

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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APPLICATION BENEFITS

- Single method for confirmation and quantification of a large number of pesticides in chili powder.
- The Xevo® TQ-S micro provides reliable, robust, and reproducible data at fast acquisition rates.
- Automated quantification of samples with Auto Addition and standard addition features.

WATERS SOLUTIONS

[ACQUITY UPLC® H-Class System](#)

[Xevo TQ-S micro](#)

[ACQUITY UPLC BEH C₁₈ Column](#)

[MassLynx® MS Software](#)

[DisQuE™ QuEChERS Dispersive](#)

[Solid Phase Extraction](#)

[LC Multiresidue Pesticide Standards Kit](#)

KEY WORDS

Pesticides, chili, QuEChERS, food safety

INTRODUCTION

Hundreds of 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 efficiently determine residue violations and protect consumers. 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. This optimizes the time spent acquiring data to maximize sensitivity while ensuring a sufficient number of data points across peaks to give good precision. One of the objectives of this method was to implement relatively wide MRM windows without any loss in performance. This removes the need to make regular checks on retention time drift, and avoids having to make adjustments to the acquisition method before each analysis.

In this application note, we describe a single, fast method for confirmation and quantification of more than 425 pesticides. All pesticides were analyzed on a reverse phase column within 12 minutes. Two MRM transitions for each pesticide were monitored using both ESI positive and negative modes (deploying polarity switching). The excellent performance of the method has been demonstrated at very low concentrations in chili powder, which is a very complex matrix. Standard curves in solvent and matrix, easy-to-use instrument and software features to calculate the incurred residues in a chili sample, and data showing robustness after a large number of injections will be presented.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY BEH C ₁₈ , 2.1 x 100 mm, 1.7 µm
Column temp.:	45 °C
Injection volume:	1 µL
Flow rate:	0.45 mL/min
Mobile phase A:	10 mM Ammonium Acetate, pH 5, (P/N 186006693) in water
Mobile phase B:	10 mM Ammonium acetate in methanol
Weak needle wash	50/50 Water/methanol (v/v)
Strong needle wash:	90/10 Methanol/water (v/v)
Seal wash:	90/10 Water/methanol

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.450	98	2	6
0.25	0.450	98	2	6
12.25	0.450	1	99	6
13.00	0.450	1	99	6
13.01	0.450	98	2	6
17.00	0.450	98	2	6

Table 1. UPLC method for pesticide analysis.

MS conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI +/-
Capillary voltage:	1 kV(+) and 0.5 kV(-)
Desolvation temp.:	500 °C
Desolvation gas flow:	1000 L/hr
Source temp.:	150 °C
Acquisition:	Multiple Reaction Monitoring (MRM)

MS methods and data acquisition

Two MRM transitions for each pesticide were generated using QUANPEDIA,[™] except for fipronil, where 1 MRM transition was used. Methods are shown in Table 2. The data were acquired using MassLynx Software, and processed using 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	4.5	10

Table 2. Methods A and B showing the number of MRM transitions and dwell times in the respective methods. Also listed are the average peak widths and data points across the peak of furalaxyl for three replicates of a chili sample spiked at 10 µg/kg.

Standards

Waters[®] LC Multiresidue Pesticide Standards Kit (P/N 186007574) was used to make a mix of standards. The standards kit has a collection of 204 compounds in 10 different ampoules at individual concentrations of 100 µg/mL. The stock solution of 10 µg/mL was prepared by combining 100 µL from each ampoule. The working standards were further diluted with acetonitrile.

For the solvent calibration curve and standard addition work, acetonitrile was spiked with 204 pesticides at 1, 5, 10, 25, 50, 100, 250, 500, and 1000 µg/kg (ppb) (concentrations equate to sample).

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 chili powder was weighed into a centrifuge tube. The sample 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 (4 g MgSO₄, 1 g NaCl and 1.5 g sodium citrate). The resulting mixture was shaken for 1 minute. The tube was then centrifuged at 4000 rpm for 5 minutes, and the supernatant was placed into vials for analysis.

