Identification and Quantitation of Pesticides in the Parts-per-Trillion Range Using Retention Time Locking and GC/MS

Application

Environmental, Food

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Abstract

The typical pesticide quantitation limit for a mass spectrometer in the Scan mode is in the sub-ppm range. By using a selected ion monitoring method, a lab can lower the target compound quantitation limit to the low parts-per-billion (pg/µL) range using a retention time locked gas chromatography/mass spectrometry method. By adding large volume injection capability to the method, target compounds at parts-per-trillion can be quantified.

A specially developed 567-compound retention time locking pesticide mass spectral library can automatically screen an acquired sample's data file for all 567 compounds in seconds. The library can also be applied for rapid screening of samples acquired in selected ion monitoring method. Using the compound library information, a selected ion monitoring method for 80 target compounds was created in less than 2 hours without running any analyses.

Introduction

Most pesticides are typically analyzed on a gas chromatograph (GC) with element-selective detectors (ESDs). Although these ESDs provide low ppb detection limits and are easy to operate, the data do not provide sufficient information to confirm a compound's presence with confidence. Due to the universal nature of mass spectrometric detection, a mass spectrometer (MS) provides additional information and increased confidence in the assignment of compound identity. With recent advances in GC/MS hardware and software and the decrease in cost of ownership, more and more laboratories are routinely analyzing pesticide residue samples with MS detection.

To match the GC/ESD detection limits and/or to eliminate sample concentration steps, a user must lower the MS detection limit by 2 to 3 orders of magnitude. This application note, discusses the following approaches.

- Run the MS in single ion monitoring (SIM) mode
- Make large volume injections (LVIs)
- Use higher electron multiplier voltage (EMV)

For compound identification, a specially developed 567-compound retention time locking (RTL) [1] pesticide library could perform the entire 567-compound screening in seconds using Scan data. A subset of the library could be screened in seconds from SIM data.

Experimental

A pesticide standard mixture was used to compare the lowest detection limits of splitless injection and LVI under Scan and SIM modes.

System Configuration for Screening and Quantitation:

- 6890 GC with a programmable temperature vaporizer (PTV) [2,3] inlet
- 5973 Mass Selective Detector (MSD)
- 7683 Automatic Liquid Sampler (ALS) tray and autoinjector
- HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), P/N 19091S-433
- G1701BA version B.00.00 MSD ChemStation software or higher
- G1049A MSD RTL Pesticide Database/Library

Table 1. GC Method Parameters

Oven	70(2)/25/150(0)/3/200(0)/8/280(10) = 41.87 min
Inlet	PTV
Inlet pressure	17.30 psi (locked to methyl chlorpyrifos at 16.593 min), constant pressure mode

Table 2. Injection Parameters

Injection mode	Solvent vent	Splitless
Injection volume (syringe)	25 µL (50-µL syringe, P/N 5183-0318)	1 µL (10-µL syringe, P/N 9301-0713)
Injection speed	Inject @ 100 µL/min Draw @ 300 µL/min Dispense @ 4500 µL/min	Fast
Inlet temp	40(0.35)/600/320 (3)/50/200 (Hold until end)	280 °C
Vent	Vent time = 0.29 min Vent flow = 150 mL/min Vent pressure = 0.00 psi	
Purge	60 mL/min @ 2 min	60 mL/min @ 2 min
Liner	Deactivated, Multi Baffled (P/N 5183-2037)	Deactivated, Multi Baffled (P/N 5183-2037)
Inlet cooling	Liquid CO_2	None

Table 3. MS Method Parameters

Solvent delay	3 min
Tune file	Atune.u
Transfer line	280 °C
MS Quad	150 °C
MS source	230 °C
Threshold	150
Sample #	2
Scan range	35 to 500 amu (in Scan mode)
Forty (40) SIM groups (in SIM mode)	

Table 4. Pesticide Screening Parameters for the SIM Method

Extraction window	±0.100 minute
Qualifier mode	Absolute
Qualifier %	30
Zero qualifiers	Included
Subtraction mode	Average start/stop
Screen database	Rtlpest.SCD

Results and Discussion

RTL [1] was used to:

1. Expedite data comparison in overlay format
2. Achieve lower target compound detection limit
3. Allow rapid pesticide screening using the RTL pesticide database/library
4. Help to differentiate isomers by their retention time (RT) differences
5. Eliminate the tedious SIM method RT updating process after column maintenance
6. Simplify the editing of the SIM ion groups

A mixture from the California Department of Food and Agriculture (CDFA) of 80 pesticides at 5000 pg/µL each was used as the stock solution for this study. The mixture contained carbamate, organochlorine, organophosphorus, and organonitrogen pesticides. Figure 1 is an offset overlay of three total ion chromatograms (TIC) with 50, 100, and 500 pg of each of the pesticides injected. These TICs were obtained in the Scan mode from 1-µL spiltless injections. For many of these pesticides the quantitation limit in the Scan mode is about 500 pg on column.

