Extraction and Analyses of Agricultural Pesticides from Oranges Using the "QuEChERS" Method

Katherine Stenerson, Robbie Wolford, and Olga Shimelis

kstenerson@sial.com

Introduction

The toxicological effects of the chemicals which humans and animals are exposed to daily are of ever-increasing concern. In the last few years, emphasis has been placed on a group of chemicals loosely referred to as endocrine disruptors; mostly man-made compounds suspected of interfering with the body's hormone system (1) by blocking or mimicking normal function. One of the avenues for human exposure to these compounds is through the consumption of agricultural products that have been treated with pesticides. These pesticides may have been used as insecticides, fungicides, or herbicides during growth, transportation and storage stages.

A number of methods currently exist for the extraction and analyses of multi-residue pesticides from a variety of food matrices (2,3). A new method, known as the "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, has recently been introduced (4) and subsequently improved (5,6). This method employs dispersive solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) techniques.

"Dispersive SPE" Procedure

With typical SPE methods, sample is passed through a tube that contains sorbent, and retained analytes are eluted with solvent. In dispersive SPE, organic solvent is mixed with a sample, and gram levels of salts and sorbent are added to drive partitioning of the analytes between the aqueous residues and the solvent. An aliquot of the organic solvent is then removed and mixed with additional salts and sorbent as an additional cleanup step. This procedure requires less time than traditional SPE, and simultaneously removes residual water and matrix interferences. After a simple vortex and centrifugation step, the supernatant is ready for analysis.

The improved QuEChERS method published by Lehotay (6) was used for the extraction of 29 different agricultural pesticides from oranges. The oranges used for the extractions were obtained from a local grocery store and were not labeled as "organic." Four extracts were prepared from orange skins according to the procedure summarized in Table 1. An extract spiked only with internal standard at 100 ppb served as a control. Three replicate extracts were spiked with each pesticide plus the internal standard (each at 100 ppb) and used to determine the accuracy and precision of the method. The final extracts were solvent exchanged from acetonitrile to toluene to increase the sensitivity of the GC-MS analysis. Vials containing pre-weighed salts and sorbent were used to perform the extraction and cleanup procedures. These vials were produced in-house, and are currently available as custom items (7).

Table 1. Extraction Procedure

1.	Weigh 15 g of ground-up orange.
2.	Add 75 µL of the internal standard stock solution (ethoprophos at 20 ppm in methanol) to all samples.
3.	Add 75 µL of the pesticide stock solution (29 pesticides, each at 20 ppm in methanol) to the "spike" samples.
4.	Add 15 mL of 1% acetic acid in acetonitrile.
5.	Add 6 g anhydrous magnesium sulfate ($MgSO_4$) and 1.5 g anhydrous sodium acetate.
6.	Shake by hand for 1 minute.
7.	Centrifuge for 2 minutes at 3300 rpm.
8.	Take a 2 mL aliquot of extract.
9.	Add 100 mg primary-secondary amine (PSA) and 300 mg anhydrous magnesium sulfate ($MgSO_4$).
10.	Centrifuge for 2 minutes at 3300 rpm.
11.	Take a 1 mL aliquot of extract and evaporate to 0.1 mL.
12.	Reconstitute in toluene to 1.0 mL using a volumetric flask.
13.	Proceed to GC analysis.

GC-MS Analyses

GC-MS analysis of the extracts described in the previous paragraph was performed on a single quadrupole GC-MS system using selective ion monitoring (SIM). Monitoring ions were chosen based on the spectra of the pesticides taken from a full mass range analysis of a high level standard. An SLB™-5ms capillary column was chosen for the analysis due to its low bleed and high inertness characteristics, resulting in its ability to detect the pesticides at a low level (8,9). Complete GC-MS conditions are listed in Figure 1. A five-point calibration using matrix-matched standards was performed prior to analyses of the extracts.

