Identification of Pesticide Residues in Essential Oils by QuEChERS Extraction and GC/MS Analysis

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Introduction

Essential oils are used throughout many industries such as pharmaceuticals, cosmetics, aromatherapy and food. Residual levels of chemicals used in citrus crops are regulated because of their impact on public health and the environment. Another aspect is illegal "organic" product distribution. Organic product misrepresentation is a disturbing and serious problem which leads to consumers paying premium prices for non-organic product. It is believed that the pesticides are mainly located in the peel of the citrus fruit, considered as a protective layer. Essential oils are extracted from the peel. It takes several kilograms of extracted fruit peel to obtain several milliliters of the essential oils. The concentration of pesticides in the essential oils is thus much higher than in the fruit itself. This application describes a new, simple and rapid extraction procedure for the pesticide residues in essential oils, lemon. The method involves the QuEChERS extraction and a modified dispersive SPE with analysis by GC/MS. Implementation of the QuEChERS extraction methodology and modified dispersive SPE (d-SPE) enabled analysis of pesticides at 60-150 ppb with RSDs less than 5% on average for the pesticides analyzed.

Experimental

Equipment and Materials

Agilent Gas Chromatogram (Agilent Technologies Inc., USA)
Agilent 5975 Mass Spectrometer (Agilent Technologies Inc., USA)
Lemon Oil (Cedarome Canada Inc., Brossard, Quebec)
SampliQ QuEChERS EN extraction kit p/n: 5982-5650 (Agilent Technologies Inc., USA)
SampliQ QuEChERS AOAC dispersive-SPE kit for fat and pigmented product p/n: 5982-5421 (Agilent Technologies Inc., USA)
Geno/Grinder (SPEX Sample Preparation Inc., USA)
Centra CL3R Centrifuge (Thermo IEC, USA)
Eppendorf microcentrifuge (Brinkman Instruments, USA)

GC Conditions

Inlet: Splitless
Inlet Liner: Helix double taper, deactivated p/n: 5188-5398
Carrier gas: Helium
Inlet pressure: 19.6 psi (constant pressure mode)
Inlet temperature: 250 ºC
Injection volume: 1.0 µL
Purge flow to split vent: 30 mL/min at 0.75 min
Oven temperature program: 70 ºC (1 min), 50 ºC/min to 150 ºC (0 min), 6 ºC/min to 200 ºC (0 min), 16 ºC/min to 280 ºC (6 min)
Column: Agilent J&W HP-5MS Ultra Inert, 15 m x 0.25 mm x 0.25 µm p/n: 19091S-431UI

MS Conditions

Tune file: Atune.u
Mode: SIM (refer to table 1 for settings in detail)
Source: quad transfer line temperature 230 ºC, 150 ºC, and 280 ºC, repectively
Solvent delay: 2.30 min
Multiplier voltage: Autotune voltage

Table 1. Instrument Acquisition Data Used for the Analysis of 26 Pesticides by GC/MS

