APPLICATION NOTE | AN0031

SOLUTIONS BY

Determination of Pesticides in Tea 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. Nevertheless, for difficult matrices such as tea, different methodologies are very often applied due to the inherent matrix effects on the ionisation in mass spectrometric systems.

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 a difficult matrix tea samples were analysed and the results for a pesticide mix with 220 compounds with LC-MS/MS measurement are shown.

2. Method Development

2.1 Reagents and Materials

- Acetonitrile (pesticide grade)
- QuEChERS-MIX I (REF 730970)
- Pesticide II, Special SPE QuEChERS Column (LCTech GmbH, P/N 16683)
- Methanol with 5 % ammonia

2.2 Sample Preparation

Homogenise 5 g of tea in a blender and weigh out 2 g into a 50 mL Falcon tube. Add 10 mL of water, shake mixture vigorously and wait 15 min. Add 10 mL of acetonitrile and internal standard and shake vigorously for 1 min. Add QuEChERS-MIX I (REF 730970) and agitate for 1 minute. Afterwards centrifuge for 10 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

2.4 Analytical Set-up

2.4.1 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

2.4.2 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))

2.4.3 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:	OFF			
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

In Fig. 5 an exemplary LC-MS/MS chromatogram of the pesticide mix under the given chromatographic conditions with 220 compounds is shown.

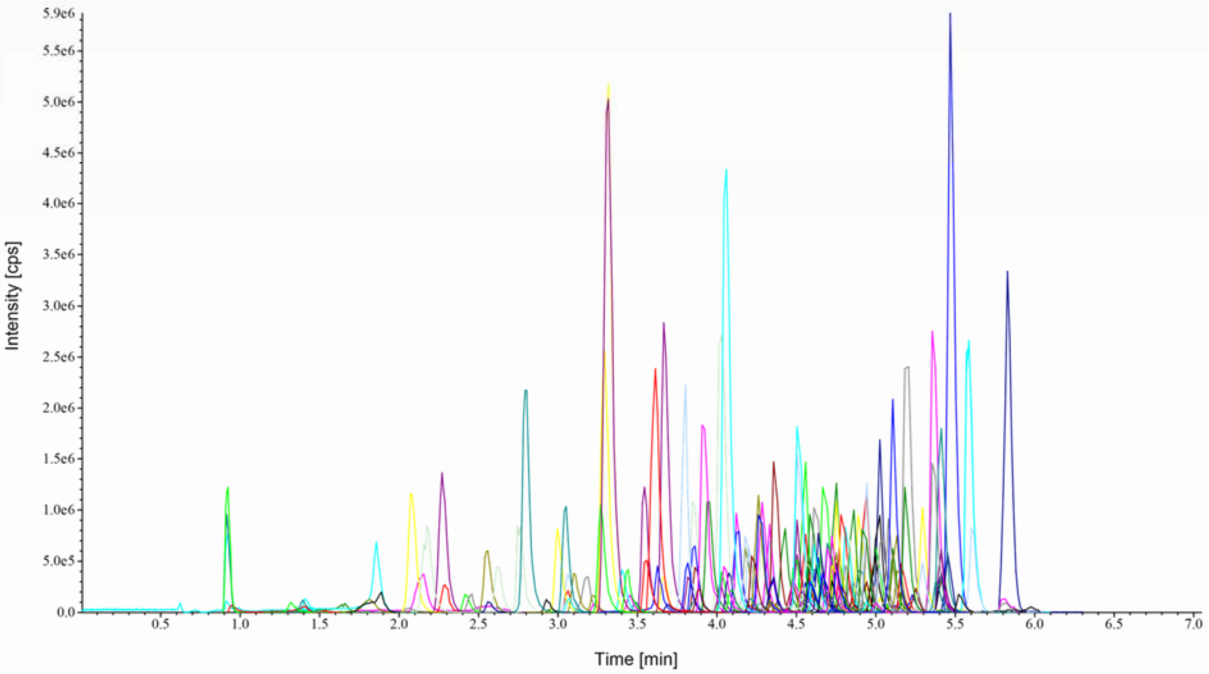

Figure 2: Chromatogram of 220 pesticides

In Tab. 1 the corresponding results of 220 pesticides are 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 %.

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 220 pesticides measured in tea.

