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Determination of Pesticides in Fruits and Vegetables Automated with FREESTYLE QuEChERS and LC-MS/MS

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Content

1. Introduction

QuEChERS has become the most important analytical method in routine pesticide labs for food and feed samples. In particular, nearly all types of fruits and vegetables are analysed with this methodology worldwide for several hundred pesticides applied in agriculture.

The original QuEChERS set-up in brief consists of two main steps, the extraction and the clean-up step. Both of them are typically manually performed and use a dispersive approach, where two different buffer/salt and clean-up mixes are added to matrix solutions, respectively, with subsequent vortexing and centrifugation steps.

The aim of this application note is to show an automated approach for the second, the clean-up step in a non-dispersive way. Automation itself is a warrantor for highly precise processing with reduced deviation of analytical results even in sequences with a high sample number.
Furthermore, using a non-dispersive approach, chromatography in general is better and unwanted matrix compounds or particles are retained on the top of the cartridge, thus leading to cleaner extracts with reduced matrix suppression in the LC-MS/MS measurement.

The FREESTYLE QuEChERS system is running in 24/7 operation with a loading capacity of up to 120 samples. It processes the clean-up step on a specific cartridge and automatically injects via a HPLC Direct Injection module into the measuring system.

As some selected matrix examples, the test were performed with apple, bell pepper, and lettuce and the results for a pesticide mix with 219 compounds with LC-MS/MS measurement are shown.

2. Method Development

2.1 Reagents and Materials

- Acetonitrile (pesticide grade)
- Citrate buffer
- Pesticide I, Classic SPE QuEChERS Column (LCTech GmbH, P/N 16301)
- Methanol with 5 % ammonia

2.2 Sample Preparation

Homogenise 20 g of fruit or vegetable in a blender and weigh out 10 g into a 50 mL Falcon tube. Add 100 µL of internal standard, 10 mL of acetonitrile and vortex for 1 min. Add QuEChERS Mix I, shake vigorously and put on ice. Afterwards centrifuge for 15 min at 4,500 rpm. Pipette 3 mL of the supernatant and fill into a 4 mL vial with septum and sealing cap and put it into the FREESTYLE QuEChERS system.

2.3 Instrumentation

2.3.1 FREESTYLE QuEChERS System

The FREESTYLE QuEChERS system consists of the xyz-robotic platform FREESTYLE BASIC and the SPE module. Additionally a HPLC Direct Injection module may be directly connected with any brand of HPLC MS/MS system.

In the following the required items for a processing of 60 samples are listed together with their corresponding part numbers.

1.	FREESTYLE BASIC, 6 solvents	P/N	12663-12
2.	FREESTYLE SPE module	P/N	12668
3.	QuEChERS-set (hardware and software)	P/N	16269
4.	Special Rack for up to 60 Miniaturized SPE Columns	P/N	15658
5.	5 x Reusable needles, stainless steel	P/N	13382
6.	2 x frame 100 mm	P/N	11915
7.	Tray, 4 mL, 60 positions	P/N	11926
8.	4 mL screw-thread vials	P/N	V0004
9.	Screw cap with hole	P/N	V0004-SL
10.	Seal G13 for 4 mL vial	P/N	V0004-D

2.5 Analytical Set-up

2.5.1 HPLC System and Settings

- Agilent Infinity II 1290 (Modules G7116B, G7167B, G7120A)
- API 5500 Triple Quad, Turbo Spray (ESI)
- Scan type: SMRM
- MRM detection window: 60 sec
- Polarity: positive
- Curtain gas: 35 psig
- Ion spray voltage: 5000 V
- Temperature: 450 °C
- Gas 1 (nebulizer): 45 psig
- Gas 2 (turbo gas): 45 psig
- CAD gas: medium

2.5.2 Chromatographic Conditions

- Column: EC 50/4.6 NUCLEOSHELL® Bluebird RP 18, 2.7 µm (REF 763432.46)
- Eluent A: 0.1% Formic acid in water
- Eluent B: 0.1% Formic acid in methanol
- Gradient: in 5 min from 5 % to 100 % B, hold for 1.0 min, in 0.1 min to 5 % B, hold 5 % B for 3.9 min
- Flow rate: 0.7 mL/min
- Temperature: 30 °C
- Injection volume: 20 µL (Concentration: 2 ng/mL in water/acetonitrile (4 + 1, v, v))

2.5.3 Software Protocol

In the following the FREESTYLE method protocol for the SPE is shown.