For the matrix match spiked (MMS) calibration, a chili sample was spiked with 204 pesticides at the same level as the solvent calibration range. These spiked levels equaled the concentrations in the chili sample.

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 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. Figure 1 shows the Xevo TQ-S micro, along with the StepWave ion guide.

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 (concentration equates to sample) and analyzed using Methods A and B, as described in Table 2. Method A contains 8 pesticides having two MRM transitions for each compound (16 MRMs total). Method B contains 430 pesticides (1 SRM, and 429 with two MRM transitions, totaling 859 MRMs). Both methods were enabled with AutoDwell functionality, at the click of a button. AutoDwell allows MassLynx MS 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. 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.

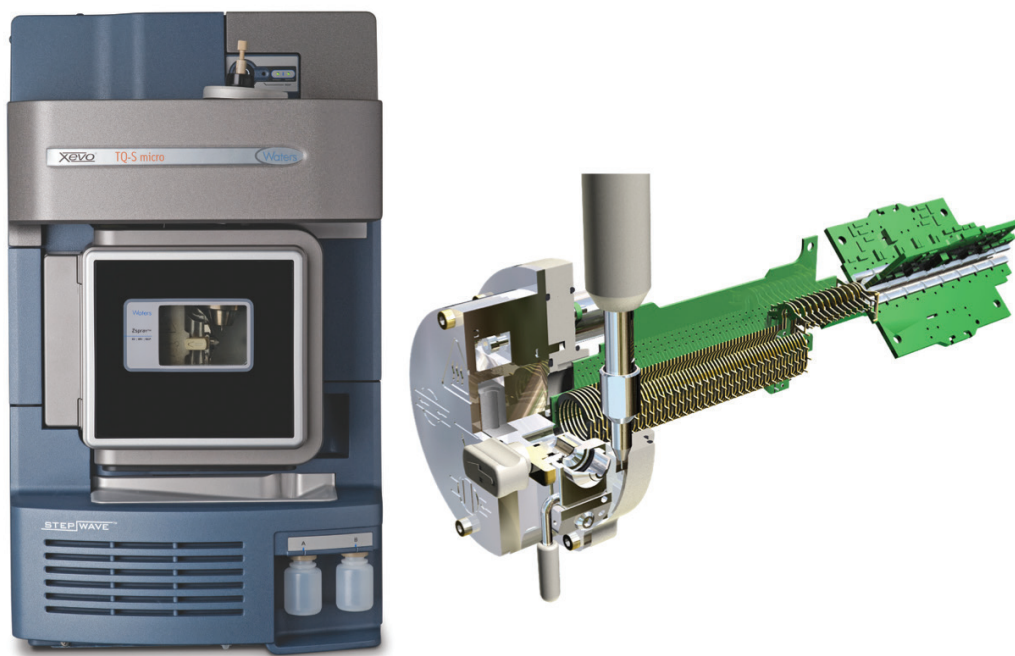


Figure 1. Xevo TQ-S micro (left) and StepWave ion guide diagram (right).

Figure 2 shows a screen shot of part of Method B. In this method, more than 100 pesticides (of the 430 being monitored) eluted between 9 and 10 minutes. Furalaxyl (RT 9.1 min) has eluted within this crowded region. As shown in Table 2, furalaxyl had an average peak width and data points across the peak of 4.4 sec and 10 data points respectively (Method B), for three replicates of the chili sample spiked at 10 µg/kg. Similar results were observed with Method A where fewer transitions (16) were monitored in the method. Despite the large number of compounds in Method B, the data quality was not compromised in the complex matrix.