Pesticide	Recovery [%]	Pesticide	Recovery [%]
3-Hydroxycarbofuran	79	Imazalil	83
Acephate	36	Imidacloprid	72
Acetamiprid	82	Indoxacarb	126
Acibenzolar-S-methyl	78	Ipconazole Isomer 1	86
Aldicarb sulfone	69	Ipconazole Isomer 2	101
Aldicarb sulfoxide	76	Iprovalicarb Isomer 1	94
Aldicarb	66	Iprovalicarb Isomer 2	94
Ametryn	86	Isocarbophos	105
Aminocarb	72	Isoprocarb	76
Amitraz	91	Isoproturon	90
Avermectin B1a		Ivermectin	125
Avermectin B1b		Kresoxim-methyl	110
Azoxystrobin	86	Linuron	79
Benalaxyl	94	Lufenuron	140
Bendiocarb	78	Mandipropamid	94
Benzoximate	117	Mefenacet	81
Bifenazate	94	Mepanipyrim	85
Bitertanol	108	Mepronil	84
Boscalid	100	Mesotrione	
Bromucanozole Isomer 1	99	Metaflumizone	173
Bromucanozole Isomer 2	91	Metalaxyl	91
Bupirimate	93	Metconazole	100
Buprofezin	112	Methabenzthiazuron	80
Butafenacil	85	Methamidophos	64
Butocarboxim	93	Methiocarb	72
Butoxycarboxim	80	Methomyl	77
Carbaryl	90	Methoprotryne	91
Carbendazim	66	Methoxyfenozide	85
Carbetamide	75	Metobromuron	77
Carbofuran	83	Metribuzin	86
Carboxin	66	Mevinphos Isomer 1	85
Carfentrazone-ethyl	84	Mevinphos Isomer 2	83
Chlorantraniliprole	80	Mexacarbate	79
Chlorfluazuron	132	Monocrotophos	90
Chloridazon	80	Monolinuron	84
Chlorotoluron	69	Moxidectin	174
Chloroxuron	79	Myclobutanil	97
Chlorpyrifos	111	Neburon	85
Chlorthalonil		Nitenpyram	50

Pesticide	Recovery [%]	Pesticide	Recovery [%]
Clethodim Isomer 1	#DIV/0!	Nuarimol	85
Clethodim Isomer 2	#DIV/0!	Omethoate	74
Clofentezine	91	Oxadixyl	83
Clothianidin	2542	Oxamyl	75
Coumaphos	91	Paclobutrazol	98
Cyamemazine	79	Penconazole	90
Cyazofamid	88	Pencycuron	88
Cycluron	79	Phenmedipham	99
Cymoxanil	80	Picoxystrobin	100
Cyproconazole Isomer 1	93	Piperonyl butoxide	103
Cyproconazole Isomer 2	96	Pirimicarb	86
Cyprodinil	92	Prochloraz	94
Cyromazine	25	Promecarb	80
Desmedipham	73	Prometon	90
Diazinon	100	Prometryne	92
Diclobutrazol	89	Propamocarb	39
Dicrotophos	89	Propargite	109
Diethofencarb	73	Propham	87
Difenoconazole Isomer 1	105	Propiconazole Isomer 1	104
Diflubenzuron	100	Propiconazole Isomer 2	99
Dimethoate	76	Propoxur	91
Dimethomorph Isomer 1	86	Prothioconazole	74
Dimethomorph Isomer 2	89	Pymetrozine	10
Dimoxystrobin	88	Pyracarbolid	83
Diniconazole	84	Pyraclostrobin	101
Dinotefuran	66	Pyridaben	145
Diuron	76	Pyrimethanil	75
Doramectin	95	Pyriproxyfen	110
Emamectin-benzoate b1a	122	Quinoxyfen	98
Emamectin-benzoate b1b	136	Rotenone	87
Epoxiconazole	91	Secbumeton	89
Eprinomectin	123	Siduron	85
Etaconazole Isomer 1	90	Simetryn	83
Ethiofencarb	59	Spinetoram	132
Ethiprole	94	Spinosad (Spinosyn A)	115
Ethirimol	55	Spinosad (Spinosyn D)	146
Ethofumesate	60	Spirodiclofen	111
Etoxazole	127	Spiromesifen	128
Famoxadone	79	Spirotetramat	83
Fenamidone	98	Spiroxamine Isomer 1	107