FREESTYLE™ AUTOMATED SAMPLE PREPARATION

Name: QuEChERS.QRS

Column:	QuEChERS.mini	Extension cannula:	yes	MINI
Conditioning 1:	ON			
Volume:	3 ml	Dispensing Speed:	10 ml / min	
Suction Speed:	20 ml / min	Waiting time:	0 min	Port : 9 acetonitrile
Conditioning 2:	OFF			
Conditioning 3:	OFF			
Load :	ON			
Volume:	1 ml	Dispensing Speed:	5 ml / min	
Suction Speed:	10 ml / min	Input Vial Type:	Type1@4	
Load in : Result vials		1 x	Type1@16	
No Quantitativ Transfer				
Elution :	ON			
Volume:	2 ml	Dispensing Speed:	5 ml / min	
Suction Speed:	20 ml / min	Waiting time:	0.1 min	Port : 9 acetonitrile
Dispense: in..				
same as Load				
Final Drying	10 ml	Dispensing Speed:	10 ml / min	

SETUP :	
Check max. pressure while loading	OFF
System - rinsing and conditioning with solvent from port:	1 acetonitrile

Figure 1: FREESTYLE method protocol for the SPE

3. Results

3.1 Clean-up Efficiency

In order to determine the clean-up efficiency of the approach to the three tested matrices, the dry weight of the corresponding matrix was measured gravimetrically in the crude extract, and after the clean-up. In Fig. 3 the overall clean-up efficiencies are compared to the corresponding crude extracts.

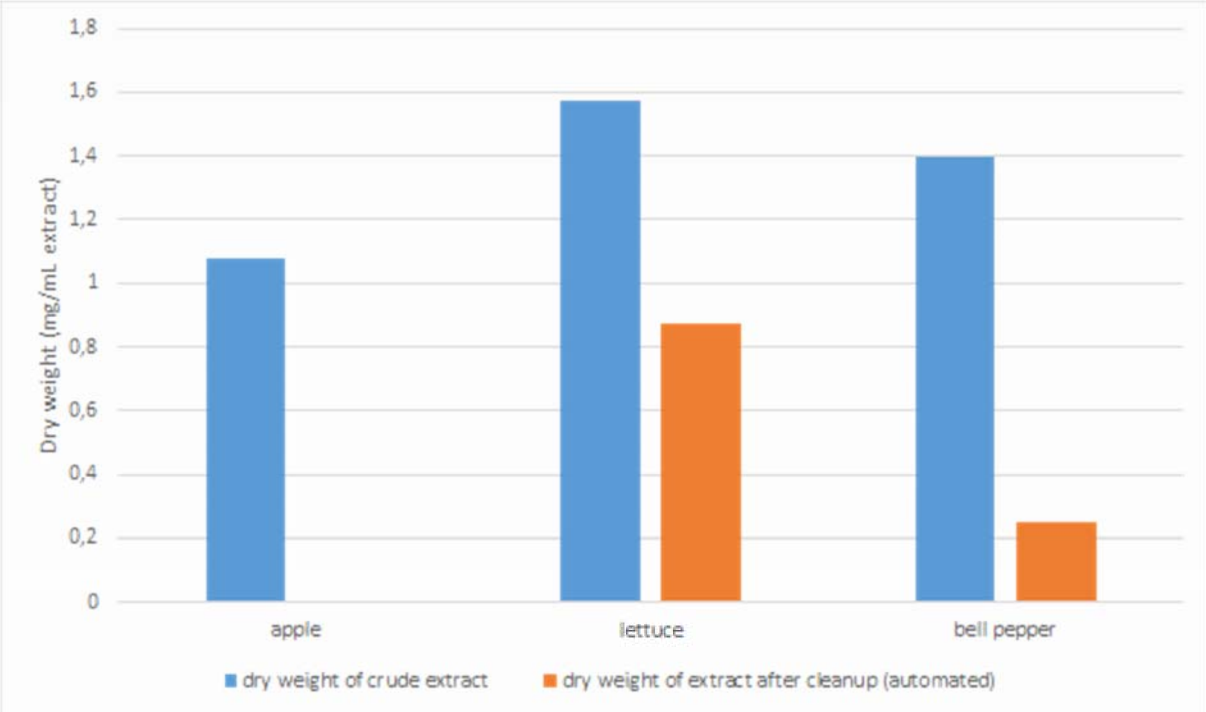

Figure 2: Gravimetric determination of dry matrix prior to and after the clean-up

Furthermore, the matrix burden was measured via UV-VIS spectroscopy in the range of 200 – 800 nm in the crude extract as well as after the clean-up.

