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Automated QuEChERS Extraction for the Confirmation of Pesticide Residues in Foods using LC/MS/MS

Virgil Settle, Fred Foster
Gerstel, Inc., 701 Digital Dr. Suite J, Linthicum, MD 21090, USA

Paul Roberts
Anatune, Ltd., Broadway House, 148-152 St. Neots Road, Hardwick, Cambridge, CB23 7QJ, UK

Peter Stone
Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051-7201, USA

Joan Stevens
Agilent Technologies, 2850 Centerville Rd., Wilmington, DE 19808, USA

Jon Wong, Kai Zhang
US FDA, 5100 Paint Branch Parkway, HFS-3 36, College Park, MD 20740, USA

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Abstract
QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation methods were developed to help monitor pesticides in a range of food samples [1]. These methods are quite labor intensive, however, since they include several manual steps, such as shaking, centrifugation, and dispersive SPE. If dispersive SPE clean up of QuEChERS type extracts could be automated, laboratory productivity for monitoring pesticide residues in food could be improved significantly.
In the work presented here, an automated dispersive SPE clean-up method for QuEChERS extracts was developed and combined with LC/MS/MS analysis of the cleaned

extracts. Extracts were prepared with commercially available kits from Agilent Technologies. Automation was achieved using a GERSTEL MPS XL PrepStation configured with an Anatune CF-100 centrifuge. Analytical methodology for confirming the presence of a variety of pesticides in a range food samples was developed using an Agilent G6460A Triple Quadrupole Mass Spectrometer. The sensitivity and selectivity of LC/MS/MS enable sufficiently low limits of determination to meet acceptance criteria for reporting the maximum residue levels (MRLs) that are established by regulatory agencies.

The ability to automate the dispersive SPE clean-up of QuEChERS extracts combined with introduction of the cleaned extract directly to the LC/MS/MS system results in improved laboratory productivity by streamlining the complete analytical process.

Introduction

Automated QuEChERS clean-up of fruit and vegetable extracts combined with LC/MS/MS determination of pesticides was previously reported [2]. Recovery and reproducibility data based upon matrices spiked at concentrations of 10 ng/g showed that the system used was suitable for automating the QuEChERS dispersive SPE clean-up.

Recently, interest was expressed in an automated method to determine the presence of pesticide residues in botanical samples. Some botanical matrices have physical properties that pose a real challenge. When using the automated QuEChERS clean-up procedure, such matrices can make it difficult to reach the low limits of determination required in order to meet acceptance criteria for reporting the maximum residue levels (MRLs) as established by regulatory agencies.

This study focuses on the automated extraction used in the second step of the QuEChERS procedure, which is followed by LC/MS/MS analysis of the extract. The aim is to provide high throughput analysis for the confirmation of pesticide residues in botanical matrices. Automated QuEChERS extractions were performed by the GERSTEL MPS XL autosampler using Agilent's SampliQ QuEChERS dispersive SPE sorbent blend suggested for fatty matrices. The resulting extracts from the automated QuEChERS process were introduced into an Agilent 6460 LC/MS/MS instrument.

Experimental

Materials. Stock solutions containing the pesticide compounds listed in Table 1 in acetonitrile were prepared and provided by the FDA. Calibration standards and matrix matched standards were prepared by making appropriate dilutions of the pesticide stock solutions using mobile phase, blank hop extract, or blank ginseng extract resulting in the following concentrations: 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, and 1000 ng/mL.

Crude acetonitrile extracts of pesticide-fortified samples, incurred samples, and blank matrix samples based on both Hops and Ginseng root were prepared and provided by the FDA. These samples were generated using QuEChERS extraction salts for the EN Method (Agilent p/n: 5982-5650) and the recommended sample preparation method supplied with the salts [3].

Instrumentation. All automated PrepSequences were performed using an MPS 2XL dual head, multi-purpose sampler configured for automated QuEChERS-LC/MS/MS analysis as shown in Figure 1.

