Veterinary Drug Residue Analysis Using the AutoMate™-Q40: An Automated Solution to QuEChERS Veterinary Residue;

使用AutoMate™-Q40 的動物用藥殘留分析：

一個QuEChERS動物用藥殘留的自動化解決方案

Application Note 應用筆記	**TELEDYNE TEKMAR** Everywhere**you**look™

Abstract 摘要

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.
QuEChERS 是一快速-簡便-廉價-高效-耐用-安全的萃取方法，已經被發展用來做農產品裏的農藥殘留的測定.
自從2003年建立之後，QuEChERS方法已被調整用於許多增加的基質.

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.
這個技術普及率的上升以及樣品測試的增加，已經推動了QuEChERS方法的自動化以增加生產力及流通量的需求.

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.
從加入乙腈(ACN)和緩衝鹽，搖盪，攪拌，離心分離樣品，移到分散式固相萃取(D-SPE)管，測量和輸送萃取物，
AutoMate™-Q40簡化了QuEChERS方法.

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.

本計劃的目標是以觀察在不同基質中的化學物質殘留, 來驗證AutoMate™-Q40的性能.

標的殘留將以液相層析串聯質譜法測定.

Introduction 簡介

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.

隨著食品工業的日益全球化有關於食品安全的結果更多的關注. 其結果,動物用藥殘留必須進行調節和監視的數量有所增加.

QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe extraction method developed in 2003 for the extraction of pesticide residues in agricultural commodities1,2,3.
Modifications to the method have expanded the scope to include many additional matrices and target analytes such as veterinary drug residues.

QuEChERS 是一個在2003年被發展用來做農產品裏的農藥殘留的快速-簡便-廉價-高效-耐用-安全的萃取方法.

這個方法經過調整後, 已經擴大了範圍以包括許多增加的基質和目標分析, 如動物用藥殘留等.

With the amounts of samples being required for residue analysis continually increasing, Teledyne Tekmar has developed the AutoMate™-Q40.

隨著需求做殘留分析的樣品量持序增加, Teledyne公司Tekmar已經開發出 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.

這一革命性的系統是為了自動化QuEChERS萃取流程而設計, 讓實驗室可以更加有效率地及時地符合其客戶快速及可靠的需求.

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.
這系統將有助於樣品製備分析工作人員保持花費在樣品製備及時間在最少量，但產出高準確度，高精密度，以及可追蹤的結果．

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.
本研究的目的是經由觀測牛肝臟裏的動物用藥殘留來評估AutoMate™-Q40的性能．
目標化合物(磺胺)將用LC-MS/ MS進行分析．

Experiment - Instrument Conditions 實驗 – 儀器條件

Sample Preparation/Extraction 樣品製備/萃取

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.
牛肝樣品購自於俄亥俄州Mason當地市場．然後將其切成細片．切碎之後的試樣放置在冰箱中直至分析時的時後．
分析當天的肝臟樣品用食物研磨機進行精細的均質化．

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 MgSO4, 150.0 mg PSA, and 150.0 mg C-18 for the dSPE cleanup step.

圖1示出了要從牛肝萃取動物用藥殘留所需的樣品製備和萃取步驟．
這個分析，AutoMate™-Q40 使用AOAC 的QuEChERS 的萃取鹽(6.0克硫酸鎂，1.5克醋酸鈉)．
AutoMate™-Q40也使用一個版本的900.0毫克硫酸鎂，150.0毫克PSA和150.0毫克的C-18於dSPE淨化步驟．

Figure 1 AutoMate™-Q40 Extraction Parameters for Bovine Liver

Weigh out 2.0 g +/-0.1 of Bovine Liver into 50.0 mL centrifuge tube
Place Samples into AutoMate-Q40

Add 8.0 ml of ACN

Add 15.0ml of 1.0% ACN

Add 100μL of IS solution and QC spike solution if necessary

ADD 7.5g of AOAC QuEChERS extraction salt

Cap and shake vigorously for 1 minute

Centrifuge for 5 minutes

Transfer 8.0 mL to dSPE 15mL centrifuge tube

Shake for 1 minute, centrifuge for 5 minutes

Transfer 4.0 ml to final extract vial

Dilute sample extract 10x into Mobile Phase A

Analyze by LC-MS/MS

Instrumentation and Analytical Conditions 儀器和分析條件

The analysis was conducted on the Shimadzu Nexera LC interfaced to an AB Sciex 4500 QTrap triple-quad mass spectrometer (LC-MS/MS).
圖1 AutoMate™-Q40牛肝萃取參數
分析工作是使用接到一部AB Sciex 4500 QTrap三重四極桿質譜儀(LC-MS/MS)的Shimadzu Nexera的液相層析儀來進行的.

