

Determinación de residuos de pesticidas en vegetales mediante el sistema robotizado AutoMate-Q40

Aplicación 052

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RESUMEN

El Método de extracción QuEChERS fue desarrollado para la determinación de residuos de pesticidas en procesado alimentario. En 2003 se creó el método no tamponado para matrices vegetales, se amplió a otros dos métodos tamponados para ampliar el tipo de matrices. El crecimiento de la popularidad del método QuEChERS ha generado la necesidad de su automatizado. El sistema robotizado modelo AutoMate-Q40 modela los pasos del método QuEChERS desde la extracción sólido-líquido a la limpieza dSPE.

Esta aplicación muestra su versatilidad valida el Automate-Q40 en matrices vegetales en partículas tomates y judías verdes. Como detección para la determinación de los residuos de pesticidas se utiliza un LCMS/MS. Las curvas de calibrado construidas sobre su matriz y el uso de patrones internos para asegurar la exactitud en la cuantificación (Document N° Sanco/12495/2011). Se avalúan muestras de control de calidad a niveles de 20.0, 40.0 y 80.0 ng/g para verificar la exactitud y presión del AutoMate-Q40. El límite de cuantificación (LOQ) obtenido es 10.0 ng/g.

Introduction

Pesticides residue laboratories are required to undertake analyses of an ever increasing number of samples. The analyses typically involve use of multi-residues methods (both GC-MS and LC-MS) to test for over 500 pesticides residues. Due to its ease of use and proven robustness, the QuEChERS extraction has become the method of choice for pesticide multi-residue analyses in wide range of matrices.

This study used laboratory spiked samples to validate the performance of the QuEChERS extraction on a fully automated system, for pesticides amenable to analysis by LC-MS/MS. Even though the QuEChERS is a simplified extraction

technique, it still requires numerous manual steps including the addition of solvent, extraction salts, centrifugation, shaking, decanting and performing the dSPE cleanup¹⁻³. Teledyne Tekmar has developed the AutoMate-Q40 to automate the QuEChERS extraction method, allowing scientist additional time to focus on other laboratory requirements. This automated platform streamlines the two part QuEChERS method from the homogenate to the final extract.

The AutoMate-Q40 will be used to perform an automated QuEChERS extraction of pesticides in green beans and tomatoes. Quantification is based on matrix-matched calibration curves with the use of an internal standard to ensure method accuracy. QC samples were analyzed at known concentrations of 20.0, 40.0, and 80.0 ng/g, precision and accuracy studies were performed to validate the AutoMate-Q40.



