Determination of Pesticides in Lemon Oil via Modified Agilent Bond Elut QuEChERS Method

Application Note

Food Safety

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Abstract

This application note describes a simple and rapid extraction procedure for pesticide residues in lemon essential oils. The method uses Agilent Bond Elut QuEChERS extraction and modified dispersive SPE coupled with GC/MS.

Introduction

Cultivation of citrus crops commonly involves the use of chemicals such as fertilizers and pesticides. It is believed that pesticide residues in citrus fruit are mainly located in the fruit peel which is considered as a protective layer. Essential oils from citrus fruits are used by food, pharmaceuticals, aromatherapy and cosmetic companies. These essential oils are extracted from the citrus peel. Since it can take several kilograms of fruit peel to extract several milliliters of essential oils, the concentration of pesticides in the essential oils could possibly be higher than in the fruit. Therefore, regulations have become increasingly strict on the residual levels of chemicals used for crop treatments because of their impact on public health and the environment. To detect these chemicals researchers have developed liquid/solid phase extraction methods with analysis by GC/MS and or LC/MS [1].

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The QuEChERS method for pesticide analysis was first introduced by USDA scientists in 2003 [2]. The method was modified to address some problematic pesticides by including a buffered extraction system, resulting in the official methods AOAC 2007.01 [3] and EN method 15662, a European variation to the QuEChERS method [4]. In summary, the method is a three step process: extraction, dispersive SPE, and analysis. In the first step, acetonitrile is added to the sample, followed by salting out of the water from the sample using anhydrous magnesium sulfate ($MgSO_4$), NaCl and buffering citrate salts to induce extraction/partitioning. The second step is dispersive solid phase extraction (d-SPE), used to minimize matrix effects with a combination of primary secondary amine (PSA) to remove organic acids, C18 EC (octyldecylsilane end-capped) for fat and lipid removal, GCB (graphitized carbon black) for pigment removal, and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugations, the upper layer is ready for analysis.

Gas chromatography/mass spectrometry (GC/MS) has been widely used for pesticide analysis. Many pesticides are volatile or semivolatile, making them very amenable for GC analysis. In this study, the Agilent EN Buffered Extraction kit (p/n 5982-5650) and Agilent AOAC dispersive SPE kit (p/n 5982-5421) for fatty and pigmented products were combined for the extraction of volatile and semivolatile pesticides in lemon oil. Analysis was performed by GC/MS. Twenty-six GC-amenable pesticides were selected that represent multiple classes, including nonpolar organochlorine pesticides (OCs), and certain organophosphorous pesticides (OPs). The US EPA has established limits for several pesticides in citrus oils (Table 1). In general, a concentration factor of 100 or 250 times is applied to the MRL (ppb) of the pesticide within the fruit to yield an indication of the MRL (ppm) of the pesticide within the citrus oil.

Table 1. US EPA Limits of Pesticides in Citrus Oils

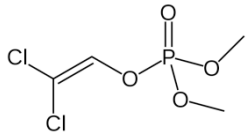
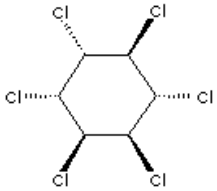
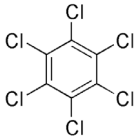
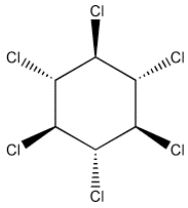
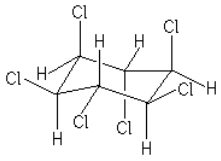
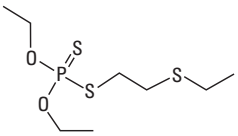
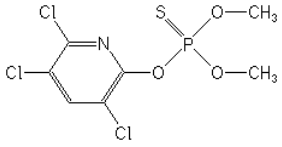
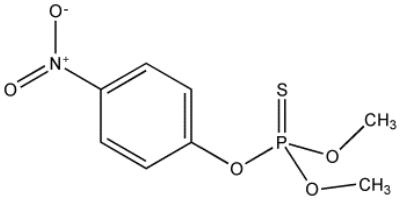
Compound	MRL (ppm)
Carbaryl	20
Chlorpyrifos	20
Dicofol (sum of p,p′, o,p′ isomers)	200
Hexythiazox	24
Imazalil	200
Metalaxyl/metalaxyl-m	7
Methidathion	420

