Automated Clean-up of Pesticides in Apple using ISOLUTE® cSPE for QuEChERS

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QuEChERS is a sample preparation technique commonly used in food safety testing for pesticide residue analysis. This two-stage extraction and clean-up technique was developed to streamline sample processing; making it **Q**uick, **E**asy, **C**heap, **E**ffective, **R**ugged and **S**afe. ISOLUTE® cSPE for QuEChERS are pre-packed columns designed to further improve the efficiency of the QuEChERS clean-up procedure.

Traditionally, clean-up of QuEChERS extracts for pesticide residue analysis relies on dispersive solid phase extraction (dSPE). The dSPE workflow requires that the sample is shaken with the dSPE media and then centrifuged to separate the clean sample extract. Alternatively, ISOLUTE® cSPE for QuEChERS columns are packed with QuEChERS 'dispersive SPE' sorbent blends allowing convenient flow-through sample processing using column solid phase extraction (cSPE). In addition, the cSPE workflow can be automated on a Biotage® Extrahera™ sample preparation workstation leading to increased throughput, reduced errors, and improved data quality.

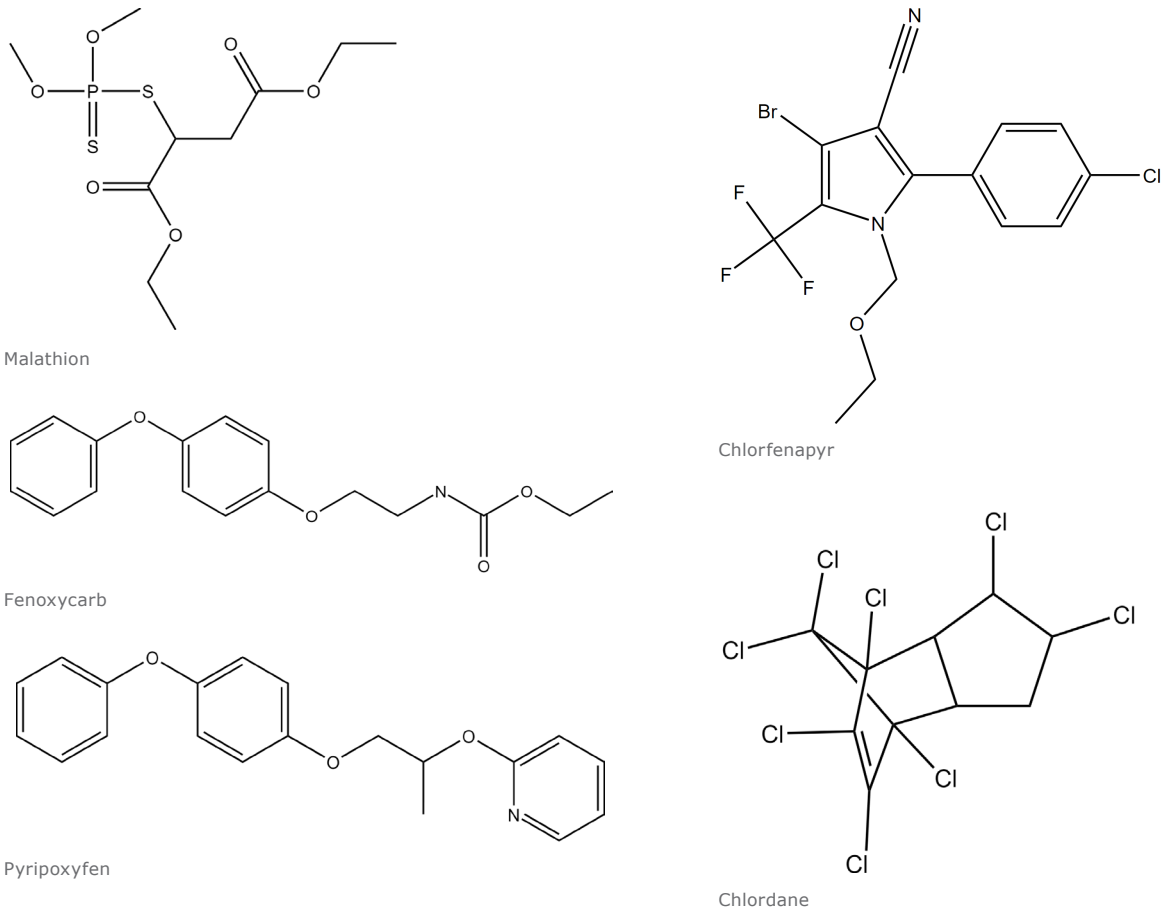

Figure 1. Example of pesticide structures.

Introduction

This application note describes the extraction of a panel of 76 commonly analyzed pesticides from apple using a two-step QuEChERS workflow. Samples are first homogenized and extracted with ISOLUTE® AOAC QuEChERS extraction salts using the Biotage® Lysera™ bead mill homogenizer. Following centrifugation, an aliquot of extract is cleaned up using ISOLUTE® cSPE for QuEChERS columns, processed using a Biotage® Extrahera™ automated sample preparation workstation. Extracts are evaporated using TurboVap™ Dual 96 and analysis is performed using GC-MS.

This application note includes optimised conditions for homogenization, extraction, extract clean-up, evaporation, and analysis.

Analytes

To demonstrate the versatility of the sample preparation approach, a panel of 76 analytes were selected to reflect a broad range of pesticide classes and chemical characteristics. The pesticides analyzed in this application note are listed below.