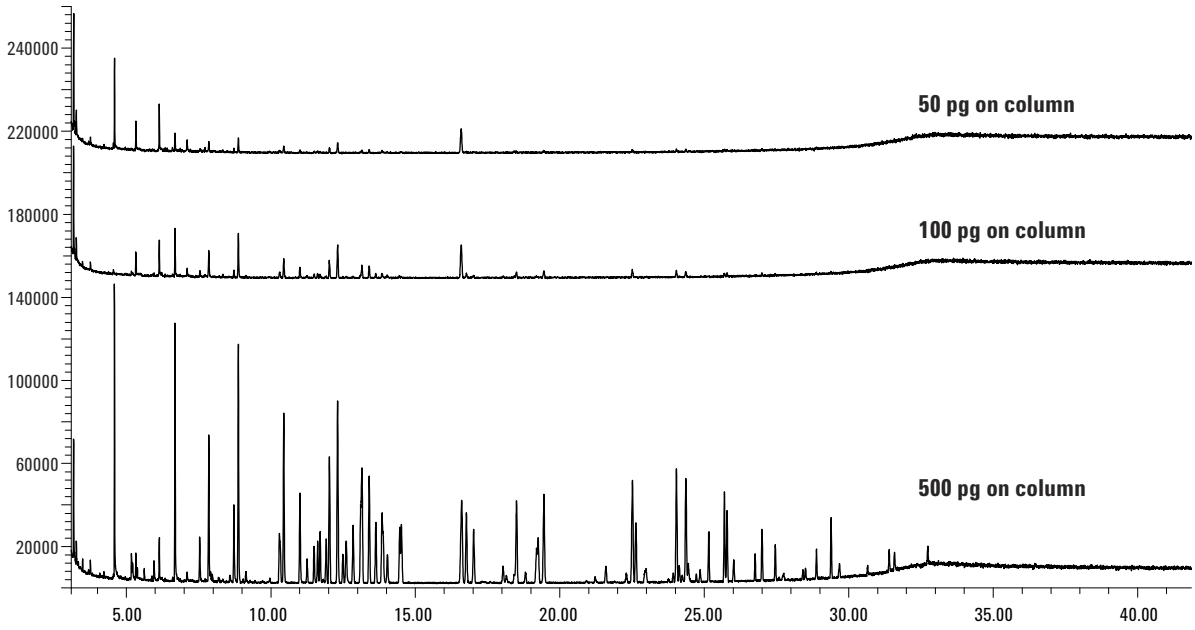


Figure 1. Total ion chromatograms from 1-µL splitless injections of 80 pesticides with 50, 100, and 500 pg of each compound injected.

SIM Mode

To lower the detection limit, a SIM method was created. Instead of the traditional way of making a SIM method, a user can use the information in the RTL Pesticide Database to build a SIM method without running an analysis. Here are the steps for editing SIM ion group parameters:

1. List the MSD RTL Pesticide Database from the ChemStation (Figure 2 is a partial listing) and paste the complete listing into a spreadsheet.

Scdlist.txt - Notepad

File Edit Search Help

SCD Compound List Report

Screen Database : C:\DATABASE\RTLPEST.SCD
Total SCD Cpnds : 567

Cpd#	Compound Name	TIon	Exp_RT	Q1	Q2	Q3
1	Diethylene glycol	45	3.39	75	76	44
2	Aniline	93	3.55	66	65	92
3	p-Dichlorobenzene	146	3.88	148	111	75
4	Dicyclopentadiene	66	4.00	132	67	65
5	Dimefox	44	4.01	110	154	42
6	o-Dichlorobenzene	146	4.09	148	111	75
7	2-Methylphenol	108	4.25	107	77	79
8	4-Methylphenol	107	4.42	108	77	79
9	m-Cresol	108	4.42	107	79	77
10	1,2-Dibromo-3-chloropropane	157	4.53	155	75	159
11	2,4-Dimethylaniline	121	5.19	120	106	77
12	2,6-Dimethylaniline	121	5.20	106	120	77
13	2,4-Dichlorophenol	162	5.19	164	63	98
14	1,2,4-Trichlorobenzene	180	5.29	182	184	145
15	Ethiolate	100	5.41	72	161	44
16	3-Chloroaniline	127	5.45	129	65	92
17	4-Chloroaniline	127	5.48	129	65	92
18	2-Ethyl-1,3-hexanediol	56	5.51	55	73	57
19	p-Nitrotoluene	91	5.57	137	65	107
20	Methamidophos	94	5.66	95	141	47
21	Dichlorvos	109	5.83	185	79	187
22	Allidochlor	41	6.18	56	138	132
23	2,3,5-Trichlorophenol	196	6.63	198	200	160
24	2,6-Dichlorobenzonitrile	171	6.75	173	136	100

Figure 2. A partial listing of the pesticide screener database. The listing includes the compound number, compound name, target ion, expected retention time, and three qualifier ions.

