Journal of Agricultural and Food Chemistry

JUNE 1995
VOLUME 43, NUMBER 6

SHORT COMMUNICATIONS

Determination of Daminozide Residues at Very Low Levels in Fruits by Gas Chromatography/Mass Spectrometry

Keywords: *UDMH; Daminozide; 2-nitrobenzaldehyde; 2-nitrobenzaldehyde 2,2-dimethylhydrazone; GC/MS; ppm; derivatization*

INTRODUCTION

Apples and peaches are consumed in large quantities in the United States and around the world. Daminozide (also known as alar or succinic acid 2,2-dimethylhydrazide) is a plant growth regulator commonly used on these fruits to improve the size, color development, and storage features. A carcinogenic residue, unsymmetrical 1,1-dimethylhydrazine (UDMH), which is a degradation product of Daminozide, has been found in the juice and sauce from Daminozide treated apples (Newsome, 1980; Conditt et al., 1988). This finding provoked a great public outcry in 1989 against the use of Daminozide on apples. The U.S. Food and Drug Administration (FDA) approved method (*Pesticide Analytical Manual*, 1991) for the determination of Daminozide is a colorimetric method with a detection limit of 1−2 ppm. Daminozide is a thermolabile substance. Therefore, several gas chromatographic methods have been reported for Daminozide (Newsome, 1980; Conditt et al., 1988; Rutschmann and Buser, 1991); these methods first hydrolyze the Daminozide to UDMH, followed by derivatization of the UDMH and quantitation of the derivative by gas chromatography (GC). Many of the reported derivatization reactions of UDMH consist of addition of a carbonyl group to the amino group of UDMH to form the corresponding hydrazone. Newsome (1980) used pentafluorobenzoyl chloride as the reagent to produce 1,1-dimethyl-2,2-bis(pentafluorobenzoyl) hydrazine and used gas chromatography with electron capture detector (GC/ECD) for the determination of the derivative. Rutschmann and Buser (1991) used the same reagent as Newsome but used GC/MS for the determination of the derivative.

Conditt et al. (1988) used salicylaldehyde as a derivatizing reagent and used GC/MS for the determination of the derivative. The reported GC-based method has a much improved detection limit of 10 ppb. UDMH was derivatized using 2-nitrobenzaldehyde (Wright, 1987) and determined by GC/ECD. The method of Wright was simple and had a sensitivity of 10 ppb. The electron capture detector has a disadvantage of generating false positive results due to interferences from the extract, even after cleanup. There is no report on further improvement of the methods for better detection limit of Daminozide. This paper reports a GC/MS method with a detection limit of 0.4 ppb. The method has advantages over the existing methodologies because it achieved the lowest detection limit for Daminozide residues.

EXPERIMENTAL PROCEDURES

Reagents. A fresh solution of 2-nitrobenzaldehyde (Aldrich Chemical Co., Inc., Milwaukee, WI) was prepared in methanol (Optima grade from Fisher Scientific, Pittsburgh, PA) so that the final concentration was 20 mg/mL. A citric acid (Aldrich) solution with a concentration of 20 mg/mL was prepared in distilled water. A matrix spike solution was prepared by accurately weighing approximately 2.0 mg of standard grade Daminozide (Chem Service, West Chester, PA) in 100 mL of methanol. A UDMH solution (1 μg/mL) was prepared by accurately weighing approximately 1.0 mg of UDMH (Aldrich) in 100 mL of air-free methanol purged with argon gas (Matheson Gas Products) in the head space of the storage container. An aqueous solution (50%) of sodium hydroxide was prepared by dissolving 500 g of sodium hydroxide pellets (Aldrich) in 1 L of distilled water. An antioxidant solution was prepared by weighing 20 g of $TiCl_3$ (Aldrich) in argon-purged distilled water, bringing the final volume to 100 mL. 2-Nitrobenzaldehyde 2,2-dimethylhydrazone standard for GC/MS was prepared using 1 g of UDMH, 0.833 g of 2-nitrobenzaldehyde, and 20 mL of absolute alcohol in a 50 mL distillation flask in a fume hood. The flask was wrapped with aluminum foil to reduce exposure to direct light. The derivatization and cleanup procedures were then followed as described below. At the end, the clean extract was dried at 40−45 °C in a rotary evaporator. The pure product was a deep

