

# Trace Level Organochlorine and Organophosphorous Pesticides Analysis in Green Tea by SBSE-GC-TOFMS

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**Key Words:** GC-TOFMS, Organochlorine and Organophosphorous Pesticides, SBSE, GERSTEL MACH/LTM, Quantitation

## 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 five different teas for the trace level presence of 22 organochlorine (OCP's) and organophosphorous (OPP's) pesticides.

GC-TOFMS was utilized to develop trace level calibration curves in tea spiked with 22 OCP/OPP pesticide standards. The Pegasus® GC-TOFMS offers high spectral acquisition rates needed for detection of trace parts-per-trillion (ppt) level residual components in complex sample matrices. A sensitive and robust calibration curve was developed from 10 to 500 ppt. Exceptional limits of detection were achieved by GC-TOFMS at or below 10 ppt for all but one of the pesticides. Stir bar sorbent extraction (SBSE) was utilized to isolate the pesticide components from brewed green tea samples prior to analysis by GC-TOFMS. Different types of green tea were qualitatively analyzed by SBSE-GC-TOFMS with subsequent quantification for the OCP/OPP pesticides.

## 2. Experimental Conditions

### SBSE-GC-TOFMS

Analytical results were generated with a LECO Pegasus time-of-flight mass spectrometer (TOFMS). The Pegasus 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 m x 0.18 mm id. x 0.20 µm film thickness MACH/LTM capillary column (Rtx-5, Restek Corp.) was used for the chromatographic separation. The GC was operated with Helium carrier gas at a corrected constant flow of 1 mL per minute. The OCP/OPP pesticide standards were spiked in various brands of brewed green tea at ppt levels from 10 to 500 ppt. A GERSTEL Twister® stir bar (10 mm x 0.50 mm PDMS) was placed in each 10 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 placed in an Automatic Tube

Exchanger (ATEX). The tube containing the stir bar was automatically placed in the Thermal Desorption Unit (TDU) by the 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. The chromatographic temperature program for the GERSTEL MACH/LTM oven module was set at an initial temperature of 40°C for 2.00 minutes then ramped at 50°C per minute to 300°C with a 1.00 minute hold time for a total chromatographic runtime of 8.2 minutes. The MS transfer line temperature was set to 280°C. The MS mass range was 50 to 510 amu with an acquisition rate of 20 spectra per second. The ion source chamber was set to 230°C and the detector voltage was 1800V with electron energy of -70eV.

## 3. Results and Discussion

Analysis of brewed green tea was carried out in duplicate by SBSE-GC-TOFMS. Six-point calibration curves (10 ppt to 500 ppt) were 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. Fresh brewed green tea samples were 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 percent recovery. Quantitative results indicate that the various green tea types tested showed no significant concentrations of organochlorine and organophosphorous pesticides. However, three different brands of green tea did show trace levels of pesticide residue at ppt to low ppb levels. This study is not intended to place any significance or implications on the toxicological effects of these findings.

Figure 1 displays the TIC chromatogram for the analysis of brewed green tea by GC-TOFMS. Approximately 550 to 600 compounds were found with a signal to noise ratio of 100 and a runtime of 8.2 minutes even with a peak overlap of the complex sample matrices. TOFMS is able to acquire full range non-skewed mass spectra at full range sensitivity. This feature allows for identification of coeluting compounds in complex sample matrices. Deconvolution is performed through algorithms which assign a unique mass for each peak that successfully identify and quantify all targeted compounds in a single run.

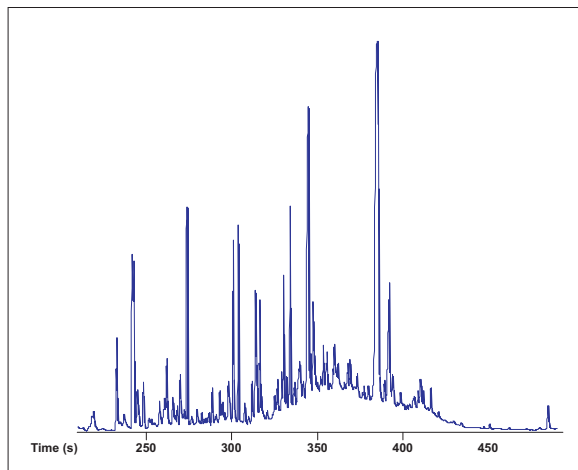


Figure 1. The Total Ion Chromatogram of a brewed green tea sample by SBSE-GC-TOFMS analysis.