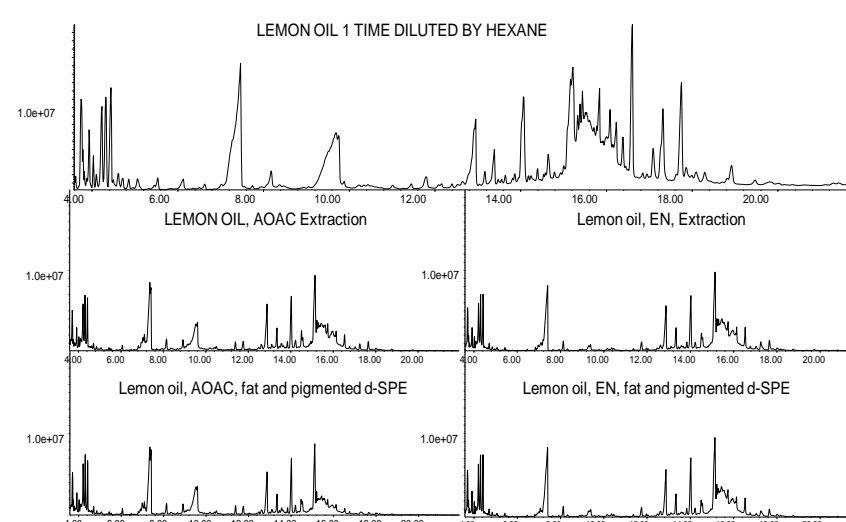| Analyte	Selected Ions for Monitoring	Acquiring window (min)	Analyte	Selected Ions for Monitoring	Acquiring window (min)
(1) Dichlorvos	**184.9**, 219.8	2.3 – 5.55	(14) Parathion	**291.0**, 138.9	8.95 – 9.8
(2) α-BHC	**218.9**, 180.9	5.55 – 6.6	(15) Procymidone	**283.0**, 284.9	9.8 – 11.2
(3) Hexachlorobenzene	**283.9**, 285.9		(16) Endosulfan I	**240.8**, 194.9	
(4) β-BHC	**218.9**, 180.9		(17) Dieldrin	**262.8**, 276.8	11.2 – 11.9
(5) γ-HCH	**218.9**, 263.8		(18) DDE	**317.9**, 245.9	
(6) Disulfoton	**88.0**, 274.0	6.6 – 7.5	(19) Endosulfan II	**194.9**, 236.8	11.9 – 12.8
(7) Chlorpyriphos methyl	**285.9**, 124.0		(20) DDD	**234.9**, 165.0	
(8) Parathion methyl	**262.9**, 233.0	7.5 – 8.3	(21) Endosulfan sulfate	**271.8**, 228.8	12.8 – 13.6
(9) Heptachlor	**271.8**, 100.0		(22) DDT	**234.9**, 165.0	
(10) Fenitrothion	**277.0**, 124.9	8.3 – 8.95	(23) Bromopropylate	**340.8**, 182.9	13.6 – 15.2
(11) Aldrin	**262.8**, 264.8		(24) λ-Cyhalothrin	**181.0**, 196.9	
(12) Malathion	**157.8**, 173.0	8.95 – 9.8	(25) Fenvalerate	**124.9**, 167.0	15.2 – 22.0
(13) Chlorpyriphos	**198.9**, 96.9		(26) Deltmethrin	**181.0**, 252.8	

Experimental
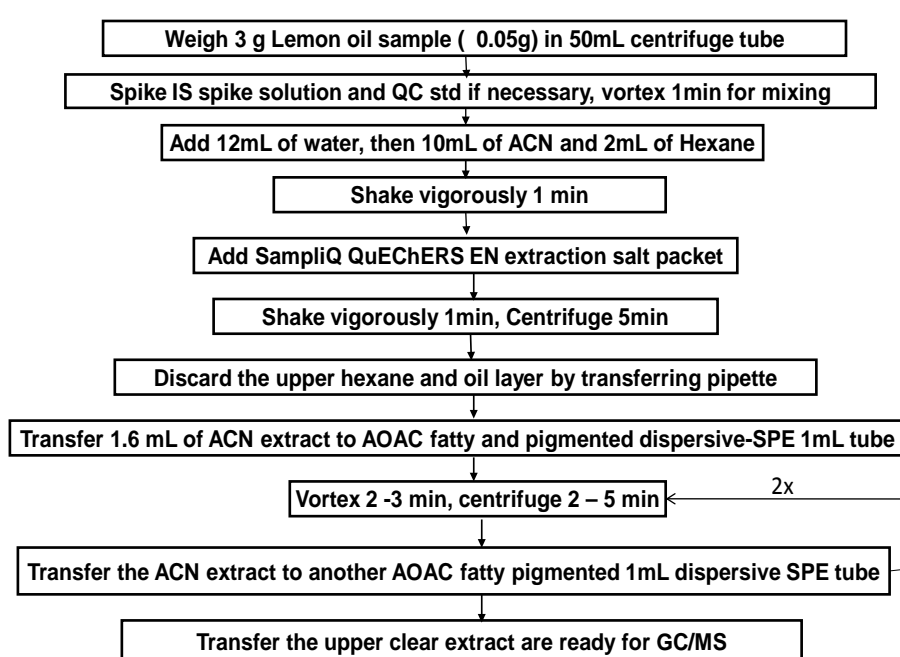
Figure 1. Flowchart of QuEChERS procedure for the determination of pesticides in essential oils