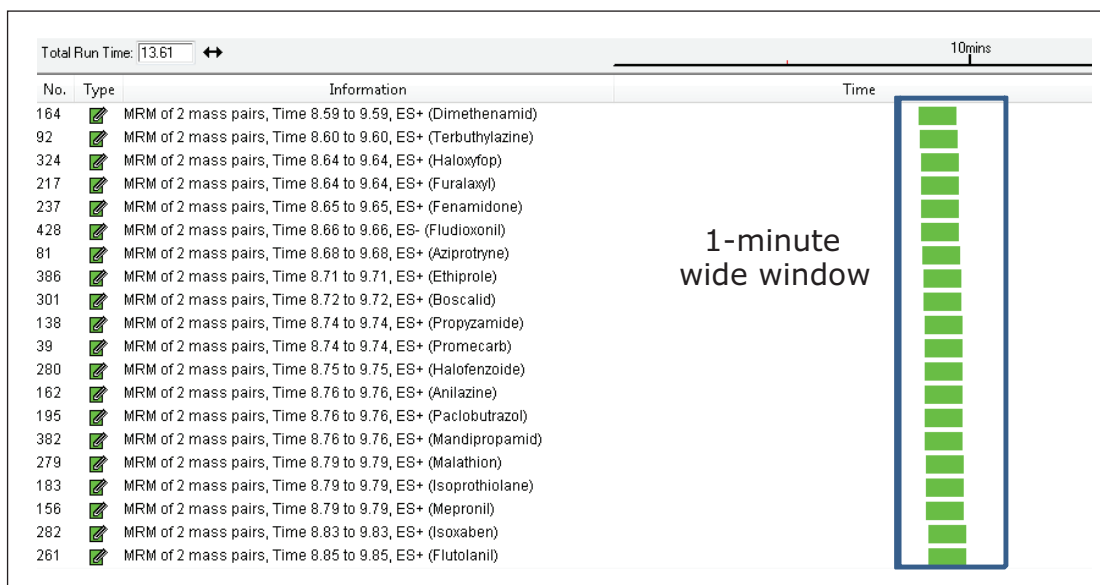


Figure 2. Screen capture of Method B.

Figure 3 shows chromatograms of furalaxyl acquired by Methods A and B. The peak area difference between Methods A and B which contained 16 and 859 MRMs respectively were minimal. Despite Method B having 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.

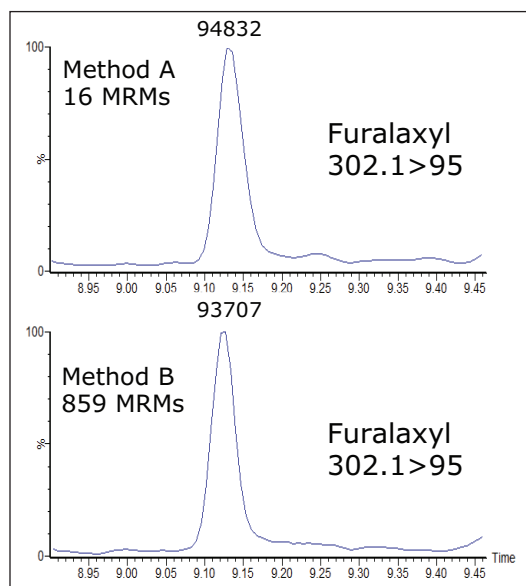


Figure 3. Chromatograms of furalaxyl showing peak area count (Methods A and B) in a spiked chili powder sample at 10 µg/kg.

Linearity

Linearity over the required working range was determined in solvent (data not shown) and matrix matched calibration curves using both MS methods. Figure 4 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. As can be seen in the Figure 4, Methods A and B showed good linearity with an r^2 value >0.999 over the entire specified range and similar slope of the equation.

