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.

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 ($MgSO_4$) 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 screening 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. 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* MRM™ 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), $MgSO_4$ (25 mg) and GCB (12.5 mg) for dispersive SPE cleanup

QuEChERS Pretreatment

1. Pipette 1 mL of the acetonitrile extract obtained following the 1st centrifugation step of the QuEChERS sample preparation method, into an autosampler vial.
2. 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 Sequence[5]

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 sec and discharged to test tube. Repeat 3 times.
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.

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

3-Hydroxycarbofuran	Acephate	Acetamiprid	Acibenzolar-S-methyl	Alanycarb	Aldicarb
Aldicarb sulfone	Aldicarb sulfoxide	Aspon	Avermectin B1a	Avermectin B1b	Azadirachtin
Azoxystrobin	Benalaxyl	Bendiocarb	Benfuracarb	Benoxacor	Benthiavalicarb
Benzoximate	Bifenazate	Bifenthrin	Bitertanol	Boscalid	Bromuconazole
Bupirimate	Buprofezin	Butafenacil	Butocarboxim	Butoxycarboxim	Cadusafos
Carbaryl	Carbendazim	Carbetamide	Carbofuran	Carboxin	Carfentrazone-ethyl
Chlordimeform	Chlorfenvinphos	Chlorfluazuron	Chlortoluron	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	Difenoconazole	Diflubenzuron	Dimethenamid	Dimethoate	Dimethomorph
Dimoxystrobin	Diniconazole	Dinotefuran	Dioxacarb	Disulfoton	Dithiopyr
Diuron	Dodemorph	Fenpyroximate	Emamectin B1a	Emamectin B1b	Epoxiconazole
Eprinomectin B1a	EPTC	Esprocarb	Ethidimuron	Ethiofencarb	Ethion
Ethiprole	Ethirimol	Ethofumesate	Ethoprophos	Etobenzanid	Etofenprox
Etoxazole	Famoxadone	Fenamidone	Fenarimol	Fenazaquin	Fenbuconazole
Fenhexamid	Fenoxanil	Fenoxycarb	Fenpropathrin	Fenpropimorph	Fenuron
Flonicamid	Flucarbazone	Fludioxonil	Flufenacet	Flufenoxuron	Flumetsulam
Flumioxazin	Fluometuron	Fluquinconazole	Flusilazole	Fluthiacet-methyl	Flutolanil
Flutriafol	Forchlorfenuron	Formetanate	Fuberidazole	Furalaxyl	Furathiocarb
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	Metalaxyl	Metconazole
Methabenzthiazuron	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	Pirimicarb-desmethyl-formamide	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
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			

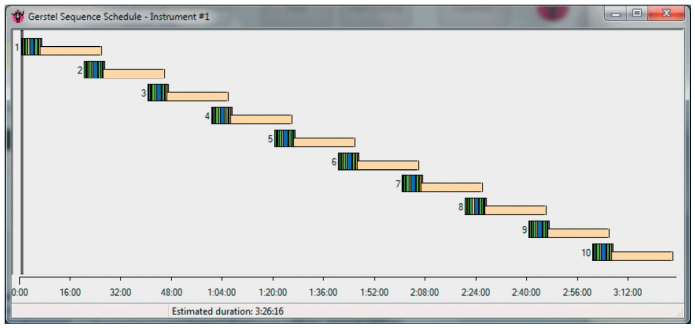

Figure 2. Example sample preparation sequence for automated DPX-QuEChERS LC-MS/MS analysis

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 µL of the respective standard stock solution into the vial and mix.

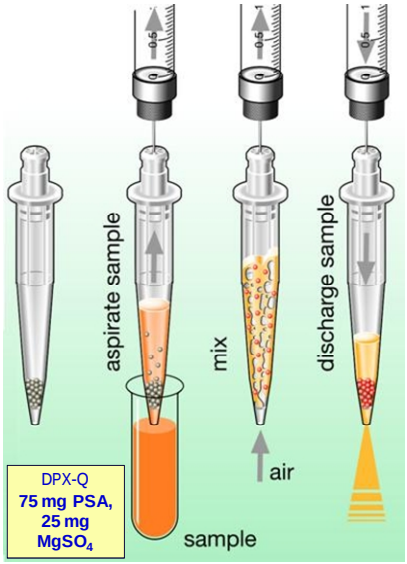

Figure 3. Example sample preparation sequence for automated DPX-QuEChERS LC-MS/MS analysis

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 µL stainless steel loop with active wash station.

