UDC 632.95:57.083.3
ISSN 1330-9862

original scientific paper

Determination of Diazinon in Fruits and Vegetables by ELISA

*Zhihua Fan and Rodney J. Bushway**

Department of Food Science and Human Nutrition,
5736 Holmes Hall, University of Maine, Orono, ME 04469-5736, USA

Received: June 26, 1997
Accepted: October 14, 1997

Summary

A commercial polyclonal plate enzyme-linked immunosorbent assay (ELISA) was applied to the analysis of diazinon in fruits and vegetables. The produce was extracted by polytroning 10 g in 20 mL of methanol for 3 min followed by a clean-up with C_{18} Sep-Paks. All samples and standards were run in 10% methanol. Reproducibility studies yielded coefficients of variation (CV) from 2.0 to 14%. Fruits and vegetables spiked at five different levels had percent recoveries ranging from 80 to 95 (CV of 2.2–15%). Comparative analysis of 59 positive diazinon samples by enzyme-linked immunosorbent assay and the gas chromatography – atomic emission detector method showed good agreement with a correlation coefficient of 0.987.

Keywords: ELISA, diazinon, fruits, vegetables, GC-AED

Introduction

Fruits and vegetables are an important part of a healthy human diet. The world's fruit and vegetable growers raise hundreds of thousands of tons annually. In 1995, 485 430 metric tons (MT) of vegetables were grown, and 394 966 MT of fruits were produced world-wide *(1)*. However, to obtain high yields of produce insecticides like the organophosphorous pesticide diazinon must be used.

Pesticide residue determination is extremely important for fruits and vegetables because of government regulations and human health. There are many methods available for analyzing diazinon in produce, the majority being screening procedures using gas chromatography (GC) equipped with a nitrogen-phosphorous detector or an atomic emission detector (AED) *(2–4)*.

The primary purpose of this work was to develop a more cost-effective and less complex method for the analysis of diazinon in produce. In order to meet these criteria ELISA was employed which has been extensively used to screen other pesticides in food *(5)*.

Experimental

Samples

A total of 278 samples of fruits and vegetables were collected from local supermarkets in Bangor, ME, USA. The fruits analyzed were apple, nectarine, peach, pear, plum, kiwi, blueberry, black, green, and red grapes; the vegetables were asparagus, beet greens, broccoli, carrots, cauliflower, celery, green pepper, lettuce, mushroom, potato, spinach, salad, and tomato.

Organic produce (apple, blueberry, green bean, lettuce, and tomato) were used for recovery and reproducibility studies. All were purchased from the University of Maine Farmer's Market in Orono, ME, USA.

Chemicals

Diazinon (88.7% pure) was a gift from Celex Corporation Plymouth, MI, USA). All solvents were ASC grade (Fisher Scientific Company, Fair Lawn, NJ, USA).

Sample preparation and extraction

Ten grams of the fresh produce was weighted into a 50 mL conical centrifuge tube followed by the addition of 20 mL of methanol. The produce was polytroned (Kinematica CH-6010 Kriens, Luzern, Switzerland) for 3 min and then centrifuged (Beckman, Sunnydale, CA, USA) for 10 min at 5000 × g. A 10 mL aliquot was removed and added to 90 mL of HPLC grade water before passing the entire 100 mL solution through an activated (5 mL methanol and 5 mL HPLC water) C_{18} Sep-Pak. The Sep-Pak was dried under light vacuum for 15 min

* To whom correspondence should be addressed

before it was eluted with one milliliter of acetonitrile. A 0.1 mL aliquot was removed and evaporated to dryness under air and the residue redissolved in 10% methanol solution for ELISA. For GC-AED analysis, a 2 μL aliquot from the 1 mL acetonitrile solution was injected.

Preparation of standard solutions

For ELISA a diazinon stock solution was prepared in methanol at a concentration of 999 μg/mL and for GC-AED a stock solution was made up in acetonitrile at a concentration of 5270 μg/mL. The ELISA stock standard was diluted to yield working standards of 30, 55, 100, 225, and 500 ppt while the GC-AED stock solution was diluted to make working standards of 26, 53, 105, 527, 1054, and 5270 ppb. All working standards were in the linear range of the corresponding technique.

