Rapid Identification and Quantitation of Diphenylamine, *o*-Phenylphenol, and Propargite Pesticide Residues on Apples by Gas Chromatography/Mass Spectrometry

Lei Yu,* Royal Schoen, Arlene Dunkin, Michael Firman, and Holly Cushman

Washington State Department of Agriculture, Pesticide Residue Laboratory, 2017 South First Street, Yakima, Washington 98903

A method has been developed to detect diphenylamine, *o*-phenylphenol, and propargite on apples. Gas chromatography mass spectrometry was used to identify and quantify three compounds in the selective ion monitoring mode. The limits of detection are 10, 9, and 15 ppb for diphenylamine, *o*-phenylphenol, and propargite, respectively. The method provides excellent recovery and linearity data with low coefficients of variation for the three pesticide residues.

Keywords: *Diphenylamine; o-phenylphenol; propargite; apple; gas chromatography/mass spectrometry; selective ion monitoring*

INTRODUCTION

Diphenylamine (DPA) and *o*-phenylphenol (OPP) are post-harvest fungicides for applications on fruits and vegetables, while propargite (PPG) is an acaricide for control of mites on crops with efficacy against citrus red mite (Figure 1). Many studies have reported that relatively high detectable DPA residue has been found on apples (Allen et al., 1980; Bramlage et al., 1996). According to the U.S. Department of Agriculture Pesticide Data Program (USDA PDP) annual reports (1994, 1995, 1996), the three compounds are mainly found on apples and selected fresh fruits and vegetables. DPA, PPG, and OPP were detected on 69.6%, 32.2%, and 18.0% of all analyzed apple samples, respectively (1996).

As a participant state, Washington State Department of Agriculture (WSDA) is currently involved in the USDA PDP to collect pesticide residue data in selected fresh fruits and vegetables. Approximately 75 pesticide residues are being simultaneously per sample screened by our pesticide laboratory utilizing California Department of Food and Agriculture (CDFA) multiresidue method (Joe, 1988; Luke et al., 1981). Typically, screened pesticide residue findings are first determined by GC and HPLC technology with various detection systems; then, the GC/MS is used to confirm the positive findings by selective ion monitoring (SIM) or full scan monitoring.

This type of screening/confirmation methodology has been used in analysis for DPA and PPG residues in the laboratory. DPA was quantified with GC/nitrogen phosphorus detector (NPD) and PPG with GC/atomic emission detector (AED). However, the sensitivity and linearity of DPA and PPG on the NPD and AED was not satisfactory. Further analytical time was also required to confirm these two compounds by GC/MS. The laboratory had not screened OPP because it was difficult to separate and detect OPP from DPA with GC/NPD analysis due to their chemical similarities.

Recently, many multiresidue methods have been developed and successfully used to detect well over 100 pesticide residues simultaneously in food samples. One of the significant techniques widely used is gas chromatography/mass spectrometry (GC/MS), which can reliably and rapidly detect pesticide residues in a very cost-effective manner (Liao et al., 1991; Fillion et al., 1995). However, these methods, in most cases, are not well suited for compounds such as diphenylamine (DPA), *o*-phenylphenol (OPP), and propargite (PPG) because of procedural and instrumental complexities as well as long analysis times. The necessity to use such complex multiresidue methods for DPA, OPP, and PPG specific analysis is not warranted. A need exists to develop a simple and rapid pesticide-specific GC/MS method for multiple compounds. This paper presents a practical method used to quantify DPA, OPP, and PPG pesticide residues on apples by GC/MS.

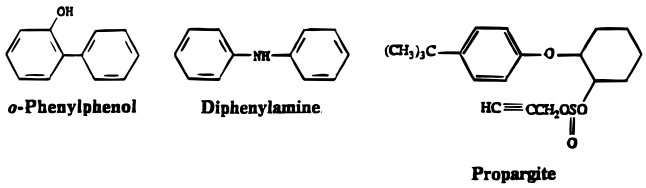

Figure 1. Chemical structures of *o*-phenylphenol, diphenylamine, and propargite.

