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アプリケーションノート

GC-MS/MS Determination of Pesticide Residues in Fruit Using the Xevo TQ-GC

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Abstract

In this application note we describe a multiresidue GC-MS/MS method for the determination of pesticide residues in fruit commodities, with extraction using the QuEChERS workflow. The Xevo TQ-GC System provided excellent, fit-for-purpose performance in terms of linearity and calibration range for both organic raisin and apple. The trueness and precision of the GC-MS/MS method determined at three QC levels was found to be acceptable with the representative compounds shown, giving %RSD <10%.

Overall the performance data indicate that the Xevo TQ-GC, when used in combination with an established extraction protocol such as QuEChERS, is suitable for checking MRL/tolerance compliance in routine laboratory testing.

Benefits

Provides a multiresidue GC-MS/MS method for the determination of pesticide residues in fruit commodities, with extraction using the QuEChERS workflow, suitable for monitoring for MRL compliance. This method which was developed on Waters Xevo TQ-GC provides fit-for-purpose performance in terms of sensitivity, linearity, and robustness.

Introduction

Pesticide residues remain a priority on the list of food safety concerns. As a result, laboratories are required to create analytical methods capable of screening the maximum amount of compounds, using a minimal number of methods, while ensuring sample turnaround times are met. The majority of countries have established clearly defined regulations[1] relating to pesticide residues, with legislation imposing Maximum Residue Limits (MRLs)[2] levels for different food commodities. To meet these criteria, laboratories require analytical techniques that are sensitive, accurate, and robust.

Multiresidue analysis is challenging due to the varying matrix complexity of different food commodities and the requirement for low part per billion (ppb or mg/kg) detection levels to achieve MRL compliance for a diverse range of pesticides. Currently there are in excess of 1000 pesticides known to be in use, and laboratories are continually under pressure to increase the scope of analytical methods employed for routine monitoring purposes. Various technologies are used to meet these challenges with the most common being liquid chromatography (LC) and gas chromatography (GC) coupled to tandem quadrupole mass spectrometry (MS/MS), which allows laboratories to cover a wide range of compounds as required by legislation.[3]

In this application note we provide example performance data from Waters Xevo TQ-GC System for pesticide residues in two different commodities. Samples were extracted by the QuEChERS CEN method and cleaned-up by dispersive solid phase extraction (d.SPE), following the protocol described in a EURL-FV multiresidue method, using QuEChERS for fruits and vegetables.[4]

Experimental

Sample preparation and extraction

Homogenized organic apple was extracted using the QuEChERS method as shown in Figure 1. Organic raisin was first rehydrated with LCMS water and then the equivalent to 5 g of sample was taken for QuEChERS extraction. The supernatant from the QuEChERS extracts were then cleaned-up by d.SPE tubes which contained 25 mg of primary secondary amine (PSA) sorbent and 150 mg $MgSO_4$, per 1 mL of sample extract. Samples were then evaporated and reconstituted in ethyl acetate, post spiked with the pesticide mix, and analyzed using the gas chromatography, mass spectrometry method highlighted below. Analyte extraction recovery was not assessed during this work, QuEChERS is a well established sample extraction method for pesticides in food and the aim of this work was to assess the GC-MS/MS method performance only.

