Analysis of Pesticide Residues in Citrus Oils by GCxGC-TOFMS with Minimal Sample Preparation

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Key Words: GCxGC-TOFMS, Classifications, Deconvolution, Quantification, Sample Preparation, Pesticides

1. Abstract

Citrus oils are widely used in consumer products. Contamination with pesticides is of particular concern because the citrus oils are derived from the outer portion of the fruit. GCxGC-TOFMS provides for analysis for many pesticides in citrus oils with minimal sample preparation. This work shows the ability of this technique to sufficiently separate these pesticides from the citrus oil components, to provide confirmation of identity, and to quantify these residues over several orders of magnitude concentration, reaching as low as part-per-billion levels.

2. Introduction

Citrus oils are traded internationally and are included in products that are traded internationally. These oils are derived from the outer portion of the fruit, as byproducts in the much more economically important production of citrus juices. And, it is the outside of the fruit that is exposed to the pesticides that are applied to protect the citrus crop and trees. The analysis of citrus oils for pesticides has become increasingly important as pesticides that are acceptable in one market may be banned in another. Even in markets where specific pesticides may be allowed, there are increasing restrictions on the permissible levels of pesticides to which humans or animals may be exposed.

While the greater portion of citrus oil may be limonene, the other compounds present form a highly complex mixture that includes many natural products. Although injection of the citrus oil directly into a GC would be highly desirable, the complexity of the oil is such that GC-MS analysis for a large number of pesticides at ppb levels is not practical. The higher-boiling compounds in the citrus oils coelute with the pesticides. These compounds are present in significant concentration (relative to pesticide residues) and provide many ions in common with the pesticides, confounding many MS detection and confirmation techniques. Additionally, the loading of some of the citrus oil components on the chromatographic column is sufficiently high that GC retention times may be shifted, again confounding the process of locating and identifying the pesticides.

This work demonstrates a GCxGC separation of citrus oil containing pesticides, and subsequent quantification using TOFMS. The first chromatographic separation is a "boiling point" separation, providing a gross separation of the complex mixture. A second separation, using a 50% phenyl phase, separates the more polar or more aromatic pesticides from the bulk of the matrix that coelutes. Compounds coeluting in the first chromatographic separation include sesquiterpines, other hydrocarbons, or derivatives of these such as alcohols, ketones, and other compounds. Even with the extremely high peak capacity provided by GCxGC, individual pesticides still may coelute with other compounds in the mixture. Mass spectral detection is required to obtain distinguishable signals for the pesticides. TOFMS is used to locate, identify, and quantitate the compounds.

3. Experimental

Unfolded citrus oils (Florida midseason orange oil and California cold-pressed lemon oil) and limonene were obtained from commercial sources. A portion of each oil was spiked to give a solution containing nominally 1000 PPB (w/v) of each pesticide. Portions of the spiked oil were mixed with unspiked oil to obtain desired concentrations. A 200 µL portion of the spiked oil was placed in a GC vial insert and 20 µL of a 20 ng/uL solution of 2-fluorobiphenyl was added as an internal standard. The sample was mixed by drawing it into a Pasteur pipette and expelling it into the insert several times.

Calibration curves were generated from pesticides spiked into limonene at concentrations of 10 to 1000 ppb to demonstrate linearity of pesticide response in an uncontaminated matrix. The citrus oils were run spiked at 100 ppb, 25 ppb, and unspiked. The 25 ppb spiked sample was run at least five times to show relative standard deviations (RSDs) at this level of spike. Where pesticides were observed in the unspiked oils, the background concentration was determined by using the added spikes as standard additions.

Analyses were performed on a LECO® Pegasus 4D GCxGC-TOFMS system under the following analytical conditions.

