Rapid Screening and Confirmational Analysis of Residual Pesticides in Agricultural Samples by GC ECD/FPD and GC-MS/MS

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Overview

Purpose: Develop and test an intelligent software to streamline the analysis of pesticides in a seed oil matrix.

Methods: An extract of seed oil was spiked with various levels of pesticides. The samples were analyzed on a TRACE™ GC Ultra gas chromatograph (GC) configured with a tandem Electron Capture Detector/Flame Photometric Detector (ECD/FPD) for the analysis of chlorinated and organophosphorus pesticides. Any samples with a detected concentration of a target compound above a predetermined limit were automatically added to a list of samples to be injected on the Polaris*Q* mass spectrometer (MS). An ion trap MS was chosen because of its superior quantitation ability in matrix via the MS/MS experiment. A robotic TriPlus™ liquid autosampler with a 150 sample tray was used to inject on either the inlet that led to the GC detectors or to the inlet that led into the MS, as directed by the Smart Screening software program.

Results: Of the 44 pesticides studied, 35 were sufficiently halogenated to be detected at a 1 pg on column load with the ECD. 15 were detectable at 10 pg column load using the FPD, and all of the pesticides were seen on the Polaris*Q* using a two stage MS experiment at a 1 pg level. The linear fits of the data showed excellent correlation constants of greater than 0.99. The linear range was from 1 pg to 100 pg injected on column for the mass spectrometer and ECD and was 10 pg to 1000 pg for the FPD. 18 replicate injections were made at the 10 pg/µL level on the Polaris*Q*. The median precision for the mass spectrometer was 8%.

Introduction

Pesticides generally fall into two groups. The first is a class of halogenated products, like p,p'-DDT which has 5 chlorine atoms per molecule, that have a strong response on the ECD. The second class is a set of organophosphorus compounds that are based on acetylcholinase inhibition. This class is not always halogenated, but it always has a phosphate moiety. These compounds are observed selectively using an FPD with a phosphorous specific photometric filter. The two GC detectors were operated in tandem with the ECD as the base detector, since it is a non-destructive detector. The various detectors used in this work are shown schematically in Figure 2. When the screening indicated the presence of a pesticide, the software then automatically added a confirming and quantitating run on the mass spectrometer. The second sequence was automatically launched with no user intervention.

FIGURE 1. Diagram of MS/MS experiment on Polaris*Q*

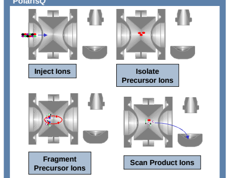
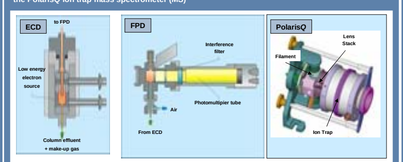


FIGURE 2. Diagrams of the electron capture detector (ECD), flame photometric detector (FPD), and the Polaris*Q* ion trap mass spectrometer (MS)



Methods:

The seed oil extracts were prepared by taking 0.2 g of sample, performing a solvent extraction, then cleaned by gel permeation chromatography (GPC). The resultant extract was reconstituted to 1 mL with iso-octane. This extract was then spiked with the pesticide mix at 1, 10, 100, and 1000 pg/µL. All injections were 1.0 µL.

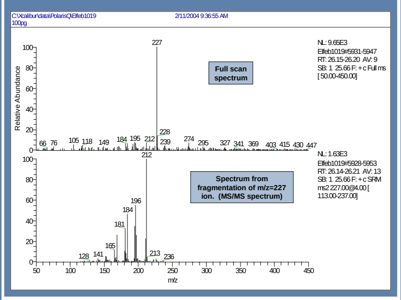
Mass Spectrometer

A diagram of what a multistage mass spectral experiment entails is shown in Figure 1. The first stage of the MS/MS experiment is the isolation of the precursor ion. The isolation is then followed by the second stage of MS, collision induced dissociation (CID), of that precursor ion to generate product ions, which are subsequently scanned out of the ion trap. The variables used in the isolation and fragmentation of the precursor ion, the trapping well depth parameter q, and the CID voltage are found in Table 1. These parameters are compound specific and must be determined experimentally. Table 1 lists the precursor ions that were isolated and the dominant fragment ions that were used for quantitation in the MS/MS experiments. A comparison of the full scan, or single stage MS, and MS/MS experiment is found in Figure 3.