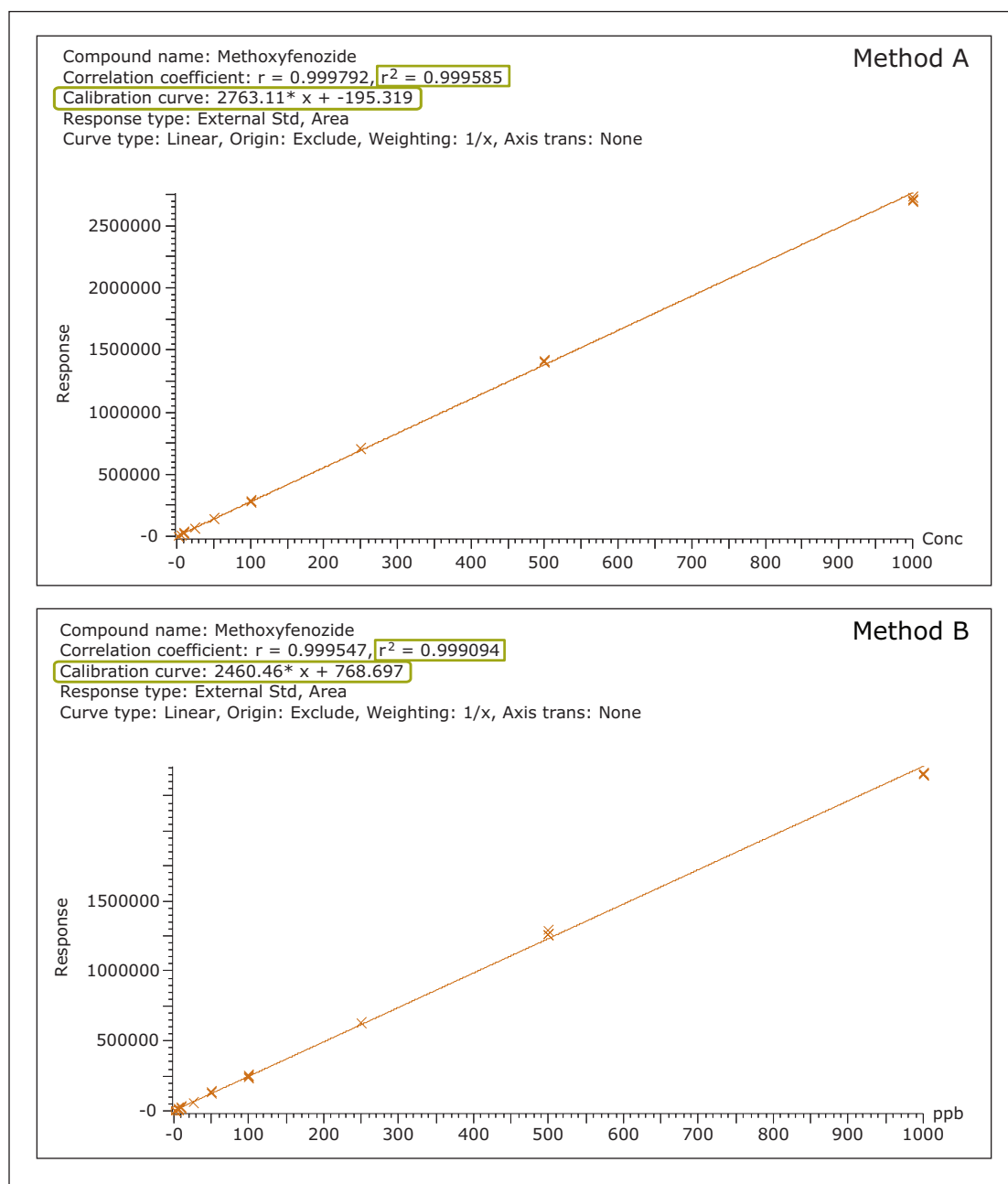


Figure 4. Matrix match spiked calibration curves of methoxyfenozide using Methods A and B.

Robustness study

To assess the repeatability and robustness of the method, 300 injections of the spiked chili sample (25 µg/kg) were analyzed by Method B. Figure 5 shows the %RSD of the peak area for example pesticide residues. As shown in Figure 5, all positive and negative ionized compound showed good %RSD (3.3 to 13.9) over 300 injections. Despite the large number of MRM transitions employing polarity switching, Method B showed excellent reproducibility and quantification at a lower concentration in a complex matrix like chili.

