Challenging Pesticide Analysis Using an Agilent J&W DB-35ms Ultra Inert GC Column

Application Note

Food Analysis and Environmental

Authors

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Abstract

As the world population has grown so has pesticide use to protect crops and increase crop yields. Traces of these pesticides are present in the food supply and the effect of long term exposure to these materials is not well understood. Interest in monitoring pesticide levels in soil, water and food matrices has risen with increased use of pesticides and the availability of highly sensitive analytical equipment.

This note shows the fast and effective separation of both organochlorine and organophosphorus pesticides using an Agilent J&W DB-35ms UI 20 m × 0.18 mm, 0.18 µm column (Agilent p/n 121-3822UI). Peaks known to coelute on a 5% phenyl phase are shown baseline-resolved on an Agilent J&W DB-35ms UI column. Peak shapes were consistently excellent and remained so when spiked into a complex fish tissue sample matrix.

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Introduction

As the world population has grown so has pesticide use to protect crops and increase crop yields [1]. Traces of these pesticides are present in the food supply and the effect of long term exposure to these materials is not well understood [2]. Interest in monitoring pesticide levels in soil, water and food matrices has risen with increased use of pesticides and the availability of highly sensitive analytical equipment.

Active sites within a gas chromatographic system can arise from the inlet liner, any metallic surface in the system flow path or the GC column [3]. The dwell time and sample exposed surface area within a column make inertness of the column a key factor in the successful analysis of active analytes such as pesticides. The Ultra Inert series of columns including the Agilent J&W DB-35ms Ultra Inert (UI) helps minimize column activity so that difficult and active analytes can be consistently analyzed at trace levels.

Pesticides tend to be active molecules that can be very challenging to analyze at trace levels particularly where difficult sample matrices are involved. Organophosphorus pesticides are a commonly-used class of active pesticides that tend to show peak tailing in active chromatographic systems. Contract laboratory pesticides (CLP) are less commonly used currently, but common use in the past has caused residues to remain in the environment. CLP pesticides, in particular endrin and DDT, have a tendency to degrade predictably on exposure to active sites within a chromatographic system. The degradation patterns for endrin and DDT can be useful in accessing system inertness. Little or no degradation indicates an inert system.

This note uses organophosphorus and CLP pesticides to illustrate the inertness performance of the Agilent J&W DB-35ms UI column. The Agilent J&W DB-35ms UI column delivers the consistent inertness required for trace level analysis of these difficult molecules. The use of a midpolar Agilent J&W DB-35ms UI phase offers more selectivity to resolve difficult analytes than a nonpolar DB-5ms UI phase. The selectivity boost can make all the difference in resolving potentially coeluting peaks such as parathion-methyl and chlorpyriphos-methyl on a 5% phenyl column, or shift a peak of interest away for matrix interference [4].

In order to achieve a shorter analysis time this work was done using an Agilent J&W DB-35ms UI 20 m × 0.18 mm, 0.18 µm high efficiency GC (HEGC) column. Columns in this format typically offer 30% speed of analysis increase with no loss of resolution [5]. Conversion to the 0.18-mm format is simplified by method translation software, which easily converts established methods on 30 m × 0.25 mm, 0.25 µm columns to 20 m × 0.18 mm, 0.18 um columns [6].

Experimental

An Agilent 7890 GC with an Agilent 5975B MSD System equipped with a multimode inlet (MMI) and an Agilent 7693 Automatic Liquid Sampler was used for this series of experiments. The GC was also fitted with a pressure controlled tee (PCT) postcolumn for automated backflush. Use of the PCT in postcolumn backflush mode helps to reduce sample cycle time by eliminating the need for system bake out between injections.

Tables 1A and 1B list the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1A Chromatographic Conditions for Organochlorine and Organophosphorus Pesticide Mix Standards

GC/MSD:	Agilent 7890 GC System/Agilent 5975B MSD System
Sampler:	Agilent 7693 Automatic Liquid Sampler, 5.0 µL syringe (Agilent p/n 5181-1273)
PCT Device:	Purged Ultimate Union (Agilent p/n G3186-60580)
Column:	Agilent J&W DB-35ms UI 20 m × 0.18 mm, 0.18 µm (Agilent p/n 121-3822UI)
Carrier:	Helium, constant flow 1.3 mL/min
Restrictor:	0.7 m × 0.15 mm id deactivated silica tubing (Agilent p/n 160-2625-10)
PCM 1:	3.8 psi constant pressure
MMI:	1 µL, splitless; 50 °C (0.02 min), 400 °C/min to 250 °C purge flow 50 mL/min at 1.5 min, gas saver 30 mL/min at 2.25 min
Oven:	50 °C (1.3 min), 50 °C/min to 135 °C, 15 °C/min to 200 °C, 20 °C/min to 310 °C (2.5 min)
Postrun Backflush:	5 min at 310 °C, backflush pressure 70 psi, inlet pressure 2 psi
MSD:	320 °C transfer line, 320 °C source, 150 °C quad, Scan mode