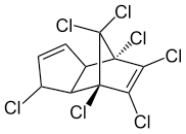
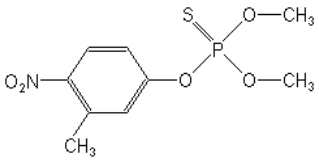
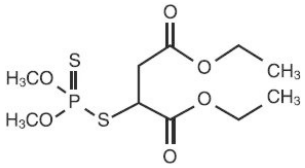
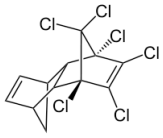
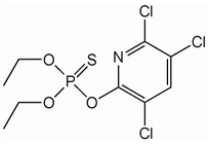
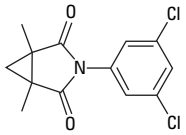
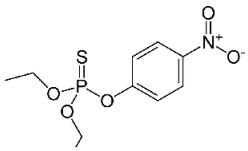
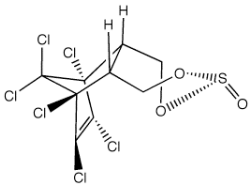
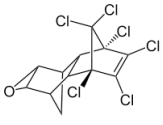
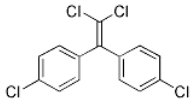
Experimental

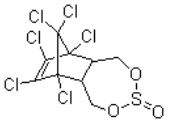
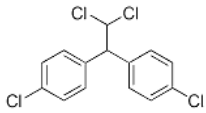
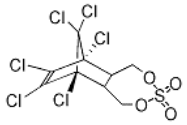
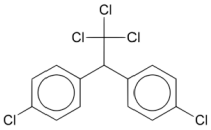
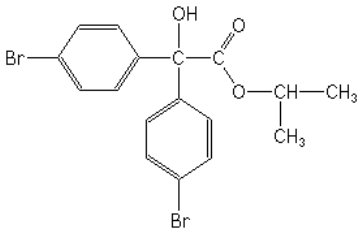
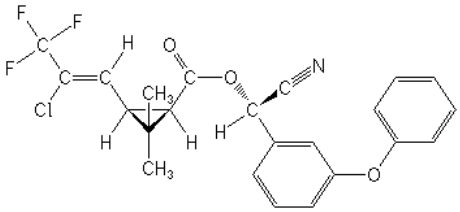
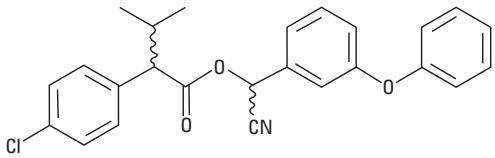
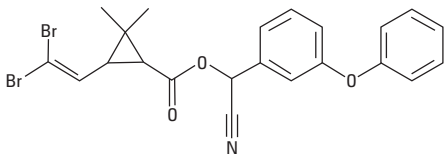
Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN), and methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The standard pesticide mix (100 ug/mL) was from Ultra Scientific (N. Kingstown, RI), Table 2. The internal standard (500 µg/mL, triphenyl phosphate, TPP) was from Agilent Technologies (p/n 5190-0503).

Table 2. Pesticide Chemical and Category

Analyte	Structure	Category
Dichlorvos		Organophosphates
α-BHC		Organochlorine
Hexachlorobenzene		Organochlorine
β-BHC		Organochlorine
γ-HCH		Organochlorine
Disulfoton		Organophosphate
Chlorpyrifos methyl		Organophosphate
Methyl parathion		Organophosphate

Analyte	Structure	Category
Heptachlor		Organochlorine
Fenitrothion		Organophosphate
Malathion		Pyrethroid
Aldrin		Organochlorine
Chlorpyrifos		Organophosphate
Procymidone		Dicarboximide Fungicide
Parathion		Organophosphate
Endosulfan I		Organochlorine
Dieldrin		Organochlorine
4,4′–DDE		Organochlorine

Analyte	Structure	Category
Endosulfan II		Organochlorine
4,4′–DDD		Organochlorine
Endosulfan sulfate		Organochlorine
4,4′–DDT		Organochloride
Bromopropylate		Bridged Diphenyl Acaracide
λ– Cyhalothrin		Pyrethroid
Fenvalerate		Pyrethroid
Deltamethrin		Pyrethroid

Standard Solutions

The standards and internal standard were made fresh daily in 1:1 ACN/H_2O (0.1% FA). A 5.0 µg/mL standard in ACN (0.1% FA) was used to prepare the calibration curves in the matrix blank and post-spiked samples, by using the appropriate dilutions. A 15 µg/mL solution of TPP spiking solution in 1:1 ACN/H_2O (0.1% FA) was used as the internal standard (IS).

