Application Note: 10013

Analysis of Pesticides and PCB's Using a Large Volume Splitless Injection Technique

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Key Words

- DSQ Single Quadrupole GC/MS
- Large Volume
- PCB
- Pesticides
- Split/Splitless

Overview

Purpose

Analysis of pesticides and polychlorinated biphenyls (PCBs) at low ppb concentrations is typically performed using traditional detectors such as the electron capture detector (ECD) or through selected ion monitoring mass spectrometry (SIM). This application explores the use of a large volume splitless injection technique for analysis of these compounds in conjunction with full scan GC/MS. The potential for increased confidence in compound identification, decreased sample preparation time, and enhanced specificity and sensitivity will be evaluated.

Methods

Standards were prepared in hexane at concentrations ranging from 1.25-250 pg/µL (1.25-250 ppb). A matrix of chrysanthemum tea was cold-extracted into hexane and spiked with 50 pg/µL of the pesticide standard. The GC/MS system was set up with a 5 m pre-column connected to a 30 m x 0.25mm x 0.25 µm analytical column. 35 µL injections were made of the curve, blank matrix, matrix spike and duplicate, as well as seven replicate injections at 2.5 pg/µL. Full scan EI+ mass spectral data was acquired with a scan range of 35-550 amu at 1500 amu/second.

Results

Typical results showed linearity from 1.25-250 pg/µL, with correlation coefficients all greater than 0.99. Full scan spectral data were consistent with NIST Library spectra. The run time was less than 20 minutes for all compounds. Precision analysis at 2.5 pg/µL (n=7) demonstrated method reproducibility, with the average %RSD (calculated amount) of 5.4%. Method detection limits were calculated at the fg/µL level, which offers ECD-level detectivity while providing full-scan mass spectral specificity.

Introduction

The analysis of pesticide and PCB residues presents analytical challenges for a number of reasons. Considerable pressure for lower detection limits of these compounds prompts laboratories to seek methods that enable them to reproducibly attain results in the pg/µL range. By combining a large volume splitless injection with full scan gas chromatography/mass spectrometry, low level analysis can be accomplished with decreased sample preparation time.

A large volume splitless injection technique was developed. This technique was then evaluated using standards containing pesticides and PCB's at concentrations ranging from 1.25-250 pg/µL (1.25-250 ppb) in hexane.

LVSL can be accomplished on the Thermo Scientific TRACE GC Ultra™ after a simple modification to the standard split/splitless injector.[1] This configuration minimizes dead volume, is forward-pressure regulated and allows for an automatic pressure surge upon the injection of the large solvent volume. Sample injection techniques typically utilize either thermospray or liquid band formation for volatilizing samples. In thermospray, the syringe barrel is heated and the plunger is depressed slowly, resulting in a "spray" of sample, which is rapidly volatilized in the injection port. Liquid band, by contrast, involves maintaining a relatively cool needle temperature and rapid ejection of the syringe plunger. When the large amount of liquid is trapped on the bottom of the liner by some means (glass wool packing material or inverted cup), sample evaporation starts, generating a large vapor cloud.

The injection port design and forward pressure regulation minimizes dead volume; therefore, the vapor cloud remains in the liner, resulting in a strong, rapid pressure increase. The solvent vapors then exit by the only available outlet: the precolumn entrance. Since the precolumn temperature is below the boiling point of the solvent, vapors quickly recondense on the uncoated precolumn. The pressure increase has the important advantage of being self-regulating and fast: pressure automatically increases and decreases again when solvent evaporation slows or ceases. The whole process, termed concurrent solvent recondensation, lasts only a few seconds.

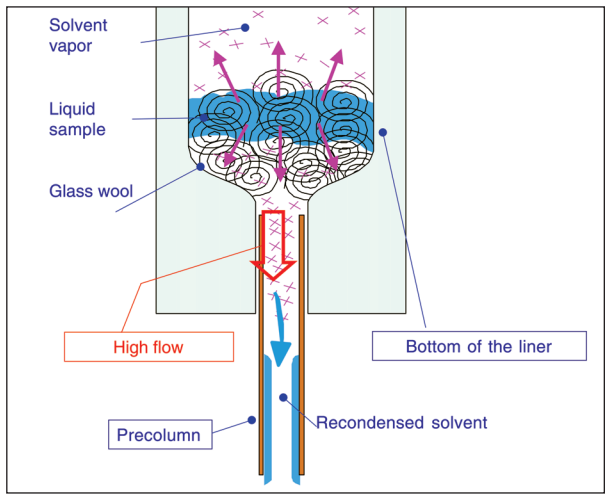

Figure 1: Schematic diagram of large volume splitless injection, showing the concurrent solvent recondensation that occurs in the pre-column.