Table 1. GC/MS SIM Mode Acquisition Parameters

compd	retn time (min)	target ion (*m*/*z*)	qualifier ions (*m*/*z*)	dwell (ms)
OPP	8.7	170	169, 168, 167	100
DPA	9.4	169	168, 167, 170	100
PPG	13.5	135	173, 201, 350	100
anthracene	10.5	188	187	100

MATERIALS AND METHODS

Materials and Apparatus. Neat pesticide standards used in this study were obtained from Chem Service (West Chester, PA). All solvents used were HPLC grade reagents from J.T. Baker, Inc. (Phillipsburg, NJ). Sodium chloride used was analytical reagent grade from J.T. Baker, Inc. Prepurified grade gases of nitrogen and helium were used.

The food processor is Model R 301 Ultra from Robot Coupe USA, Inc. (Ridgeland, MS). The homogenizer is Model 17105 from Omni International (Gainesville, VA). The nitrogen evaporator is Model Meyer N-evap 111 from Organomation Assoc., Inc. (Berlin, MA).

* To whom correspondence should be addressed [telephone (509) 575-2759; FAX (509) 454-7699].

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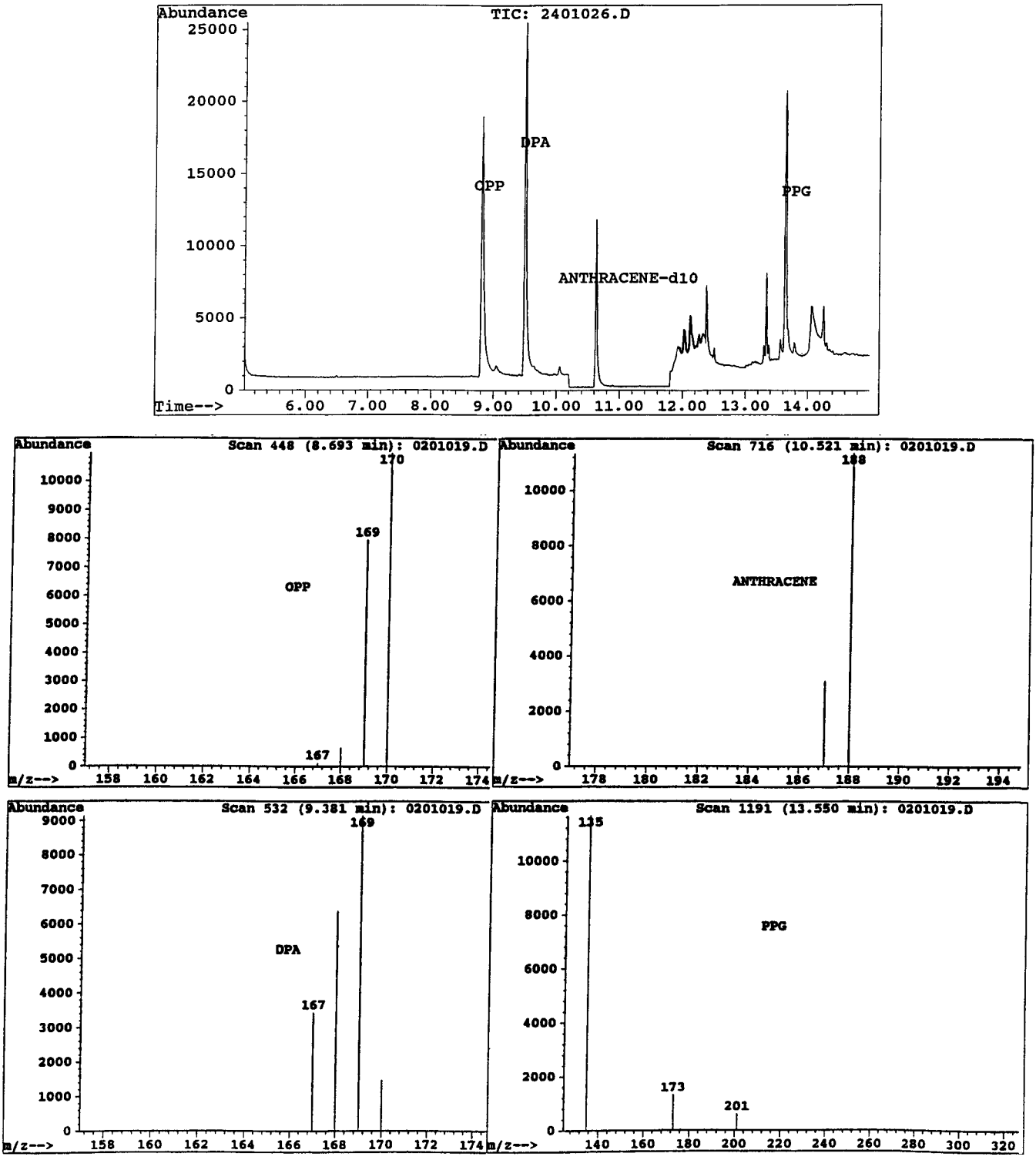