Carbofuran 1, Propamocarb, Mevinphos, Acephate, Captan, Carbaryl 1, Methiocarb 1, Propoxur, Ethoprophos, Dimethoate, Carbofuran 2, Atrazine, Quintozene, Pyrimethanil, Diazinon, Parathion-methyl, Carbaryl 2, Metalaxyl, Pirimiphos-methyl, Methiocarb 2, Malathion, Chlorpyrifos, Oxamyl, Cyprodinil, Thiabendazole, Fluopyram, Fipronil, Chlordane, Prallethrin 1, Prallethrin 2, Imazalil, Isoprothiolane, Buprofezin, Myclobutanil, Fludioxonil, Trifloxystrobin, Cyflufenamid, Chlorfenapyr, Penthiopyrad, Quinoxyfen, Propiconazole, DDT, Kresoxim-methyl, Fluopicolide, Tebuconazole, Propargite, Piperonyl butoxide, Iprodione, Fenoxycarb, Bifenthrin, Fluxapyroxad, Acetamiprid, Fenpropathrin, Etoxazole, Fenamidone, Cyflumetofen, Pyriproxyfen, Lambda-Cyhalothrin, Ametoctradin, Permethrin 1, Pyridaben, Permethrin 2, Coumaphos, Cyfluthrin 1, Fenbuconazole, Cyfluthrin 2, Boscalid, Cypermethrin 1, Cypermethrin 2, Cypermethrin 3, Etofenprox, Difenoconazole, Indoxacarb, Azoxystrobin, Famoxadone, Dimethomorph.

Sample preparation procedure

Format

Step 1: QuEChERS Extraction: ISOLUTE® AOAC QuEChERS extraction salts (6 g $MgSO_4$ + 1.5 g sodium acetate) p/n Q0010-15V

Step 2: cSPE Clean-up: ISOLUTE® AOAC General 200 mg/3 mL (Tabless), p/n Q0030-0020-BG

Homogenization

15 g of manually diced apple was weighed into a 50 mL centrifuge tube and frozen. 5 g of ceramic beads were added to the centrifuge tube and three samples were homogenized simultaneously using the Biotage® Lysera and 50 mL tube carriage with the following settings:

- Speed: 6 m/s
- Processing Time: 30 seconds
- Number of Cycles: 3
- Dwell Time: 10 seconds

Note: Freezing the sample aids in a more successful homogenisation and reduces the risk of increased temperatures. Many pesticides are thermally sensitive to any heat generated during sample processing which may result in loss of recoveries for these compounds. Use of the Biotage® Lysera with optional Cryo Cooling Unit can prevent increase of sample temperature, and may protect heat sensitive samples during homogenization.

Step 1: QuEChERS Extraction

15 mL of acetonitrile and the QuEChERS AOAC extraction salts (containing 6 g $MgSO_4$ and 1.5 g sodium acetate) were added to the homogenized apple (in the same 50 ml centrifuge tube) and shaken using the Lysera with the following settings:

- Speed: 2.4 m/s
- Processing Time: 15 seconds
- Number of Cycles: 1
- Dwell Time: N/A

The samples were then centrifuged for 5 minutes at 5000 RCF. To evaluate the performance of cSPE for QuEChERS clean-up, the supernatant was spiked at a concentration of 96 ppb with a mixture of 76 pesticides (see Additional Information). No internal standards were used.

Step 2: Automated Clean-up Procedure

An excess of 1 mL supernatant from step 1 was transferred into 12 x 75 mm test tubes and placed into the upper processing shelf of the Biotage® Extrahera™ Classic, (position 4, sample rack 12 x 75 mm, 24 positions). The ISOLUTE® AOAC General cSPE columns (p/n Q0030-0020-BG) were also placed onto upper processing shelf of the Extrahera™ Classic (position 3, column rack 24 x 3 mL), while collection vials were placed in the lower collection carousel (position B, collection rack 12 x 75 mm equipped with rack insert). Using the Extrahera™, 1 mL of QuEChERS extract was loaded onto each column. The columns were processed automatically by applying pressure (0.6 bar for 210 seconds, followed by 5 bar for 15 seconds and a 15 second plate dry). Extracts were collected directly into high recovery

microsampling GC vials. The GC vials were then transfered directly to the evaporation system.

For options to increase batch size and throughput with minimal sample transfer steps, see Appendix 2.

Post Extraction

Samples were evaporated using the TurboVap® 96 Dual with the following parameters:

- 24 Configuration, Single Mode
- Gas Temp: 40 °C
- Plate Temp: 40 °C
- Gas Flow: 25 L/min
- Plate Height: 56 mm
- Time: 30 minutes

Extracts were reconstituted with 30 µL of toluene (added directly to the GC vial) and vortexed prior to analysis.

Analytical Conditions

Table 1. GC conditions

Instrument	Agilent 7890A GC
Column	Restek Rtx-5MS 30 m x 0.25 mm ID x 0.25 µm
Carrier gas	Helium 1.2 mL/min
Inlet	Splitless, 280°C, 50 mL/min at 1 min
Injection volume	1.3 µL
Wash solvent	Methanol
Oven settings	Available on request
Transfer line	300°C

Table 2. MS Conditions

Instrument	Agilent 5975C MSD
Source temp	230°C
Quadrupole temp	150°C
MSD mode	SIM

The monitored ions for each compound are listed in table 3 below. Ions were acquired using Selected Ion Monitoring (SIM) mode.