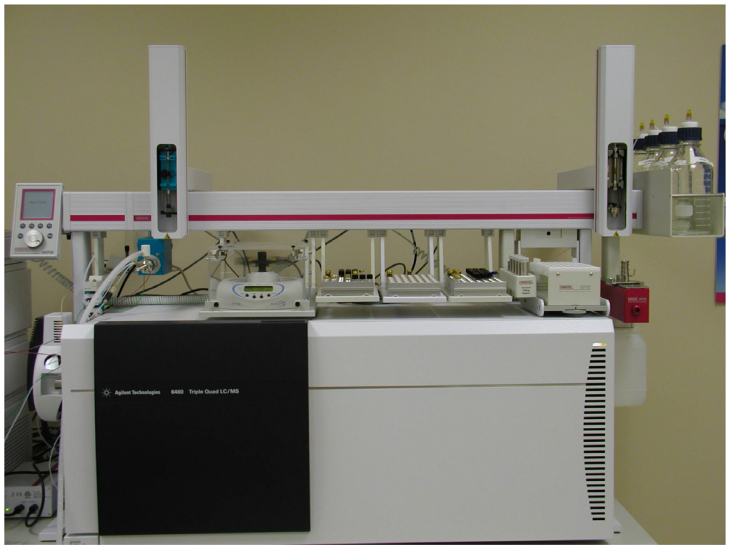

Figure 1. MPS 2XL dual head, multi-purpose sampler configured for automated QuEChERS-LC/MS/MS analysis.

Table 1. 200+ pesticides monitored using the automated QuEChERS-LC/MS/MS method.

3-Hydroxycarbofuran	Acephate	Acetamiprid	Acibenzolar-S-methyl	Alanycarb
Aldicarb	Aldicarb sulfone	Aldicarb sulfoxid	Aspon	Avermectin B1a
Avermectin B1b	Azadirachtin	Azoxystrobin	Benalaxyl	Bendiocarb
Benfuracarb	Benoxacor	Benthiavalicarb	Benzoximate	Bifenazate
Bifenthrin	Bitertanol	Boscalid	Bromuconazole-1	Bromuconazole-2
Bupirimate	Buprofezin	Butafenacil	Butocarboxym	Butoxycarboxim
Cadusafos	Carbaryl	Carbendazim	Carbetamid	Carbofuran
Carboxine	Carfentrazone-ethyl	Chlordimeform	Chlorfenvinphos-beta	Chlorfluazuron
Chlorotoluron	Chloroxuron	Clethodim	Clofentezine	Clothianidin
Coumaphos	Cumyluron	Cyanazine	Cyanophos	Cyazofamid
Cycluron	Cymoxanil	Cyproconazole	Cyprodinil	Cyromazine
d10-Diazinon	d6-Dichlorvos	d6-Dimethoate	d6-Diuron	d6-Linuron
d6-Malathion	Daimuron	Dazomet	Deltamethrin	Diazinon
Dichlorvos	Dicrotophos	Diethofencarb	Difenoconazol	Diflubenzuron
Dimethenamid	Dimethoat	Dimethomorph A	Dimethomorph B	Dimoxystrobin
Diniconazole	Dinotefuran	Dioxacarb	Disulfoton	Dithiopyr
Diuron	Dodemorph 1	Dodemorph 2	E-Fenpyroximate	Emamectin B1a
Emamectin B1b	Epoxiconazole	Eprinomectin B1a	EPTC	Esprocarb
Ethidimuron	Ethiofencarb	Ethion	Ethiprole	Ethirimol
Ethofumesate	Ethoprop	Etobenzanid	Etofenprox	Etoxazole
Famoxadone	Fenamidone	Fenarimol	Fenazaquin	Fenbuconazol
Fenhexamid	Fenoxanil	Fenoxycarb	Fenpropathrin	Fenpropimorph
Fenuron	Flonicamid	Flucarbazone	Fludioxinil	Flufenacet
Flufenoxuron	Flumetsulam	Flumioxazin	Fluometuron	Fluquinconazole
Flusilazol	Fluthiacet-methyl	Flutolanil	Flutriafol	Forchlorfenuron
Formetanate	Fuberidazole	Furalaxyl	Furathiocarb	Heptenophos
Hexaconazol	Hexaflumuron	Hexythiazox	Hydramethylnon	Imazalil
Imazapyr	Imibenconazole	Imidacloprid	Indanofan	Indoxacarb
Ipconazole	Iprovalicarb	Isocarbamid	Isofenfos	Isopropalin
Isoproturon	Isoxaben	Isoxaflutole	Kresoxim-methyl	Lactofen
Leptophos	Linuron	Lufenuron	Mandipropamid	Mefenazet
Mepanipyrim	Mepronil	Metalaxyl	Metconazole	Methabenzthiazuron
Methamidophos	Methiocarb	Methomyl	Methoprotryne	Methoxifenozid
Metobromuron	Metribuzin	Mevinphos	Mexacarbate	Molinate
Monocrotophos	Monolinuron	Moxidectin	Myclobutanil	Neburon
Nitenpyram	Norflurazon	Novaluron	Nuarimol	Omethoate
Oxadixyl	Oxamyl	Paclobutrazol	Penconazole	Pencycuron
Phenmedipham	Picoxystrobin	Piperonyl butoxide	Pirimicarb	Prochloraz
Promecarb	Prometon	Prometryn	Propachlor	Propamocarb
Propargite	Propazine	Propham	Propiconazole	Propoxur
Pymetrozine	Pyracarbolid	Pyraclostrobin	Pyridaben	Pyrimethanil
Pyriproxyfen	Quinoxyfen	Rotenone	Sebuthylazine	Secbumeton
Siduron	Simazine	Simetryn	Spinosyn A	Spinosyn D
Spirodiclofen	Spiromesifen	Spiroxamin	Sulfentrazone	Tebuconazole
Tebufenozide	Tebufenpyrad	Tebuthiuron	Teflubenzuron	Temephos
Terbumeton	Terbutryn	Terbutylazine	Tetraconazole	Tetramethrin cis
Thiabendazole	Thiacloprid	Thiametoxam	Thiazopyr	Thidiazuron
Thiobencarb	Thiofanox	Thiophanate-methyl	Triadimefon	Triadimenol
Trichlamide	Trichlorfon	Tricyclazole	Trifloxystrobin	Triflumizole
Triflumuron	Triticonazole	Uniconazole	Vamidothion	Zoxamide

