

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

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Overview

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 cleanup, making it a quite labor-intensive process. There is a need for automating parts of QuEChERS extraction in order to improve laboratory productivity for monitoring pesticide residue in foods.

In this publication, we describe an automated sample preparation and analysis workflow for the screening of over 200 pesticides in different food matrices by LC-MS/MS. The automated cleanup of the QuEChERS extracts was performed using Disposable Pipette Extraction (DPX) with a GERSTEL MultiPurpose Sampler (MPS) 2XL interfaced to an AB SCIEX QTRAP® 4500 LC/MS/MS system. The sensitivity and selectivity of the LC-MS/MS system enabled us to identify and quantify with limits of detection which meet acceptance criteria for reporting Maximum Residue Levels (MRL) as established by regulatory agencies. The ability to automate the dispersive SPE cleanup of QuEChERS extracts followed by direct LC-MS/ MS analysis resulted in improved laboratory productivity by streamlining the complete analytical process.

Introduction

QuEChERS protocols are widely used to prepare samples for the monitoring of pesticide residues in food. These methods require many manual steps, such as shaking, centrifugation, and dispersive SPE cleanup, making it a quite labor-intensive process. A laboratory's productivity and efficiency can be greatly improved by automating parts of the QuEChERS procedure, the dispersive SPE cleanup step and subsequent dilution prior LC-MS/MS analysis.

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A simpler and more practical way to perform the dispersive SPE cleanup method is to use 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 (MgSO₄) 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 screening of food samples using DPX-QuEChERS with LC-MS/MS. The use of QuEChERS tips has been reported previously¹⁻³ and has been found to provide comparable results to those obtained using manual methods based on dispersive SPE. A GERSTEL MPS 2XL equipped with DPX option coupled to an ABSCIEX QTRAP[®] 4500 system was used for the automated cleanup of QuEChERS extracts and extract dilution. The LC-MS/MS method utilized the *Scheduled* MRMTM algorithm to obtain the best data quality in combination with fast polarity switching to cover the broadest range of pesticides possible. In addition, QTRAP[®] full scan MS/MS spectra were acquired to allow library searching in order to increase confidence in identification.

The method was successfully applied to identify and quantify over 200 pesticides in QuEChERS extracts of fruit, vegetable, herb and spice samples.



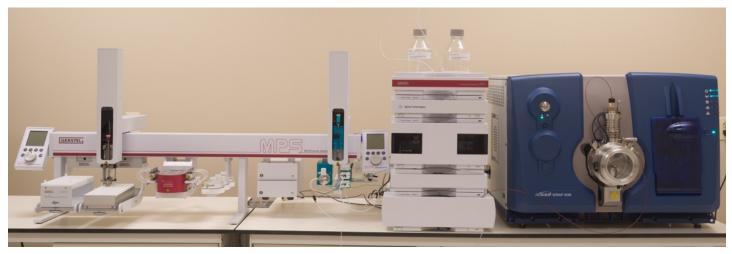


Figure 1. GERSTEL MPS 2XL with DPX option coupled to an QTRAP® 4500 system

Experimental

Materials

- Fruit, vegetable, herb, and spice samples, including organic produce, from a local supermarket
- SCIEX iD Quant[™] standards kit for pesticide analysis plus additional pesticides of interest (Table 1)
- Serial dilutions to prepare calibration standards with concentration of 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL
- Acetonitrile extracts of blank matrix samples, incurred samples and fortified samples using commercial QuEChERS kits following the AOAC method 2007.1
- DPX QuEChERS tips provided by DPX labs containing PSA (75 mg), MgSO₄ (25 mg) and GCB (12.5 mg) for dispersive SPE cleanup

QuEChERS Pretreatment

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

Automated QuEChERS Sample Preparation Sequence⁵

- 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 sec and discharged to test tube. Repeat 3 times.
- 4. MPS transfers 50 μ L of cleaned extract to a sealed vial, where it is diluted with 450 μ L 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.