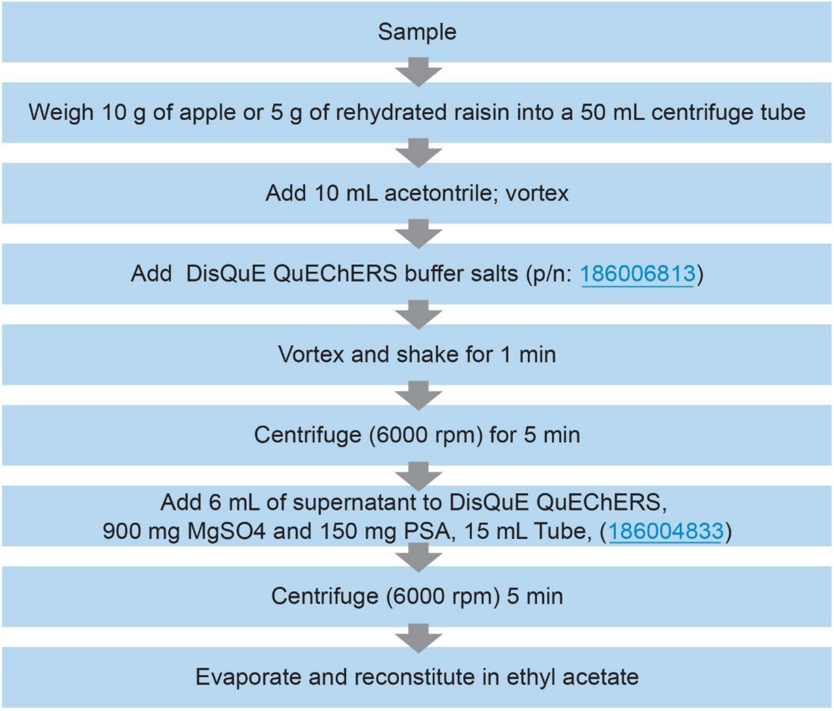


Figure 1. Sample preparation and QuEChERS workflow used to prepare the samples.

The performance of the GC-MS/MS step of the method was assessed using SANTE guidelines[5] for 150 pesticides. Solutions of matrix-matched standards were prepared over the range 0.001 to 0.050 mg/kg (1.0 to 50.0 ppb in vial concentration) in apple, and 0.002 to 0.100 mg/kg (1.0 to 50.0 ppb in vial concentration) in raisin. Replicate injections at three concentration levels were run between bracketed calibration curves to assess the performance of the method. An internal standard (triphenylphosphate) was added to all samples prior to the analysis to correct for any injection variability which may have occurred.

GC conditions

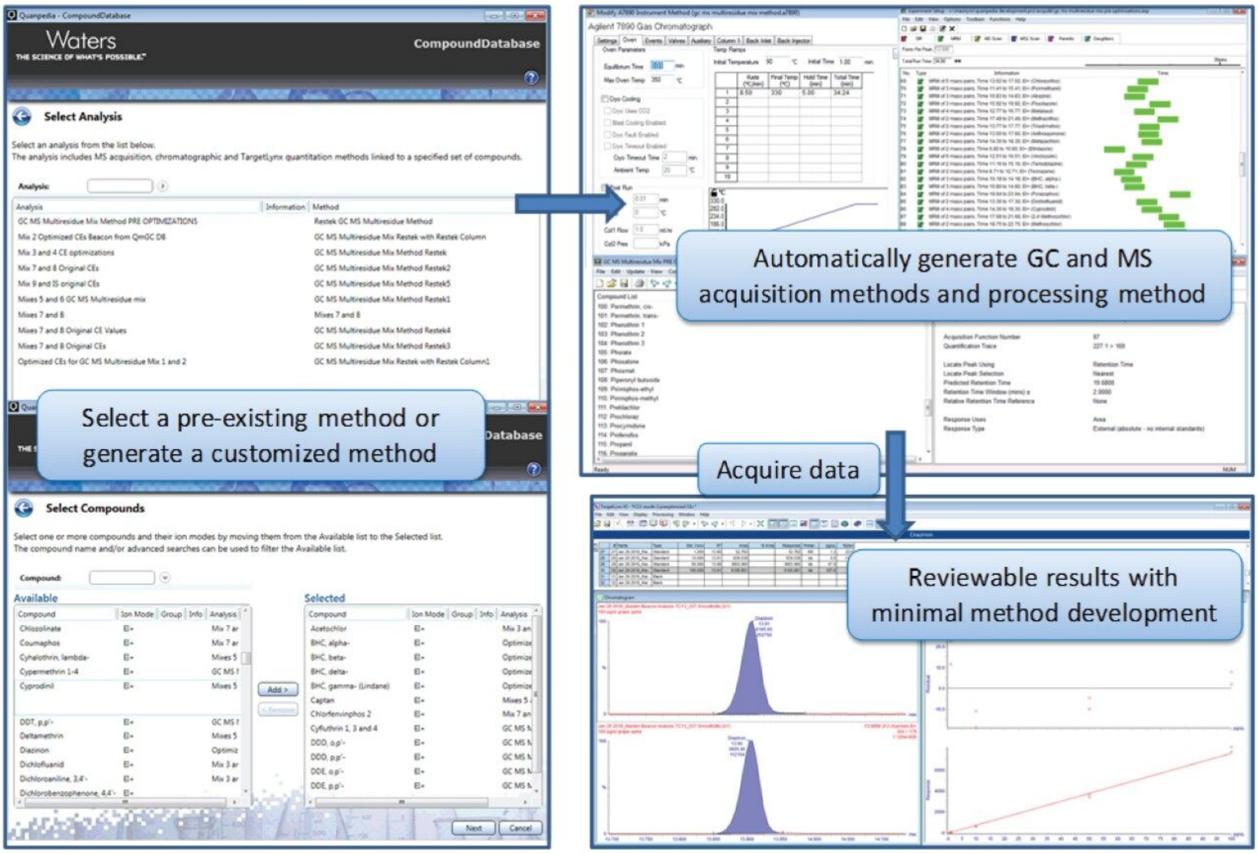
GC column:	Rxi-5MS 30 m x 0.25 mm id × 0.25 µm (Restek)
Carrier gas:	Helium
Gas flow rate:	1.4 mL/min
Injection type:	Pulsed splitless
Inlet temp.:	280 °C
Pulse time:	1.10 min
Pulse pressure:	170 kPa
Purge flow:	30 mL/min
Septum purge flow:	3 mL/min
Inlet liner:	Gooseneck splitless liner 4 mm × 6.5 × 78.5 (Restek)
Wash solvent:	Hexane (A) and ethyl acetate (B)
Oven program:	91 °C (hold 1.0 min) to 330 °C at 8.5 °C/min and hold for 5 min, run
Run time:	34.12 min
Injection volume:	1 µL

