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Automated Sample Preparation and Analysis Workflows for Pesticide Residue Screenings in Food Samples using DPX-QuEChERS with LC/MS/MS

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Pesticide residue monitoring, QuEChERS, LC/MS/MS, Sample Preparation, DPX, Lab Automation

Abstract
QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation methods have been developed to help monitor pesticides in a range of food samples. These methods require many manual steps, such as shaking, centrifugation, and dispersive SPE, making it a quite labor-intensive process. There is a need for automating the dispersive SPE technique to clean up QuEChERS type extracts in order to improve laboratory productivity for monitoring pesticide residue in foods.

In this report, we describe an automated sample preparation and analysis workflow for the screening of pesticides residues in different food matrices (fruits, vegetables and spices) by LC/MS/MS. The automated cleanup of the QuEChERS extracts methodology was performed using disposable pipette extraction (DPX). Analytical methodology for confirming the presence of a variety of pesticides in various food samples was developed using a GERSTEL MultiPurpose

Sampler (MPS), a combined autosampler and liquid handling robot, interfaced to an AB SCIEX QTRAP® 4500 LC/MS/MS System. Two transitions per parent compound were monitored using a single *Scheduled* MRM™ method. The sensitivity and selectivity of the LC/MS/MS system enabled us to reach detection limits, which meet acceptance criteria for reporting maximum residue levels (MRLs) as established by regulatory agencies.

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

Introduction

QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation methods have been developed to help monitor pesticides in a range of food samples. These methods require many manual steps, such as shaking, centrifugation, and dispersive SPE, making it a quite labor-intensive process. There is a need for automating the dispersive SPE technique to clean up QuEChERS type extracts in order to improve laboratory productivity for monitoring pesticide residue in foods.

A simpler and more practical way to perform the dispersive SPE cleanup method is to use Disposable Pipette Extraction (DPX) tips. These tips have a screen that retains loose sorbent material inside the pipette tip. The DPX tips used for this project contain anhydrous magnesium sulfate ($MgSO_4$) and primary and secondary amine (PSA) as cleanup sorbents and are denominated "QuEChERS Tips".

Here we present a new automated sample preparation and analysis workflow for pesticide residue screenings of food samples using DPX-QuEChERS with LC/MS/MS. The use of QuEChERS Tips has been reported previously [1-3] and has been found to provide comparable results to those obtained using manual methods based on dispersive SPE tubes. A GERSTEL MPS autosampler equipped with DPX option coupled to an ABSCIEX QTRAP® 4500 System was used for the automated cleanup of QuEChERS extracts. The LC/MS/MS method utilized the *Scheduled* MRM™ algorithm in combination with fast polarity switching. The method was successfully applied to quantify and identify 200+ pesticides in a number of QuEChERS extracts of fruit, vegetables, and spices. In addition full scan MS/MS spectra were acquired to allow library searching in order to increase confidence in identification.

Experimental

Materials. Blended mixtures in acetonitrile of serially diluted concentrations of the pesticide compounds listed in Table 1 were prepared using the SCIEX iDQuant™ Standards Kit for Pesticide Analysis. These were used for method setup and preparation of calibration standards to give concentrations equivalent to 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, and 1000 ng/mL. Additional pesticides were added to cover all compounds of interest.

Acetonitrile crude extracts of pesticide fortified samples, incurred samples, and blank matrix samples were prepared using Restek QuEChERS kits (Q110, Q210 and Q213) and the recommended sample preparation method supplied with the salts.

QuEChERS Tips containing PSA (75 mg), $MgSO_4$ (25 mg) and graphitized carbon black (GCB, 12.5 mg) were used to perform the automated cleanup method according to AOAC method 2007.1 (6 g $MgSO_4$, 1.5 g NaOAc) [4]. DPX tips were provided by DPX Labs.

Instrumentation. All automated PrepSequences were performed using a dual head MultiPurpose Sampler (MPS XL), configured for automated QuEChERS-LC/MS/MS analysis as shown in Figure 1.