Gas Chromatograph

A GC method for the separation of 44 pesticides was developed using the tandem ECD/FPD to detect both chlorinated and organophosphorus pesticides in a single injection. This injection was made on a 100% dimethylsiloxane stationary phase column. The oven was held at 80°C for 1.0 minute followed by a ramp of 30°C/min to 150°C which was held for 10 minutes. A final ramp of 7°C/min reached the final temperature of 320°C, which was then held for 10 minutes. The injection was made in a 200°C splitless injector with a splitless time of 1.0 minute. The carrier gas for both columns was helium flowing at 1 cc/min. Next, a method was developed for the analysis of the same pesticides on the Polaris*Q* by MS/MS. The oven was held at 90°C for 1.5 minutes. The temperature was ramped at 30°C/min until 190°C was reached. This temperature was held for 10 minutes and then ramped at 5°C/min until 320°C was reached. This temperature was held for 10 minutes. The injection technique for the Polaris*Q* used programmable temperature vaporization. It was used in a splitless mode for 1.0 minutes. The initial temperature of the injector was 80°C and was held for 6 seconds. The injector was ramped at 10°/sec to a final temperature of 290°C, where it was held for 1.5 min. The injector was cleaned between samples by holding the temperature at 320°C for 10 minutes. Table 1 lists the retention times of the pesticides for both of the chromatographic conditions.

FIGURE 3. Methoxychlor spectra in full scan mode and MS/MS mode



Smart Screening Software

Smart Screening makes intelligent sequence decisions automatically. Using the defined objects in the XDK, a programming system for the Xcalibur™ data system, the software reviews the results of the GC ECD/FPD analyses to determine if any samples showed the presence of the target analytes above a threshold defined by the user. These limits typically relate to the limit of detection, limit of quantification, or a regulatory limit. These limits can be established on a compound-by-compound basis so each compound can have a unique limit for confirmation. If the GC analysis of a sample shows the presence of any target compounds above the threshold, that sample is added to a new sequence to be used for GC-MS/MS analysis to provide confirmation and quantitation.

Once the data review for the entire GC sequence has been completed, the GC-MS/MS confirmatory sequence is started automatically by the software. No user intervention is required to initiate this sequence, and the TriPlus Autosampler will, as required, access the correct injector with no system hardware modification. Both the GC and the GC-MS/MS analyses will be completed in a completely automated manner. The decision flow chart for the Smart Screening software is shown in Figure 4.

FIGURE 4. Smart Screening decision flow chart

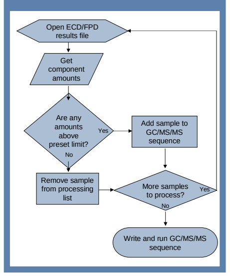


FIGURE 5. Comparison of FPD, ECD, and MS/MS analysis of Chlorpyrifos at an equivalent concentration of 50 ppb in matrix