2. In the spreadsheet, delete the rows of the compounds not needed in the method.
3. Separate target compounds into groups (see the added "Group #" column on Figure 3) using these criteria:
   - One to three compounds in each group, and
   - The RTs of the adjacent compounds in adjacent groups are at least 0.2 minute apart. For example, compounds 42 and 51 are more than 0.2 minute apart, so they are in different groups. Compounds 51 and 55 are less than 0.2 minute apart, so they are in the same group.
4. Use the average RT of the adjacent compounds in adjacent groups as the SIM group RT (see the added "Group RT" column on Figure 3). For example, the average retention time of compound 42 (7.91 min, in group 2) and compound 51 (8.78 min, in group 3) is 8.35 minute which is used as the starting retention time of group 3. When all the group numbers and respective starting retention times are determined, make a hardcopy of the spreadsheet for easy entry into the "MS SIM/Scan Parameters" in the next step.
5. Enter the target ion and qualifier ion(s) (Q1, Q2, and/or Q3) of all compounds into the respective ChemStation SIM group (Figure 4). Notice that all the information for building the SIM groups came from Figure 3.

#	Compound Name	MSD_RT	T	Q1	Group #	Group RT
24	2,6-Dichlorobenzonitrile	**6.75**	171	173	**1**	**3.00**
35	Mevinphos	**7.60**	127	192		
42	Propham	**7.91**	93	179	**2**	**7.75**
51	o-Phenylphenol	**8.78**	170	169	**3**	**8.35**
55	Pentachlorobenzene	**8.95**	250	252		
76	Propoxur	**10.35**	110	152	**4**	**9.60**
82	Diphenylamine	**10.52**	169	168		
92	Chlorpropham	**11.05**	127	213	**5**	**10.76**
98	Ethalfluralin	**11.28**	276	316		
102	Bendiocarb	**11.54**	151	126	**6**	**11.41**
103	Trifluralin	**11.64**	306	264		
104	Benfluralin	**11.73**	292	264		
111	Phorate	**11.96**	75	121	**7**	
113	BHC alpha isomer	**12.09**	181	219		
117	Hexachlorobenzene	**12.38**	284	286		
120	Dicloran	**12.56**	206	176		
122	Demeton-S	**12.63**	88	60		
124	Dimethoate	**12.68**	87	93		
129	Simazine	**12.91**	201	186		

Figure 3. A spreadsheet of target compounds separated into different SIM groups with RTs of the adjacent compounds in adjacent groups at least 0.2 minute apart. The starting retention time of each group was determined by calculating the average RT of the adjacent compounds in adjacent groups.

The number of qualifier ions used in a SIM method depends on the number of analytes of interest. For a method monitoring 20 to 30 compounds, all three qualifier ions should be used in the SIM method. As the list of target compounds grows, fewer qualifier ions should be used in the method to maintain a reasonable and comparable ion dwell time and sampling rate.

In general, 10 scans (cycles) per peak are recommended for quantitation purposes. For example, if an analyte peak is 6 seconds wide, about 1.7 cycles per second should be maintained for that SIM ion group. Once the number of cycles per second is determined, the dwell time of the ions can be varied to meet that. As the dwell time is entered for each ion, the ChemStation automatically shows the number of cycles per second. In Figure 4, Group 6 has 3.03 cycles per second.

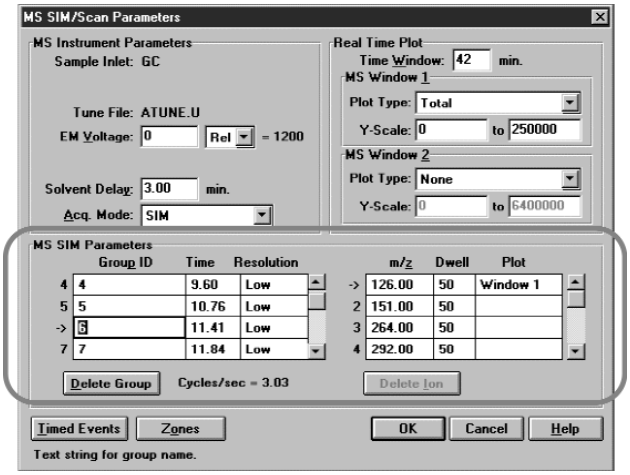


Figure 4. A screen capture of the MSD ChemStation showing the MS and SIM parameters. The SIM parameters (group ID, group retention time, and ions) were all derived from Figure 3.

