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High Performance Liquid Chromatographic Method for the Determination of Aldicarb Sulfoxide in Watermelon

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Aldicarb sulfoxide (2-methyl-2-(methylsulfinyl) propanal O-{(methylamino)carbonyl}oxime) is a metabolite of aldicarb. Aldicarb is marketed by Union Carbide under the trade name Temik and is not registered for use in watermelons. It is a broad-spectrum soil-applied systemic pesticide used for the control of insects, mites, and nematodes. Aldicarb sulfoxide is highly water soluble and very toxic. The mode of action is reversible carbamylation of the acetylcholinesterase enzyme. The consequence is the accumulation of acetylcholine at cholinergic neuroeffector junctions, at skeletal muscle myoneural junctions and in autonomic ganglia. Generally, the symptons of poisoning are diarrhea, nausea, vomiting, abdominal pain, profuse sweating, salivation and blurred vision (Recognition and Management of Pesticide Poisonings 1982).

On July 4, 1985, a poisoning epidemic caused by eating California watermelons containing aldicarb sulfoxide was reported. The cases of illness were estimated to be as many as 250; fortunately, there were no deaths. Immediately, the California State Department of Food and Agriculture in conjunction with the California State Health Services, the U.S. Food and Drug Administration, and the County Agricultural Commissioners ordered the recall of all California watermelons distributed in 10 states and parts of Canada. In addition, the California Department of Food and Agriculture began an adhesive sticker program to reassure the public that watermelons with the attached sticker, which stated "Passed California Agriculture", were safe to eat. In essence, watermelons with the sticker had come from a field which was sampled and tested by the Departmental Chemistry Laboratories. These watermelons were found to be free of aldicarb sulfoxide, and an affidavit stating that aldicarb had not been used on the field in the past 12 months was signed by the grower.

On July 5, 1985, this laboratory was assigned to certify all the watermelon fields in Southern California and part of the San Joaquin Valley. Based on the protocol from the headquarters in Sacramento, a group of 20 melons were randomly sampled from each acre field and 5 melons constituted a composite sample for the test. If aldicarb sulfoxide was found at the detection limit (0.2 ppm) or higher, each watermelon in the group was required to be individually tested.

Traditionally, aldicarb sulfoxide is first oxidized to aldicarb sulfone with peracetic acid. Then, a gas chromatographic (GC) unit with sulfur-mode flame photometric detector (S-FPD) is used for determination of the derivative (Zweig 1974, Cochrane et al. 1982, Hill et al. 1984). However, this method is time consuming and impossible to handle with a large number of samples in a short time span. With the single goal of satisfying the certification in mind, we made a few changes in our Six N-Methylcarbamate method (Ting et al. 1984), and aldicarb sulfoxide was successfully determined by the high performance liquid chromatographic (HPLC) unit with post-column derivatization.

MATERIALS AND METHODS

Reagents

(a) Water - High purity distilled water filtered by a Millipore water filtration kit.
(b) Acetonitrile - MCB OmniSolv AX 0142 (Spectrum Chemical Mfg. Corp., Gardena, CA 90248).
(c) Methanol - MCB OmniSolv MX 0488.
(d) 2-mercaptoethanol - Reagent grade (Fisher Scientific Co., Fair Lawn, NJ 07410).
(e) Sodium hydroxide solution - 5 and 0.05 N.
(f) Sodium tetraborate solution 0.1 M -ACS grade sodium tetraborate decahydrate (Fisher Scientific Co.).
(g) OPA - O-phthalaldehyde (Fisher Scientific Co.).
(h) Aldicarb sulfoxide standard solutions -
    (1) Stock slution - 1 mg/mL in methanol.
    (2) Working solution - 1 ug/mL in methanol.

Apparatus

(a) Analytical column - Cyclohexyl (CH) 5 um, 25 cm * 4.6 mm I.D. column (Analytichem International, Harbor City, CA 90710).
(b) Food chopper - Model 8181-D (Hobart Mfg. Company, Troy, OH 45374).
(c) Homogenizer - Omni-Mixer (DuPont Co. BioMedical Division, Newton, CT 06410).
(d) Water bath - Model WBT-100, equipped with thermostat (Barnstead Still & Steriliqer Co.

Boston, MA 02132).

(e) Water filtration kit - Filter holder, Teflon-faced glass 47 mm and membrance disc filter, pore size 0.45 um (Millipore Corp., Bedford, MA 01730).

(f) Centrifuge - Model CS (International Equipment Co., Boston, MA).