MS conditions

MS system:	Xevo TQ-GC
Software:	MassLynx v4.2
Ionization mode:	EI+, 70eV
Source temp.:	250 °C
GC interface:	320 °C
MRM:	All transitions were imported from Waters Quanpedia Database, and further information can be obtained in Waters technology brief no.: 720006248en. IntelliStart Custom Resolution settings were used.

Results and Discussion

Method development or method transfer on a new GC-MS/MS system can be time consuming, requiring parameters such as MRM transitions, collision energies, chromatography conditions, and processing methods to be optimized. To speed up this process, Quanpedia Database was used to automatically establish these parameters with minimal manual optimization required. An example of the Quanpedia workflow is shown in Figure 2.



Select Analysis

Select an analysis from the list below.
The analysis includes MS acquisition, chromatographic and TargetLynx quantitation methods linked to a specified set of compounds.

Analysis:

Analysis	Information	Method
GC MS Multiresidue Mix Method PRE OPTIMIZATIONS		Restek GC MS Multiresidue Method
Mix 2 Optimized CEs Beacon from QmGC DB		GC MS Multiresidue Mix Restek with Restek Column
Mix 3 and 4 CE optimizations		GC MS Multiresidue Mix Method Restek
Mix 7 and 8 Original CEs		GC MS Multiresidue Mix Method Restek2
Mix 9 and IS original CEs		GC MS Multiresidue Mix Method Restek5
Mixes 5 and 6 GC MS Multiresidue mix		GC MS Multiresidue Mix Method Restek1
Mixes 7 and 8		Mixes 7 and 8
Mixes 7 and 8 Original CE Values		GC MS Multiresidue Mix Method Restek4
Mixes 7 and 8 Original CEs		GC MS Multiresidue Mix Method Restek3
Optimized CEs for GC MS Multiresidue Mix 1 and 2		GC MS Multiresidue Mix Restek with Restek Column1

Select Compounds

Select one or more compounds and their ion modes by moving them from the Available list to the Selected list.
The compound name and/or advanced searches can be used to filter the Available list.

