

## Application Note

### Abstract

The QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) sample extraction method was developed for the determination of pesticide residues in agricultural commodities. While originally QuEChERS has been developed for plant matrices the technique has since been adapted to be used in several applications and in many additional matrices such as dry tea samples.

With the rise in popularity of this extraction technique and due to its reliable multiresidue methods it has driven the need for automation of the QuEChERS extraction to increase productivity and throughput. The AutoMate-Q40 streamlines the QuEChERS method from adding Acetonitrile (ACN) and buffering salts, shaking, mixing, centrifugating the sample, transferring to a dispersive solid phase extraction (d-SPE) tube, measuring and delivering the extract.

This application note validates the extraction performance of the AutoMate-Q40 by monitoring pesticides in tea. The target residues will be determined by Liquid Chromatography tandem mass spectrometry.

### Introduction

In 2012, Americans consumed well over 79 billion servings of tea, which is just over 3.60 billion gallons.<sup>1</sup> If you factor in the rest of the world it makes tea the most consumed traditional beverage worldwide. Black, green, oolong and white teas all come from the same plant, but the differences among the types come from a diverse degree of processing and level of oxidization.<sup>1</sup>

As with any plant based commodity, it has the potential to be vulnerable to pests and diseases. In which these pests and diseases are controlled by applying pesticides to the tea crop. Thus, meaning a potential for having pesticide residues in the final product.

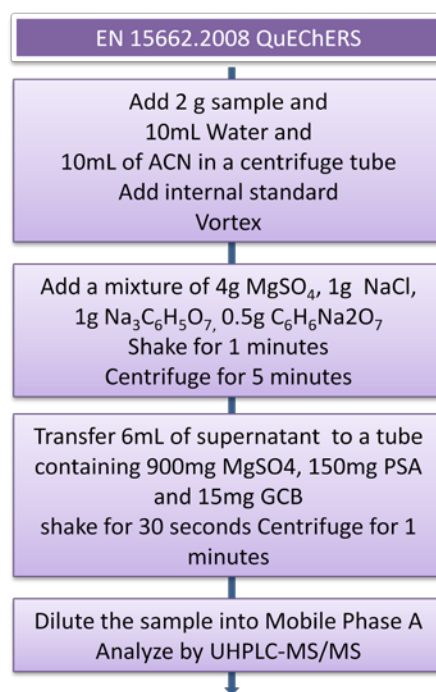
The QuEChERS extraction method offers superb selectivity, and sensitivity when extracting pesticide residues in tea. This application note evaluates the performance and versatility of the AutoMate-Q40 for the extraction of pesticides in tea. Liquid Chromatography coupled to a triple-quadrupole mass spectrometry (LC-MS/MS) was employed for the detection of pesticides in tea. Quantification was based on matrix-matched calibration curves

### Experimental Instrument Conditions

Dry tea samples (2.0 g) were weighted into a 50.0 mL centrifuge tubes. These tubes were then placed into the AutoMate-Q40 sample prep workstation, which then automates the QuEChERS procedure (EN15662:2008) (Figure 1) developed and defined by the European Reference Laboratory.<sup>2</sup>

For this analysis, the AutoMate-Q40 used 6.5 g of EN15662.2008 QuEChERS extraction salts. The AutoMate-Q40 also used the EN version of MgSO<sub>4</sub> (900.0 mg), PSA (150.0 mg) and GCB (15.0 mg) for the dSPE cleanup step.



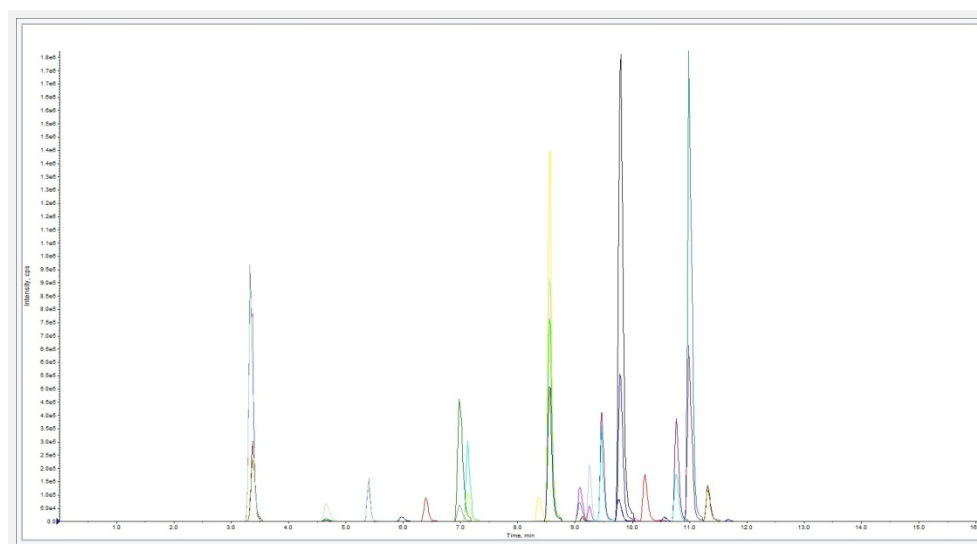
**Figure 1** Modified EN 15662:2008 QuEChERS Extraction