QuEChERS acetonitrile extract pretreatment.

- Pipette 1mL of the acetonitrile extract obtained following the 1st centrifugation step of the QuEChERS sample preparation method into a 2 mL glass autosampler vial containing the sorbent blend from Agilent's SampliQ QuEChERS dispersive SPE kit for fatty samples, AOAC (p/n: 5982-5122).
- Place the sample onto a tray on the GERSTEL MPS 2XL dual head, multi-purpose sampler configured for automated QuEChERS-LC/MS/MS.

The automated QuEChERS extraction consisted of the following steps:

Automated QuEChERS Prep Sequence.

- Agitate the sample vial for 1 minute using the Anatune CF-100 centrifuge.
- Centrifuge the sample vial at 575 g for 3 minutes using the Anatune CF-100 centrifuge.
- Filter 500 µL of the resulting supernatant through a 0.45 µm GERSTEL format syringe filter.
- Combine 100 µL of the resulting filtrate with 400 µL of mobile phase A in a clean 2 mL vial.
- Agitate the sample vial using the Anatune CF-100 centrifuge for 30 seconds.
- Inject 2 µL into the LC/MS/MS system.

Figure 2 shows a Prep Sequence used to perform an automated QuEChERS-LC/MS/MS run.

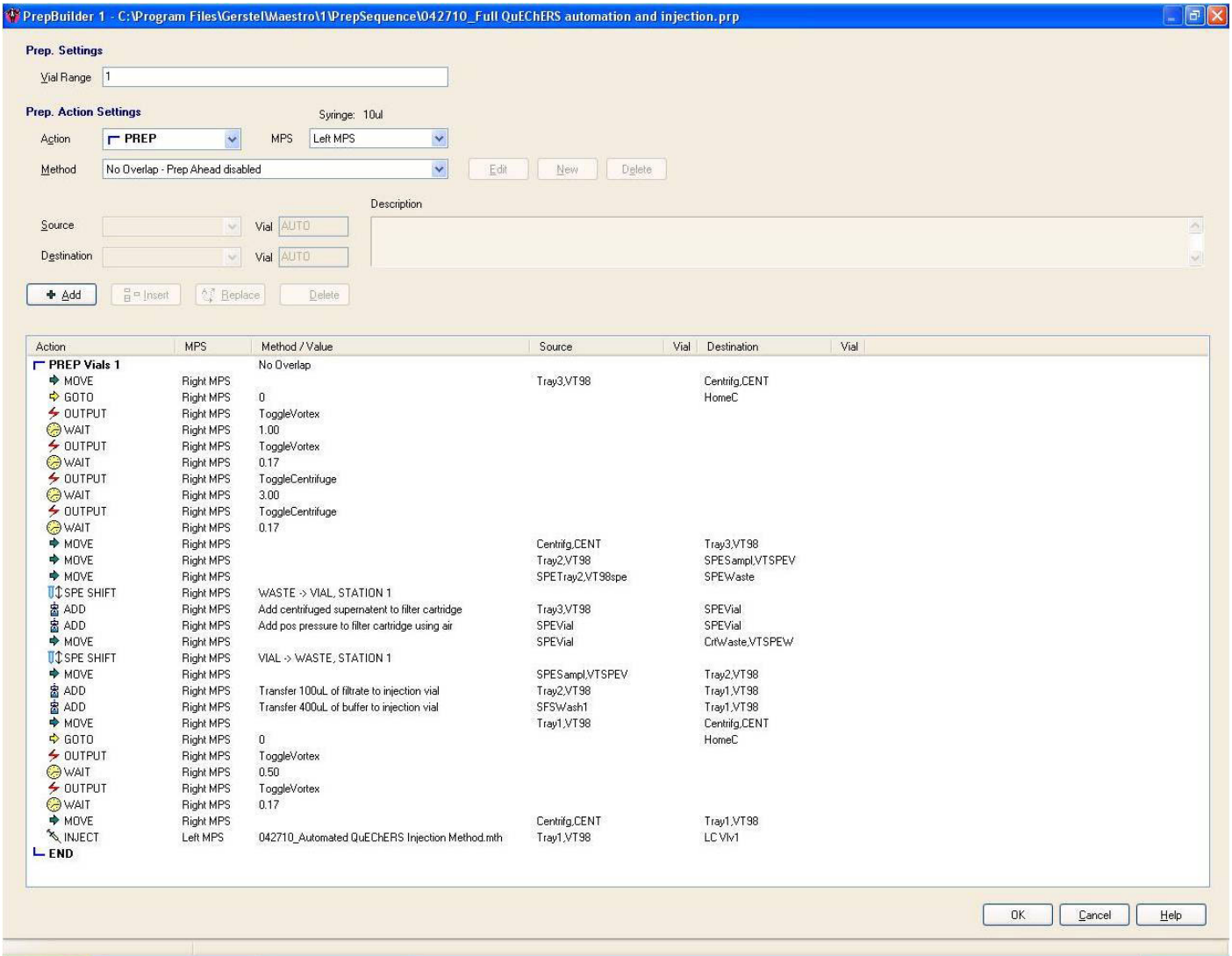

Figure 2. Example Prep Sequence for Automated QuEChERS LCMSMS Analysis.

Preparation of all standards was automated using the MPS 2XL dual head, multipurpose sampler configured for automated QuEChERS-LC/MS/MS analysis as follows:

Preparation of Calibration Standards and Matrix Matched Standards.

- Transfer 100 µL of previously extracted matrix blank or 100 % acetonitrile into an empty 2 mL autosampler vial.
- Transfer 250 µL of mobile phase A into the vial.
- Transfer 150 µL of the respective standard stock solution into the vial.
- Agitate the vial using the Anatune CF-100 centrifuge for 30 seconds.

