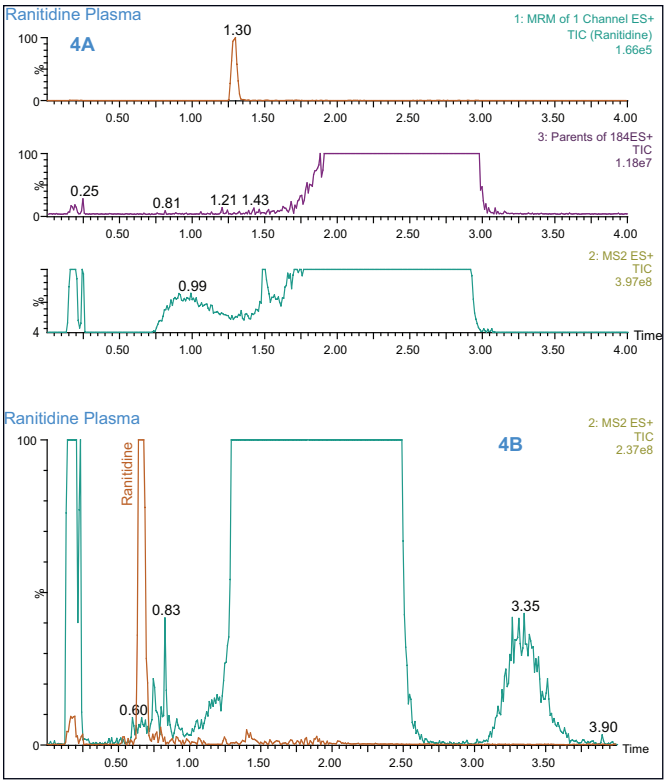

Figure 4: LC/MS/MS analysis of ranitidine hydrochloride in rat plasma using aqueous basic methanol gradients. 4A shows the initial method and ranitidine coeluting with matrix components in the full scan TIC trace. The second trace shows parents of m/z 184 ions which are characteristic of phospholipids. 4B shows the final method resulting in ranitidine being resolved from the matrix components.

SUMMARY

Accessing high quality quantitative data with every injection is a key advantage to the analytical laboratory. The benefits of having information-rich, RADAR data may be summarized as:

- Detect contaminants in samples while performing sensitive, reproducible and accurate quantitative analysis and retrospectively analyze historical data for contaminants.
- Discover any co-eluting matrix compounds so that matrix variation between samples can be assessed and therefore data quality is increased.
- Collect full scan MS data for analytes that may be of interest but are not included in targeted MRM screening methods.
- Monitor the background matrix for interferences to reduce matrix effects enabling fast method development.

References

1. Monitoring Matrix Complexity During Ultra-Trace Level Multi-Residue Pesticide Analysis. Waters Technology Brief 720003428EN.
2. The Use of Xevo TQ MS with RADAR for Simultaneous Acquisition Of Routine MRM's and Full Scan Background Data. Waters Technology Brief 720003806EN.
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5. RADAR, a Xevo TQ-S function, combined with Low-High pH UPLC Screening for Fast, Simple Bioanalysis Methods Development. Paul D. Rainville and Robert S. Plumb. Waters Application Note 7200034174EN.
6. Monitoring the Matrix Background Through the Sample Preparation Process in Complex Matrices Using the Xevo TQD with RADAR Functionality. Waters Technology Brief 720003979EN.

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