Equipments and Materials

- Agilent Gas Chromatography (Agilent Technologies Inc., Santa Clara, CA, USA)
- Agilent 5975C Series GC/MSD (Agilent Technologies Inc., Santa Clara, CA, USA)
- Lemon oil (Cedarome Canada, Inc, Brossard, Quebec)
- Agilent Bond Elut QuEChERS EN Extraction kit, p/n 5982-5650 (Agilent Technologies Inc., Wilmington, DE, USA)
- Agilent Bond Elut QuEChERS AOAC dispersive SPE kit for fatty and pigmented product , p/n 5982-5421 (Agilent Technologies Inc., Wilmington, DE, USA)
- Centra CL3R Centrifuge (Thermo IEC, MA, USA)
- Bottle top dispenser (VWR, So Plainfield, NJ, USA)
- 2010 Geno/Grinder (Spex sampleprep, LLC, Metuchen, NJ)
- Eppendorf microcentrifuge (Brinkman Instruments, Westbury, NY, USA)

Instrument Conditions

GC conditions

An Agilent GC/MS method for pesticides analysis was used for this study [8].

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 °C
Injection volume	1.0 µL
Purge flow to split vent	30 mL/min at 0.75 min
Over temperature program	70 °C (1 min) 50 °C/min to 150 °C (0 min) 6 °C /min to 200 °C (0 min) 16 °C/min to 280 °C (6 min)
Column	Agilent J&W HP-5MS Ultra Inert 15 m × 0.25 mm, 0.25 µm (p/n: 19091S-431UI)

MS conditions

Tune file	Atune.u
Mode	SIM (refer to Table 3 for settings in detail)
Source, quad, transfer line temperature	230 °C, 150 °C and 280 °C, respectively
Solvent delay	2.30 min
Multiplier voltage	Autotune voltage

Table 3. Selected Ion Monitoring for the Analytes Used in the QuEChERS Extraction

GC/MS SIM Conditions for Quantitation

Analyte	Selected Ions for Monitoring*	Retention time (min)	Acquiring window (min)	Analyte	Selected Ions for Monitoring	Retention time (min)	Acquiring window (min)
(1) Dichlorvos	**184.9,** 219.8	2.83	2.3–5.55	(14) Parathion	**291.0,** 138.9	9.26	8.95 – 9.8
(2) α-BHC	**218.9,** 180.9	5.74	5.55–6.6	(15) Procymidone	**283.0,** 284.9	10.54	9.8 – 11.2
(3) Hexachlorobenzene	**283.9,** 285.9	5.86		(16) Endosulfan I	**240.8,** 194.9	10.86	
(4) β-BHC	**218.9,** 180.9	6.26		(17) Dieldrin	**262.8,** 276.8	11.52	11.2 – 11.9
(5) γ-HCH	**218.9,** 263.8	6.37		(18) 4,4′-DDE	**317.9,** 245.9	11.64	
(6) Disulfoton	**88.0,** 274.0	6.91	6.6–7.5	(19) Endosulfan II	**194.9,** 236.8	12.21	11.9 – 12.8
(7) Chlorpyrifos methyl	**285.9,** 124.0	7.89	7.5–8.3	(20) 4,4′-DDD	**234.9,** 165.0	12.51	
(8) Parathion methyl	**262.9,** 233.0	7.89		(21) Endosulfan sulfate	**271.8,** 228.8	13.06	12.8 – 13.6
(9) Heptachlor	**271.8,** 100.0	7.97		(22) 4,4′-DDT	**234.9,** 165.0	13.19	
(10) Fenitrothion	**277.0,** 124.9	8.62	8.3–8.95	(23) Bromopropylate	**340.8,** 182.9	14.04	13.6 – 15.2
(11) Aldrin	**262.8,** 264.8	8.81		(24) λ-Cyhalothrin	**181.0,** 196.9	14.96	
(12) Malathion	**157.8,** 173.0	9.06	8.95–9.8	(25) Fenvalerate	**124.9,** 167.0	17.04	15.2 – 22.0
(13) Chlorpyrifos	**198.9,** 96.9	9.25		(26) Deltmethrin	**181.0,** 252.8	17.59	

