The Determination of Pesticides in Wine

Application Note

Abstract

According to the state institute for chemical and veterinary analysis of food, “Conventionally grown wine grapes are one of the crops most extensively treated with pesticides” (CVUA Stuttgart). The use of different fungicides and insecticides is commonplace in vineyards across the world to improve crop yields. Maximum residue levels (MRLs) have been established for grapes in many areas in the world but do not necessarily apply to processed grape products such as wines. The European Union has established MRLs for 65 pesticide residues in table grapes and wine grapes. The most common sample preparation method for pesticide residue analysis is the QuEChERS technique. QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe method that has been developed for the determination of multiple pesticide residues in various agricultural commodities. The rise in popularity of this technique and the increase in sample testing have driven the need for automation to increase productivity and throughput. The AutoMate-Q40 instrument streamlines the QuEChERS method, from adding Acetonitrile (ACN) and buffering salts, shaking, mixing, centrifuging the sample, transferring to a dispersive solid phase extraction (d-SPE) tube, and finally measuring and delivering the extract. These advances in automation will greatly improve laboratory production.

This study will evaluate the performance of the AutoMate-Q40 by monitoring multiresidue pesticides in wine. The target pesticides found in the wine will be determined by High Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC-MS/MS).

Introduction

The continual increase of globalization in the food industry has led to increased concerns about food safety. With recent advancements in multiresidue pesticide screening, the methods have been simplified from using the Luke extraction which uses large volumes of dichloromethane (DCM) that generates a lot of chlorinated waste per sample. Since 2003, the Luke method has been simplified by the introduction of the QuEChERS method.

Even though the QuEChERS is a simplified extraction technique, it still requires many manual steps ranging from adding solvent, extracting salts, centrifuging, shaking, decanting and performing the dSPE cleanup[1-3]. To modernize the traditional QuEChERS extraction through the use of automation, Teledyne Tekmar has developed the AutoMate-Q40. This automated platform will streamline the two part QuEChERS method from the liquid extraction through the cleanup.

This study will evaluate the performance of the AutoMate-Q40 by monitoring multiresidue pesticides in red and white wine. Pesticide residues were extracted from the wine by using the AutoMate-Q40. Quantification was based on matrix-matched calibration curves. QC samples were evaluated at levels of 15.0 and 75.0 ng/g to ensure the precision and accuracy of the AutoMate-Q40. The target pesticides found in the wine will be determined by High Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC-MS/MS).

Procedure

Sample Preparation/Extraction

A red and white wine sample was purchased from a local grocery store in Mason, Ohio. The samples were prepared according to the procedure described in the "*AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate*"[1].

Figure 1 shows the steps that are taken by the AutoMate-Q40 to extract the pesticides residues from the wine sample. For this analysis, a 20.0 mL sample of wine was used and the AutoMate-Q40 used 7.5 g of AOAC QuEChERS extraction salts ($MgSO_4$ and NaOAc). The AutoMate-Q40 also used the AOAC version of $MgSO_4$, and PSA for the dSPE cleanup step.

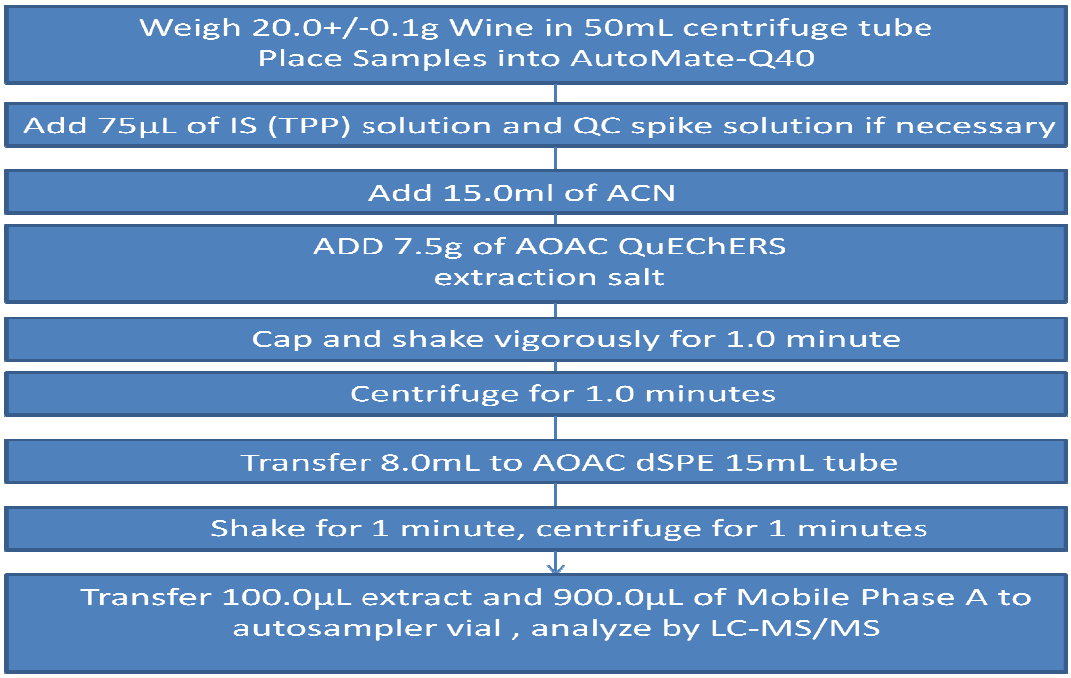

Figure 1: AutoMate-Q40 extraction parameters.