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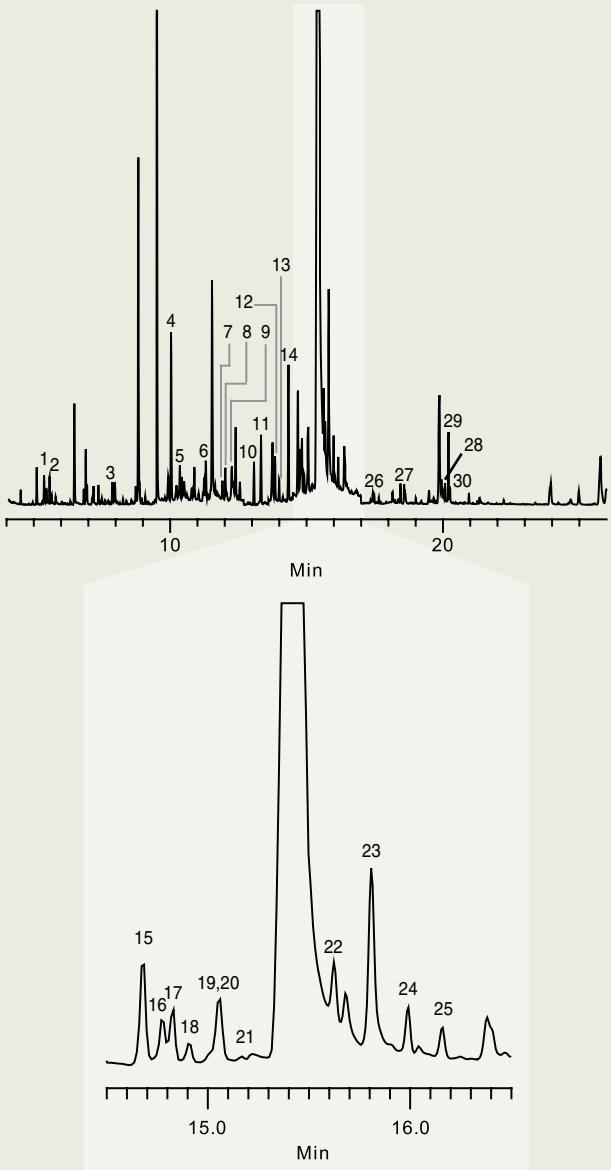
Figure 1. Extract of Spiked Orange Sample

column: SLB-5ms, 30 m x 0.25 mm I.D, 0.25 µm (28471-U)
oven: 100 °C (1 min.), 10 °C/min. to 300 °C (5 min.)
inj.: 250 °C
MSD interface: 300 °C
scan range: selected ion monitoring (SIM), 7 monitoring groups used
carrier gas: helium, 1 mL/min constant
injection: 1 µL, pulsed (20 psi until 0.20 min.), splitless (1.0 min.)
liner: 4 mm I.D., single taper

1. Methamidiphos
2. Dichlorvos
3. Acephate
4. Propoxur
5. Ethoprophos (I.S.)
6. Hexachlorobenzene
7. γ-BHC
8. Diazinon
9. Chlorothalonil
10. Methyl chlorpyrifos
11. Carbaryl
12. Dichlofluanid
13. Chlorpyrifos
14. p-Dichlorobenzophenone
15. Cyprodinil
16. Pencanazole
17. Tolylfluanid
18. Heptachlor epoxide
19. Captan
20. Thiabendazole
21. Folpet
22. cis-Chlordane
23. Imazalil
24. 4,4'-DDE
25. Dieldrin
26. Endosulfan sulfate
27. Dicofol
28. cis-Permethrin
29. trans-Permethrin
30. Coumaphos



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Results

Chromatograms of the spiked orange samples are presented in Figure 1. Several background peaks eluting prior to nine minutes are due to impurities in the toluene. Despite extract cleanup, matrix peaks are also present in the chromatograms. Further sample cleanup may be possible by increasing SPE sorbent weight. Nevertheless, all pesticides were detected. Calibration, recovery, and precision data are presented in Table 2. A first order fit was used for calibration. Linearity for the five-point calibration curves was excellent, with 28 of the 29 pesticides having r^2 values >0.995 at a range of 50-500 ppb. Proper calibration of imazalil was not possible due to its presence in the orange blanks.

Several pesticides were tentatively detected in the orange blanks. The identity of imazalil was confirmed spectrally by re-analyzing the sample in the full scan mode. The peak was beyond calibration range, and was

Table 2. Calibration and Recovery Results

Analyte	r^2 Value	Average Recovery (%)	% RSD n=3
Methamidiphos	0.999	86	5
Dichlorvos	0.999	96	13
Acephate	0.998	94	4
Propoxur	0.999	98	6
Hexachlorobenzene	0.998	98	20
γ-BHC	0.998	105	14
Diazinon	0.999	105	19
Chlorothalonil	0.997	90	10
Methyl chlorpyrifos	0.999	108	13
Carbaryl	0.999	113	12
Dichlofluanid	0.999	102	8
Chlorpyrifos	0.999	109	11
p-Dichlorobenzophenone	0.999	103	7
Cyprodinil	0.999	103	12
Pencanazole	0.999	109	7
Tolylfluanid	0.998	91	13
Heptachlor epoxide	0.998	102	18
Captan	0.997	91	42
Thiabendazole	0.999	76	34
Folpet	0.999	111	13
cis-Chlordane	0.998	102	22
Imazalil	0.969	335	26
4,4'-DDE	0.998	100	17
Dieldrin	0.998	104	11
Endosulfan sulfate	0.998	102	12
Dicofol	0.995	151	27
cis-Permethrin	0.999	118	6
trans-Permethrin	0.999	112	5
Coumaphos	0.999	114	7

therefore, not quantified. Imazalil is a post-harvest fungicide that is commonly used on citrus, so it is not unreasonable for it to be present. Peaks corresponding to the retention times of dicofol and captan were detected in the orange blank extracts but their low levels did not allow mass spectral confirmation in a subsequent full scan mode analysis. Because of their possible presence in the oranges prior to spiking, the recovery values for imazalil and dicofol were much higher than expected (335% and 151%, respectively).