*SIM: **qualifying ion**, target ion

Sample Preparation

A 3 g (± 0.05 g) sample of essential lemon oil was placed into a 50 mL centrifuge tube. QC samples were fortified with the appropriate QC spiking solution. The internal IS spiking solution (15 ug/mL of TPP) was added to all the samples to yield a 100 ng/g concentration. A 12 mL amount of water was added to the 50 mL tube. Tubes were capped and vortexed for 1 min. Then 10 mL of ACN and 2 mL of hexane were added, and the tubes vortexed again for 1 min. An Agilent Bond Elut QuEChERS EN extraction salt packet (p/n 5982-5650) containing 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g NaCitrate, and 0.5 g disodium citrate sesquihydrate, was added directly to each tube. Tubes were sealed tightly and shaken for 1 min. The upper hexane and oil layer (top layer above the ACN layer) was discarded by a transfer pipette.

Dispersive SPE Cleanup

A 1.6 mL amount of the ACN extract was transferred into an Agilent Bond Elut QuEChERS AOAC dispersive SPE 2 mL tube (p/n 5982-5421) containing 50 mg of PSA, 50 mg of GCB, 50 mg C18 EC, and 150 mg of $MgSO_4$. The tubes were capped tightly and vortexed for 2-3 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min. Then the upper extract was completely transferred to another dispersive SPE tube (as stated previously) followed by vortexing and centrifugation. The above d-SPE step was repeated a third time to achieve an even cleaner extract. The upper clear extract was transferred into an autosampler vial for GC/MS analysis. Figure 1 shows the flow chart of the modified QuEChERS extraction of lemon oil.

Results and Discussion

Pesticide extraction in essential oil has been considered difficult due to the complicated oily matrix. Literature suggests the use of very selective detection methods such as negative GC/MS, or GC×GC/TOF-MS in order to address this issue. These selective detection methods are limited, and specific for a certain group of pesticides [4,5]. As demonstrated before, QuEChERS is a very good sample extraction and cleanup method that is suitable for broad varieties of pesticides [4]. With some modifications, QuEChERS can also be used for pesticides analysis in olive oil [7]. Therefore, a QuEChERS sample preparation approach has been developed for the analyses of different groups of pesticides in lemon oil by GC/MS.