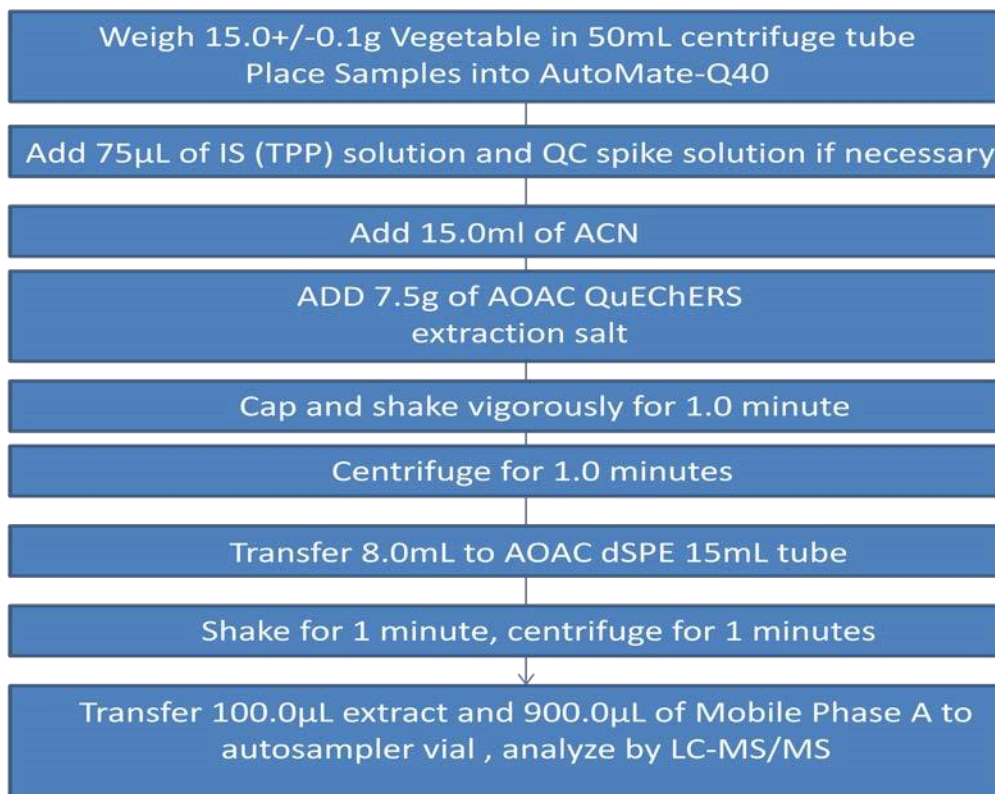
Instrument Conditions

Extraction Parameters

Green beans and tomato samples were prepared following “AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate”¹. The fresh samples were stored at room temperature. Once homogenize, the samples were stored in the refrigerator.

Figure 1 shows the sample preparation and extraction steps that are needed to extract the pesticide residues from vegetables. For this analysis, the AutoMate-Q40 used AOAC QuEChERS buffered extraction salts (Anhydrous MgSO₄ and NaOAc). The AutoMate-Q40 also used the AOAC version of MgSO₄, and PSA for the dSPE cleanup step. (1200mg MgSO₄, 400mg PSA)

Figure 1 AutoMate-Q40 Extraction Parameters for Green Beans and Tomato



Instrumentation and Analytical Conditions

A Shimadzu Nexera LC interfaced to an AB Sciex 4500 QTrap, triple-quad mass spectrometer (LCMS/MS) was used to separate and detect the pesticide residues. A Phenomenex Synergi 4u Fusion RP

80Å column was used to separate the pesticides of interest. Table I and Table II documents the critical LC-MS/MS analysis parameters for chromatographic separation and optimal analyte transitions. Figure 2 shows the scheduled MRM chromatogram for a sample spiked at the 400.0 ng/g level.

