SINGLE LC-MS/MS METHOD FOR CONFIRMATION AND QUANTIFICATION OF OVER 400 PESTICIDES IN A COMPLEX MATRIX WITHOUT COMPROMISING DATA QUALITY

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INTRODUCTION

More than 500 compounds are routinely used for crop protection across the globe. With increasing global trade there is a requirement for rapid multi-residue screening and quantification methods to determine residues. Effective multi-residue methods rely on management of the acquisition of a large number of MRM transitions. Setting up overlapping MRM windows based around the retention time of each analyte ensures that no time is wasted acquiring other transitions for compounds that have yet to elute. One of the objectives of this method was to implement relatively wide MRM windows, without any loss in performance, removing the need to make regular checks on retention time drift thus avoiding the need to make adjustments to the acquisition method before each analysis.

METHODS

Conditions

LC system : ACQUITY UPLC® H-Class
Column : ACQUITY® BEH C18 2.1 x 100 mm 1.7 µm
Mobile phase A : 10 mM ammonium acetate in water, pH 5
Mobile phase B : 10 mM ammonium acetate in methanol, pH 5
MS system : Xevo® TQ-S micro
Ionization mode : ESI +/-
MRM transitions: Quanpedia™
UPLC® method : see application note 720005559EN
Data acquisition: MassLynx® 4.1
Data process: TargetLynx™ XS Application manager

Method	MRM transitions	Dwell time	Average peak width (sec) n=3	Average data points across peak n=3
A	16	Autodwell	4.5	10
B	859	Autodwell	4.4	10

Table 1. Methods A and B showing number of MRM transitions and dwell time in the respective methods. Also listed are the average peak width and data points across the peak of furalaxyl for 3 replicates of chilli sample spiked at 10 µg/kg.

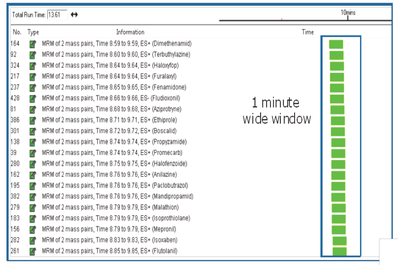


Figure 1. Screen capture of Method B

Standards

Waters® LC multi-residue pesticide standards kit (p/n 186007574) was used to make a mix of standards. The working standards were diluted with acetonitrile.

Sample preparation

The sample preparation followed the protocol described in a previous application note[1]. A DisQuE™ QuEChERS (CEN method 15662)[2] was used to prepare all samples.

Briefly, two grams of chilli powder was mixed with 8 mL of water and vortexed for 30 seconds. The mixture was extracted with 10 mL of acetonitrile followed by the addition of QuEChERS CEN material. The resulting mixture was shaken for one minute and centrifuged at 4000 rpm for 5 minutes. The supernatant was placed into vials for analysis.

RESULTS AND DISCUSSION

The chili sample extract was analyzed for the presence of pesticides using an ACQUITY UPLC H-Class System coupled with the Xevo TQ-S micro Mass Spectrometer.

Novel SpaceWire technology of the Xevo TQ-S micro facilitates faster acquisition speeds with Xcellerate Ion Transfer (XIT™). To achieve the additional sensitivity, the instrument is integrated with StepWave™ Ion Guide Technology. StepWave effectively removes the neutral molecules, providing additional sensitivity, and improving robustness.

In order to demonstrate the fast acquisition rate and excellent data quality of the MS instrument, a chili extract was post spiked with pesticides at 10 µg/kg and analyzed using Methods A and B (Table 1).

Method A contains 8 pesticides having two MRM transitions for each compound (16 MRMs total). While Method B contains 430 pesticides (429 with two MRM transitions and 1 with one MRM, totaling 859 MRMs). Both methods were enabled with AutoDwell functionality, at the click of a button which allows MassLynx Software to optimize the dwell time automatically for each compound depending on its retention time, as well as the peak width and required data points across the peak, as defined by the user.

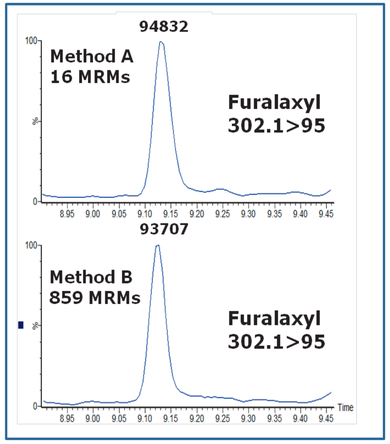


Figure 2. Chromatograms of furalaxyl showing peak area count (Methods A and B) in spiked chilli powder sample at 10 µg/kg.

In this experiment, 1-minute wide acquisition windows were selected for both methods which eliminates the regular checks for retention time drift (due to matrix interferences) and simplifies inter- and/or intra- laboratory method transfer.