Sample analysis was conducted using a Shimadzu Nexera UHPLC system coupled to an AB Sciex 4500 QTrap tandem mass spectrometer (MS/MS) via electrospray ionization (ESI). For separation of the compounds of interest, a Phenomenex Kinetex 2.6um Biphenyl (50 x 2.1mm) column was used. [Table I](#) and [Table II](#) demonstrates the optimized LC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. Figure 2 shows the scheduled MRM chromatogram spiked at 400.0 ng/g.

Table I UHPLC-MS/MS Parameters	
Shimadzu Nexera	
Column	Kinetix 2.6 u Biphenyl 100Å
Column Temp	40°C
Column Flow	0.4500 mL/min
Mobile Phase	A: 5mM Ammonium Acetate in Water B: 5mM Ammonium Acetate in Methanol
Injection Volume	10 uL
AB Sciex 4500 QTrap	
Curtain Gas	30
Ion Spray (V)	4500
Temperature (°C)	500
GS1	50
GS2	60

**Table II LC-MS/MS Transitions**

Compounds	Q1 Mass (Da)	Q3 Mass (Da)	RT	DP	CE	CXP	Compounds	Q1 Mass (Da)	Q3 Mass (Da)	RT	DP	CE	CXP
Boscalid	342.897	307.0	9.21	66	27	24	Metalaxyl	279.995	220.0	8.49	21	19	16
	342.897	271.0	9.21	66	43	20		279.995	192.0	8.49	21	25	14
Buprofezin	306.002	201.0	10.97	21	17	18	Methiocarb	225.920	168.9	8.51	41	13	14
	306.002	115.9	10.97	21	21	10		225.920	121.0	8.51	41	25	10
Carbaryl 1	202.014	144.9	7.08	11	13	12	Methomyl	162.981	87.9	3.29	6	13	12
	202.014	127.0	7.08	11	37	12		162.981	106.0	3.29	6	13	16
Carbendazim	191.977	159.9	4.56	40	23	16	Metolachlor	283.976	252.0	9.77	16	21	20
	191.977	131.9	4.56	40	41	10		283.976	176.0	9.77	16	33	14
Chlorpyrifos	349.76	197.8	11.28	56	25	16	Metribuzin	214.943	187.0	6.34	46	25	14
	349.76	96.9	11.28	56	47	14		214.943	168.8	6.34	46	13	14
Cyprodinil	225.977	93.1	9.71	31	47	8	Monocrotophos	223.934	192.8	3.25	41	11	14
	225.977	108.0	9.71	31	33	10		223.934	126.9	3.25	41	21	12
Imazalil	296.908	158.9	10.15	30	31	11	Myclobutanil	288.931	70.0	9.02	16	47	10
	296.908	160.9	10.15	30	23	15		288.931	124.9	9.02	16	39	10
Imidacloprid	255.886	208.9	5.34	46	21	16	Profenofos	372.850	302.7	10.72	56	25	22
	255.886	175.0	5.34	46	25	14		372.850	344.8	10.72	56	17	26
Iprodione	329.89	244.8	10.00	54	21	22	Pyrimethanil	200.017	107.0	8.31	70	31	10
	329.89	287.8	10.00	54	16	19		200.017	82.0	8.31	70	21	11
Malathion	330.909	126.9	9.44	11	17	10	Simazine	201.965	131.9	5.89	46	25	10
	330.909	98.9	9.44	11	31	10		201.965	124.0	5.89	46	25	10
							Thiodicarb	355.808	107.9	9.07	30	21	10
								355.808	163.0	9.07	30	11	12

**Figure 2** Tea MRM chromatogram spiked at 400.0 ng/g.


## Experimental Results

By automating the QuEChERS extraction, it enables an easy, reliable and more reproducible extraction. The AutoMate-Q40 offer significant labor savings, while it improves the repeatability and consistency between the samples.