All analyses were performed using an Agilent 1290 HPLC, an Agilent 6460 Triple Quadrupole Mass Spectrometer with electrospray source and Jet Stream Option and a GERSTEL MPS 2XL autosampler configured with Active WashStation. Sample injections were made using a 6 port (0.25 mm) Cheminert C2V injection valve fitted with a 2 µL stainless steel sample loop.

Analysis conditions LC.

Mobile Phase:	A - 5 mM ammonium formate in water with 0.01 % formic acid B – 0.01 % formic acid in acetonitrile	
Gradient:	Initial	94 % A / 6 % B
	0.3 min	94 % A / 6 % B
	14 min	5 % A / 95 % B
	17 min	5 % A / 95 % B
Pressure:	600 bar	
Flowrate:	500 µL/min	
Runtime:	17 min	
Post time:	2.5 min	
Column:	2.1 mm x 100 mm, 1.8 µm, Zorbax Eclipse Plus C18 RRHT (Agilent)	
Oven:	55°C	
Inj. volume:	2 µL	

Analysis conditions MS.

Operation:	Electrospray positive mode (Jet Stream)
Time Filter Width:	0.04 min
Scan Type:	Dynamic MRM
Delta EMV:	0 V
Cycle Time:	660 ms
Gas Temperature:	225 °C
Gas Flow (N2):	10 L/min
Nebulizer pressure:	25 psi
Sheath Gas (N2):	350 °C
	11 L/min
Capillary voltage:	4500 V
Nozzle Voltage:	500 V

The mass spectrometer acquisition parameters and respective quantifier/qualifier ion transitions were chosen using the pesticide database option available for the MassHunter B.03.01 software. Table 1 provides a list of the more than 200 pesticides that were monitored using this single LC/MS/MS method. A retention time window value of 0.5 minute was used for each positive ion transition being monitored during the course of the dynamic MRM experiment.

Results and Discussion

Figure 3 shows a representative overlay mass chromatogram resulting from a QuEChERS extract of a pesticide-fortified hop sample. More than 200 different pesticides were successfully determined in this botanical matrix using the automated QuEChERS-LC/MS/MS method.