Compound:

Available

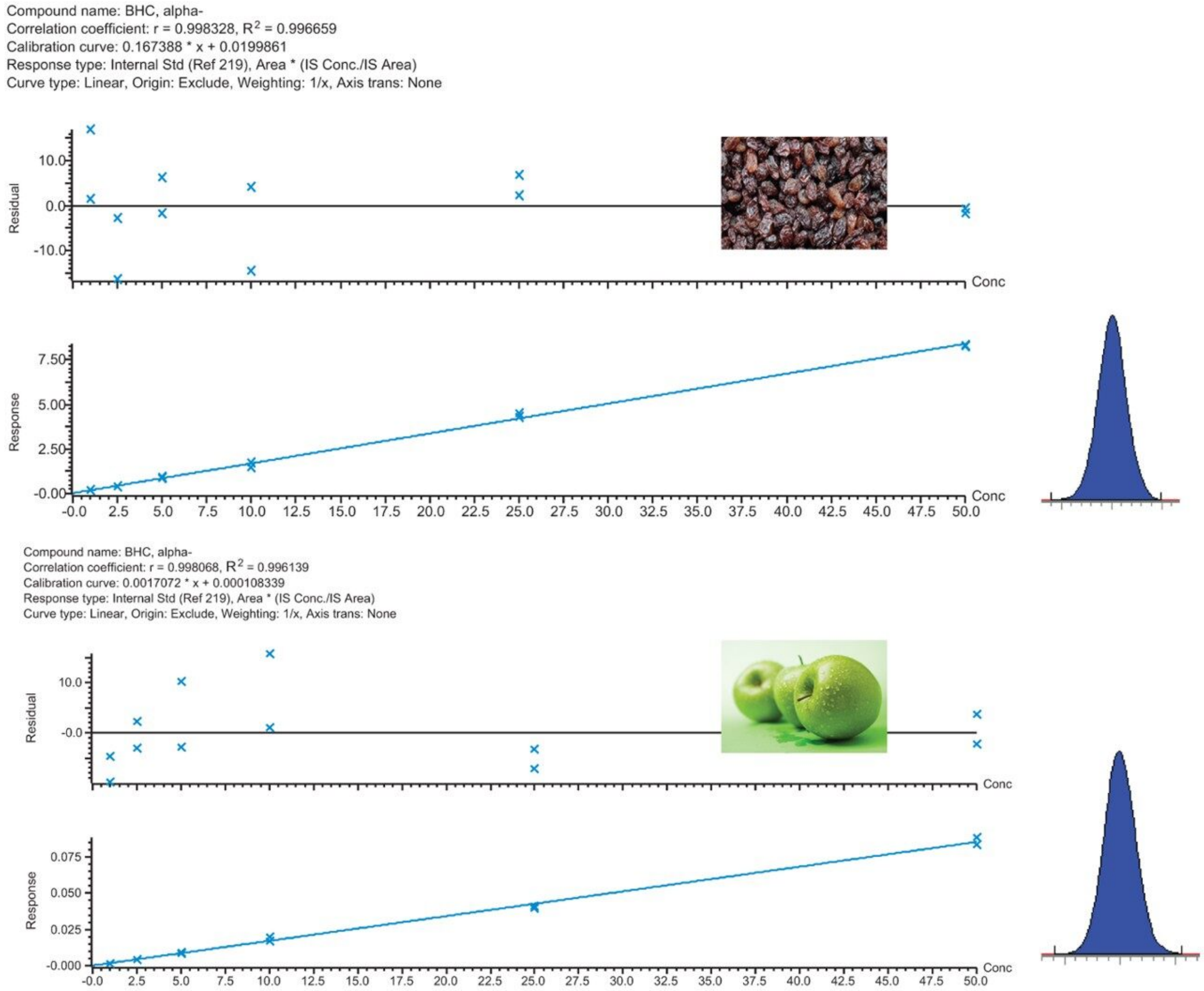
Compound	Ion Mode	Group	Info	Analysis
Chlozolinate	EI+			Mix 7 ar
Coumaphos	EI+			Mix 7 ar
Cyhalothrin, lambda-	EI+			Mixes 5
Cypermethrin 1-4	EI+			GC MS I
Cyprodinil	EI+			Mixes 5
DDT, p,p'-	EI+			GC MS I
Deltamethrin	EI+			Mixes 5
Diazinon	EI+			Optimiz
Dichlofluanid	EI+			Mix 3 ar
Dichloroaniline, 3,4'-	EI+			Mix 3 ar
Dichlorobenzophenone, 4,4'-	EI+			