Table 3. Ions monitored for each analyte

Compound	Quantifier Ion	1st Qualifier Ion	2nd Qualifier Ion
Carbofuran 1	164	149	122
Propamocarb	58	42	44
Mevinphos	127	109	192
Acephate	136	42	94
Captan	79	80	77
Carbaryl 1	144	115	116
Methiocarb 1	168	153	109
Propoxur	110	152	27
Ethoprophos	158	43	97
Dimethoate	87	93	125
Carbofuran 2	164	149	122
Atrazine	200	215	58
Quintozene	237	249	295
Pyrimethanil	198	77	199
Diazinon	137	179	152
Parathion-methyl	263	125	109
Carbaryl 2	144	115	116
Metalaxyl	45	132	206
Pirimiphos-methyl	290	276	305
Methiocarb 2	168	153	109
Malathion	173	125	93
Chlorpyrifos	197	97	199
Oxamyl	72	42	69
Cyprodinil	224	77	225
Thiabendazole	201	174	63
Fluopyram	173	145	223
Fipronil	367	369	213
Chlordane	373	375	377
Prallethrin 1	123	81	77
Prallethrin 2	123	81	77
Imazalil	215	41	173
Isoprothiolane	118	162	189
Buprofezin	105	106	172
Myclobutanil	179	152	181
Fludioxonil	248	127	154
Trifloxystrobin	116	131	59
Cyflufenamid	91	55	118
Chlorfenapyr	59	60	247
Penthiopyrad	177	302	152
Quinoxyfen	237	272	307
Propiconazole	173	69	259
DDT	235	237	176
Kresoxim-methyl	116	131	206
Fluopicolide	209	210	211
Tebuconazole	125	250	70
Propargite	135	81	57
Piperonyl butoxide	176	149	177
Iprodione	56	316	43
Fenoxycarb	116	88	255
Bifenthrin	181	165	
Fluxapyroxad	159	160	
Acetamiprid	56	152	126
Fenpropathrin	97	55	181
Etoxazole	204	300	141
Fenamidone	238	237	239
Cyflumetofen	173	145	
Pyriproxyfen	136	78	77
Lambda-Cyhalothrin	181	197	208
Ametoctradin	176	190	204
Permethrin 1	183	41	27
Pyridaben	147	117	148

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Permethrin 2	183	41	27
Coumaphos	362	97	109
Cyfluthrin 1	163	165	206
Fenbuconazole	129	198	125
Cyfluthrin 2	163	165	206
Boscalid	140	112	142
Cypermethrin 1	163	181	165
Cypermethrin 2	163	181	165
Cypermethrin 3	163	181	165
Etofenprox	163	135	107
Difenoconazole	265	323	267
Indoxacarb	59		
Azoxystrobin	344	388	345
Famoxadone	330	329	331
Dimethomorph	301	165	303

Results

Using the automated cSPE procedure described in this application note, a panel of 76 pesticides spiked into apple extracts were cleaned up using cSPE. 72 pesticides demonstrated recovery between the acceptable limits of 70-130%, with the majority between 85 and 90%. Figure 2 shows recoveries obtained from apple extract spiked prior to cSPE clean-up at a concentration equivalent to 96 ppb in raw apple (n=4). Excellent reproducibility was achieved, 74 analytes demonstrating relative standard deviation (RSD) lower than 20% (n=4) with most being lower than 5%. Figure 3 shows %RSD obtained from apple extract spiked prior to cSPE clean-up at a concentration equivalent to 96 ppb in raw apple. Automation of the method ensures variation is kept to a minimum.

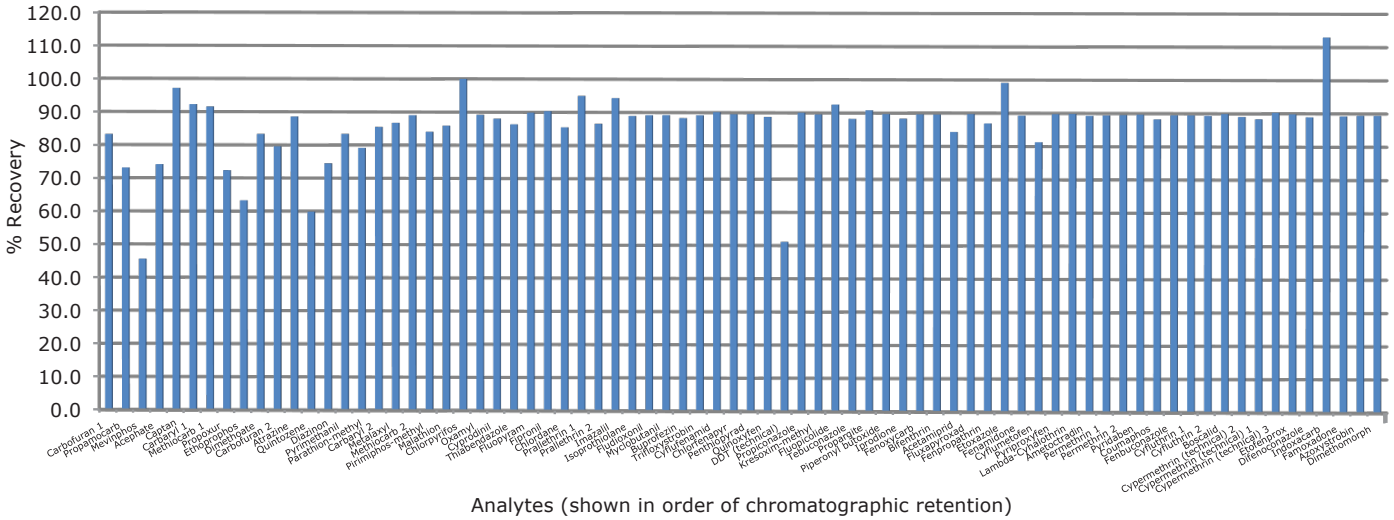

Figure 2. Average analyte % recoveries obtained from apple extract spiked at a concentration equivalent to 96 ppb in raw apple (n=4).