A precision and accuracy study was performed using the AutoMate-Q40. A 0.2 and 2.0 µg/mL stock pesticide solution was used to fortify the tea samples. Using the AutoMate-Q40, the system is able to spike the following samples with 50.0 and 100.0 µL of the pesticide standard that yielded a 10.0, 50.0 and 100.0 ng/g check samples. These QC samples were quantitated against their corresponding matrix matched calibration curve.

Table III documents the AutoMate-Q40 ability to extract pesticide residues from tea samples with 90% of all the recoveries falling within 70-120%. These spike recoveries fall well within the recommended values in **Document N° Sanco/12495/2011**,<sup>3</sup> which states the mean recoveries must fall within 70% to 120% and a RSD <20%. The AutoMate-Q40 also demonstrated great precision on average of 6.6%RSD for the spiked QC samples.

**Table III Recovery and Reproducibility of the AutoMate-Q40 in Fortified Tea**

Compounds	Low Spike 10ng/g		Mid Spike 50ng/g		High spike 100ng/g	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Atrazine	99.7	4.5	87.3	3.3	89.8	2.1
Boscalid	65.7	14.3	95.6	5.0	103.2	4.2
Buprofezin	97.2	4.7	88.9	3.2	92.0	1.8
Carbaryl	81.2	7.6	89.8	3.3	92.2	4.6
Carbendazim	Nd	Nd	74.6	19.0	93.1	14.3
Chlorpyrifos	94.0	9.9	91.5	4.3	98.2	2.4
Cyprodinil	100.6	5.9	90.4	5.9	95.9	0.7
Imazalil	61.0	11.4	84.4	6.3	89.5	5.4
Imidacloprid	96.4	6.2	90.6	5.9	93.9	3.9
Malathion	97.4	4.6	97.8	3.3	101.3	5.6
Metalaxyl	93.0	5.1	93.3	4.4	97.4	4.1
Methiocarb	92.4	5.2	89.7	4.8	92.9	2.5
Methomyl	88.4	8.7	87.9	6.6	96.2	1.9
Metolachlor	97.3	3.9	92.5	2.5	96.9	3.1
Metribuzin	63.0	19.9	82.5	4.9	91.9	2.9
Monocrotophos	100.9	6.3	91.3	4.8	96.6	0.9
Myclobutanil	94.9	16.2	89.6	8.2	91.7	5.8
Profenofos	59.9	14.2	82.8	5.9	92.2	2.2
Pyrimethanil	77.9	11.4	85.8	8.2	88.3	4.1
Thiodicarb	115.6	17.9	96.7	6.1	87.7	4.9

Nd = not detected

This developed method was then applied to the quantitation of pesticides in real tea. Figure 3 shows an example of the tea chromatogram that was collected. Table IV shows the pesticides concentration in said tea samples (n=3).

