Tandem GC/MS Analysis of Pesticides in Food Oils by Large Volume Injection with a Back-flush Option

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Overview

Purpose
This work is designed to detect pesticides at very low concentrations in challenging food matrices on a routine use gas chromatograph/mass spectrometer (GC/MS). The target concentration was 1 pg/µL injected on-column, or 50 ppt equivalent in matrix.

Methods
Method development was undertaken to create a robust and sensitive way to analyze pesticides in a complicated food matrix. A back-flush and a large volume injection option were employed to prevent late eluting components of the matrix from entering the analytical column. Minimal sample clean up and preparation was performed before analysis. The use of tandem mass spectral analyses allowed for the elimination of matrix interference.

Results
A lower limit of detection for most of the pesticides was found to be 5 ppt in matrix.

Introduction

Pesticide analysis is an important aspect of food safety. As the global sourcing of foodstuffs becomes more common, more work will be done to test for compliance with various regulations. The sheer number of analyses dictates that the methods used must be reliable, robust, and, given the competitive nature of the food industry, the cost per analysis must be low. Food is a complicated matrix for an analysis, and the chemical complexity of the food makes co-elution of matrix peaks with residues of interest inevitable. Even when using the mass spectrum of a compound of interest, there can be difficulty in determining pesticide contamination, especially at low levels, because of isobaric interferences. In this work, a tandem GC/MS/MS analytical method was developed using a quadrupole ion trap to provide a robust and sensitive analysis of over 45 pesticides in a food oil extract. This particular food extract, because of high concentrations of triglycerides, is especially challenging. A calibration curve was run in the matrix spiked with typically 1-100 pg of various pesticides per 0.2 g of initial matrix. Over 20 injections of the 10 pg standard spiked matrix, the equivalent of 50 ppt of pesticide in the original sample, were run to check system reproducibility.

Methods

The goal of having a robust analytical method which incorporated minimal sample cleanup necessitated the selection of advanced injection techniques. Because of the sensitivity requirements and the heavy matrix involved, it was decided that a tandem mass spectrometer was needed. All experiments were performed on a Polaris*Q* quadrupole ion trap mass spectrometer coupled to a TRACE GC Ultra™.

Other published work suggests that a traditional single quadrupole operating in selected ion monitoring (SIM) mode would have difficulty in reliably detecting the pesticides at these low concentrations in matrix.[1] Since the method called for a rugged system and an inexpensive analysis, a quadrupole ion trap was selected. However, these traps have a limited ion capacity. In order to prevent the trap from being flooded with residual solvent tailing off of the column, which would have reduced the number of analytically useful ions in the trap, a back-flush system was installed on the GC. This allowed for the solvent to be purged from the system before it entered the analytical column. A schematic diagram of the back-flush system is shown in Figure 1. A back-flush injection technique operates by reversing the flow through a 2 meter guard column attached through a valve to the analytical column.
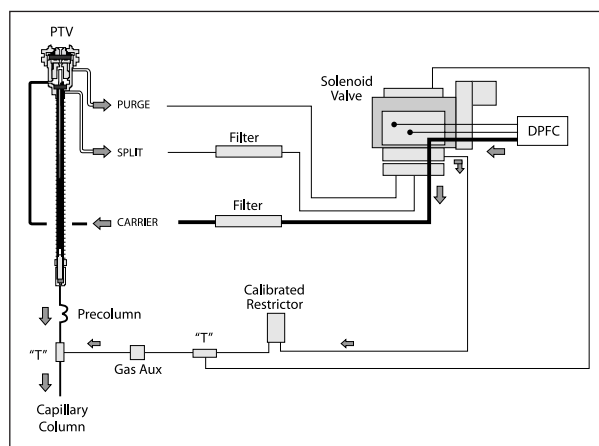