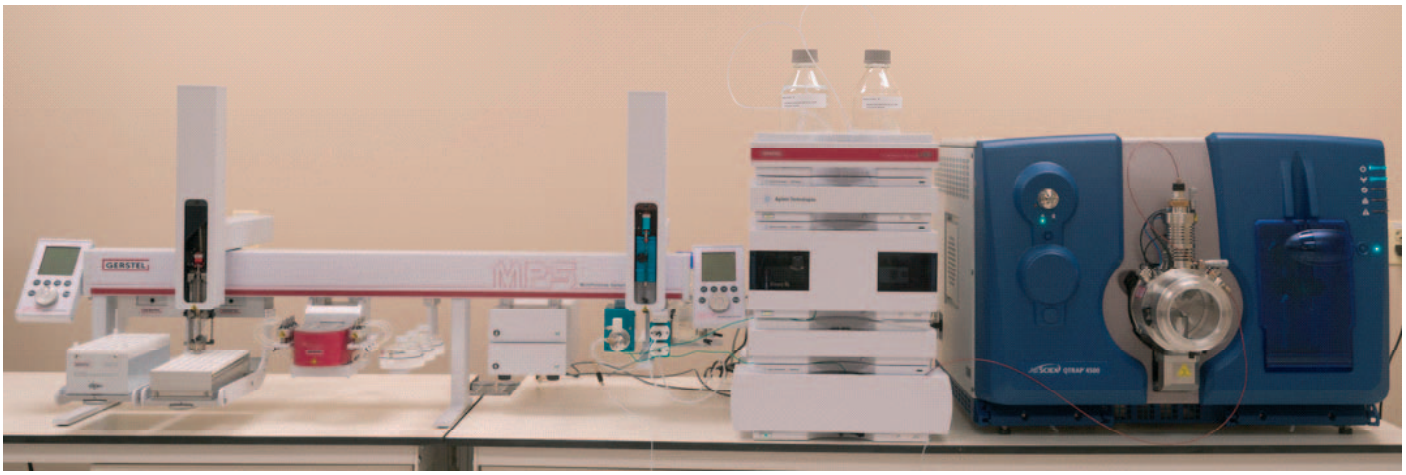


Figure 1. System used for automated pesticide residue screening: GERSTEL MultiPurpose Sampler MPS XL configured with DPX Option; Agilent 1200 HPLC system, and AB SCIEX QTRAP® 4500 LC/MS/MS.

Table 1. 200+ pesticides monitored using the automated DPX-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
d_{10}-Diazinon	d_6-Dichlorvos	d_6-Dimethoate	d_6-Diuron	d_6-Linuron
d_6-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

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

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 1 mL of the acetonitrile extract obtained following the 1st centrifugation step of the QuEChERS sample preparation method, into a 2 mL glass vial.
- Place the sample onto a tray on the dual head GERSTEL MPS XL configured for automated DPX-QuEChERS-LC/MS/MS.

The automated QuEChERS extraction consisted of the following steps:

Automated QuEChERS Prep Sequence:

1. MPS transfers 500 µL of QuEChERS extract to an open test tube.
2. DPX-QuEChERS tip is picked up and transported to the test tube for sample cleanup.
3. Sample is aspirated into the tip, mixed for 30 s and discharged to test tube. Repeat x3.
4. MPS transfers 50 µL of cleaned extract to a sealed vial, where it is diluted with 450 µL of mobile phase A.
5. The diluted cleaned extract is injected to the LC/MS/MS for analysis.

A schematic of the automated DPX-QuEChERS procedure is shown in Figure 2.

Figure 3 shows the automated Prep Sequence used to perform an automated DPX-QuEChERS-LC/MS/MS run. Preparation of all standards was automated using the dual head MPS XL configured for automated DPX-QuEChERS-LC/MS/MS analysis as follows:

Calibration Standard and Matrix Matched Standard preparation:

1. Transfer 100 µL of previously extracted matrix blank or 100 % acetonitrile into an empty 2 mL autosampler vial,.
2. Transfer 250 µL of mobile phase A into the vial.
3. Transfer 150 µL of the respective standard stock solution into the vial and mix.

All analyses were performed using LC separation, performed using an Agilent 1200 Series LC pump configured with a Phenomenex Synergi 2.5 µm Fusion-RP (100 Å, 50 x 2.0 mm) column, an AB SCIEX QTRAP® 4500 System and GERSTEL MPS XL autosampler configured with Active Washstation. Sample injections were made using a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 10 µL stainless steel sample loop.

