GC/MS analysis of pesticides in grapes using QuEChERS sample extraction

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Overview

Purpose: To demonstrate QuEChERS dispersive SPE as a simple, fast and quantitative sample preparation method for the GC/MS analysis of pesticides in grapes. Additionally, GC column selection is considered by comparing the method performance of a generic 5% phenyl polysilphenylene-siloxane phase column with a pesticide dedicated column.

Introduction

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive Solid Phase Extraction (SPE) technique for extracting multi-residues of pesticides from fruits and vegetables. The advantages of this methodology are speed,ease of execution, minimal solvent requirement and cost to perform when compared with conventional solid phase extraction techniques.

The QuEChERS methodology was developed by Anastassiades et al and has become widely used in food safety analyses.[1] The method is:

- Quick – high sample throughput, typically 8 samples can be prepared in just under 30 min
- Easy – it requires less handling of extracts than other techniques and no laborious steps are involved
- Cheap – less sorbent material is needed and less time is need to perform compared to other techniques
- Effective – the simple technique gives high and accurate recovery levels for a range of different compound types e.g polar pesticides, pH dependent compounds
- Rugged – the method can detect a large number of pesticides including pH dependent and polar pesticides
- Safe – unlike other techniques, it does not require any chlorinated solvents. Extraction is typically carried out using acetonitrile, which is both GC and LC amenable.

The QuEChERS procedure is usually a two stage process: sample extraction, followed by dispersive SPE. In the sample extraction stage, the food sample is homogenized to maximize the available surface area of the sample for better extraction efficiencies. The homogenized sample is placed in the extraction tube containing magnesium sulphate and sodium acetate. 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, an exothermic reaction occurs between the magnesium sulphate and water, which can lead to low recoveries of the pesticides. This effect can be reduced by adding the salt and sample to the extraction tube while this is immersed in an ice bath or by weighing the sample into a FEP tube and then adding the solvent and salts slowly. The tube is then capped, shaken vigorously and centrifuged. 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. This may be followed by solvent exchange to improve compatibility of samples to GC analysis, and additional sample clean-up to reduce matrix effects and therefore improve method robustness. Internal standards are used to minimize errors that might be introduced in the different steps of the QuEChERS method, as well as compensate for GC injection variability.

The pesticides analyzed are a mixture of organophosphate, organochlorine, pyretheroid, benzenoid, triazole and dicarboximide compounds. Lehotay reviewed the LC and GC analyses of pesticides in produce and the type of pesticide that is likely to be found in each matrix.[2] The requirements for pesticide residue analysis in fruit and vegetables are established by organizations such as World Health Organization, Japanese Food Chemical Research Foundation, EEC Directives, and the US-EPA.[3, 4, 5, 6] These organizations establish which pesticides need to be determined in different produce and the Method Regulatory Limits (MRLs). The pesticides determined in this study are all listed by the four regulatory organizations and all have minimum MRLs of 50 ng/g (ppb). The recoveries of the pesticides in grapes are based on this value.

Methods

Sample Preparation

Reagents

Green grapes obtained from the local supermarket; Acetonitrile and Glacial acetic acid (HPLC grade); Hexane and Acetone (GC grade).

Apparatus

Thermo Scientific Reacti-Therm III Heating/Stirring System; Thermo Scientific Reacti-Vap III Evaporator; Thermo Scientific Reacti-Block; Thermo Scientific Reacti-Vial small reaction vials 10 mL; Centrifuge; Vortex.

QuEChERS Tubes

Stage 1: 50 mL PP tubes containing 6g MgSO4,1.5 g anhydrous CH3COONa

Stage 2: 15 mL ENVIRO tubes containing 900 mg MgSO4, 300 mg PSA (primary/secondary amine) 150 mg C18

Stage 3: 2 mL tubes containing 150 mg MgSO4, 50 mg PSA

Methodology

The methodology described in this application note is for the preparation of calibration standards and produce sample spike (Figure 1). For a fully validated QuEChERS method in extracting multi-residue pesticide, see technical note 10222.7

Extraction

(Steps 1 to 5 in the flow diagram in Figure 1)

The sample was homogenized using a blender and 15 g of it was weighed into the FEP extraction tube, followed by the addition of 15 mL of 1% acetic acid in acetonitrile.