Selected

Compound	Ion Mode	Group	Info	Analysis
Acetochlor	EI+			Mix 3 an
BHC, alpha-	EI+			Optimize
BHC, beta-	EI+			Optimize
BHC, delta-	EI+			Optimize
BHC, gamma- (Lindane)	EI+			Optimize
Captan	EI+			Mixes 5
Chlorfenvinphos 2	EI+			Mix 7 an
Cyfluthrin 1, 3 and 4	EI+			GC MS N
DDD, o,p'-	EI+			GC MS N
DDD, p,p'-	EI+			GC MS N
DDE, o,p'-	EI+			GC MS N
DDE, p,p'-	EI+			GC MS N

Figure 2. Example workflow using Quanpedia to generate a new pesticide multiresidue analysis and Targetlynx XS processing method for routine analysis.

The linearity of the method was assessed using bracketed matrix-matched calibration curves, which were internal standard corrected. Figure 3 shows the calibration curves for alpha-BHC in both organic apple and raisin.



Compound name: BHC, alpha-
Correlation coefficient: r = 0.998328, R^2 = 0.996659
Calibration curve: 0.167388 * x + 0.0199861
Response type: Internal Std (Ref 219), Area * (IS Conc./IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Compound name: BHC, alpha-
Correlation coefficient: r = 0.998068, R^2 = 0.996139
Calibration curve: 0.0017072 * x + 0.000108339
Response type: Internal Std (Ref 219), Area * (IS Conc./IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 3. Matrix matched calibration curves at 1 ng/mL to 50 ng/mL, and 0.010 mg/kg chromatograms for alpha-BHC in organic raisin and apple.

The linearity of response and calibration range in organic raisin for all 150 pesticides assessed in this study are shown in Figure 4. The calibration curve data for all 150 compounds can be found in Appendix 1. The concentration levels take into account that only 5 grams of rehydrated raisin sample was taken for extraction. All compounds gave excellent linear response and only one compound, trans-nanochlor, gave a linear response <0.990. All residuals (back calculated concentrations) were within the 20% tolerance of the SANTE guidelines, with calibration ranges between either 0.002 mg/kg or 0.005 mg/kg to 0.100 mg/kg.

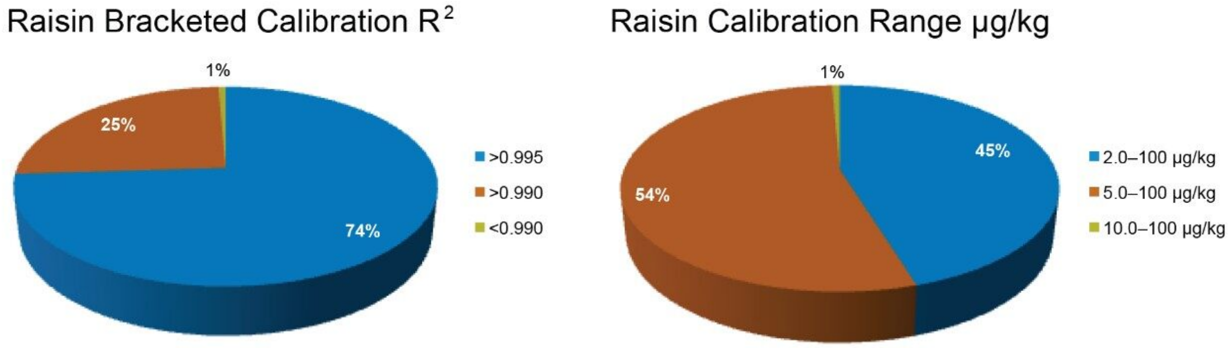


Figure 4. Matrix-matched bracketed calibration data for 150 pesticides spiked into QuEChERS extracted organic raisin.

Replicate (n=6) injections were run for three levels, 0.005 mg/kg, 0.010 mg/kg, and 0.020 mg/kg in each matrix. The calculated mean concentrations and precision for representative pesticides in organic raisin are shown in Figures 5 and 6.