Figure 3: Crude (left) and cleaned extract (right) of apple for UV-VIS measurement

Figure 4: Crude (left) and cleaned extract (right) of lettuce for UV-VIS measurement

Figure 5: Crude (left) and cleaned extract (right) of bell pepper for UV-VIS measurement

For all three matrices a significantly reduced matrix burden is measured, which leads to a reduced ion-suppression during ionisation for the MS-measurement (Fig. 3 to Fig. 5).

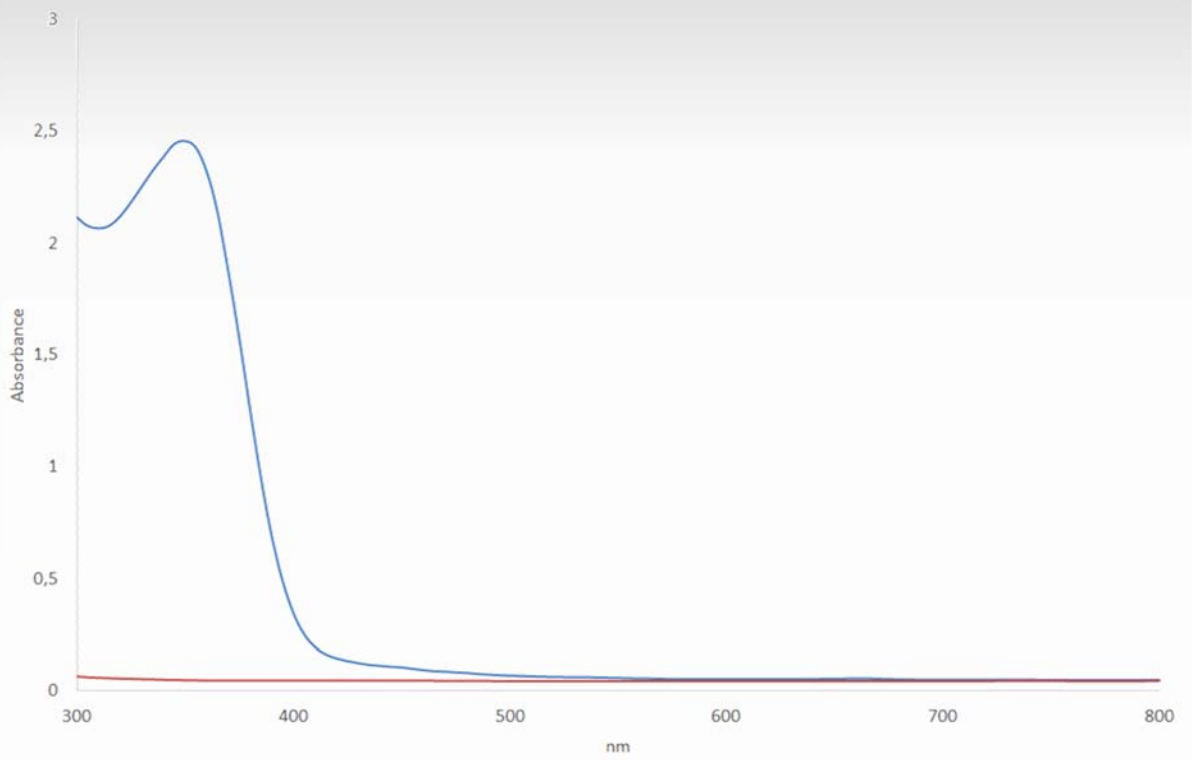

Figure 6: UV-VIS spectra of crude apple extract (blue) and extract after clean-up (red-brown)