HPLC Chromatographic Condition and Installation

Flow rate of the mobile phase was set at 1.5 mL/min. The isocratic elution was 10% acetonitrile and 90% water for 9 min per test. The volume of each injection was 20 uL. The details of installation of the HPLC and post-column derivatization are given in Ting et al. (1984).

Sample Preparation

A sharp knife was used to cut a quarter portion of a watermelon. Five pieces from five watermelons were chopped in a Hobart food chopper to make a composite sample. Exactly 100 g was weighed into a one-pint Mason jar; and then, 100 mL of acetonitrile was poured into the jar. The Mason jar was put on an Omni-Mixer and the contents were blended for 2 min. The liquid portion was filtered through a sharkskin filter paper in a funnel and collected in a 4-oz glass bottle. About 15 g of NaCl was added into the bottle. The bottle was corked and shaken vigorously for 1 min. Next, the bottle was placed in a centrifuge for 2 min at 1,500 rpm. After centrifuging, the acetonitrile extract was visible on the top. Exactly 10 mL of the acetonitrile extract layer was pipetted into a 50-mL beaker. The extract in the beaker was concentrated on a 100°C water bath with the aid of a gentle air stream. The final concentration to dryness was by evaporation at ambient temperature with a gentle air stream.

For the 0.1 ppm recovery study, 20 mL of acetonitrile extract layer was pipetted into a 50-mL beaker. The concentration procedure was the same as above. Exactly 5 mL of methanol was put into the dried beaker which was rotated gently for dissolving the solids. Approximately, 1.5-mL aliquot was transfered into a 2-mL vial. Then, the vial was sealed with a cap and ready to put on a HPLC's auto sampler for the determination.

RESULTS AND DISCUSSION

The chromatograms obtained for watermelon with 0.2 ppm aldicarb sulfoxide added, 20 ng of aldicarb sulfoxide standard, and watermelon control are presented in Fig. 1. It illustrated that the retention time of aldicarb sulfoxide is 6.91 min under the isocratic condition, which is 10% acetonitrile and 90% water with 1.5 mL/min flow rate. It is also clearly demonstrated that the CH-

Table 1. Recovery (%) results of 0.1, 0.2 and 0.4 ppm aldicarb sulfoxide added in the watermelon.

	Recovery, %		
Aldicarb sulfoxide added	0.1 ppm	0.2 ppm	0.4 ppm
No.1	76	73	76
No.2	71	70	72
No.3	71	75	79
No.4	65	75	76
No.5	65	75	76
No.6	81	75	72
No.7	81	75	76
No.8	74	75	76
No.9	81	75	79
No.10	81	75	79
Mean	75	74	76
S.D.	6.47	1.64	2.56
Cv	8.6%	2.2%	3.4%

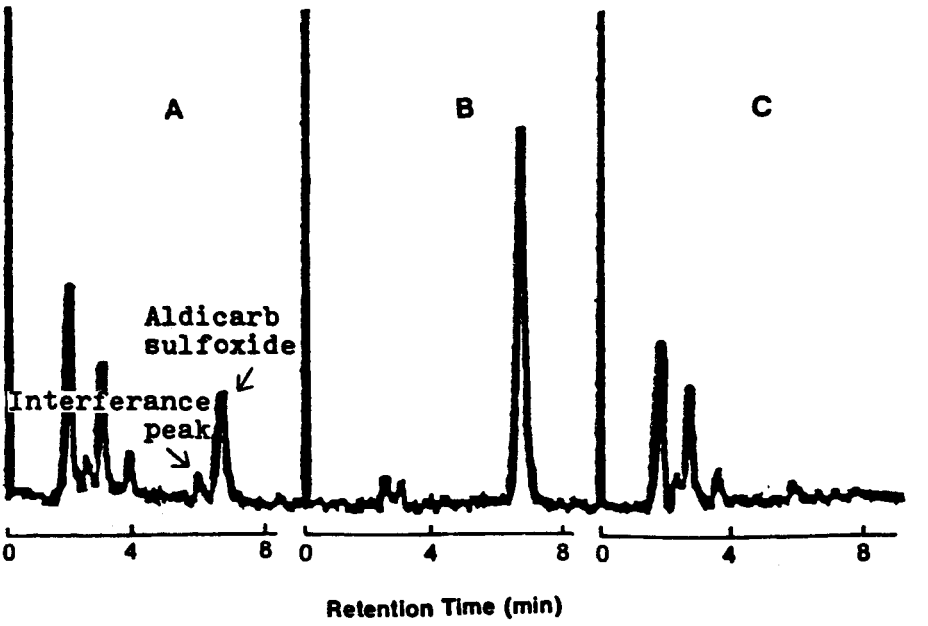

Figure 1. HPLC chromatograms. A. Control watermelon with 0.2 ppm aldicarb sulfoxide added. B. 20 ng aldicarb sulfoxide standard. C. Watermelon control.