The MgSO4 and sodium acetate mixture in the QuEChERS extraction tube was poured very slowly into the FEP tube. No significant difference in the recoveries was observed when the homogenized grapes were directly weighed into the extraction tube containing the salts, that had been placed in an ice bath. This was demonstrated for the grapes matrix only but it may differ for other fruit and vegetable matrices. The pesticides standard mixture was spiked into the FEP tube for calculating recoveries for produce sample spike. For the calibration standards, the pesticide standard mixture was not spiked at this first stage of the QuEChERS method. The tube was capped, shaken vigorously for 5 minutes, and centrifuged at 3000 rpm for 5 minutes. The solids are separated from the supernatant, which contains the pesticides.

Dispersive SPE

The dispersive SPE stage consists of three steps: the initial clean up (steps 6 to 7 in the flow diagram in Figure 1), the solvent exchange (steps 8 to 5) and the final clean up (steps 12 to 14). 11mL of the supernatant (acetonitrile extract) was transferred to a stage 2 clean up tube containing the C18 sorbent, MgSO4 and PSA. The sample tube was capped and centrifuged at 3000 rpm for 5 minutes. 5 mL of the supernatant was transferred to a 10 mL vial, which was placed into a Dry Block Sample Incubation System for evaporation of the solvent under a stream of nitrogen at 40 °C for 1 hour. The extract was reconstituted in hexane/acetone 9:1, which is a more acquiescent solvent for GC splitless injection. For the produce sample spike 0.9 mL of hexane/acetone 9:1 was added and for calibration sample 0.8 mL was added. This was followed by the addition of internal standard. At this point, calibration standards were also prepared by spiking pesticide standards in various concentrations to each vial. The produce sample spike was prepared in triplicates whereas seven point calibration standards were prepared. In the final clean up step, 1 mL of sample was transferred to a clean up dispersive SPE tube containing C18, MgSO4 and PSA. This extra clean up step was performed to reduce the matrix effect. The tube was capped, vortexed centrifuged. 500 µL of the supernatant was transferred to a silanised GC vial for analysis.

GC/MS Instrumentation and Methodology

Thermo Scientific Instruments: TRACE GC Ultra, DSQ II single quadrupole mass spectrometer, TriPlus autosampler Xcalibur software was used to process the data.

Columns

Thermo Scientific TRACE TR-5MS 30 m x 0.25 mm x 0.25 µm analytical column with 5 m x0.25 mm guard, and TRACE TR-Pesticide 30m x 0.25mm x 0.25µm analytical column with 5m integral guard.

GC/MS Method

Autosampler: Sample volume: 2 µL; Sample rinses: 5; Pre and post Injection solvent wash cycles: 5; Pre inject solvent A: 1:1 Hexane/Acetone; Post inject solvent B: Acetonitrile/;

GC

Oven program: 40 °C (hold for 1.5 min), 25 °C/min to 150 °C; 7 °C/min to 225 °C; 25 °C/min, to 290 °C (hold for 10 min).

Injector: 250 °C, Splitless with pressure surge 250 kPa; Inject time: 0.5 min; Split flow: 50 mL/min

Column flow: 1 mL/min, constant flow

Transferline temperature: 290 °C

MS

Source temperature: 250 °C; Ion volume: Closed EI; Electron energy: -70 V;

Scan parameters:

Seg 1: Start 14.80 min, m/z 125, 197, 286, Dwell 100 ms;

Seg 2: Start 15.10 min, m/z 115, 114, 160, 201, 249, 279,Dwell 50 ms

Seg 3: Start 15.80 min, m/z 127, 158, 173, 197, 258, 314, Dwell 50 ms

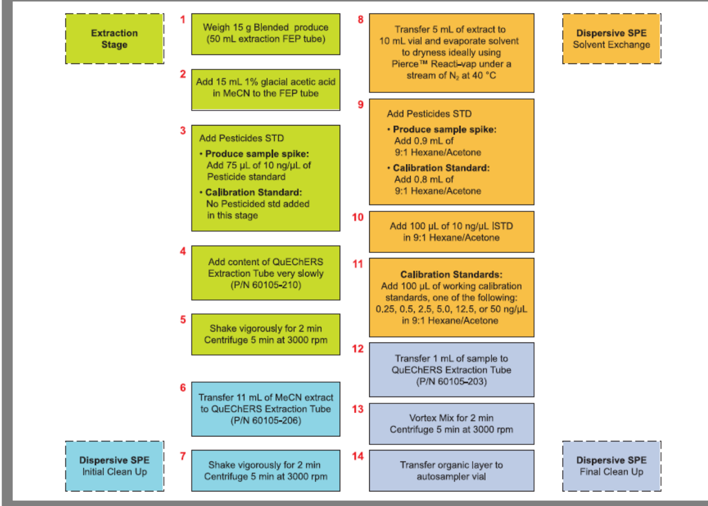
Seg 4: Start 17.00 min, m/z 96, 159, 213, 248, 255, 283, Dwell 50 ms

Seg 5: Start 19.40 min, m/z 169, 233, 326, Dwell 100 ms

Seg 6: Start 20.00 min, m/z 111, 139, 251, Dwell 100 ms

Seg 7: Start 21.10 min, m/z 127, 163, 183, Dwell 100 ms

FIGURE 1. Flow diagram of QuEChERS methodology used in this application.