Figure 5 shows two chromatograms obtained from 1-µL splitless injections at 50 pg/µL using both Scan and SIM modes. The Scan mode has significantly higher baseline noise than the SIM mode. Some of the compounds, especially the late eluters, were not detected in the Scan mode. When the Scan method was changed to a SIM method at this concentration, the signal-to-noise ratio (S/N) increased by a factor of 100. It is worth pointing out that a SIM method does not record background ions from the sample matrix, therefore minimizing the baseline noise and improving the S/N.

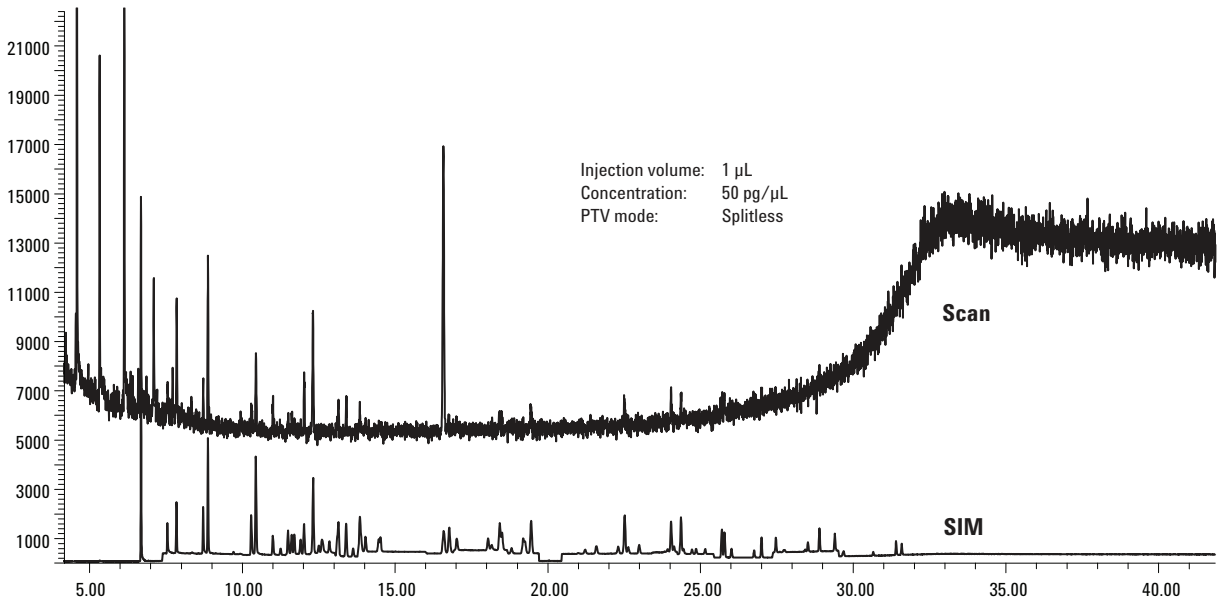


Figure 5. Chromatograms of 1-µL splitless injections at 50 pg/µL from Scan and SIM modes.

In a SIM method, the retention times of the ion groups normally need updating after column maintenance. By using RTL, a user can not only eliminate the tedious RT updating process [4] but also decrease the detection limit. With reproducible known RTs of target compounds, the start and end time of each ion group can be determined optimally. By narrowing the time windows of an ion group to monitor only one or two compounds at a time, the MS can monitor fewer ions in each window, allowing more sampling time for the target ions.

Ideally, a SIM method will have the maximum number of ion groups and the minimum number of ions in each group. In this way, each ion group can get more scans per unit time resulting in better peak shape and more accurate quantitation.

LVIs

To decrease the detection limit further, a user can put more sample on column using the LVI technique. The typical “solvent-vent” approach is to inject the sample slowly into a PTV inlet at a temperature just below the solvent boiling point and let solvent evaporate before ramping up the inlet temperature to move the compounds onto the capillary column. Figure 6 compares a 1-µL splitless injection with a 25-µL solvent-vent injection. Both injections resulted in 50 pg per compound on column. Note that the solvent-vent ion chromatogram is plotted upside down for ease of comparison with the splitless ion chromatogram. It is obvious from the figure that the two techniques provide very similar results. This demonstrates that the solvent-vent technique is a viable approach for sample introduction.

