SBSE-GCxGC-TOFMS Trace Level Organochlorine and Organophosphorous Pesticides Analysis in Brewed Green Tea

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1. Introduction

Chemical pesticide, fungicide, and herbicide trace level residues found in Green Tea is a concern that has gained international attention. The European Union (EU) adopted regulations in 2001 reducing residual pesticide tolerance levels in tea by one hundred times. The Japanese Positive list for maximum residual limits of agricultural chemicals in food was made effective in 2006. The United States EPA also regulates pesticide limits in food. Given the economic impact of tea crops worldwide and the widespread consumption of green tea for health benefits, this study examines a specific brand of green tea for trace level presence of 22 organochlorine (OCP's) and organophosphorous (OPP's) pesticides.

SBSE coupled with comprehensive two-dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC-TOFMS) was conducted on one type of green tea spiked with OCP/OPP pesticides at 10 to 500 parts-per-trillion (ppt) levels. GCxGC offers dual column orthogonal separation which delivers enhanced resolution, increased detectability, and more available peak capacity. The benefit of coupling GCxGC with TOFMS includes acquiring full range non-skewed mass spectral information for all chromatographic peaks. This paper presents the results of the GCxGC-TOFMS trace level pesticides analysis.

The Pegasus® 4D GCxGC-TOFMS offers high spectral acquisition rates up to 500 spectra/second needed for detection of trace ppt level residual components in complex sample matrices. A sensitive and robust calibration curve was developed from 10 to 500 ppt. Stir-bar sorbent extraction (SBSE) was utilized to isolate the pesticide components from brewed green tea samples prior to analysis by GCxGC-TOFMS. One brand of green tea was qualitatively analyzed by SBSE-GCxGC-TOFMS with subsequent quantification for the OCP/OPP pesticides.

2. Experimental Conditions

The OCP/OPP pesticides standards were spiked in brewed green tea at ppt levels from 10 to 500 ppt. A GERSTEL Twister® stirbar (10 mm x 0.50 mm PDMS) was placed in each 2.0 mL aliquot of different brewed green tea brands spiked with OCP/OPP pesticides and stir bar extracted at 1000 RPM for 60 minutes. The individual stir bars were placed in a clean quartz tube and then inserted into an Automatic Tube Exchanger (ATEX). The tube containing the stirbar was automatically placed in the Thermal Desorption Unit (TDU) by the multipurpose auto sampler (MPS2) for the sample desorption and analysis. Thermal desorption was followed by sample focusing in a cryogenically cooled inlet (CIS4) prior to introduction onto the MACH/LTM GC column.

GCxGC-TOFMS results were generated with a LECO Pegasus 4D time-of-flight mass spectrometer (TOFMS). The Pegasus 4D GC-TOFMS instrument was equipped with an Agilent 6890 gas chromatograph featuring a multipurpose auto sampler (MPS2), a thermal desorption unit (TDU), a cryogenic inlet system (CIS4), and a GERSTEL/MACH low thermal mass (LTM) oven. LECO ChromaTOF® software was used for all acquisition control, calibration curve development, peak identification, and data quantification. A 10 meter Rtx-5 MACH/LTM capillary column was used as the primary column for the GCxGC-TOFMS analysis. In the GCxGC configuration a second column (1 M x 0.10 mm id x 0.10 mm film thickness, Rtx-17, Restek Corp.) was placed inside the GC oven after the thermal modulator. Helium carrier gas flow rate was set to 1.5 mL/minute at a corrected constant flow via pressure ramps. The MACH/LTM column was programmed with an initial temperature of 40°C for 2 minutes and ramped at 8°C/minute to 300°C for 1 minute. The GC oven was set to an initial temperature of 45°C for 2 minutes and then ramped at 8°C/minute to 305°C with a 1 minute hold time. The thermal modulator was set to +30°C relative to the primary oven and a modulation time of 4 seconds was used. The MS mass range was 45 to 550 amu with an acquisition rate of 150 spectra/second. The ion source chamber was set to 230°C and the detector voltage was 1800V with an electron energy of -70eV. Processing of the GCxGC data was carried out in ChromaTOF software.

