Optimization of the Injection Phase of the Analysis of Pesticides in Produce by GC/MS

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Abstract

Purpose: To examine GC injection techniques when using programmable temperature vaporizing (PTV) large volume injections during pesticides analysis.

Methods: We used a DSQ™ II bent-optics quadrupole mass spectrometer with the TRACE GC Ultra™. The GC was set up with a conventional split/splitless (S/SL) injector and PTV injector. The effects of temperature and surface area activity during the injection phase of the analysis of a short list of pesticides were studied. Apple samples were prepared using the QuEChERS sample preparation protocol for this study.[1] (*Figure 1*)

Results: GC/MS sensitivity improved during the PTV large volume injection for the method by eliminating most of the polar extraction solvent, acetonitrile, in the evaporation phase. The basic optimization tips for setting up a large volume injection on the PTV are listed in *Figure 2*.

Introduction

When analyzing pesticides in various matrices, the scientist selects a sample preparation method that offers the best extraction efficiency for the pesticides while minimizing the matrix interference. A matrix that does remain in the extracted sample must be dealt with during the injection phase, separation phase, or at the detection phase. For the purpose of this study, only the injection phase will be studied. Assuming that the sample preparation was successful and >70% of the pesticides present are recovered, the injection phase must transfer as close to 100% of the pesticides in solution in the extract without altering the chemical structure of the pesticide.

Setting up ideal injection parameters can be difficult because the list of studied pesticides (*Table 1*) cover a wide range of boiling points, polarity, and thermal stability. In fact, the optimum injection parameters are a compromise that result in the best injection efficiency for as many pesticides as possible. While the conventional constant temperature splitless injection is viewed as being the easiest to set up, it may not provide the best transfer efficiency of polar thermally labile compounds. Ideally, a true cold on-column injection would offer the ultimate in injection efficiency (100%) regardless of physical properties. Since the build-up of matrix associated with the true cold on-column injection requires daily trimming the guard column, the PTV offers the next best injection technique. Some of the advantages of the injection using a Split/splitless and PTV will be demonstrated with examples of performance for the analysis of a short list of pesticides in apples using the QuEChERS sample preparation protocol.

Materials & Methods

Method performance for analysis of pesticides is measured by determining the precision and accuracy of spiking a known amount of pesticides in a control matrix. Since the matrix is difficult to control and can adversely affect the results, the initial evaluation in this study was performed in solvent with the addition of analyte protectants.[2] Some studies were done by spiking apples. The Thermo Scientific DSQ II bent-optics quadrupole mass spectrometer coupled with the TRACE GC Ultra gas chromatograph provides a wide linear range with excellent precision and sensitivity. The pesticides listed in *Table 1* were tested for linearity over a working range from 2 pg/µL to 2000 pg/µL in reagent with protectants at two different concentrations. Each of the following injection techniques were evaluated by testing the presence of endrin breakdown products:

- Splitless 1 µL
- PTV constant temperature splitless
- PTV cold splitless
- PTV cold splitess with surge pressure
- PTV Large volume 10 µL

Figure 1. Injection port Cross- Sectional Views:

a.) Split/Splitless Inlet b.) PTV

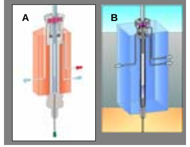


Figure 2. Optimization Tips for PTV LVI

Injection Temperature: -10°C of boiling point of solvent

Time: Dependent on injection time for volume to be injected and at what speed of injection

Evaporation: +10°C of boiling point of highest boiling solvent

Time: Dependent on volume of solvent injected and volatility of first analyte

If evaporation is incomplete adverse effects of peak shapes will be seen and the foreline pressure of the pump will show an elevated pressure for several seconds.

No Surge Pressure: surge pressure tends to impede evaporation, not effective in transfer phase as with standard splitless injections

Transfer Temperature: +25°C of S/SL method unless using packing material like carbofrit (+50°C higher)

Transfer Time: 1 minute

Cleaning: Transfer Temperature = Temperature required to remove matrix.