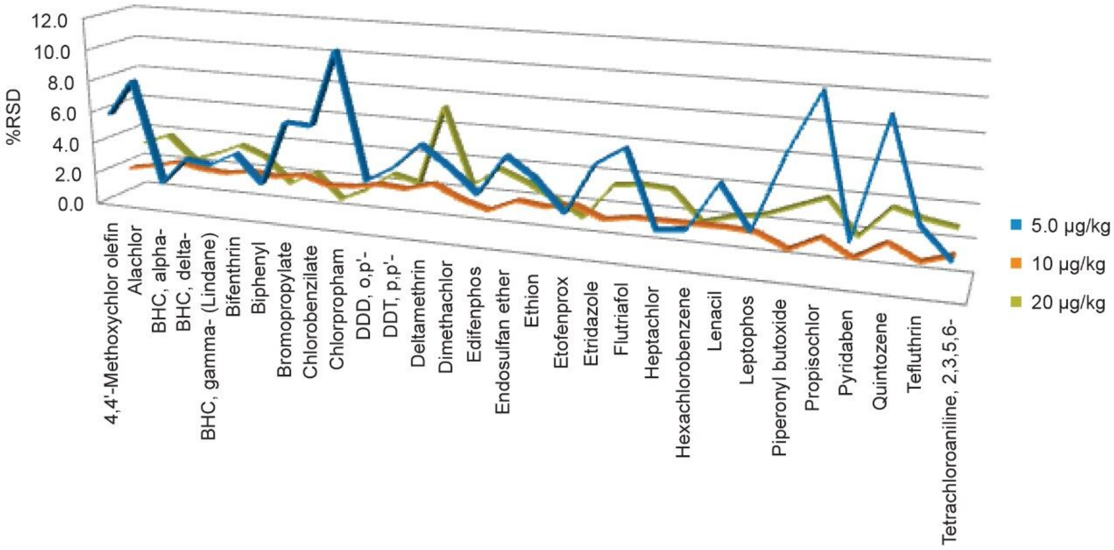


Figure 5. Repeatability (%RSD) data for representative pesticides in organic raisin using matrixmatched QC points (n=6) at 0.005 mg/kg, 0.010 mg/kg, and 0.020 mg/kg.