Table 1B. Chromatographic Conditions for CLP Pesticides Standard

GC/MSD:	Agilent 7890 GC System/Agilent 5975B MSD System
Sampler:	Agilent 7693 Automatic Liquid Sampler, 5.0 µL syringe (Agilent p/n 5181-1273)
PCT Device:	Purged Ultimate Union (Agilent p/n G3186-60580)
Column:	Agilent J&W DB-35ms UI 20 m × 0.18 mm, 0.18 µm (Agilent p/n 121-3822UI)
Carrier:	Helium, constant flow 1.3 mL/min
Restrictor:	0.7 m × 0.15 mm id deactivated silica tubing (Agilent p/n 160-2625-10)
PCM 1:	3.8 psi constant pressure
MMI:	0.5 µL, splitless; 50 °C (0.02 min), 400 °C/min to 250 °C purge flow 50 mL/min at 1.25 min, gas saver 30 mL/min at 2 min
Oven:	50 °C (0.85 min), 50 °C/min to 135 °C, 15 °C/min to 310 °C (1 min)
Postrun Backflush:	5 min at 310 °C, backflush pressure 70 psi, Inlet pressure 2 psi
MSD:	320 °C transfer line, 320 °C source, 150 °C quad, Scan mode

Table 2 Flow Path Supplies

Vials:	Amber crimp top glass vials (Agilent p/n 5183-4496)
Vial Caps:	Crimp caps (Agilent p/n 5181-1210)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liner:	2 mm dimpled deactivated liner (Agilent p/n 5190-2296)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
PCT fittings:	Internal nut (Agilent p/n G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)

Solutions and Standards

A 10 µg/mL 20-component, pesticide analyzer checkout solution (Agilent p/n 5190-0468) was diluted in acetone to yield standard solutions of 1 and 10 µg/mL. A 26-component mix at a concentration of 10 µg/mL, and an 11-component mix at a concentration of 1000 µg/mL were purchased from Ultra Scientific and diluted to final concentration in acetone. A fourth standard containing CLP pesticides at a nominal concentration of 2000 µg/mL was obtained from Supelco (Bellefonte, PA) and diluted in acetone to yield standard solutions of 1 and 10 µg/mL. The acetone used was JT Baker Ultra Resi grade purchased through VWR International (West Chester, PA).

Spiked Sample Preparation

A red snapper fish sample was purchased from a local fish market. The fish was chopped into small cubes and frozen at –80 °C overnight. The samples were then comminuted thoroughly to achieve sample homogeneity. The sample extraction method used the QuEChERS method followed by dSPE [7]. The spiked samples were then prepared by appropriate dilution of the pesticide standards in the fish matrix blank extract to yield either 250 ng/mL or 500 ng/mL.

Results and Discussion

The pesticide analyzer checkout solution (Agilent p/n 5190-0468) represents a range of pesticides including organophosphorus and organochlorine pesticides that are known to degrade or tail on active sites within the GC flow path including the inlet liner and the column. In Figure 1 no evidence of peak tailing is indicated. This confirms that the system is working properly and activity on the column and in the inlet is minimal.