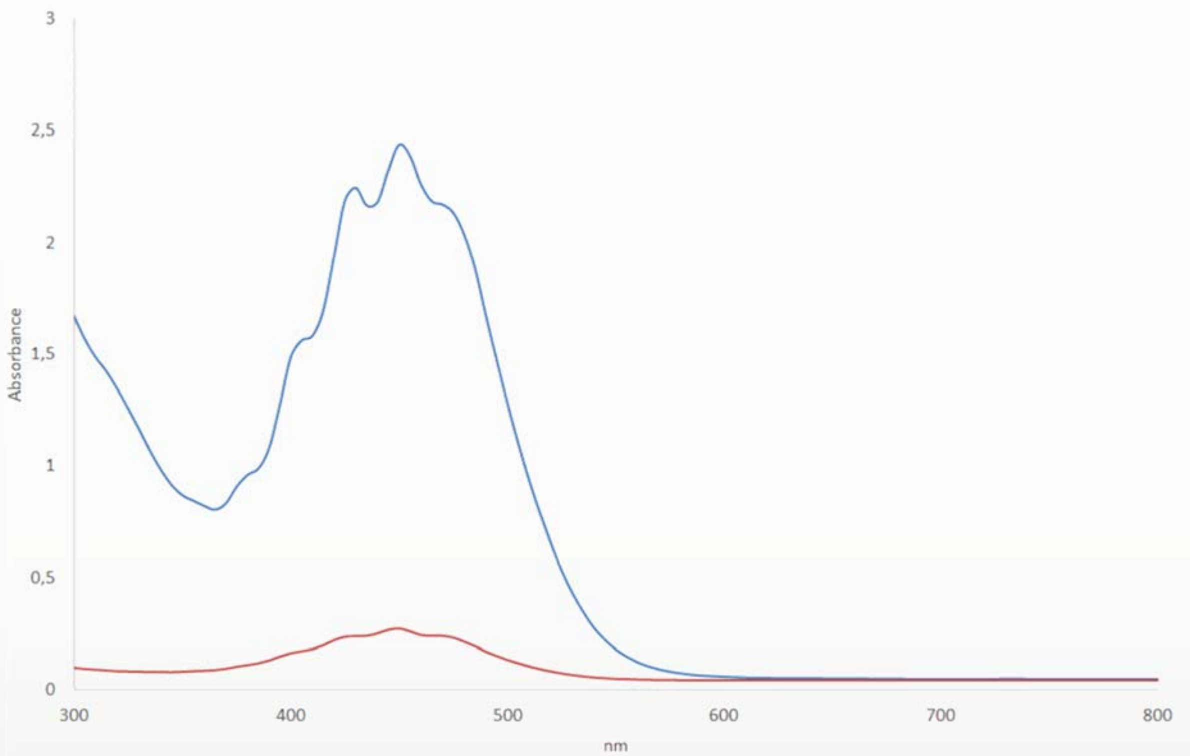

Figure 7: UV-VIS spectra of crude bell pepper extract (blue) and extract after clean-up (red-brown)