**Figure 3** Tea sample (extract 10x dilution) screen for pesticides

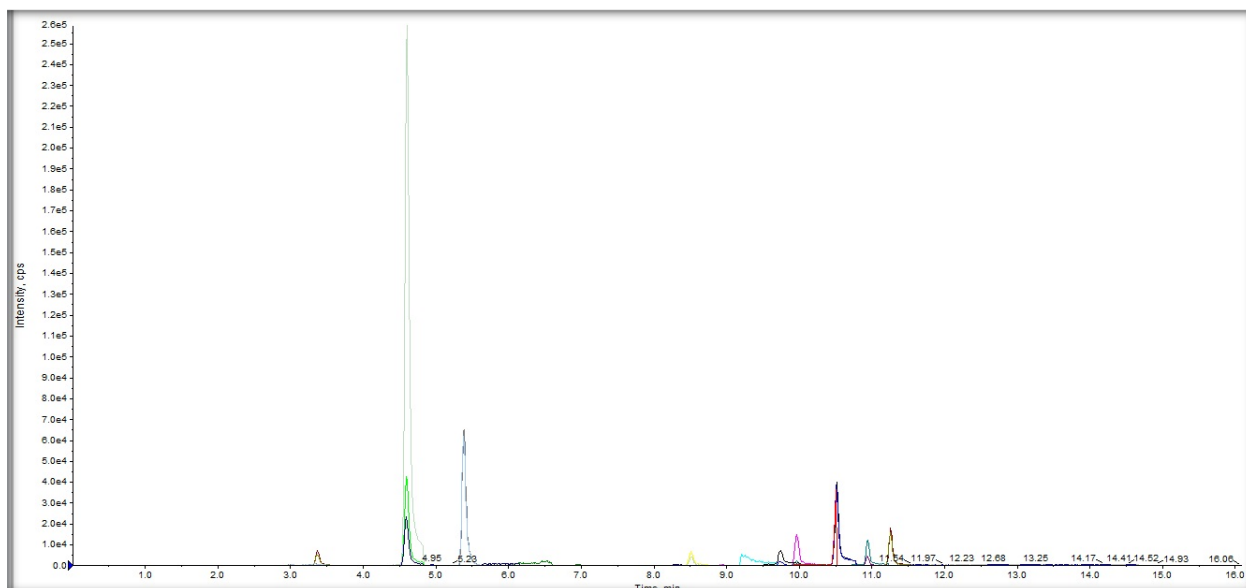
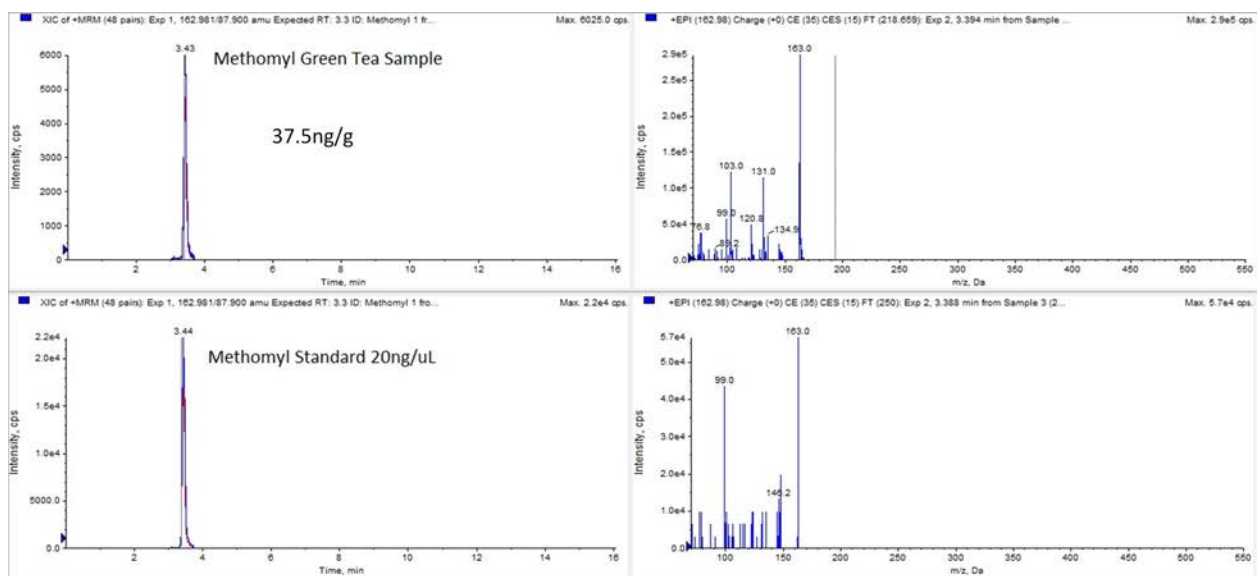