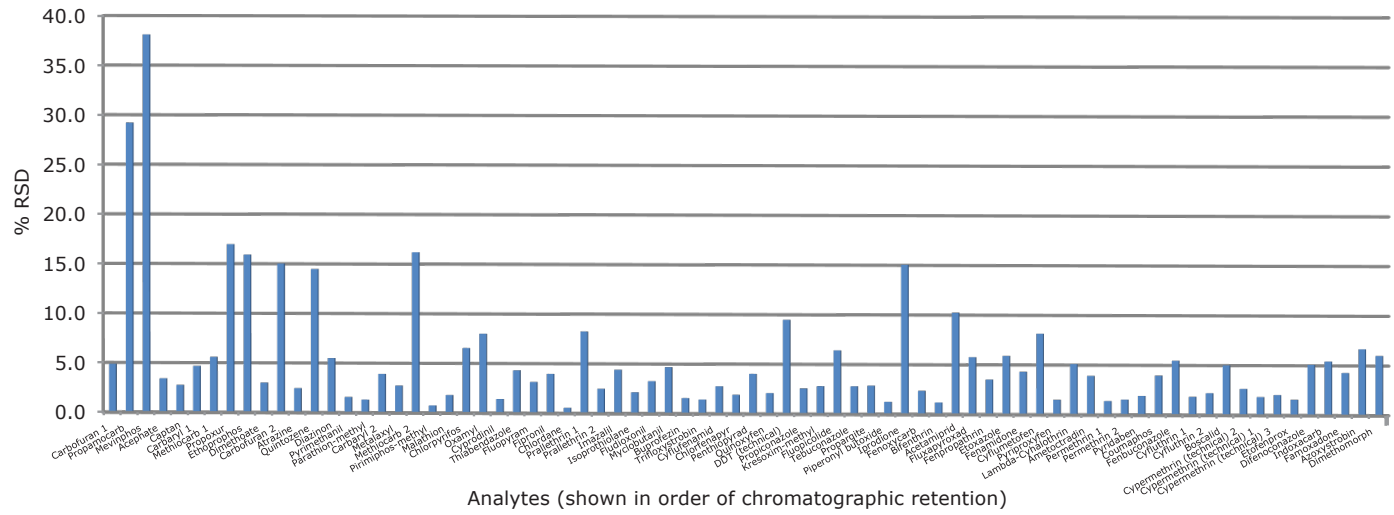

Figure 3. % RSDs obtained from apple extract spiked at a concentration equivalent to 96 ppb in raw apple (n=4).

Linearity and LOQ

Calibration curves were produced for apple extract spiked with pesticides, either within the concentration range 0.8 ppb – 160 ppb or 4 ppb to 800 ppb (equivalent in raw apple) depending on analyte limit of quantitation (LOQ).

To ensure that calibration samples reflect real samples as closely as possible, samples were prepared using pooled apple extract (from QuEChERS step 1). The highest-level spiked sample was prepared at a concentration of 800 ppb. The remaining spiked samples were prepared by a serial dilution of the highest-level spiked sample using additional blank apple extract. 1 mL of each concentration of calibration samples were then cleaned-up using cSPE columns.

LOQs were estimated at the lowest concentration of which the analyte gave a signal to noise ratio of 10:1. LOQs of analytes varied across the large panel with most being between 0.8 and 4 ppb. Good linearity was observed for most analytes, typically giving r^2 values greater than 0.99. Table 4 below shows the r^2 and LOQ values of all analytes.

Table 4. Linearity and LOQ

Compound	r^2	LOQ (ppb)
Carbofuran 1	0.999	0.8
Propamocarb	0.999	0.8
Mevinphos	0.992	4
Acephate	0.999	4
Captan	0.995	4
Carbaryl 1	0.999	0.8
Methiocarb 1	0.999	0.8
Propoxur	0.999	0.8
Ethoprophos	0.999	0.8
Dimethoate	0.990	8*
Carbofuran 1	0.999	0.8
Atrazine	0.999	0.8
Quintozene	0.998	0.8
Diazinon	0.999	4
Pyrimethanil	0.999	0.8
Parathion-methyl	0.998	4
Metalaxyl	0.999	1.6
Carbaryl 2	0.992	4
Methiocarb 2	0.991	4
Pirimiphos-methyl	0.999	0.8
Malathion	0.999	0.8
Chlorpyrifos	0.999	0.8
Oxamyl	0.999	40*
Cyprodinil	0.999	0.8
Thiabendazole	0.998	40*
Fluopyram	0.999	0.8
Fipronil	0.999	0.8
Chlordane	0.999	0.8
Prallethrin 1	0.999	4
Prallethrin 2	0.998	4
Imazalil	0.999	0.8
Isoprothiolane	0.999	0.8
Fludioxonil	0.994	4
Buprofezin	0.999	0.8
Myclobutanil	0.999	0.8
Trifloxystrobin	0.999	0.8
Cyflufenamid	0.999	0.8
Chlorfenapyr	0.999	0.8
Penthiopyrad	0.999	0.8
Quinoxyfen	0.999	0.8
Propiconazole	0.998	4
DDT	0.998	0.8
Kresoxim-methyl	0.999	0.8
Fluopicolide	0.999	0.8
Tebuconazole	0.998	4
Propargite	0.991	4
Piperonyl butoxide	0.999	0.8
Iprodione	0.998	4
Fenoxycarb	0.998	4
Acetamiprid	0.997	4
Bifenthrin	0.999	0.8
Fluxapyroxad	0.999	0.8
Fenpropathrin	0.999	0.8
Etoxazole	0.999	0.8
Fenamidone	0.999	0.8
Cyflumetofen	0.999	0.8
Pyriproxyfen	0.999	0.8
Lambda-cyhalothrin	0.999	1.6
Ametoctradin	0.999	0.8
Permethrin 1	0.999	0.8
Pyridaben	0.999	0.8
Permethrin 2	0.999	0.8
Coumaphos	0.999	0.8
Fenbuconazole	0.999	0.8
Cyfluthrin 1	0.999	8*
Cyfluthrin 2	0.999	8*
Boscalid	0.999	0.8
Cypermethrin 1	0.999	8*
Cypermethrin 2	0.999	4
Cypermethrin 3	0.999	8*
Etofenprox	0.999	0.8
Difenoconazole	0.992	4
Indoxacarb	0.995	8*
Azoxystrobin	0.996	4
Famoxadone	0.987	4
Dimethomorph	0.999	1.6