ELISA systems and operating conditions

Immunoassay analysis of diazinon was performed using EnviroGard plate kits from Strategic Diagnostics Inc. (Newark, DE, USA). Three strips of 12 wells each were run simultaneously in a strip holder. First, 100 μL of negative control (10% methanol), standards and samples were added to each well followed by 100 μL of enzyme conjugate. The contents were mixed by covering the strips with tape and moving them in a circular motion, after which they were incubated for 1 h at room temperature. After incubation, the wells were washed four times under tap water and then blotted dry on a paper towel. A 100 μL substrate-chromogen mixture was added to each well. The strips were incubated for 30 min before adding 100 μL of 1M HCl to stop the reaction. The color in the wells changed from blue to yellow and the wells were read immediately at 450 nm using a Molecular Devices Emax Precision Microplate Reader (Menlo Park, CA, USA). Once the absorbances (A) of each set of calibrators (control) (A for control was 1.4) and samples (everything analysed in duplicate) were determined, they were averaged and employed to calculate the B_0 using the following equation: B_0 = (average A of calibration or sample/average A of negative control). Finally, a semi-log plot of B_0 *vs.* concentration was prepared to calculate the amount of diazinon in the samples.

GC-AED system and operating conditions

The GC-AED analyses were performed with a Hewlett-Packard (HP) Model 5890 Series II gas chromatograph interfaced with a HP 5921 atomic emission detector and a HP 7673 autosampler. A capillary column HP-5 (crosslinked 5% PhMe silicon) 25 m × 0.32 mm × 0.17 μm film thickness was employed for the separation. Data were processed with a HP AED Chemstation 5890. GC-AED conditions were as follows: carrier gas, He, flow-rate, 1.0 mL/min; make-up gas, He, flow-rate, 75 mL/min; reagent gas, O_2, H_2; helium supply pressure, 30 psi; cavity pressure, 1.5 psi; column head pressure, 20 psi; injection temperature, 100 °C; injection volume, 2 μL; injection mode, splitless; oven program, 100(0.5)[30](5)250(4) (the column temperature was started at 100 °C and was programmed to hold for 0.5 min, then increased at a rate of 30 °C per min until it reached 250 °C and was held at this value for 4 min); detector temperature, 250 °C; attenuation, 0; peak width, 0.04; threshold, –1.

Recovery studies

Recovery studies were carried out by spiking organic produce with a known amount of diazinon. The fortification amounts were 5.3, 53, 105, 527, 1054 ppb diazinon. Four spikes of each level were allowed to set for 1 h before extracting. These samples were then extracted and analysed by ELISA.

Reproducibility studies

Samples from five different spiking levels, the same as in recovery studies, were extracted and analysed once a day for six different days.

Correlation studies

To verify the ELISA, 278 fruit and vegetable samples were taken from local supermarkets. To confirm and ascertain the accuracy of ELISA the results were compared to those obtained by a classical GC-AED technique.

Results and Discussion

Linearity study

The linear range for the diazinon ELISA kit was from 30 to 500 ppt (Fig. 1) with a correlation coefficient of 0.9975 and an IC_{50} (diazinon concentration at $B_0 = 50\%$) value of 150 ppt.