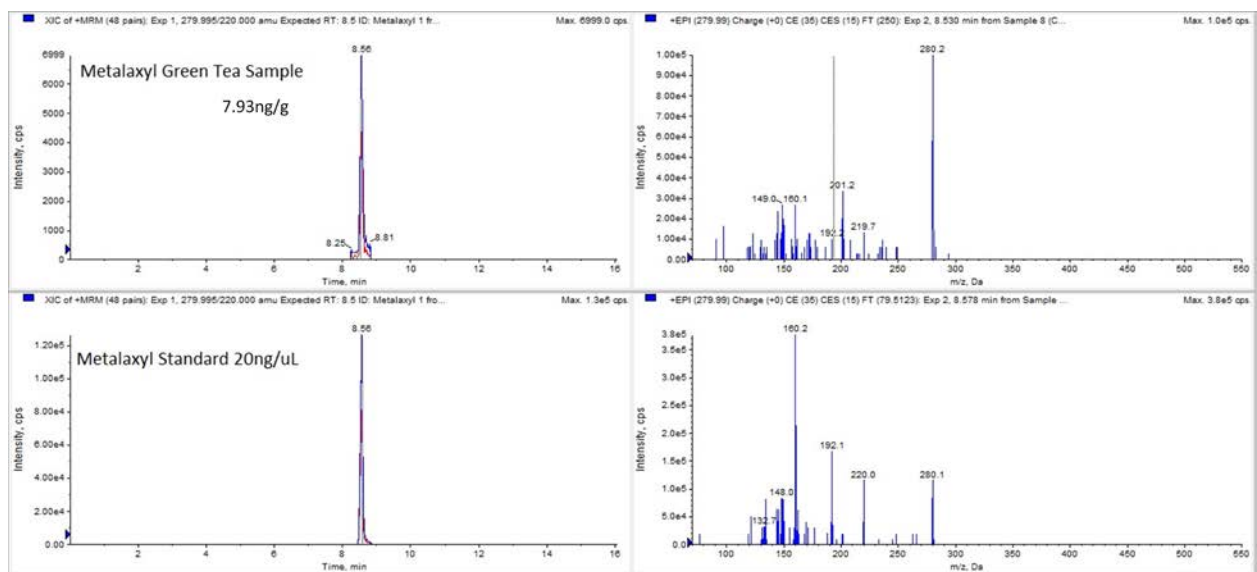
Table IV Summary of Pesticide Findings in Real Tea Samples		
Sample	Pesticide	Concentration (ng/g)
Tea	Methomyl	37.5
	Metalaxyl	7.90
	Imidacloprid	861
	Metolachlor	1.50
	Buprofezin	9.00
	Chlorpyrifos	176

Despite having the high selectivity of the MRM detection there is always a risk for false positives due to matrix interference. For improved accuracy and identification of the pesticides, the MS/MS was run in Enhanced Product Ion (EPI) mode of the 4500 QTRAP. This allowed for spectral library matching to improve the confidence of detection. Figures 4-9 show the found pesticide MRM and spectra compared to a known standard MRM and spectra.