0021-8561/95/1443-1421$09.00/0

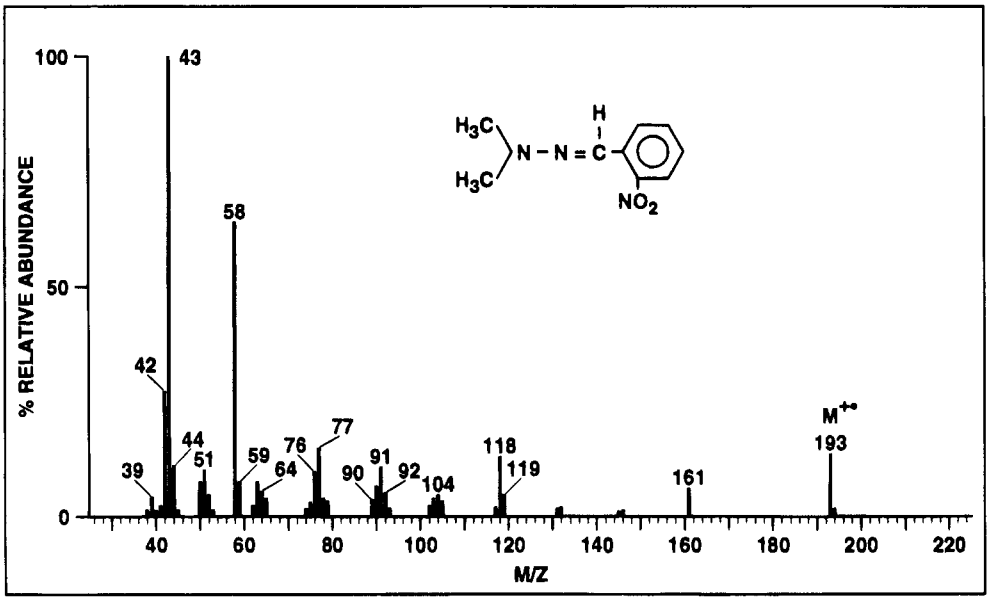


Figure 1. 70 eV EI mass spectrum of 2-nitrobenzaldehyde 2,2-dimethylhydrazone.

orange liquid. Purity of the compound was 100% as determined by GC/MS. 2-Nitrobenzaldehyde 2,2-dimethylhydrazone is light sensitive and hence was stored in an amber vial wrapped with aluminum foil. Fresh working standards in the concentration range of 10–200 ng/mL in toluene were prepared before analysis.

Extraction. Hydrolysis of Daminozide from apples was performed using a modified form of the FDA method (*Pesticide Analytical Manual*, 1991). Apple homogenate (100 g) was mixed with 100 mL of 50% NaOH solution, 37 g of NaOH pellets, 5 mL of 20% $TiCl_3$, and 4 drops of antifoam A. Matrix spikes were performed by fortifying the homogenate with Daminozide solution (200 ng) and then mixing with the reagents. Method blanks were processed by adding all of the reagents in 100 mL of distilled water (no apple homogenate or Daminozide was used in the blank).

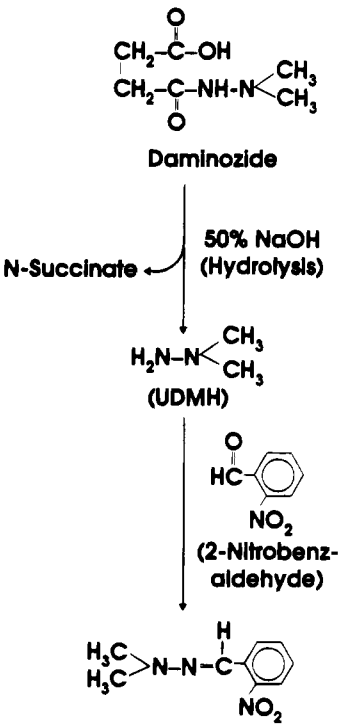
The reaction mixture was then distilled, and 100 mL of distillate was collected in 10 mL of 2% citric acid and 1 drop of phenolphthalein. Any basic pink color that appeared during distillation was cleared by dropwise addition of citric acid solution. After the distillation, the pH of the distillate was adjusted to 5 using 1 N NaOH solution.