Results and Discussion

Figure 2. Determination of the appropriate extraction salts for the essential oil sample, AOAC and EN buffered extraction salts

- EN extraction kit works better than AOAC extraction kit on the matrix clean-up.
- Please note the big interference bump shown at ~RT 10min. It is much more significant in sample extracted by AOAC extraction kit (acetate salt) relative to the by EN extraction kit.(chloride, and citrate salts)
- Data were acquired under TIC mode.
- EN extraction kit will be used based on the above data
- High amount of GCB (50mg) can remove the late eluted matrix at ~ 16min
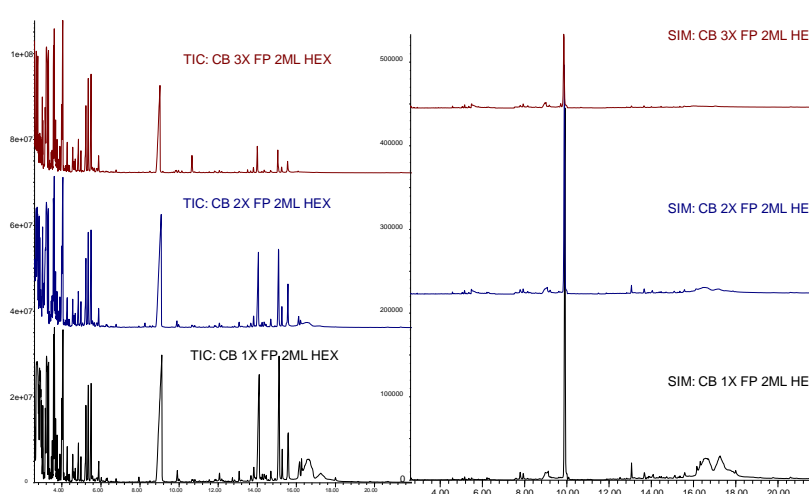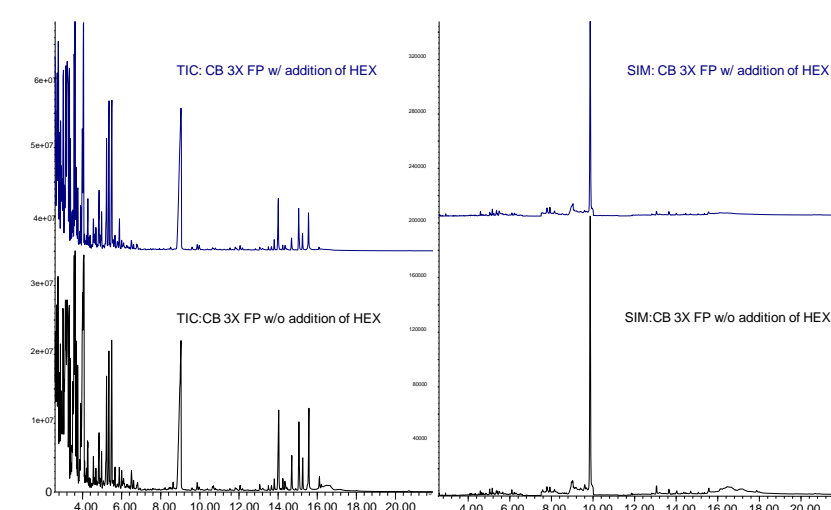

Figure 3. Method development: multiple d-SPE clean-up with AOAC fats and pigmented d-SPE. The late eluting matrix material is significantly reduced with 3 separate AOAC fats and pigmented d-SPE. CB: control blank, FP: fats and pigmented AOAC d-SPE, Hexane: hexane

- Addition of hexane in the extraction tube is helpful in removing late eluted interference.
- 2mL of hexane was added in the extraction tube before adding EN extraction salt packet.
- Addition of hexane changed the density of oil, thus move the oil layer up to the top of ACN layer.
- Addition of hexane may cause some loss of non-polar pesticides, chlorinated pesticides.