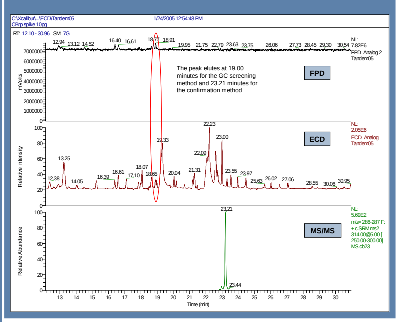


TABLE 1. Mass spectrometer and ECD/FPD detector information

Compound Name	MS Retention Time (min)	Precursor Ion (amu)	Isolation Notch Width (amu)	Q Value	CID Energy (V)	Quantitation Product Ions	GC Detector Retention Time (min)	Functional Moiety (Halogenated or Phosphate)
DDVP	5.98	185	4	0.225	3	93,109,131	3.41	Both
trichloroanisole	7.07	195	6	0.225	3	167,169	4.04	Halogenated
tecnazene	11.99	261	6	0.225	4	203,201,205,229,231,000	6.38	Halogenated
tetrachloroanisole	13.51	246	6	0.225	5	231,229,233	7.34	Halogenated
phorate	13.86	231	4	0.225	3	203,175,185	9.76	Phosphate
alpha-BHC	15.37	183	6	0.450	6	145,146,147,148,149	8.84	Halogenated
pentachloroanisole	15.82	265	6	0.450	4	237,235,239	10.94	Halogenated
HCB	15.93	284	6	0.450	6	249,247,251	10.60	Halogenated
terbufos	16.63	231	4	0.45	3	203,175	13.10	Both
diazinon	16.78	179	4	0.45	4.5	137,164,96,122	14.50	Phosphate
dicloran	17.00	176	6	0.45	4.5	148,150,140	9.57	Halogenated
fonofos	17.38	246	4	0.225	3	109,137,202	12.93	Both
lindane	17.70	183	6	0.225	3.5	145,146,147,148,149	10.41	Halogenated
PCNB	17.80	295	6	0.45	6	265,263,267	12.37	Halogenated
beta-BHC	19.75	183	6	0.45	3.5	145,147,148	11.55	Halogenated
pentachloroaniline	20.50	265	6	0.45	6	228,229,230,265,193,203	15.25	Halogenated
methyl chlorpyrifos	20.76	286	6	0.45	4.5	271,273,208,210	16.51	Both
delta-BHC	21.19	181	6	0.45	3.5	145,146,147,148	11.97	Halogenated
heptachlor	21.33	272	6	0.45	4	237,260	17.11	Halogenated
methyl parathion	21.40	263	4	0.45	5	246,233,216,153	16.62	Both
methyl pirimiphos	21.68	290	4	0.45	4	233,262	18.40	Phosphate
chloroathalonil	21.75	266	6	0.45	2		13.28	Halogenated
malathion	22.38	173	4	0.225	2	127,145	18.64	Phosphate
fenitrothion	22.53	277	4	0.225	3	260	17.89	Phosphate
pentachlorothioanisole	23.07	296	6	0.45	5	263,261,265	18.07	Halogenated
aldrin	23.07	263	6	0.45	6	228,226,230,191	18.07	Halogenated
chlorpyrifos	23.16	314	6	0.45	5	286,258,288	18.99	Both
fenthion	23.29	278	4	0.45	4	245,246,263	18.78	Phosphate
ethyl parathion	23.80	291	4	0.225	3	263,274,261	18.92	Phosphate
HE	25.46	353	6	0.45	4	263,317,315,335	20.04	Halogenated
o,p-DDE	26.51	246	6	0.45	6	176,211	21.19	Halogenated
methidathion	26.70	145	4	0.225	3	85,58	20.71	Phosphate
endosulfan I	27.37	195	6	0.45	4.5	159cluster	21.26	Halogenated
p,p-DDE	28.26	246	6	0.45	6	176,211	22.23	Halogenated
dieldrin	28.58	263	6	0.45	6	228cl,191cl	22.10	Halogenated
perthane	29.22	223	4	0.45	5	167,196	23.00	Halogenated
endrin	29.59	263	2	0.45	5.5	191,193,228,226	22.63	Halogenated
ethion	29.65	231	4	0.225	4.5	203,175,185	23.59	Phosphate
o,p-DDT	30.00	235	6	0.45	4.5	165,199,200	23.31	Halogenated
p,p-DDD	30.58	235	6	0.45	4.5	165,199,200	23.56	Halogenated
endosulfan II	30.85	195	6	0.45	4.5	159cluster	22.74	Halogenated
4,4'-DDT	31.86	235	6	0.45	4.5	165,199,200	24.49	Halogenated
thiodan sulfate	32.56	272	6	0.45	4.5	237,235,239	23.97	Halogenated
methoxychlor	33.84	227	4	0.45	4.5	212,196,195,181,184	26.03	Halogenated

