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Determination of Pesticides in Tobacco 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 tobacco, 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 tobacco 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 tobacco 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.

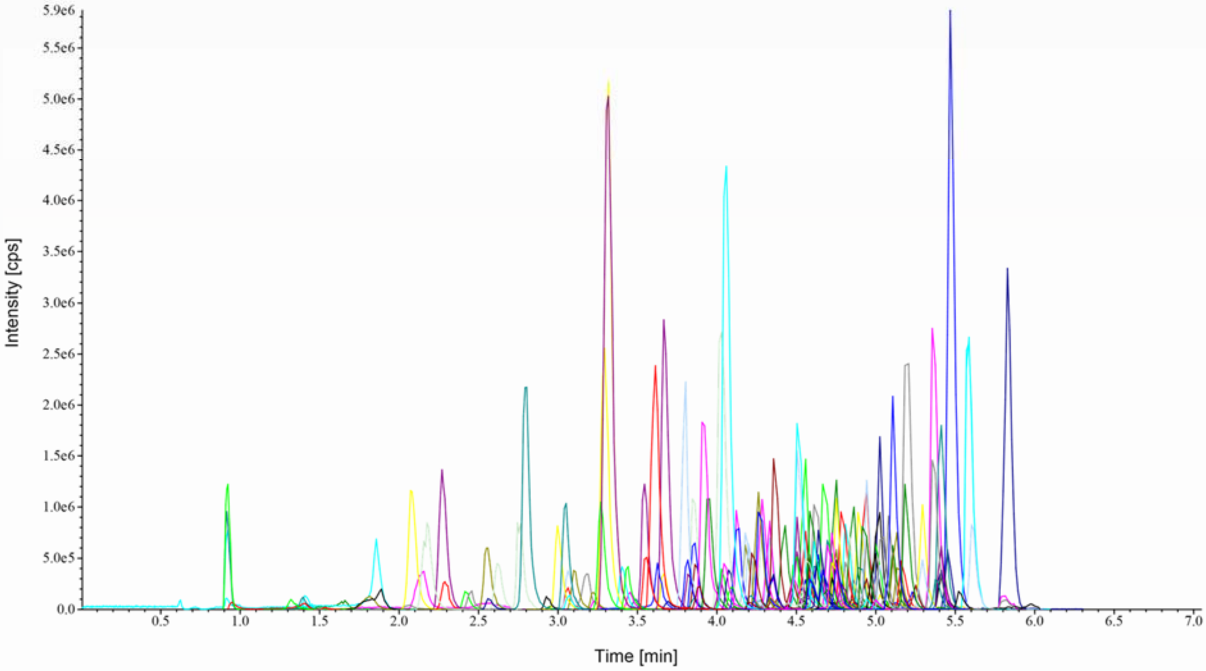

Figure 2: Chromatogram of 220 pesticides

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 tobacco.

Pesticide	Recovery [%]	Pesticide	Recovery [%]
3-Hydroxycarbofuran	69	Imazalil	99
Acephate	24	Imidacloprid	21
Acetamiprid	12	Indoxacarb	70
Acibenzolar-S-methyl	93	Ipconazole Isomer 1	114
Aldicarb	69	Ipconazole Isomer 2	101
Aldicarb sulfone	60	Iprovalicarb Isomer 1	
Aldicarb sulfoxide	67	Iprovalicarb Isomer 2	
Ametryn	94	Isocarbophos	
Aminocarb	73	Isoprocarb	84
Amitraz	107	Isoproturon	89
Avermectin B1a		Ivermectin	
Avermectin B1b		Kresoxim-methyl	42
Azoxystrobin	16	Linuron	82
Benalaxyl	121	Lufenuron	73
Bendiocarb	83	Mandipropamid	105
Benzoximate	151	Mefenacet	102
Bifenazate	108	Mepanipyrim	105
Bitertanol	86	Mepronil	87
Boscalid	96	Mesotrione	
Bromucanozole Isomer 1	101	Metaflumizone	240
Bromucanozole Isomer 2	91	Metalaxyl	42
Bupirimate	112	Metconazole	109
Buprofezin	127	Methabenzthiazuron	81
Butafenacil	103	Methamidophos	51
Butocarboxim	89	Methiocarb	89
Butoxycarboxim	87	Methomyl	67
Carbaryl	98	Methoprotryne	92
Carbendazim		Methoxyfenozide	104
Carbetamide	80	Metobromuron	97
Carbofuran	89	Metribuzin	102
Carboxin	91	Mevinphos Isomer 1	74
Carfentrazone-ethyl	100	Mevinphos Isomer 2	77
Chlorantraniliprole	42	Mexacarbate	70
Chlorfluazuron	118	Monocrotophos	69
Chloridazon	66	Monolinuron	88
Chlorotoluron	86	Moxidectin	
Chloroxuron	113	Myclobutanil	80
Chlorpyrifos	115	Neburon	141
Chlorthalonil	34	Nitenpyram	69