The oven temperature is maintained below the boiling point of the solvent through the evaporation phase, which reconcentrates volatile solutes through solvent trapping. Higher-boiling components, which are spread within the uncoated precolumn, are focused by the retention gap effect.[1,2] After the initial hold time, set by using a software tool that provides GC settings, detection and analysis of the samples is performed using the Thermo Scientific DSQ™ quadrupole mass spectrometer. The system operated in full scan EI mode, provides mass spectral data for identification and quantification of compounds.

Ret. Time (Min)	Component Name	Quantitation mass (*m/z*)	Calibration Range (pg/µL)	r^2	Precision (%RSD)
7.62	naphthalene-D8	136			13.2 (n=1)
8.38	Hexachlorocyclopentadiene	237	1.25-250	0.9990	6.9
9.10	acenaphthene-D10	162			9.9 (n=1)
9.14	2-chloroBP	188	1.25-50	1.0000	3.5
9.98	2,3-dichloroBP	222	1.25-125	0.9999	3.1
10.05	hexachlorobenzene	284	1.25-250	0.9993	12.9
10.07	simazine	201	5-250	0.9937	Below Cal Range
10.15	atrazine	200	1.25-250	0.9985	2.1
10.25	γ-BHC	181	1.25-250	0.9982	15.6
10.51	2,4,5-trichloroBP	256	1.25-250	0.9997	2.7
10.68	alachlor	160	1.25-250	0.9998	4.9
10.75	heptachlor	100	1.25-250	0.9999	5.1
10.9	2,2',4,4'-tetrachloroBP	292	1.25-250	0.9993	3.4
11.02	aldrin	263	1.25-250	0.9999	4.5
11.27	heptachlor epoxide	353	1.25-250	0.9998	6.5
11.27	2,2',3',4,6-pentachloroBP	326	1.25-250	0.9984	8.8
11.43	γ-chlordane	373	1.25-250	1.0000	2.9
11.53	α-chlordane	373	1.25-250	0.9998	2.8
11.56	trans-nonachlor	409	1.25-250	0.9992	3.9
11.69	2,2',4,4',5,6-hexachloroBP	360	1.25-250	0.9987	5.0
11.72	dieldrin	263	1.25-250	0.9996	8.3
11.90	endrin	263	1.25-250	0.9987	5.7
12.04	cis-nonachlor	409	1.25-250	0.9993	3.4
12.72	methoxychlor	227	1.25-125	0.9915	2.4
12.77	2,2',3,3',4,4',6-heptachloroBP	394	1.25-125	0.9996	5.5
12.83	2,2',3,3',4,5',6,6'-octachloroBP	430	1.25-250	0.9971	6.2

Table 1: Summary of results for LVSL GC/MS analysis of pesticides and PCB's in hexane. The r^2 value is for linear fit. Precision as %RSD for the pesticides and PCB's is based upon calculated amount (n=7). Precision for the internal standard based on area count (n=31). Simazine was not included in the replicate analysis, as it fell below the calibration range for that compound.

Methods

Sample Preparation

Pesticide and PCB standards were obtained from Supelco (Bellefonte, PA), at concentrations of 500 µg/mL of each of the pesticides and PCB's listed in Table 1. A working standard of 250 pg/µL was prepared, and from this solution, 1.0 mL standards for the curve were created with points at 1.25, 2.5, 5, 10, 50, 125, and 250 pg/µL. A matrix blank was prepared by an overnight cold-extraction of 20 grams of chrysanthemum tea with approximately 200 mL hexane.

The extracted matrix was not concentrated further by evaporation. A matrix spike and the duplicate were prepared by adding 200 µL of the working standard to 800 µL of matrix blank, for a spike amount of 50 pg/µL. 1000 µL of unspiked matrix served as a matrix blank. Additional standards at 2.5 pg/µL were prepared for precision analyses. To each vial, 10 µL of working internal standard solution (Cerilliant Corporation, Round Rock, TX) were added for a final internal standard amount of 50 pg/µL. The autosampler vials were capped and prepped for analysis.