Figure 4. Method development: addition of hexane during the extraction step
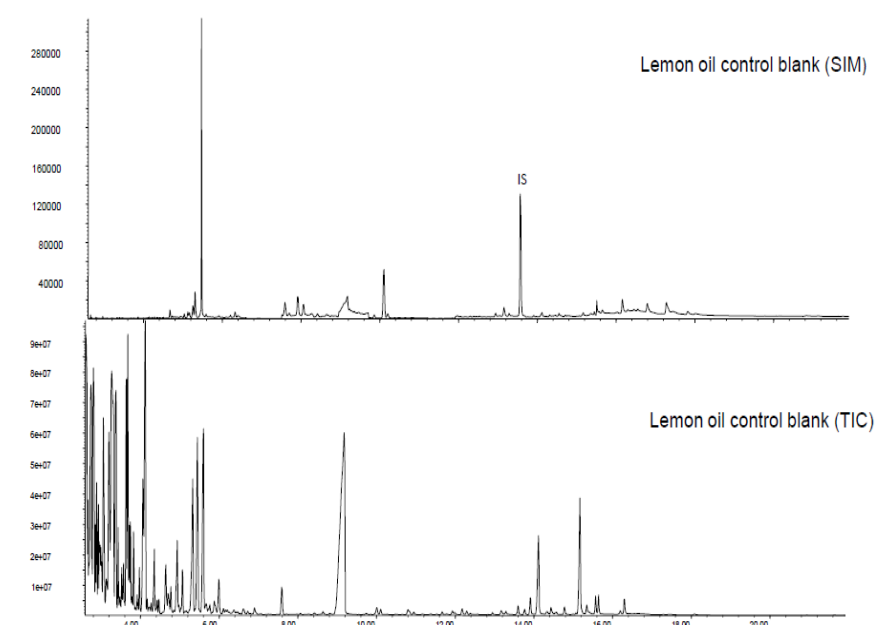
Results and Discussion

Figure 5. GC/MS chromatogram of the lemon oil matrix blank after EN buffered extraction and 2 d-SPE (fats and pigments)
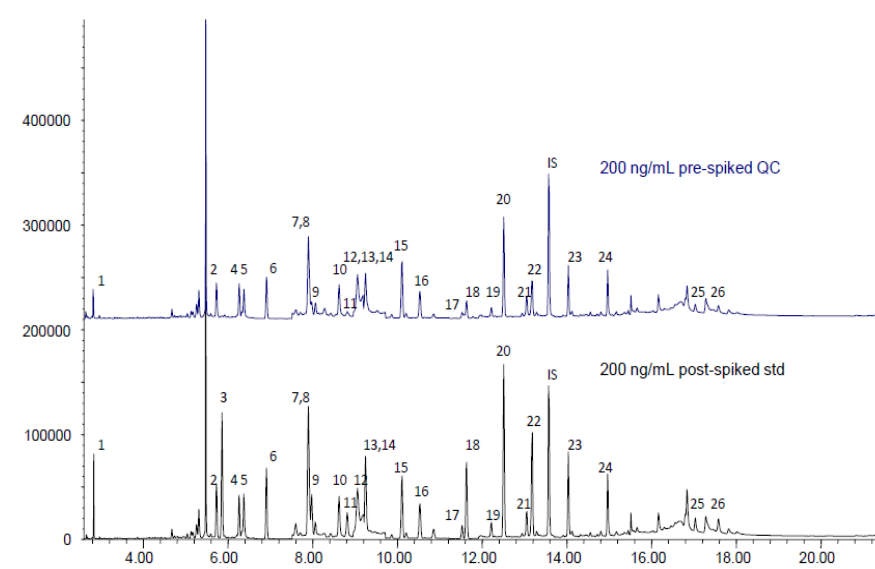

