Pulsed Splitless and Large-Volume Injection in Capillary Gas Chromatography Mass Spectrometry for the Determination of Ultra-Trace Level Pesticide Residues from Lake Waters

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Introduction

A collaborative research effort between two branches of U.S. EPA's National Exposure Research Laboratory investigates airborne transport of pesticides from the San Joaquin Valley (SJV), CA into the high-elevation lakes of the southern Sierra Nevada in the Sequoia and Kings Canyon National Parks. Exposure of the alpine lakes to these pesticides could have impacted these sensitive ecosystems and thus, have contributed to the extinctions of the mountain yellow-legged frog from some of its historic range. The research project will measure pesticide concentrations in this area of the Sierra Nevada, investigate temporal and spatial trends in the concentrations, and use those trends to test the hypothesis that current use pesticides are being transported by near-surface winds to the area from the SJV.

The anticipated concentrations of pesticides in the lakes are lower than the detection limits of conventional analytical approaches [i.e., field collection of water, laboratory extraction, and analysis of less than 1% of the extract by capillary gas chromatography mass spectrometry (GCMS)]. Our approach to achieving lower detection limits is to extract analytes in situ from 100 L of water, inject a substantial fraction of the extract into a programmable temperature vaporizer (PTV) and analyze by GCMS in selected ion monitoring mode (SIM). Initial method development optimized the chromatographic conditions using conventional pulsed splitless injection (PSI) method with 1-µL sample. Once chromatographic separation using PSI method was optimal, large-volume injection (LVI)-GCMS was developed, using the PSI parameters as a starting point.

This poster presents experimental results for the two sample introduction techniques, pulsed splitless injection (PSI) and large-volume injection with solvent venting (LVI). PSI introduces 1 µL of sample into the inlet liner at a high temperature and pulsed high pressure. LVI slowly injects at least 20 µL of sample into the GC inlet liner at a temperature below the solvent's boiling point, while venting the bulk of the sample solvent away from the GC column.

Selection of target analytes was based on the annual usage of these pesticides in the SJV. Table 1 lists the pesticides of interest, which include a range of pesticide classes. The oxygen analogs and transformation products listed in Table 2 are also determined.

Table 1. Pesticides targeted in this study.*

Compound	Class	Annual Use (kg)	Season of Peak Use
Alachlor	aniline herbicide	7,259	spring
Azinphos-methyl	organophosphorus (OP) insecticide	78,543	summer
Butylate	thiocarbamate herbicide	20,549	spring
Carbaryl	carbamate insecticide	208,877	summer
Chlorothalonil	organochlorine (OC) fungicide	211,521	spring/summer
Chlorpyrifos	OP insecticide	650,598	summer
Cymazine	triazine herbicide	183,568	summer
Diazinon	OP insecticide	145,766	winter
Dicofol	OC pesticide	164,026	summer
Disulfoton	OP insecticide	10,309	spring/summer
Endosulfan (I and II)	OC insecticide	54,430	summer
EPTC	thiocarbamate herbicide	96,144	spring
Ethalfluralin	aniline herbicide	5,070	spring
Lindane (γ-HCH)	OC insecticide	463	spring
Linuron	substituted urea herbicide	18,836	fall/spring
Malathion	OP insecticide	65,331	summer/spring
Methidathion	OP insecticide	92,307	winter/summer
Methyl parathion	OP insecticide	24,892	spring
Metolachlor	aniline herbicide	22,914	spring
Napropamide	amide herbicide	18,372	winter/spring
Pebulate	thiocarbamate herbicide	25,926	spring
Pendimethalin	aniline herbicide	131,569	spring
Permethrin (*cis* and *trans*)	synthetic pyrethroid pesticide	23,574	summer
Phorate	OP insecticide	36,546	spring
Phosmet	OP insecticide	135,764	summer/spring
Propargite	sulfonic acid acaricide	556,508	summer
Simazine	triazine herbicide	216,616	winter
Tribufos	OP defoliant	250,774	fall
Trifluralin	aniline herbicide	232,891	spring

*Use information extracted from State of California (1999) for the period 1996-1998. Annual use data are active ingredient applied in Fresno, Kern, Kings, Madera, and Tulare Counties in the year of median use of individual chemical during that three-year period.

Table 2. List of target oxygen analogs and transformation products.

Azinphos-methyl oxon	Methyl parathion oxon
Chlorpyrifos oxon	Paraoxon
Diazoxon	Phosmet oxon
Malaoxon	4,4'-Dichlorobenzophenone

Experimental

Chemicals and Reagents

All mixtures of pesticide standards, internal standards (acenaphthene-d10, phenanthrene-d10, and chrysene-d12), and triphenyl phosphate (surrogate) were purchased from ChemService (West Chester, PA). Deuterated pesticide standards (trifluralin-d14, diazinon-d10, chlorpyrifos-d10) for use as surrogates were purchased from Cambridge Isotope Laboratories (Andover, MA). Working standards were prepared by appropriate dilution with Ultra Resi-Analyzed® ethyl acetate (J.T. Baker, Phillipsburg, NJ). Solvents were used without further cleanup.