TABLE 2. Tandem ECD/FPD Results

Compound Name	R^2	Blank concentration (pg/µl)	Spiked with 10 pg/µl	Detected on:
DDVP	1.0000	13	40	ECD
DDVP	0.9988	<10	18	FPD
trichloroanisole	1.0000	12	23	ECD
tetrachloroanisole	0.9996	< 1	13	ECD
phorate	0.9985	<10	<10	FPD
alpha-BHC	1.0000	5	17	ECD
dicloran	0.9989	4	10	ECD
fonofos	0.9990	<10	24	FPD
lindane	1.0000	< 1	13	ECD
HCB	1.0000	< 1	13	ECD
terbufos	0.9983	<10	47	FPD
diazinon	0.9998	<10	56	FPD
pentachloroanisole	0.9995	< 1	12	ECD
beta-BHC	0.9991	< 1	11	ECD
delta-BHC	0.9985	3	10	ECD
PCNB	0.9997	< 1	11	ECD
chlorothalonil	0.9998	56	71	ECD
malathion	0.9988	<10	32	FPD
pentachloroaniline	0.9999	4	6	ECD
methylchlorofos	1.0000	< 1	13	ECD
heptachlor	0.9997	< 1	11	ECD
methyl-parathion	0.9983	<10	26	FPD
pentachlorothioanisole	1.0000	64	75	ECD
chlorpyrifos	0.9999	< 1	11	ECD
chlorpyrifos	0.9989	<10	24	FPD
Fenthion	0.9984	<10	31	FPD
ethyl-parathion	0.9982	<10	21	FPD
HE	0.9998	5	10	ECD
o,p-DDE	0.9999	42	51	ECD
methidathion	0.9981	<10	39	FPD
endosulfan I	0.9999	< 1	9	ECD
dieldrin	0.9998	10	8	ECD
p,p'-DDE	0.9998	10	10	ECD
endrin	0.9999	25	11	ECD
ethion	0.9891	<10	28	FPD
endosulfan II	0.9999	5	11	ECD
perthane	0.9998	< 1	4	ECD
o,p-DDT	0.9993	< 1	10	ECD
p,p'-DDD	1.0000	< 1	10	ECD
thiodan sulfate	0.9999	3	5	ECD
p,p'-DDT	0.9996	< 1	9	ECD
methoxychlor	0.9999	< 1	18	ECD

Results

The linearity of response was excellent for all detectors. The correlation coefficients exceeded 0.99 for all compounds. The 10 pg/µL concentration was the lowest sample that could be seen on the FPD. As a result, the calibration curves were run from 10 to 1000 pg/µL for this detector. The ECD was an order of magnitude more sensitive and was able to detect the 1 pg/µL samples. However, as is seen in Figure 5, the response factor of the Polaris*Q* is significantly higher at this same concentration. As a test of the reliability of the analysis, 18 replicate samples at 10 pg injected on column were run in matrix on the Polaris*Q*. The average precision was 9.3%. The limits of detection (LOD) for the Polaris*Q* is well below the 50 ppb equivalent concentration in matrix. As has been stated, the LOD was 10 pg/µL for the FPD and 1 pg/µL for the ECD. As a measure of the robustness of the analysis on the Polaris*Q*, after all the samples were run, a final injection of the 100 pg/µL pure standard was run. The calculated amount was within 20% of the injected amount for nearly all compounds. The exceptions were caused by chromatographic difficulties such as non-Gaussian peak shapes. The results are summarized in Tables 2 and 3.

TABLE 3. Polaris*Q* MS/MS Results

Compound Name	R^2	Blank concentration (pg/µl)	%RSD (n=18)	Recovery of 100 pg/µl check standard
DDVP	0.9985	ND	10.9	92%
trichloroanisole	0.9997	ND	6.2	97%
tecnazene	1.0000	ND	7.5	129%
tetrachloroanisole	0.9998	ND	6.1	104%
phorate	1.0000	ND	8.0	125%
alpha-BHC	0.9998	ND	6.9	106%
pentachloroanisole	0.9999	ND	4.3	110%
HCB	0.9999	ND	5.0	100%
terbufos	0.9995	ND	6.4	132%
diazinon	1.0000	ND	10.8	133%
dicloran	0.9999	ND	11.2	117%
fonofos	1.0000	ND	9.3	111%
lindane	0.9996	ND	5.5	105%
PCMB	0.9999	ND	7.7	123%
beta-BHC	1.0000	ND	6.4	110%
pentachloroaniline	0.9999	1.4	6.9	121%
methyl chlorpyrifos	0.9989	ND	7.9	109%
delta-BHC	0.9983	ND	8.4	71%
heptachlor	0.9998	ND	4.9	109%
methyl parathion	0.9976	ND	14.3	94%
methyl pirimiphos	0.9996	ND	8.2	120%
chlorothalonil	0.9978	ND	16.3	37%
malathion	0.9990	ND	10.4	93%
fentrothion	0.9990	ND	9.9	100%
aldrin	0.9998	ND	7.8	111%
pentachlorothioanisole	0.9998	ND	8.2	112%
chlorpyrifos	0.9994	ND	8.9	112%
fenthion	0.9993	ND	11.9	118%
ethyl parathion	0.9998	ND	12.9	126%
HE	1.0000	ND	6.3	117%
o,p-DDE	0.9998	ND	5.9	111%
methidathion	0.9967	5.5	25.6	34%
endosulfan I	0.9999	ND	6.5	120%
p,p"-DDE	0.9999	1.9	7.2	116%
dieldrin	0.9999	ND	7.0	109%
perthane	0.9989	ND	7.2	113%
endrin	0.9999	ND	10.4	122%
ethion	0.9992	2.6	11.0	110%
o,p-DDT	0.9991	ND	11.6	94%
p,p'-DDD	0.9997	ND	6.1	119%
Endosulafan II	0.9996	ND	11.1	117%
p,p'-DDT	0.9985	ND	17.1	74%
thiodan sulfate	0.9977	3.8	15.5	58%
methoxychlor	0.9987	ND	15.0	81%