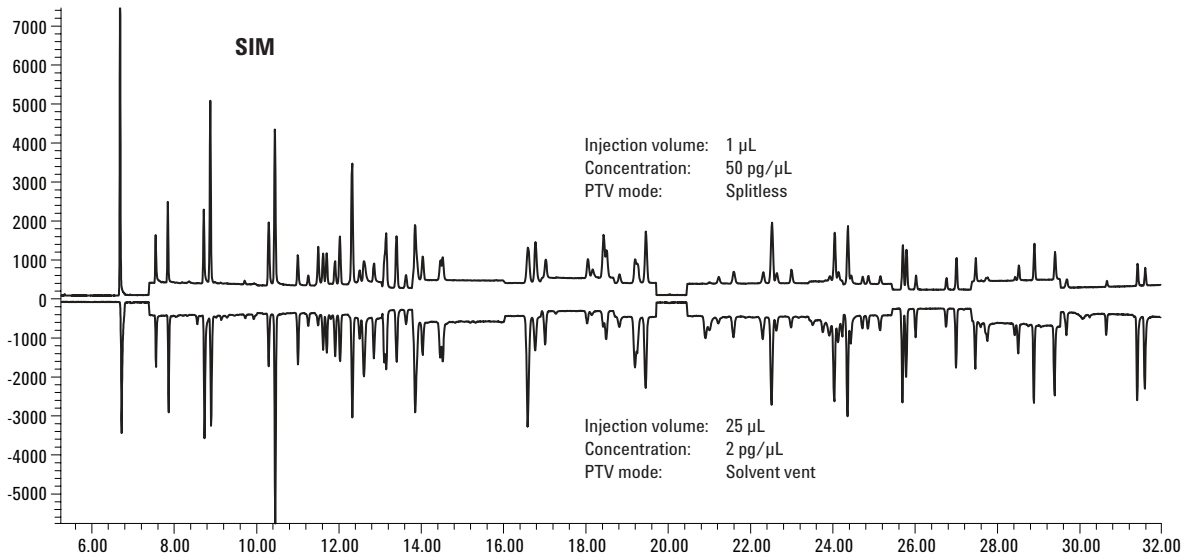


Figure 6. SIM results of 50 pg on column using either a 1-µL splitless or a 25-µL solvent-vent injection.

Higher EMV

It is known that the signal increases with higher EMV on the MS. In Figure 7, the upper signal, after 10-fold magnification, is a 25-µL LVI of 0.5 pg/µL at tune voltage. The bottom signal is the same injection with the electron multiplier set to tune +400 V. Adding 400 V to the EMV increases the signal by 10X, which makes the integration more accurate. However, the baseline noise also increases by 10X, so the S/N stays the same.

Although increasing the EMV does help to bring small peaks over the detection threshold, it shortens the life of the multiplier. In general, the EMV should be kept at the tune voltage.

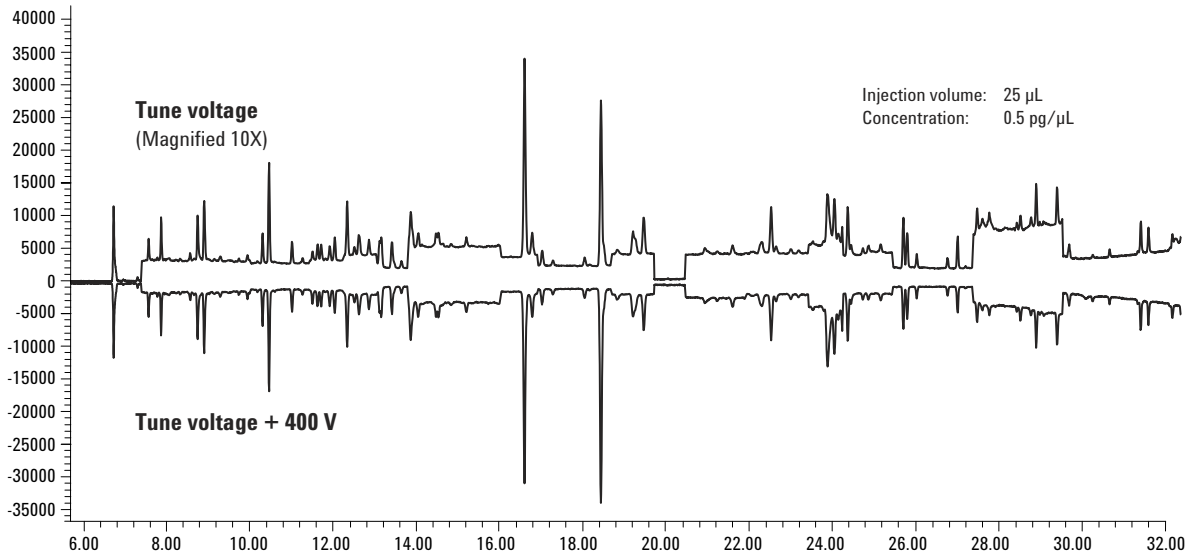


Figure 7. SIM results of 12.5 pg on column using either EMV at Tune voltage or Tune +400 V.