Figure 1: Schematic of the back-flush system

During the injection, the solvent is transferred to the guard column. The back-flush valve is enabled to controllably allow gas flow back through the guard column so the solvent is eliminated through the split vent in the injector. After the bulk of the solvent is gone, the purge valve is closed to allow the carrier gas to flow in a forward direction through the guard column and then to the analytical column. The programmable temperature vaporizing inlet (PTV) temperature is then increased to allow the analytes to be transferred to the guard column. It was found that there were a number of triglycerides and other low volatility compounds that eluted an additional 12 minutes after the last pesticide. These heavy matrix compounds had to be removed from the system between injections to improve the analytical precision. Since the back-flush vent can be opened multiple times, after the last pesticide had been transferred to the analytical column, the back-flush valve was again enabled, reversing the flow through the guard column and out the split vent, sweeping the heavy matrix out of the system. An example of the effect of using a back-flush activation is shown in Figure 2.
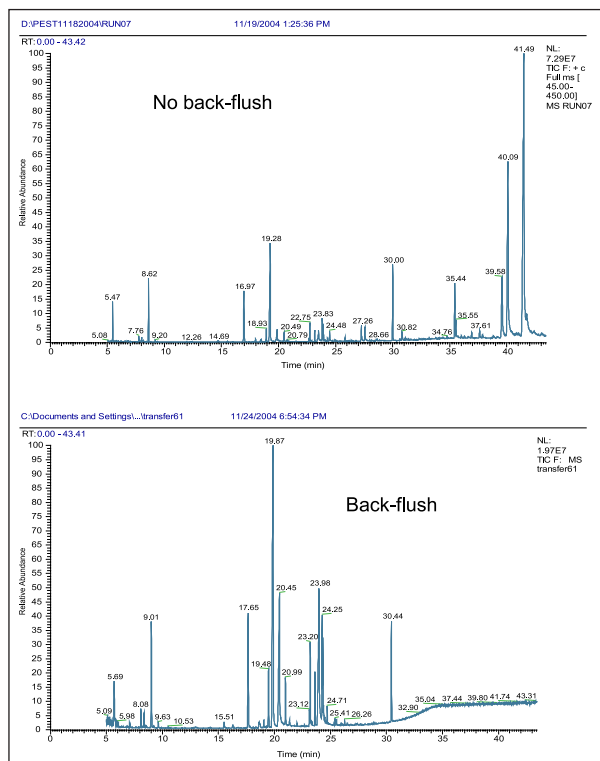
Figure 2: Chromatogram of the food oil matrix showing the elimination of matrix components with and without the back-flush activation

It is known that several of the pesticides studied are also thermally labile. By using an initially cold injector, the degradation of those compounds is minimized. The PTV also allows for large volume techniques to be used to increase the sensitivity of the method. In this method, a 10 µL cold, large volume injection was performed. In general, it is found that the solvent boiling point should be at least 50°C less than the boiling point of the most volatile component to maximize transfer efficiency. The injector and valve profile are shown in Figure 3, and the oven temperature profile is shown in Figure 4.
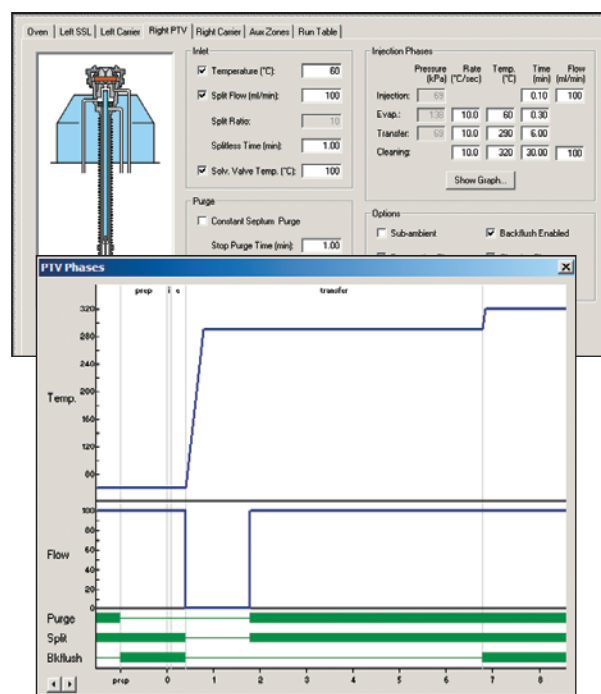

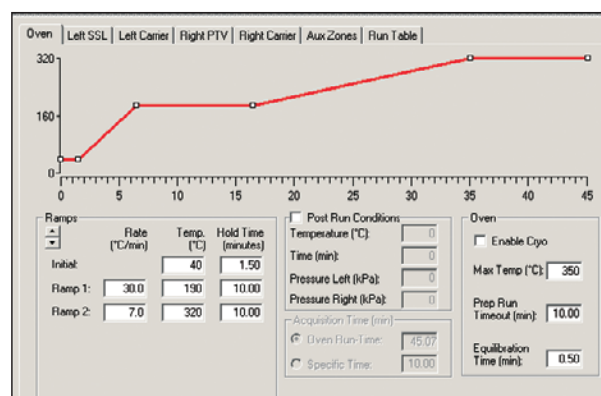Figure 3: PTV inlet conditions used and diagram showing valve state