Injection

Volume:	1 µL
Liner:	Splitless Liner with Wool (Restek #22401)
Temp:	250°C
Mode:	Splitless

Column

#1	J&W Scientific DB-PONA (50 m x. 0.2 mm x 0.5 µm)
#2	SGE BPX-50 (2 m x 0.1 mm x 0.1 µm)
Carrier:	He
Flow Rate:	0.5 mL/minute

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Temperature Ramp

Primary Oven	
Initial Temp:	150°C; Duration: 3 minutes
Ramp:	3°C/minute
Final Temp:	330°C; Hold: 5 minutes
Secondary Oven	
Initial Temp:	160°C; Duration: 3 minutes
Ramp:	3°C/minute
Final Temp:	335°C; Hold: 5 minutes

Modulator

Temp Offset:	30°C
Modulation Time:	10 seconds
Hot Pulse Time:	1.5 seconds

Mass Spectrometer

Acquisition Range:	29 to 390u
Acquisition Rate:	100 spectra/second
Source Temp:	200°C
Transfer Line Temp:	300°C

4. Results

Chromatography of the pesticides in limonene shows separation of the pesticides from the higher boiling materials normally found in commercial limonene (Figure 1).

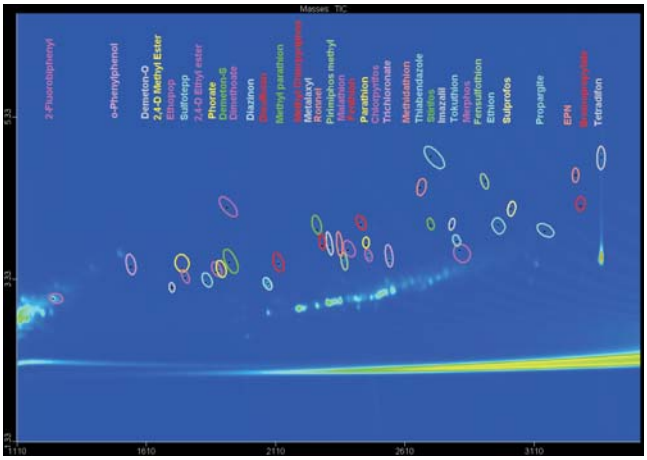

Figure 1. Pesticides in the 1000 ppb standard made in commercial limonene. Note that limonene elutes from the column set before 1100 seconds under the conditions used. Higher boiling impurities from the limonene are seen in the contour plot. Peak markers are shown only for pesticides present in the chromatogram.

The response of the compounds is linear over the range of interest. A typical calibration curve is shown in Figure 2.

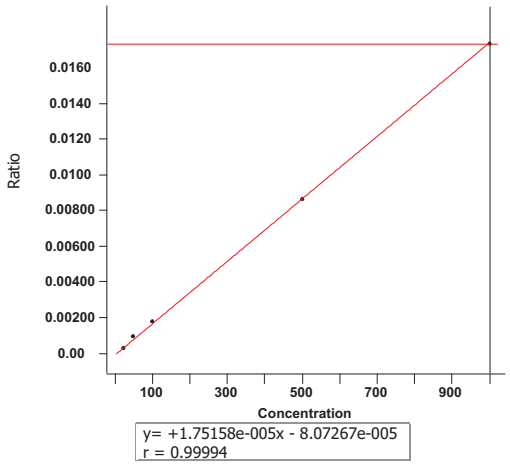

Figure 2. Calibration plot for Parathion in limonene. Concentration range is 10 to 1000 ppb.

The GCxGC chromatogram of orange oil shows the presence of significant quantities of materials coeluting with the pesticides in the first chromatographic dimension. The addition of the second chromatographic dimension provides sufficient separation to allow the identification and quantitation of the pesticides. Figure 3 shows the locations of pesticides in an orange oil chromatogram. Two peaks, identified as chinomethionate and fenpropathrin, were not included in any standard, but were identified in a review of the chromatographic peak table generated by the Automated Peak Find and Spectral Deconvolution capability of LECO's ChromaTOF® software. Chinomethionate (also known as Morestan) is used as an acaride, fungicide, fumigant, and insecticide on citrus. The mass spectrum from this peak and its library-matched spectrum are shown in Figure 4. Fenpropathrin, a pyrethroid, is used as an acaride and insecticide. The mass spectrum from this peak and its library-matched spectrum are shown in Figure 5.