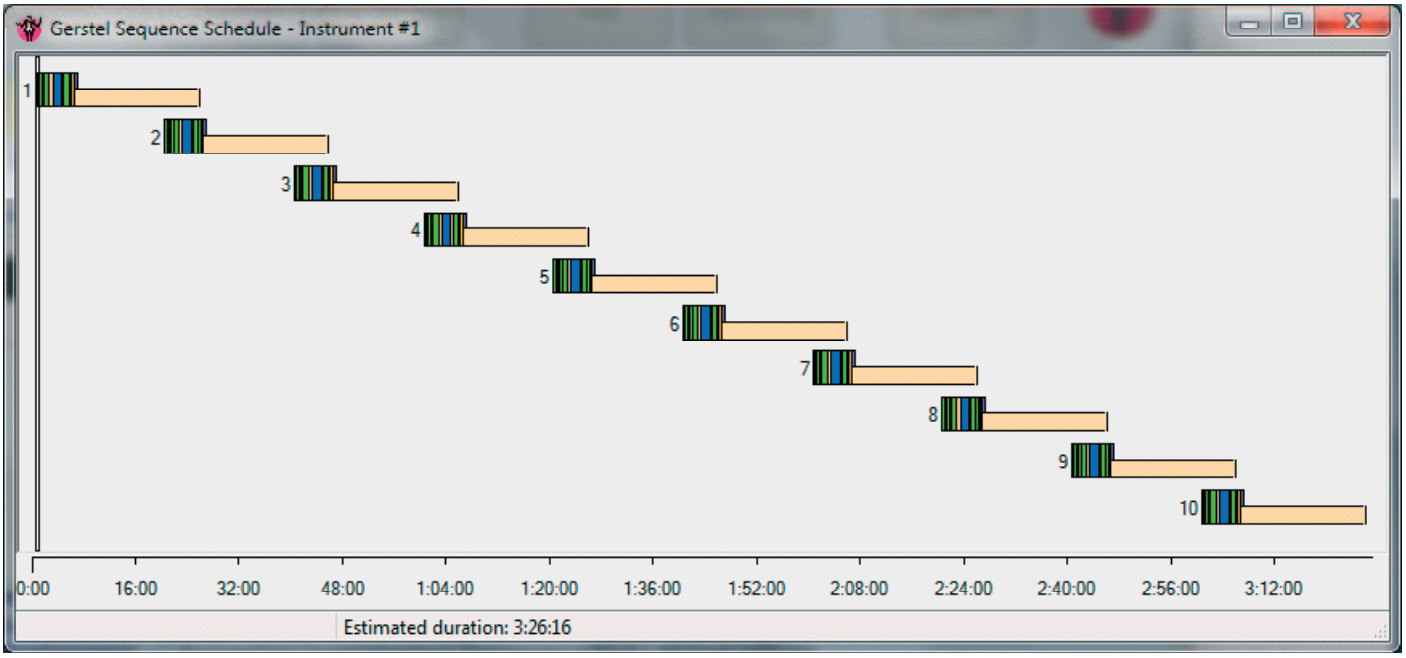


Figure 2. Example prep sequence for automated DPX-QuEChERS-LC/MS/MS analysis.