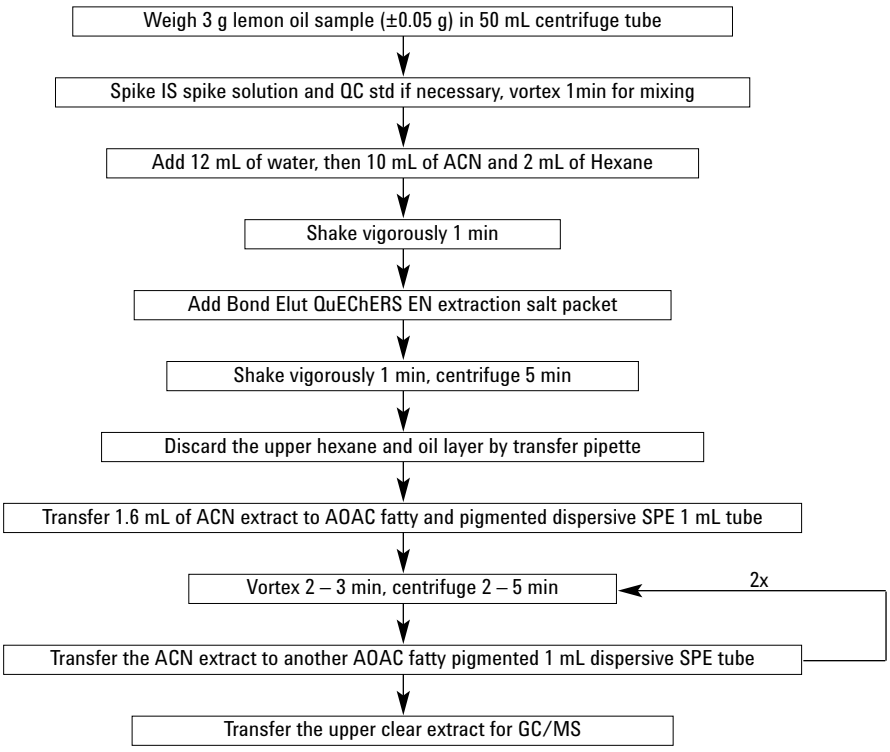


Figure 1. Flow chart of the Agilent modified QuEChERS EN extraction procedure.

Commercially available QuEChERS kits offer a procedure that is fast, easy, and saves time and labor. An analyst can process 40-50 samples in just a few hours. Agilent's extraction salts are uniquely prepared in an anhydrous nitrogen-filled package. The anhydrous salts packaging allows the salts to be added after the addition of organic solvents to the sample, as specified in the original QuEChERS methodology. The final QuEChERS prepared samples still contain some matrix impurities, which can be observed in the GC/MS chromatogram of the blank lemon oil. Therefore, it is critical to carefully choose the selected ions of each compound for monitoring when setting up the SIM method. In general, the most abundant ions were selected to achieve the best sensitivity; however, in a few instances the sensitivity was compromised to obtain better selectivity by using a more unique but less abundant ion.

The original sample preparation method diluted the lemon oil 1:1 with hexane. Figure 2 shows the chromatogram of a lemon oil sample that has been diluted with hexane 1:1 and injected into the GC/MS. As shown in the chromatogram, substantial interferences are observed that significantly inhibit identification and quantitation of pesticides in the lemon oil sample.

In order to minimize or eliminate matrix interferences, different modifications were performed to remove the late eluters. The EN and AOAC extraction kits were evaluated relative to their effects on the matrix interferents. The EN extraction salt kit yielded a cleaner extract; therefore it was chosen for the lemon oil extraction. The addition of hexane to the initial QuEChERS extraction step changed the polarity of the solvent system, and was helpful in removing lipid interfering components from the lemon oil. Different dispersive kits were also tested, and the AOAC fatty pigmented dispersive kit was selected because it yielded the cleanest matrix.

Figure 3 illustrates the results achieved from the method development experiments. It was observed that adding 2 mL of hexane to the first step of the QuEChERS extraction and repeating the dispersive SPE FP (fatty and pigmented products) step, two or three times yielded the cleanest lemon extract. This was observed by the TIC and SIM chromatograms of the CB (control blanks). Since the cleanest extract was achieved by repeating the dispersive step on the same sample, a dispersive kit containing twice as much of the individual components (100 mg of PSA, GCB, C18 EC, and

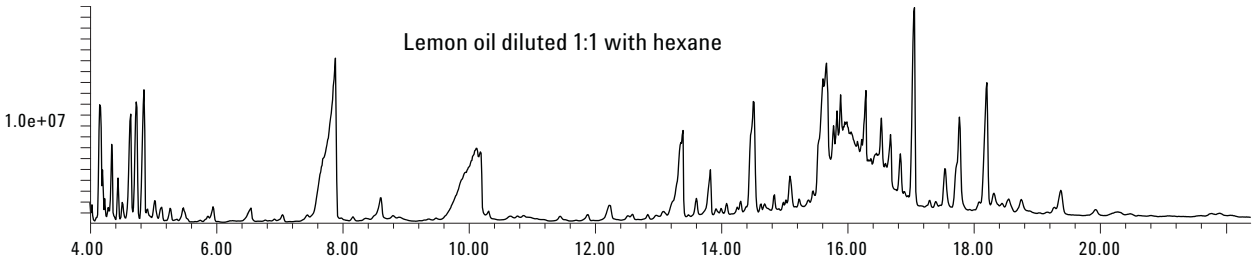


Figure 2. GC/MS chromatogram of lemon oil using a 1:1 dilution with hexane.