Figure 4: GC oven temperature profile used in this method

The matrix sample cleanup consisted of a common food oil source that was run though a gel permeation column (GPC) to eliminate many of the salts and other highly polar compounds. The matrix sample was then eluted from the column with a 50:50 mixture of dichloromethane and hexane. There was no other sample preparation performed. The final concentration of the matrix injected into the system was approximately 200 µg/µL. It was found that a 5-fold increase in sensitivity was obtained when using a damping gas flow of 3 mL/min. This higher flow into the trap increased the efficiency of both ion trapping and ion fragmentation. Since a tandem MS experiment was used for the analysis, a check was performed to ensure that the isolation of the precursor ions before fragmentation did not inadvertently remove the desired ions. In this work, the isolation step was always greater than 80% efficient. Since an ion trap will store all masses that are injected into it, there are times during the experimental runs where large intensities of matrix ions would limit the capacity of the ions formed from pesticides. Since the mass spectrometer uses an external ionization source, it is possible to prevent the initial storage of

unwanted masses by creating RF fields within the ion trap that continuously eject the matrix ions. This was not done at all times because the application of these waveforms can also reduce the storage capacity for low mass ions. The waveforms were applied from 21 to 26 minutes in the chromatographic run. Tests with the pure standards showed no change in response regardless of the presence or absence of excitation waves. However, a significant improvement was seen when the waveforms were present for the samples in the matrix. After the optimization of the ionization and isolation of the precursor ions, a final series of experiments were performed to optimize the collisionally induced dissociation (CID) parameters for an efficient second stage of MS.

COMPOUND NAME	PRECURSOR ION (Da)	QUANTITATION ION(S) (Da)	CONCENTRATIONS INJECTED (pg/μL)	LINEAR CORRELATION COEFFICIENT, R²	PEAK AREA PRECISION AT 50 (%RSD, n=20)
DDVP	185	93	2-200	0.9991	19.0%
trichloroanisole	195	167, 169	5-100	0.9994	9.0%
acenaphthene-d10	164	163, 164	Internal Standard (I.S.)	I.S.	I.S.
tecnazene	203	83, 107, 117, 141, 143, 177	5-100	0.9998	10.5%
tetrachloroanisole	246	229, 231, 233	1-100	0.9999	14.3%
phorate	231	175, 185, 203	1-100	0.9999	16.9%
alpha-BHC	183	145, 146, 147, 148	1-100	0.9999	7.5%
pentachloroanisole	265	235, 237, 239	1-100	0.9999	7.2%
HCB	284	247, 249, 251	1-100	0.9999	9.4%
terbufos	231	175, 185, 203	10-1000	0.9999	7.7%
diazinon	179	164, 137	1-100	1.0000	12.0%
dicloran	176	148, 150	5-100	0.9996	10.0%
fonos	137	109	2-200	0.9990	11.0%
lindane	219	181, 183	1-100	0.9999	10.0%
PCNB	237	141, 143	1-100	0.9999	11.0%
phenanthrene-d10	188	188	I.S.	I.S.	I.S.
beta-BHC	181	145, 146, 147, 148	1-100	1.0000	9.0%
pentachloroaniline	265	194, 230	1-100	0.9996	12.0%
methly chlorpyrifos	286	208, 271, 273	2-200	1.0000	9.8%
delta-BHC	181	145, 146, 147, 148	1-100	0.9898	9.3%
heptachlor	272	235, 237, 239	1-100	1.0000	6.9%
methyl parathion	263	246	10-200	1.0000	12.0%
methyl pirimiphos	290	233, 262	2-200	0.9992	8.4%
chloroathalonil	266	194, 203, 229	10-200	0.9780	12.0%
malathion	173	127, 145	10-200	0.9983	19.0%
fenitrothion	260	228, 232	1-100	0.9991	13.0%
pentachlorothioanisole	246	174, 176, 209, 211	1-100	0.9995	13.0%
aldrin	291	220, 255	1-100	0.9998	9.8%
chlorpyrifos	314	286, 258	1-100	0.9999	5.1%
fenthion	278	245, 263	2-200	0.9998	11.0%
ethyl parathion	291	263, 274	2-200	0.9999	9.2%
HE	353	263, 317, 335	1-100	1.0000	5.5%
o,p-DDE	246	176, 211	1-100	1.0000	7.2%
methidathion	145	85	2-200	0.9954	25.0%
endosulfan I	195	159, 133	5-100	1.0000	12.0%
p,p-DDE	246	176, 211	1-100	0.9998	6.3%
oxyfluoren	252	224, 196, 146	1-100	0.9994	13.0%
dieldrin	277	239, 241, 207	1-100	0.9999	8.1%
endrin	263	191, 193, 226, 228	20-2000	0.9997	6.8%
perthane	223	168, 194, 204	20-2000	0.9992	24.0%
ethion	153	125, 97	2-200	0.9899	12.0%
o,p-DDT	235	165, 199, 200	1-100	0.9997	6.9%
p,p-DDD	235	165, 199, 200	1-100	1.0000	7.9%
endosulfan II	195	157, 159, 160	1-100	0.9998	7.4%
4,4'-DDT	235	165, 199, 200	1-100	0.9998	7.9%
thiodan sulfate	272	235, 237, 239	1-100	1.0000	8.2%
methoxychlor	227	165, 184, 196, 212	1-100	0.9999	7.0%
chrysene-d12	240	240	I.S.	I.S.	I.S.

