Application Note 20549

QuEChERS Dispersive Solid Phase Extraction for the GC-MS Analysis of Pesticides in Cucumber

Anila I Khan, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
QuEChERS, pesticide residue analysis, cucumber, food safety

Abstract
QuEChERS dispersive SPE is a simple, fast and quantitative sample preparation method. This application demonstrates the effectiveness of this technique in the GC/MS analysis of pesticides in cucumber, using a Thermo Scientific TraceGOLD TG-5MS GC column for analysis.

The recoveries for the spiked pesticides in cucumber matrix at 50 ng/g were between 75.2 to 119.6% with relative standard deviations ranging from 2.1 to 8.9% using the QuEChERS method described in EN15662.

Introduction
QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive Solid Phase Extraction (SPE) technique for extracting multi-residue pesticides from fruits and vegetables. The advantages of this methodology are speed, ease of execution, minimal solvent requirement and cost. The QuEChERS methodology was developed by Anastassiades et al[1] and has become widely used in food safety analyses.

The method is:

- **Quick** – high sample throughput, typically 8 samples can be prepared in under 30 min
- **Easy** – it requires less handling of extracts than other techniques i.e. fewer steps are required
- **Cheap** – less sorbent material is needed and less time is required to process samples compared to other techniques
- **Effective** – the simple technique gives high and accurate recovery levels for a range of different compound types.
- **Rugged** – the method can detect a large number of pesticides including charged and polar pesticides
- **Safe** – unlike other techniques, it does not require the use of chlorinated solvents. Extraction is typically carried out using acetonitrile, which is both GC and LC compatible.

The sample preparation approach described in the European EN15662 QuEChERS procedure[2] was used for extracting pesticides from cucumber. This is a two stage process: sample extraction, followed by dispersive SPE.

In the sample extraction stage, the food sample is homogenized to increase the available surface area of the sample to provide optimal extraction efficiencies. The homogenized sample is placed in the extraction tube containing magnesium sulfate and salts (sodium chloride, sodium citrate tribasic dihydrate, sodium citrate dibasic sesquihydrate). Magnesium sulfate ensures that, upon addition of acetonitrile, a phase separation is induced between water and organic solvent with the pesticides of interest being extracted into the organic phase. When acetonitrile is poured into the extraction tube containing the homogenized sample,

Thermo SCIENTIFIC

an exothermic reaction occurs between the magnesium sulfate and water. This step may lead to reduced recoveries of the pesticides. To overcome this problem, the sample can be weighed directly into an empty centrifuge tube followed by the addition of acetonitrile.The tube can then be immersed in an ice bath with slow addition of salts.

The second stage of the QuEChERS method uses dispersive SPE, which involves transferring a portion of the acetonitrile extract to a clean-up tube containing a combination of sorbents for removal of unwanted sample components. The sample clean-up also reduces matrix effects and therefore improves method robustness.

The pesticides analyzed in the cucumber matrix include mixtures of herbicides, fungicides, organophosphorus pesticides and pyrethroids. Six extractions of 50 ng/g spiked level were used for the recovery experiments.

Experimental Details

Consumables		Part Number
Column:	TraceGOLD TG-5MS, 30 m x 0.25 mm x 0.25 µm	26098-1420
Septum:	BTO, 17 mm	31303211
Liner:	Splitless Straight Liner, 3 x 8 x 105 mm	45350033
Column ferrules:	100% Graphite ferrules for TRACE injector 0.1-0.25 mm ID	29053488
	Graphite/vespel for transfer line 0.1-0.25 mm ID	29033496
Injection syringe:	10 µL Fixed needle syringe for a TriPlus Autosampler	36500525
Thermo Scientific Chromacol 9mm screw 2 mL vial -clear		2-SVW
Thermo Scientific Chromacol 9mm screw caps with PTFE/Silicone/PTFE		9-SC(B)-TST1

Chemicals and Reagents		Part Number
QuEChERS Extraction Stage:	Metalized Pouch containing 6g $MgSO_4$, 1.5 g sodium chloride, 1.5 g sodium citrate tribasic dihydrate, 0.75 g sodium citrate dibasic sesquihydrate and empty centrifuge tube with plug seal cap	60105-337
QuEChERS Dispersive SPE Stage:	15 mL centrifuge tube with 900 mg $MgSO_4$, 150 mg PSA, 150 mg C18	60105-227
Fisher Scientific HPLC grade Acetonitrile	A/0626/17	

Sample Preparation

The methodology described in this application note is for the preparation of calibration standards and sample spike (Figure 1).