Table 1. Pesticides monitored using the automated DPX-QuEChERS-LC-MS/MS method

Addicarb sulfone Addicarb sulfoxide Aspon Avermectin B1a Avermectin B1b Azadirachtin Azaxystrobin Benalasyi Bendicarb Benduracarb Benoxacor Benthiavastearb Benoximate Blenzate Bifenatare Bifenthrin Bitertanol Bosalid Bronuconazole Buprimate Buprofezin Butafenacil Buccarboxim Butoxycarboxim Cadusaldos Carbaryl Carbendazim Carbetamide Carbofuran Carboxin Cardendracone-ethyl Chlordimeform Chlorfenvinphos Chloriluszuron Chloroturon Chloroxuron Clethodim Colorenterion Chloroxuron Chloroxuron Clorenterion Chlorilus Cycluron Cymoxanil Cyproconazole Cyproconazole Cyprophos Cynazdanid Cyproconazole Cyproconazole Cyproconazole Cyprophos Cyprophos Cynazdanid Cyproconazole Cypr	3-Hydroxycarbofuran	Acephate	Acetamiprid	Acibenzolar-S-methyl	Alanycarb	Aldicarb
Benzoximate Bifenazate Bifenthrin Bitertanol Boscald Bromuconazole Bupirimate Buprofezin Butafenacil Butocarboxim Carboxim Cadualos Garbaryl Carbendazim Carbetamide Carboturan Carboxim Carforace-entryl Chiordimeform Chlordivinghos Chiordivazuron Chloroxuron Clethodim Clofentezine Clothianidin Coumaphos Cumyturon Cyanazine Cyanazine Cyanaphos Cyazofamid Cycluron Cymoxanil Cyproconazole Cyprodinil Cyanazine Cyanaphos Cyazofamid Cycluron Cymoxanil Cyproconazole Cyprodinil Cyanaphos	Aldicarb sulfone	Aldicarb sulfoxide	Aspon	Avermectin B1a	Avermectin B1b	Azadirachtin
Bupirimate Buprofezin Butafenacii Butocarboxim Butoxycarboxim Cadusafos Carbaryl Carbendazim Carbetamide Carbofuran Carboxin Carfentrazone-ethyl Chlordimeform Chlorfenvirphos Chlorfluazuron Chlorduuron Chloroxuron Citethodim Cloraciane Chloridinidin Comaphos Curnylturon Cyanazine Cyanopalos Cyazofamid Cyclron Oymonanil Cyprocanzole Cyprodinil Cyronapalos Disposario De-Dichloros De-Dimathose De-Diumonanilo De-Linuron De-Malathion Diamuron Dazomet Deltamethrin Diazinon Dichloros Dicrotophos Diethofencarb Difenoconazole Diflubenzuron Dimethenamid Dimethoate Dimethomorph Dimonystrobin Diniconazole Pinoproximate Emamectin B1a Emamectin B1a <td>Azoxystrobin</td> <td>Benalaxyl</td> <td>Bendiocarb</td> <td>Benfuracarb</td> <td>Benoxacor</td> <td>Benthiavalicarb</td>	Azoxystrobin	Benalaxyl	Bendiocarb	Benfuracarb	Benoxacor	Benthiavalicarb
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Ciofentezine Ciothianidin Coumphos Cumyluron Cyanazine Cyanophos Cyazofamid Cycluron Cymoxanil Cyproconazole Cyprodinil Cyromazine Dıp-Diazinon De-Dichlorvos De-Dimethoate De-Diuron De-Linuron De-Malathion Daimuron Dazomet Deltamethrin Diazinon Dichlorvos Dicrotophos Diethofencarb Diffenconazole Diffueluran Dioacarb Disulfoton Direthomorph Dimoxystrobin Diniconazole Dinofeluran Dioacarb Disulfoton Dithipoyr Diuron Dodemorph Fenproximate Emamectin B1a Emamectin B1a Eprococanazole Eprinomecin B1a EPTC Esprocarb Ethidimuron Ethiofencarb Ethion Ethiprole Ethirinol Ethotumesate Ethoprophos Etobenzanid Etofenprox Etoxazole Famoxadone Fenamidone Fenarimol Fenazaquin Fenbuconazole Fenhexamid Fenoxycari Fenarimol Fenazaquin Fenbuconazole	Carbaryl	Carbendazim	Carbetamide	Carbofuran	Carboxin	Carfentrazone-ethyl