300 mg of $MgSO_4$) relative to the standard dispersive SPE for fatty and pigmented matrix (50 mg PSA, GCB, C18EC, and 150 mg $MgSO_4$) was made. However, the results obtained by the new kit were not comparable to repeating the dispersive SPE step on the same sample. In fact, it yielded a dirtier matrix. Therefore, the study continued to process a single extract by two dispersive SPE steps in series prior to analysis by GC/MS.

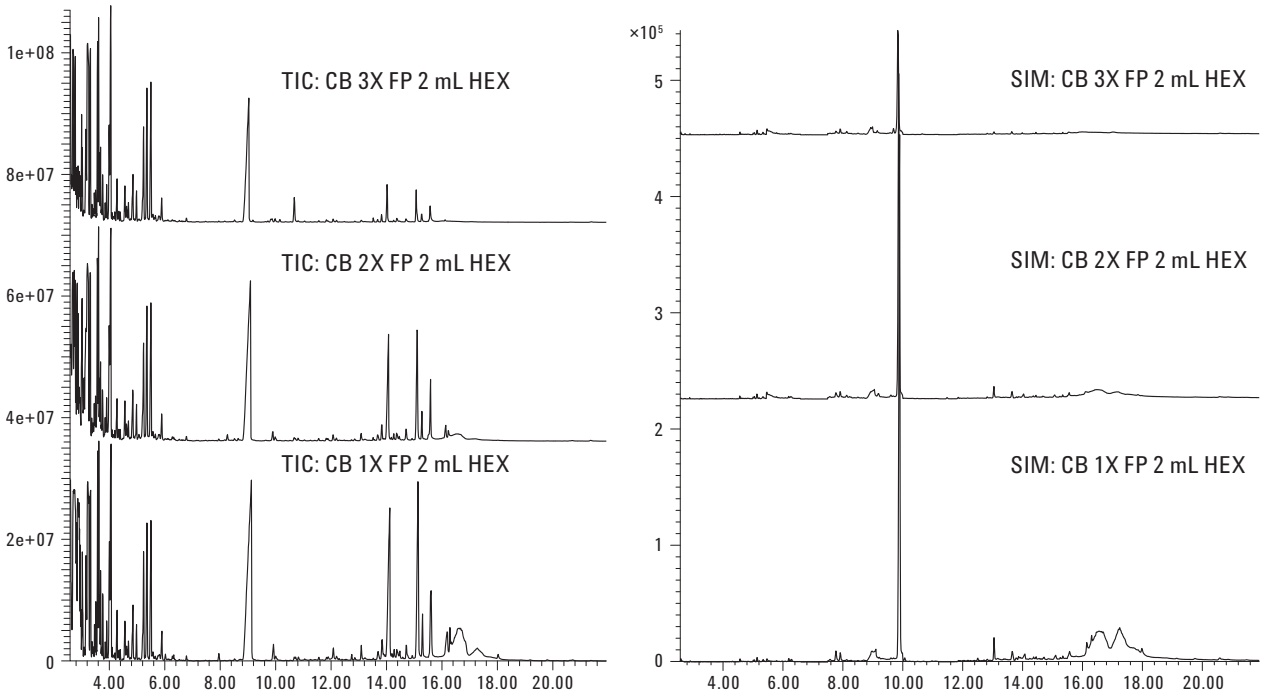


Figure 3. Optimized extraction from the method development experiments involving the addition of hexane and multiple dispersive SPE steps. CB (control blank), FP (AOAC fatty pigmented dispersive SPE), HEX (Hexane).

Figures 4a and 4b show the GC/MS chromatograms (SIM and TIC) of the blank lemon oil extract after following the optimized QuEChERS extraction procedure shown in Figure 1. Comparison of the lemon oil diluted with hexane 1:1 (Figure 2) to the lemon oil after QuEChERS extraction (Figure 4 a,b) demonstrated substantial decreases in matrix interferences with the QuEChERS extraction.