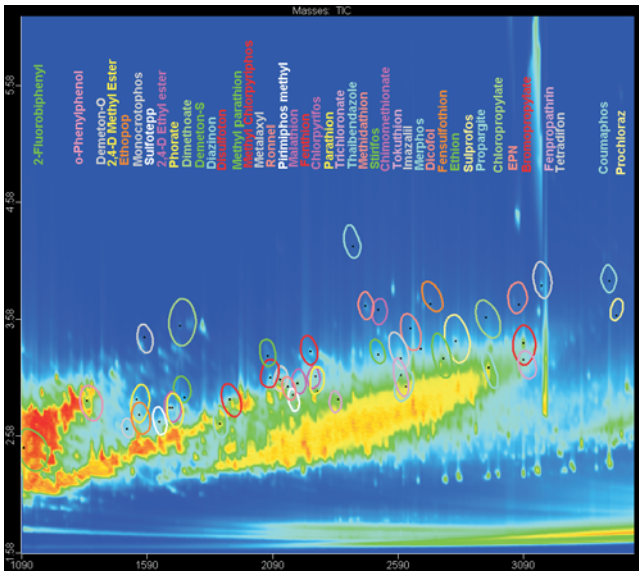

Figure 3. Pesticides in chromatogram of spiked orange oil, 1 ppm spike.

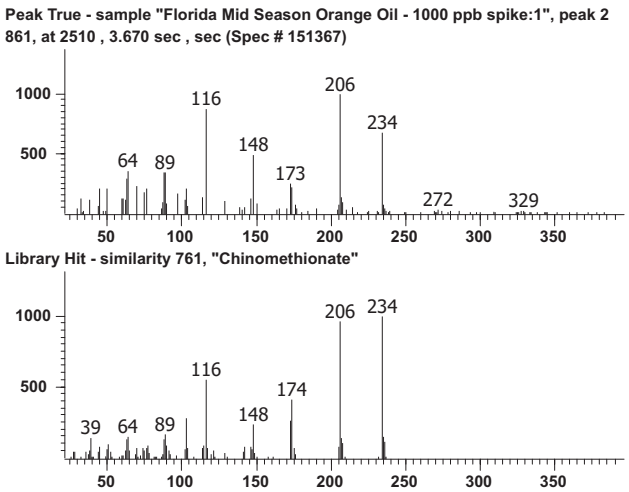

Figure 4. Sample and library mass spectra for peak identified as chinomethionate.

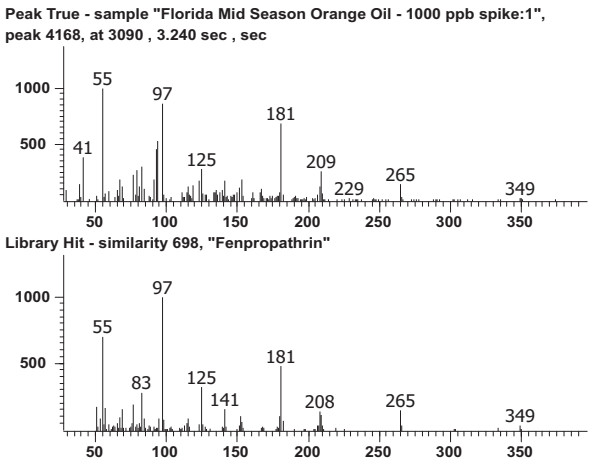

Figure 5. Sample and library mass spectra for peak identified as fenpropathrin.