Figure 2. Total ion chromatogram from fortified apple sample (top) and mass ion spectrum of OPP, DPA, anthracene, and PPG eluting at 8.69, 9.38, 10.52, and 13.55 min, respectively (bottom).

GC Parameters. The GC/MS instrumental system consists of a Hewlett-Packard (HP) 5890 Series II GC coupled with Model 5971A quadropole mass spectrometer with a 30 m × 0.25 mm × 0.25 μm film thickness HP-5 MS column. Operating conditions were as follows: split-splitless injector with electronic pressure control 7.0 psi at 60 °C under constant flow, injector temperature 220 °C, oven temperature from 60 °C (1 min hold) to 265 °C (2 min hold) at 18 °C/min. The mass spectrometer is operated in selective-ion monitoring (SIM) mode with the GC/MS interface at 280 °C. SIM parameters for all compounds are listed in Table 1 (Worthing, 1987), and their mass spectra are illustrated in Figure 2.

The GC/NPD instrumental system consists of an RGS HP 5890 GC series II and HP NPD with a 30 m × 0.53 mm × 1.0 μm film thickness DB-17 column. The GC/AED instrumental system consists of an RGS 5890 GC series II and HP 5921A AED with a 25 m × 0.32 mm × 0.17 μm film thickness HP-1 column.

Sample Preparation. The sample extraction procedure employed in this method is the CDFA multiresidue method (Joe, 1988). Other multiresidue screening techniques can be accomplished simultaneously. The procedure is as follows: Weigh 50.0 g of homogenized apple sample into a pint Mason jar. Add 100 mL of acetonitrile and blend at high speed for 3 min. Using filter paper transfer blended mixture into screw cap Erlenmeyer flask containing 10 g of sodium chloride. Shake for 1 min and allow separation for at least 30 min, then transfer a 10.0 mL aliquot of the acetonitrile layer into a small beaker and dry it on the steam bath under air to 0.5−1.0 mL. Remove and gently evaporate solvent traces under air or

