Organophosphorus Pesticides Analysis Using an Agilent J&W DB-5ms Ultra Inert Capillary GC Column

Application Note

Environmental

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Abstract

Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. This new testing procedure employs deliberately aggressive probes to thoroughly investigate and verify column inertness and quality. In challenging separations, knowing that the GC column has been thoroughly investigated for column inertness gives analysts higher confidence in the accuracy of their results.

Trace- and ultra trace-level pesticide analyses are important tools for accessing food supply and environmental quality worldwide. In this application note, trace-level organophosphorus pesticide analysis is demonstrated using electron impact single quadrupole scanning mass spectrometry. Agilent's J&W DB-5ms Ultra Inert capillary GC column provides excellent peak shape for even the most problematic pesticides.

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Introduction

Pesticides are commonly used in agricultural and residential applications throughout the world. Organophosphorus pesticides make up approximately 70 percent of the insecticides currently in use. Unfortunately, these highly toxic materials have three main routes of human exposure: inhalation, ingestion, and skin penetration. Sources of these exposures include consumption of foodstuff containing pesticide residues, aerosol inhalation, and dermal contact during pesticide application. [1]

Organophosphorus pesticides use the same mechanism of action as deadly nerve agents such as sarin, soman, and VX. These pesticides affect the nervous system of insects, mammals, and wildlife by inhibiting the enzyme cholinesterase, important in helping regulate nerve impulses. Inactivation of cholinesterase leads to the accumulation of the neurotransmitter acetylcholine in the central and peripheral nervous system, which leads to depressed motor function and respiratory depression. Human toxicities for this class of molecules have shown acute as well as chronic effects from pesticide poisoning. [2,3]

Organophosphorus pesticides tend to be difficult to quantify due to poor peak shape, as evidenced by broad, asymmetrical peaks. An EPA Method 525.2 standard containing organophosphorus pesticides along with a custom pesticide mix acquired from Ultra Scientific (North Kingstown, RI) were analyzed to highlight the value of using a 30-m Agilent J&W DB-5ms Ultra Inert capillary GC column for difficult pesticide analysis. Many pesticides are sensitive to chromatographic system activity and will readily breakdown. The Ultra Scientific custom mix contains several types of these pesticides, which are useful in quickly evaluating system performance with particularly challenging pesticide analytes. Capillary GC column activity as a potential source of result uncertainty has been virtually eliminated with the Ultra Inert series of columns. [4]

Experimental

An Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1A. Chromatographic Conditions for EPA Method 525.2 Calibration Standards

GC	Agilent 6890N/5975B MSD
Sampler	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0-µL splitless injection
Carrier	Helium 44 cm/sec, 1.5 mL/min constant flow
Inlet	Pulsed splitless; 250 °C, 40 psi until 0.75 min, purge flow 50 mL/min at 1.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven	40 °C (1 min) to 110 °C (50 °C/min), 7 °C/min to 190 °C, 12 °C/min to 285 °C, hold 2 min.
Detection	MSD source at 250 °C, quadrupole at 150 °C, transfer line at 280 °C, EI mode, scan range 45–450 amu

Table 1B. Chromatographic Conditions for Ultra Scientific Calibration Standards

GC	Agilent 6890N/5975B MSD
Sampler	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0-µL splitless injection
Carrier	Helium 52 cm/s, constant flow
Inlet	Pulsed splitless; 250 °C, 40 psi until 0.75 min, purge flow 50 mL/min at 1.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven	75 °C to 175 °C (15 °C/min), 10 °C/min to 275 °C (1 min)
Detection	MSD source at 250 °C, quadrupole at 150 °C, transfer line at 280 °C, EI mode, scan range 45–450 amu

Table 2. Flow Path Supplies

Vials	Amber crimp-top glass vials (Agilent p/n 5183-4496)
Vial caps	Crimp caps with 11-mm septa (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 liners	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier	20x magnifier loupe (Agilent p/n 430-1020)

