Application Note

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 installment in 2003, QuEChERS has been adapted for use with many additional matrices.

The rise in popularity of this technique and the increase in sample testing have driven the need for automation of the QuEChERS method 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.

The aim of this project is to validate the performance of AutoMate-Q40 by monitoring various chemical residues in different matrices. The target residues will be determined by Liquid Chromatography tandem mass spectrometry.

Introduction

With the increasing globalization of the food industry there are consequently more concerns about food safety. As a result, the number of veterinary drug residue which must be regulated and monitored has increased. QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe extraction method developed in 2003 for the extraction of pesticide residues in agricultural commodities[1,2,3]. Modifications to the method have expanded the scope to include many additional matrices and target analytes such as veterinary drug residues.

With the amounts of samples being required for residue analysis continually increasing, Teledyne Tekmar has developed the AutoMate-Q40. This revolutionary system is designed to automate the QuEChERS extraction workflow, allowing laboratories to be more efficient and timely in meeting their customer requirements for fast and reliable results. This system will help keep sample preparation and the time an analyst spends on sample prep at a minimum, while producing highly accurate, precise, and traceable results.

The intent of this study is to evaluate the performance of the AutoMate-Q40 by monitoring veterinary drug residues in bovine liver. The target compounds (Sulfonamides) will be analyzed using LC-MS/MS.

Experiment - Instrument Conditions

Sample Preparation/Extraction

Bovine liver samples were purchased from a local market in Mason, Ohio. It was then chopped into fine pieces. Once chopped the sample was placed in the freezer until the time of analysis. The day of analysis the liver sample was homogenized meticulously with a food grinder.

Figure 1 shows the sample preparation and extraction steps that are needed to extract the veterinary drug residue from bovine liver. For this analysis, the AutoMate-Q40 used QuEChERS AOAC extraction salts (6.0 g MgSO4; 1.5 g NaAcetate). The AutoMate-Q40, also, used a version of 900.0 mg $MgSO_4$, 150.0 mg PSA, and 150.0 mg C-18 for the dSPE cleanup step.

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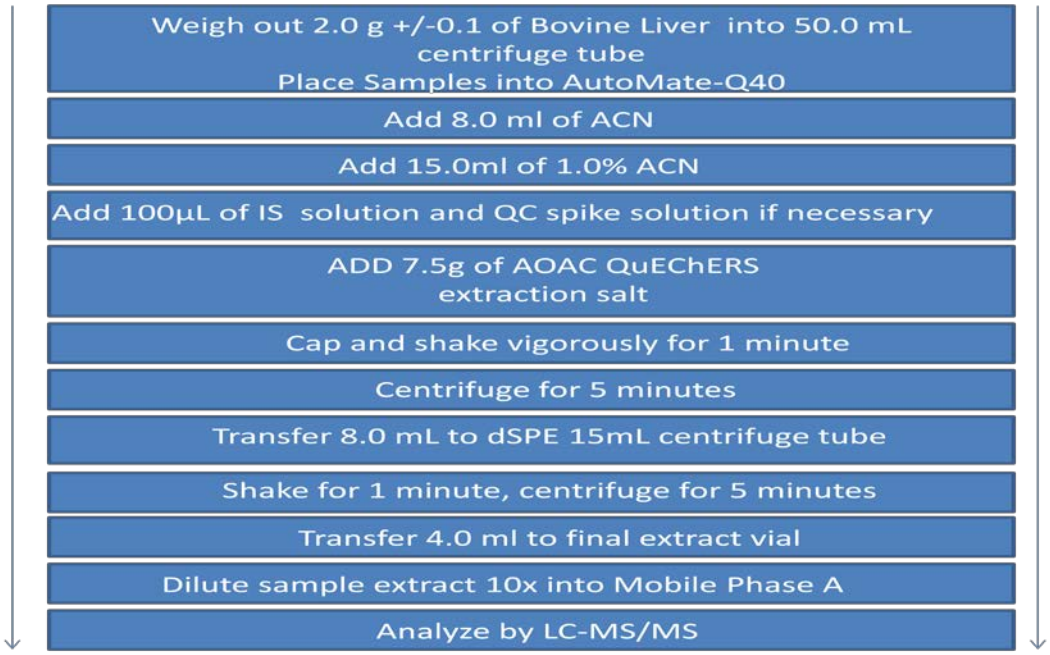
Figure 1 AutoMate-Q40 Extraction Parameters for Bovine Liver



Instrumentation and Analytical Conditions

The analysis was conducted on the 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 XB-C18 100A (50 x 2.1 mm) 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 µL/L.

Table I 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)	Confirmation product ion (m/z)	DP(V)	CE(V)	CXP(V)
Sulfapyridine (IS)	2.52	249.87	91.9	51	33	10	107.9	51	31	10
Sulfamethoxazole	3.36	253.87	155.6	56	21	14	91.9	56	35	8
Sulfamethazine	3.10	278.91	91.9	11	19	12	123.9	11	29	12
Sulfadimethoxine	4.28	310.9	155.7	71	27	14	91.9	71	43	8
Sulfadiazine	2.10	250.8	107.9	46	31	10	91.8	46	33	8