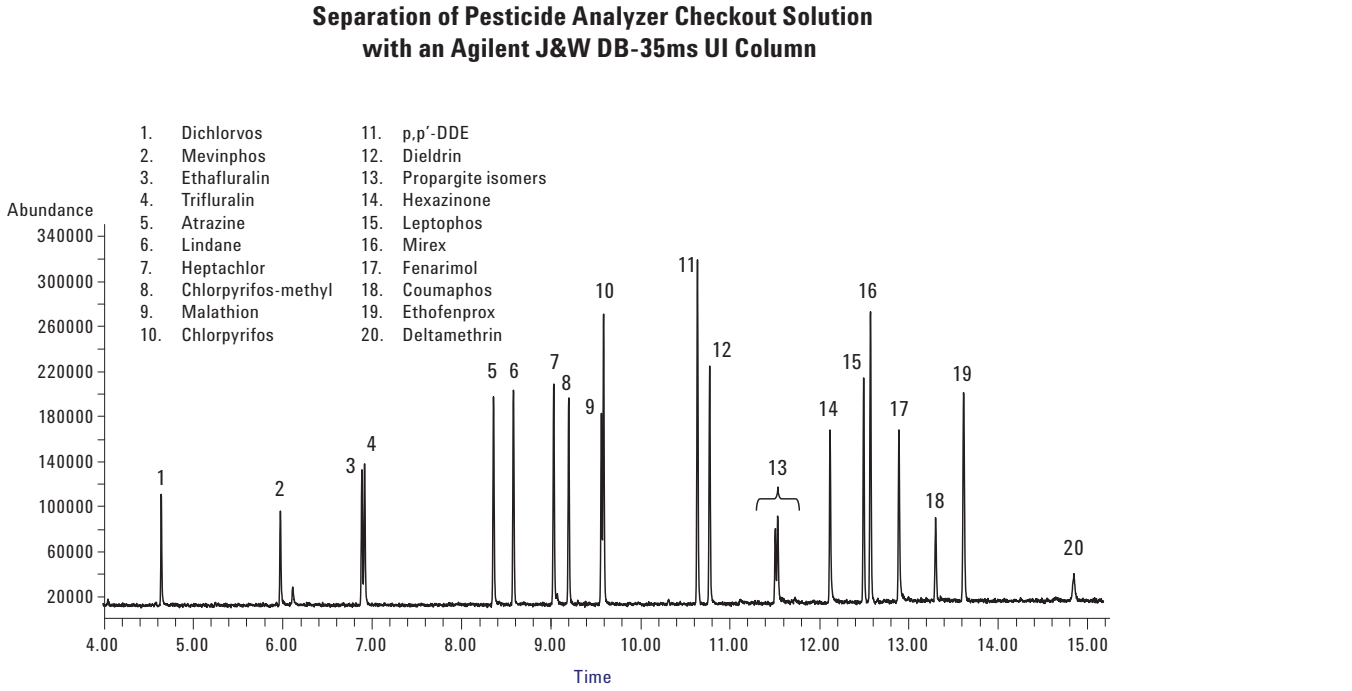

Figure 1. This GC/MS TIC shows the separation of the 20 components in the pesticide analyzer checkout solution (Agilent p/n 5190-0468) on an Agilent J&W DB-35ms UI column (Agilent p/n 121-3822UI). This mix represents a range of pesticides that have either predictive degradation patterns or are known to tail. These effects are absent in this GC/MS scan trace indicating excellent inertness.

The 11-component pesticide mix shown in Figure 2 shows good peak shapes for the organophosphorus pesticides in the mix and no indication of endrin or DDT breakdown products. This system behaves well with the midpolarity Agilent J&W DB-35ms UI column in place. This same mix was previously analyzed on a Agilent J&W DB-5ms UI column [8]. The selectivity of the Agilent J&W DB-35ms UI shifts the retention of these analytes, which is useful to shift a peak of interest away from potential sample matrix interferences.