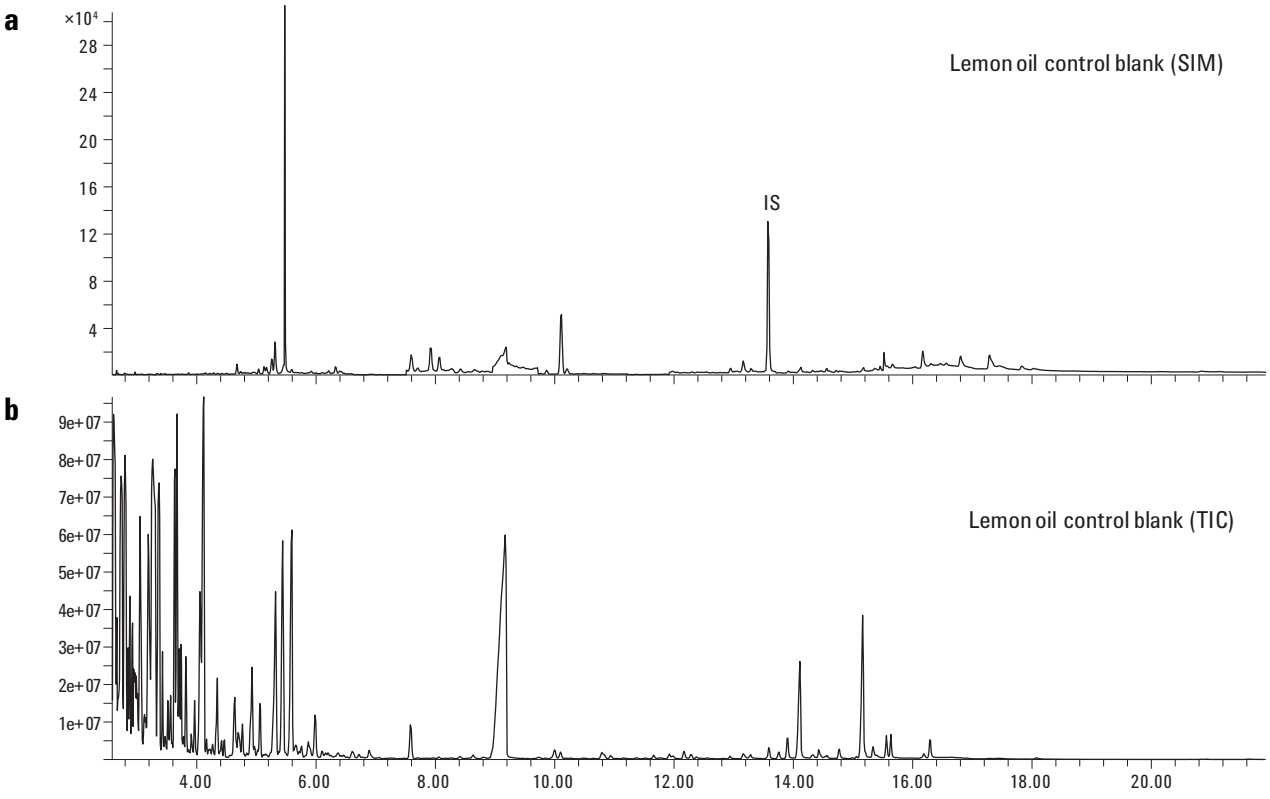


Figure 4 (a,b). Blank lemon oil extract after QuEChERS extraction, SIM and TIC.

Figure 5 shows the chromatograms for a prespiked and postspiked lemon oil extract. Twenty-five of the 26 pesticides gave repeatable results with RSD < 5% on average.