The ability to separate a compound from interfering matrix is clearly demonstrated by Methyl Parathion. The mass spectrum of Methyl Parathion shows three intense ions (109, 125, and 263), as well as many weaker ions. GCxGC surface plots (Figure 6) show the well-resolved peak in the chromatographic plane. A reconstructed one-dimensional trace is shown in each of these figures (as the chromatogram in the background). The reconstructed one-dimensional trace shows that this 1000 ppb standard would be difficult to detect, confirm, and quantify based on examination of ion traces and ion ratios from one-dimensional chromatography. And, this compound would be even more difficult to detect and quantify at lower concentrations.

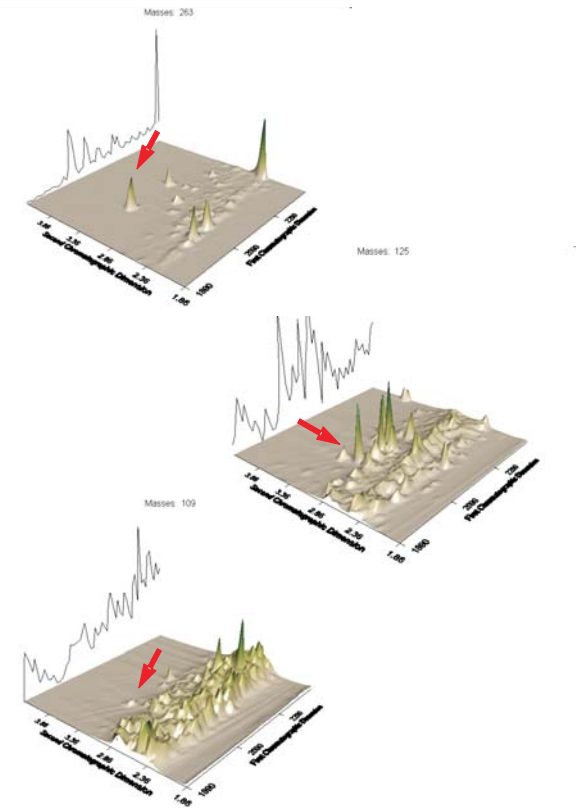

Figure 6. Methyl Parathion separated from interferences in GCxGC, shown for m/z 263, 125, and 109.

Table 1 shows the variability of the 25 ppb spike. (In cases where a 50 ppb spike was used, it was because that was the spike level afforded by the standard solutions used.) With five replications, the RSDs obtained when there was no pesticide originally present ranged from about 10% to 20%. Examination of the results of the spiking study showed a number of pesticides to be present in the orange oil prior to spiking. When pesticides were already present in the oil, the spike, corrected for additional material present, gave RSDs in the same range. In cases where pesticides were already present in the oil, the regression through the results for the spike at 100 ppb, the multiple results at 25 ppb, and the unspiked sample gave resulted in the measurement of the pesticide already present in the oil. With background concentrations included in the calculation, RSDs for the measured pesticides typically ranged from about 5% to about 20%.

Bromopropylate is clearly present in the orange oil, but other compounds present in the oil offer to confound ion ratios if they are to be used in one-dimensional GC-MS to confirm identity. Figure 7 shows the surface plot for mass 183 with a peak for bromopropylate. In the GCxGC plane, the bromopropylate peak is well resolved from other peaks. The reconstructed ion trace shows that in a one-dimensional trace, coelutions would adversely affect the ability to integrate the m/z 183 peak. The ion for m/z 185 does not show similar interference in the one-dimensional trace, thus the ratio of intensities of m/183 and 185 in this sample would not be expected to match the ratio for these masses in the pure compound, with the risk of reporting bromopropylate as being undetected.