Instrumentation

A Thermo Scientific DSQ with a 250 L/s turbomolecular pump was used in positive electron ionization (EI) mode for collection of full scan data, with a mass range of 35-550 amu, at a scan rate of 1500 amu/sec (~2.7 scans/sec). The emission current was set to 50 µA, and a detector gain of 1.0 x 10^5 was used. All other lens and prefilter settings were from Autotune values. The solvent (filament) delay was set to 7.0 minutes.

The injection volume was 35 µL, with a splitless duration of 1.0 minute. The use of LVSL requires that the constant septum purge be turned off during the splitless time, to ensure that all valves are closed and to force the vapor cloud into the precolumn. The initial oven temperature and time were programmed in accordance with the accompanying software tool, which is designed to optimize the large volume splitless (LVSL) technique. Run time was less than 20 minutes for all compounds. The mass spectrometer acquired data in full scan mode, from 35-550 amu at a scan rate of 1500 amu/sec.

A TRACE GC Ultra with a split/splitless inlet was modified for use with the large volume splitless injection technique. A special injection port head designed to keep the syringe needle cooler than the injection port during sample injection replaced the standard inlet septum nut, and a 5mm splitless liner with a plug of deactivated glass wool at the gooseneck was used as a liner. A 5 m x 0.32 mm i.d. uncoated pre-column was inserted approximately 58 mm into the injection port, so that the column head was just below the glass wool (Figure 1). A 30 m x 0.25 mm i.d. x 0.25 µm df Rtx®-5MS analytical column was connected to the pre-column with a Siltek™ Press-Tight® union.[3] The entire system was checked manually for leaks prior to analysis.

An autosampler was programmed to inject 35 µL of sample at a speed of 99 µL/sec. The needle depth into the injection port was set up at 13 mm to allow the syringe needle to come below the septum but remain outside of the main heated injection port body. This technique ensured delivery of a liquid droplet to the injection port.

The oven temperature program was set up through the use of the Large Volume Splitless Assistant software tool. Figure 2 shows the method used, with an injection volume of 35 µL of hexane, constant flow of He at 1.0 mL/min, and the appropriate column K-factor entered. From this tool, the GC oven program was set to an initial temperature of 67 °C for an initial hold time of 3.85 minutes. Following the isothermal evaporation phase, the analytical oven run began. The oven temperature was ramped at 30 °C/min to 290 °C, held for 0.5 min, then ramped again at 30 °C/min to 320 °C, with a final hold time of 3.0 minutes. Each sample was injected twice, except for the precision samples.

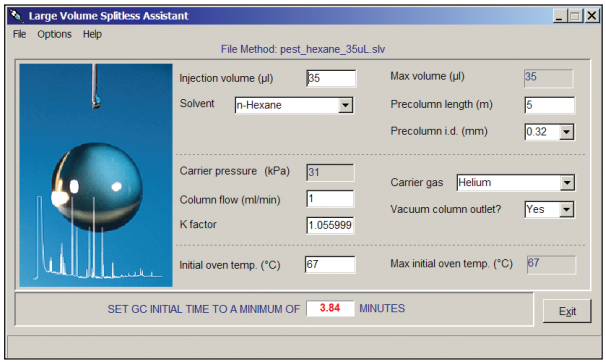

Figure 2: Large Volume Splitless Assistant software tool, depicting method for injection of 35 µL of hexane using a 5 m x 0.32 pre-column at constant flow of He of 1.0 mL/min.

Results

The analysis of 24 pesticides and PCB's by LVSL shows good linearity from 1.25 to 250 pg/µL for 20 compounds, with a run time of less than 20 minutes. A precision analysis at 2.5 pg/µL showed good reproducibility in the calculated amounts for the compounds, with % RSD's of the concentrations ranging from 2.1 to 15.6% (n=7). The area count precision for the two internal standards, naphthalene-D8 and acenaphthene-D10, were 13.2 and 9.9% RSD respectively (n=31). Correlation coefficients for standards in this evaluation ranged from 0.9915 for methoxychlor to 1.000 for 2-chlorobiphenyl and γ-chlordane. Reproducibility data was obtained through repeated injections of seven standards at 2.5 pg/µL. Chrysanthemum tea extracts were also analyzed, both spiked and blank, prior to the precision analyses to demonstrate method ruggedness. These data are summarized in Table 1.