3. Results and Discussion

The GCxGC-TOFMS chromatogram shown in Figure 1 clearly illustrates the increased peak capacity as opposed to one-dimensional GC-TOFMS for the OCP/OPP pesticides analysis. Over 1200 peaks were found in this sample utilizing a data processing method with a S/N ratio of 100. Both planes of the separation are clearly labeled with a 1st dimension retention time (x-axis) of approximately 2200 seconds and a 2nd dimension retention time (y-axis) of 4 seconds. In general, GCxGC analytes are separated by volatility in the first dimension and by polarity in the second dimension. Peaks are displayed on the contour plot with blue being the baseline and red being the most intense peaks. The contour plot shows the ability of GCxGC to separate and resolve additional components in the second dimension that would otherwise be coelutions.

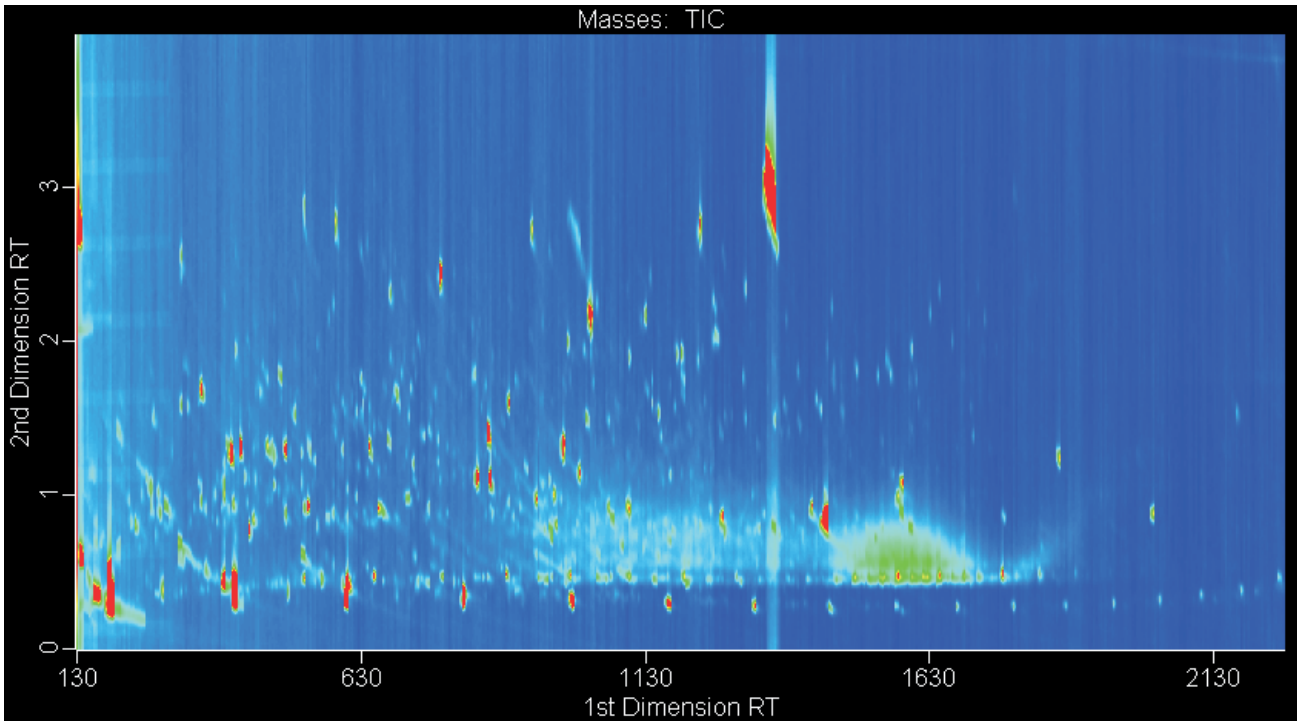

Figure 1. GCxGC-TOFMS contour plot of brewed green tea with OCP/OPP pesticides showing over 1200 peaks at a S/N ratio of 100.

Figure 2 shows the reconstructed 1st dimension chromatograms of the quantitation masses for Sulfotepp and Lindane. The quantitation masses of Lindane (mass=181) and Sulfotepp (mass=97) clearly illustrates these two pesticides coelute in the first dimension. The reconstructed first dimension chromatogram represents the combined peaks in the 8-second time window from 1181 to 1197 seconds.