Cyazofamid Cyduron Cymoxanil Cyproconazole Cyprodinil Cyromazine D₁₀-Diazinon D₀-Dichlorvos D₀-Dimethoate D₀-Diuron D₀-Linuron D₀-Malathion Daimuron Dazomet Deltamethrin Diazinon Dichlorvos Dicrotophos Diethofencarb Difenconazole Difubenzuron Dimethenamid Dimethoate Dimethomorph Diuron Dodemorph Penpyroximate Emamectin B1a Emamectin B1b Epocionazole Eprinomectin B1a EPTC Esprocarb Ethidimuron Ethidinecarb Ethion Ethiprole Ethifinol Ethodrumesate Ethoprophos Etobenzanid Etofenprox Etoxazole Famoxadone Fenamidone Fenarimol Fenazaquin Fenbuconazole Fenhaxamid Fenoxanil Fenoxycarb Fenpropathrin Fenpropimorph Fenuron Flumitosazin Fluoreturon Fludiconnil Fludenazet Fludenazet Fludenoxuron Flumetsulam Flutriafol Fluoreturon Formetanate Flube	Chlordimeform	Chlorfenvinphos	Chlorfluazuron	Chlortoluron	Chloroxuron	Clethodim
D _{ic} -Diazinon D _{ic} -Dichiorvos D _{ic} -Dimethoate D _{ic} -Diuron D _{ic} -Malathion Daimuron Dazomet Deltamethrin Diazinon Dichtorvos Dicrotophos Diethofencarb Difenconazole Diffubenzuron Dimethenamid Dimethoate Dimethomorph Dimoxystrobin Diniconazole Dinotefuran Dioxacarb Disulfoton Dithiopyr Diuron Dodemorph Fenpyroximate Emamectin B1a Emamectin B1b Epoxiconazole Eprinomectin B1a EPTC Esprocarb Ethidimuron Ethiofencarb Ethion Ethizorol Ethirinol Ethofumesate Ethoprophos Etobenzanid Etofenprox Etoazole Famoxadone Fenamidone Fenarimol Fenazaquin Fenotocanazole Fenhexamid Fenoxanil Fenoxycarb Fenpropathrin Fenopropimorph Fenuron Fludiacatini Fludiacatini Fenoxycarb Fenuron Fludiacatini Fludiacatini Fludiacatini Fenuron Fludiacatini Flutiriatol	Clofentezine	Clothianidin	Coumaphos	Cumyluron	Cyanazine	Cyanophos
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Flutriafol Forchlorfenuron Formetanate Fuberidazole Furalaxyl Furathiocarb Heptenophos Hexaconazole Hexaflumuron Hexythiazox Hydramethylnon Imazalil Ilmazapyr Imibenconazole Imidacloprid Indanofan Indoxacarb Ipconazole Iprovalicarb Isocarbamid Isofenphos Isopropalin Isoproturon Isoxaben Isoxaflutole Kresoxim-methyl Lactofen Leptophos Linuron Lufenuron Mandipropamid Mefenacet Mepanipyrim Mepronil Metalaxyl Metconazole Methabenzthiazuron Metribuzin Mevinphos Mexacarbate Molinate Monocrotophos Monolinuron Moxidectin Myclobutanil Neburon Nitenpyram Norflurazon Novaluron Nuarimol Omethoate Oxadixyl Oxamyl Paclobutrazol Penconazole Pencycuron Phenmedipham Picoxystrobin Piperonyl butoxide Pirimicarb Pirimicarb-desmethyl Pirimicarb-desmethyl-formamide Proparoler Propamocarb Propargite Propazine Propham Propiconazole Propoxur Pymetrozine Pyracarbolid Pyraclostrobin Pyridaben Pyrimethanil Pyriproxyfen Quinoxyfen Rotenone Sebuthylazine Secbumeton Spiromesifen	Flonicamid	Flucarbazone	Fludioxonil	Flufenacet	Flufenoxuron	Flumetsulam