Discussion

By using selective detectors for screening, the likelihood of co-eluting matrix interferents is greatly reduced. The FPD detector is selective to phosphorus containing compounds only. For screening, the detector was able to find levels near 10 pg injected on column. This is an order of magnitude larger than for both the ECD detector and the Polaris*Q*. The ECD detector is also selective; it is only sensitive to halogenated compounds. Since the 10 pg/µL concentration in matrix is the lowest level that can be seen by the FPD, its utility as a screening tool is limited to higher concentrations. In fact, there were three samples that were flagged for re-analysis by MS/MS because they contained ECD sensitive pesticides. The Polaris*Q* found and quantified them, and it also found organophosphorus compounds at levels below the 10 pg/µL cutoff concentration for the FPD. The power of the MS/MS experiment easily pulled out the target pesticides in matrix in the confirming analysis. As can be seen in Table 4, the blank matrix sample had 15 compounds detected on the ECD. These presumptive false positives were then analyzed on the Polaris*Q*. Of the 15 compounds detected on the ECD, three were confirmed in the blank on the mass spectrometer. As can be seen in Table 5, there were two other pesticides found in the blank that were organophosphorus compounds. Methidithion and ethion were not seen in the blank because the concentrations were below the LOD for the FPD. Upon analysis with the Polaris*Q*, they were seen at concentrations of 5.5 and 2.6 pg/µL. This highlights both the high sensitivity of the Polaris*Q*, as well as the need for a more sensitive screening technique.

An example of the different chromatograms is shown in Figure 6. The compound of interest is eluting at 19.00 minutes for the top two traces. For the MS/MS run, the elution time is obvious. This is a remarkable increase in signal-to-noise. The equivalent concentration in the original sample, assuming perfect recovery into the analyzed sample, is 50 ppb. The amount injected was 1 pg.

The chromatographic run times are comparable for both methods. For the GC detector run, the final compound, methoxychlor, elutes at 26 minutes. For the GC/MS/MS run, methoxychlor is still the final compound, and it elutes at 34 minutes. Future work will focus on decreasing the chromatographic run time of the GC detector based method in order to improve the sample throughput for screening. In addition, a long rail version of the autosampler will be implemented to allow for screening injections to be made on two different gas chromatographs with mass spectral confirmation made on the one Polaris*Q* in the system. This system will have 2 GCs, 3 injectors, 2 stacks of GC detectors, and the Polaris*Q* MS. This will effectively double the throughput of the screening system.

Conclusions

A series of proof-of-concept experiments have shown that an intelligent sequencing software can use a screening analysis to flag samples for confirmation and quantitation. In this work, a series of 44 pesticides were spiked into a seed oil matrix. The samples were screened using a tandem ECD/FPD setup. This dual detector allowed for the simultaneous screening of halogenated and organophosphorus based pesticides. Any positives from the screening were then automatically added to a sequence for confirmation on the Polaris*Q* ion trap mass spectrometer. The Polaris*Q* used MS/MS to remove matrix based interference. A TriPlus Autosampler was used to inject the samples on either of the two inlets on the TRACE GC Ultra. In addition, the sensitivity of the Polaris*Q* is significantly higher than that of the screening techniques. The ideas presented here will also reduce sample preparation time because the same samples are used for both the screening and confirming method.