

# The Extraction and Analysis of Multi-Residue Pesticides in Orange Using Dispersive SPE and GC-MS

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# Introduction

- Multi-residue surveillance of pesticides in agricultural products is an ongoing project for regulatory agencies, contract laboratories, and industrial laboratories worldwide (1).
- A number of methods exist for extraction and analysis.
- Further clean-up using SPE typically necessary to decrease background levels for trace pesticide detection, reduce matrix-induced signal enhancement, and relieve stress/reduce downtime on the GC system (2).
- In 2003, Anastassiades et al. introduced a novel sample prep approach called “dispersive SPE” (3).
- In a follow up study, Lehotay et al. demonstrated the method’s effectiveness by extracting over 200 pesticides from lettuce and orange for both GC-MS and LC-MS (4).



## Introduction (contd.)

- In “dispersive SPE”, a food sample is initially extracted with solvent (e.g. acetonitrile).
- Gram levels of salt (magnesium sulfate, sodium sulfate, sodium citrate, and/or sodium chloride) is added to drive phase separation between aqueous residues and acetonitrile layer.
- SPE clean-up is done by mixing bulk SPE and magnesium sulfate with an aliquot of the acetonitrile layer.
- After a simple vortex and centrifugation step, the supernatant is ready for further GC-MS analysis.
- In this study, we demonstrate the effectiveness of this method by extracting 29 agricultural pesticides using pre-weighed salt and SPE vials that are commercially available from Supelco (Figure 1).



# Figure 1. Supelco Pre-Weighed Salt/Sorbent Vials for Dispersive SPE



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## Experimental

- “Non-organic” oranges were purchased from a local grocery store.
- Four extracts were prepared from orange skins according to procedure summarized in Table 1.
- An extract spiked only with I.S. (100 ppb) served as a blank control.
- Three replicate extracts were spiked with each pesticide plus I.S. (each at 100 ppb) and used to determine method accuracy and precision.
- Final extracts were solvent exchanged from acetonitrile to toluene prior to GC-MS to improve sensitivity.



## Table 1. Extraction Procedure

1. Weigh 15 g of ground orange
2. Add 75  $\mu$ L I.S. stock (ethoprophos at 20 ppm in methanol) to all samples
3. Add 75  $\mu$ L of pesticide stock solution (29 pesticides, each at 20 ppm in methanol) to the “spike” samples
4. Add 15 mL 1% acetic acid in acetonitrile
5. Empty “dispersive” vial containing 6 g anhydrous magnesium sulfate and 1.5 g anhydrous sodium acetate into extract
6. Shake by hand for 1 min. Centrifuge for 2 minutes at 3300 rpm



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## Table 1. Extraction Procedure (contd.)

7. Empty “dispersive” vial containing 100 mg PSA (primary-secondary amine) SPE and 300 mg anhydrous magnesium sulfate into 2 mL aliquot of acetonitrile supernatant
8. Vortex and centrifuge for 2 min. at 3300 rpm
9. Evaporate 1 mL aliquot of supernatant to 0.1 mL
10. Reconstitute in toluene to 1 mL using a volumetric flask
11. Proceed to GC-MS analysis



# GC-MS Analysis

- Analysis performed on a single quadrupole GC-MS system using selective ion monitoring (SIM).
- Monitoring ions were chosen based on spectra of the pesticides taken from a full mass range analysis of a high level standard.
- A Supelco SLB™-5ms capillary column was chosen due to its low bleed and high inertness characteristics allowing for lower LOQ.
- A five point calibration using matrix-matched standards was performed prior to analyses of the extracts.
- Complete GC-MS conditions are listed in Table 2.