Heptenophos Hexaconazole Hexaflumuron Hexythiazox Hydramethylnon Imazalil Imazapyr Imibenconazole Imidacloprid Indanofan Indoxacarb Ipconazole Iprovalicarb Isocarbamid Isofenphos Isopropalin Isoproturon Isoxaben Isoxaflutole Kresoxim-methyl Lactofen Leptophos Linuron Lufenuron Mandipropamid Mefenacet Mepanipyrim Mepronil Methapenzthiazuron Methamidophos Methiocarb Methomyl Methoprotryne Methoxyfenozide 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 Pirimicarb-desmethyl formamide Propargite Propazine Propham Propiconazole Propoxur Pymetrozine Pyracarbolid Pyraclostrobin Pyridaben Pyrimethanil Pyriproxyfen Quinoxyfen Rotenone Sebuthylazine Secbumeton Spiromesifen	Flumioxazin	Fluometuron	Fluquinconazole	Flusilazole	Fluthiacet-methyl	Flutolanil
ImazapyrImibenconazoleImidaclopridIndanofanIndoxacarbIpconazoleIprovalicarbIsocarbamidIsofenphosIsopropalinIsoproturonIsoxabenIsoxaflutoleKresoxim-methylLactofenLeptophosLinuronLufenuronMandipropamidMefenacetMepanipyrimMepronilMetladaxylMetconazoleMethabenzthiazuronMethamidophosMethiocarbMethomylMethoprotryneMethoxyfenozideMetobromuronMetribuzinMevinphosMexacarbateMolinateMonocrotophosMonolinuronMoxidectinMyclobutanilNeburonNitenpyramNorflurazonNovaluronNuarimolOmethoateOxadixylOxamylPaclobutrazolPenconazolePencycuronPhenmediphamPicoxystrobinPiperonyl butoxidePirimicarbPirimicarb-desmethylPirimicarb-desmethyl-formamideProphorazPromecarbPrometonPrometrynPropachlorPropamocarbPropargitePropazineProphamPropiconazolePropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Flutriafol	Forchlorfenuron	Formetanate	Fuberidazole	Furalaxyl	Furathiocarb
IprovalicarbIsocarbamidIsofenphosIsopropalinIsoproturonIsoxabenIsoxaflutoleKresoxim-methylLactofenLeptophosLinuronLufenuronMandipropamidMefenacetMepanipyrimMepronilMetalaxylMetconazoleMethabenzthiazuronMethamidophosMethiocarbMethomylMethoprotryneMethoxyfenozideMetobromuronMetribuzinMevinphosMexacarbateMolinateMonocrotophosMonolinuronMoxidectinMyclobutanilNeburonNitenpyramNorflurazonNovaluronNuarimolOmethoateOxadixylOxamylPaclobutrazolPenconazolePencycuronPhenmediphamPicoxystrobinPiperonyl butoxidePirimicarbPirimicarb-desmethyl-formamideProphamariaPromecarbPrometonPrometrynPropachlorPropamocarbPropargitePropazineProphamPropiconazolePropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Heptenophos	Hexaconazole	Hexaflumuron	Hexythiazox	Hydramethylnon	Imazalil
IsoxaflutoleKresoxim-methylLactofenLeptophosLinuronLufenuronMandipropamidMefenacetMepanipyrimMepronilMetalaxylMetconazoleMethabenzthiazuronMethamidophosMethiocarbMethomylMethoprotryneMethoxyfenozideMetobromuronMetribuzinMevinphosMexacarbateMolinateMonocrotophosMonolinuronMoxidectinMyclobutanilNeburonNitenpyramNorflurazonNovaluronNuarimolOmethoateOxadixylOxamylPaclobutrazolPenconazolePencycuronPhenmediphamPicoxystrobinPiperonyl butoxidePirimicarbPirimicarb-desmethyl formamideProphorazPromecarbPromecarbPrometonPrometrynPropachlorPropamocarbPropargitePropazineProphamPropiconazolePropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Imazapyr	Imibenconazole	Imidacloprid	Indanofan	Indoxacarb	Ipconazole