Figure 2 shows a 3 second zoomed-in retention time window of a single dimension chromatogram with the quantification mass  $m/z = 353$  for Heptachlor Epoxide (Peak 486) and 4 other unique masses of matrix interferences. Peak 486 at 408.3 seconds represented by the red peak marker at mass  $m/z = 353$  is a 100 ppt spike of Heptachlor Epoxide in brewed green tea. Interfering unique masses  $m/z = 174, 100, 204,$  and  $223$  are shown for coeluting and matrix interference peaks. Notice that Heptachlor Epoxide is completely buried in the peak with mass  $m/z = 174$ . Heptachlor Epoxide (Peak 486) is submerged with matrix interference compounds making identification difficult without deconvolution. This demonstrates the ability to locate and deconvolute trace concentrations of analytes at low ppt levels in complex matrices.

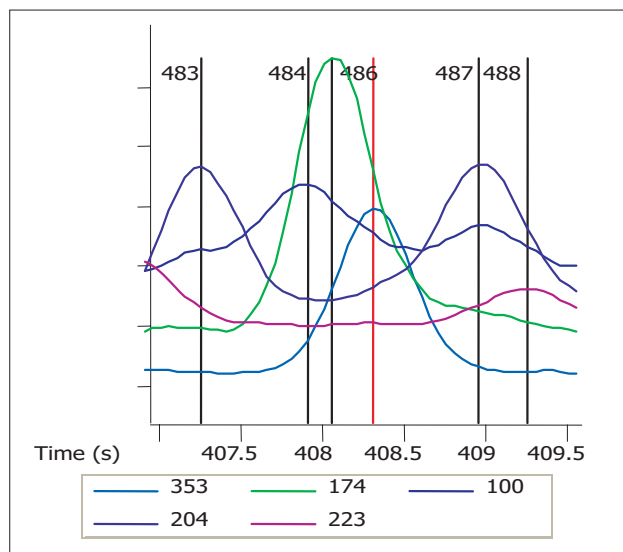


Figure 2. Shown above is a 3 second zoomed-in window of 100 parts per trillion for Heptachlor Epoxide with a quant. mass of  $m/z = 353$  (Peak 486).

Figure 3 shows in detail the automated mass spectral deconvolution process for Heptachlor Epoxide. Figure 3(A) the Total Ion Chromatogram (Caliper) mass spectrum shows the full range of all masses collected at 408.3 seconds. Figure 3(B) the Deconvoluted (Peak True) mass spectrum is the true mass spectrum for the peak identification with the matrix interference masses subtracted. Figure 3(C) displays the (Library Hit) mass spectrum for Heptachlor Epoxide. It is the deconvoluted mass spectrum (B) that is searched against the library. The deconvoluted mass spectra (B) clearly shows the fragment ions such as  $m/z = 353, 317, 263,$  and  $81$  that are observed in the library spectral match (C). The matrix interfering masses shown in the Caliper spectra (A) are removed from the deconvoluted Peak True mass spectra (B) using the deconvolution algorithm that combines only the non-skewed mass spectral data together. A similarity match score of 805 out of 1000 was returned for Heptachlor Epoxide at 100 ppt.

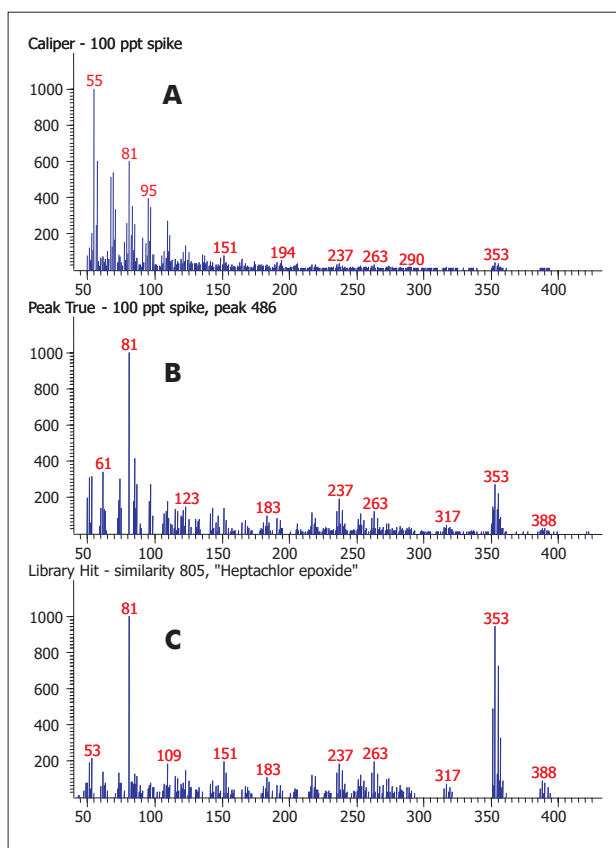


Figure 3. Shown in succession is the Caliper - Total Ion Mass Spectrum (A), The Peak True - Deconvoluted Mass Spectrum (B), and the Library Hit - Mass Spectrum (C) for Heptachlor Epoxide.