Derivatization. To the distillate was added 20 mL of 2-nitrobenzaldehyde (20 mg/mL), and the content was refluxed at 30–35 °C for 2 h while being stirred.

Solvent Extraction. The reaction mixture was then transferred to a 250 mL separatory funnel extracted three times with 50 mL of pure toluene (Optima grade from Fisher Scientific). The volume of the extract was reduced to approximately 5 mL using a turbo evaporator with nitrogen stream at 40–45 °C and used for cleanup. Loss during derivatization followed by extraction and cleanup was monitored by reacting 100 μL of UDMH (10 μg/mL) with all other reagents. A method blank for the derivatization followed by other steps was processed by adding all of the reagents except UDMH in a separate container. The extraction and derivatization procedures are shown in Scheme 1.

Cleanup. The cleanup column (Pyrex, 2 cm i.d. and 30 mL long) was prepared by adding 25 g of basic alumina (alumina-B-Super I, ICN Biomedicals, Germany) with 1 in. of anhydrous sodium sulfate (Mallinkrodt Chemical Co. Inc., Paris, KY) at the top. The column was washed with 50 mL of toluene (Optima grade from Fisher Scientific). The extract was diluted with 20 mL of toluene and quantitatively transferred to the column. The extract was eluted with 100 mL of 50% ether in toluene. The extract was concentrated to a final volume of 2 mL at 40–45 °C using a turbo evaporator with dry nitrogen stream. The extract was stored in an amber vial to protect the analyte from direct light.

Scheme 1



GC/MS Analysis. A Fisons MD-800 GC/MS (Fisons Instruments, Denvers, MA) equipped with Lab-Base software was used for the analysis. A 30 m × 0.25 mm i.d. (J&W Scientific, Folsom, CA) DB-5 capillary column was employed. The following GC oven temperature program was used: initial temperature was 120 °C for 1 min, ramped at the rate of 15 °C/min to a final temperature of 295 °C. The final temperature was maintained for 2 min to complete the process. The ion source temperature of the mass spectrometer was maintained at 200 °C during the analysis. Ionization was performed at 70 eV, and the instrument was scanned in selected-ion monitoring mode to achieve good sensitivity and selectivity. The molecular ion m/z 193 (Figure 1) was used as the quantitation mass, and ions with m/z 77 and 58 were used for confirmation. Phenanthrene-d_{10} was used as an internal standard, and quantitation was performed using a five-point

calibration curve using 2-nitrobenzaldehyde 2,2-dimethylhydrazone as the standard in the concentration range of 10–200 ng/mL.

RESULTS AND DISCUSSION

Derivatization of Daminozide was necessary for the GC/MS method because of the high polarity and thermolabile nature of the target analyte (Newsome, 1980). It can be determined directly without derivatization using LC/MS method (Kim et al., 1990), but the detection limit is much higher [25 ppb reported by Kim et al. (1990)]. The purpose of this study was to determine the presence of not only Daminozide but also its residue UDMH. The target analyte for the GC-based methods is the resultant product of both Daminozide and its residue UDMH.

The five-point calibration standard curve used for quantitation was linear within the concentration range 0.01–0.20 ng/μL. The relative standard deviation of 2-nitrobenzaldehyde 2,2-dimethylhydrazone in the curve varied from 2 to 3.5 for different calibrations. A 70 eV electron impact mass spectrum (Figure 1) of benzaldehyde 2-dimethylhydrazine showed a molecular ion at m/z 193 with a relative abundance of 20%. This ion was found to be free from any matrix interference.

