AutoMate-Q40 Automated QuEChERS Extraction for Pesticide Residues in Botanicals

Application Note

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Abstract

QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe extraction method that has been developed for the determination of pesticide residues in agricultural commodities. Since its development, QuEChERS has been applied to many different classes of commodities ranging from fruits and vegetables to dry grains and teas. In 2007, the United States Food and Drug Administration (USFDA) announced that the makers of dietary supplements (botanicals) must adhere to stricter regulations.[1] Since a majority of the dietary supplements are sourced from botanicals, it called for the determination of pesticide residues in samples. It was determined that the QuEChERS extraction was suitable to extract pesticide residues from the botanical samples.

This study will evaluate the performance and versatility of the AutoMate-Q40, an automated QuEChERS extraction platform. Liquid Chromatography coupled to a triple-quadrupole Mass Spectrometer (LC-MS/MS) was used to determine pesticide residues from the botanical samples. Quantification was based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy. QC samples were evaluated at various levels to ensure precision and accuracy of the AutoMate-Q40 extraction.

Introduction

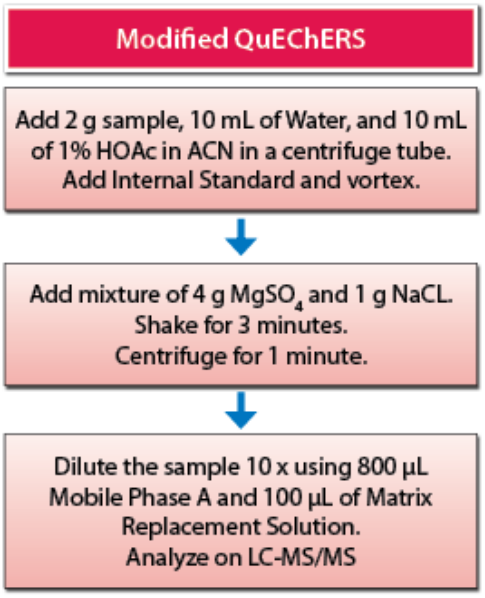
Botanical products are used to improve health and energy, and in 2015 reached sales of $93.15 billion worldwide. [2] Often the popular perception is that botanicals are harvested “in the wild”, but in reality many botanicals are farmed using conventional agricultural practices, including the application of pesticides and fungicides.[3] Consequently, in 2007 the United States Food and Drug Administration announced that makers of dietary supplements (botanicals) must adhere to more stringent regulations.[4]

The QuEChERS extraction method offers superb selectivity and sensitivity when extracting pesticide residues in botanical samples. This study evaluates the performance and versatility of the AutoMate-Q40 for the extraction of pesticides in botanicals using an automated QuEChERS extraction method. Liquid Chromatography coupled to a triple-quadrupole Mass Spectrometer (LC-MS/MS) was employed for the detection of the pesticides. Quantification was based on matrix-replacement calibration curves.

Experimental Conditions

“Real World” Pepin and Ginkgo samples were submitted to Teledyne Tekmar for this study. For method development, a pesticide-free green coffee matrix was used to establish the method validation. The samples were stored at ambient temperature until the time of extraction. Figure 1 lists the sample preparation and extraction steps used for this analysis. The AutoMate-Q40 performed a modified unbuffered QuEChERS method using 5.0 g of unbuffered QuEChERS extraction salts (4.0 g $MgSO_4$ and 1.0 g NaCl). [5,6]

Figure 1 AutoMate-Q40 Sample Preparation and Extraction



Sample analysis was conducted using a Shimadzu Nexera® LC interfaced to an AB Sciex™ 4500 QTrap® Triple-Quad Mass Spectrometer (LC-MS/MS). For separation of the compounds of interest, a Phenomenex® Kinetex® 2.6u Biphenyl column was used. Table I and Table II show the optimized LC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. Figure 2 shows the scheduled Multiple Reaction Monitoring (MRM) chromatogram spiked at 0.4 mg/kg.

Table I Critical LC-MS/MS SRM Transitions and Parameters for AB Sciex™ 4500 QTrap®			
Curtain Gas (CUR)			20
Ion Spray Voltage (IS)			+/-4500
Temperature (TEM)			500 ˚C
Collision Gas (CAD)			High
Analyte Transitions			
Compounds	**RT (min)**	**Precursor Ion (m/z)**	**Quantization Product Ion (m/z)**
Acetamiprid	5.66	223.2	125.9
Azoxystrobin	8.86	404.3	371.9
Boscalid	8.12	343.2	306.8
Chlorpyrifos	9.75	349.5	197.7
Clothianidin	3.71	250.1	131.9
Difenoconazole	9.54	406.2	187.9
Monocrotophos	3.20	224.2	126.9
Myclobutanil	7.97	289.2	70.0
Propiconazole	8.92	342.2	158.8
Pyraclostrobin	9.54	388.1	193.9