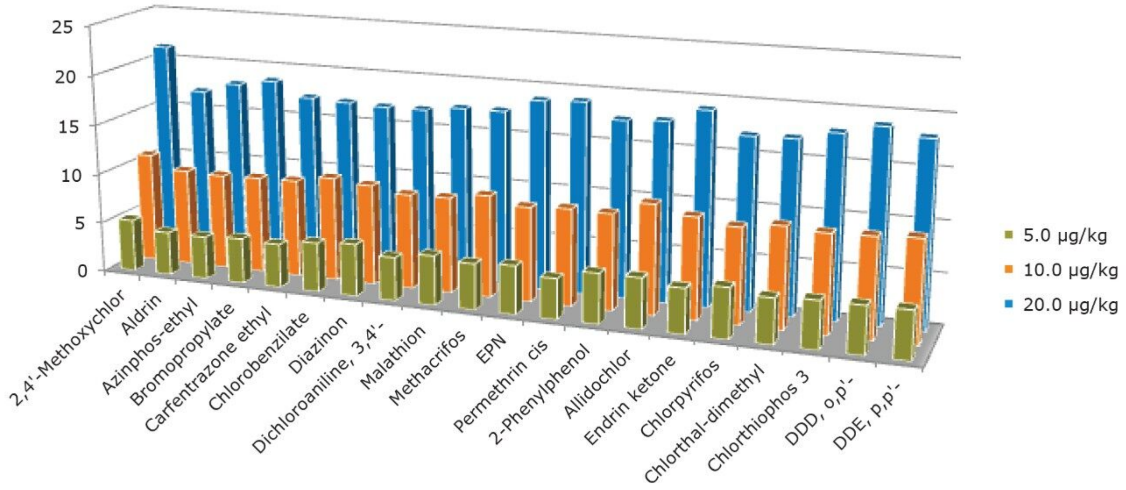
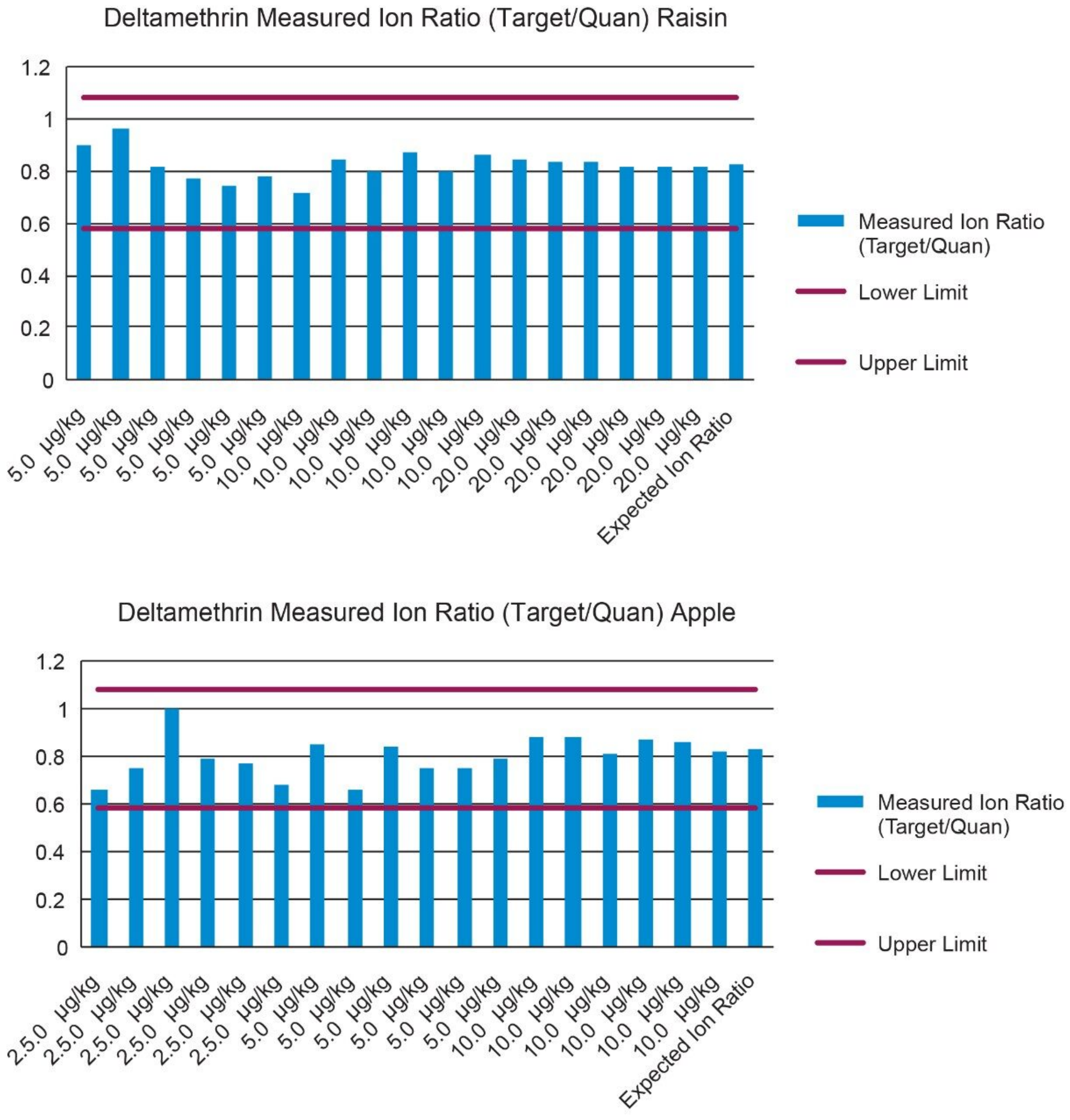


Figure 6. Calculated mean concentration data for representative pesticides in matrix-matched QC points (n=6) for organic raisin at 0.005 mg/kg, 0.010 mg/kg, and 0.020 mg/kg.

Ion ratios and retention times from the matrix-matched standards agreed well with the reference values and most were within the required tolerances (±30 % and ±0.1 min, respectively). An example of the ion ratios given by each of the QC levels for deltamethrin in organic raisin and apple can be seen in Figure 7.


Deltamethrin Measured Ion Ratio (Target/Quan) Raisin

Deltamethrin Measured Ion Ratio (Target/Quan) Apple

Figure 7. Ion ratios for the three QC levels of deltamethrin in A. raisin, and B. apple.