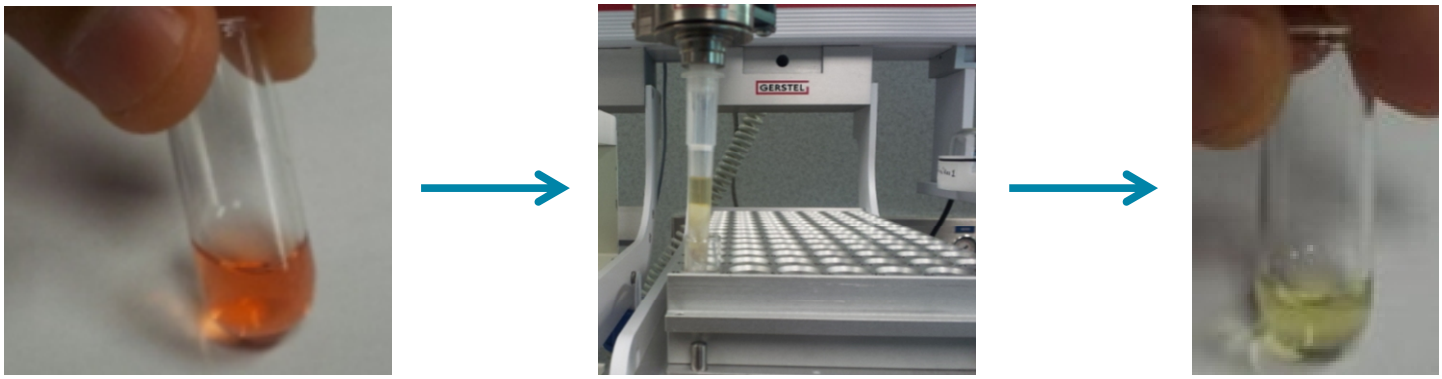

Figure 4. Schematic of the automated DPX-QuEChERS procedure, 500 µL of apple extract (left) and after DPX-QuEChERS cleanup (right)

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 ($MgSO_4$) and fatty acids (PSA) is necessary to ensure reproducible peak intensities for quantitative analysis. GCB is used to remove pigments, particularly chlorophyll and carotenoids.

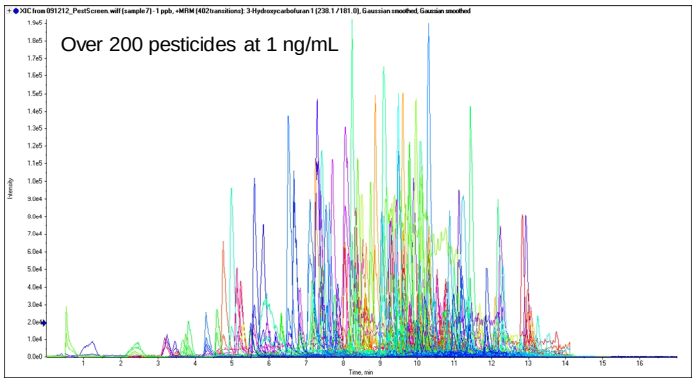

Figure 5. Detection of over 200 pesticides in a fortified cucumber sample at 1 ng/mL

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.