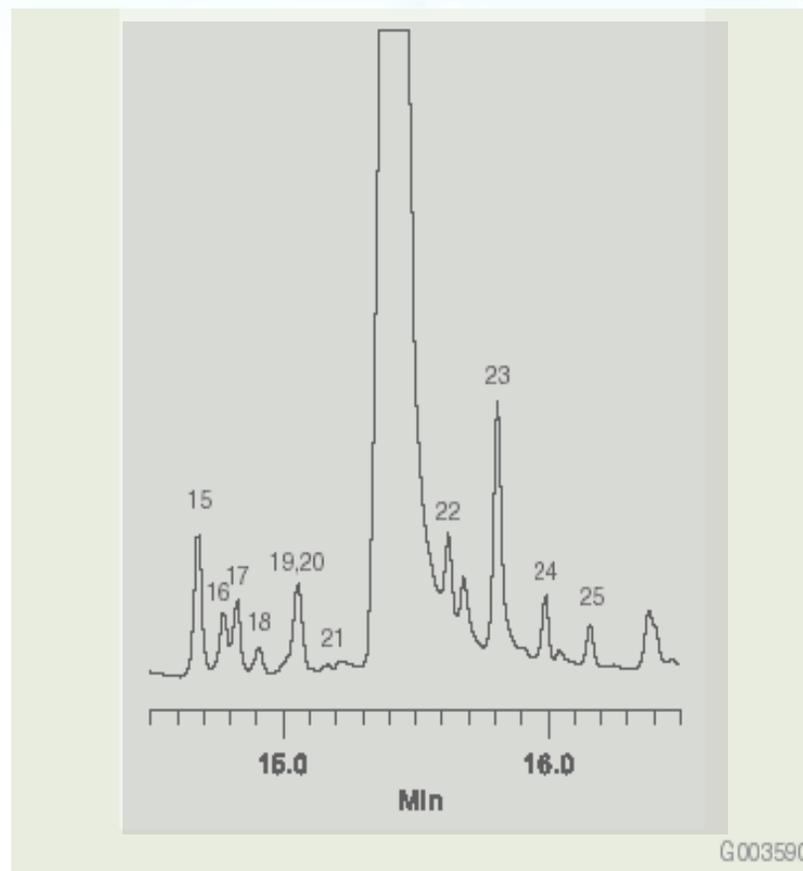
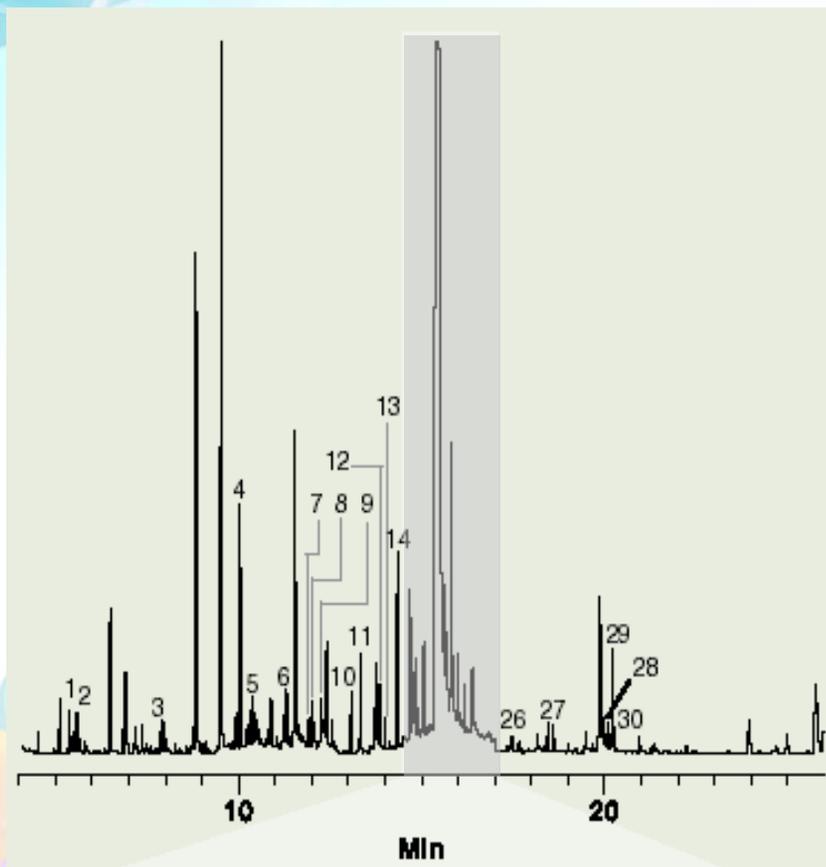


## Table 2. GC-MS Conditions

column:	SLB-5ms, 30 m x 0.25 mm I.D.
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 min.)
liner:	4 mm I.D., single taper