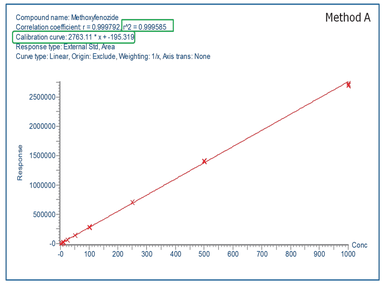
Figure 1 shows a screen shot of part of Method B. In this method, more than 100 pesticides (of the 430 being monitored) eluted between 9 to 10 minutes. Furalaxyl (RT 9.1 min) elutes within this crowded region with an average peak width and data points across the peak of 4.4 sec and 10 respectively (Table 1 –Method B).

Similar results were observed with Method A where fewer number of transitions (16) were monitored in the method. Despite the large number of compounds in Method B, the data quality was not compromised for the complex matrix.

Figure 2 shows chromatograms of furalaxyl acquired by Methods A and B. The peak area difference between Methods A and B were minimal. Despite Method B containing significantly more MRMs than method A, both methods yielded similar area counts (<2 % deviation), which is an acceptable deviation for injection of replicate matrix. Both methods have shown a minimum of 10 data points across the peak, a typical requisite for accurate quantification.

Linearity

Figure 3 shows excellent agreement and linearity for matrix matched calibration curve of methoxyfenozide (RT 9.56 min) from 1 to 1000 µg/kg (ppb) using Methods A and B.



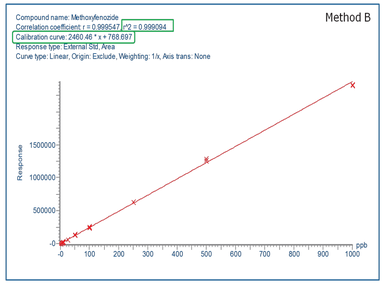


Figure 3 Matrix match spiked calibration curves of methoxyfenozide using Method A and B.. Calibration range 1 to 1000 µg/kg (ppb) [concentrations equate to sample].

Robustness study

To assess the repeatability and robustness of the method, 300 injections of the spiked chilli sample (25 µg/kg) were analyzed by Method B. All positive and negative ionized compound showed good %RSD (3.3 to 13.9) over 300 injections.

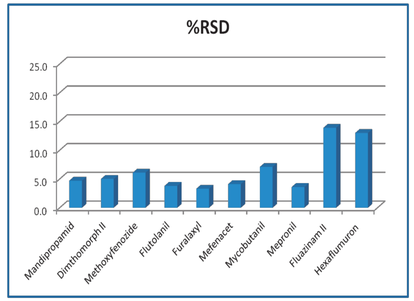


Figure 4. % RSD of 300 injections of the example pesticides spiked in chill sample at 25 µg/kg and analyzed by Method B

Standard addition using Auto Addition feature

The standard addition method was employed to calculate the concentration of incurred residues in the chilli sample. Matrix matched samples were prepared using the Auto Addition functionality of the UPLC system, automatically enabling the repeatable mixing of multiple aliquots from several vials within a single injection. Figure 5 shows the Auto Addition setup sample list created in MassLynx.

Figure 5. Sample list created in MassLynx showing Auto Addition setup

Figure 6 shows an example of calculated concentration of pyraclostrobin (12.35 ppb) in chilli sample using the TargetLynx XS standard addition approach.

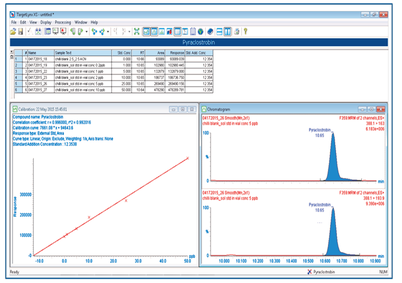


Figure 6. TargetLynx XS showing quantification of incurred pyraclostrobin in a chilli sample using the standard addition method.

CONCLUSION

- **A large number of MRM transitions with a one minute wide acquisition window in Method B eliminates the manual retention time check process and allows for easy transfer of methods between laboratories.**
- **Fast scanning speed of the XEVO TQ-S micro provides enough data points across the peak for accurate quantification within high volume multi-residue analysis.**
- **A combination of the Auto Addition and standard addition features facilitates automated quantification of incurred residues which reduces labor and the need for a blank sample.**
- **Excellent linearity, robustness and sensitivity were achieved in a complex matrix such as chilli powder.**

References

1. Rapid analysis of sudan and other prohibited dyes in chilli powder using the ACQUITY UPLC H-Class system with Xevo TQD . http://www.waters.com/webassets/cms/library/docs/720004975en.pdf
2. http://www.waters.com/webassets/cms/library/docs/720003048en.pdf

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