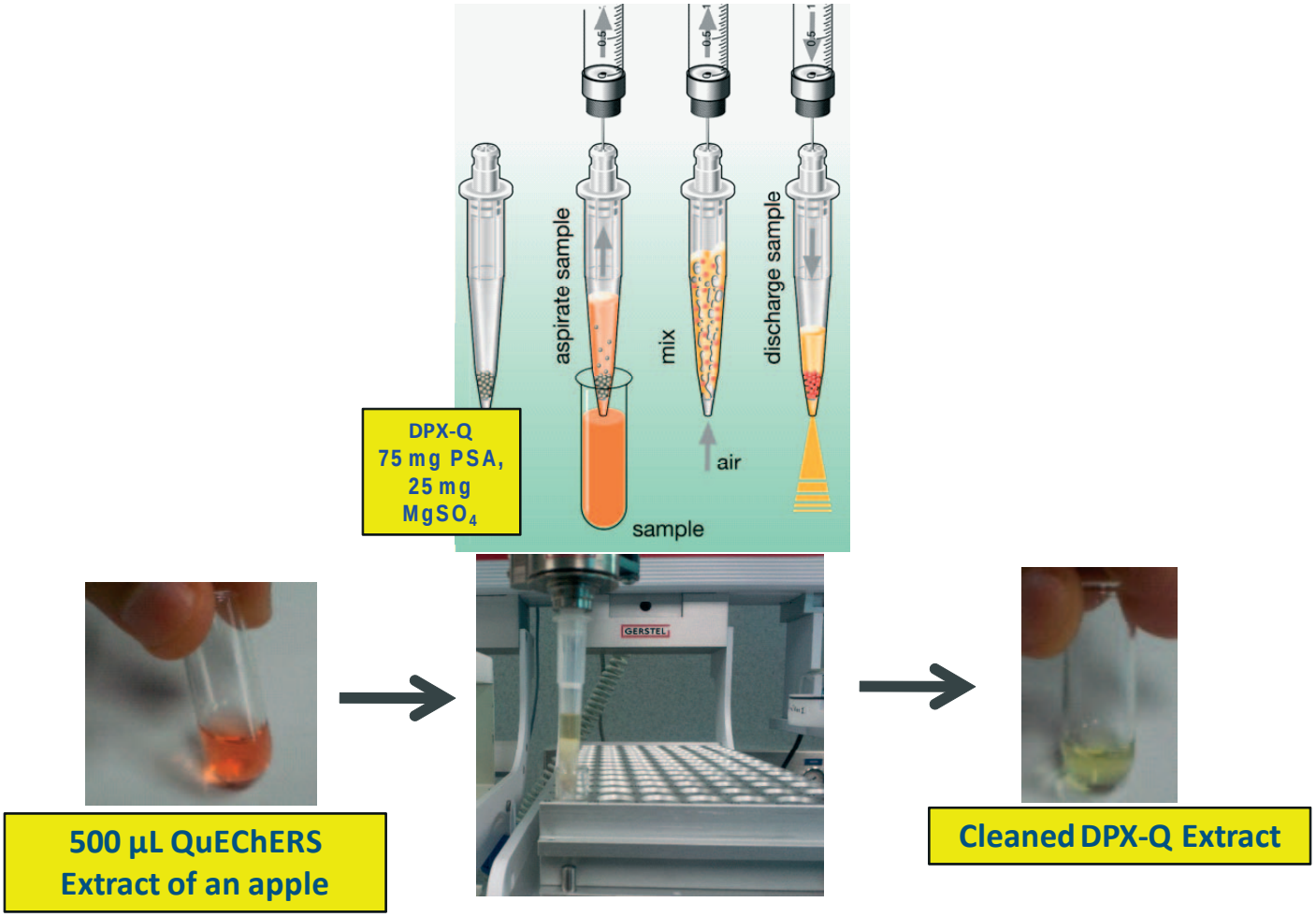


Figure 3. Schematic of the automated DPX-QuEChERS procedure.

LC/MS/MS conditions obtained from iMethod™ Test for Pesticides Screening

Mobile Phase: A – Water/Methanol (90:10)
+ 5 mM ammonium formate
B – Methanol/Water (90:10)
+ 5 mM ammonium formate

LC Gradient:

Time (min)	Flow (mL/min)	% B
0	0.4	0
1	0.4	0
15	0.4	100
18	0.4	100
18.05	0.4	0
20	0.4	0

Run time: 20 minutes.
Injection volume: 15 µL (loop overfill)
Column Temperature: 40°C

Mass Spectrometer Parameters:

Operation: Electrospray positive mode
Temperature: 400°C
Ion Source Gas 1: 50
Ion Source Gas 2: 50
IonSpray Voltage: 5500 V
Curtain Gas: 30
CAD: High

The AB SCIEX QTRAP® 4500 System was operated with Turbo V™ source and Electrospray Ionization (ESI) probe. Approximately 400 MRM transitions were monitored in both positive and negative polarity. Optimized transitions for all compounds were obtained through the MRM catalogue of the iMethod™ Test for Pesticide Screening version 2.1.

The *Scheduled* MRM™ algorithm was used in combination with fast polarity switching using Analyst® 1.6.1 Software. MultiQuant™ 2.1 Software was used for quantitative data processing.

Results and Discussion

The automated DPX-QuEChERS cleanup method was performed to remove sample matrix from extracts prior to chromatographic analysis. The removal of water ($MgSO_4$) and fatty acids (PSA) is necessary to ensure reproducible peak intensities for quantitative analysis. GCB is used to remove pigments, particularly chlorophyll and carotenoids, with its strong affinity toward planar molecules.

Figure 4 shows an overlay of representative MRM chromatograms from a pesticide-fortified cucumber sample QuEChERS extract at 1 ppb. More than 200 different pesticides were successfully determined in this sample matrix using the automated DPX-QuEChERS-LC/MS/MS method.