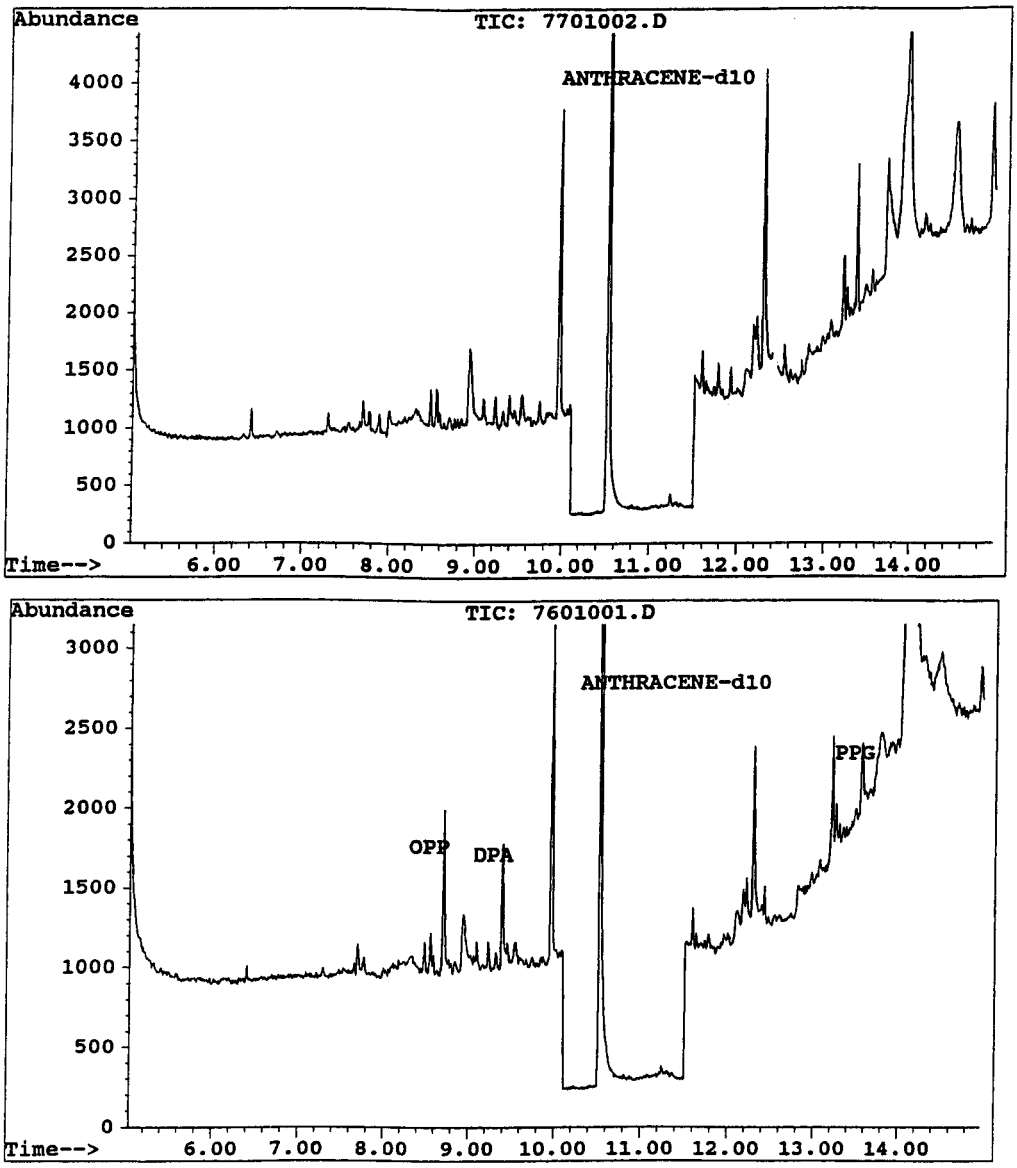

Figure 3. Total ion chromatograms from blank matrix sample (top) and fortified apple sample with 10 ppb OPP, 8 ppb DPA, and 15 ppb PPG (bottom).

nitrogen, just to dryness. Immediately add 1 mL of acetone and quantitatively transfer to a graduated centrifuge tube with several approximately 0.5 mL acetone rinses. Fortify all samples with 100 μL of 0.005 ng/μL internal standard anthracene-d_{10}, which is used to normalize the analytes during data analysis using Model HP 5971A GC/MS chemstation software. Adjust samples to a 5.0 mL final volume in acetone and transfer to an injection vial for GC/MS analysis or GC/NDP and GC/AED analysis.