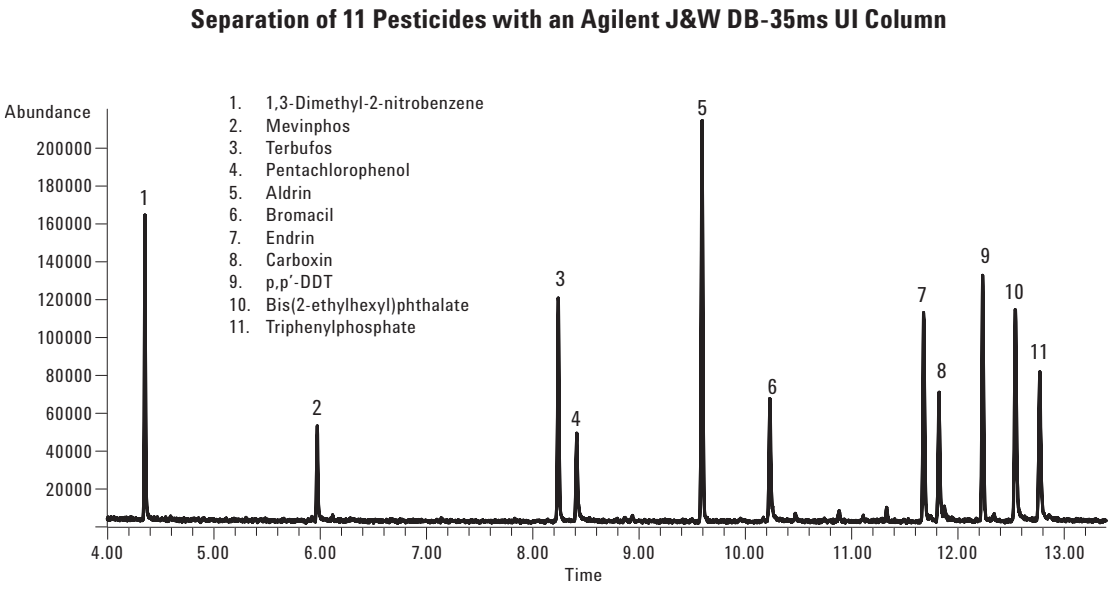

Figure 2. This GC/MS TIC shows the separation of 11 pesticides on an Agilent J&W DB-35ms UI (Agilent p/n 121-3822UI) including endrin and DDT that have distinct breakdown patterns in response to inlet or column activity. Breakdown products are not in evidence indicating a high level of inertness.

The separation shown in Figure 3 is more challenging in terms of selectivity. Chlorpyrifos-methyl and methyl parathion have been reported as a coeluting critical pair on 5 % phenyl columns [4]. Here, using the additional selectivity of the Agilent J&W DB-35ms UI column they are baseline-resolved in a 15-min analysis with sharp peaks. The enhanced selectivity of these analytes toward the Agilent J&W DB-35ms UI phase also aids in the resolution of positional isomers for pesticides such as propargite, cypermethrin, fluvalinate, and fenvalerate.

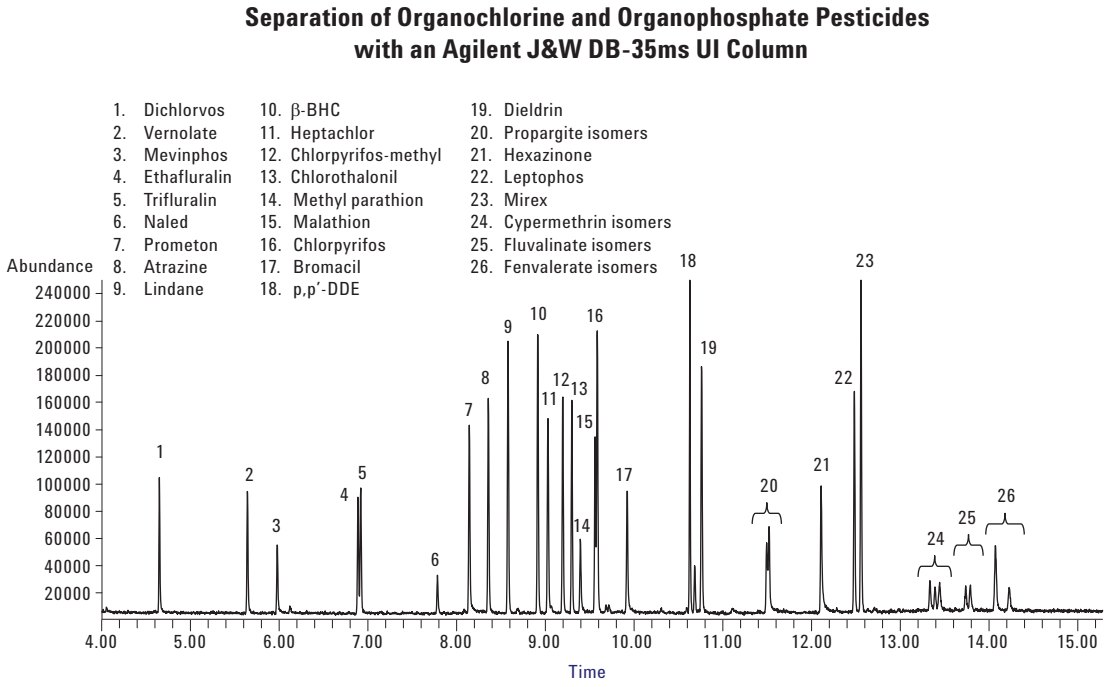

Figure 3. This GC/MS TIC highlights the selectivity available using an Agilent J&W DB-35ms UI column (Agilent p/n 121-3822UI) with components that coelute on a 5 % phenyl phase. Here chlorpyrifos-methyl and methyl parathion are baseline resolved.