Conclusion

The goal of this study was to evaluate the performance of the Xevo TQ-GC for the determination of pesticide residues in fruit commodities prepared by QuEChERS sample preparation methodology. The Xevo TQ-GC provided excellent, fit-for-purpose performance in terms of linearity and calibration range for both organic raisin and apple. The trueness and precision of the GC-MS/MS method determined at three QC levels was found to be acceptable with the representative compounds shown, giving %RSD <10%. Overall the performance data indicate that the Xevo TQ-GC, when used in combination with an established extraction protocol such as QuEChERS, is suitable for checking MRL/tolerance compliance in routine laboratory testing.

References

1. European Union (2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and 91/414/EEC.
2. European Commission (2016) EU Pesticide Database.
3. K Organtini, G Cleland, E McCall, S Hird. (2017) UPLC and APGC Multiresidue Pesticide Analysis on a Single Tandem Quadrupole Mass Spectrometer Platform. Waters application note no. 720006013en.
4. European Union Reference Laboratory – Fruits and Vegetables, ‘Multiresidue Method Using QuEChERS Followed by GC-QqQ/MS/MS and LC-QqQ/MS/ MS for Fruits and Vegetables’.
5. European Union (2017). Document No. SANTE 11813/2017. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed.

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DisQuE Quechers, 1 g Trisodium Citrate Dihydrate, 0.5 g Disodium Hydrogencitrate Sesquilhydrate, 1 g NaCl and 4 g MgSO4, 50 mL Pouch, 50/pkg <https://www.waters.com/186006813>

720006378, October 2018

Appendix

Pesticides assessed in this study and calibration results for organic raisin.

Appendix

Pesticides assessed in this study and calibration results for organic raisin.

Name	Coeff. of determination (Raisin)	Calibration range µg/kg (Raisin)
2,4'-Methoxychlor	0.999	2.0–100
2-Phenylphenol	0.997	2.0–100
4,4'-Methoxychlor olefin	0.997 (2nd order)	2.0–100
Acetochlor	0.997	2.0–100
Acrinathrin	0.993	5.0–100
Alachlor	0.998	5.0–100
Aldrin	0.996	2.0–100
Atrazine	0.996	5.0–100
Azinphos-ethyl	0.991	5.0–100
Benfluralin	0.997	5.0–100
BHC, alpha-	0.997	2.0–100
BHC, beta-	0.995	2.0–100
BHC, delta-	0.997	2.0–100
BHC, gamma- (Lindane)	0.995	2.0–100
Bifenthrin	0.998	5.0–100
Biphenyl	0.995	2.0–100
Bromfenvinphos	0.996	5.0–100
Bromfenvinphos-methyl	0.996	5.0–100
Bromophos-methyl	0.997	5.0–100
Bromopropylate	0.994	2.0–100
Bupirimate	0.995	2.0–100
Carfentrazone ethyl	0.995	5.0–100
Chlordane, trans-	0.994	5.0–100
Chlorfenson	0.994	5.0–100
Chlorobenzilate	0.996	2.0–100
Chloroneb	0.995	2.0–100
Chlorpropham	0.996	2.0–100
Chlorpyrifos	0.995	5.0–100
Chlorpyrifos-methyl	0.995	5.0–100
Chlorthal-dimethyl	0.997	2.0–100
Chlorthiophos 3	0.998	5.0–100
Chlozolinate	0.995	5.0–100
Clomazone	0.996	2.0–100
Coumaphos	0.991	5.0–100
Cyfluthrin I	0.995	5.0–100
Cyfluthrin II	0.994	5.0–100
Cyfluthrin III	0.991	5.0–100
Cyfluthrin IV	0.990	5.0–100
Cyfluthrin I-IV	N/A	5.0–100
Cyhalothrin, lambda-	0.994	2.0–100
Cypermethrin I	0.997	5.0–100
Cypermethrin II	0.991	5.0–100
Cypermethrin III	0.990	5.0–100
Cypermethrin IV	0.993	5.0–100
Cypermethrin Peaks I-IV	N/A	5.0–100
Cyprodinil	0.995	5.0–100
DDD, o,p'-	0.999	2.0–100
DDD, p,p'-	0.996	2.0–100