#### 4. Calibration Curve Development

Pesticide calibration standards were prepared and spiked into brewed green tea at ppt levels. Two injections of each SBSE extracted standard were made to develop calibration curves at the following concentrations: 10 ppt, 25 ppt, 50 ppt, 100 ppt, 250 ppt, and 500 ppt. The calibration curves were utilized to examine and quantify trace level OCP/OPP pesticide residues in different types of green tea. A complete list of all the OCP/OPP pesticides with retention time and correlation coefficients are in Table 1 for the SBSE-GC-TOFMS analysis.

Shown in Figure 4 is the full range six point calibration curve acquired by GC-TOFMS for Ethoprophos and developed from two data points for each concentration level. The inset shows the low end linearity for the concentrations from 10 to 500 ppt. The correlation coefficient for Ethoprophos is 0.9994.

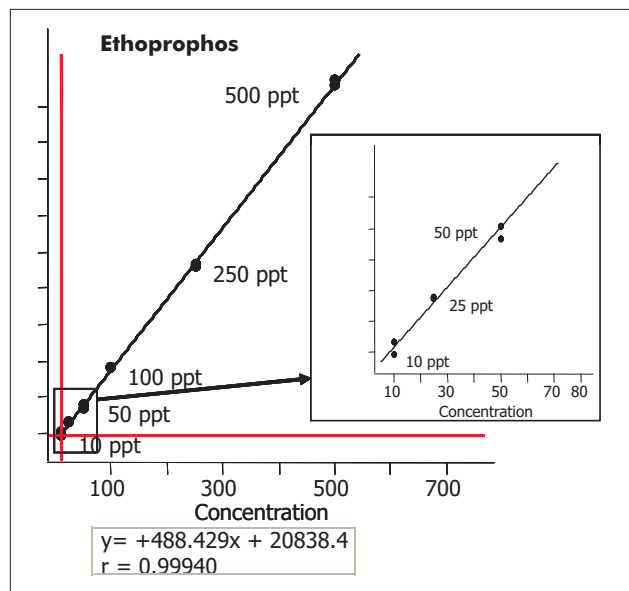


Figure 4. Calibration curve for Ethoprophos covering 10 ppt to 500 ppt.

Table 1. Correlation Coefficients and retention times for 22 OCP/OPP pesticides

Pesticide Name	Absolute R.T. (s)	Correlation Coefficients
Ethoprophos	354.5	0.9994
Sulfotepp	360.2	0.9969
à-Lindane	365.8	0.9972
Demeton-s	366.4	0.9987
b/g-Lindane	374.0	0.9966
Diazinone	374.1	0.9988
Disulfoton	377.0	0.9876
ë-Lindane	379.5	0.9976
Methyl parathion	387.8	0.9948
Ronnel	390.9	0.9977
Heptachlor	391.2	0.9954
Parathion	398.2	0.9974
Trichloronat	401.4	0.9970
Heptachlor epoxide	407.9	0.9947
Chlordane	413.1	0.9957
Chlordane:2	416.3	0.9976
Dieldrin	421.9	0.9981
Endrin	427.0	0.9906
Endosulfan II:2	428.7	0.9949
o,p'-DDT	436.1	0.9957
Methoxychlor	448.0	0.9983
Endrin ketone	449.4	0.9927

Table 1 lists the absolute retention time and correlation coefficient values for the calibration curves of OCP/OPP pesticides conducted by SBSE-GCTOFMS. 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 5 points of the calibration curve. Review and assessment of all peak assignments, integrations, and calibration curve linearity was conducted using ChromaTOF software. For the GC-TOFMS analysis 21 pesticides exhibited correlation coefficient values of greater than 0.9900. Disulfoton is known to give poor chromatographic performance, however a correlation coefficient of 0.9876 for Disulfoton was calculated at a concentration range of 50 ppt to 500 ppt. Excellent linearity was achieved over the concentration range of 10 ppt to 500 ppt for the other 21 pesticides.

### 5. Conclusions

This study conducted with the LECO Pegasus HT GC-TOFMS demonstrates a novel sampling method requiring no sample preparation utilizing SBSE and automated sample introduction. The GC-TOFMS analysis results show that low trace level pesticides can be accurately identified and measured quantitatively. The analysis conducted by GC-TOFMS presents a fast, accurate, and sensitive method for trace-level quantitation of organochlorine and organophosphorous pesticide residues found in five types of green tea. Fast acquisition TOFMS acquires simultaneous non-skewed full range spectra which allows Deconvolution algorithms to successfully assign unique ions for all compounds in a sample, even where serious chromatographic overlap occurs. The ability to successfully identify and deconvolute target compounds is imperative for qualitative and quantitative studies in heavy sample matrix. This experimental approach demonstrates how the integration of a novel sampling method SBSE with thermal desorption coupled with a LTM column in combination with GC-TOFMS provides a highly efficient method for the quantitative evaluation of OCP/OPP pesticides in complex sample matrices such as green tea.