Initial oven hold time: Equal to duration of total injection phase minus cleaning phase

Results: Endrin Breakdown Products

Endrin has been used to measure activity in the injector for other methods.[3] By monitoring the amount of the breakdown products for endrin, endrin aldehyde and endrin ketone the overall activity of the inlet liner can be determined. The breakdown products for 4,4'-DDT are 4,4'-DDD and 4,4'-DDE, which were not found in any of the tests performed. Two solutions of endrin and 4,4'-DDT were made at 500 pg/µL for the study. One was made with analyte protectants added and the other without.[2] The following protectants were added: sorbitol (0.5 µg/µL) and 3-ethoxy-12-propanediol (10 µg/µL). Several modes of injection were run on the Split/Splitless and PTV Injector. The study was run to monitor the need for protectants and their ability to eliminate the adverse effects of activity in the injection phase. To test the effect of residence time in the injector, surge pressure was added as a variable. The following modes of injection were studied:

1. S/SL Splitless: 5 mm Splitless liner,3 mm Splitless, Liner Focus Liner (*Figure 3*)
2. PTV Constant Temperature (CT) splitless: glass liner with silanized glass wool, Siltek™ baffled liner with and without Siltek wool, the sintered glass liner (*Figure 4*)
3. PTV Cold Splitless: glass liner with silanized glass wool (*Figure 5*)
4. PTV Cold Splitless with Surge Pressure: glass liner with silanized glass wool (*Figure 5*)
5. PTV LVI 5 µL: glass liner with silanized glass wool, Siltek beveled liner, sintered liner (*Figure 6*)
6. PTV LVI 5 µL with Surge Pressure: glass liner with silanized glass wool (*Figure 6*)

A TIC of the pesticide standard with protectants is shown in *Figure 7*.

Figure 3. S/SL Splitless

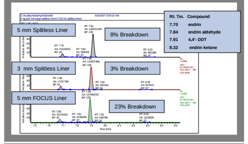


Figure 4. PTV Constant Temperature Splitless (1 µL)

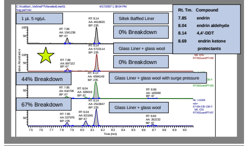


Figure 5. PTV Cold Splitless (1 µL)

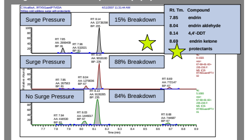


Figure 6. PTV LVI (5 µL) MeCN

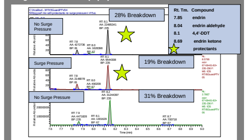


Figure 7. PTV TIC of 2 ng/µL pesticide Mix with Protectants

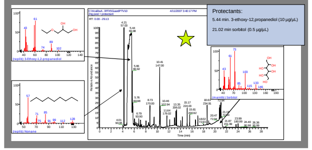


Table 1. Pesticides Studied

#	compound	Ret. Tm.	Quan Ion	MW	structure
1	methamidophos	6.55	94	141	$C_2H_8NO_2PS$
2	dichlorophos	6.66	109	220	$C_4H_7Cl_2O_4P$
3	acephate	7.92	136	183	$C_4H_{10}NO_3PS$
4	2-phenylphenol	8.69	170	170	$C_{12}H_{10}O$
5	omethoate	9.37	156	213	$C_5H_{12}NO_4PS$
6	diazinone	11.63	179	304	$C_{12}H_{21}N_2O_3PS$
7	chlorothalonil	12.12	266	264	$C_8Cl_4N_2$
8	vinclozolin	12.95	286	285	$C_{12}H_9Cl_2NO_3$
9	sevin	13.15	144	201	$C_{12}H_{11}NO_2$
10	metalaxyl	13.32	206	279	$C_{15}H_{21}NO_4$
11	dichlofluanid	14.02	123	332	$C_9H_{11}Cl_2FN_2O_2S_2$
12	d10-parathion	14.28	301		
13	fenthion	14.32	278	278	$C_{10}H_{15}O_3PS_2$
14	cyprodinil	15.14	224	225	$C_{14}H_{15}N_3$
15	thiabendazole	15.55	201	201	$C_{10}H_7N_3S$
16	endosulfan_I	16.59	241	404	$C_9H_6Cl_6O_3S$
17	imazalil	17	215	296	$C_{14}H_{14}Cl_2N_2O$
18	4,4'-DDT	18.61	235	352	$C_{14}H_9Cl_5$
19	TPP	18.9	326		
20	cis-permethrin	20.71	183	390	$C_{21}H_{20}Cl_2O_3$
21	trans-permethrin	20.82	183		$C_{21}H_{20}Cl_2O_3$
22	coumaphos	20.98	362	362	$C_{14}H_{16}ClO_5PS$
23	deltamethrin	23.94	181	503	$C_{22}H_{19}Br_2NO_3$
24	iprodione*	19.09	187	329	$C_{13}H_{13}Cl_2N_3O_3$
	*peak shape broad, not analyzed				

Results: Linearity of PTV LVI Injection

To measure the method linearity, a calibration curve was run in an reagent with protectants from 2 ng/g to 2000 ng/g in 3-ion SIM. We used a Siltek beveled liner and injected 5 µL. All solutions were prepared in acetonitrile (MeCN). Two curves were run with protectants at 0.250 µg/µL sorbitol and 5 µg/µL of 3-ethoxy-1,2-propanediol and 0.005 µg /µL sorbitol and 0.1 µg/µL of 3-ethoxy-1,2-propanediol. The generated Full Scan TIC shown in *Figure 8* at the two different concentrations of protectants. The instrument detection parameters are listed in *Table 2*. The results of the linearity studies are shown in *Table 3*. Iprodione was not analyzed due to the poor peak shape generated using PTV large volume injection with MeCN. The effect of the concentration of the protectants on the linearity of omethoate and thiabendazole are shown in *Figure 9*.