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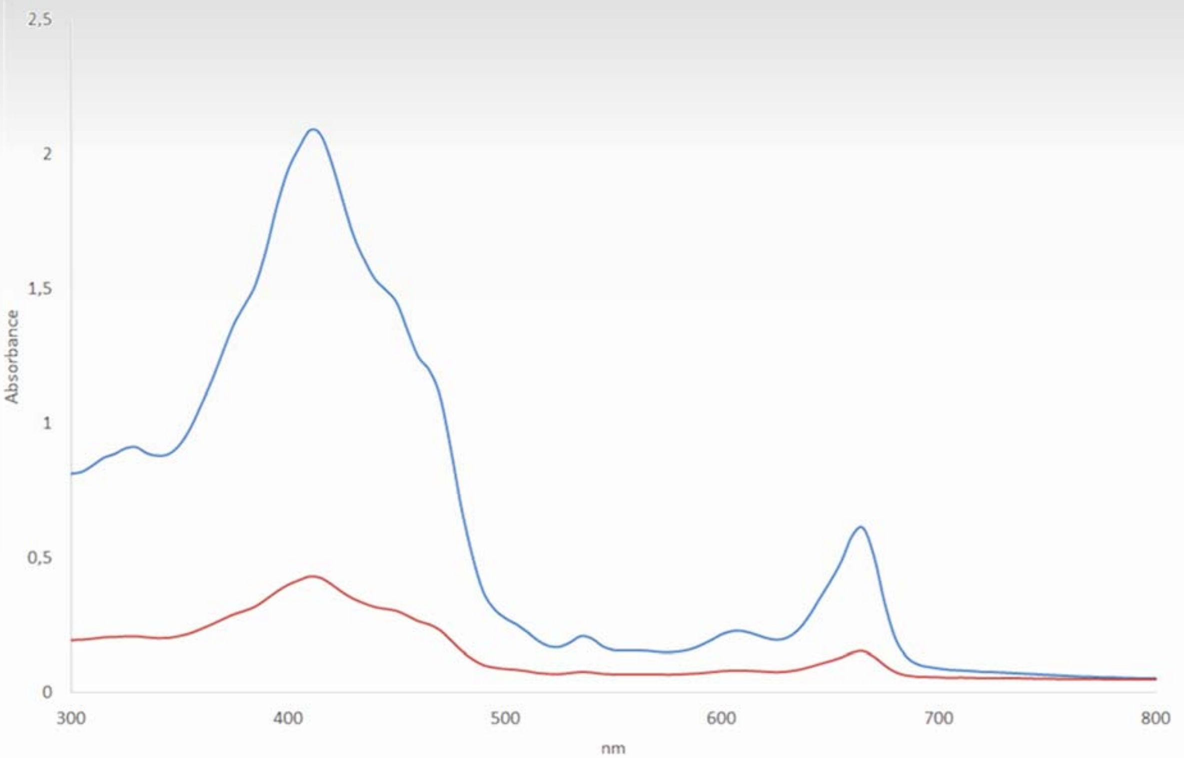

Figure 8: UV-VIS spectra of crude lettuce extract (blue) and extract after clean-up (red-brown)

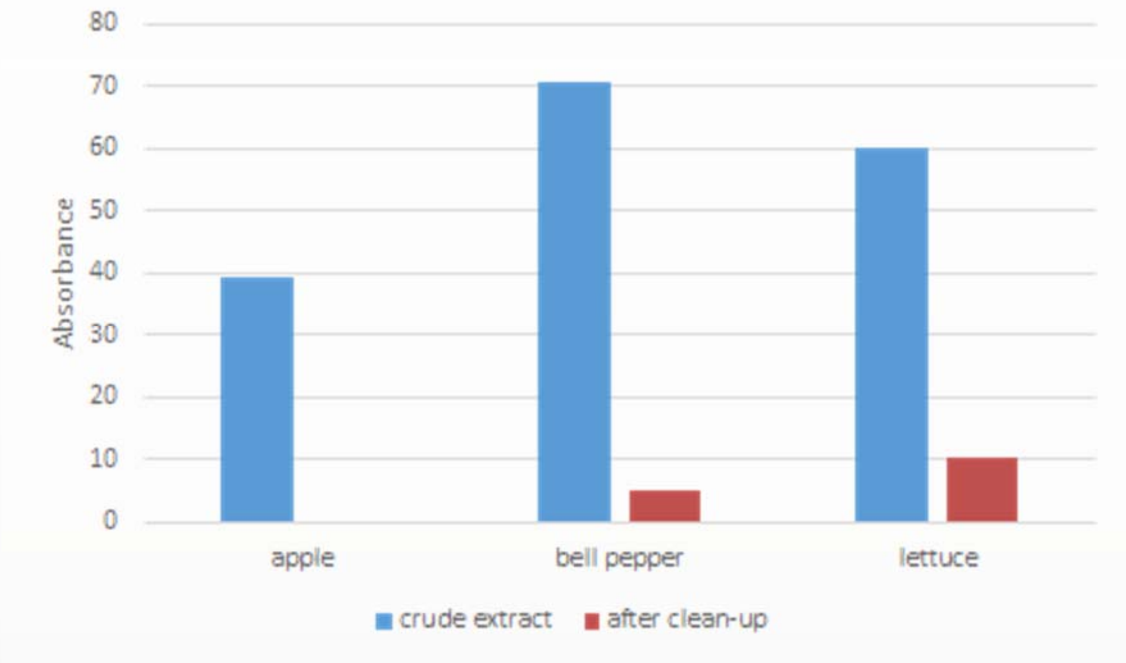

Figure 9: Integrated absorbance values for the individual matrices calculated from 200 to 800 nm

3.1 Analytical Results

In Fig. 10 an exemplary LC-MS/MS chromatogram of the pesticide mix under the given chromatographic conditions with 219 compounds is shown.