Instrumentation and Analytical Conditions

The analysis was conducted on the Shimadzu Nexera LC interfaced to an AB Sciex 4500 QTrap triple-quad mass spectrometer HPLC-MS/MS. For separation of the analytes, a Restek Ultra AQ C18 (100 x 2.1mm, 3um) column was used. Table 1 and 2 shows the optimized HPLC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. LC samples were prepared by adding 100 µL of final extract into 900 µL of HPLC grade water.

AB Sciex 4500 QTrap			
Compounds	Precursor Ion (m/z)	Quantization Product Ion (m/z)	Confirmation Product Ion (m/z)
Azoxystrobin	403.9	372.0	343.9
Boscalid	344.8	306.7	139.9
Carbendazim	191.9	159.9	131.9
Chlopyrifos	349.7	96.9	197.5
Cyprodinil	225.9	93.0	77.0
Kresoxim-methyl	313.9	205.7	115.9
Myclobutanil	288.9	70.0	124.9
Pyraclostrobin	387.9	193.7	162.9
Pyrimethanil	200.0	107.0	77.0
Quinoxyfen	307.8	196.8	161.9
Tebuconazole	308.0	69.9	124.8
Thiabendazole	201.8	174.9	130.9
Thiophanate-methyl	342.9	150.8	311.0
Trifloxystrobin	408.9	185.6	144.7
Triflumizole	345.9	277.6	72.8

Table 1: SRM transitions for HPLC-MS/MS parameters.

Shimadzu Nexera LC Parameters		
Column	Restek Ultra AQ C18	
Dimensions	100 x 2.1mm, 3um	
Mobile Phase	A 4mM Ammonium Formate in H_2O	
	B 4mM Ammonium Formate in MeOH	
Gradient	Time (min)	%B
	0.10	20
	8.00	90
	12.00	100
	15.00	100
	15.10	20
	17.00	Stop

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Injection Vol (µL)	10.0
Flow Rate (mL/min)	0.31
Column Temperature (C˚)	30.0

Table 2: HPLC conditions parameters.

Experimental Results

By automating the QuEChERS extraction, it enables a fast, 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 6 µg/mL stock pesticide solution was used to fortify the wine samples. Using the AutoMate-Q40, it was able to spike the following samples with 50.0 and 250.0 µL of the pesticide standard that yielded a 15.0 and 75.0 µg/L check samples. These QC samples were quantitated against their corresponding matrix matched calibration curve.

Compounds	Red Wine				White Wine			
	Low Spike		High Spike		Low Spike		High Spike	
	Avg %Recovery	Avg %RSD	Avg %Recovery	Avg %RSD	Avg %Recovery	Avg %RSD	Avg %Recovery	Avg %RSD
Azoxystrobin	95.19	5.00	106.83	2.67	nd	nd	nd	nd
Boscalid	83.76	9.05	98.84	3.50	101.88	14.77	98.92	6.81
Carbendazim	nd	nd	62.80	3.51	69.25	8.76	98.76	6.05
Chlopyrifos	94.30	3.16	98.70	3.45	97.51	8.50	104.31	1.64
Cyprodinil	94.99	3.38	93.97	3.43	97.63	8.63	102.71	3.67
Kresoxim-methyl	96.97	6.37	99.91	2.62	94.96	11.34	100.71	2.93
Myclobutanil	95.32	5.57	98.55	3.49	93.72	11.98	103.22	3.11
Pyraclostrobin	99.34	4.26	99.83	4.40	91.35	7.86	102.77	3.04
Pyrimethanil	98.29	4.81	97.52	3.69	92.42	5.95	97.32	3.37
Quinoxyfen	93.68	4.53	100.70	4.73	94.74	6.41	98.22	7.71
Tebuconazole	98.52	4.01	96.34	2.93	95.90	11.35	98.44	3.27
Thiabendazole	102.43	7.91	94.26	1.11	84.88	12.12	102.59	4.88
Thiophanate-methyl	103.29	3.10	98.70	2.80	94.17	7.96	97.66	4.83
Trifloxystrobin	101.86	2.95	94.96	3.82	98.89	10.32	99.74	2.31
Triflumizole	93.50	4.05	92.59	4.08	98.07	8.95	98.72	2.47

Table 3: Red and White Wine sample extracted using AutoMate-Q40

Table 3 shows when using the AutoMate-Q40 to extract pesticide residues from red and white wine, it exhibits

excellent recoveries averaging 97.5%. These recoveries fall within the methods guidelines of 70-120% recovery for most pesticides. The AutoMate-Q40 also demonstrates great precision on average of 5.4%RSD which falls within the method guide lines of RSD <20%.

Conclusion

This study demonstrates the feasibility of automating the QuEChERS extraction method using the AutoMate-Q40. By automating the liquid handing, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision™, the extraction process is faster, more reliable, and easier. This enables time and labor savings, while improving consistency and repeatability of the extraction. As shown above in Table 3, all pesticides gave excellent spike recoveries, on average of 97.5% and excellent precision with an average %RSD of 5.4%.

Reference

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. M. Anastassiades: QuEChERS a mini-multiresidue method for the analysis of pesticide residues in low-fat products

4. Method Validation and Quality Control Procedure for Pesticide Residues Analysis in Food and Feed (Document N˚ SANCO/12495/2011)