Cyclohexyl, 25 cm, column separated the watermelon coextractives very well. In the watermelon, there is a coextractive which could interfere with the aldicarb sulfoxide; with this method, the interference peak is shown slightly ahead of aldicarb sulfoxide and has the retention time at 5.91 min (Fig. 1). If a 15 cm, C-18 column is used, this interference peak would have the same retention time as aldicarb sulfoxide and would be misidentified as aldicarb sulfoxide. This is because the 25 cm, CH-Cyclohexyl column is slightly polar and 10 cm longer than the 15 cm, C-18 column; both features help to separate the very polar aldicarb sulfoxide from the coextractive interference in the watermelons.

The recovery data of watermelon fortified at 0.1, 0.2 and 0.4 ppm aldicarb sulfoxide are presented in Table 1. We found that the mean recovery results ranged from 74 to 76%. In the case of the 0.1 ppm recovery study, 20 mL of acetonitrile extract was used in the concentration step instead of 10 mL used in the others. Therefore, the signal and noise ratio for the 0.1 ppm recovery study stayed the same as the 0.2 ppm recovery study.

Aldicarb sulfone (2 methyl-2-(methylsulfonyl)propanal O-{(methylamino)carbonyl}oxime) may be detected by this method also. It's retention time was 11.0 min. In this work, the aldicarb sulfone wasn't of interest for two reasons: 1) We didn't find aldicarb sulfone in the watermelons, and 2) Aldicarb sulfone could be easily detected by GC methods.

In the California watermelon certification program, we entirely depended on this method to detect aldicarb sulfoxide due to its all around balanced characteristics in accuracy, precision and speed. However, other methods were also established for the purpose of confirmation. In the confirmation methods, we used two instruments: 1) A Varian 3400 GC with enhanced S-FPD and a Hewlett Packard, 530 u series, 10 m, 50% phenylmethyl silicone capillary column, and 2) A Varian 3700 GC with nitrogen mode electrolytic conductivity detector and a Hewlett Packard 530 u series, 3.3 m, 50% phenylmethyl silicone column. As known, aldicarb sulfoxide was thermally labile and poor volatile compound; therefore we set the high temperature (250°C) at the injector port for degrading the compound, and then separated the unknown degradation compound in the column at the temperature of 130°C. Finally, the compound was detected by the sulfur and/or nitrogen mode selective detectors. In our experience, the GC with S-FPD had about one half of the minimum detectability of the HPLC method. It was

fair to use for confirming the positive samples, as a large sample load was applied into the GC. However, when the laboratory had as many samples as the certification program (55 samples in 20 hr per day) the GC could be contaminated quickly after heavy usage and down-time became a problem. Therefore, we couldn't rely on this unit to be a primary instrument. In the case of using a GC with nitrogen mode electrolytic conductivity detector, it is initially a sensitive tool. The problem was that its noise level increased quickly after 10 to 20 consecutive sample applications; consequently, the linearity of peak height or area decreased and precision became poor. In addition, it is interesting to note that the nitrogen-mode electrolytic conductivity detector is practically useless when certain commodities, such as broccoli, are analyzed without a clean up procedure. This is because, broccoli contains nitrogenous compounds; some of these compounds release slowly in a GC column and the conductivity cell of the detector would be flooded with these compounds for a period of time. Therefore, it is impossible to determine aldicarb sulfoxide or other chemicals in broccoli by this instrument without a clean up procedure. However, the clean up procedure is time consuming, and the recovery may be sacrificed.

Because of the possible litigation involved during the watermelon contamination investigation by the State enforcement division, unused watermelons with positive results were stored in the freezer. Also, the sample extracts from those watermelons were ordered to be stored in a refrigerator for a possible repeat test by other laboratories. The possibility of degradation in the acetonitrile extraction was of concern; therefore 10 extracts were prepared from 5 uncontaminated watermelons with 0.4 ppm aldicarb sulfoxide added. These extracts were analyzed on 0,1,2,5,6,7,8,9 days. There was no degradation of aldicarb sulfoxide found in any of them.

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