Table 2. Instrument Parameters for Detection

#	compound	Ret. Tm.	Quan Ion	MW	structure	seg. #	Start Tm.	scan event#	SIM Ion	Width	Qualifier Ions	Dwell Time (ms)
1	methamidophos	6.55	94	141	$C_2H_8NO_2PS$	1	6.00	1	94	0.5	95,141	50
2	dichlorophos	6.66	109	220	$C_4H_7Cl_2O_4P$			2	109	0.5	145,185	50
3	acephate	7.92	136	183	$C_4H_{10}NO_3PS$	2	7.20	1	136	0.5	94,125	20
4	2-phenylphenol	8.69	170	170	$C_{12}H_{10}O$			2	170	0.5	141,169	20
5	omethoate	9.37	156	213	$C_5H_{12}NO_4PS$			3	156	0.5	110,126	20
6	diazinone	11.63	179	304	$C_{12}H_{21}N_2O_3PS$	3	10.80	1	179	0.5	137,152	50
7	chlorothalonil	12.12	266	264	$C_8Cl_4N_2$			2	266	0.5	264,268	50
8	vinclozolin	12.95	286	285	$C_{12}H_9Cl_2NO_3$	4	12.50	1	285	0.5	198,212	20
9	sevin	13.15	144	201	$C_{12}H_{11}NO_2$			2	144	0.5	115,116	20
10	metalaxyl	13.32	206	279	$C_{15}H_{21}NO_4$			3	206	0.5	160,220	20
11	dichlofluanid	14.02	123	332	$C_9H_{11}Cl_2FN_2O_2S_2$	5	13.60	1	123	0.5	167,224	20
12	d10-parathion	14.28	301					2	301			20
13	fenthion	14.32	278	278	$C_{10}H_{15}O_3PS_2$			3	278	0.5	125,169	20
14	cyprodinil	15.14	224	225	$C_{14}H_{15}N_3$	6	14.80	1	224	0.5	210,226	50
15	thiabendazole	15.55	201	201	$C_{10}H_7N_3S$			2	201	0.5	129,174	50
16	endosulfan_I	16.59	241	404	$C_9H_6Cl_6O_3S$	7	16.20	1	241	0.5	195,339	50
17	imazalil	17	215	296	$C_{14}H_{14}Cl_2N_2O$			2	215	0.5	173,217	50
18	4,4'-DDT	18.61	235	352	$C_{14}H_9Cl_5$	8	17.80	1	235	0.5	165,237	50
19	TPP	18.9	326					2	326			50
20	cis-permethrin	20.71	183	390	$C_{21}H_{20}Cl_2O_3$	9	20.20	1	183	0.5	163,165	50
21	trans-permethrin	20.82	183		$C_{21}H_{20}Cl_2O_3$			2	183	0.5	163,165	50
22	coumaphos	20.98	362	362	$C_{14}H_{16}ClO_5PS$			3	362	0.5	109,226	50
23	deltamethrin	23.94	181	503	$C_{22}H_{19}Br_2NO_3$	10	22.50	1	181	0.5	209,253	100
24	iprodione*	19.09	187	329	$C_{13}H_{13}Cl_2N_3O_3$							
	*peak shape broad, not analyzed											

Figure 8. 200 ng/g Full Scan (5 µL PTV)

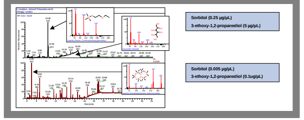
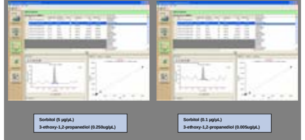


Table 3. Linearity (2ng/g to 2000 ng/g)