Pesticide	Recovery [%]	Pesticide	Recovery [%]
Fenarimol	101	Spiroxamine Isomer 2	112
Fenazaquin	127	Sulfentrazone	
Fenbuconazole	95	Tebuconazole	99
Fenhexamid		Tebufenozide	94
Fenobucarb	73	Tebufenpyrad	116
Fenoxycarb	102	Tebuthiuron	75
Fenpropimorph	110	Teflubenzuron	123
Fenpyroximate	139	Temephos	123
Fenuron	79	Terbumeton	89
Fipronil	111	Terbutryn	97
Flonicamid	86	Terbutylazin	90
Flubendiamide	78	Terbutylazin-desethyl	91
Fludioxinil	98	Tetraconazole	96
Flufenacet	85	Thiabendazole	59
Flufenoxuron	124	Thiacloprid	71
Fluometuron	78	Thiamethoxam	62
Fluoxastrobin	79	Thidiazuron	45
Fluquinconazole	112	Thiobencarb	88
Flusilazole	96	Thiofanox	107
Flutolanil	91	Thiophanate-methyl	44
Flutriafol	93	TPP	97
Forchlorfenuron	86	Triadimefon	89
Formetanate HCl	65	Triadimenol	97
Fuberidazole	73	Trichlorfon	72
Furalaxyl	87	Tricyclazole	52
Furathiocarb	107	Trifloxystrobin	106
Halofenozide	91	Triflumizole	117
Hexaconazole	103	Triflumuron	97
Hexaflumuron	146	Triticonazole	93
Hexythiazox	100	Vamidothion	82
Hydramethylnon	97	Zoxamide	88

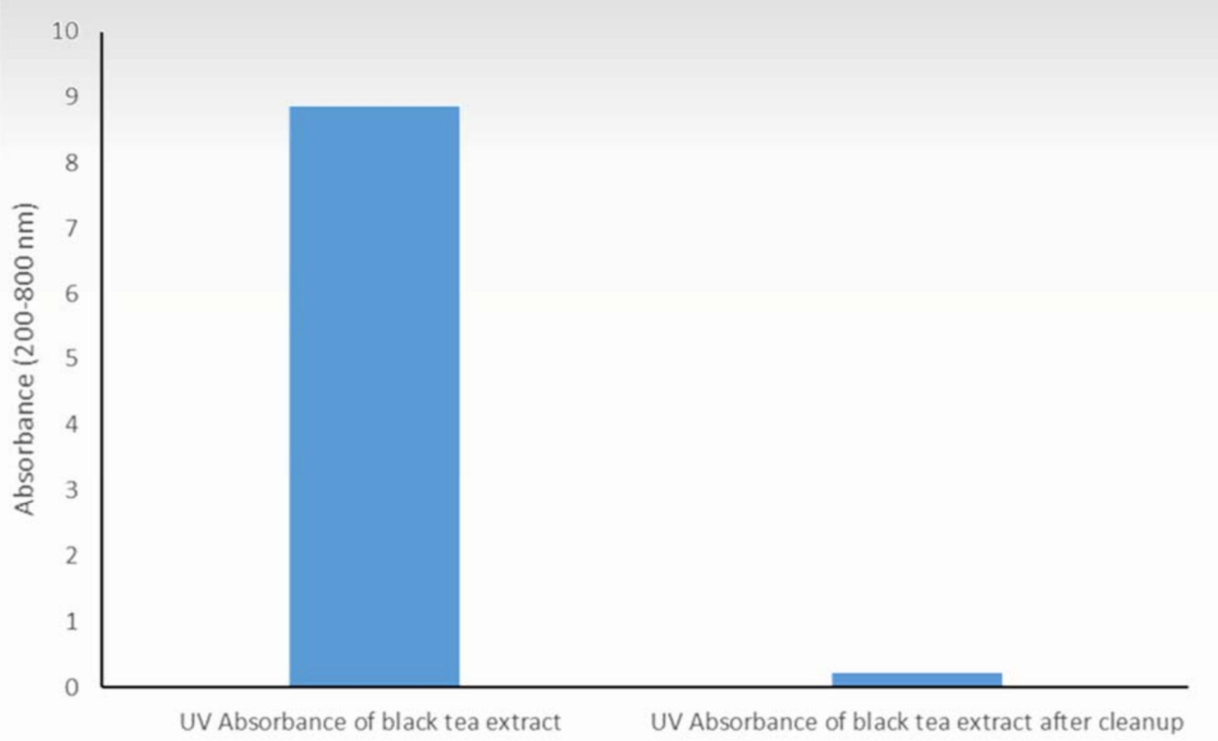

Figure 3: UV absorbance (200-800 nm) of cleaned and crude tea extracts.