Sample Preparation

A six-component EPA Method 525.2 pesticide standard mix and internal/surrogate standard mix were purchased from Accu-Standard (New Haven, CT) and used to prepare a six-level calibration standard set. The stock pesticide solution as delivered had a nominal concentration of 1,000 µg/mL. The internal/surrogate solution as delivered had a nominal concentration of 500 µg/mL. The calibration standards were prepared with component concentrations of 10, 5, 2, 1, 0.5, and 0.1 µg/mL and a constant level of 5 µg/mL of internal/surrogate standard as per EPA Method 525.2. All solutions were prepared in acetone using class A volumetric pipettes and flasks. Acetone used was JT Baker Ultra Resi Grade purchased thorough VWR International (West Chester, PA). Acetone was used as a reagent blank and syringe wash solvent.

An 11-component pesticide standard mix was purchased from Ultra Scientific and used to prepare a seven-level calibration standard set. The stock pesticide solution as delivered had a nominal concentration of 1,000 µg/mL. The calibration standards were prepared with component concentrations of 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 µg/mL. All solutions were prepared in 2,2,4-trimethylpentane using class A volumetric pipettes and flasks. The 2,2,4-trimethylpentane used was JT Baker Ultra Resi Grade purchased thorough VWR International (West Chester, PA). 2,2,4-Trimethylpentane was used as a reagent blank and syringe wash solvent.

Results and Discussion

Baseline Inertness Profile for Ultra Inert Columns

The basic approach for inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is testing with aggressive active probes at low concentration and low temperature. [5] This is a rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert GC column series. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species that tend to adsorb onto active sites, particularly at trace level, like the organophosphorus pesticides in this application example. A more detailed description of the test mix and additional application examples can be found in references 6 through 8.

Organophosphorus Pesticide Analysis

In this application note, a multilevel pesticide calibration curve set was evaluated over the concentration range of 0.1 to 10 µg/mL on an Agilent J&W Ultra Inert DB-5 ms 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI). Separate calibration curves were developed for both the EPA 525.2 organophosphorus and Ultra Scientific standards. The standard levels used for the 525.2 calibration were 0.1, 0.5, 1, 2, 5, and 10 µg/mL, while the Ultra Scientific calibration levels were 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 µg/mL. The custom pesticide standard from Ultra Scientific was used to determine system performance by analyzing difficult pesticides, such as endrin and p,p'-DDT, which are prone to analyte breakdown.

No tailing was observed for any of the organophosphorus pesticide peaks across the range studied in either standard set. Sharp, symmetrical peak shapes were noted for all the organophosphorus pesticides analyzed. Good resolution was obtained for each of the pesticides investigated.

Linearity for the 525.2 standard components was excellent across the range studied, giving R^2 values of 0.997 or greater in all cases but fenamiphos, which had an R^2 value of 0.978. This value increases to 0.991 at the midlevel concentrations as suggested by EPA Method 525.2 Sec. 13.2.3.3. Figure 5 indicates the correlation coefficients for each of the individual pesticides and shows an example linear regression plot for disulfoton.

Linearity for the Ultra Scientific standard components was also quite good across the range studied. R^2 values of 0.990 or greater were obtained for the organophosphorus pesticides. Figure 6 indicates the correlation coefficients for each of the individual pesticides and shows an example linear regression plot for mevinphos.