Table 1: Summary results

Results

The time savings created by the use of a back-flush to prevent the late eluting triglycerides from entering the analytical column were demonstrated in a reduction of run time from 45 to 35 minutes. In addition, required maintenance was minimized since less matrix enters in the analytical column and mass spectrometer. Both of these reduce the analysis cost. The utility of using tandem mass spectrometry is seen in Figure 5. The matrix interference
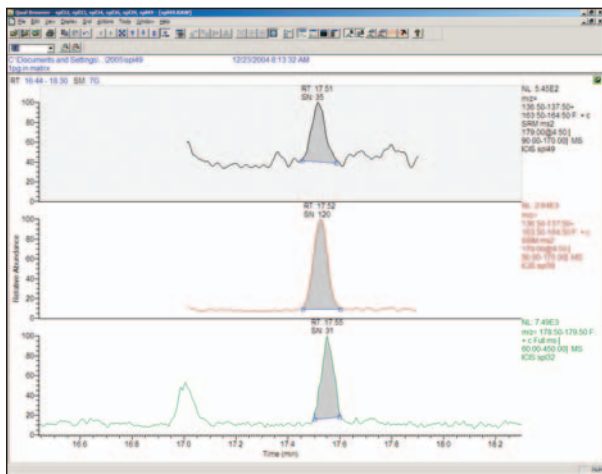

Figure 5: Chromatograms of diazinon spiked in matrix at 1 pg/μL (microliter) and 10 pg/μL (microliter) in Full Scan and MS/MS modes. The 1 pg/μL (microliter) sample was not detected in Full Scan mode.

prevents the lowest level compounds from being seen if only one stage of mass spectrometry is used. However, by using the uniqueness of the precursor fragmentation, the pesticide can be readily pulled out of the background and quantitated when using two stages of MS. Because the pesticide mixture did not have equal concentrations of all the components, some of the lowest limits of the calibration curve do not meet the stated goals of 1 pg/μL. This is not the result of the instrument or the method, but is due to sample size constraints. A sample calibration curve is shown in Figure 6. The correlation coefficient is greater
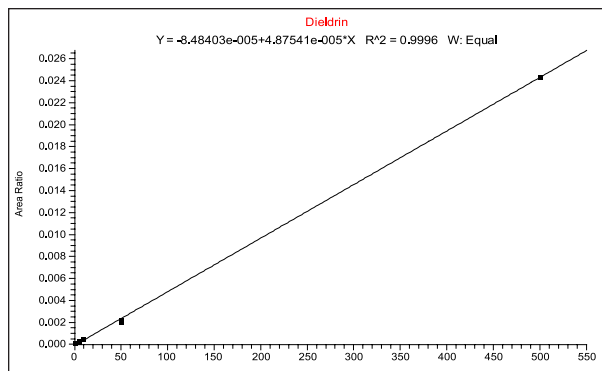

Figure 6: Representative calibration curve for a pesticide in matrix

than 0.978 in all cases, and is greater than 0.99 for all the compounds except chloroathalonil (0.978) and delta-BHC (0.989). The retention drift was within +/- 0.008 minutes (+/- 0.5 seconds) for most compounds. The precision of the mass spectrometer is quite good as well. For a 50 ppt sample with 20 replicate injections, the precision was generally less than 15%. The exceptions were for some thermally labile and poorly chromatographed compounds. A summary of MS/MS ions used along with the retention time and replicate sample precisions are shown in Table 1. The robustness of the instrument is quite good. During the course of these experiments, which was over 700 injections over a one month period, the ion volume of the MS was cleaned only once, the injector liner was replaced once, and the injector port septa was changed once every 50 injections. The guard column was never trimmed or replaced, which indicates that the back-flush system did a thorough job of preventing matrix contamination from entering the analytical column and building up in the guard column. No other system maintenance was needed.

Conclusions

- Linear, reproducible data were shown to levels of 50 ppt in matrix.
- The sample preparation consisted solely of a GPC cleanup.
- For over 700 injections, the ionization volume was cleaned once and the injection port liner was replaced once. The injection port septum was replaced every 50 injections.
- Mass spectrometer tuning was automatically performed only once at the start on the experiments.
- The robustness of the method and the instrument allowed for the transfer of the method to multiple instruments without alterations in the method or the system performance.

Future work will include additional experiments to show the limit of detection for all the compounds at 1 pg/μL. In addition, future studies will test the method extraction efficiency by adding the pesticide spikes before the GPC cleanup.

Reference

1. *Comparison of GC/MS/MS to GC/MS Analysis of Pesticides in Vegetables,* Butler, J. Thermo Fisher Scientific Application Note AN10017.

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