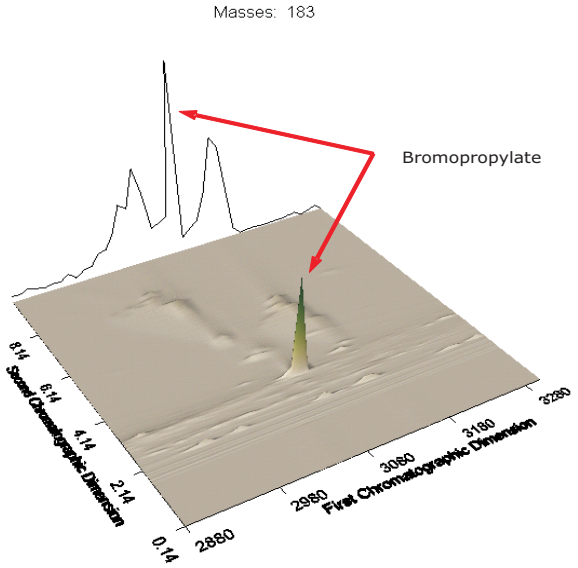

Figure 7. Extracted ion surface plot for m/z 183 showing Bromopropylate in orange oil. Note first dimension coelution that would confound ion ratio calculations for one-dimensional chromatography and selected ion monitoring MS.

The coelution of fenpropathrin with bromopropylate would not have been likely to have been noticed with techniques such as selected ion monitoring (SIM) or MS/MS unless this compound was on a list of target analytes. Including ions for this compound in SIM or MS/MS target lists could reduce the overall sensitivity in this region, as detection time would have to be divided between these two coeluting compounds.

Table 1. Results of spiking orange oil with pesticides. With background levels included in the calculation, RSDs for five injections range from 4% to 24%.

Pesticide	Spike (ppb)	Average Found value (ppb)	Standard deviation	RSD %	Concentration in blank (by regression line, ppb)	RSD % for corrected conc.
o-Phenylphenol	25	25[1]	Appears to be High ppb or low ppm level			
2,4-D methyl ester	25	25[1]	2.0	7.9		
Ethoprop	25	25[1]	2.6	10		
Sulfotepp	25	25[1]	2.1	8.5		
Phorate	25	27	6.4	23	5	20
Dimethoate	25	25[1]	3.0	12		
Methyl parathion	50	50[1]	2.6	5.2		
Methyl chlorpyrifos	50	50[1]	2.2	4.4		
Metalaxyl	25	25[1]	0.95	3.8		
Ronnel	25	20	2.4	12	3	11
Pirimiphos methyl	25	25[1]	1.5	6.0		
Malathion	50	51	13	25	13 (peak is too poorly shaped for practical use)	20
Fenthion	25	27	3.8	14	8	11
Parathion	25	26	6.7	26	7 (peak is too poorly shaped for practical use)	20
Chlorpyrifos	50	45	15	33	217	5.6
Trichloronate	25	23	4.1	17	3	15
Thiabendazole	25	22	5.0	22	16	13
Methidathion	25	25[1]	Present in "blank" at level above 100 ppb			
Stirifos	25	25[1]	3.9	29	6	20
Tokuthion	25	25[1]	5.1	22	4	19
Merphos	25	25[1]	9.1	34	Background peak clearly not Merphos, but contributes to signal.	
Fensulfothion	25	25[1]	1.8	8.9	6	6.8
Ethion	25	25[1]	6.4	27	67	7.1
Sulprofos	25	25[1]	6.1	27	9	19
Bromopropylate	25	25[1]	In excess of 500 ppb in background			
Tetradifon	25	25[1]	0.46	1.8	Small peak present in blank, but requires additional standards to account for curvature in response curve.	
Prochloraz	25	25[1]	4.6	18		

[1] *In cases where no pesticide was present in the blank, there were only two levels in the regression, the low and high spikes. In this case, deviation from linearity will not be observed.*

Lemon oil again shows significant quantities of material coeluting with the pesticides in the first chromatographic dimension. With the addition of the second chromatographic dimension, the pesticides are separated from the bulk of the matrix. Figure 8 shows the location of pesticides in lemon oil.