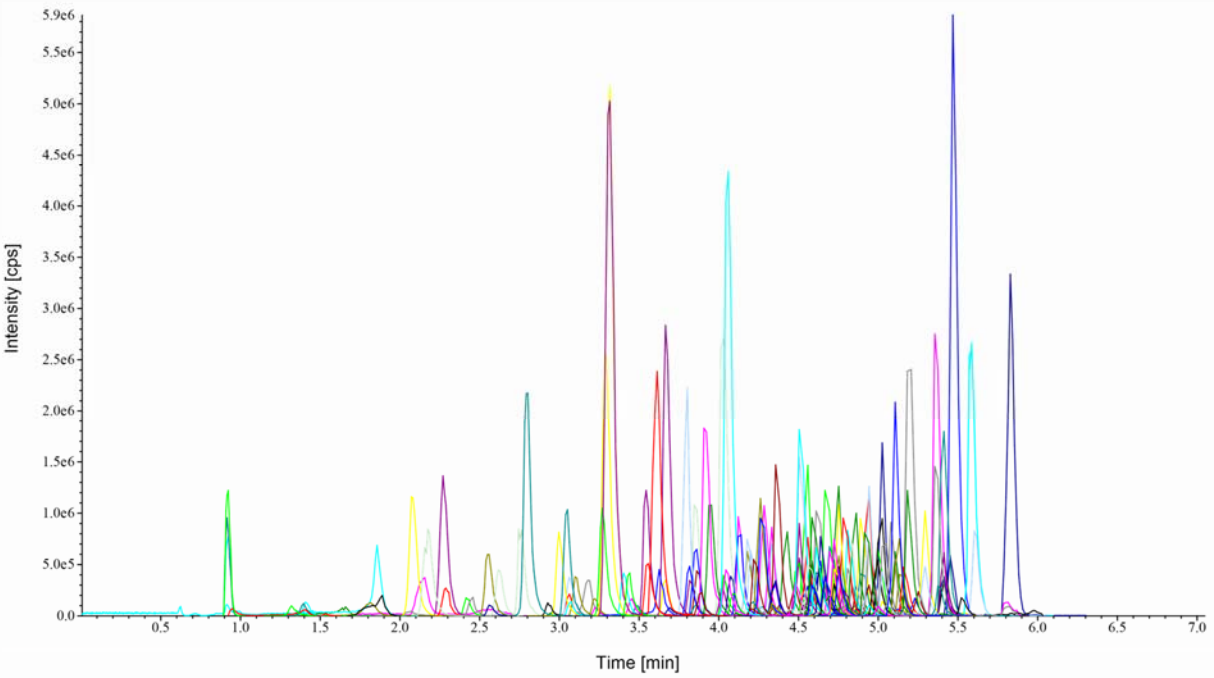

Figure 10: Chromatogram of 219 pesticides

In Tab. 1 the corresponding results of 219 pesticides in the four tested matrices is shown. In general, most of the analytes are found in the commonly accepted range of 60 to 120 % recovery. For some of the pesticides it can be seen that depending on the matrix influence a matrix ion suppression or enhancement took place resulting in recovery values < 60 or > 120 %. For apple 12 analytes were below 60 % and 11 were > 120 %, for bell pepper these figures were 12 and 13, and for lettuce 8 and 10, respectively. Therefore, 89 % were in the accepted range for apple, 89% for bell pepper, and 92% for lettuce.

Nevertheless, as the fully automated approach is highly reproducible and in general shows standard deviations < 20 %, a matrix-specific correction factor can be applied.

For pesticides where no recovery data are shown, the chromatographic evaluation did not allow a proper integration.

Table 1: Recovery data of the 219 pesticides measured in the three matrices.