Figure 6. GC/MS chromatograms of 200 ng/mL pre and post-matrix fortified sample. Peak identification: 1. Dichlorvos, 2. α-BHC, 3. Hexachlorobenzene, 4. β-BHC, 5. γ-HCH, 6. Disulfoton, 7. Chlorpyriphos methyl , 8. Parathion methyl, 9. Heptachlor, 10. Fenitrothion, 11. Aldrin, 12. Malathion, 13. Chlorpyriphos, 14. Parathion, 15. Procymidone, 16. Endosulfan I, 17. Dieldrin, 18. DDE, 19. Endosulfan II, 20. DDD, 21. Endosulfan sulfate, 27. DDT, 23. Bromopropylate, 24. λ-Cyhalothin, 25. Fenvalerate, 26. Deltmethrin.

Table 2. Lemon Essential Oil Recovery and Repeatability Results

Pesticides	Low QC (60 ppb)		Mid QC (200ppb)		High QC (800ppb)	
	recovery	RSD % (n=6)	recovery	RSD % (n=6)	recovery	RSD % (n=6)
Dichlorvos	108.7	5.5	74.1	11.5	74.9	12.7
α-BHC	65.0	6.1	69.9	4.7	66.0	2.2
β-BHC	105.6	7.4	76.3	3.4	81.2	3.4
γ-HCH	79.6	12.1	70.6	3.2	67.4	4.0
Disulfoton	75.7	3.0	65.7	3.2	63.9	3.5
Chlorpyriphos methyl	45.1	1.5	46.6	4.0	46.2	3.8
Parathion methyl	74.1	2.6	76.4	2.3	83.6	2.1
Heptachlor	27.6	1.9	31.0	3.2	29.6	3.4
Fenitrothion	70.7	1.1	73.4	2.3	81.0	1.6
Aldrin	13.4	6.5	18.0	2.4	18.0	5.6
Malathion	72.1	5.1	82.9	1.9	86.1	2.4
Chlopyrifos	36.4	4.9	36.0	6.1	36.4	4.1
Parathion	60.9	0.8	68.9	2.0	76.2	2.4
Procymidone	79.8	1.9	77.6	2.0	77.5	1.8
Endosulfan I	38.2	8.1	40.2	2.4	41.0	1.5
Dieldrin	41.4	3.1	47.0	4.2	47.3	2.9
DDE	23.6	1.2	23.3	2.1	23.0	2.8
Endosulfan II	59.3	8.7	64.2	3.1	63.2	1.4
DDD	69.1	0.8	57.0	1.7	56.7	1.6
Endosulfan sulfate	79.5	4.2	84.8	1.9	81.9	1.0
DDT	58.6	1.6	35.2	1.6	34.6	2.4
Bromopropylate	68.8	1.6	59.6	1.8	60.4	1.4
λ-Cyhalothrin	107.4	2.5	68.7	2.5	70.8	2.1
Fenvalerate	80.1	2.3	60.1	2.9	63.1	1.3
Deltamethrin	87.8	0.8	52.8	3.5	56.8	3.7

Conclusions

Agilent's SampliQ QuEChERS EN extraction and AOAC d-SPE for fats and pigmented product provide a simple, fast and effective method for the extraction of pesticides from lemon essential oil. The recovery and reproducibility based on matrix spiked standards were acceptable for multi-class, multi-residue pesticide determination in essential oils. Since the selected pesticides represented a broad variety of different classes and properties, the Agilent SampliQ QuEChERS EN extraction and AOAC d-SPE kit for fats and pigmented products is an excellent choice for pesticides in similar product matrices.