Table I Critical LC-MS/MS SRM Transitions and Parameters for AB Sciex 4500 QTrap

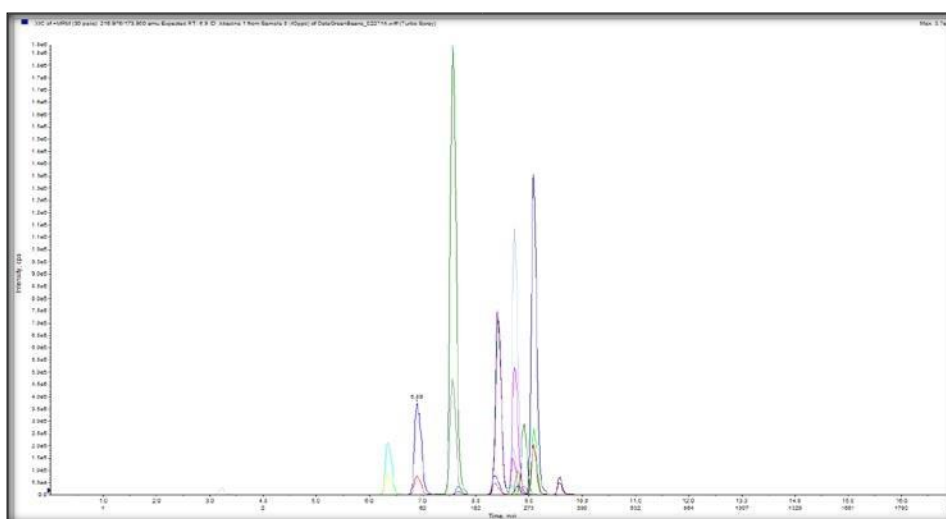
Curtain Gas (CUR)					20	
Ion Spray Voltage (IS)					5500	
Temperature (TEM)					350	
Collision Gas (CAD)					Medium	
Analyte Transitions						
Compounds	RT (min)	Precursor Ion (m/z)	Quantization product Ion (m/z)	DP(V)	CE(V)	CXP(V)
Atrazine 1	6.9	215.97	173.9	11	25	14
Atrazine 2	6.9	215.97	103.9	11	39	14
Azoxystrobin 1	7.6	403.88	372.0	41	21	10
Azoxystrobin 2	7.6	403.88	343.9	41	35	26
Bitertanol 1	8.7	337.95	268.8	16	13	22
Bitertanol 2	8.7	337.95	99.00	16	19	10
Boscalid 1	7.7	344.80	306.7	76	25	10
Boscalid 2	7.7	344.80	139.9	76	23	14
Carbaryl 1	6.3	201.90	144.9	11	13	22
Carbaryl 2	6.3	201.97	126.9	11	39	10
Chlorpyrifos 1	9.6	351.95	96.90	51	43	10
Chlorpyrifos 2	9.6	351.95	199.6	51	27	18
Clofentezine 1	8.8	302.78	137.8	46	21	12
Clofentezine 2	8.8	302.78	101.9	46	51	14
Cyprodinil 1	8.4	225.94	92.9	111	47	8
Cyprodinil 2	8.4	225.94	76.9	111	65	8
Difenoconazole 1	8.9	405.78	250.8	46	33	18
Difenoconazole 2	8.9	405.78	187.9	46	61	14
Pyaclostrobin1	8.7	387.86	193.8	46	17	18
Pyaclostrobin 2	8.7	387.86	162.8	46	33	14
Tebufozide 1	8.4	353.02	132.9	56	25	14
Tebufozide 2	8.4	353.02	296.9	56	11	22
TPP 1	8.7	326.91	214.7	91	35	16
TPP 2	8.7	326.91	167.7	91	47	14
Trifloxystrobin 1	9.1	408.91	185.6	56	27	18
Trifloxystrobin 2	9.1	408.91	144.7	56	61	12
Triflumazole 1	9.1	345.88	277.6	20	15	24
Triflumazole 2	9.1	345.88	72.80	20	21	6

Table II Shimadzu Nexera LC Parameters

Column	Synergi 4u Fusion RP 80Å
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Dimensions	50 X 2.00 mm	
Mobile Phase	A:5mm Ammonium Acetate w/0.5% Acetic in H ₂ O	
	B:5mm Ammonium Acetate w/0.5% Acetic in MeOH	
Gradient	Time	%B
	0.10	5%
	9.00	90%
	12.00	100%
	15.00	100%
	15.10	20%
17.00	STOP	
Flow Rate (mL/min)	0.300	
Column Temperature (°C)	40	

Figure 2 400.0 ng/g Spike Pesticides in Tomato and Green Beans



Experimental Results

Automating the QuEChERS extraction, enables fast, easy, reliable and more reproducible extractions. The AutoMate-Q40 offers significant labor savings, while improving the repeatability and consistency of the sample extractions.

A precision and accuracy study was performed using the AutoMate-Q40. A 6.0 µg/mL stock pesticide solution was used to fortify the vegetable samples. The AutoMate-Q40 spiked the samples with 50.0, 100.0 and 200.0 µL of the pesticide standard, to produce 20.0, 40.0 and 80.0 µg/L check samples. The AutoMate-Q40 was also used to spike 75.0 µL of the internal standard, Triphenylphosphate, (TPP) in each sample. These QC samples were then quantitated against the corresponding matrix matched calibration curve.