GC/MS Calibration and Quantitation. Stock standards were prepared in acetone and maintained at 4 °C. Intermediate standard solutions were directly diluted from the stock solutions in acetone. The spiking solutions were prepared from intermediate standard solutions and diluted to suitable volumes with acetone. The five-point working calibration standards were prepared in acetone to establish calibration curves over the target compound concentration range of interest. Calibration curves were constructed for each compound with resultant regression coefficient $r \geq 0.990$. Compounds were identified according to their retention times and mass spectra as defined by Table 1. Confirmation was made by comparison of response ratios of selected ions utilizing upper and lower ratio limits. Each compound was quantified by measuring the response ratio of a target ion with respect to the calibrated internal standard (HP, 1992).

RESULTS AND DISCUSSION

Method Sensitivity and Limits of Detection. Limit of detection (LOD) is here defined as approximately three times the system noise in the matrix. To determine GC/MS LOD for the three compounds, two blank apple samples were spiked at 0.010, 0.009, and 0.015 ppm for DPA, OPP, and PPG, respectively, based upon previously characterized compound sensitivities. The blank apple matrix was fortified with internal standard anthracene prior to injection and analyzed to ensure detection of the compounds of interest at these fortified levels. Figure 3 showed that the three compounds could be well resolved and detected from noise at these fortification levels, and there was no detection of any compounds of interest in the blank apple matrix. It is also noted that the signal-to-noise ratio of compounds was actually considerably greater than three times the noise in this method. Thus LOD's were conservatively established pending future applications of the method to more highly matrix effected commodities.

Method Quantitation, Recovery, and Linearity. The USDA PDP annual reports (1996) showed residue findings of DPA, OPP, and PPG on apples to be from

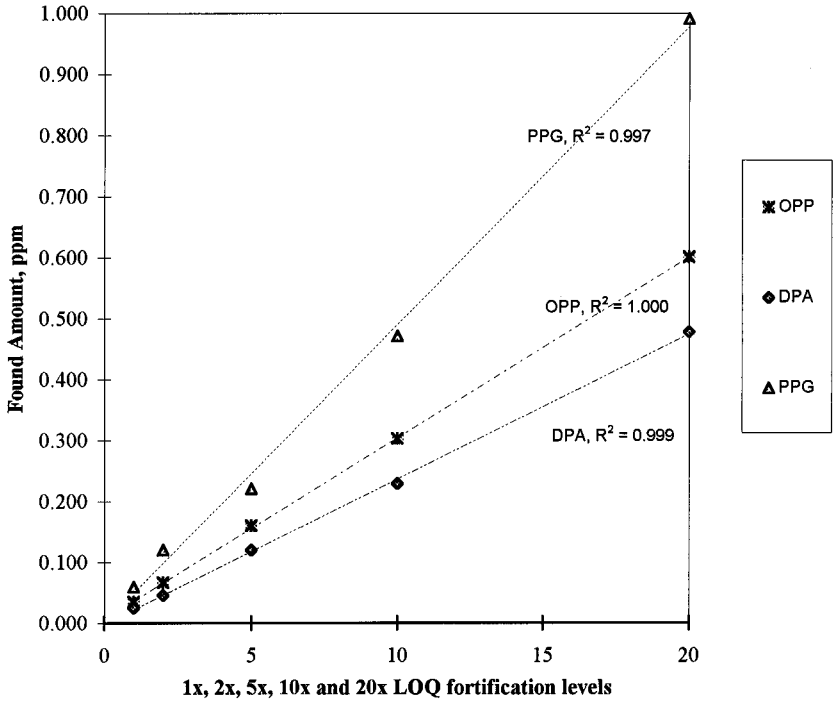

Figure 4. Method linearity regression curves of OPP, DPA, and PPG for the five fortified levels.