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MonolinuronMoxidectinMyclobutanilNeburonNitenpyramNorflurazonNovaluronNuarimolOmethoateOxadixylOxamylPaclobutrazolPenconazolePencycuronPhenmediphamPicoxystrobinPiperonyl butoxidePirimicarbPirimicarb-desmethylPirimicarb-desmethyl-formamideProchlorazPromecarbPrometonPrometrynPropachlorPropamocarbPropargitePropazineProphamPropiconazolePropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Methabenzthiazuron	Methamidophos	Methiocarb	Methomyl	Methoprotryne	Methoxyfenozide
NovaluronNuarimolOmethoateOxadixylOxamylPaclobutrazolPenconazolePencycuronPhenmediphamPicoxystrobinPiperonyl butoxidePirimicarbPirimicarb-desmethylPirimicarb-desmethyl-formamideProchlorazPromecarbPromecarbPrometonPrometrynPropachlorPropamocarbPropargitePropazineProphamPropiconazolePropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Metobromuron	Metribuzin	Mevinphos	Mexacarbate	Molinate	Monocrotophos
PenconazolePencycuronPhenmediphamPicoxystrobinPiperonyl butoxidePirimicarbPirimicarb-desmethyl formamideProchlorazPromecarbPrometonPrometrynPropachlorPropamocarbPropargitePropazineProphamPropiconazolePropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Monolinuron	Moxidectin	Myclobutanil	Neburon	Nitenpyram	Norflurazon
Pirimicarb-desmethyl formamideProchlorazPromecarbPrometonPrometrynPropachlorPropamocarbPropargitePropazineProphamPropiconazolePropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Novaluron	Nuarimol	Omethoate	Oxadixyl	Oxamyl	Paclobutrazol
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	Penconazole	Pencycuron	Phenmedipham	Picoxystrobin	Piperonyl butoxide	Pirimicarb
PropoxurPymetrozinePyracarbolidPyraclostrobinPyridabenPyrimethanilPyriproxyfenQuinoxyfenRotenoneSebuthylazineSecbumetonSiduronSimazineSimetrynSpinosyn ASpinosyn DSpirodiclofenSpiromesifen	Pirimicarb-desmethyl		Prochloraz	Promecarb	Prometon	Prometryn
Pyriproxyfen Quinoxyfen Rotenone Sebuthylazine Secbumeton Siduron Simazine Simetryn Spinosyn A Spinosyn D Spirodiclofen Spiromesifen	Propachlor	Propamocarb	Propargite	Propazine	Propham	Propiconazole
Simazine Simetryn Spinosyn A Spinosyn D Spirodiclofen Spiromesifen	Propoxur	Pymetrozine	Pyracarbolid	Pyraclostrobin	Pyridaben	Pyrimethanil
	Pyriproxyfen	Quinoxyfen	Rotenone	Sebuthylazine	Secbumeton	Siduron
Spiroxamine Sulfentrazone Tebuconazole Tebufenozide Tebufenpyrad Tebuthiuron	Simazine	Simetryn	Spinosyn A	Spinosyn D	Spirodiclofen	Spiromesifen
	Spiroxamine	Sulfentrazone	Tebuconazole	Tebufenozide	Tebufenpyrad	Tebuthiuron