Figure 4 shows the organochlorine and organophosphorus pesticide mix spiked at the 500 ng/mL level into a fish tissue matrix sample. The matrix was prepared using a QuEChERS sample prep approach followed by dispersive SPE. A GC/MS blank matrix trace is shown below the analyte trace to indicate the level of potential matrix interference with the analytes of interest. Potential interference with the analytes of interest was minimal. Peaks shapes for the organochlorine and organophosphorus pesticides are still just as sharp and well-resolved, indicating excellent performance on the Agilent J&W DB-35ms UI column in a challenging fish tissue matrix.

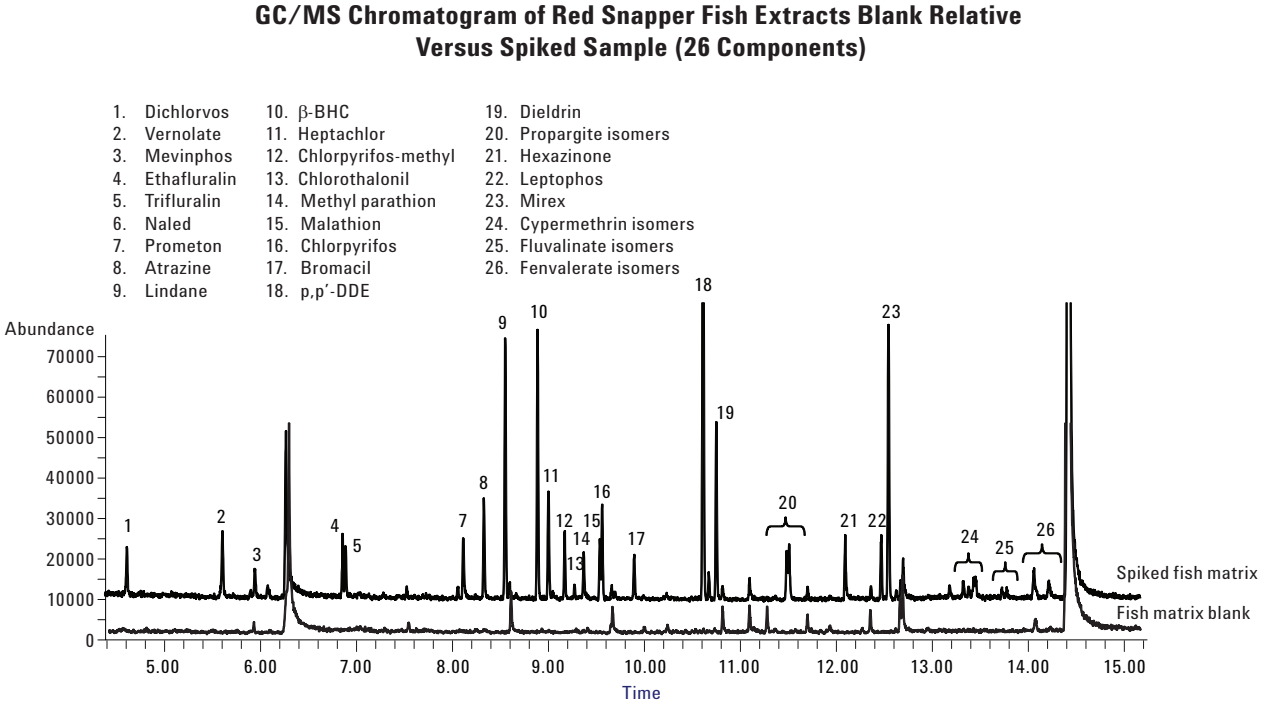

Figure 4. This GC/MS TIC compares the blank fish extract after QuEChERS extraction and dSPE (bottom) versus the same extract spiked with the 26-component pesticide mix. The oncolumn loading of the spiked pesticides shown is 0.5 ng/ component. Separation and excellent peak shapes were maintained in this complex matrix.

Figure 5 show an organochlorine or contract laboratory pesticide (CLP) mix 250 ng/mL solution spiked into a red snapper fish extract matrix. The matrix was prepared using a QuECHERS sample prep approach followed by dispersive SPE. A GC/MS blank matrix trace is shown below the analyte trace to indicate the level of potential matrix interference with the analytes of interest. In this case potential interference with the analytes of interest is minimal. Peak shapes for the organochlorine pesticides are still quite sharp and well-resolved indicating excellent performance on the Agilent J&W DB-35ms UI column in a challenging fish tissue matrix.