Table III LC-MS/MS Values for Tomatoes Checks Samples Extracted using AutoMate-Q40						
Compound	Low Spike 20.0 ng/g		Medium Spike 40.0 ng/g		High Spike 80.0 ng/g	
	%Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
Azoxystrobin	92.2	7.3	99.8	1.7	101.2	7.4
Bitertanol	88.8	7.4	93.6	2.4	90.4	6.2

Boscalid	92.5	8.7	98.4	2.8	94.9	8.1
Carbaryl	94.8	7.8	98.7	2.1	100.6	5.5
Chlorpyrifos	90.8	7.0	91.5	2.6	91.7	6.3
Clofentezine	88.9	8.6	91.8	1.9	93.7	8.6
Cyprodinil	84.6	9.4	89.1	2.2	87.2	6.7
Difenoconazole	88.2	9.2	91.0	3.6	89.6	6.7
Pyraclostrobin	88.3	10.0	94.1	2.0	94.4	7.4
Tebufozide	90.6	9.4	94.7	1.6	94.1	6.8
Trifloxystrobin	87.1	10.1	95.5	4.4	93.2	7.8
Triflumazole	84.5	9.5	87.9	3.3	87.0	7.6

Table IV LC-MS/MS Values for Green Bean Checks Samples Extracted using AutoMate-Q40

Compound	Low Spike 20.0 ng/g		Medium Spike 40.0 ng/g		High Spike 80.0 ng/g	
	%Recovery	%RSD	%Recovery	%RSD	% Recovery	%RSD
Atrazine	82.7	7.7	88.6	3.4	94.5	1.2
Azoxystrobin	97.5	6.1	105.3	4.1	114.1	2.0
Bitertanol	88.6	7.38	93.5	4.7	100.6	2.4
Boscalid	94.2	6.5	101.6	4.7	109.4	2.2
Carbaryl	84.8	7.2	89.8	4.4	98.5	1.7
Chlorpyrifos	89.3	6.3	92.8	3.1	98.5	3.4
Clofentezine	92.3	6.5	98.2	5.8	106.4	1.2
Cyprodinil	90.5	6.4	94.7	4.0	101.7	2.3
Difenoconazole	89.0	6.7	92.8	4.2	99.8	1.6
Pyraclostrobin	96.4	4.7	102.7	4.1	110.8	2.0
Tebufozide	96.8	5.2	107.5	5.1	118.0	1.6
Trifloxystrobin	96.5	5.5	101.2	4.0	110.7	2.2
Triflumazole	90.4	4.8	94.0	3.6	100.8	1.1

The data from tables III and Table IV documents the AutoMate-Q40 ability to extract pesticide residues from vegetable samples with recoveries ranging from 82.8% to 118.1%. These spike recoveries fall well within the recommended mean values in **Document N° Sanco/12495/2011**⁵ which states the mean recoveries must fall within 70% to 120% and a RSD <20%. The AutoMate-Q40, also, demonstrated great precision ranging from 1.7% to 10.1%RSD for the spiked QC samples.

Conclusion

The AutoMate-Q40 successfully processes vegetable samples for pesticide residue utilizing the QuEChERS method. By automating the liquid handling, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using patent pending VialVision™. Today's scientists are freed from laborintensive extraction methods and exposure to unhealthy chemicals.

This creates an extraction process that is faster, more reliable, and easier than the traditional manual based procedure. As seen above in Tables III and IV, excellent precision and accuracy can be achieved without consuming valuable analysts' time.

Additionally, the combined pesticide spike recoveries of 95.0%, at an average RSD of 5.17%, exceed the requirement outlined in **The Document N° Sanco/12495/2011**⁵, validating the performance of the AutoMate-Q40 to perform the QuEChERS pesticide extraction method for vegetables.

References

1. 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.
2. AOCA Official Method 2007.07 Pesticide Residues in Food by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry, First Action 2007
3. Anastassiades M.; QuEChERS a mini-multiresidue method for the analysis of pesticide residues in lowfat products
4. Mastoska, K.; Dorweiler K.; Leotay S.; Pesticide Multiresidue Analysis in Cereal Grains using Modified Quechers Method Combined with Automated direct Sample Introduction GC-TOFMS and UHPLC-MS/MS Techniques
5. Method Validation and Quality Control Procedure for Pesticide Residues Analysis in Food and Feed (Document N° SANCO/12495/2011)