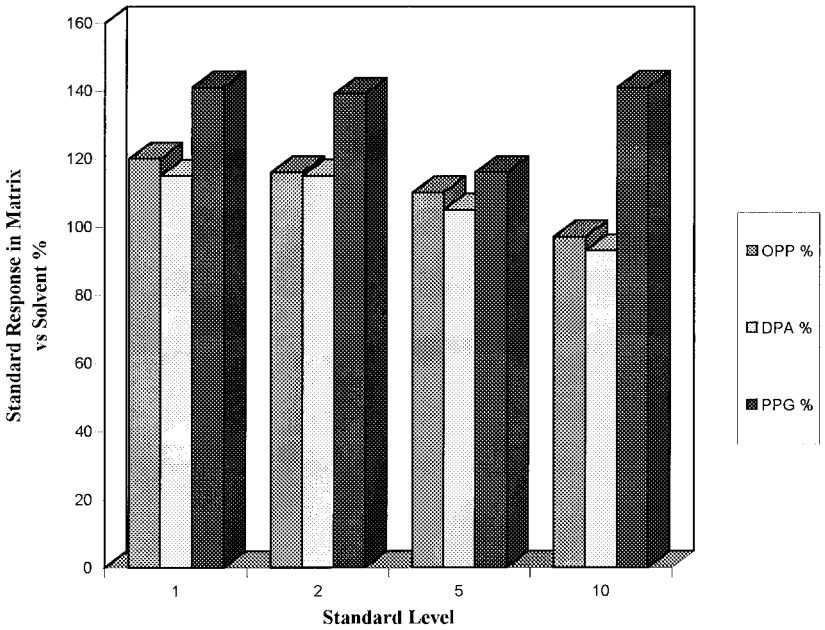

Figure 5. Matrix effect on OPP, DPA, and PPG (left to right) at the four standard levels.

Table 2. Average Validation Recoveries for Three Compounds

fortification level, DPA (ppm)	DPA % recovery (mean ± %RSD, *n*)	fortification level, OPP (ppm)	OPP % recovery, (mean ± %RSD, *n*)	fortification level, PPG (ppm)	PPG % recovery (mean ± %RSD, *n*)
0.025	99 ± 3.8, $n = 3$	0.032	109 ± 2.3, $n = 3$	0.044	136 ± 8.4, $n = 3$
0.051	90 ± 5.2, $n = 8$	0.064	105 ± 5.2, $n = 8$	0.089	135 ± 9.6, $n = 8$
0.127	94 ± 4.3, $n = 3$	0.160	100 ± 4.1, $n = 3$	0.222	100 ± 8.2, $n = 3$
0.254	90 ± 3.0, $n = 3$	0.320	95 ± 4.1, $n = 3$	0.444	106 ± 5.5, $n = 3$
0.508	94 ± 6.3, $n = 3$	0.640	94 ± 5.5, $n = 3$	0.888	112 ± 2.0, $n = 3$

13 ppb to 5.4 ppm (DPA), 14 ppb to 0.61 ppm (OPP), and 18 ppb to 2.8 ppm (PPG). From these results, the method fortification ranges for the three compounds were determined.

The limits of quantitation (lowest fortification level) (LOQ) were determined as 0.025, 0.032, and 0.044 ppm for DPA, OPP, and PPG, respectively, for our study. Data were collected at five fortifications from LOQ to 20× LOQ. The reported results in Table 2 are from running triplicate samples (except 2× LOQ) at each fortification level. The average results, expressed in the percent of recovered spiked compounds over the fortified levels, range from 90% to 99% for DPA, 94% to 109% for OPP, and 100% to 136% for PPG. The relative standard deviation was equal to or lower than 9.6% for each of the three compounds over the five fortified levels. Figure 4 plots method linearity for each compound over the fortified range.