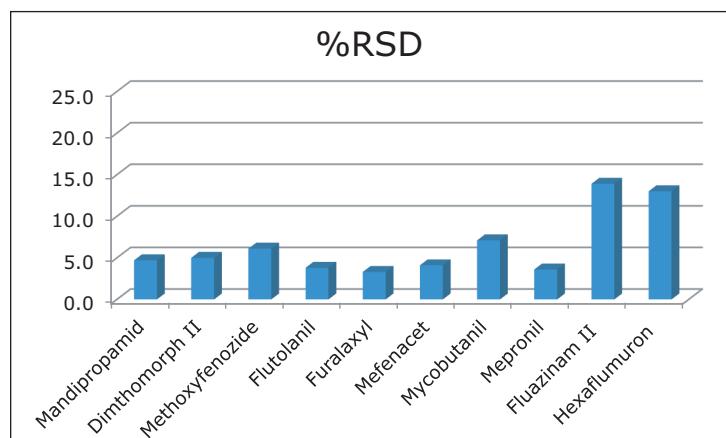


Figure 5. %RSD of 300 injections of the example pesticides spiked in chili sample at 25 µg/kg and analyzed by Method B.

Standard addition using the Auto Addition feature

A few incurred residues were found when the chili sample was screened with Method B. The standard addition method was employed to calculate the concentration of incurred residues in the chili 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. The extract was diluted directly in the sample loop before injection. In this study, Auto Addition provided an automated calibration curve for the chili sample by spiking blank chili extract with various concentration of pesticides. Figure 6 shows the Auto Addition setup sample list created in MassLynx Software.

File Name	File Text	MS File	MS Tune File	Inlet File	Bottle	Inject Volume	Auto Addition	Sample Type	Conc A
04172015_15	Acetonitrile (ACN)	430 pest_autodwell_one min window	MS Switch	LC_17 minute	1:6	5.000		Blank	
04172015_16	Non-spiked chili extract 2.5 uL_2.5 uL ACN	430 pest_autodwell_one min window	MS Switch	LC_17 minute	1:4	2.500	1:6,2.5	Standard	0
04172015_19	Non-spiked chili extract 2.5 uL_2.5 uL of 1 ppb	430 pest_autodwell_one min window	MS Switch	LC_17 minute	1:4	2.500	1:1,2.5	Standard	1
04172015_22	Non-spiked chili extract 2.5 uL_2.5 uL of 5 ppb	430 pest_autodwell_one min window	MS Switch	LC_17 minute	1:4	2.500	1:7,2.5	Standard	5
04172015_23	Non-spiked chili extract 2.5 uL_2.5 uL of 10 ppb	430 pest_autodwell_one min window	MS Switch	LC_17 minute	1:4	2.500	1:13,2.5	Standard	10
04172015_26	Non-spiked chili extract 2.5 uL_2.5 uL of 25 p...	430 pest_autodwell_one min window	MS Switch	LC_17 minute	1:4	2.500	1:19,2.5	Standard	25
04172015_27	Non-spiked chili extract 2.5 uL_2.5 uL of 50 ppb	430 pest_autodwell_one min window	MS Switch	LC_17 minute	1:4	2.500	1:25,2.5	Standard	50

↑ 1st vial
↑ 1st volume
↑ 2nd vial
↑ 2nd volume

Figure 6. Sample list created in MassLynx showing Auto Addition setup.

As shown in Figure 6, vials 1:6 and 1:4 contain acetonitrile and a blank chili extract respectively. A mix of pesticide standards in acetonitrile, ranging from 1 to 50 µg/kg (ppb), were placed in individual vials (1:6 to 1:25). Figure 6 also shows the injection order that starts with drawing 2.5 µL of non-spiked extracted chili sample followed by 2.5 µL of acetonitrile. The rest of the injections start by drawing 2.5 µL of the non-spiked extracted chili sample and 2.5 µL of various concentrations of pesticide standards. In this instance, a 5-µL injection total volume was kept constant to ensure accurate and reproducible injections. It also maintains a constant amount of matrix to allow for accurate quantification.

After the data acquisition, incurred residues were then automatically quantified using the Standard Addition functionality in TargetLynx XS Software. Figure 7 shows an example of the calculated concentration of pyraclostrobin (12.35 ppb) in a chili sample using the standard addition approach.