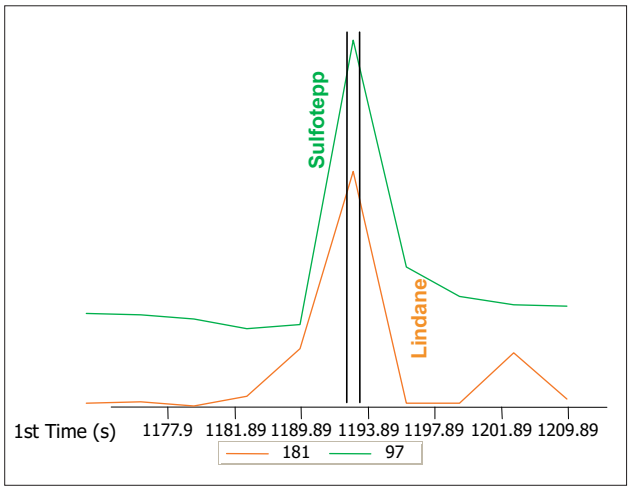

Figure 2. The GCxGC-TOFMS reconstructed 1st dimension chromatogram shows coelution of Lindane and Sulfotepp in the 1st dimension.

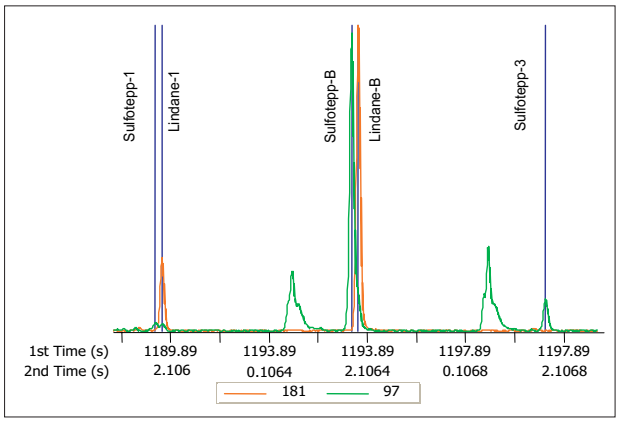

Figure 3. Figure 3 shows that the integration of each slice equals the total area observed in the 3D view. The linear chromatogram shows the uncombined peaks and labelled peak markers for Sulfotepp and Lindane over the GCxGC modulations of 4 seconds each from 1189 to 1197 seconds in the first dimension.

Figure 4 shows a surface plot view for Sulfotepp and Lindane in the 4-second modulation period of the second chromatographic dimension which is compared to the coelution observed in the first dimension chromatograms illustrated in Figure 2 and Figure 3. The quantitation masses for Lindane (mass=181) and Sulfotepp (mass=97) indicate that the two pesticides are mostly resolved in the second dimension. Enhanced resolution and increased peak capacity using multi-dimensional chromatography is illustrated by the partially resolved peaks of the 500 ppt spiked standards of Lindane and Sulfotepp depicted in Figure 4. The inset shows the modulated slice of the base peaks with the peak markers for both pesticides with a resolution of 132 milliseconds at the 2nd dimension retention times of 1.789 seconds for Sulfotepp and 1.921 seconds for Lindane.

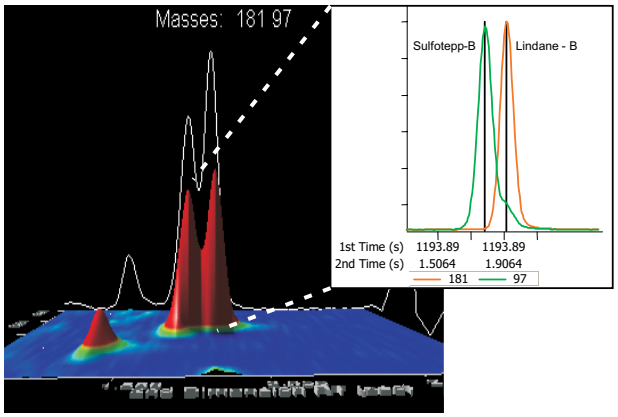

Figure 4. The GCxGC-TOFMS surface plot of 500 ppt spiked standards of Sulfotepp (mass=97) and Lindane (mass=181) illustrates the resolution in the second dimension of the chromatographic plane. The inset is the zoomed in linear chromatogram of the modulated slice for the base peaks of Sulfotepp and Lindane. These two pesticides coelute at 1193 seconds in the 1st dimension. However Sulfotepp and Lindane are separated by 132 milliseconds in the second dimension.