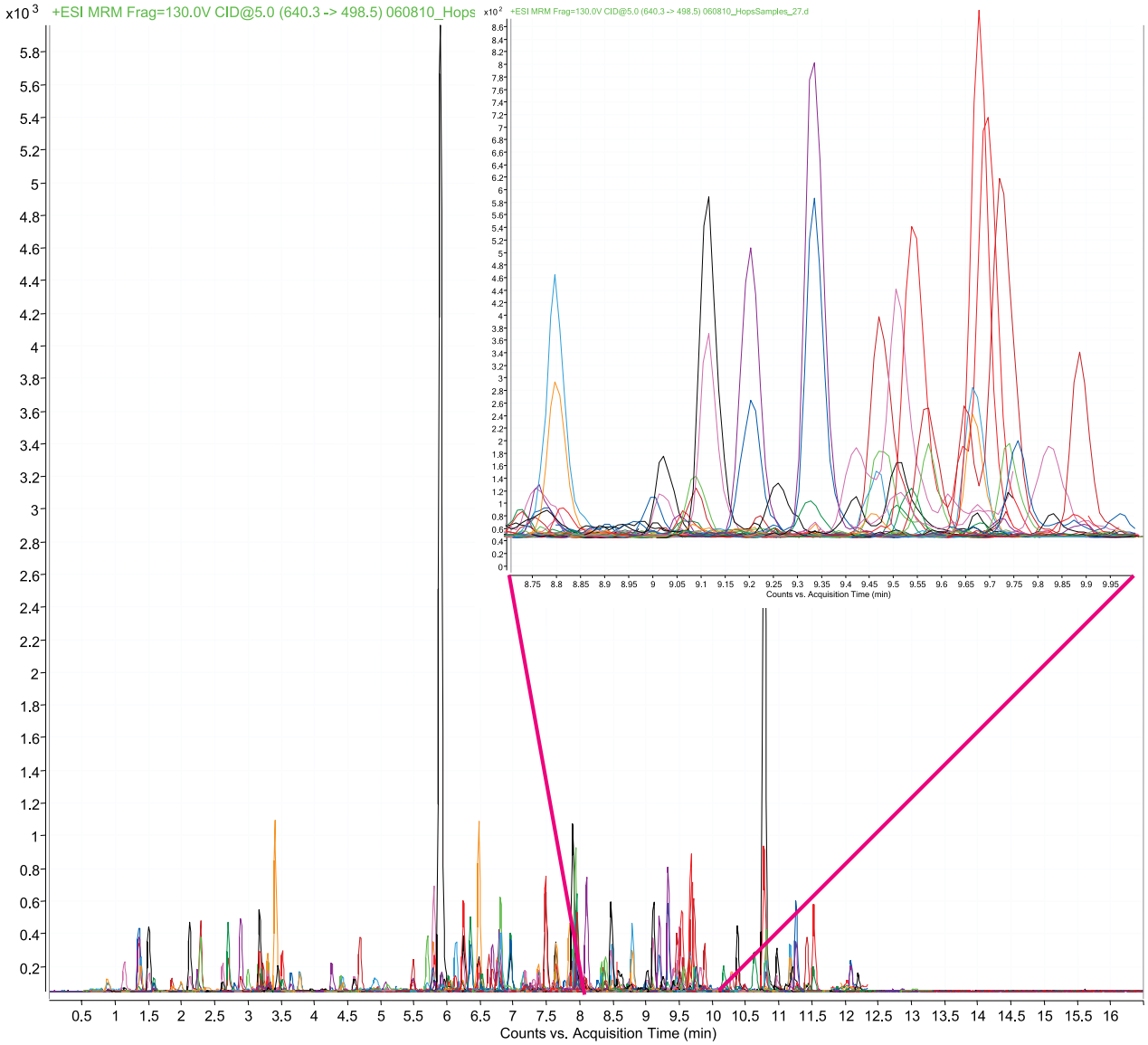

Figure 3. Representative Mass Chromatograms for low QC sample.

Figure 4 shows the a representative calibration curve resulting from automated preparation of neat standards. The calibration curves were shown to be linear from at least 1.00 to 200 ppb for the pesticides monitored, using a linear, 1/x regression method.