# Figure 2. Extract of Spiked Orange Sample



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## Table 3. Calibration and Recovery Results

Analyte	r <sup>2</sup> value	Average Recovery (%)	% RSD (n=3)
1. Methamidiphos	0.999	86	5
2. Dichlorovos	0.999	96	13
3. Acephate	0.998	94	4
4. Propoxur	0.999	98	6
5. Ethoprophos (I.S.)			
6. Hexachlorobenzene	0.998	98	20
7. $\gamma$ -BHC	0.998	105	14
8. Diazinon	0.999	105	19
9. Chlorothalonil	0.997	90	10
10. Methyl chlorpyrifos	0.999	108	13
11. Carbaryl	0.999	113	12



## Table 3. Calibration and Recovery Results (contd.)

Analyte	r <sup>2</sup> value	Average Recovery (%)	% RSD (n=3)
12. Dichlofluanid	0.999	102	8
13. Chlorpyrifos	0.999	109	11
14. p-Dichlorobenzophenone	0.999	103	7
15. Cyprodinil	0.999	103	12
16. Pencanazole	0.999	109	7
17. Tolyfluanid	0.998	91	13
18. Heptachlor epoxide	0.998	102	18
19. Captan	0.997	91	42
20. Thiabendazole	0.999	76	34
21. Folpet	0.999	111	13
22. cis-Chlordane	0.998	102	22



## Table 3. Calibration and Recovery Results (contd.)

Analyte	r <sup>2</sup> value	Average Recovery (%)	% RSD (n=3)
23. Imazilil	0.969	335	26
24. 4,4'-DDE	0.998	100	17
25. Dieldrin	0.998	104	11
26. Endosulfan sulfate	0.998	102	12
27. Dicofol	0.995	151	27
28. cis-permethrin	0.999	118	6
29. trans-permethrin	0.999	112	5
30. Coumaphos	0.999	114	7



## Results

- Chromatograms of spiked orange samples presented in Figure 2.
- Several background peaks eluting prior to 9 min. due to impurities in toluene.
- Despite extract cleanup, matrix peaks 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 presented in Table 3.



## Results (contd.)

- A first order fit was used for calibration.
- Linearity for the five-point calibration was excellent, with 28 of the 29 pesticides have  $r^2$  values  $> 0.995$  at a range of 50-500 ppb.
- Several pesticides tentatively detected in the orange blanks.
- Identity of imazilil was confirmed spectrally by re-analyzing the sample in full scan mode.
- The imazilil peak was beyond calibration range, and was therefore, not quantified.
- Imazilil is a post-harvest fungicide that is commonly used on citrus.



## Results (contd.)

- Peaks corresponding to the retention times of dicofol and captan were detected in the orange blank extracts but their low levels did not allow for mass spectral confirmation in the full scan mode.
- As a result, recovery values for imazilil and dicofol were much higher than expected (335% and 151%, respectively)
- Overall, recovery and precision were generally good averaging at  $101.6 \pm 13.4\%$  for 27 of the 29 pesticides tested.



## Conclusion

- The QuEChERS method (aka, “dispersive” SPE) is an emerging extracting approach within the area of food quality/safety analysis.
- Table 4 lists the available sorbents and salts commonly used in dispersive SPE.
- Although the technique offers some advantages over conventional methods, it requires the analyst to weigh a pre-determined amount of salts and SPE sorbents for each sample.
- The use of commercially available pre-weighed salts and SPE sorbents can alleviate this potential time consuming bottleneck when greater throughput is required.
- Capillary columns of adequate inertness and low bleed is necessary for low level detection of pesticides in food samples.



## Table 4. Common SPE Sorbents and Salts Commonly Used in Dispersive SPE

Florisil (57209)	Magnesium sulfate (23,039-1)
NH <sub>2</sub> SPE (57212-U)	Sodium chloride (S 9888)
SAX SPE (57214-U)	Sodium citrate (32320)
PSA SPE (52738-U)	Sodium acetate (24,124-5)
ENVI-Carb (graphitized carbon black) (57210-U)	Sodium sulfate (23,859-7)
C18 SPE (52600-U)	



## References

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