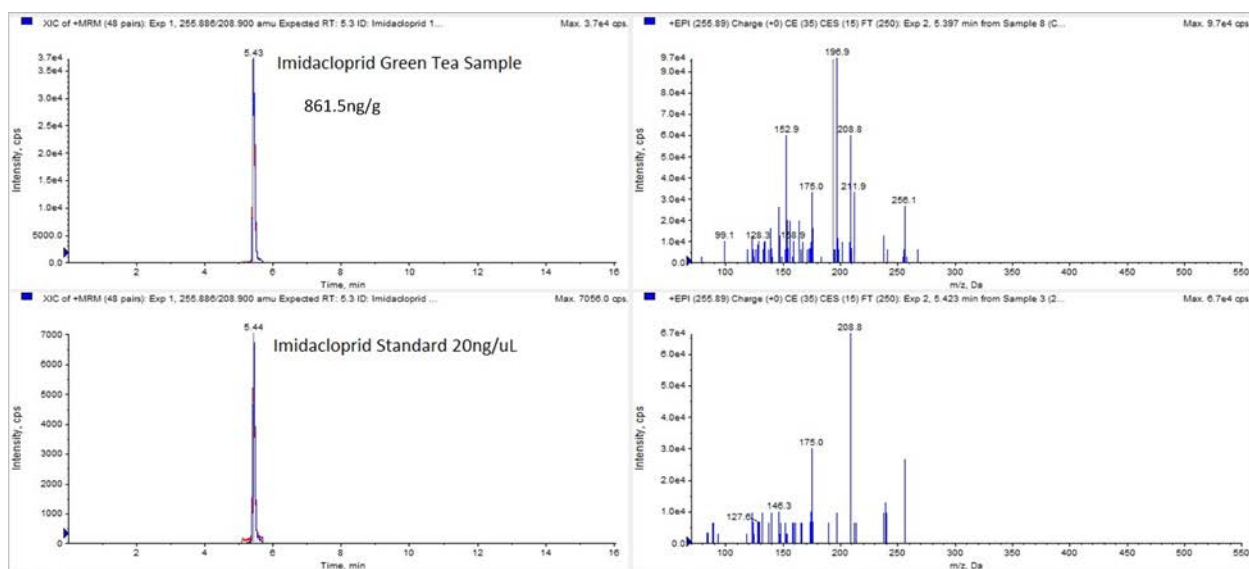
**Figure 4** Methomyl Example Spectra



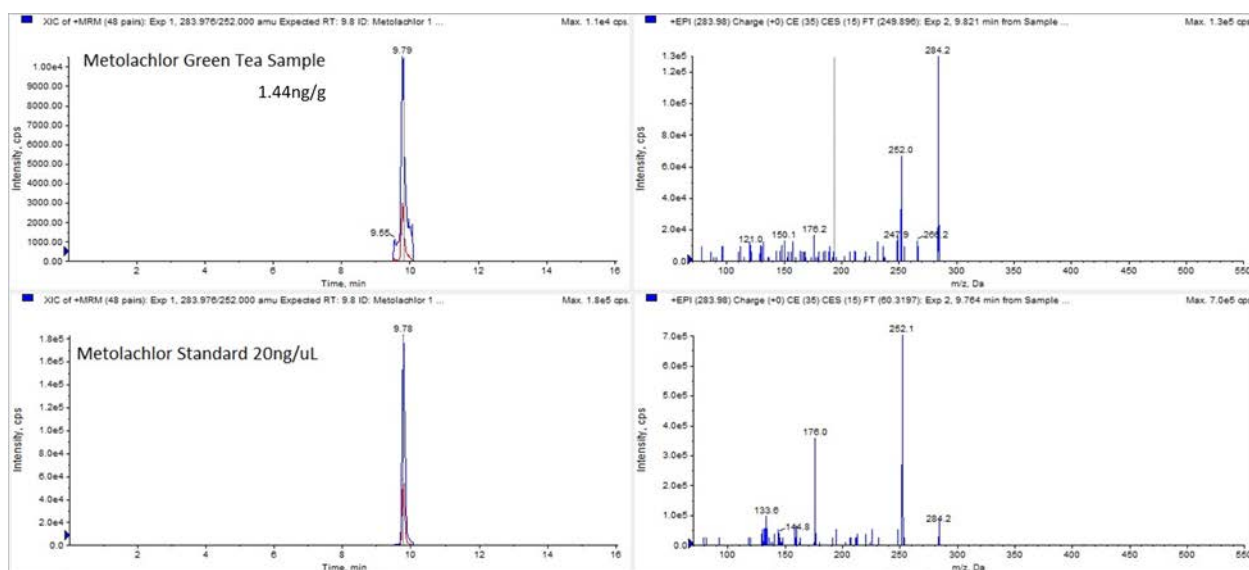
**Figure 5** Metalaxyl Example Spectra



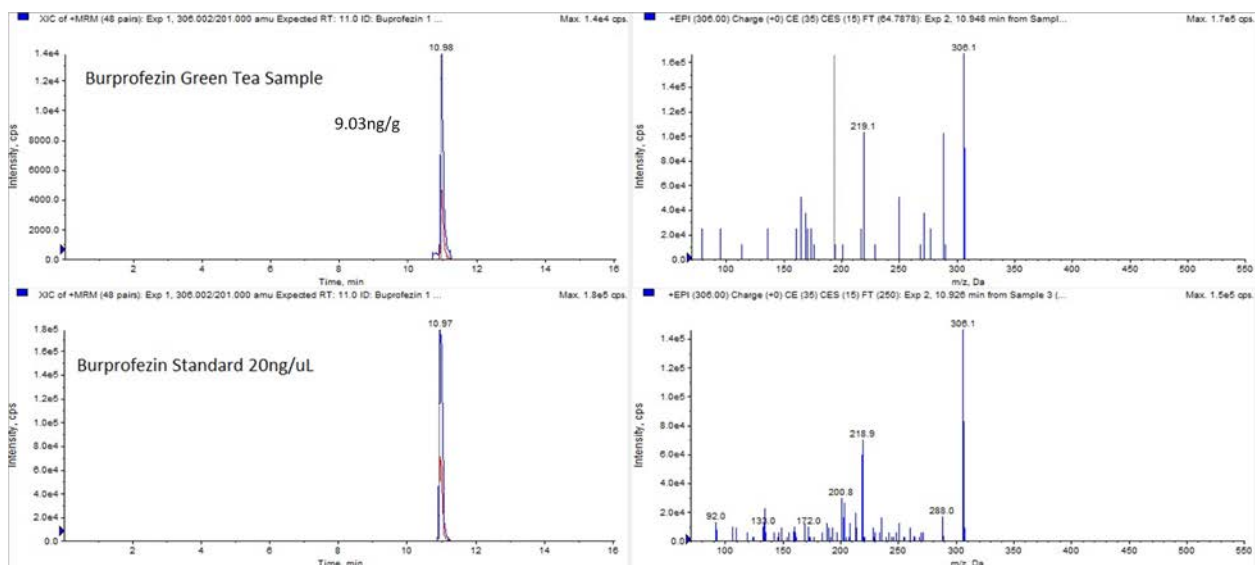
**Figure 6** Imidacloprid Example Spectra