Table 1. (cont.)

Teflubenzuron	Temephos	Terbumeton	Terbutryn	Terbuthylazine	Tetraconazole
Tetramethrin	Thiabendazole	Thiacloprid	Thiamethoxam	Thiazopyr	Thidiazuron
Thiobencarb	Thiofanox	Thiophanate-methyl	Triadimefon	Triadimenol	Trichlamide
Trichlorfon	Tricyclazole	Trifloxystrobin	Triflumizole	Triflumuron	Triticonazole
Uniconazole	Vamidothion	Zoxamide			

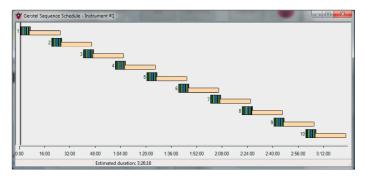


Figure 2. Example sample preparation sequence for automated DPX-QuEChERS LC-MS/MS analysis

Figures 3 and 4 show the automated sample preparation sequence used to perform DPX-QuEChERS.

Preparation of Solvent Standards and Matrix Matched Standards

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

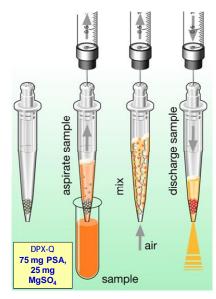


Figure 3. Example sample preparation sequence for automated DPX-QuEChERS LC-MS/MS analysis

LC-MS/MS Analysis

All analyses were performed using an Agilent 1200 Series LC system and a GERSTEL MPS MPS 2XL equipped with DPX option and a 10 μ L stainless steel loop with active wash station.

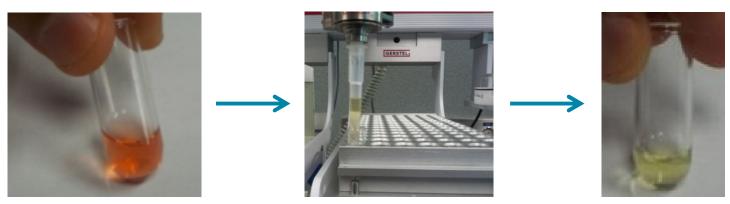


Figure 4. Schematic of the automated DPX-QuEChERS procedure, 500 µL of apple extract (left) and after DPX-QuEChERS cleanup (right)



A Phenomenex Synergi-Fusion 2.5u (50x2 mm) column was used with a gradient of water / methanol + 5 mM ammonium formate at a flow rate of 0.4 mL/min was used with a total run time of 20 min.

The AB SCIEX QTRAP[®] 4500 system was used with Turbo V[™] source and Electrospray Ionization (ESI) probe operated in both positive and negative polarity. The *Scheduled* MRM[™] algorithm was used for enhanced Signal-to-Noise (S/N), accuracy and reproducibility.⁶

Optimized MRM transitions for all pesticides were obtained through the MRM catalogue of the iMethod™ application for pesticide screening version 2.1. Two MRM transitions were monitored for each target pesticide to allow quantitation and identification using the MRM ratio. In addition, QTRAP® full scan MS/MS spectra were acquired to allow library searching in order to increase confidence in identification.

MultiQuant[™] and LibraryView[™] software was used for qualitative and quantitative data processing.

Results and Discussion

The automated DPX-QuEChERS cleanup method was performed to automatically remove matrix components from sample extracts prior to LC-MS/MS analysis. The removal of water (MgSO₄) and fatty acids (PSA) is necessary to ensure reproducible peak intensities for quantitative analysis. GCB is used to remove pigments, particularly chlorophyll and carotenoids.

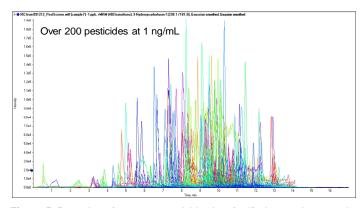


Figure 5. Detection of over 200 pesticides in a fortified cucumber sample at 1 ng/mL

Figure 5 shows a representative MRM chromatogram from a pesticide-fortified cucumber sample QuEChERS extract at 1 ng/mL. Over 200 pesticides were successfully detected in this

sample matrix using the automated DPX-QuEChERS LC-MS/MS method.

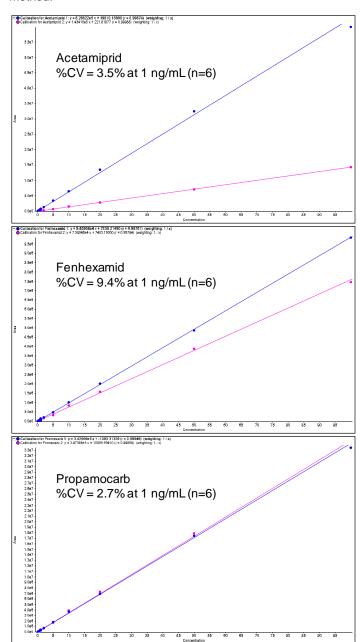


Figure 6. Representative calibration lines for Carbendazim, Propiconazole, and Thiacloprid from 0.1 to 100 ng/mL with an regression coefficient $r^2 > 0.997$ with excellent repeatability of %CV < 10%

Figure 6 shows calibration curves obtained using automated solvent standards. The resulting calibration curves were shown to be linear from at least 0.1 to 100 ng/mL with excellent repeatability for the pesticides monitored.