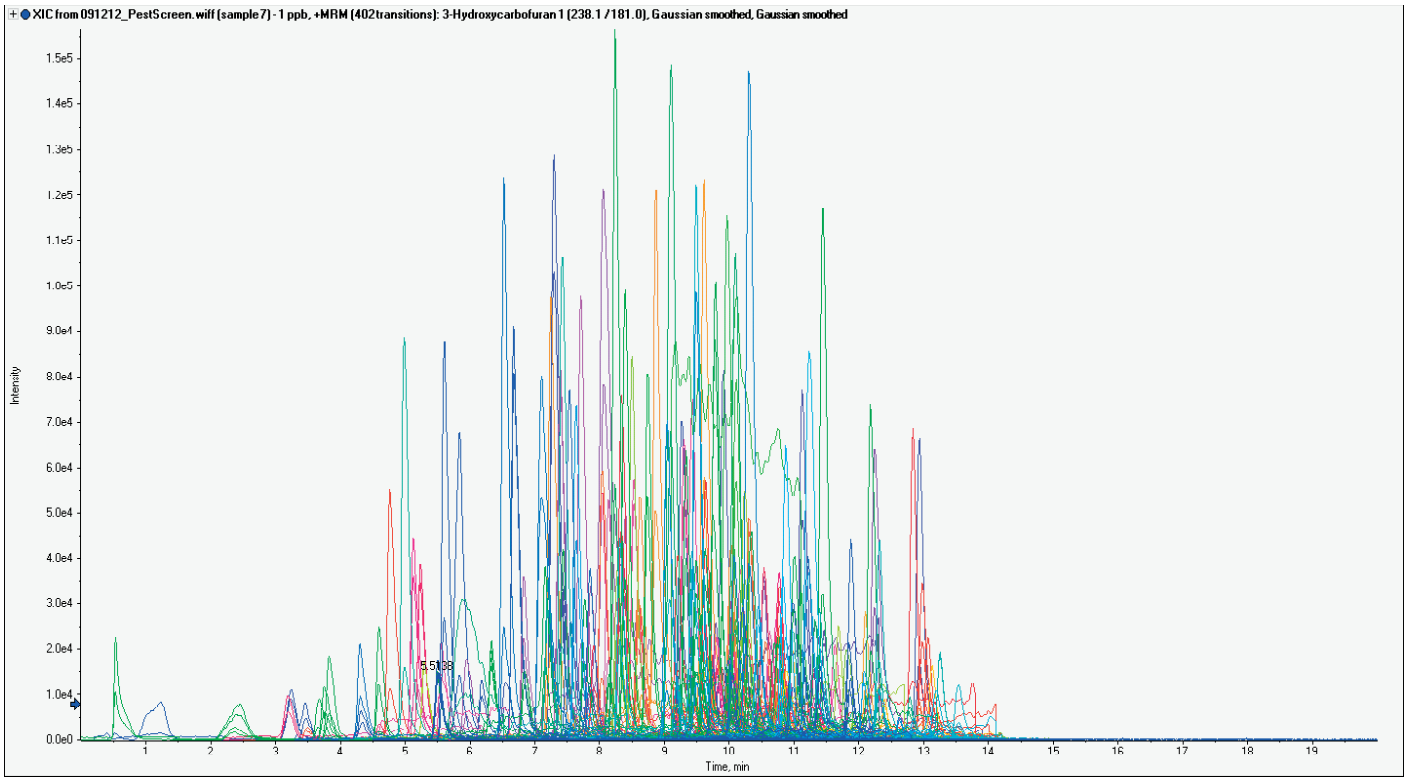
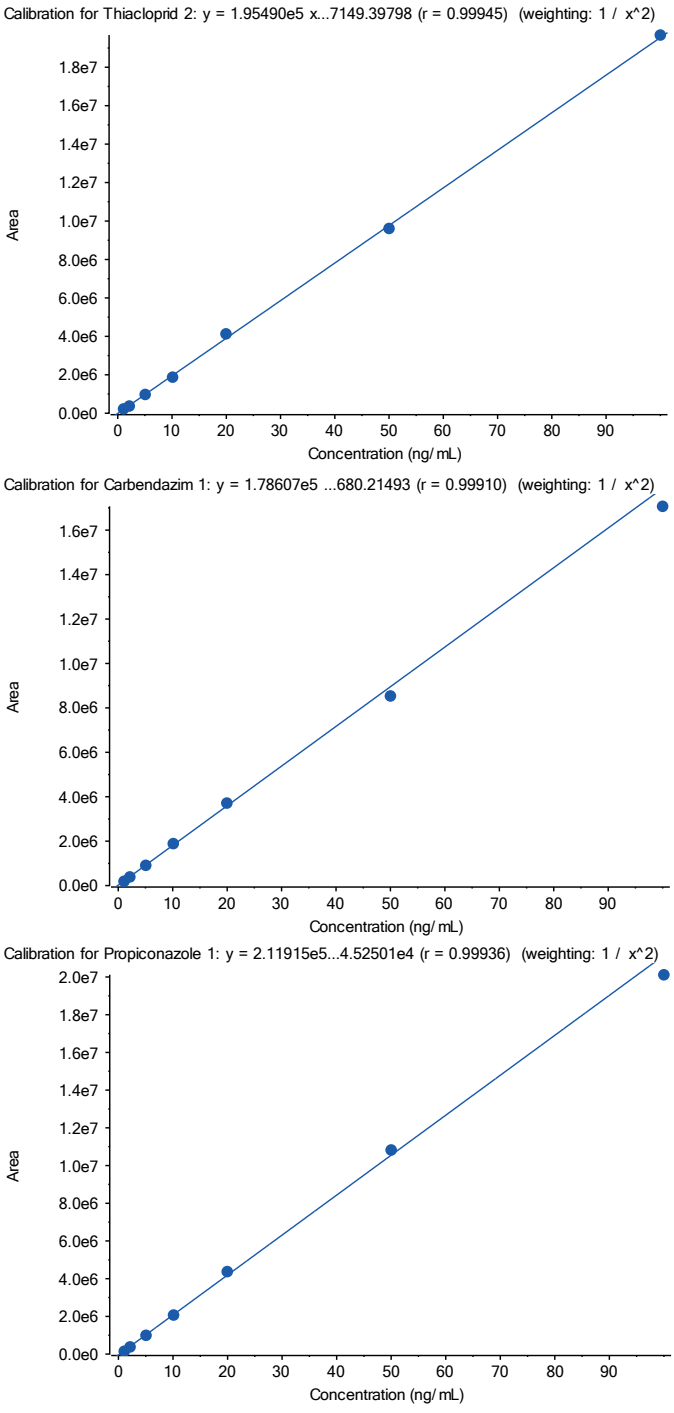