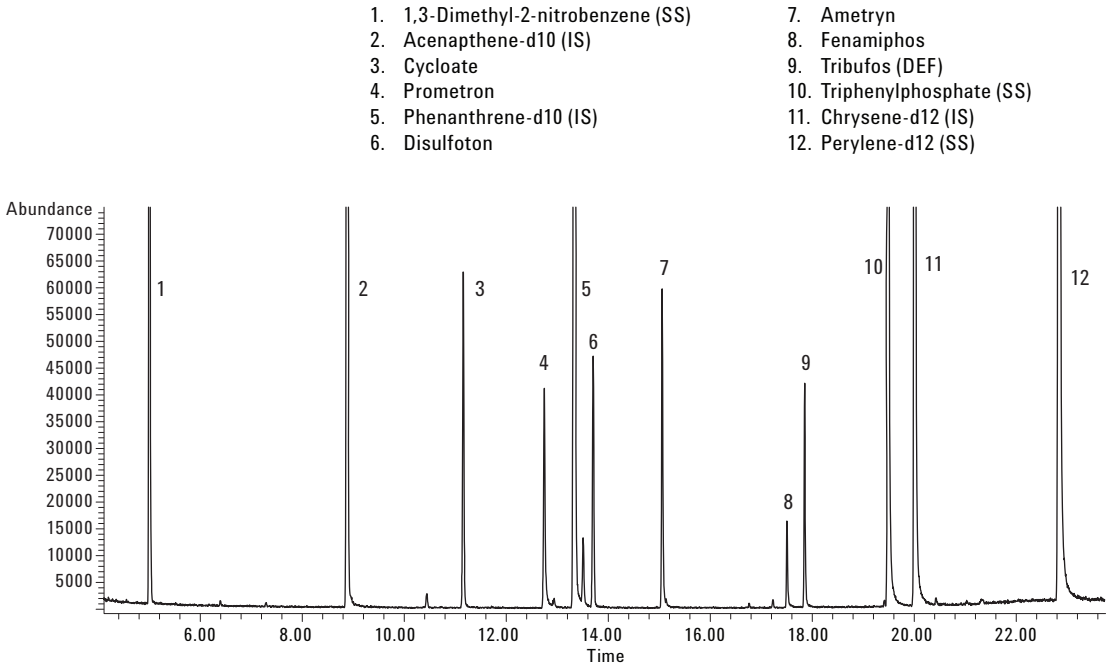

Figure 1. Total ion chromatogram (scan mode) of the 1-ng on-column EPA Method 525.2 standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1A.

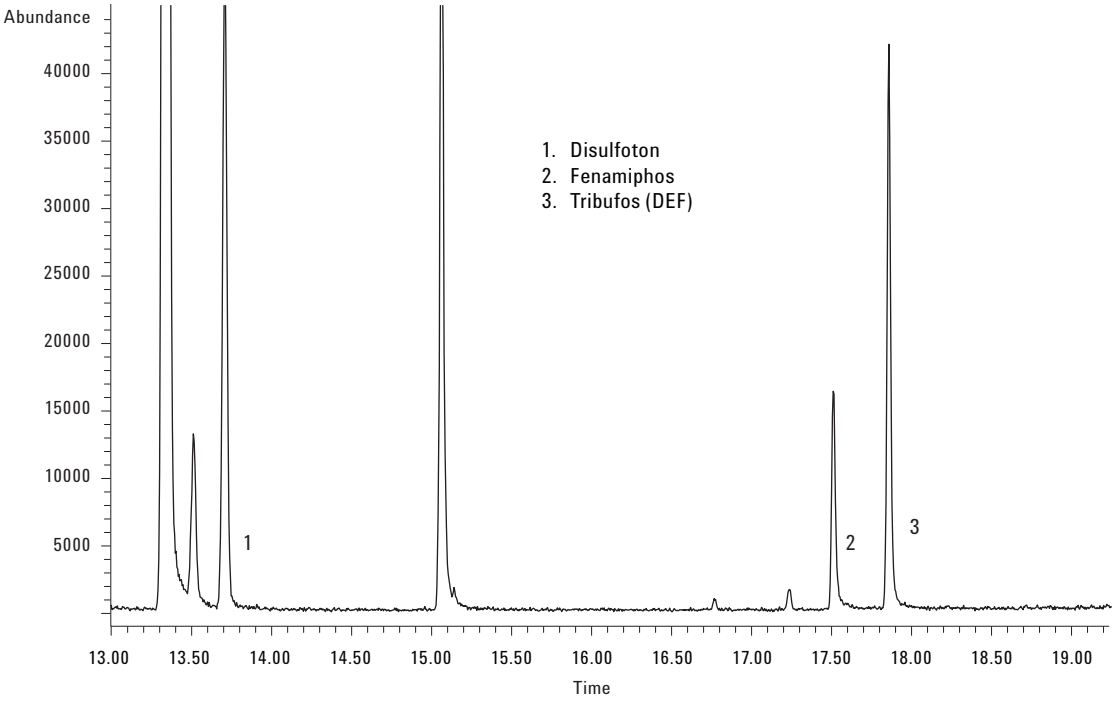

Figure 2. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 1.0 µg/mL EPA Method 525.2 standard pesticide mix. The peaks noted in the figure are the three organophosphorus pesticides of interest. Chromatographic conditions are listed in Table 1A.