Overall, recovery and precision were generally good averaging at 101.6 ± 13.4% for 27 of the 29 pesticides tested.

Conclusion

The QuEChERs method is an emerging extraction approach within area of food quality/safety analysis, and proved to be fairly simple and easy to perform. Table 3 lists the available sorbents and salts commonly used in dispersive SPE. The use of vials containing pre-weighed salts and SPE sorbent eliminated the need for a chemist to spend time performing this task. Sorbent weighing could be a time consuming bottleneck for food safety laboratories that need to perform hundreds of these extractions. For the GC-MS analysis, the SLB-5ms column provided adequate inertness and low bleed, allowing for low level detection of these pesticides.

Table 3. Available SPE Sorbents and Salts Commonly Used in Dispersive SPE[1]

Florisil® (57209)	C18 SPE (52600-U)
NH_2 SPE (57212-U	PSA SPE (52738-U
SAX SPE (57214-U)	ENVI-Carb™ (graphitized carbon black) (57210-U)
Sodium acetate (24,124-5)	Magnesium sulfate (23,039-1)
Sodium sulfate (23,859-7)	Sodium chloride (S 9888)

[1] Catalog numbers in parentheses are for bulk quantities of 100 g or greater.

References

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Featured Products

Description	Qty.	Cat. No.
SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm	1	**28471-U**

Related Products

Description	Qty.	Cat. No.
Supelclean™ ENVI-Carb II/PSA SPE Tubes		
300 mg/600 mg/6 mL	30	**54058-U**
500 mg/300 mg/6 mL	30	**55119-U**
500 mg/500 mg/6 mL	30	**54067-U**
500 mg/500 mg/20 mL	20	**54217-U**
Supelclean ENVI-Carb II/SAX/PSA SPE Tubes		
500 mg/500 mg/500 mg/12 mL	20	**52574-U**
Supelclean SAX/PSA SPE Tubes		
250 mg/250 mg/6 mL	30	**52576-U**
500 mg/500 mg/6 mL	30	**52577-U**
Supelclean PSA SPE Tubes		
200 mg/3 mL	54	**52578-U**
500 mg/6 mL	30	**52579-U**
SLB-5ms Capillary Columns		
10 m x 0.10 mm I.D., 0.10 µm	1	**28465-U**
15 m x 0.10 mm I.D., 0.10 µm	1	**28466-U**
20 m x 0.18 mm I.D., 0.18 µm	1	**28564-U**
12 m x 0.18 mm I.D., 0.30 µm	1	**28566-U**
30 m x 0.18 mm I.D., 0.30 µm	1	**28575-U**
20 m x 0.18 mm I.D., 0.36 µm	1	**28576-U**
30 m x 0.20 mm I.D., 0.20 µm	1	**28513-U**
30 m x 0.25 mm I.D., 0.10 µm	1	**28467-U**
15 m x 0.25 mm I.D., 0.25 µm	1	**28469-U**
60 m x 0.25 mm I.D., 0.25 µm	1	**28472-U**
15 m x 0.25 mm I.D., 0.50 µm	1	**28577-U**
30 m x 0.25 mm I.D., 0.50 µm	1	**28473-U**
60 m x 0.25 mm I.D., 0.50 µm	1	**28474-U**
30 m x 0.25 mm I.D., 1.0 µm	1	**28476-U**
15 m x 0.32 mm I.D., 0.25 µm	1	**28557-U**
30 m x 0.32 mm I.D., 0.25 µm	1	**28482-U**
30 m x 0.32 mm I.D., 0.32 µm	1	**28532-U**
15 m x 0.32 mm I.D., 0.50 µm	1	**28597-U**
30 m x 0.32 mm I.D., 0.50 µm	1	**28484-U**
30 m x 0.32 mm I.D., 1.0 µm	1	**28487-U**

Related Information

For more information on Supelco Low Bleed SLB-5ms capillary columns, request T405130 (IKA) or visit **sigma-aldrich.com/slb**

For more information on SPE products from Supelco, see page 14.

Did you know...?

Supelco can provide vials containing pre-weighed amounts of the salts and SPE sorbent(s) mentioned in this article. For example, two sets of vials can be prepared to support the method described in this article. Each vial in the first set would contain 6 g anhydrous magnesium sulfate plus 1.5 g anhydrous sodium acetate. Each vial in the second set would contain 50 mg primary-secondary amine plus 150 mg anhydrous magnesium sulfate. Simply contact 800-SUPELCO (US and Canada only) or your local Sigma-Aldrich sales office to request a quotation for these, or any other custom dispersive SPE products.