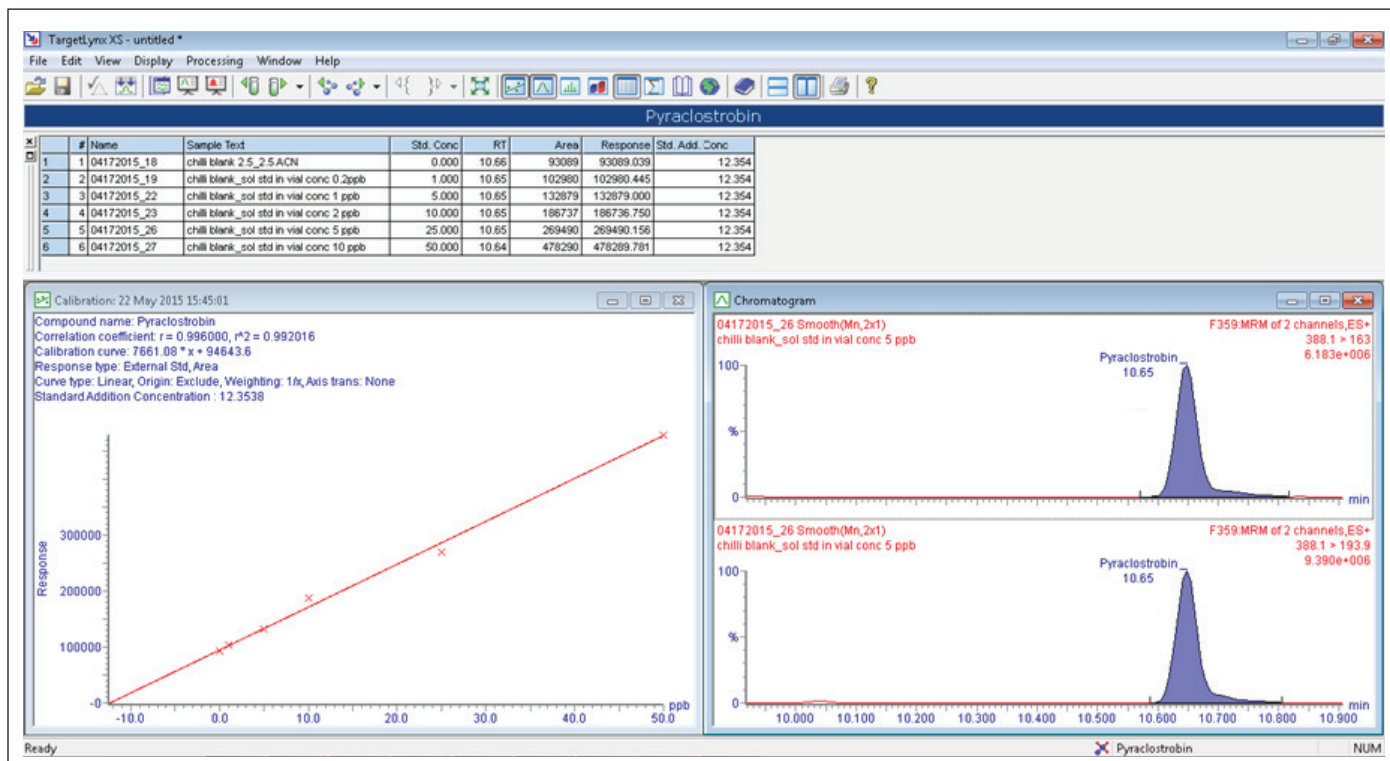


Figure 7. TargetLynx XS showing quantification of incurred pyraclostrobin in a chili sample by standard addition method.

CONCLUSIONS

- Waters LC Multi-Residue Pesticide Standards Kit is a quality assured collection of 204 compounds that eliminates the need for monotonous manual measurement of individual standards, thus saving the chemist time and laboratory resources.
- 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.
- The 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 chili powder.

References

1. D Shah, E Goh, J Burgess. Rapid analysis of Sudan and other prohibited dyes in chili powder using the ACQUITY UPLC H-Class System with Xevo TQD. Waters application note no. [720004975en](#). March, 2014.
2. DisQuE Dispersive Sample Preparation brochure no. [720003048en](#). July, 2015.

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