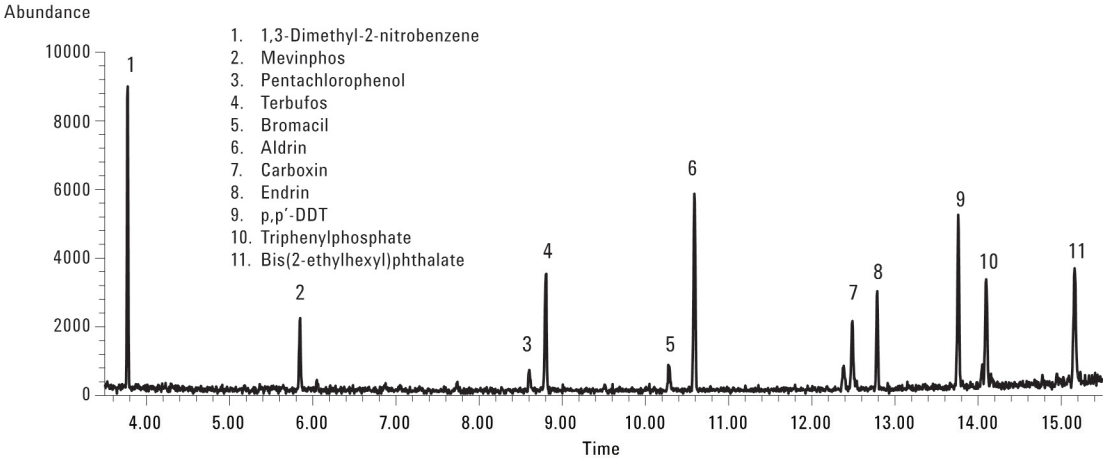

Figure 3. Total ion chromatogram (scan mode) of the 0.1-ng on-column Ultra Scientific standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1B.

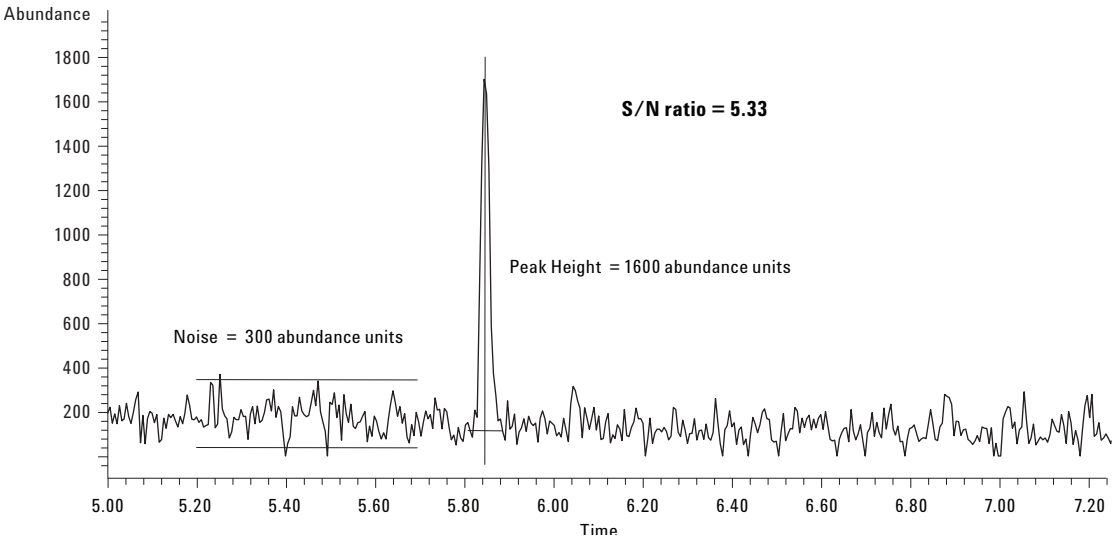

Figure 4. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 0.1 µg/mL Ultra Scientific standard pesticide mix on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). The peak in the figure is mevinphos, an organophosphorus pesticide of interest. This injection represents an on-column loading of 0.1 ng per component. Chromatographic conditions are listed in Table 1B.