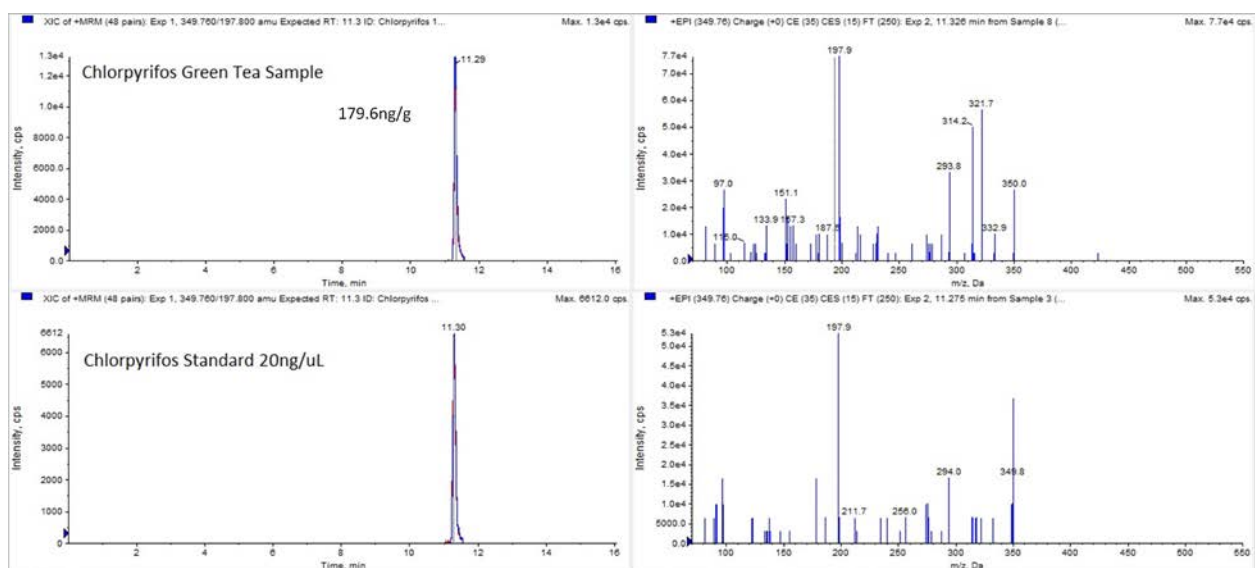
**Figure 7** Metolachlor Example Spectra



**Figure 8** Bupropfezin Example Spectra



**Figure 9** Chlorpyrifos Example Spectra



## Conclusion

This study demonstrates the Automate-Q40's ability to successfully process tea samples for pesticide residue by the QuEChERS extraction method. By automating the liquid handling, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision™, the AutoMate-Q40 frees the scientist from a labor-intensive extraction method and exposure to unhealthy chemicals.

The automated extraction process enables an easy, reliable and more reproducible extraction. This enables time and labor savings, while improving consistency and reproducibility of the extraction. As shown above in Table III the combined pesticide spikes recoveries of 90.71%, with an average RSD of 6.61% exceed the requirement outlined in The Document N° Sanco/12495/2011. These numbers indicate superb precision and accuracy thus validating the performance of the AutoMate-Q40 to adequately perform the QuEChERS pesticide extraction method for tea.



## References

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2. European Committee for Standardization/Technical Committee CEN/TC275 (2008),  
Foods of plant origin: Determination of pesticide residues using GC-MS and/or LC-  
MS/MS following acetonitrile extraction/ partitioning and cleanup by dispersive SPE  
QuEChERS-method.
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and Feed (Document N° SANCO/12495/2011)