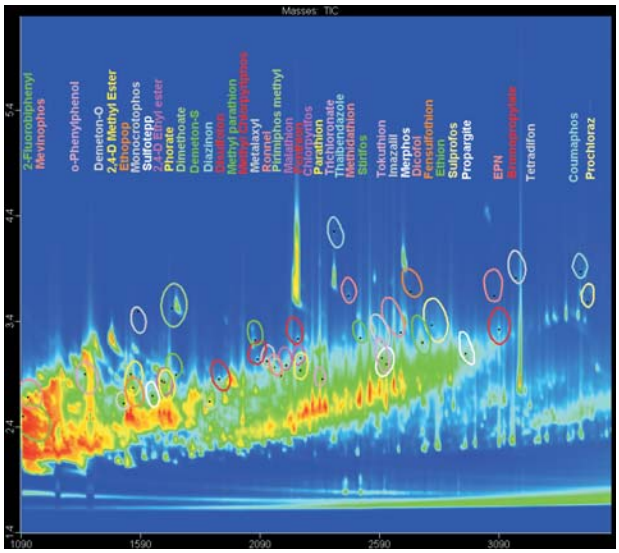

Figure 8. Pesticides in the chromatogram of spiked lemon oil, 1 ppm spike.

As with the orange oil, pesticides were found to be present in the unspiked lemon oil. Tetradifon is present in the oil at about 44 ppb. The GCxGC separation gives ion traces that clearly allow for accurate integration of the peak (Figure 9). The spectrum obtained unequivocally identifies the compound (Figure 10).

Examination of the GCxGC plots for individual ions (Figure 11) shows a clearly identifiable peak for several ions, while in the one-dimensional trace, m/z 159 might be a distinguishable peak. Confirming ions would be difficult to find in a one-dimensional analysis, and if found, ion ratios may be compromised. The GCxGC separation offers the ability to identify and quantify Tetradifon at even lower concentrations than found here.

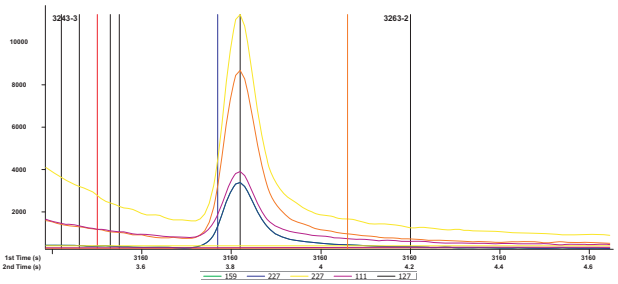

Figure 9. Extracted ion chromatograms for Tetradifon in unspiked lemon oil.

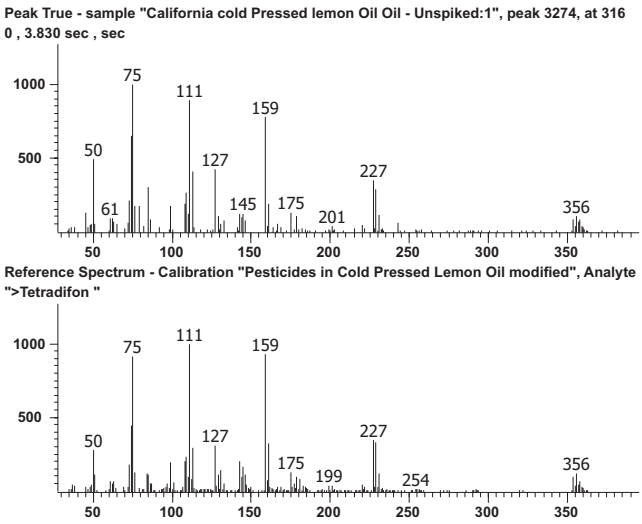

Figure 10. Mass spectrum of Tetradifon found in unspiked lemon oil with comparison to Reference Spectrum.