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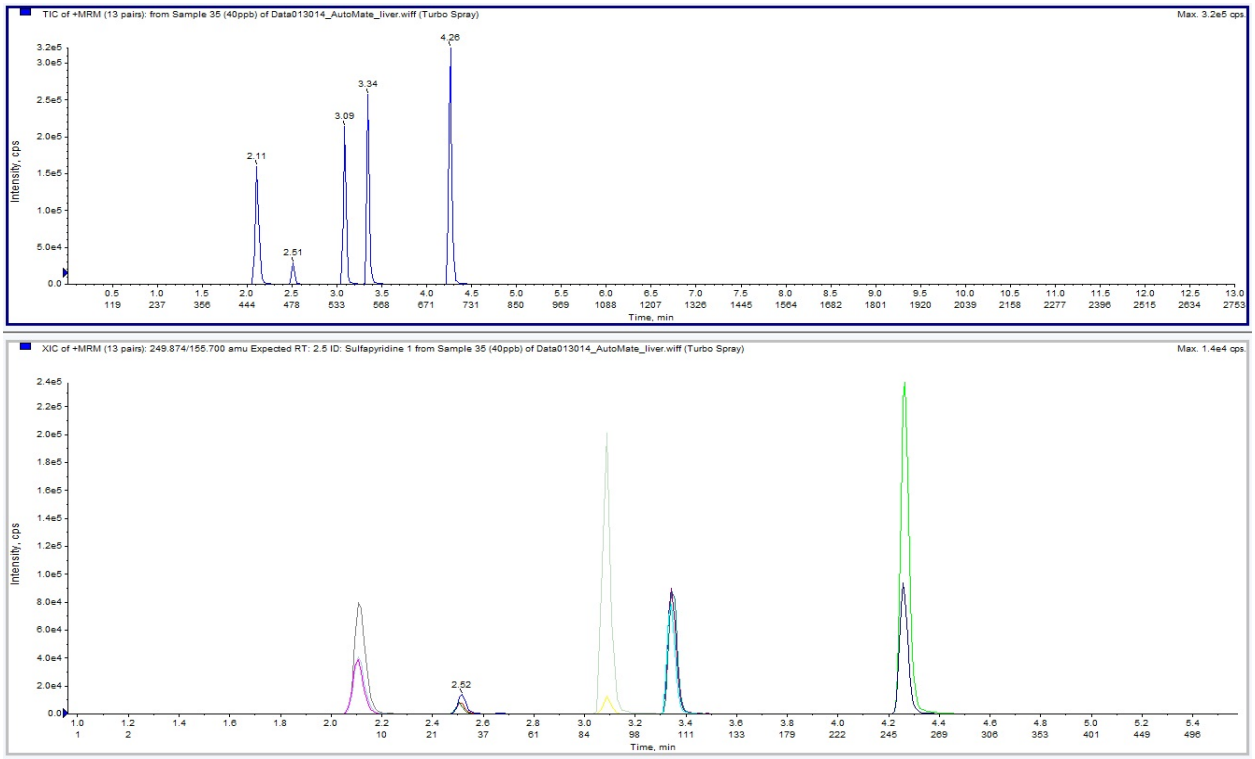
Table II Shimadzu Nexera LC Parameters		
Column	Kinetex 2.6u XB-C18 100Å	
Dimensions	50.0 X 2.1mm	
Mobile Phase	A: 1.0% Formic Acid in H2O	
	B: 1.0% Formic Acid in MeOH	
Gradient	Time (min)	%B
	0.1	5.0
	0.3	90.0
	7.27	100.0
	7.37	100.0
	8.27	20.0
	13.00	Stop
Flow Rate (mL/min)	0.5	
Column Temperature (°C)	40.0	

Figure 2 400.0 ng/g Spike of Sulfonamides in Bovine Liver



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Experimental Results

Automation of the QuEChERS extraction allows for a fast, easy reliable and more reproducible extraction. By using the AutoMate-Q40, it offers significant labor savings, while improving the repeatability and consistency between the samples.

A precision and accuracy study was performed on the bovine liver samples using the AutoMate-Q40. A 2.0 µg/mL stock solution was used to fortify the liver samples. Using the AutoMate-Q40, the system is able to spike the following samples with 50.0, 100.0 and 250.0 µL of the standard that yielded a 50.0, 100.0, and 250.0 ng/g check samples. These QC samples were quantitated against a corresponding matrix matched calibration

Table III Data Table For Veterinary Drug Residues from Bovine Liver Samples

		50.0 ng/g Spike		100.0 ng/g Spike		250.0 ng/g Spike	
Compounds	R2	% Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
Sulfapyridine (IS)			8.5		8.5		8.5
Sulfamethoxazole	0.9992	99.56	7.1	85.08	4.9	74.22	1.7
Sulfamethazine	0.9995	103.53	5.6	87.38	9.4	84.81	6.5
Sulfadimethoxine	0.9992	103.57	2.2	86.95	2.7	77.61	2.1
Sulfadizine	0.9995	102.24	5.6	89.88	3.6	78.70	2.2

Table III shows that when using the AutoMate-Q40 to extract veterinary drug residues from bovine liver samples, it exhibits recoveries ranging from 74.22% to 103.57%. These spiking recoveries fell within the recommend values for the Document N˚ Sanco/12495/2011[4]. This document also states that the %RSD must be below 20%. By using the AutoMate-Q40 for the QuEChERS extraction it showed great precision ranging from 1.17% to 9.4%RSD for the spiked QC samples.

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 III combined veterinary residues spikes recovered at 89.46% with an average RSD of 4.45%RSD. These numbers indicate superb precision and accuracy thus validating the performance of the AutoMate-Q40 and its use as an excellent analytical tool.

References

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