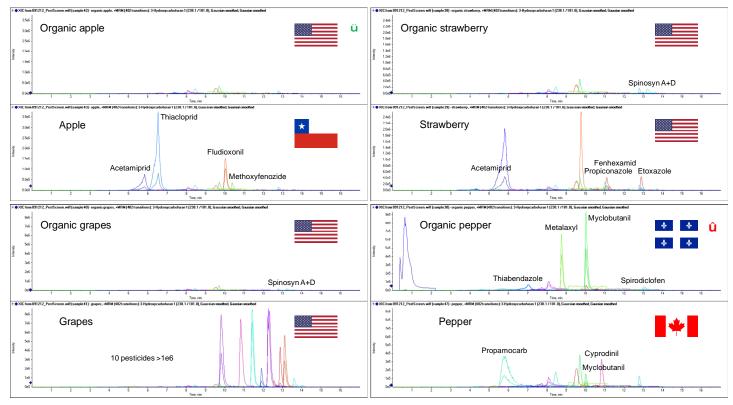


Figure 7. MRM chromatograms of pesticides identified in different food samples, including organic produce, from a local super market

The developed method was applied to the detection of pesticides in extracts of real food samples obtained from a local supermarket. QuEChERS extracts were cleaned using the DPX-QuEChERS method and diluted 10x for LC-MS/MS analysis (Figure 7).

Sample data was processed using MultiQuant™ software with the 'Multicomponent' query. Query files are customizable commands to perform custom querying of the result table. Figures 8a and b show examples of using the 'Multicomponent' query to flag pesticides present in sample extracts above a user

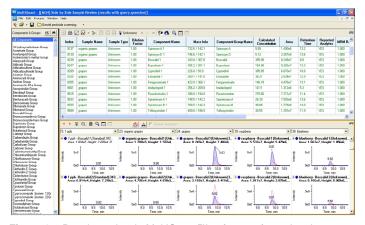


Figure 8a. Results review in MultiQuant™ software after using the 'Multicomponent' query with peak review for the pesticide Boscalid (bottom): 1 ng/mL standard, organic grapes, grapes, raspberry, and blueberry

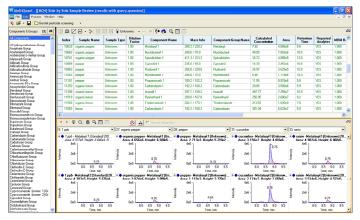


Figure 8b. Results review in MultiQuant[™] software after using the 'Multicomponent' query with peak review for the pesticide Metalaxyl (bottom): 1 ng/mL standard, organic pepper, pepper, cucumber, and raisin



specified concentration level and with positive identification using the MRM ratio.

To increase confidence in identification additional full scan MS/MS experiments were performed and spectra were searched against the iMethod™ pesticide library. Extracted spectra and library search Purity score values using the LibraryView™ software are shown in Figures 9a and b for an paprika and tarragon with low analyte concentrations.



Figure 9a. Automated library identification for Pyrimethanil butoxide detected in a paprika extract after DPX-QuEChERS LC-MS/MS

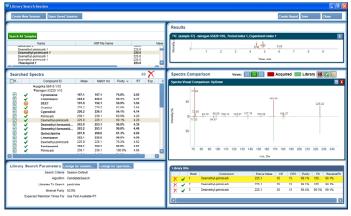


Figure 9b. Automated library identification for Desmethyl-pirimicarb detected in a paprika extract after DPX-QuEChERS LC-MS/MS

Summary

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

- The described DPX-QuEChERS LC-MS/MS workflow using the GERSTEL MPS 2XL equipped with DPX option coupled to an AB SCIEX QTRAP[®] 4500 system enabled automated cleanup and analysis of QuEChERS extracts for screening and confirmation of over 200 pesticides in a single LC-MS/MS run.
- Quantitative analysis was performed in the same run allowing for both quantitation and qualitative data to be collected simultaneously. Linear calibration curves resulting in r² values of 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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