Figure 4. Representative MRM chromatograms from a pesticide-fortified cucumber sample QuEChERS extract at 1 ppb, 402 MRM transitions screened.

Figure 5 shows calibration curves obtained using automated neat standard preparation. The resulting calibration curves were shown to be linear from at least 1 to 200 ppb for the pesticides monitored, using a linear, $1/x^2$ regression method. Sample data was processed using MultiQuant™ software version 2.1 with the 'Multicomponent' query. Query files are customizable commands to perform custom querying of the result table. The 'Multicomponent' query automatically calculates and compares MRM ratios for compound identification and highlights concentrations above a user specified maximum residue level.



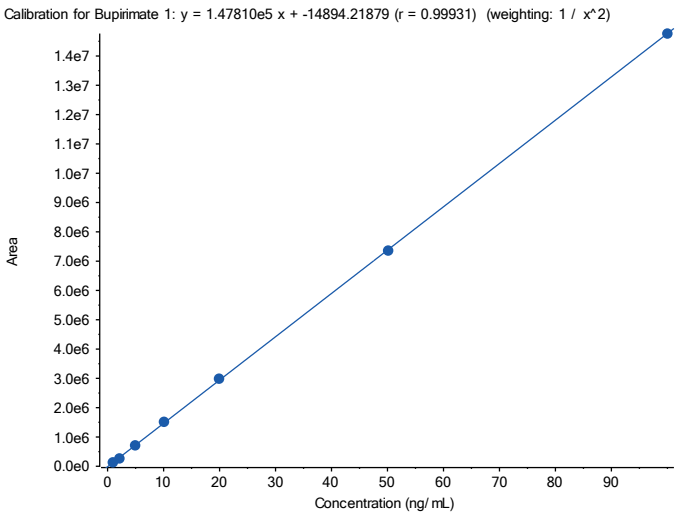


Figure 5. Representative calibration curves from automated neat standard preparation: Thiacloprid, Carbendazim, Propiconazole, Bupirimate, calibration range 1 – 100 ppb.

The developed method was applied to the determination of pesticides in extracts of real food samples obtained from a local supermarket. QuEChERS extracts of fruits and vegetables were cleaned using the DPX-QuEChERS method and diluted 1:10 for LC/MS/MS analysis. Figure 6 shows representative MRM chromatograms of pesticides determined in different food matrices. To increase confidence in identification full scan MS/MS experiments can be performed and spectra can be searched against mass spectral libraries. Here MS/MS spectra were acquired in the EPI mode of the QTRAP® 4500 system and searched against the iMethod™ pesticide library (version 1.1). Extracted spectra and library search Purity Score values using an MS/MS library search algorithm are shown in Figure 7 for an apple extract with low analyte concentrations.