The 10 ppt spiked calibration standard in brewed green tea analyzed by SBSE-GCxGC-TOFMS is described by the mass spectra for Sulfotepp in Figure 5. Figure 5 shows the Caliper–Total Ion Mass Spectrum (A), the Peak True Deconvoluted Mass Spectrum (B), and the Reference Mass Spectrum (C) for Sulfotepp. The Caliper (A) shows the mass spectra for all ions acquired or the total ion mass spectra before deconvolution. The Peak True (B) shows the deconvoluted mass spectra for 10 ppt of Sulfotepp found with the ChromaTOF software Peak Find algorithm. The Reference Mass Spectrum (C) shows the mass spectrum for Sulfotepp used as the reference for identification and calibration curve generation. Figure 5 illustrates that the SBSE-GCxGC-TOFMS analysis of brewed green tea exhibited excellent detectability for 10 ppt of Sulfotepp even when partially coeluted with the pesticide Lindane.

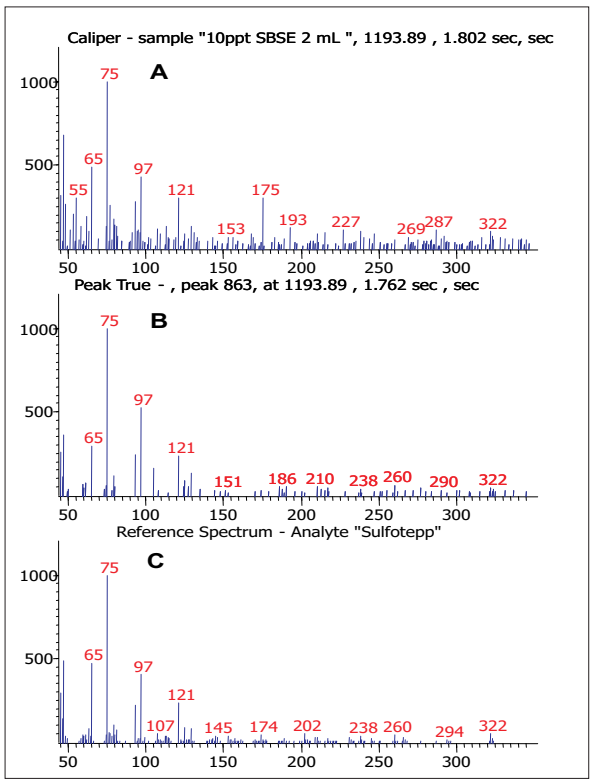

Figure 5. Shown in succession is the Caliper–Total Ion Mass Spectrum (A), The Peak True–Deconvoluted Mass Spectrum (B), and the Reference–Mass Spectrum (C) for 10 ppt of Sulfotepp spiked in brewed green tea.

4. Calibration Curve Development

Analysis of brewed green tea was carried out by SBSE-GCxGC-TOFMS. A three point calibration curve (10 ppt to 500 ppt) was developed by spiking known concentrations of OCP and OPP pesticides in the post brewed green tea prior to SBSE extraction. Extraction efficiency of the SBSE is based on the Log Kow of each compound. Analytical results shown here do not account for the loss in extraction efficiency. The fresh brewed green tea was then extracted and analyzed. Correctly identified pesticides were quantified against the reference calibration curves. Calculated concentrations are based upon the initial concentration in solution in ppt and extracted results are back calculated assuming 100% recovery. Quantitative results indicate that the green tea tested showed no significant concentrations of organochlorine and organophosphorous pesticides. This study is not intended to place any significance or implications on the toxicological effects of these findings.

Pesticide calibration standards were prepared and spiked into brewed green tea at ppt levels. Injections of each SBSE extracted standard were made to develop calibration curves at the following concentrations: 10 ppt, 250 ppt, and 500 ppt. The calibration curves were utilized to examine and quantify trace level OCP/OPP pesticide residues in one brand of brewed green tea. A complete list of all the OCP/OPP pesticides with retention time and correlation coefficients are in Table 1 for the SBSE-GCxGC-TOFMS analysis.

Shown in Figure 6 is the three point calibration curve acquired by SBSE-GCxGC-TOFMS for Sulfotepp developed from each concentration level at 10 to 500 ppt. Excellent linearity is attained with a correlation coefficient value of 0.9978 for Sulfotepp.