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.
對於感興趣的化合物的分離, 是用Phenomenex Kinetex 2.6u XB-C18100A(50×2.1mm)分離管柱.
表Ⅰ和表Ⅱ中展示了層析分離和最佳的分析物躍遷兩者的最佳的LC-MS/MS分析參數.
Figure 2 shows the scheduled MRM chromatogram spiked at 400.0 μL/L.
圖2示出了在摻入400.0μL/L時的安排下的MRM層析圖.

Table I LC-MS/MS SRM Transitions and Parameters for AB Sciex 4500 QTrap
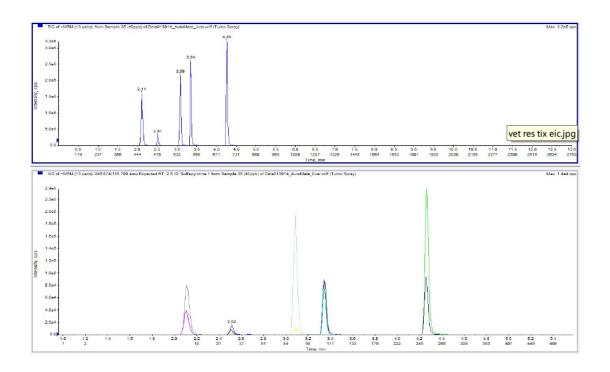
表I LC-MS/MS SRM躍遷和AB SCIEX4500 QTrap 的參數

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

Table II Shimadzu Nexera LC Parameters
表二 Shimadzu Nexera 液相層析參數

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
圖2 摻入400.0ng/g時的牛肝裏的磺胺類藥物

Experimental Results 實驗結果

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.
QuEChERS萃取的自動化允許一個快速，簡單可靠及更具有再現性的萃取.
AutoMate™-Q40的使用,提供顯著勞動力的節省,同時提高了樣品之間的可重複性和一致性.

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.
精密度和準確度的研究是用牛肝臟樣品，以AutoMate™-Q40進行的.
將2.0µg/ mL的儲備溶液用於加強肝臟的樣品.

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
使用AutoMate™-Q40，此系統能夠摻入下列50.0, 100.0 和250.0µL的標準品

到樣品中, 這產生了**50.0, 100.0, 和250.0ng/g** 的查檢查用樣品.
這些QC樣品與基質匹配的校正標準樣品對應地進行定量.

Table III Data Table For Veterinary Drug Residues from Bovine Liver Samples
表三 牛肝樣品的動物用藥殘留的數據表

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%.
表三顯示, 當使用AutoMate™-Q40萃取牛肝樣品的動物用藥殘留時, 它表現出從74.22％到103.57％回收率.

These spiking recoveries fell within the recommend values for the Document N˚ Sanco/12495/20114 .
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.
這些標準品摻入的回收率落於 N˚Sanco/12495/20114 文獻的推薦值之內.
此文件還指出, ％RSD必須低於20％.
經由AutoMate™-Q40的QuEChERS萃取的使用, 在這些摻入標準品的QC樣品, 它表現出了從1.17% to 9.4%RSD的極大精準度的範圍.

Conclusion 結論

This study demonstrates the feasibility of automating the QuEChERS extraction method using the AutoMate™-Q40.
這項研究表明了用AutoMate™-Q40的QuEChERS萃取方法的可行性.

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.

經由液體處理，鹽/緩衝液添加，樣品混合，移液的自動化，以及採用正在申請專利的VialVision™液位感測，萃取過程更快，更可靠，更容易.
這使能節省時間和勞力，同時提高了萃取的一致性和可重複性.

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.

如上表III所示，組合的動物用藥殘留標準品摻入，回收率在89.46％, RSD 4.45％. 這些數字表明了極好的精密度和準確度，因而驗證了AutoMate™-Q40的性能及其作為一個很好的分析工具的使用.

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