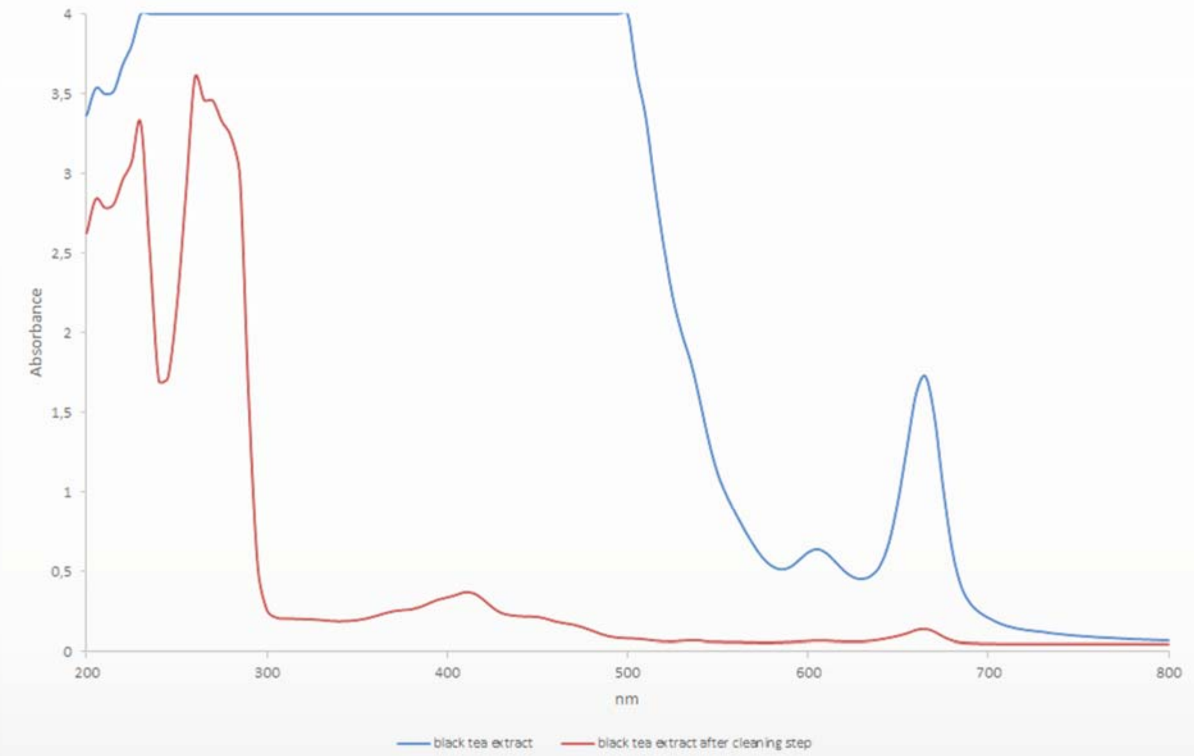

Figure 4: UV-Spectra (200-800 nm) of cleaned and crude tea extracts.

Figure 6 and 7 show the UV absorbance and the spectra of crude tea extracts and of tea extracts after automated QuEChERS clean-up. The absorbance from 200 nm to 800 nm allows to summarise the amounts of all UV-VIS observable compounds in crude extracts and cleaned sample extracts. The decrease of amount of matrix compounds is clearly evident and indicates the benefit using automated QuEChERS methodology for gaining clean sample extracts.

4. Conclusion

In the application note tea matrices were tested on the new FREESTYLE QuEChERS automation in combination with a specifically adapted cartridge.

In general, 82 % of the analytes could be detected within the accepted recovery range. 11 and 21 pesticides were below or higher as the accepted range, but some very close to the acceptance limits, and 7 seem not to be detectable under the given conditions. 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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