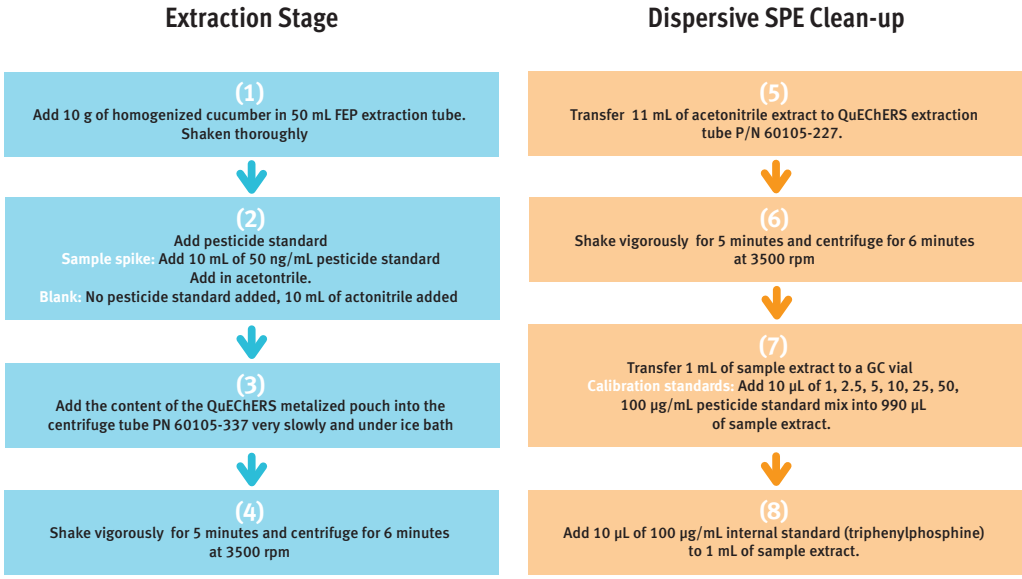

Figure 1: Flow diagram of QuEChERS methodology used in this application

Separation Conditions	Part Number
Instrumentation:	Thermo Scientific TRACE GC Ultra
Carrier gas:	Helium
Column flow:	1.0 mL/min, Constant flow
Oven temperature:	90 °C (1 min), 30 °C/min, 180 °C (0.5 min), 5 °C/min, 280 °C (5 min), 30 °C/min, 320 °C (10 min)
Injector type:	Programmable Temperature Vaporizer (PTV)
Injector mode:	PTV Splitless (0.75 min)
Injector conditions:	80 °C (0.05 min), evaporation 14.5 °C/sec, 180 °C (1 min), transfer 2.5 °C/sec, 300 °C (3 min), 14.5 °C/sec, 330 °C (20 min) 50 mL/min flow rate

MS Conditions	
Instrumentation:	Thermo Scientific ISQ Single Quadrupole mass spectrometer
Transfer line temperature:	282 °C
Source temperature:	280 °C
Ionization conditions:	EI
Electron energy:	70 eV

Segment	Compound	Start time (min)	m/z (Quan) Qual ions	Dwell time/ sec
1	Dichlobenil	3.50	(171), 173, 100, 136	0.05
2	Tribromoanisol	6.20	(346), 344, 329, 331	0.05
3	Sulfotep	6.80	(322), 97, 202, 146	0.05
4	Hexachlorobenzene	7.40	(284), 282, 283, 214	0.05
5	Parathion	10.40	(291), 109, 97	0.05
6	Triphenylphosphine (IS)	13.00	(262), 183, 108	0.05
7	EPN	18.90	(157), 169, 141, 110	0.05
8	Azinphos methyl	19.00	(160), 77, 132	0.05
9	Permethrin	20.90	(183), 163, 165, 153	0.05
10	Fenvalerate	23.00	(125), 167, 225	0.05
11	Deltamethrin	25.60	(253), 181, 251, 152	0.05

Table 1: SIM Scan Parameters

Injection Conditions	
Instrumentation:	Thermo Scientific TriPlus Autosampler
Injection Volume:	2 µL

Data Processing	
Software:	Software: Thermo Scientific XCalibur™

Results

In order to assess the method linearity, a calibration curve was constructed for each of the ten pesticides spiked in the sample matrix, using triphenylphosphine as the internal standard (IS). The concentration range studied for the selected pesticides in Table 2 was 25 to 1000 ng/g. The coefficient of determination (R^2) between area ratio of sample and internal standard for all pesticides were higher than 0.99 (Table 2), demonstrating good method linearity.