Instrumentation

All GCMS analyses were performed using an Agilent 6890A capillary gas chromatograph and a 5973N mass selective detector (Agilent Technologies, Palo Alto, CA) in electron impact mode. The gas chromatograph was fitted with a 30 m x 0.25 mm ID fused silica capillary column coated with a 0.25-µm film of crossbonded 5% diphenyl – 95% dimethyl polysiloxane (Restek Corporation, Bellefonte, PA). A deactivated guard column of 5 m x 0.25 mm ID was fused to the front end of the analytical column. LVI was performed in a Gerstel CIS4 inlet (Mühlheim, Germany), a programmable temperature vaporizer, equipped with a 71 mm x 2.0 mm ID deactivated baffled liner. Speed-controlled injection for LVI was performed using a 100-µL syringe in a Gerstel MPS2 autosampler. Ultrapure helium was used as carrier gas.

Sample Collection/Extraction

- Collect 100-L water sample thru an 8-g Nexus column
- Remove excess water under N_2
- Elute column with appropriate solvent
- Dry eluent over $NaSO_4$
- Reduce sample to ~ 0.5 mL
- Add internal standard and adjust volume to 1 mL with ethyl acetate
- Clean up extract on silica gel

Pulsed Splitless Injection Method

In the PSI method, a 1-µL sample is injected into the liner at 200ºC and a pulsed pressure of 174 kPa, which is held for 0.75 min. The pulsing effect maximizes sample introduction into the column while narrowing the sample bandwidth. Additionally, the sample has a very short residence time in the liner and thus minimizes losses of active compounds such as disulfoton and dicofol. After the initial pressure pulse, the carrier gas flow is held constant at 1 mL/min. The oven temperature program is presented below.

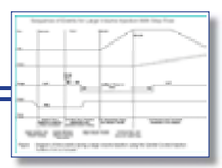
Oven:

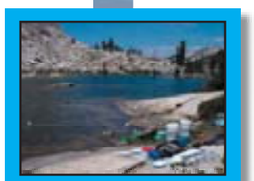
Initial temp: 50ºC Initial time: 1.0 min

Ramps:	#	Rate	Final T	Hold time	Run time
	1	35.0	150	0.0	
	2	7.0	290	0.0	
	3	100.0	300	2.0	25.96

Large-Volume Injection with Solvent Venting

Introduction of 20 µL of sample extract into the inlet liner while venting the solvent away from the analytical column preconcentrates the analytes in the liner. They are subsequently swept into the column in a narrow band by rapidly heating the inlet. The sample is introduced at a rate of 1 µL/sec. CIS4 is held at 20ºC during injection/venting and held for another 0.1 min to complete the venting process before ramping the CIS4 temperature to 300ºC. The oven ramping used in LVI is similar to the PSI method except for the initial oven temperature hold time of 0.0 min. Figure 1 (adapted from Gerstel Application Notes Rev. 1 RJC 11/95) shows the sequence of events during LVI.



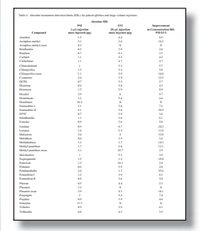


Large-Volume Injection with Solvent Venting vs. Pulsed Splitless Injection

Table 3 shows the absolute sensitivity (response per ng injected) for the PSI and LVI/SV methods. For the same mass of analyte injected, LVI generally produces an increase in analyte response compared to PSI method, except for disulfoton and dicofol. LVI appears to be 30-60% more efficient than PSI at transferring most analytes to the analytical column. The low LVI absolute sensitivity for disulfoton and dicofol may be due to some active sites in the liner. The sample has a longer contact time with the liner during LVI, and if the liner is dirty or otherwise activated, losses of these active compounds could be exacerbated relative to PSI. Of course, the concentration sensitivity (response per ng/L) for LVI is 15-40 times that of PSI for any of the analytes.

Instrument Detection Limit

The absolute detection limits of LVI, based on the signal-to-noise ratio of seven replicate 20-pg injections, are generally slightly higher than those of PSI, despite the former's better absolute sensitivities. This reflects significantly higher variance in the LVI response, which might be expected, given the more complex injection process. Nevertheless, concentration IDLs, which are more relevant to detection limits in lake-water extracts, are much better for LVI. The detection limits shown for LVI in Table 4 would correspond to detection limits in lake-water extracts of 0.5-5 pg/L. The PSI detection limits would correspond to 5-100 pg/L.



Cleanup of Sample Extract

Figure 2 shows ion chromatograms (1-µL PSI) for the quantitation ion (m/z 195) and qualifying ions of endosulfan II in an extract of 100-L blank reagent water as taken directly from the Nexus column. The background for the quantitation ion is elevated and the qualifying ion at m/z 237 is essentially unusable due to interferences. The extract was cleaned up by sequential extraction from silica gel and spiked with 10 pg/µL endosulfan II. The resulting chromatograms in Figure 3 show that the backgrounds of the four ions were greatly reduced and all of the peaks for the pesticide are easily distinguishable. Cleanup of real samples is essential for trace analysis, especially for analysis by LVI, where the load of interfering components in a raw extract would degrade the sample introduction system and eventually the analytical column.

Figure 2. Ion chromatograms showing the background noise levels of endosulfan II ions in uncleaned 100-L DI extract.

Figure 3. Ion chromatograms showing the background noise levels of endosulfan II ions in cleaned 100-L DI extract spiked with 10 pg/µL mixed standards.

Conclusions

- Absolute sensitivity of LVI is increased relative to PSI.
- Absolute detection limits of PSI and LVI comparable, while concentration detection limits for LVI are much better than those of PSI.
- Clean-up procedure is needed to allow LVI-GC/MS determination in extracted lake-water samples.