LVIs in Combination with SIM Methods

Combining LVI and SIM, Figures 8 and 9 show quantifiable peaks of three compounds at as low as 5 pg on column. In Figure 8, ion chromatograms of endosulfan sulfate and p,p'-DDT at 0.2 and 500 pg/µL are shown. The top chromatogram was from a 25-µL solvent-vent SIM method and the bottom chromatogram was from a 1-µL splitless Scan method. By using LVI and SIM, it is interesting to see that similar S/N ratios were achieved even with a 2500-fold decrease (from 500 to 0.2 pg/µL) in sample concentration.

By increasing the injection volume to 100 µL, samples at concentration as low as 0.05 pg/µL can also be quantified as shown in Figure 9. The top portion shows the chlorthal-dimethyl extracted ion chromatograms (EIC) of mass 299 and 301 from a 100-µL full Scan run. The bottom portion shows the same ions from a 100-µL SIM run. The SIM method shows better peak shape and lower baseline noise.

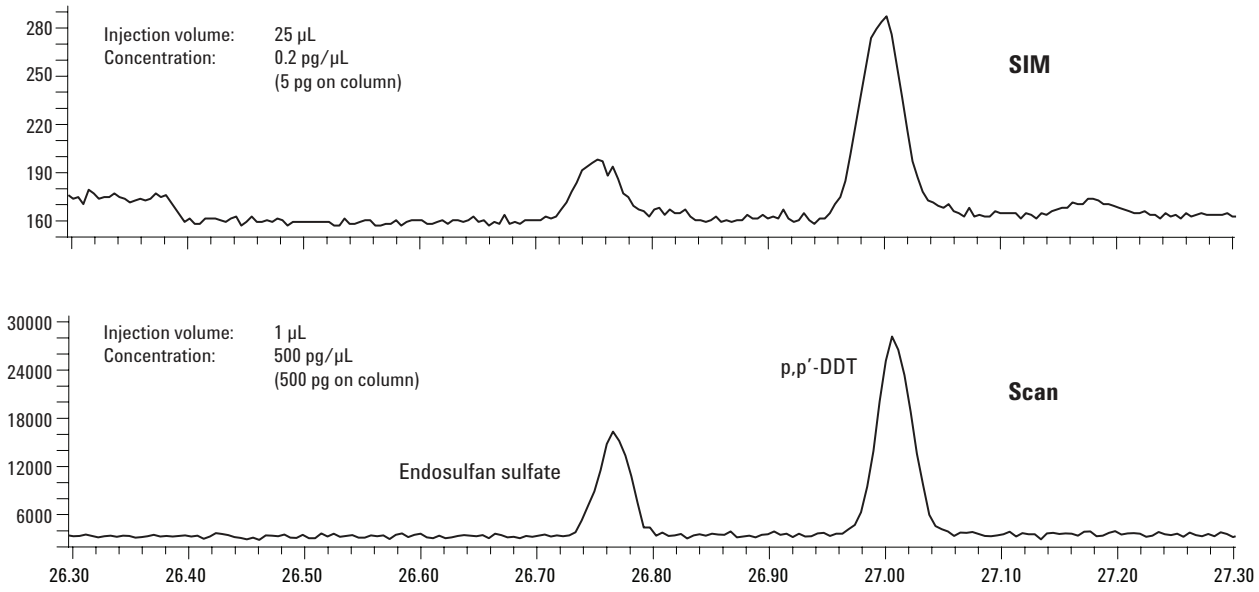


Figure 8. Ion chromatograms of endosulfan sulfate and p,p'-DDT at 0.2 and 500 pg/µL. The top chromatogram was from a 25-µL solvent-vent SIM method and the bottom chromatogram was from a 1-µL splitless Scan method.