*analytes have higher LOQs than most of the panel so have less than 6 calibration points

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Calibration Curves

Calibration curves for selected pesticides are show in figures 4-7 below.

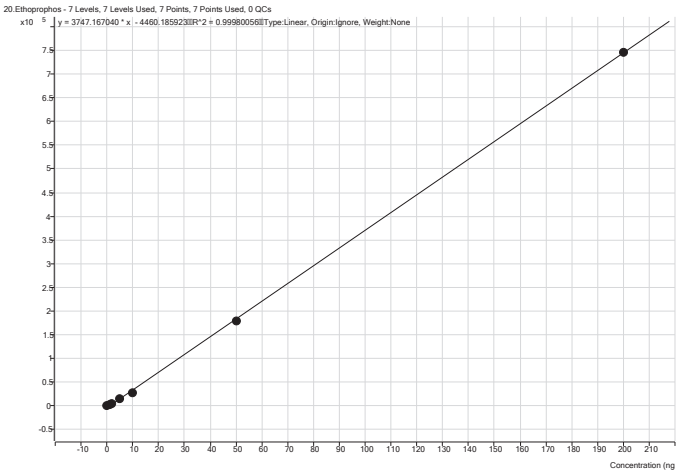

Figure 4. Ethoprophos, calibration range 0.8 – 160 ppb

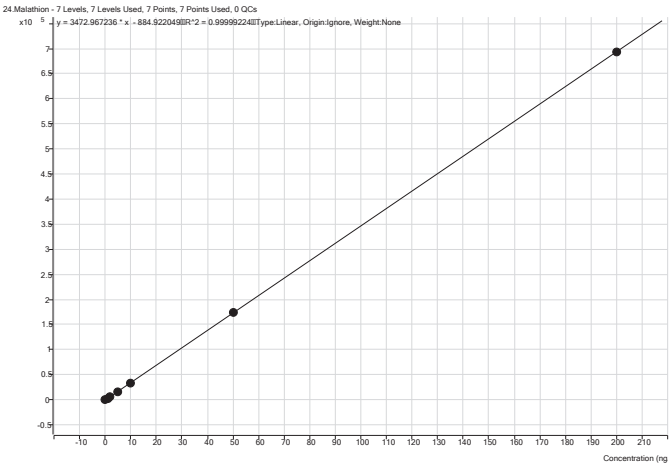

Figure 6. Malathion, calibration range 0.8 – 160 ppb

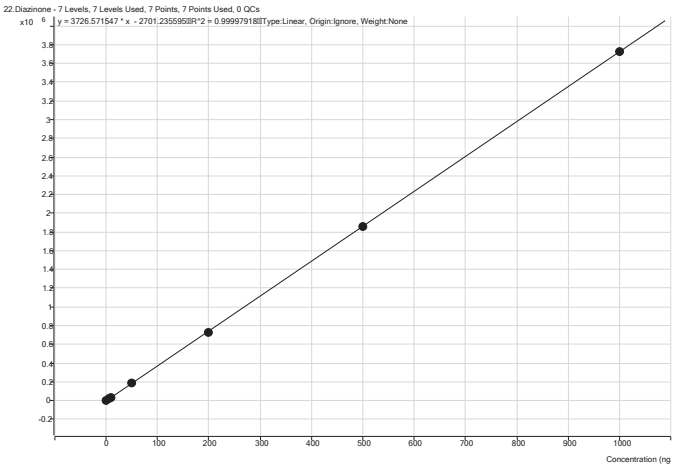

Figure 5. Diazinon, calibration range 4 – 800 ppb

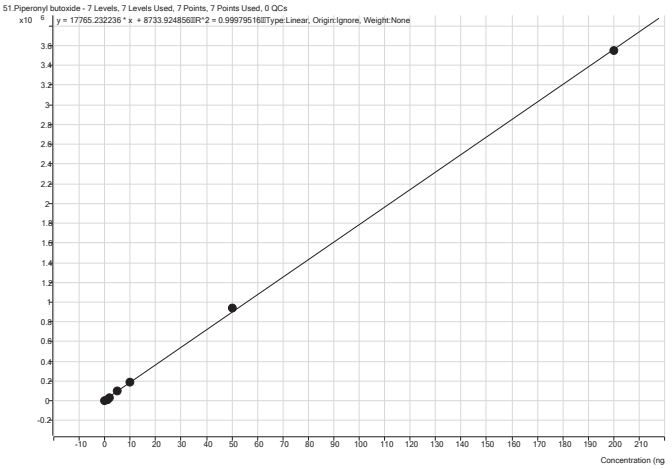

Figure 7. Piperonyl butoxide, calibration range 0.8 – 160 ppb

Discussion and Conclusion

The use of ISOLUTE® cSPE for QuEChERS columns provides a simple, robust flow-through clean-up of QuEChERS extracts from apple samples for pesticide analysis. The columns deliver high recoveries, low RSDs and exceptionally clean extracts.