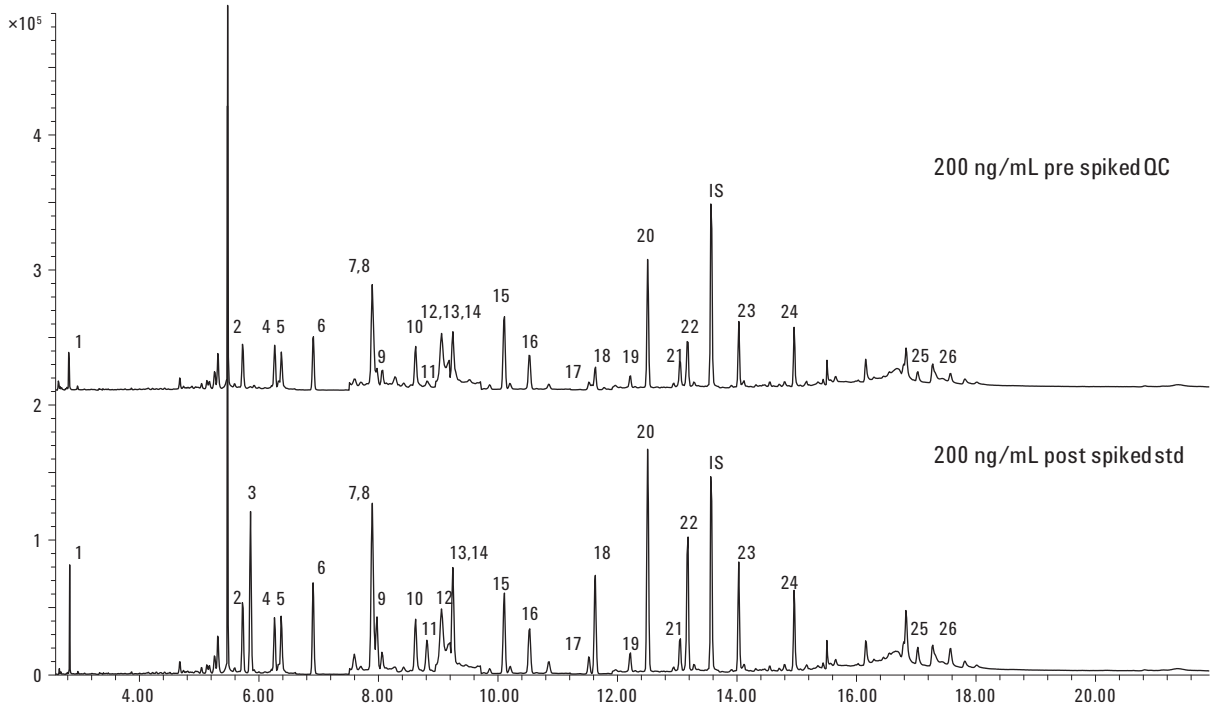


Figure 5. GC/MS chromatograms of 200 ng/mL pre and postmatrix fortified samples. Peaks identification: 1. Dichlorvos, 2. α-BHC, 3. Hexachlorobenzene, 4. β-BHC, 5. γ-HCH, 6. Disulfoton, 7. Chlorpyrifos methyl, 8. Parathion methyl, 9. Heptachlor,10. Fenitrothion, 11. Aldrin, 12. Malathion, 13. Chlorpyrifos, 14. Parathion, 15. Procymidone, 16. Endosulfan I, 17. Dieldrin,18. 4,4′-DDE, 19. Endosulfan II, 20. 4,4′-DDD, 21. Endosulfan sulfate, 22. 4,4′-DDT, 23. Bromopropylate, 24. λ-Cyhalothrin, 25. Fenvalerate, 26. Deltmethrin.

The planar pesticide hexachlorobenzene was totally lost due to the addition of GCB in the dispersive SPE clean-up step. The addition of 2 mL of hexane facilitates the removal of late-eluted matrix interferences. It can also cause the loss of certain nonpolar organochlorine pesticides, which negatively impacts the recovery of these pesticides. However, the extraction precision for these pesticides is good (< 5% RSD) and consistent recoveries are achieved from low to high concentration with good sensitivity. Recoveries and RSDs for 25 of the 26 pesticides are shown in Table 4. Although an organochlorine pesticide such as aldrin had low recovery, which is expected when using hexane, the RSDs are within the acceptable range of 15% and consistent throughout the range of 60 – 800 ppb.

Table 4. Lemon Essential Oil Recovery and Repeatablility Results

	Low QC (60 ppb)		Mid QC (200 ppb)		High QC (800 ppb)	
Pesticides	**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
Chlorpyrifos 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	2	47.3	2.9
4,4′-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
4,4′-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
4,4′-DDT	8.6	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 Bond Elut QuEChERS EN extraction and AOAC dispersive SPE for fatty pigmented product kit provides a simple, fast and effective method for the extraction of pesticides from lemon essential oil. The recovery and reproducibility, based on matrix spiked standards, are acceptable for multiclass, multiresidue pesticide determinations in essential oils. Since the selected pesticides represented a broad variety of different classes and properties, the Agilent Bond Elut QuEChERS EN extraction and AOAC dispersive SPE kits for fatty pigmented products is an excellent choice for pesticides in similar product matrices.

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