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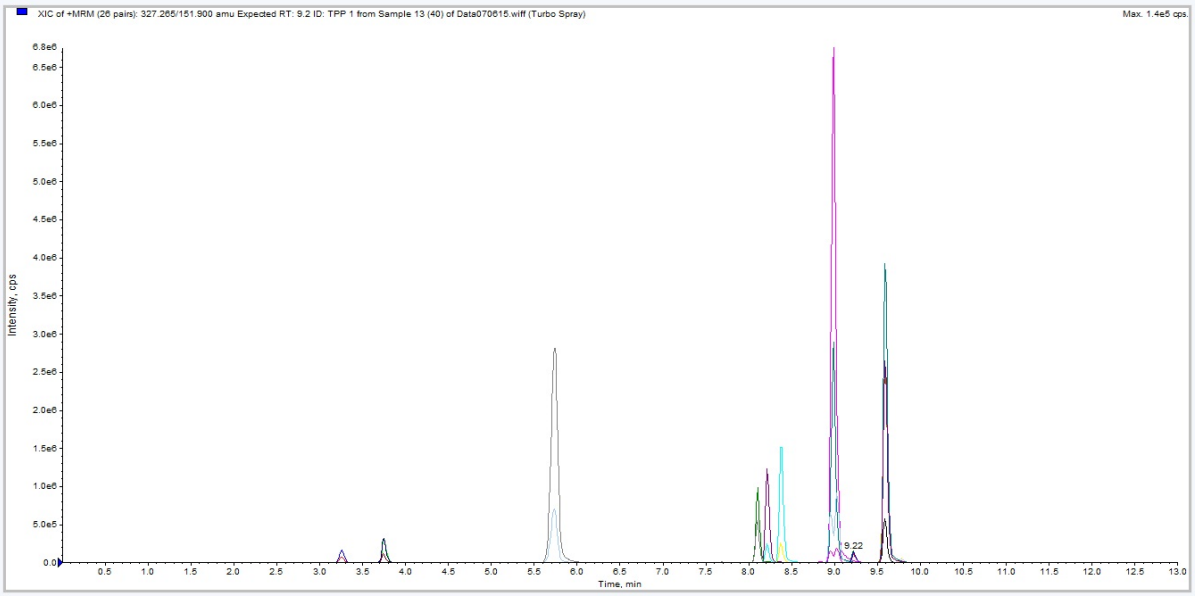
Table II Shimadzu Nexera® LC Parameters		
Column	Phenomenex® Kinetex® 2.6u Biphenyl	
Dimensions	50 X 2.1 mm	
Mobile Phase	A: 5 mM Ammonium Acetate in Water B: 5 mM Ammonium Acetate in Methanol	
Gradient	Time	%B
	0.0	10%
	1.0	10%
	10.0	90%
	15.0	90%
	15.1	10%
	20.0	10%
	20.1	Stop
Flow Rate	0.500 mL/min	
Column Temperature	35 °C	

Figure 2 Spiked Matrix Match Calibration Sample 0.400 mg/kg



Results

By automating the QuEChERS extraction, an easy, reliable and more reproducible extraction is possible. The AutoMate-Q40 offers significant labor savings, while improving the repeatability and consistency between samples.

A precision and accuracy study was performed using the AutoMate-Q40. A 2.0 µg/mL stock pesticide solution was used to fortify the botanical samples. Using the AutoMate-Q40's ability for spike addition, 25.0, 50.0 and 100.0 µL of the pesticide standard was added to each sample, yielding 5.0, 10.0 and 20.0 ng/g Quality Control (QC) samples. These QC samples were quantitated against their corresponding matrix replacement calibration curve.

Table III exhibits the AutoMate-Q40's ability to extract pesticide residues from botanical samples. All recoveries fall easily within the recommended 70-120% mean recovery and <20% RSD. The AutoMate-Q40 also demonstrated great precision, averaging 5.0%RSD for the spiked QC samples.

Table III Spike Recovery Table for Botanical Samples

Compound	5.0 ng/g		10.0 ng/g		20.0 ng/g	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Acetamiprid	105.5	4.6	119.9	14.0	102.1	2.6
Azoxystrobin	76.1	4.6	100.4	10.2	97.5	3.1
Boscalid	104.7	6.6	110.1	8.6	96.2	1.3
Chlorpyrifos	78.4	16.7	82.3	3.8	87.5	3.3
Clothianidin	105.1	2.1	104.9	12.3	93.8	3.3
Difenoconazole	100.1	4.0	99.4	1.4	98.7	4.2
Monocrotophos	101.2	4.8	102.3	3.3	98.7	2.8
Myclobutanil	99.1	3.1	99.5	4.5	95.7	1.6
Propiconazole	102.7	4.2	102.8	7.6	98.9	1.6
Pyraclostrobin	96.0	2.9	94.8	1.7	93.9	5.5
Tebuconazole	115.0	7.5	110.0	6.5	96.0	5.4

The developed method was then applied to the quantitation of pesticides in the submitted, "real-world" botanical samples. Figure 3 shows an example of the Pepin chromatogram that was collected. Table IV shows the pesticides concentration in the "real world" botanical samples (n=3).