Excellent linearity was achieved across a wide calibration range for all analytes, demonstrating that the flow-through cSPE approach provides consistent analyte recovery and matrix removal at all concentration levels, with LOQ typically < 1 ppb.

When automated using a Biotage® Extrahera™ workstation, a batch of 24 samples can be processed in 10.3 minutes (15.1 minutes for 48 samples). ISOLUTE® cSPE for QuEChERS columns provide a simplified extract clean-up workflow when compared to the dSPE equivalent (see figure 8 , page 7). This results in fewer manual transfer steps and higher throughput. In comparison, the equivalent dSPE clean-up procedure takes 23.5 minutes for a batch of 24 samples (47 minutes for 48 samples).

Traditionally QuEChERS using dSPE for clean-up does not utilize a concentration step. However, standard QuEChERS methods (AOAC, EN and mini-multiresidue) allow for an evaporative concentration step where large volume injection GC-MS analysis is not used, or where solvent exchange from acetonitrile is beneficial. Typically, a concentration factor of x 4 is utilized.

In order to improve our working sample concentration range we incorporated a post clean-up evaporation and reconstitution step. After cSPE clean-up the extract was evaporated to dryness under highly controlled conditions using the TurboVap® 96 Dual, and reconstituted in a total volume of 30 µL of toluene, from which 1.3 µL was injected. No significant evaporative

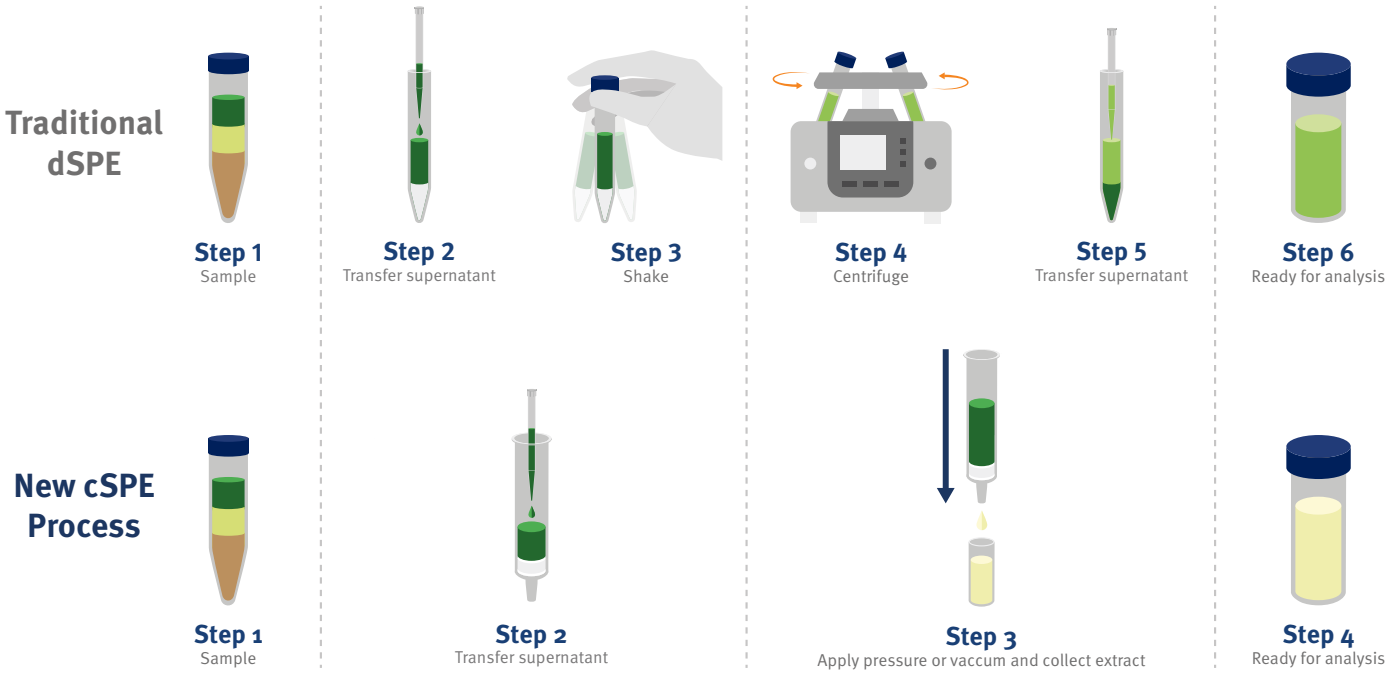

Figure 8. Comparison of dSPE vs cSPE clean-up workflow

losses were observed. This represents a 30 x increase in analyte concentration injected into the GC-MS system, and allows for a more appropriate injection solvent for GC-MS analysis. The higher concentration factor allows for improved sensitivity (lower analyte LOQ) using the single quadrupole GC-MS system. It is expected that LOQs could be further improved using a higher sensitivity / more selective GC-MS/MS system.

The improved cleanliness of the extract from the flow through cSPE process (more efficient matrix removal) compared with dSPE means that the more concentrated sample extract (x 30 compared with x 4) can be injected without compromising the GC system or analyte separation.

Chemicals and Reagents

- All pesticide stock solutions were purchased from LGC Ltd. (Middlesex, UK) and stored at -20°C.
- California Pesticide Class 1, Class 2A, Class 2B mixes were purchased at 100 µg/mL. All other compounds were purchased as individual stocks at 1 mg/mL and diluted to 100 µg/mL with acetonitrile.
- A spiking solution was prepared weekly in acetonitrile at a concentration of 10 µg/mL and stored at -20°C.
- Acetonitrile was purchased from Rathburn Chemicals Ltd. (Walkerburn, UK).