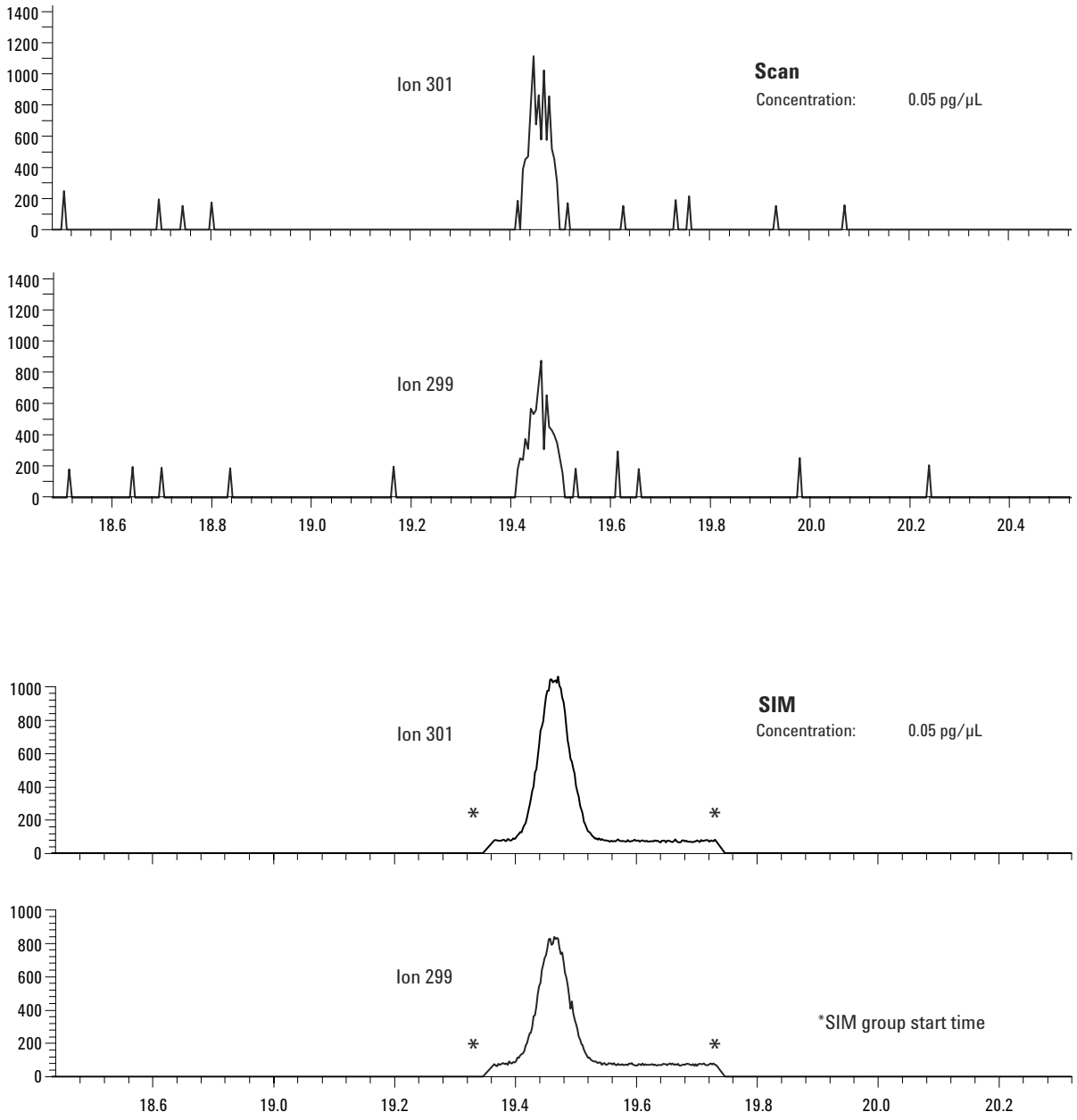
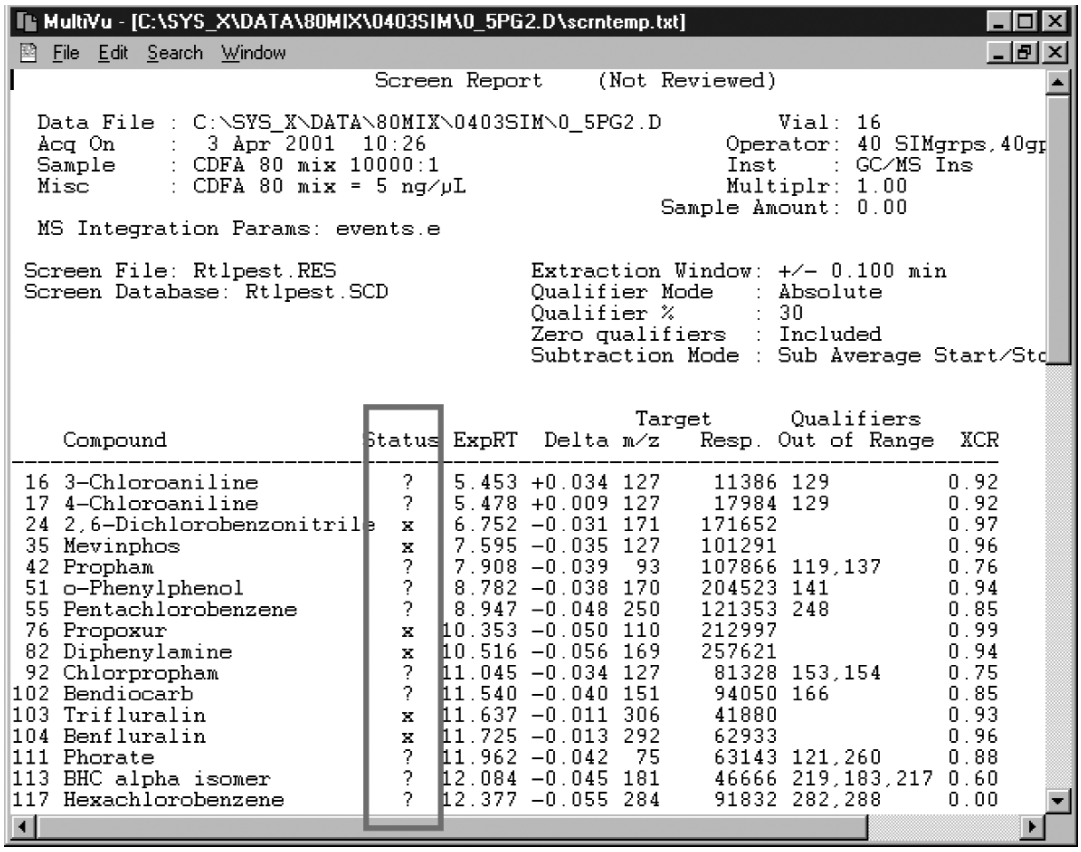