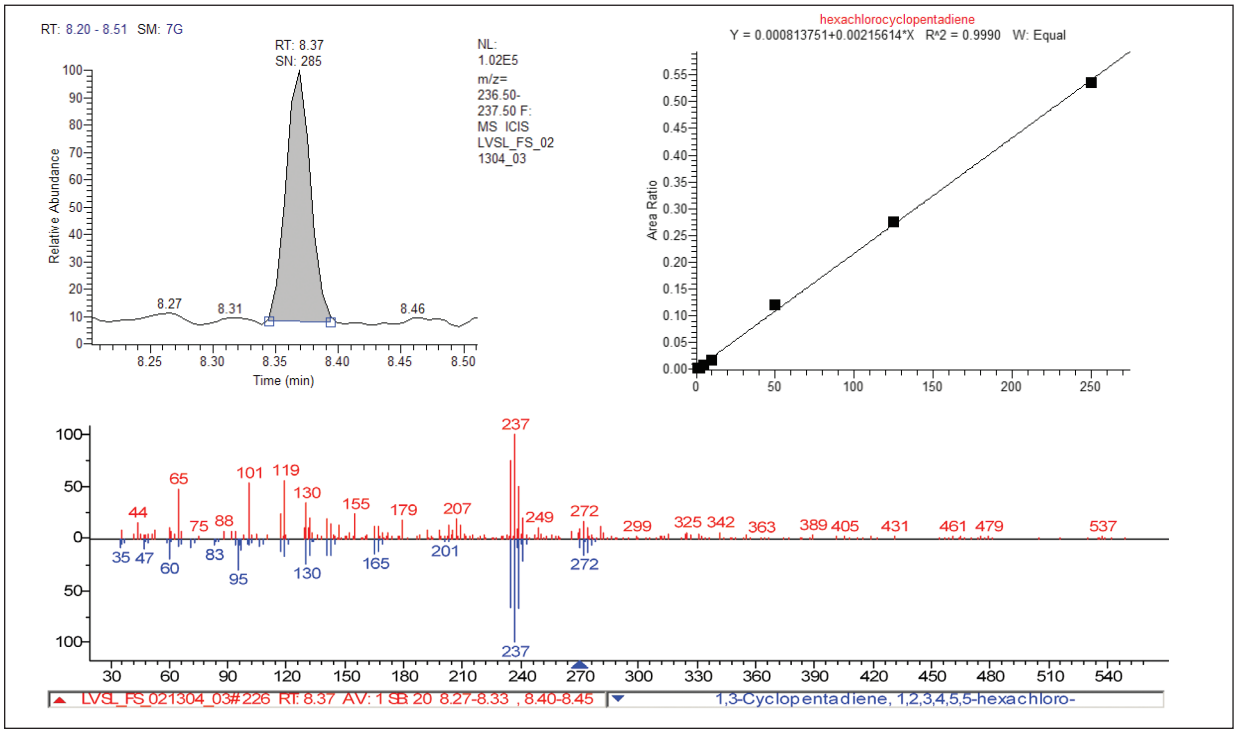

Figure 3: Data for hexachlorocyclopentadiene at 1.25 pg/µL, showing an extracted ion chromatogram (*m/z* 237), calibration curve from 1.25-250 pg/µL (r^2=0.9990) and full scan spectrum compared to the NIST Library reference spectrum for this compound. The library reverse match quality was 851, with a probability of 81.6% (top hit).