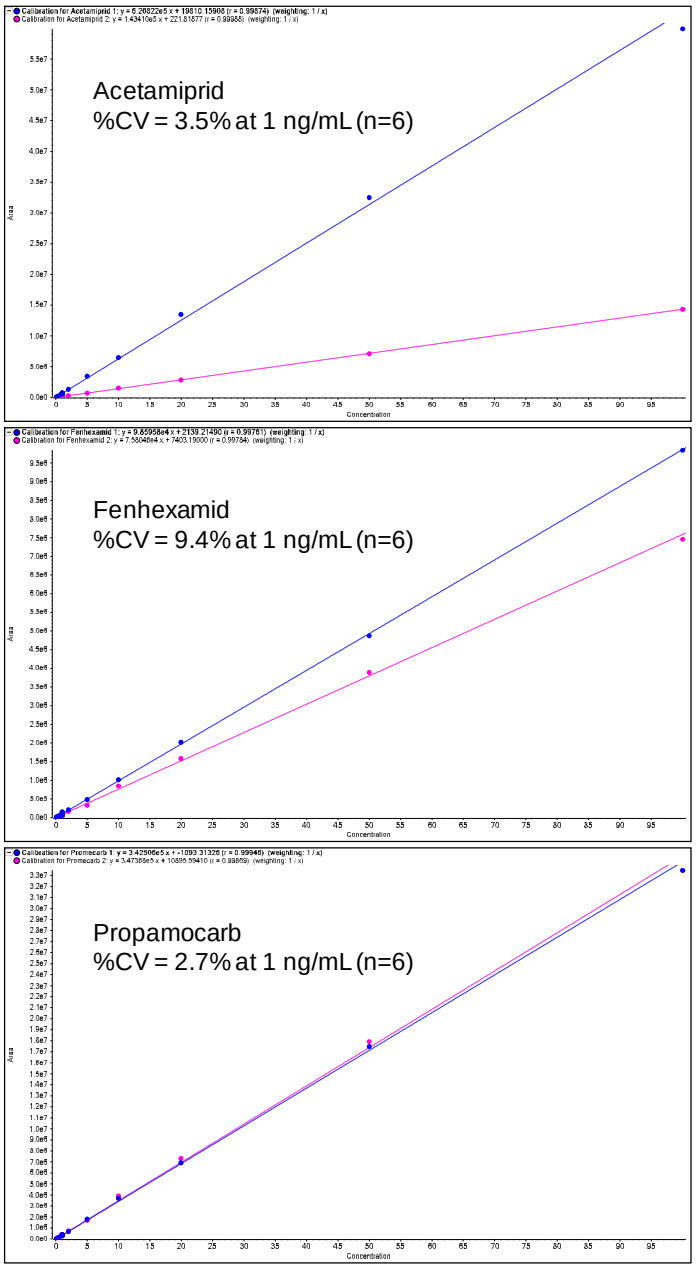

Figure 6. Representative calibration lines for Carbendazim, Propiconazole, and Thiacloprid from 0.1 to 100 ng/mL with an regression coefficient $r^2 > 0.997$ with excellent repeatability of %CV < 10%

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.