Figure 9. Ion chromatograms of 100-µL chlorthal-dimethyl injected at 0.05 pg/µL. The top portion was from a full Scan run and the bottom portion was from a SIM run.

Target Compound Screening

Combing RTL and the G1049A MSD RTL Pesticide Database/Library, a user can screen for 567 pesticides and suspected endocrine disrupters from any Scan run [5]. A user can screen a subset of the library with improved sensitivity using a SIM method. The MSD ChemStation can generate a 567-compound screening report automatically in less than 30 seconds. Figure 10 is a report of the 0.5 pg/µL sample (25 µL injected in SIM mode) that lists the "probable hits" (marked with an x) and "possible hits" (marked with a ?). All target compounds at this 12.5 pg on column level were found by the software.



MultiVu - [C:\SYS_X\DATA\80MIX\0403SIM\0_5PG2.D\scrntemp.txt]

File Edit Search Window

Screen Report (Not Reviewed)

Data File : C:\SYS_X\DATA\80MIX\0403SIM\0_5PG2.D Vial: 16
Acq On : 3 Apr 2001 10:26 Operator: 40 SIMgrps,40gp
Sample : CDFA 80 mix 10000:1 Inst : GC/MS Ins
Misc : CDFA 80 mix = 5 ng/µL Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: events.e

Screen File: Rtlpest.RES Extraction Window: +/- 0.100 min
Screen Database: Rtlpest.SCD Qualifier Mode : Absolute
Qualifier % : 30
Zero qualifiers : Included
Subtraction Mode : Sub Average Start/Sto

	Compound	Status	ExpRT	Delta	Target m/z	Resp.	Qualifiers Out of Range	XCR
16	3-Chloroaniline	?	5.453	+0.034	127	11386	129	0.92
17	4-Chloroaniline	?	5.478	+0.009	127	17984	129	0.92
24	2,6-Dichlorobenzonitrile	x	6.752	-0.031	171	171652		0.97
35	Mevinphos	x	7.595	-0.035	127	101291		0.96
42	Propham	?	7.908	-0.039	93	107866	119,137	0.76
51	o-Phenylphenol	?	8.782	-0.038	170	204523	141	0.94
55	Pentachlorobenzene	?	8.947	-0.048	250	121353	248	0.85
76	Propoxur	x	10.353	-0.050	110	212997		0.99
82	Diphenylamine	x	10.516	-0.056	169	257621		0.94
92	Chlorpropham	?	11.045	-0.034	127	81328	153,154	0.75
102	Bendiocarb	?	11.540	-0.040	151	94050	166	0.85
103	Trifluralin	x	11.637	-0.011	306	41880		0.93
104	Benfluralin	x	11.725	-0.013	292	62933		0.96
111	Phorate	?	11.962	-0.042	75	63143	121,260	0.88
113	BHC alpha isomer	?	12.084	-0.045	181	46666	219,183,217	0.60
117	Hexachlorobenzene	?	12.377	-0.055	284	91832	282,288	0.00

Figure 10. Typical report from the GC/MS pesticide screener showing probable "hits" (marked with an x) and possible hits (marked with a ?). Other information includes the library retention time followed by the RT difference in this chromatogram, the target ion, its abundance, out of range qualifier(s), and a cross correlation value with the library spectrum.

Conclusions

Using the information (compound names, retention times, and ion masses) in the RTL pesticide database, a SIM method of 80 target compounds can be created in less than 2 hours without running any analyses. The examples show that both LVI and SIM are effective techniques to decrease the quantitation limit of target compounds from sub-ppm to ppt.

Any lab can decrease the quantitation limit by a factor of 100 without any hardware modification. Lowering the quantitation limit from 500 pg down to 5 pg on column can be done using a SIM method and RTL. By adding LVI to the system, target compounds in femtogram/µL can be quantified.

Acknowledgement

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References

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