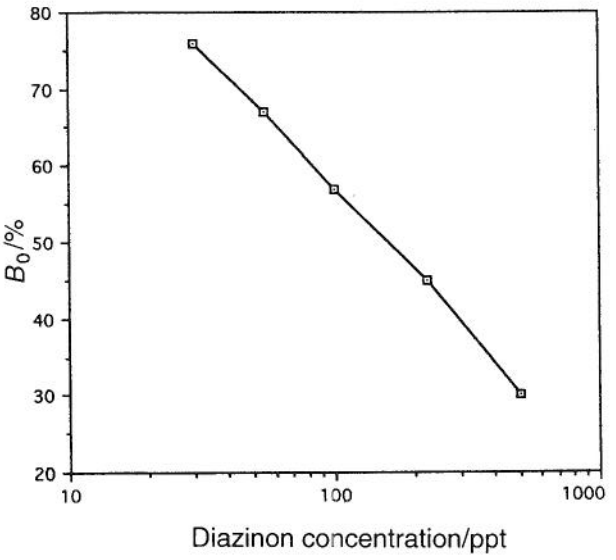

Fig. 1. ELISA standard curve for diazinon

Detection limit

Analytical results are always approximations of the actual concentration in a sample and the errors of these results increase close to or at the detection limits. A detection limit is broadly defined as the smallest solute mass which yields a discernible signal upon completion of analysis that is statistically significant at a defined

level *(6)*. The detection limit for diazinon in produce was ascertained to be 0.5 ppb.

Cross reactivity

The EnviroGard diazinon antibody is very specific for diazinon and is essentially non-reactive with the exception of pirimiphos-ethyl (lowest detectable limit, LLD = 125 ppb), pirimiphos-methyl (LLD = 900 ppb), and diazoxon (LLD = 200 ppt).

Recovery study

In order to determine the efficacy of the extraction method and the accuracy of the assay for the produce, five fruits and vegetables were fortified with diazinon (5.3–1054 ppb). Percent recovery results (Table 1) were as follows: 84–87 (CV/%: 4.7–14) for apples, 84–90 (CV/%: 2.2–13) for blueberries, 85–95 (CV/%: 3.4–8.4) for green beans, 84–95 (CV/%: 5.4–15) for lettuce, and 80–89 (CV/%: 6.5–13) for tomato. The average percent recovery for all produce at all fortification levels was 87 (range 80–95 and CV ranging from 2.2–15%). Thus the recoveries were good.

Table 1. Percent Recovery of Diazinon by ELISA

Spiking level/ppb	Apple	Blueberry	G. beans	Lettuce	Tomato
5.3	86(14)[1]	89(2.2)	85(8.4)	86(8.5)	86(6.5)
53.0	87(9.4)	86(5.9)	89(3.4)	90(5.9)	89(8.1)
105.0	84(4.7)	84(7.5)	90(4.9)	90(5.4)	80 (12)
527.0	85(7.1)	85(13)	94(6.1)	95(8.6)	89 (13)
1054.0	87(6.5)	90(4.6)	86(6.0)	84(15)	88(9.2)

[1]Mean and (CV/%) values based on four determinations

It should be mentioned that when C_{18} Sep-Paks are being used care must be taken to make sure that they are conditioned properly and that in the final drying stage they are only allowed to dry for 15 min. Diazinon is volatile and it was observed initially when samples were dried for 30–40 min that recoveries were only 45–60%. This agrees with the work done by Schombur and Seiber *(7)* and Goedicke *(8)*.

Reproducibility study

With any analytical technique, the reproducibility of the results is very important. Five different fruits and vegetables (apple, blueberry, green bean, lettuce, and tomato) were used in these studies, with five different fortification levels for each produce. The ranges of CV/% (Table 2) obtained were as follows: 3.3–7.5 for apple; 3.7–7.9 for blueberry; 8.9–14 for green beans; 2.0–8.3 for lettuce and 3.4–8.2 for tomato. The overall reproducibility for six different days looks excellent with the average CV of 6.5% (range 2.0–14%), with the majority below 6%.

Correlation study

Correlation studies between ELISA and GC-AED methods were performed. Two hundred and seventy eight fruit and vegetable samples were analysed for diazinon by GC-AED in the sulfur mode and by ELISA. Of these 278 samples, 59 were found to contain diazinon by both techniques while the other 219 samples were shown to be negative by both methods. The equation obtained was $y = 1.02\,x - 1.97$ with a correlation coefficient of 0.987 (Fig 2). A slope of 1.02 demonstrates that there is no bias between the techniques.

Table 2. Reproducibility of Diazinon by ELISA

Spiking level/ppb	CV/%				
	Apple	Blueberry	G. beans	Lettuce	Tomato
5.3	3.3	5.4	11.0	4.6	3.7
53.0	5.5	4.8	13.0	7.0	3.8
105.0	5.0	3.7	14.0	8.3	5.2
527.0	7.5	7.9	8.9	5.0	8.2
1054.0	4.6	5.1	10.0	2.0	3.4

CV values based on six determinations performed on six different days.