The total DPX-QuEChERS extraction time per sample was 5 minutes. This means that while a sample is being analyzed, the next sample can be prepared "just in time" to be ready for injection when the LC/MS/MS system becomes ready - without adding to the overall analysis time. The MAESTRO PrepAhead function enables optimal timing of the entire process. Automation of the cleanup procedure helps save cost while improving productivity, throughput and reproducibility.

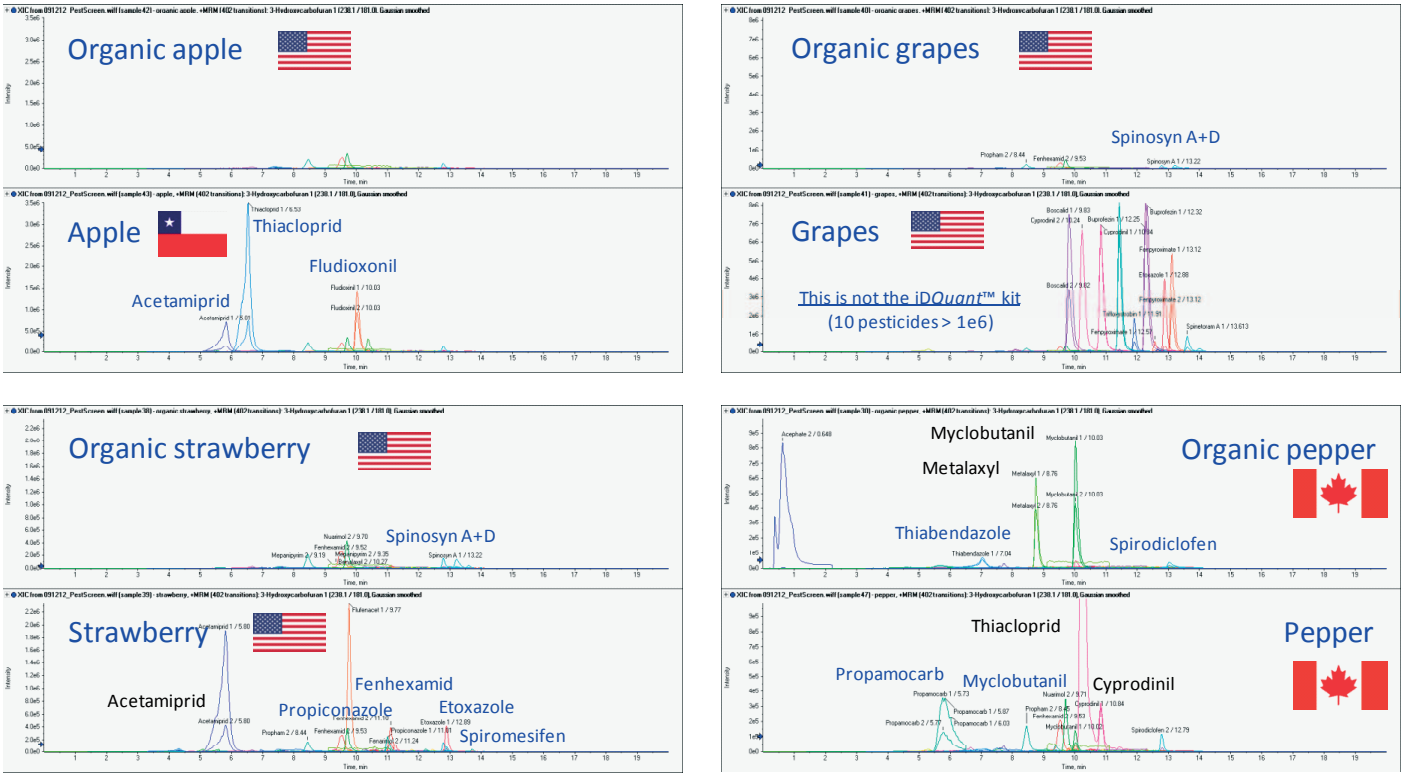


Figure 6. MRM chromatograms of pesticides identified in different food matrices.