Component	R^2
Cycloate	1.000
Prometon	0.999
Disulfoton	0.999
Ametryn	0.999
Fenamiphos	0.978
Tribufos (DEF)	0.997

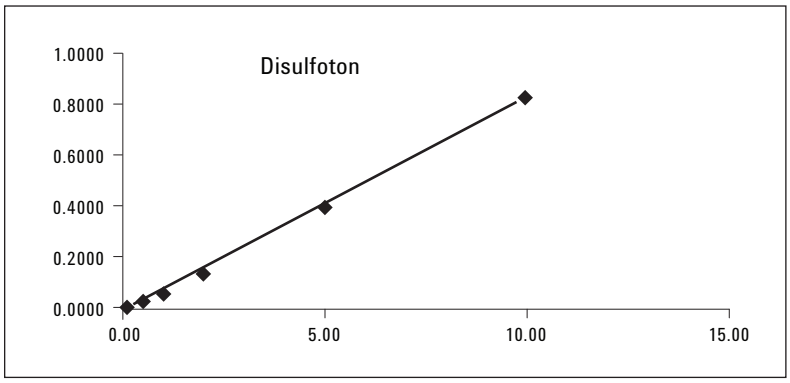

Figure 5. Correlation coefficients for the EPA Method 525.2 pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for disulfoton.

Component	R^2
1,3-Dimethyl-2-nitrobenzene	0.999
Mevinphos	0.990
Pentachlorophenol	0.989
Terbufos	0.996
Bromacil	0.988
Aldrin	0.999
Carboxin	0.996
Endrin	0.998
p,p'-DDT	0.996
Triphenylphosphate	0.997
DEHP	0.996

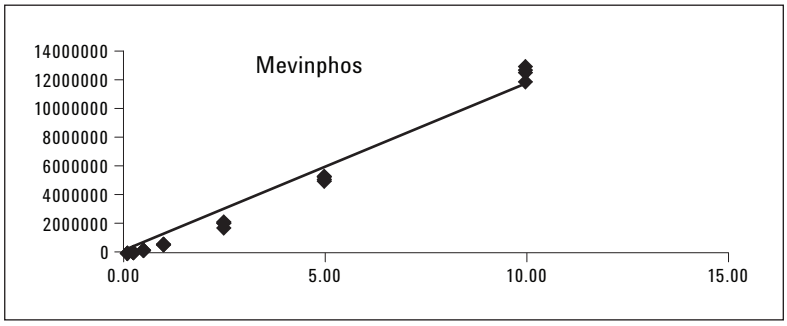

Figure 6. Correlation coefficients for the Ultra Scientific pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for mevinphos.

Conclusions

This application successfully demonstrates the use of an Agilent J&W DB-5ms Ultra Inert capillary GC column for trace-level organophosphorus pesticides. Linearity was excellent for all organophosphorus pesticides studied, yielding 0.99 or greater R^2 values down to a 0.1-ng on-column loading of each component. One of the reasons for excellent linearity and high R^2 values is the highly inert surface of the column. The lack of chemically active sites makes these columns an excellent choice for trace-level applications.

This study was done using scan mode on an Agilent 6890/5975B GC/MSD equipped with an inert electron impact source. The signal-to-noise ratio for a 0.1-ng on-column loading of mevinphos was greater than 5 to 1 with this system. This result shows clearly the power of using an Agilent J&W DB-5ms Ultra Inert column for trace-level organophosphorus pesticides analysis. Lower limits of quantification are expected when using one of Agilent's latest GC/MS offerings, such as the 7890/5975C GC/MSD Triple-Axis Detector coupled with an Agilent J&W DB-5ms Ultra Inert GC capillary column.

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