Figure 3 Pepin (A) Sample (extract 10X Dilution)

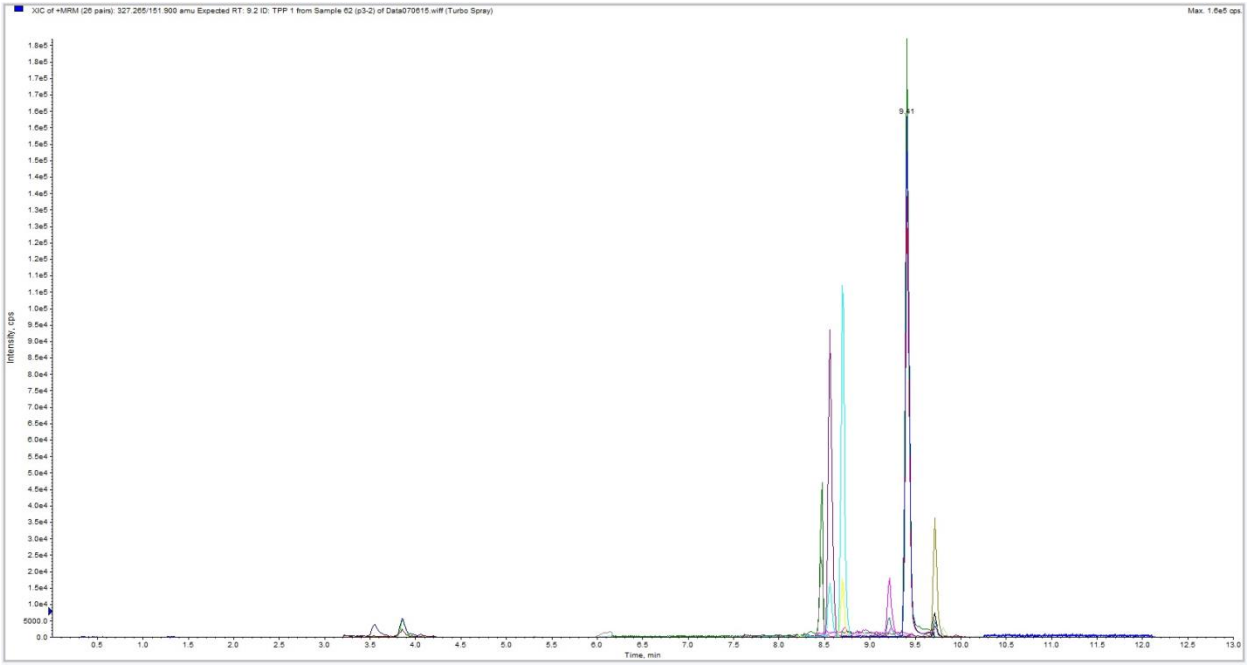


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Table IV Summary of Pesticide Findings in "Real World" Botanical Samples (ng/g)						
Sample Name	Ginkgo A	Ginkgo B	Ginkgo C	Pepin A	Pepin B	Pepin C
Acetamiprid	-	-	-	-	-	-
Azoxystrobin	-	-	-	-	-	-
Boscalid	-	-	-	11.74	17.13	25.24
Chlorpyrifos	-	-	-	5.31	-	-
Clothianidin	-	-	-	4.58	6.68	8.96
Difenoconazole	-	-	-	2.70	2.51	4.69
Monocrotophos	-	-	-	-	-	-
Myclobutanil	-	-	-	-	-	-
Propiconazole	-	-	-	-	-	-
Pyraclostrobin	-	-	-	-	-	-
Tebuconazole	-	-	-	21.35	16.86	28.13

Conclusion

This study demonstrates the Automate-Q40's ability to successfully process botanical 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 VialVision feature, the AutoMate-Q40 frees the analyst from the labor-intensive extraction method as well as exposure to unhealthy chemicals.

The automated extraction process enables an easy, reliable and more reproducible extraction, significantly saving time and labor and improving consistency and reproducibility of the extraction. As shown in Table III the combined pesticide spike recoveries of 98.3% with an average RSD of 5.0% exceed the requirement for this study. These numbers indicate superb precision and accuracy, thus validating the performance of the AutoMate-Q40 to perform the QuEChERS pesticide extraction method for botanical samples.

References

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