Pesticide	Apple	Bell Pepper	Lettuce
3-Hydroxycarbofuran	108	131	90
Acephate	73	79	87
Acetamiprid	106	91	79
Acibenzolar-S-methyl	114	87	97
Aldicarb sulfone	108	94	72
Aldicarb sulfoxide	89	87	88
Aldicarb	101	103	91
Ametryn	97	97	87
Aminocarb	93	98	92
Amitraz	107	97	90
Avermectin B1a	52	122	92
Avermectin B1b			
Azoxystrobin	116	94	92
Benalaxyl	116	82	85
Bendiocarb	110	121	88
Benzoximate	100	108	94
Bifenazate	77	55	44
Bitertanol	94	91	101
Boscalid	97	94	107
Bromucanozole Isomer 1	108	87	67
Bromucanozole Isomer 2	106	99	52
Bupirimate	102	94	95
Buprofezin	98	97	90
Butafenacil	114	86	83
Butocarboxim	83	94	100
Butoxycarboxim		104	95
Carbaryl	104	101	83
Carbendazim	94	85	90
Carbetamide	106	93	85
Carbofuran	101	91	86
Carboxin	108	93	79
Carfentrazone-ethyl	98	98	95
Chlorantraniliprole	105	94	96
Chlorfluazuron	97	84	82
Chloridazon	116	91	82
Chlorotoluron	107	154	104
Chloroxuron	100	96	90
Chlorpyrifos	109	92	74

Pesticide	Apple	Bell Pepper	Lettuce
Chlorthalonil	104	2	98
Clethodim Isomer 1	43	141	115
Clethodim Isomer 2	91	82	90
Clofentezine	111	95	135
Clothianidin	102	80	78
Coumaphos	109	89	86
Cyamemazine	81	81	109
Cyazofamid	99	92	88
Cycluron	102	90	104
Cymoxanil	104	197	88
Cyproconazole Isomer 1	96	91	89
Cyproconazole Isomer 2	93	101	92
Cyprodinil	106	96	88
Cyromazine	50	45	60
Desmedipham	100	276	107
Diazinon	93	101	94
Diclobutrazol	98	90	97
Dicrotophos	99	96	90
Diethofencarb	115	80	84
Difenoconazole Isomer 1	92	97	105
Diflubenzuron	104	99	85
Dimethoate	101	105	84
Dimethomorph Isomer 1	107	99	90
Dimethomorph Isomer 2	110	93	90
Dimoxystrobin	102	91	85
Diniconazole	89	102	87
Dinotefuran	105	97	74
Diuron	107	90	79
Doramectin	91	91	85
Emamectin-benzoate b1a	15	11	45
Emamectin-benzoate b1b			82
Epoxiconazole	58	98	130
Eprinomectin	96	129	87
Etaconazole Isomer 1	110	94	96
Ethiofencarb	125	74	76
Ethiprole	104	97	79
Ethirimol	85	77	83
Ethofumesate	94	98	96
Etoxazole	104	103	130
Famoxadone	102	107	92

Pesticide	Apple	Bell Pepper	Lettuce
Fenamidone	103	92	93
Fenarimol	112	88	90
Fenazaquin	94	94	84
Fenbuconazole	114	100	99
Fenhexamid	67	68	64
Fenobucarb	112	99	98
Fenoxycarb	94	92	74
Fenpropimorph	91	88	87
Fenpyroximate	98	94	94
Fenuron	96	89	86
Fipronil	99	100	162
Flonicamid	97	96	90
Flubendiamide	120	116	95
Fludioxinil	77	105	79
Flufenacet	104	86	84
Flufenoxuron	108	85	75
Fluometuron	107	88	86
Fluoxastrobin	103	83	95
Fluquinconazole	99	90	105
Flusilazole	114	91	92
Flutolanil	110	96	90
Flutriafol	102	89	92
Forchlorfenuron	95	101	94
Formetanate HCl	75	72	92
Fuberidazole	129	187	146
Furalaxyl	109	94	88
Furathiocarb	101	94	91
Halofenozide	101	95	95
Hexaconazole	113	107	97
Hexaflumuron	89	88	96
Hexythiazox	94	83	64
Hydramethylnon	750	52	153
Imazalil	64	64	50
Imidacloprid	110	103	85
Indoxacarb	113	112	101
Ipconazole Isomer 1	77	81	110
Ipconazole Isomer 2	83	93	104
Iprovalicarb Isomer 1	109	94	151
Iprovalicarb Isomer 2	111	100	133
Isocarbophos	187	140	108