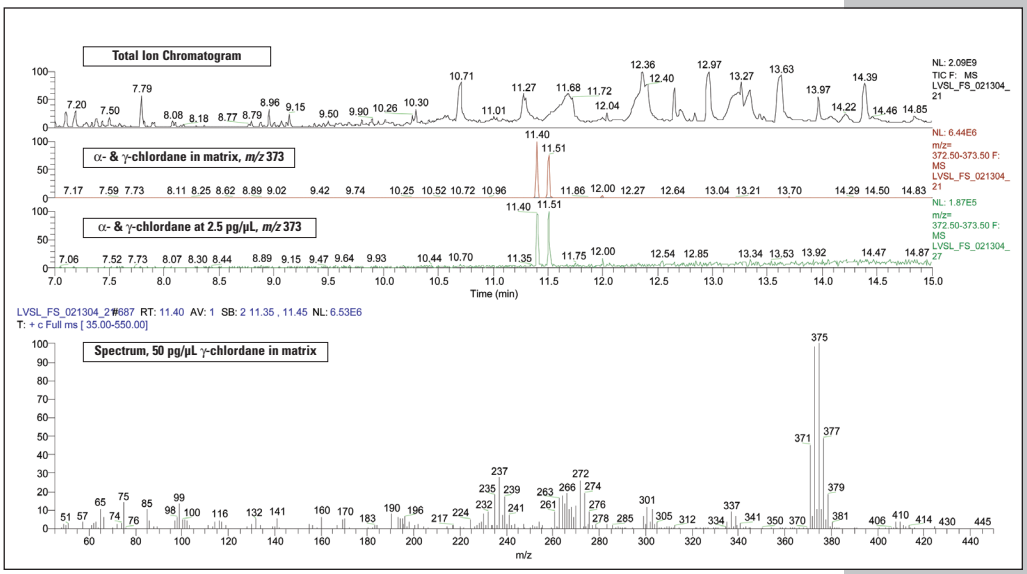

Figure 4: Chrysanthemum tea hexane extract spiked with 50 pg/µL of pesticide and PCB standard. The top trace is the total ion chromatogram. The middle traces show the extracted ion chromatograms (EIC) for γ- and α-chlordane (*m/z* 373) at 50 pg/µL in matrix (red) and at 2.5 pg/µL in hexane (green). The bottom trace is the spectrum for γ-chlordane at 50 pg/µL in matrix.

The use of a 35 µL injection volume results in an effective on-column concentration of the standards from 43.75 pg up to 8.75 ng. The concentration for the low standard of 1.25 pg/µL, based on a 20g sample size, is 62.5 pg/kg. This enables the use of full scan acquisitions even at very low concentrations. Figure 3 depicts the extracted ion chromatogram for *m/z* 237, which served as the quantitation ion for hexachlorocyclopentadiene. The EI spectrum at 1.25 pg/µL is also shown for this compound. The spectrum produced the number one library match using NIST MS Search 2.0 2002 Library.

Six injections of matrix (2 each of blank matrix, spike and duplicate) preceded the precision samples. This afforded the opportunity to evaluate method ruggedness. The top trace in Figure 4 shows the total ion chromatogram for the spiked sample matrix. This spike contained 50 pg/µL of the pesticide/PCB standard. The extracted ion chromatograms (EIC) for *m/z* 373, for γ-chlordane and α-chlordane, are shown as well. The maroon trace reflects the 50 pg/µL spike. The green trace is the EIC at 2.5 pg/µL, which followed the matrix injections, and which was proceeded by two blank injections. The spectrum shown is for γ-chlordane at 50 pg/µL. LVSL injections allowed a 100-fold decrease in method detection limits, when compared to previously published results for standard splitless injections.[4] Utilizing the LVSL technique and an injection volume of 35 µL, detection limits of 341 fg/µL, 300 fg/µL, and 470 fg/µL were calculated for hexachlorocyclopentadiene, 2-chlorobiphenyl, and heptachlor.

Conclusions

Ultimately, the combination of LVSL and the Thermo Scientific DSQ allows analysts to confirm low-level pesticides and PCB's using full-scan mass spectrometry. These full-scan data offer greater specificity than GC detectors or SIM methodologies. The technique offers other advantages as well. LVSL yields MDLs in the fg/µL range, which represents a 100-fold decrease in detection limits compared to standard splitless injections. The analysis of 24 pesticides and PCB's by GC/MS was accomplished successfully through the use of a large volume splitless injection technique. Full-scan data were acquired from 1.25 to 250 pg/µL in solution with good linearity, reproducibility and system robustness.

The LVSL technique simplifies sample preparation by minimizing evaporation and concentration techniques. Additionally, LVSL uses the standard split/splitless injection port. The modifications for using the technique are easily reversible, which allows interchangeable operation between standard SSL and LVSL. The DSQ, when equipped with a 250 L/s turbo pump, is able to tolerate the solvent, and the Ion Bright source enhances system performance and minimizes maintenance needs.

References

1. P. Magni, T. Porzano. Concurrent Solvent Recondensation Large Sample Volume Splitless Injection. *Journal of Separation Science*. 2003, **26**.1491 –1498.
2. P. Magni, T. Porzano, in: Proc. 25th Int. Symp. On Capillary Chromatography, Riva del Garda, 2002.
3. Rtx-5MS, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

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