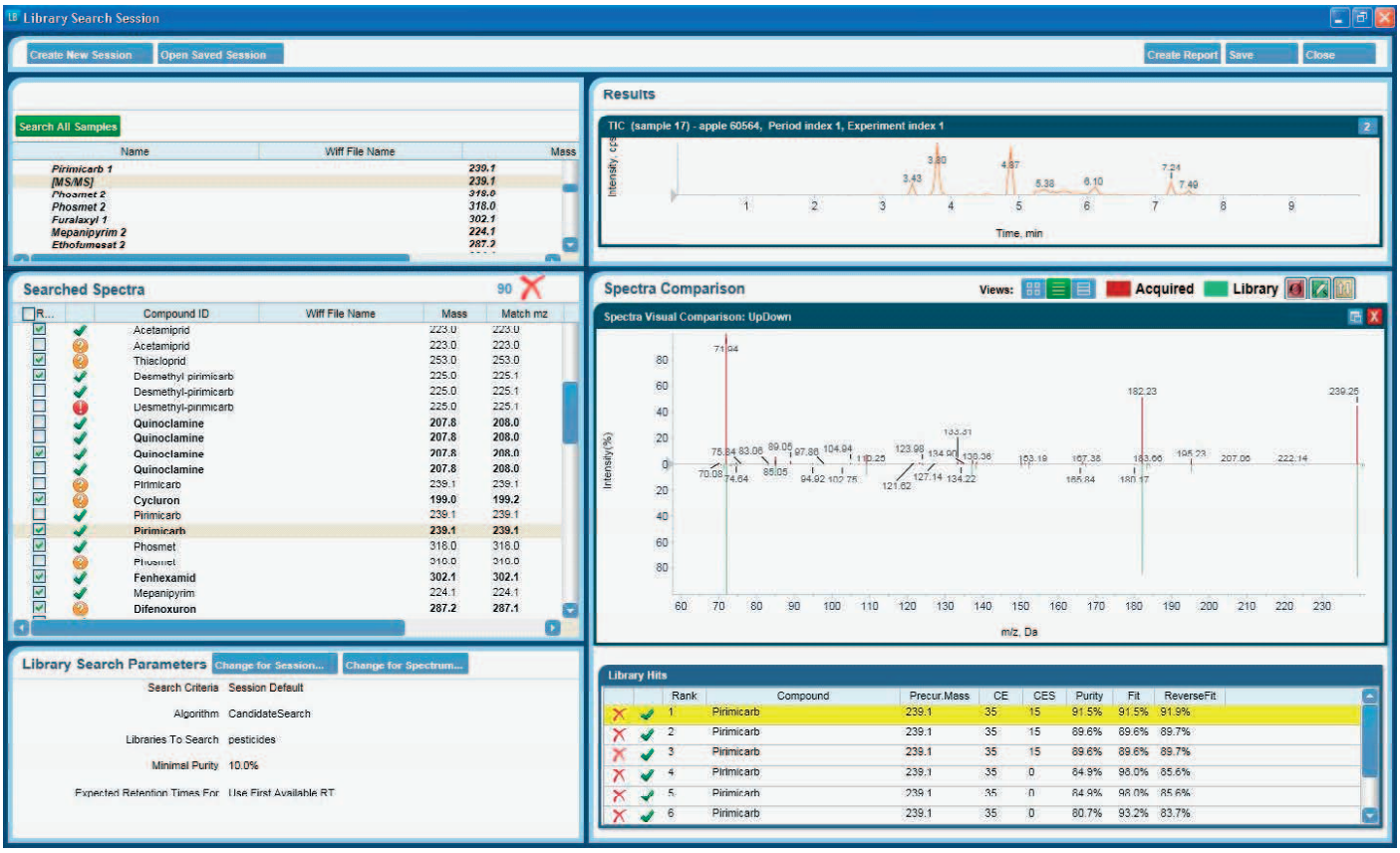


Figure 7. Automated library confirmation for Pirimicarb found in an apple extract after DPX-QuEChERS cleanup.

Conclusions

As a result of this study, we were able to show:

- The described new DPX-QuEChERS-LC/MS/MS workflow using the dual head GERSTEL MPS XL autosampler coupled to an AB SCIEX QTRAP® 4500 LC/MS/MS System enabled automated cleanup and analysis of QuEChERS extracts for screening and confirmation of 200+ pesticides in a single run.
- Quantitative analysis was performed in the same run allowing for both quantification and qualitative data to be collected simultaneously. Linear calibration curves resulting in R^2 values 0.99 or greater were achieved for the samples analyzed.
- With this configuration a 15 min/sample cycle time is achieved, including "just-in-time" PrepAhead sample preparation, for LC/MS/MS analysis of QuEChERS extracts.

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