There were other fragment ions with high percent relative abundance such as m/z 43 and 58. These ions in the low mass region of the mass spectrum are not very specific. Use of high abundance ions could have increased the sensitivity of the determination with the sacrifice of specificity. Since specificity is very important in any quantitative analysis, the molecular ion m/z 193 was used for the quantitation. The instrument detection limit using the molecular ion was 0.038 ppb (calculated according to the procedure mentioned in 40CFR, Chapter 1, part 136, Appendix B). Precision of the method was 9%.

Since a low-level detection limit was the intention, crushed apples were spiked with Daminozide at a concentration of 2 ppb in the matrix. The recovery of the matrix spike was 90 ± 7% (mean ± SD; $n = 6$). The detection limit of the method calculated from these recovery data was 0.4 ppb. A maximum recovery loss of 3% was observed during derivatization followed by solvent extraction and cleanup. The maximum loss during the initial distillation procedure was 4%. Daminozide was absent in 16 of 17 batches of apples and was detected in only 1 batch at a level of 0.94 ppb, which was much lower than the EPA-approved tolerance of 20 ppm for Daminozide. However, an adjusted tolerance for children may be needed based upon their dietary intake and the potential carcinogenicity of UDMH; the current EPA tolerance may be too high (*Pesticides in the Diets of Infants and Children*, 1993). This low level was probably due to contamination of the apples with alar (Daminozide) applied to other crops by the farmer who grew the batch of apples. The absence of Daminozide in most of the batches is probably because Daminozide was not used on apples after the public outcry of 1989.

This method achieved the lowest detection limit for Daminozide reported to date. The overall procedure was simple and does not need any extra care during the distillation process because the product UDMH vapor generated during the process is stable under the experimental condition. This method has good selectivity and is applicable to other fruits and vegetables for the determination of Daminozide. This study showed that the use of Daminozide on apples has been probably discontinued in recent years.

ACKNOWLEDGMENT

The assistance of Tom McIntire, Kevin Villalobos, and Herbert Schattenberg is acknowledged.

LITERATURE CITED

Conditt, M. K.; Baumgardner, J. R.; Hellmann, L. M. Gas chromatographic/mass spectrometric determination of Daminozide in high protein food products. *J. Assoc. Off. Anal. Chem.* **1988**, *71* (4), 735–739.

Detection Limit is (Std. Dev.) × 2.821. *Code of Federal Regulations*, Chapter 1, Part 136, Appendix B.

Kim, S. I.; Sasinos, F. I.; Stephens, R. D.; Brown, M. A. Analysis for Daminozide in apple juice by anion-exchange chromatography-particle beam mass spectrometry. *J. Agric. Food Chem.* **1990**, *38*, 1223–1226.

Newsome, W. H. Determination of Daminozide residues on foods and its degradation to 1,1-dimethylhydrazine by cooking. *J. Agric. Food Chem.* **1980**, *28*, 319–321.

Pesticide Analytical Manual. U.S. Food and Drug Administration: Washington, DC, 1991; Vol. 2, pp 781–790.

Pesticides in the Diets of Infants and Children. National Research Council, National Academy Press: Washington, DC, 1993.

Rutschmann, M. A.; Buser, H-R. Determination of Daminozide and dimethylhydrazine residues in Swiss apple juice concentrates using gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **1991**, *39* (1), 176–181.

Wright, D., Jr. New method for the determination of 1,1-dimethylhydrazine residues in apples and peaches. *J. Assoc. Off. Anal. Chem.* **1987**, *70* (4), 718–720.

Received for review January 6, 1995. Revised manuscript received April 4, 1995. Accepted April 11, 1995.

Tapan K. Majumdar,* Paul W. Geno, and Alice Yau

Department of Environmental Chemistry, Southwest Research Institute, P.O. Box 28510, San Antonio, Texas 78228-0510

JF950011P

* Author to whom correspondence should be addressed.