Additional Information

Preparation of pooled sample for cSPE evaluation

The focus of this application note is evaluation of the automated clean-up step using cSPE columns. Therefore, to provide homogenous samples for comparison purposes, supernatants prepared in step 1 were pooled and spiked appropriately with pesticide standards. 1 mL aliquots of the pooled supernatants were used for evaluation of the cSPE clean-up step.

For recovery testing, the pooled sample was spiked at a concentration of 120 ng/mL which equates to 96 ppb in raw apple matrix. This is calculated with the assumption that 15 g of apple matrix with QuEChERS salts results in 12 mL of supernatant for cSPE clean up. Supernatant volumes will be dependent on matrix type and initial QuEChERS salt used.

For determination of linearity and LOQ, the highest-level spiked sample was prepared at a concentration of 800 ppb. The remaining spiked samples were prepared by a serial dilution of the highest-level spiked sample with the pooled blank apple extract.

Use of internal standards

No internal standardisation was used in this application note, however if used, we would recommend that appropriate internal standards were added to the homogenised matrix along with acetonitrile and QuEChERS salts during step 1, prior to mixing and subsequent centrifugation.

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Evaporation and reconstitution

The use of the tapered bottom microsampling vials (Agilent p/n 5184-3550) for extract collection, evaporation and reconstitution reduces variability in this step. Pre- and post-evaporation samples in tapered bottom vials are illustrated in figure 9 below. Pre-evaporation sample (left) consists of 1 mL of apple extract in acetonitrile after passing through the cSPE column (Q0030-0020-BG). Post-evaporation sample (right) is the same extract after evaporation to dryness and reconstitution in 30 µL toluene (30 x concentration factor). Direct to GC vial elution eliminates the need for sample transfer for evaporation, and further reduces the risk of analyte loss.

This approach allows us to enhance the sensitivity of our GC-MS single quad, with 30 x higher on column concentration range vs raw sample range.

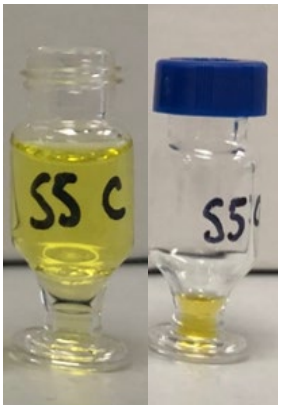


Figure 9. Pre (left)- and post (right)-evaporation and reconstitution samples in tapered bottom vials.

Ordering Information

Biotage® Consumables Ordering Information

Part Number	Description	Pack Size
Homogenization and QuEChERS extraction used in this application note		
Q0010-15V	Extraction salts: ISOLUTE® QuEChERS AOAC 15 g/15 mL Extraction Tube	25
19-6650	Bulk 50 mL Tubes with Leak Proof Screw Caps	100
19-646	Bulk 2.8 mm Ceramic Beads, 325 g	1
QuEChERS Clean-up used in this application note		
Q0030-0020-BG	cSPE: ISOLUTE® AOAC General 200 mg/3 mL (Tabless)	50
414141	1000 µL Clear Tips	960

Biotage® Equipment & Accessories Ordering Information

Part Number	Description	Pack Size
Homogenization and QuEChERS extraction		
19-060	Biotage® Lysera	1
19-345-050	50 mL Tube Carriage Kit	1
QuEChERS Clean-up		
414001	Biotage® Extrahera™ Classic	1
417002	Biotage® Extrahera™ HV-5000	1
414174SP	Column Rack 24 x 3 mL	1
415555SP	Sample/Collection Rack 12 x 75 mm, 48 Positions	1
414511SP	Collection Rack 12 x 75 mm, 24 Positions	1
414578SP	Inserts For 12 x 32 mm Vials For Collection Rack 12 x 75 mm 24 positions	24
414256SP	Sample Rack 12 x 75 mm, 24 Positions	1
Evaporation		
418000	TurboVap® 96 Dual	1
418319SP	Rack 12 x 32 mm, 24 Positions	1

ISOLUTE® cSPE for QuEChERS Range

Selection of the appropriate product depends on regulation (AOAC or EN) and matrix type.

Part Number	Description	Pack Size
QuEChERS extraction		
Q0010-15V	Extraction salts: ISOLUTE® QuEChERS AOAC 15 g/15 mL Extraction Tube	25
Q0020-15V	Extraction salts: ISOLUTE® QuEChERS EN 10 g/15 mL Extraction Tube	25
QuEChERS Clean-up		
Q0030-0020-BG	ISOLUTE® AOAC General 200 mg/3 mL (Tabless)	50
Q0035-0020-BG	ISOLUTE® EN General 200 mg/3 mL (Tabless)	50
Q0050-0035-BG	ISOLUTE® AOAC Waxed 350 mg/3 mL (Tabless)	50
Q0060-0035-BG	ISOLUTE® EN Waxed 350 mg/3 mL (Tabless)	50
Q0070-0015-BG	ISOLUTE® AOAC Pigment 150 mg/ 3 mL (Tabless)	50
Q0080-0030-BG	ISOLUTE® EN Pigment 300 mg/3 mL (Tabless)	50
Q0090-0050-BG	ISOLUTE® EN High Pigment 500 mg/3 mL (Tabless)	50