Pesticide	Apple	Bell Pepper	Lettuce
Isoprocarb	107	107	92
Isoproturon	110	94	90
Ivermectin	100	88	89
Kresoxim-methyl	103	108	77
Linuron	104	97	80
Lufenuron	125	100	92
Mandipropamid	105	105	91
Mefenacet	102	99	79
Mepanipyrim	88	90	85
Mepronil	105	89	89
Mesotrione	21	30	27
Metaflumizone	436	110	88
Metalaxyl	102	94	85
Metconazole	91	108	90
Methabenzthiazuron	107	98	91
Methamidophos	83	78	66
Methiocarb	98	97	95
Methomyl	74	92	90
Methoprotryne	102	90	90
Methoxyfenozide	102	104	90
Metobromuron	97	93	79
Metribuzin	99	97	97
Mevinphos Isomer 1	102	97	86
Mevinphos Isomer 2	103	98	88
Mexacarbate	103	91	89
Monocrotophos	99	98	83
Monolinuron	108	92	75
Moxidectin	202	87	95
Myclobutanil	109	103	91
Neburon	112	105	87
Nitenpyram	97	90	82
Nuarimol	97	93	96
Omethoate	87	93	93
Oxadixyl	108	102	77
Oxamyl	100	100	85
Paclobutrazol	100	101	95
Penconazole	102	99	90
Pencycuron	111	90	83
Phenmedipham	97	201	100
Picoxystrobin	95	98	88

Pesticide	Apple	Bell Pepper	Lettuce
Piperonyl butoxide	103	92	71
Pirimicarb	100	98	91
Prochloraz	89	100	85
Promecarb	95	92	96
Prometon	98	91	98
Prometryne	97	97	94
Propamocarb	92	87	146
Propargite	104	96	73
Propham	87	133	122
Propiconazole Isomer 1	102	103	100
Propiconazole Isomer 2	95	95	101
Propoxur	108	95	89
Prothioconazole	71	73	54
Pymetrozine	28	46	45
Pyracarbolid	107	97	85
Pyraclostrobin	96	96	89
Pyridaben	100	82	78
Pyrimethanil	100	90	87
Pyriproxyfen	105	101	93
Quinoxyfen	95	93	79
Rotenone	111	102	89
Secbumeton	99	95	92
Siduron	101	85	85
Simetryn	100	97	94
Spinetoram	43	32	55
Spinosad (Spinosyn A)	57	50	83
Spinosad (Spinosyn D)	50	35	60
Spirodiclofen	101	99	95
Spiromesifen	128	97	87
Spirotetramat	95	95	97
Spiroxamine Isomer 1	51	57	74
Spiroxamine Isomer 2	51	48	72
Sulfentrazone	83	95	106
Tebuconazole	94	93	99
Tebufenozide	108	107	94
Tebufenpyrad	98	90	89
Tebuthiuron	101	92	100
Teflubenzuron	89	117	78
Temephos	98	92	87
Terbumeton	124	90	105

Pesticide	Apple	Bell Pepper	Lettuce
Terbutryn	100	93	95
Terbutylazin	103	100	99
Terbutylazin-desethyl	107	93	93
Tetraconazole	107	96	91
Thiabendazole	93	114	103
Thiacloprid	111	89	73
Thiamethoxam	120	92	67
Thidiazuron	87	93	102
Thiobencarb	111	84	84
Thiofanox	124		
Thiophanate-methyl	153	112	65
Triadimefon	97	99	77
Triadimenol	97	93	93
Trichlorfon	103	164	82
Tricyclazole	95	96	91
Trifloxystrobin	109	99	85
Triflumizole	95	105	79
Triflumuron	98	95	86
Triticonazole	94	94	90
Vamidothion	107	100	87
Zoxamide	116	91	86

4. Conclusion

In the application note fruit and vegetable matrices were tested on the new FREESTYLE QuEChERS automation in combination with a specifically adapted cartridge.
In general, over all three matrices 90 % of the analytes could be detected within the accepted recovery range. Additionally it can be seen that no analyte did not work at all, so in general the recovery data obtained were mainly depending on the individual matrix composition.

Due to the high level of automation and the non-dispersive approach, the extracts were cleaner compared to a standard QuEChERS approach and showed good reproducibility. As the system can work fully unattended over night or the weekend it is a great support for any routine pesticide lab.

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