Pesticide	Recovery [%]	Pesticide	Recovery [%]
Clethodim Isomer 1		Nuarimol	108
Clethodim Isomer 2		Omethoate	59
Clofentezine	110	Oxadixyl	83
Clothianidin	50	Oxamyl	70
Coumaphos	134	Paclobutrazol	98
Cyamemazine	92	Penconazole	99
Cyazofamid	101	Pencycuron	126
Cycluron	81	Phenmedipham	93
Cymoxanil	67	Picoxystrobin	91
Cyproconazole Isomer 1	93	Piperonyl butoxide	102
Cyproconazole Isomer 2	92	Pirimicarb	79
Cyprodinil	46	Prochloraz	110
Cyromazine	48	Promecarb	92
Desmedipham	80	Prometon	90
Diazinon	116	Prometryne	94
Diclobutrazol	110	Propamocarb	76
Dicrotophos	80	Propargite	138
Diethofencarb	93	Propham	88
Difenoconazole Isomer 1	123	Propiconazole Isomer 1	110
Diflubenzuron	103	Propiconazole Isomer 2	107
Dimethoate	72	Propoxur	94
Dimethomorph Isomer 1	91	Prothioconazole	108
Dimethomorph Isomer 2	86	Pymetrozine	23
Dimoxystrobin	112	Pyracarbolid	78
Diniconazole	96	Pyraclostrobin	58
Dinotefuran	53	Pyridaben	138
Diuron	76	Pyrimethanil	84
Doramectin		Pyriproxyfen	137
Emamectin-benzoate b1a	159	Quinoxyfen	138
Emamectin-benzoate b1b	169	Rotenone	
Epoxiconazole	102	Secbumeton	90
Eprinomectin		Siduron	95
Etaconazole Isomer 1	101	Simetryn	87
Ethiofencarb	89	Spinetoram	151
Ethiprole	106	Spinosad (Spinosyn A)	163
Ethirimol	56	Spinosad (Spinosyn D)	226
Ethofumesate		Spirodiclofen	228
Etoxazole	142	Spiromesifen	
Famoxadone	97	Spirotetramat	105
Fenamidone	107	Spiroxamine Isomer 1	118

Pesticide	Recovery [%]	Pesticide	Recovery [%]
Fenarimol	102	Spiroxamine Isomer 2	122
Fenazaquin	174	Sulfentrazone	#DIV/0!
Fenbuconazole	120	Tebuconazole	27
Fenhexamid		Tebufenozide	140
Fenobucarb	98	Tebufenpyrad	163
Fenoxycarb	107	Tebuthiuron	80
Fenpropimorph	121	Teflubenzuron	
Fenpyroximate	156	Temephos	144
Fenuron	75	Terbumeton	91
Fipronil	125	Terbutryn	96
Flonicamid	75	Terbutylazin	86
Flubendiamide	34	Terbutylazin-desethyl	86
Fludioxinil	23	Tetraconazole	93
Flufenacet	108	Thiabendazole	59
Flufenoxuron	248	Thiacloprid	73
Fluometuron	86	Thiamethoxam	22
Fluoxastrobin	107	Thidiazuron	28
Fluquinconazole	95	Thiobencarb	104
Flusilazole	116	Thiofanox	
Flutolanil	107	Thiophanate-methyl	33
Flutriafol	89	TPP	125
Forchlorfenuron	89	Triadimefon	89
Formetanate HCl	75	Triadimenol	96
Fuberidazole	70	Trichlorfon	64
Furalaxyl	98	Tricyclazole	41
Furathiocarb	142	Trifloxystrobin	69
Halofenozide	106	Triflumizole	130
Hexaconazole	132	Triflumuron	123
Hexaflumuron	241	Triticonazole	101
Hexythiazox	97	Vamidothion	73
Hydramethylnon	186	Zoxamide	100