	Ret. Tm.	*Curve**	*Curve#*
methamidophos	6.55	0.9998	0.9996
dichlorphos	6.66	0.9998	1.0000
acephate	7.92	0.9996	0.9995
2-phenylphenol	8.69	0.9998	0.9998
omethoate	9.37	1.0000	0.9979
diazinone	11.63	0.9994	0.9990
chlorothalonil	12.12	0.9999	0.9987
vinclozolin	12.95	0.9999	0.9986
sevin	13.15	0.9999	0.9998
metalaxyl	13.32	0.9999	0.9997
dichlofluanid	14.02	1.0000	0.9978
fenthion	14.32	0.9999	0.9996
cyprodinil	15.14	0.9997	0.9999
thiabendazole	15.55	0.9995	0.9956
endosulfan_I	16.59	0.9999	0.9992
imazalil	17.01	0.9999	0.9964
4,4'-DDT	18.61	0.9979	0.9999
cis-permethrin	20.71	0.9995	0.9992
trans-permethrin	20.82	0.9993	0.9987
coumaphos	20.98	0.9987	0.9966
delmethrin_I	23.94	0.9980	0.9974
deltamethrin_II	19.09	0.9978	0.9970
**sorbitol 0.25ug/L & 3-ethoxy-1,2-propanediol 5 ug/L*			
#sorbitol 0.005ug/L & 3-ethoxy-1,2-propanediol 0.1 ug/L			

Figure 9. Effect of Concentration of Protectants on Linearity



Results: QC Spikes in Matrix

Matrix samples were studied to test the recovery of analytes in matrix vs. reagents by spiking the complete target list in 15 g of apple at 200 ng/g. The sample preparation was performed as shown in the flow chart in *Figure 10*. The full scan TIC of the unspiked apple matrix is shown in *Figure 11*. The recovery values are listed in *Table 4*.

Figure 10. Sample Preparation

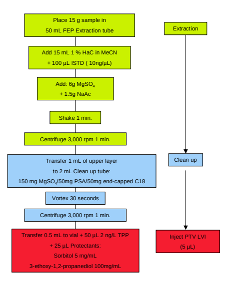
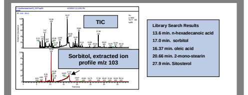


Table 4. Apple Matrix Spike Recovery (200ng/g)

#	compound	Ret. Tm.	apple(ng/g)	apple spike 200ng/g	% Recovery
1	methamidophos	6.55		159	80
2	dichlorophos	6.66		201	101
3	acephate	7.92	315	517	101
4	2-phenylphenol	8.69		117	59
5	omethoate	9.37		59	30
6	diazinone	11.63		220	110
7	chlorothalonil	12.12		88	44
8	vinclozolin	12.95		215	108
9	sevin	13.15	1.1	119	59
10	metalaxyl	13.32	2.9	198	98
11	dichlofluanid	14.02	4.4	193	94
12	d10-parathion	14.28		181	91
13	fenthion	14.32		195	98
14	cyprodinil	15.14		198	99
15	thiabendazole	15.55		173	87
16	endosulfan_I	16.59		201	101
17	imazalil	17		322	161
18	4,4'-DDT	18.61		210	105
19	TPP	18.9		220	110
20	cis-permethrin	20.71	9.1	206	98
21	trans-permethrin	20.82		227	114
22	coumaphos	20.98		214	107
23	deltamethrin I	23.54		227	114
23	deltamethrin II	23.93		241	121
	average recovery				95

Figure 11. Full Scan TIC – Apple Matrix



Conclusions

The evaluation of various injection techniques showed that the addition of analyte protectants can remedy the adverse effects of the lengthened residence time and exposure to surface area in the inlet during a temperature programmed injection. Since the preferred solvent of extraction is acetonitrile, the PTV LVI injection mode offers the option to remove the bulk of the solvent and minimized the adverse effects of this polar solvent on the column. This is not possible in a standard splitless injection on a Split/splitless inlet. A solvent exchange step to toluene is required. PTV LVI with 5 µL injection was used to study the linearity and matrix spike in apple for the application. The linearity study with protectants was good with a R^2 fit of > 0.999 from 2 to 2000 ng/g. The amount and selection of protectants added makes a significant difference in the linear fit with LVI PTV in MeCN.[2] The 200ng/g matrix spike for the pesticide list studied showed an average recovery of 95%. Future work will be performed on this method with other matrices. An evaluation of the method performance using a conventional splitless injection with the Split/Splitless inlet and solvent exchange to toluene with a concentration factor of 5 will be studied.

References

(1) Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, Michelangelo Anastassiades and Steven Lehotay, et al. *Journal of AOAC International* Vol. 86, No. 2, 2003.

(2) Combination of Analyte Protectants To Overcome Matrix Effects in Routine GC Analysis of Pesticide Residues in Food Matrixes, Katreina Mastovska, Steven Lehotay, and Michelangelo Anastassiades, *Anal. Chem*. 2005, 77. 8229-8137.

(3) EPA Method 525.2: Determination of Organic Compounds in Drinking Water by Liquid/Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry. Rev 2. 1995.

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