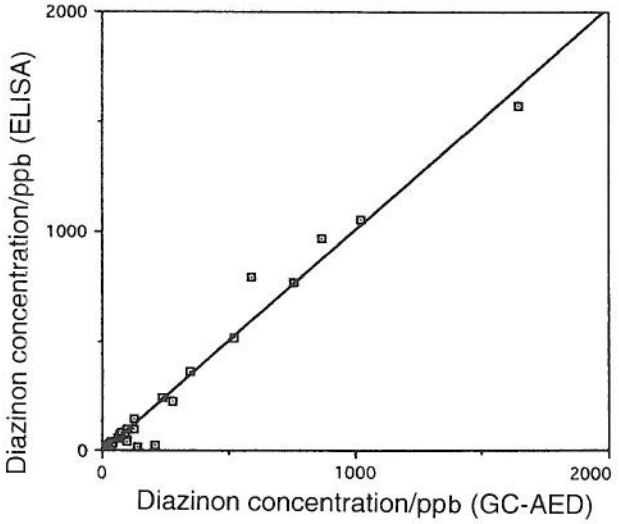

Fig. 2. Diazinon residue correlation between ELISA and GC-AED sulfur mode

Of the 59 positive samples, 98.3% or 58 samples were below the Environmental Protection Agency's (EPA) tolerances, making only one sample – mushrooms 1.7% above tolerance. Sixty-six percent of the positive samples were fruit – apples, nectarines, peach, pear, plum, and grapes, while 34 percent of the positive samples were vegetables made up of lettuce, mushrooms, spinach, and tomatoes.

Conclusion

The results of the recovery, reproducibility, and sample comparison studies indicate that ELISA is an acceptable cost-effective technique to screen diazinon in produce. Futhermore, it can be used in the laboratory or in the field with good sensitivity, accuracy, and reproducibility. It is also free from interferences since the antibody is quite specific.

Acknowledgement

This manuscript is #2502 of the Maine Agricultural Experiment Station.

References

1. Food and Agriculture Organization, *Quarterly Bulletin of Statistics, 9* (1996) 16.
2. S. Lee, P. L. Wylie, *J. Agric. Food Chem. 39* (1991) 2192.
3. C. Lentza-Rizos, *J. AOAC Int. 77* (1994) 1096.
4. P. L. Wylie, K. Uchiyama, *J. AOAC Int. 79* (1996) 571.
5. R. J. Bushway, T. S. Fan, *Food Technol. 50* (2) (1995) 108.
6. M. Dressler, *J. Chromatogr. 36* (1986) 7.
7. C. J. Schombur, J. N. Seiber, *Environ. Sci. Technol. 25* (1991) 155.
8. H. J. Goedicke, *Z. Gesamte Hyg. ihre Grenzgeb. 35* (1989) 146.

Određivanje diazinona ELISA-postupkom u voću i povrću

Sažetak

Komercijalni ELISA-postupak s poliklonskim antitijelom na mikrotitracijskim pločama primijenjen je za analizu diazinona u voću i povrću. Uzorak od 10 g ekstrahiran je homogeniziranjem u 20 mL metanola 3 minute, a zatim pročišćen propuštanjem kroz kolonu C_{18} Sep-Pak. Svi uzorci i standardi analizirani su u 10%-tnom metanolu. Ispitivanjem reproducibilnosti ustanovljen je koeficijent varijacije (CV) od 2,0 do 14%. U voću i povrću, koje je sadržavalo pet različitih koncentracija diazinona, postotak iskorištenja bio je od 80 do 95% (CV=2,0-15%). Usporedna analiza 59 uzoraka s diazinonom ELISA-postupkom i postupkom plinske kromatografije povezane s određivanjem atomske apsorpcije (GC-AED), pokazala je dobru podudarnost s koeficijentom korelacije od 0,987.