Two calibration curves were established to calculate the different fortified recoveries. One was used to

Table 3. Sample Result Comparisons between GC/MS, GC/NPD (DPA), and GC/AED (PPG)

	DPA (ppm)		PPG (ppm)	
sample no.	GC/MS	GC/NPD	GC/MS	GC/AED
1	0.031	0.02	0.242	0.203
2	0.074	0.06	0.528	0.676
3	0.085	0.09	0.504	0.482
4	0.098	0.10	0.914	0.989
5	0.105	0.12		
6	0.128	0.12		
7	0.128	0.15		
8	0.170	0.18		
LOD	0.008	0.024	0.015	0.028
LOQ	0.025	0.079	0.044	0.093

calculate 1× and 2×LOQ recoveries with $^1/_2$×, 1×, and 5× LOQ working standard. The other was based on 5×, 10×, and 50× LOQ working standard to determine 5×, 10×, and 20× LOQ recoveries. If one calibration table was made from all working standards, the results near the low fortified level would be affected rather than that of the high fortified level. The data acquisition procedure in this method was also applied to the analysis of real apple samples.

Precision and Accuracy of Method Quantitation. Eight apple samples were fortified at the 2× LOQ level to demonstrate precise and accurate recoverability. The mean recoveries for DPA, OPP, and PPG are 90%, 105%, and 135%, with the RSD's 5.2%, 5.2%, and 9.6%, respectively (Table 2).

Matrix effect was studied comparing variation of standard response in acetone solvent to blank apple matrix. Four levels of standard (from 1× to 10× LOQ) were compared between the matrix and solvent. The response ratio was expressed as the response of standard in the matrix versus the response in solvent. Figure 5 showed that the matrix had a bigger effect on PPG response than OPP and DPA. This matrix enhancement could be observed in PPG recovery at its low level, which may explain the relatively high recovery and RSD% at the 1× and 2× LOQ fortification levels. No obvious effect on the internal standard anthracene response between the solvent and matrix was noted.

Due to sample load and laboratory efficiency, a freezer storage stability study was not done. Generally, samples in our laboratory are run through homogenization, extraction, and cleanup within 72 h of sample arrival. A second run linearity study was made 3 days after the first run, with no significant difference between the two runs.

Data Results Comparison and Discussion. Several apple sample positive findings for DPA and PPG compare GC/MS with GC/NPD and GC/AED quantitation results, respectively (Table 3). Data comparison showed this GC/MS method has well-correlated results with GC/NPD and GC/AED. Some of the NPD and AED data was not comparable to the GC/MS for DPA and PPG due to poor instrument sensitivity and performance. The GC/MS quantitation method can provide equivalent or superior results with simultaneous confirmation, which is an obvious advantage over AED and NPD. As we mentioned before, OPP is difficult to separate from DPA due to their similar chemical structure and properties. Therefore, confirming and quantifying via this GC/MS method is ideal for our laboratory.

Since we developed this GC/MS method in March 1996, 110 apple samples analyzed were DPA positive, which accounts for about 66% of the total 167 apple samples. The minimum and maximum values detected were 18 ppb and 2.4 ppm. Our distribution of DPA concentration ranges for apples showed that the high frequent occurrences were 0.10−1.0 ppm, correlating with previous data reports by USDA PDP (1994, 1995).

Prospective Study for This Method. On the basis of current EPA pesticide tolerances for 1996 commodities, monitoring for DPA, OPP, and PPG would be done on apples, oranges, spinach, and peaches at the WSDA lab. With relatively low LOD's, monitoring for these residues via this GC/MS method can enhance detection, confirmation, and quantitation over various commodities.

ABBREVIATIONS USED

DPA, diphenylamine; OPP, *o*-phenylphenol; PPG, propargite; GC/MS, gas chromatography/mass spectrometry; USDA PDP, U.S. Department of Agriculture Pesticide Data Program; WSDA, Washington State Department of Agriculture; CDFA, California Department of Food and Agriculture; NPD, nitrogen phosphorus detector; AED, atomic emission detector; HP, Hewlett-Packard; SIM, selective-ion monitoring; LOD, limit of detection; LOQ, limit of quantitation; RSD, relative standard deviation.

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