Results

The application specific column, TR-Pesticide provided better quantitative data than the generic column, particularly for the organochlorinated pesticides, chlorpyrifos and chlorpyrifos methyl. The pesticide column is specifically deactivated for this type of analyte. Comparative total ion chromatograms on the two columns for the injection of a sample spike at 1ng/µL level are shown in Figure 2. Further data presented was obtained with the pesticide specific column.

In order to assess the method linearity, a calibration curve was constructed for each of the eight pesticides spiked in the sample matrix, using triphenylphosphate as the internal standard. The concentration range studied was 0.005 to 5 ng/µL. The correlation coefficients (R^2) between area ratio of sample and internal standard for all pesticides were higher than 0.99 (Table 1), demonstrating good method linearity. Example of calibration plot shown in Figure 3.

FIGURE 2. TIC for the GC/MS analysis of grapes spiked with1 ng/µL of each pesticide: (a) On TR-Pesticide column (b) On TR-5MS column.

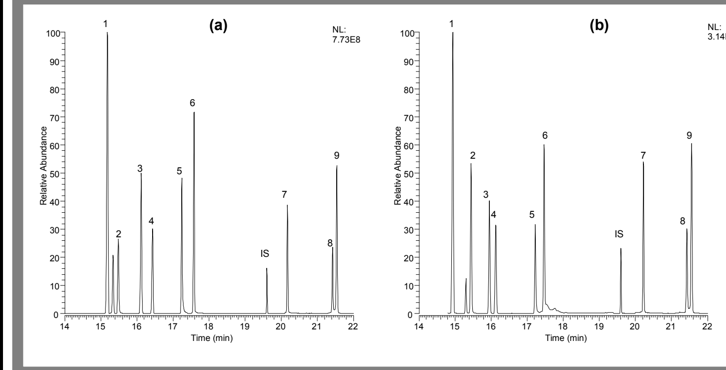
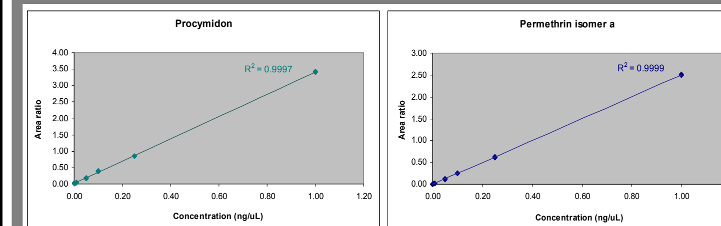


Table 1. Summary of results on a TRACE TR-Pesticide column

Pesticide	Retention time (min)	Linearity	%Recovery (n=3)	%RSD (n=3)
1. Chlorpyrifos methyl	15.16	0.9942	107	14.8
2. Metalaxyl	15.88	0.9967	110	6.7
3. Malathion	16.10	0.9980	109	17.7
4. Chlorpyrifos	16.42	0.9925	106	9.5
5. Penconazole	17.28	0.9925	103	9.9
6. Procymidon	17.56	0.9997	102	6.9
7. Dicofol	20.16	0.9933	112	9.4
8. Permethrin Isomer a	21.42	0.9999	104	8.2
9. Permethrin Isomer b	21.53	0.9998		

FIGURE 3. Calibration plot for procymidon and permethrin.



The average extraction recoveries of the pesticides analysed in the TR-Pesticide column was 107% with an average %RSD of 10.4.

Conclusions

- 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 0.005 to 5 ng/µL spiked matrix which includes the MRLs of 50 ppb (0.05 ng/µL).
- The application specific TRACE TR-Pesticide column provided good chromatographic resolution of the pesticides studied and demonstrated higher inertness towards the analytes than the generic 5% phenyl polysilphenylene-siloxane column.

References

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7. Jessie Butler, David Steineger and Eric Philips. Thermo Fisher Scientific, Austin, TX, USA. Technical note 10222.

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