The analysis was performed in SIM. Figure 2 shows the TIC chromatogram of spiked pesticides in cucumber matrix at 10 µg/g in full scan. Six extractions of ten pesticides in sample matrix spiked at 50 ng/g were measured. These values do not appear to correlate with those in table 2.

The recoveries for spiked pesticides were between 75 and 120%, with an relative standard deviation (RSD) under 9% (Table 2).

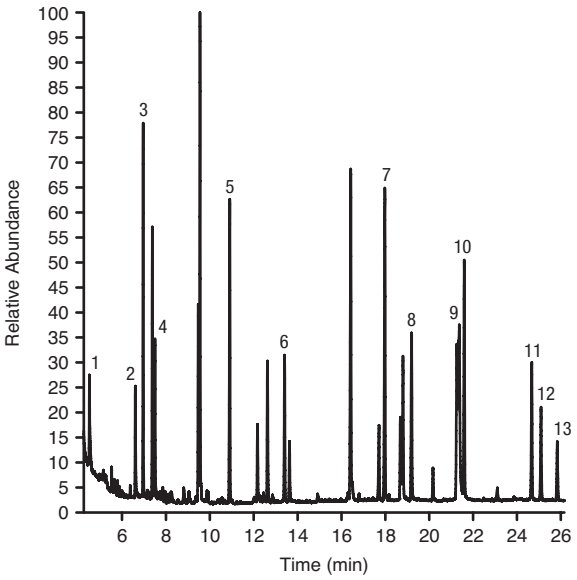

Figure 2: TIC for the GC/MS analysis of cucumber matrix spiked with 10 µg/g of each pesticide in full scan (50-450 m/z). See Table 2 for identified peaks. Unidentified peaks are matrix peaks

Conclusion

The QuEChERS sample preparation method provided high recoveries and good reproducibility. The QuEChERS – GC/MS method was found to be linear in the concentration range of 25 to 1000 ng/g spiked matrix. The TraceGOLD TG-5MS GC column provided good chromatographic resolution of the pesticides studied.

References

1. M. Anastassiades, S.J. Lehotay, D. Stajnbaher and F.J. Schenck, J AOAC Int 86 (2003) 412.

2. Foods of plant origin - Determination of pesticide residues using GC/MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE –QuEChERS method. European Standard EN15662:2008

Pesticides	t_R (min)	Linearity	Nominal concentration ng/g	Measured concentration (n=6) ng/g.	Average % Recovery (n=6)	Recovery %RSD (n=6)
1. Dichlobenil	4.52	0.9988	50	58.7	117.4	2.1
2. Tribromoanisol	6.60	0.9990	50	54.3	108.5	6.0
3. Sulfotep	6.95	0.9984	50	59.8	119.6	2.3
4. Hexachlorobenzene	7.49	0.9983	50	55.2	110.4	2.8
5. Parathion	10.90	0.9979	50	53.0	106.0	5.9
6. Triphenylphosphine (IS)	13.41	-	-	-	-	-
7. EPN	17.90	0.9985	50	46.1	92.1	6.7
8. Azinphos methyl	19.20	0.9984	50	37.6	75.2	4.9
9. Permethrin isomer a	21.38	0.9987	50	49.8	99.5	8.9
10. Permethrin isomer b	21.58	0.9985	50	50.9	101.9	4.8
11. Fenvalerate isomer a	24.60	0.9985	50	47.7	95.4	8.9
12. Fenvalerate isomer b	25.02	0.9973	50	50.6	101.2	7.2
13. Deltamethrin	25.84	0.9949	50	51.7	103.3	6.8

Table 2: Summary of Results
Unidentified peaks in Figure 2 are impurity/matrix peaks

thermoscientific.com/tracegold

USA and Canada +1 800 332 3331
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20 or 21
United Kingdom +44 (0)1928 534110
Japan +81 3 5826 1615

China +86 21 68654588 +86 10 84193588
+86 20 83145199 800 810 5118
India +91 22 6742 9494 +91 27 1766 2352
Australia 1 300 735 292 (free call domestic)
New Zealand 0800 933 966 (free call domestic)
All Other Enquiries +44 (0) 1928 534 050

Technical Support
North America +1 800 332 3331
Outside North America +44 (0) 1928 534 440

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

AN20549_E 07/12S