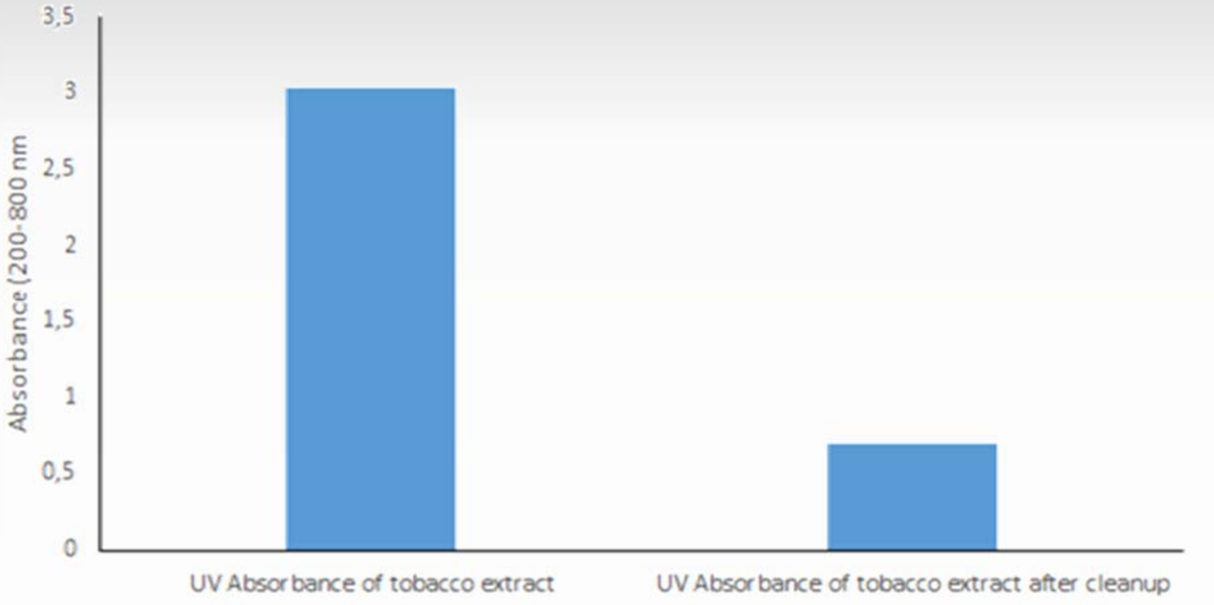

Figure 3: UV absorbance (200 - 800 nm) of cleaned and crude tobacco extracts.

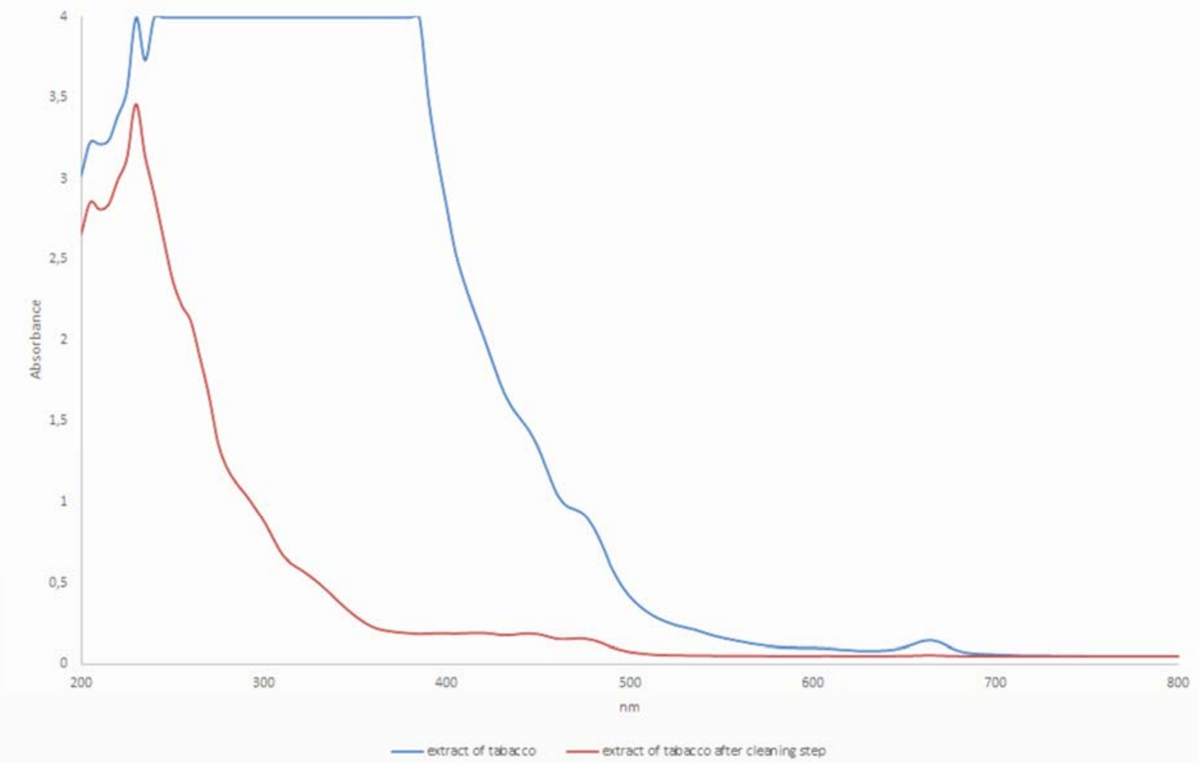

Figure 4: UV-Spectra (200 - 800 nm) of cleaned and crude tobacco extracts.

Figure 6 and 7 show the UV absorbance and the spectra of crude tobacco extracts and of tobacco 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 tobacco matrices were tested on the new FREESTYLE QuEChERS automation in combination with a specifically adapted cartridge.

In general, 64 % of the analytes could be detected within the accepted recovery range. 25 and 35 pesticides were below or higher as the accepted range, but some very close to the acceptance limits, and 20 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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