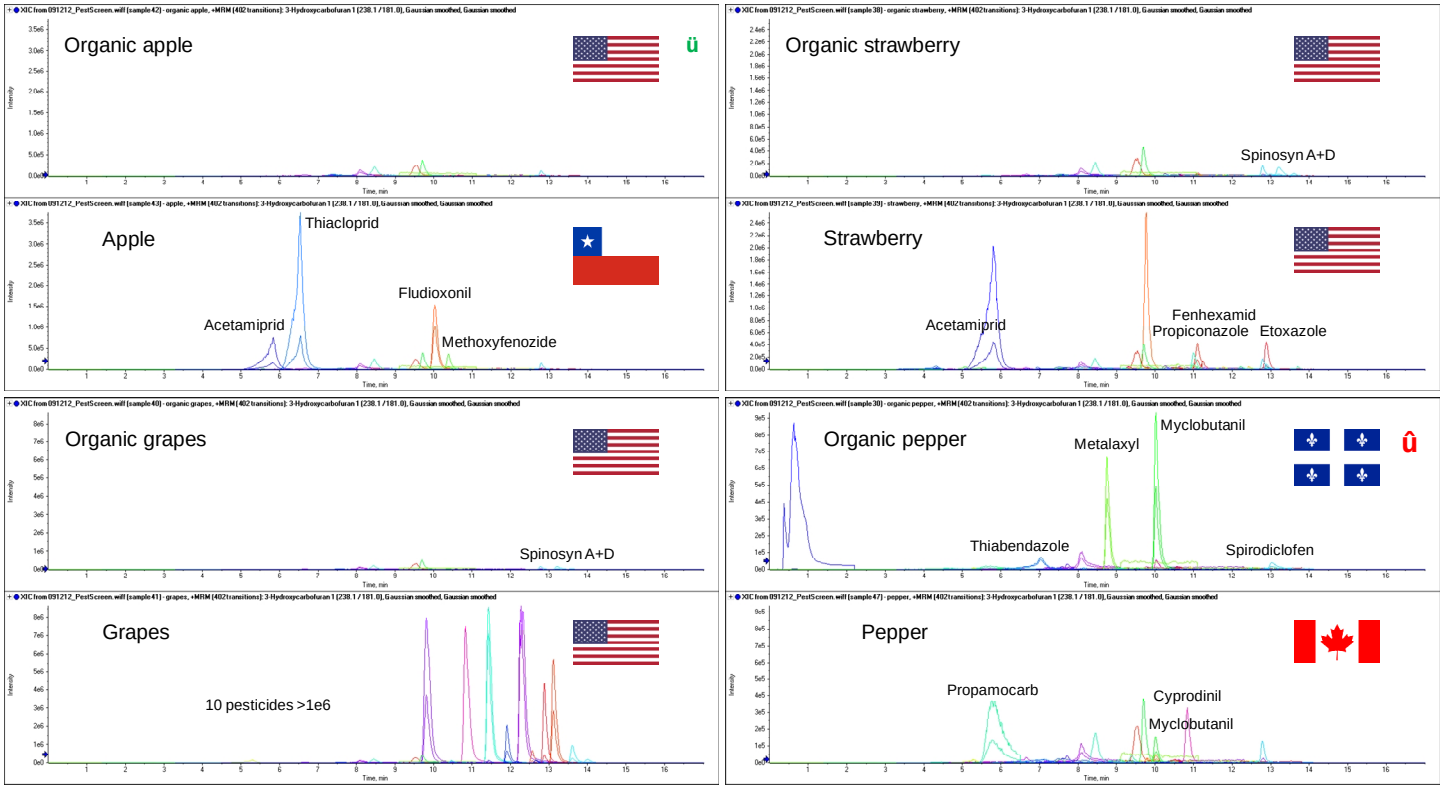

Figure 7. MRM chromatograms of pesticides identified in different food samples, including organic produce, from a local super market

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

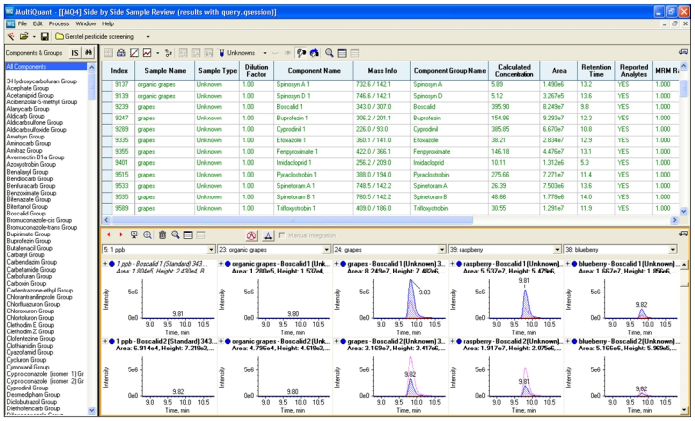

Figure 8a. Results review in MultiQuant™ software after using the 'Multicomponent' query with peak review for the pesticide Boscalid (bottom): 1 ng/mL standard, organic grapes, grapes, raspberry, and blueberry

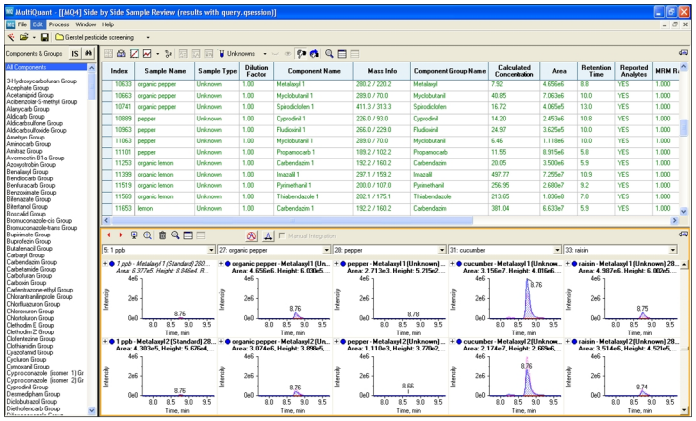

Figure 8b. Results review in MultiQuant™ software after using the 'Multicomponent' query with peak review for the pesticide Metalaxyl (bottom): 1 ng/mL standard, organic pepper, pepper, cucumber, and raisin

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 r^2 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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