The evaluation of pesticides in lemon oil was focused primarily on those pesticides noted as already present in the oil before spiking. RSDs are similar to those obtained for pesticides in orange oil. Again, when background levels are included in the calculation, RSDs for seven replicates are typically in the 10% to 20% range.

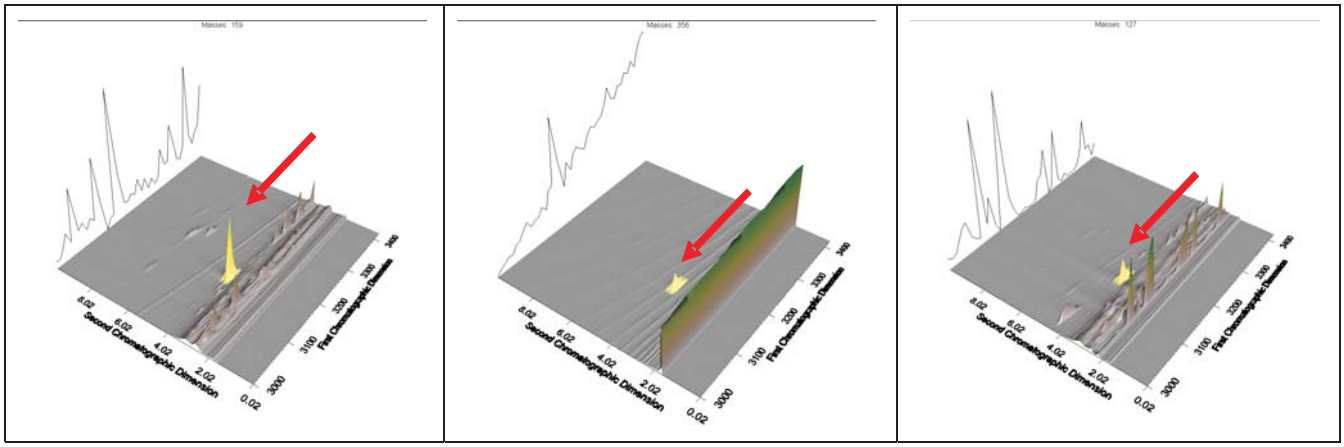

Figure 11. Peaks for Tetradifon shown among interferences in lemon oil. Masses shown are 159, 356, and 127.

Table 2. Results for selected pesticides in lemon oil. With background concentrations taken into account, RSDs for seven replicates range from 5% to 22% for the pesticides measured.

Pesticide	Spike (ppb)	Average Found value (ppb)	Standard deviation	RSD %	Concentration in blank (by regression line, ppb)	RSD % for corrected conc.
o-Phenylphenol	25	25[1]	14		22	
2,4-D methyl ester	25	25[1]	5	21		
Ethoprop	25	25[1]	3	12		
Phorate	25	25[1]	1.3	5		
Methyl parathion	50	25[1]	9	18	7	
Methyl chlorpyrifos	25	26	5	18	1	17
Parathion	25	25[1]	2	17	1	
Chlorpyrifos	50	40	12	31	115	8.0
Methidathion	25	20	11	54	34	20
Stirifos	25	25[1]	1	5		
Tokuthion		18	3	16	8	11
Ethion	25	25	6	31	51	8.5
Bromopropylate	25	25	7	49	39	11
Tetradifon	25	25	10	41	44	22
Prochloraz	25	25[1]	2	9		

[1]*In cases where no pesticide was present in the blank, there were only two levels in the regression, the low and high spikes. In this case, deviation from linearity will not be observed.*

5. Conclusions

GCxGC-TOFMS provides the capability to determine pesticides in citrus oils with no sample preparation other than the addition of internal standard. Detection limits below 25 ppb for many pesticides are clearly achievable. Even at low concentrations, when spectral information becomes difficult to obtain, the retention times obtained on two stationary phases serves to confirm pesticide identity. The full mass range capability of the TOFMS, coupled with spectral deconvolution, affords the ability to identify pesticides not originally included in a target analysis list.

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