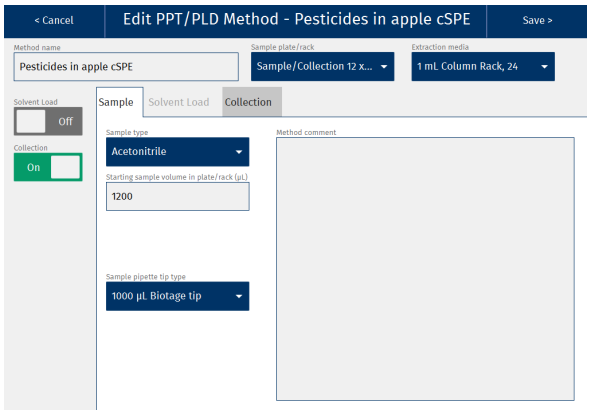
Appendix 1

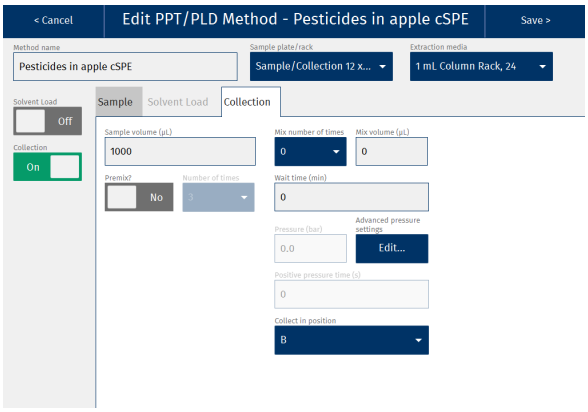
Biotage® Extrahera™ Classic method

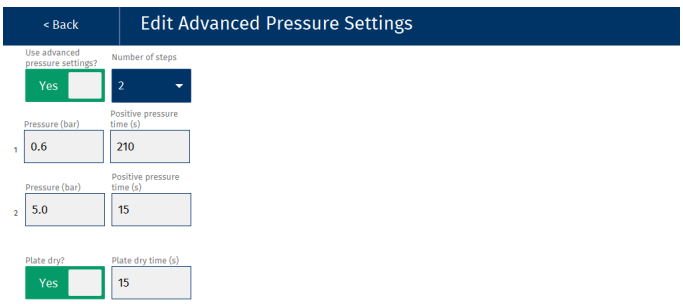
The cSPE clean-up method described in this application note was automated on the Biotage® Extrahera™ Classic. This appendix contains the software settings required to configure Extrahera™ Classic to run this method for a 1 mL sample aliquot.

Sample plate/rack	12 x 75 mm, 24
Extraction media	1 mL Column Rack, 24 (ISOLUTE® AOAC General)
Sample Load	Off
Collection	On

Screenshot







Settings

Sample	
Sample type	Acetonitrile
Start vol, µL	1200
Sample tip	1000 µL Biotage Tip

Collection	
Sample vol, µL	1000
Premix	No
Mix number of times	0
Mix volume, µL	0
Wait time, min	0
Collect in position	B

Advanced Pressure Settings	
Use advanced pressure settings	Yes
Number of steps	2
1. Pressure (bar)	0.6
1. Positive pressure time (s)	210
2. Pressure (bar)	5.0
2. Positive pressure time (s)	15
Plate dry	Yes
Plate dry time (s)	15

Appendix 2

Options for streamlining the Automated QuEChERS Process

Homogenization & Extraction

As part of the QuEChERS workflow, use of the Biotage® Lysera for initial sample pre-treatment and extraction (QuEChERS step 1) reduces the need for manual steps, saving time and reducing the possibility of manual handling errors. The need to clean a blending device in-between each sample is eliminated and an even homogenisation between each sample is ensured.

In this application note, ISOLUTE® AOAC QuEChERS extraction salts (p/n Q0010-15V) optimized for extraction of 15 g of sample matrix were used to prepare samples in 50 mL tubes, for the initial extraction step.

In place of manually shaking samples by hand, 3 x 50 mL tubes can be shaken simultaneously in 15 seconds using the Biotage™ Lysera.

Clean-up

Using the Biotage® Extrahera™ HV-5000 platform for automated clean-up (instead of the Extrahera™ Classic platform used for this application note), 50 mL extraction tubes from the initial homogenization and extraction step can be transferred directly to the Extrahera™ HV-5000 after centrifugation, eliminating a manual sample transfer step. The supernatant can then be aspirated directly from the 50 mL centrifuge tube to the cSPE column utilizing the Extrahera's 'Smart Pipetting' function, ensuring no particulate is transferred. A maximum of 12 samples can be processed using 50 mL tubes.

Increasing batch size and sample throughput with minimal manual sample transfer

As an alternative to 50 mL tubes for homogenization and extraction, Biotage® Lysera can be used to homogenize samples in smaller tubes (30 mL, 7 mL) simultaneously processing batch sizes of up to 12 samples.

Reduction of the starting mass of sample, for example from 15 g to 5 g or 2 g, extracted with an appropriately reduced wight of extraction salt, and utilizing smaller extraction tubes can lead to increased batch size and further reduction in overall processing time.

After centrifuging these can be transferred directly to the Extrahera™ HV-5000 for cSPE clean-up, with no additional manual sample transfer steps. These options are summarized in the table below.

Options for increasing batch size with minimal manual sample transfer

Matrix sample size	Extraction tube volume	Biotage® Lysera capacity	Maximum batch size using Biotage® Extrahera™ 5000
10-15 g	50 mL	3 tubes	12
5-10 g	30 mL	6 tubes	12
1.5-2.5 g	7 mL	12 tubes	24

Alternatively, a manual transfer of the supernatant into test tubes can be included after QuEChERS extraction, which can then be processed in a batch size of up to 48.

Manual Processing Options

Clean-up of pre-extracted samples using ISOLUTE® cSPE for QuEChERS columns can be performed manually using the Biotage® Pressure+ 48 Positive Pressure Manifold or Biotage® VacMaster™-20. Processing parameters are available on request.

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