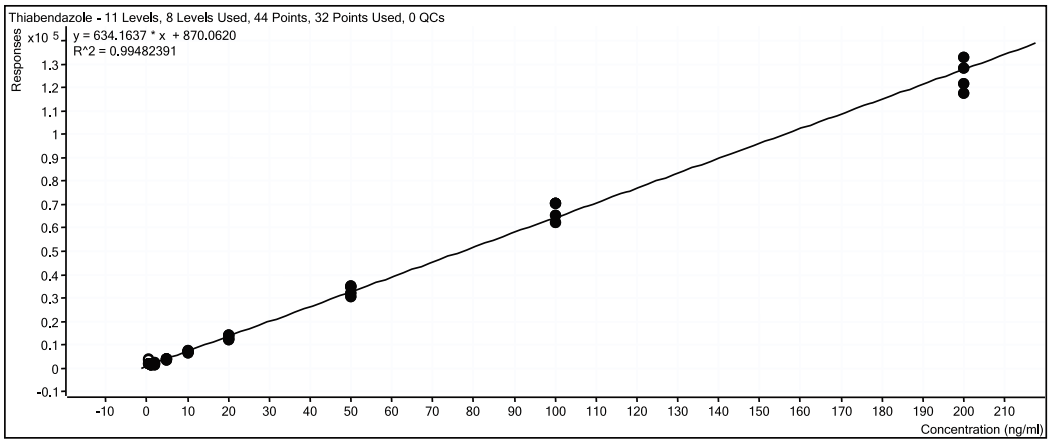

Figure 4. Representative calibration curve from automated neat standard preparation: thiabendazole.

Figures 5 through 7 show representative overlay mass chromatograms of neat, hop matrix matched, and ginseng matrix matched calibration standards respectively, all at a concentration of 10 ppb. The calibration standards were prepared automatically by the MPS 2XL.

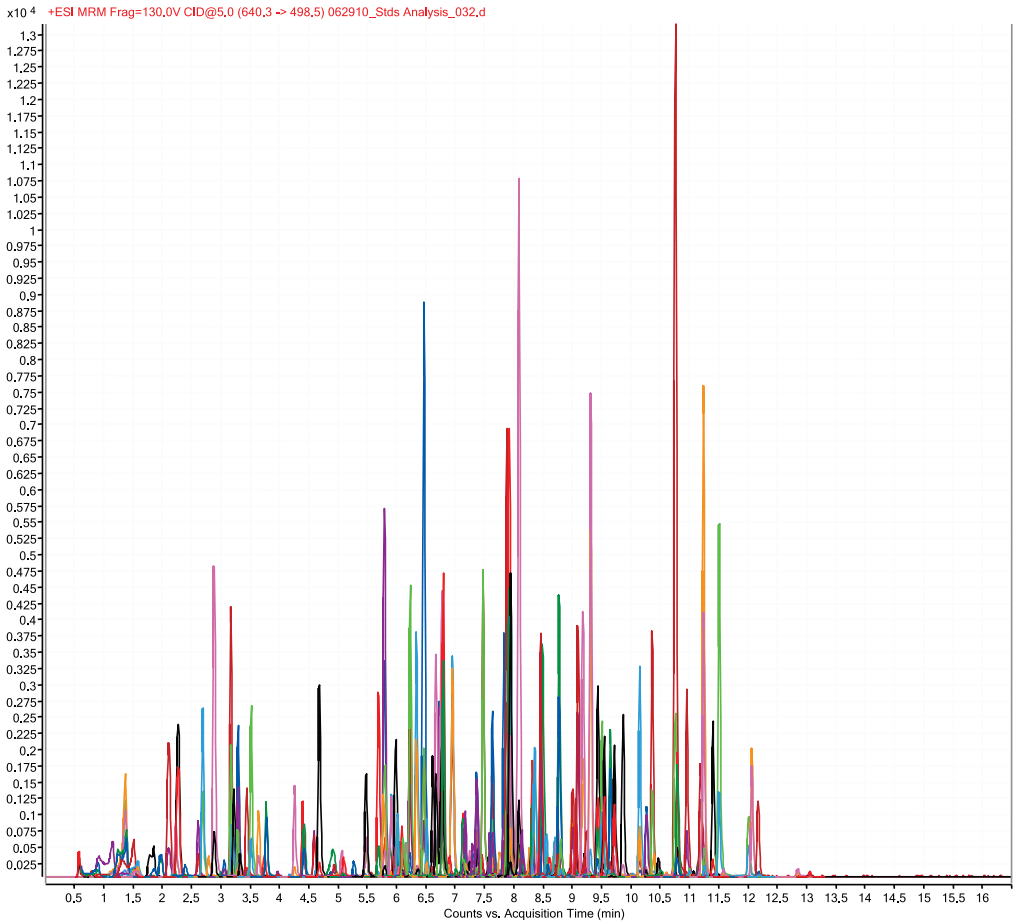

Figure 5. Representative overlay mass chromatogram for a 10 ppb neat standard.