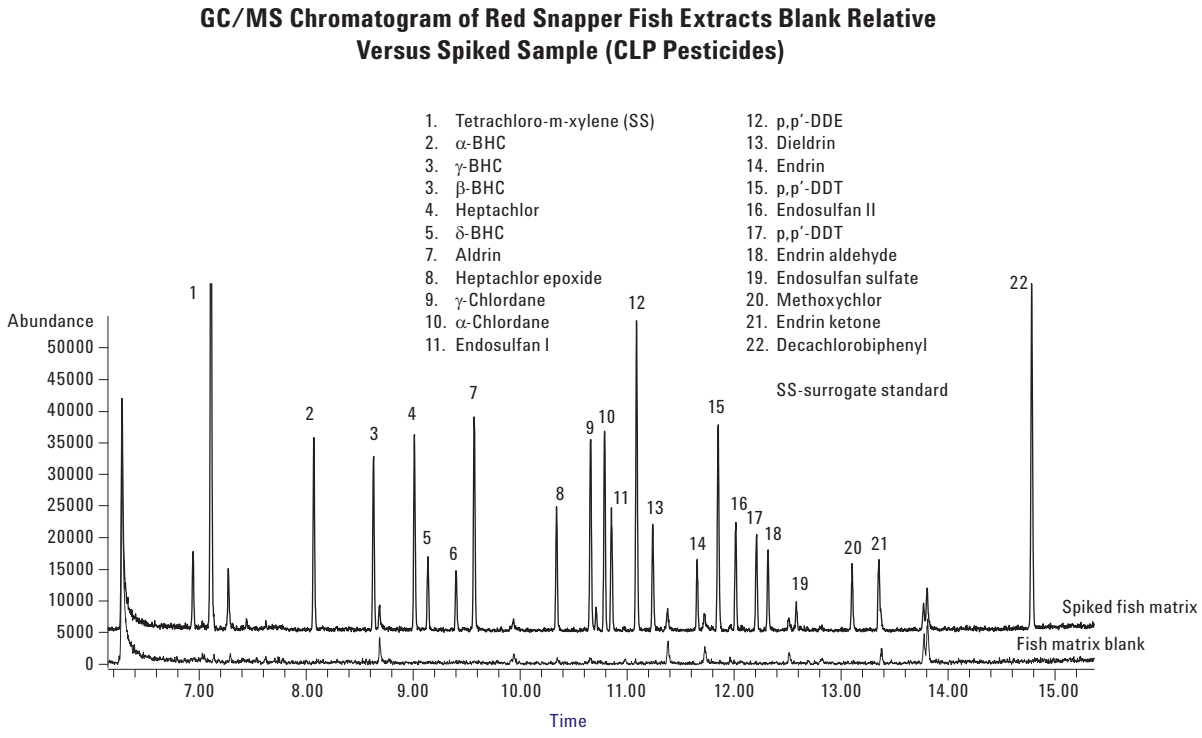

Figure 5. This GC/MS TIC compares the blank fish extract after QuEChERS extraction and dSPE (bottom) versus the same extract spiked the CLP pesticide mix. On-column loading of the spiked pesticides is 0.25 ng/ component. Separation and peak shapes were maintained in this complex matrix.

Conclusions

Effective separation of both organochlorine and organophosphorus pesticides was demonstrated using an Agilent J&W DB-35ms UI 20 m x 0.18 mm, 0.18 µm column (Agilent p/n 121-3822UI). Peak shapes were consistently excellent and remained so when spiked into a complex fish tissue sample matrix. No DDT or endrin degradation patterns were observed indicating excellent inertness of the system and the column.

The 0.18 mm id or high efficiency GC (HEGC) column format used in these experiments shows effective separation of a broad range of pesticides in a 15-min analysis. GC columns in this format allow faster separations with no loss of resolution when compared to 30 m x 0.25 mm, 0.25 µm columns often used for pesticides in food applications.

The availability of an exceptionally inert midpolar Agilent J&W DB-35ms UI column provides more selectivity for resolving active analytes such as pesticides. The selectivity to shift peaks of interest away from potential interferences and to resolve critical pairs that co-elute on 5 % phenyl columns are powerful tools for effective method development. Chlorpyrifos-methyl and methyl parathion were baseline-resolved using a midpolar Agilent J&W DB-35ms UI column.

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