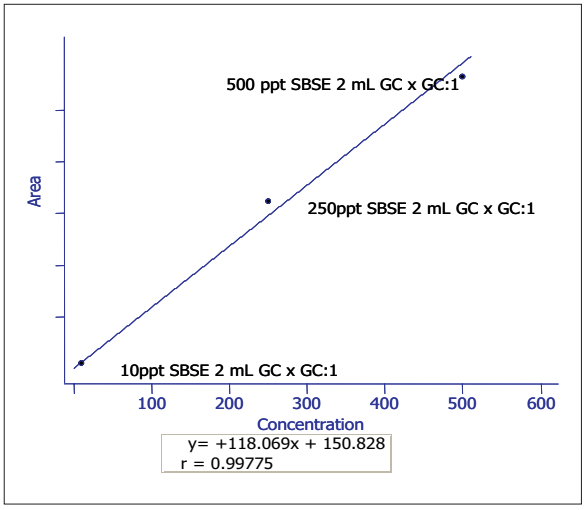

Figure 6. Calibration curve for Sulfotepp covering 10 ppt to 500 ppt.

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Table 1. SBSE-GCxGC-TOFMS correlation coefficients and retention times for 22 OCP/OPP pesticides.

Pesticide Name	GCxGC-TOFMS Absolute R.T. (sec , sec)	GCxGC-TOFMS Correlation Coefficients
Ethoprophos	1137.90 , 1.756	0.9922
Sulfotepp	1193.89 , 1.789	0.9978
à-Lindane	1193.89 , 1.921	0.9917
Demeton-s	1229.89 , 1.914	0.9982
b/g-Lindane	1249.89 , 2.257	0.9918
Diazinone	1301.88 , 1.525	0.9968
Disulfoton	1301.88 , 1.762	0.9975
ë-Lindane	1301.88 , 2.376	0.9945
Methyl parathion	1373.88 , 2.171	0.9907
Ronnel	1397.87 , 1.795	0.9965
Heptachlor	1373.88 , 1.584	0.9978
Parathion	1457.87 , 2.226	0.9977
Trichloronat	1473.87 , 1.544	0.9944
Heptachlor epoxide	1497.86 , 1.841	0.9968
Chlordane	1537.86 , 1.736	0.9992
Chlordane:2	1561.86 , 1.789	0.9931
Dieldrin	1593.86 , 1.980	0.9985
Endrin	1629.85, 2.191	0.9990
Endosulfan II:2	1645.85 , 2.323	0.9981
o,p'-DDT	1725.84 , 1.905	0.9940
Methoxychlor	1813.83 , 2.383	0.9908
Endrin ketone	1777.84 , 2.937	0.9922

Table 1 lists the first and second dimension absolute retention time and correlation coefficient values for the calibration curves of OCP/OPP pesticides conducted by SBSE-GCxGC-TOFMS. The spiked green tea calibration standards data was automatically processed by ChromaTOF software with a user-defined data processing method. Data review was conducted on a single midpoint standard. The midpoint standard was used as a reference to process the remaining points of the calibration curve. Review and assessment of all peak assignments, integrations, and calibration curve linearity was conducted using ChromaTOF software. The GCxGC-TOFMS analysis of 22 OCP/OPP pesticides exhibited correlation coefficient values of greater than 0.9900.

5. Conclusions

This study demonstrates a novel sampling method requiring no sample preparation utilizing SBSE and automated sample introduction. The GCxGC-TOFMS analysis results show that low trace level pesticides can be accurately identified and measured quantitatively. The analysis conducted by GCxGC-TOFMS presents an accurate and sensitive analysis for trace level quantitation of OCP/OPP pesticide residues found in various types of green tea. Excellent results for the GCxGC-TOFMS analysis were achieved for the calibration linearity. The increased peak capacity of GCxGC-TOFMS was apparent with results showing an average of 1200 identified peaks per sample.

TOFMS acquires simultaneous non-skewed full-mass range spectra that allows for fast acquisition combined with the deconvolution needed to identify and quantify low level targeted components in complex samples. This experimental approach demonstrates how the integration of sample handling using the GERSTEL thermal desorption unit and the CIS-4 inlet along with the GERSTEL/MACH LTM column oven in combination with GCxGC-TOFMS provides a highly efficient method for the quantitative evaluation of OCP/OPP pesticides in complex sample matrices such as green tea.

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