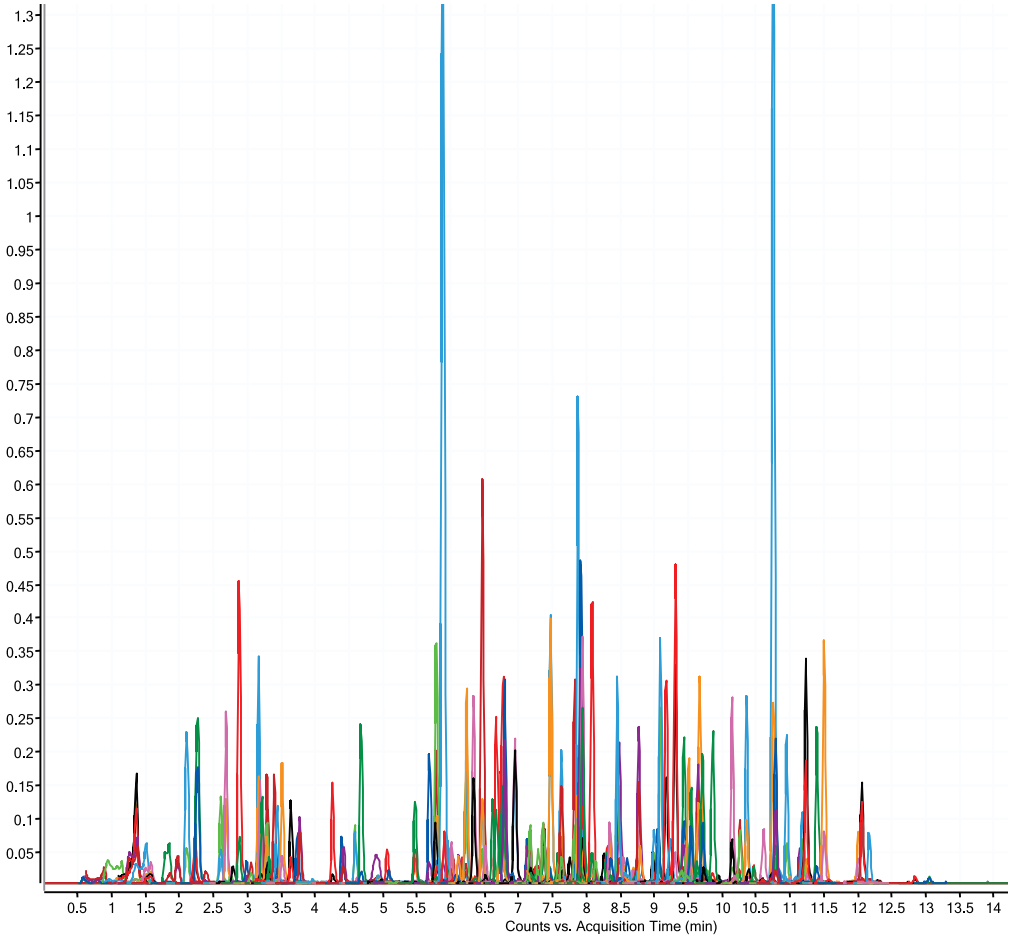

Figure 6. Representative overlay mass chromatogram for a 10 ppb hops matrix matched standard.

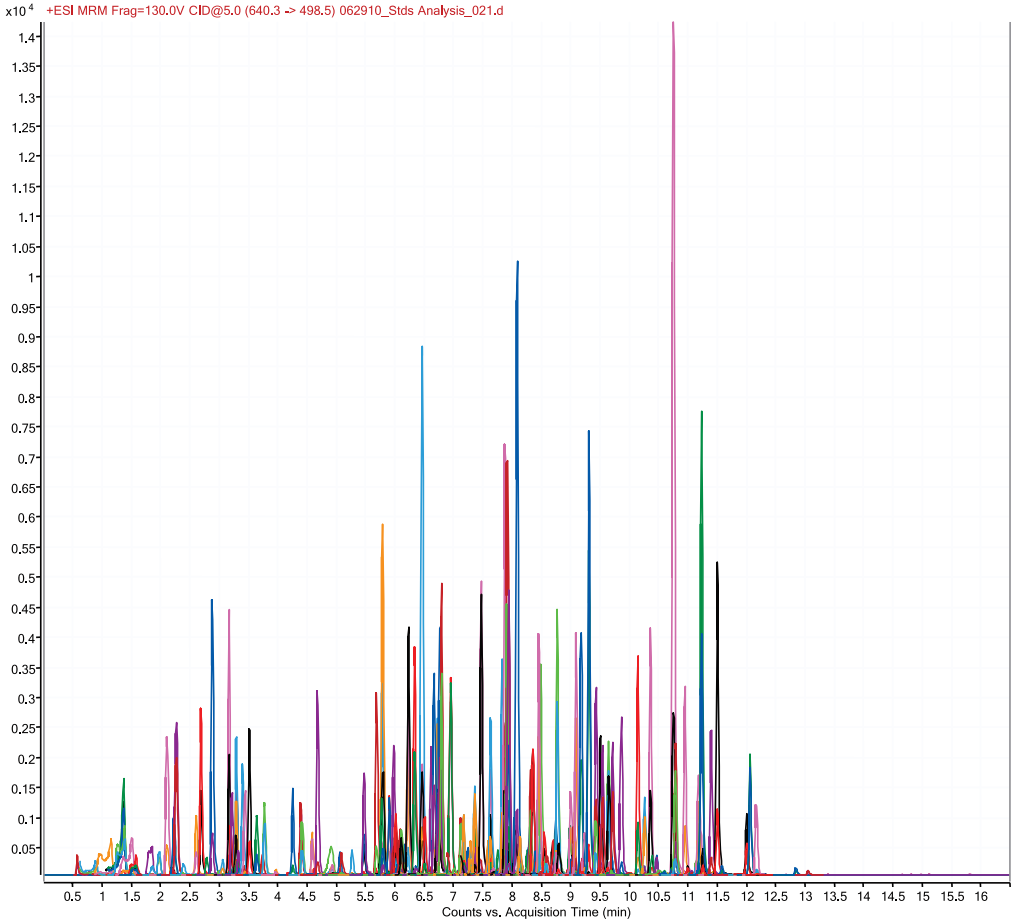

Figure 7. Representative overlay mass chromatogram for a 10 ppb ginseng matrix matched standard.

The total time required per sample to perform the QuEChERS extraction was 15 minutes. This was shorter than the LC-MS/MS analysis run, enabling the MPS system to complete preparation of the next sample during the LC-MS/MS run. Sample preparation and analysis are easily synchronized using the MAESTRO software providing "just in time" sample preparation and maximizing sample throughput.

Conclusions

In this study, we were able to demonstrate:

- Successful monitoring of more than 200 pesticides in botanical matrix samples using an automated QuEChERS extraction method coupled to LC/MS/MS analysis using the Agilent 6460 Triple Quadrapole Mass Spectrometer.
- Automation of both the QuEChERS method and the preparation of standards using the GERSTEL MPS 2XL dual head robotic sampler.
- The "just-in-time" sample preparation capability included in the MAESTRO software enables highly efficient QuEChERS extraction and analysis.

References

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[2] Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. Lehotay, SJ., J. AOAC Int. 90, 485 (2007)

[3] Agilent SampliQ QuEChERS Kit brochure. Document number 5990-3562EN www.chem.agilent.com

GERSTEL GmbH & Co. KG
Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany
+49 (0) 208 - 7 65 03-0
+49 (0) 208 - 7 65 03 33
gerstel@gerstel.com
www.gerstel.com

GERSTEL Worldwide

GERSTEL, Inc.
701 Digital Drive, Suite J
Linthicum, MD 21090
USA
+1 (410) 247 5885
+1 (410) 247 5887
sales@gerstelus.com
www.gerstelus.com

GERSTEL AG
Wassergrabe 27
CH-6210 Sursee
Switzerland
+41 (41) 9 21 97 23
gerstelag@ch.gerstel.com
www.gerstel.ch

GERSTEL K.K.
1-3-1 Nakane, Meguro-ku
Tokyo 152-0031
SMBC Toritsudai Ekimae Bldg 4F
Japan
+81 3 5731 5321
+81 3 5731 5322
info@gerstel.co.jp
www.gerstel.co.jp

GERSTEL LLP
Level 25, North Tower
One Raffles Quay
Singapore 048583
+65 6622 5486
+65 6622 5999
SEA@gerstel.com
www.gerstel.com

GERSTEL Brasil
Av. Pascoal da Rocha Falcão, 367
04785-000 São Paulo - SP Brasil
+55 (11)5665-8931
+55 (11)5666-9084
gerstel-brasil@gerstel.com
www.gerstel.com.br

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