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Note

High-performance liquid chromatographic determination of carbaryl in fruit juices

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Carbaryl (Sevin) is a broad-spectrum carbamate insecticide that is till widely applied to fruit and vegetable crops because of its low acute mammalian toxicity (the oral LD_{50} to rats is 560 mg/kg) and its effectiveness against insects. However research during the last few years has shown that carbaryl has possible chronic toxic effects[1–4]. In fact the United States Department of Agriculture has recently changed the health hazard status of carbaryl to a higher risk category[5]. Because of possible chronic toxic effects and because of it wide distribution, there is a need to know if and to what extent carbaryl may be present in process foods and in particular fruit juices.

Methods available to analyze carbaryl focus on the use of high-performance liquid chromatography (HPLC) because of the sensitivity and non-thermal porperties. Numerous HPLC methods have been developed for carbaryl analysis (reviewed by Bushway[6] and Kawai *et al.*[7]) in water, soil, biological samples and raw fruit and vegetable crops, but no procedure for fruit juices was described.

This paper describes an HPLC method that is rapid, accurate and sensitive for the determination of carbaryl in several kinds of fruit juices at the low ppb* level.

EXPERIMENTAL

Solvents and pesticides

All solvents were HPLC grade. The acetonitrile and methanol were purchased from VWR (Bridgeport, CT, U.S.A.). HPLC water was prepared by passing glass-distilled water through a Barnstead water purifying system (Fisher Scientific, Pittsburg, PA, U.S.A.). Carbaryl with a purity of 99.9% was obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC, U.S.A.).

Juice samples

All juices were commercial samples obtained from local supermarkets in Bangor, ME, U.S.A.

Standard preparation

Stock solutions of carbaryl were prepared in methanol at 0.94 mg/ml. Spiking solutions were made by removing a 0.25-ml aliquot of stock solution and placing it

* Throughout the article the American billion (10^9) is meant.

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into a 50-ml volumetric flask. The solution was brought to volume with methanol and appropriate aliquots were removed to spike juice samples.

Apparatus

The HPLC system consisted of a Valco injector (Vici Instruments, Houston, TX, U.S.A.) with 20- and 50-μl loops, a Waters 510 pump (Waters Assoc., Milford, MA, U.S.A.), a Hewlett-Packard 1040A photodiode array detector/integrator system (Hewlett-Packard, Avondale, PA, U.S.A.). The column was an Ultremex C_{18}, 5-μm, 150 mm × 4.6 mm I.D. (Phenomenex, Palos Verdes, CA, U.S.A.). The mobile phase was methanol–water–acetonitrile (40:45:15) at a flow-rate of 1 ml/min. Detection was at 224 nm and 0.04 absorbance units full scale (a.u.f.s.). Temperature was ambient and pressure 2500 p.s.i.

Analytical procedure

Direct analysis. Juice samples as received and/or spiked were filtered (1 ml) through a 0.45-μm, 25-mm nylon filter (Gelman, Ann Arbor, MI, U.S.A.) and injected 20 or 50 μl directly into the HPLC system. Juices containing more than 70 ppb carbaryl can be analyzed by direct injection.

Trace enrichment. Juice samples (60 ml) as received and/or spiked were passed through a C_{18} Sep-Pak cartridge using a syringe. The Sep-Pak cartridge was activated by prewetting it with 4 ml of methanol followed by 5 ml of water. To elute the carbaryl adsorbed on the cartridge, 5 ml acetonitrile–water (25:75) were passed through first and discarded followed by 2 ml of acetonitrile–water (75:25). This fraction was injected (20–50 μl) into the HPLC system.

Recovery studies

Juice samples were spiked at levels ranging from 12.6 to 157.8 ppb and either passed through the C_{18} Sep-Pak cartridges or directly injected to test recovery.

Purity check

UV spectral scans from 190 to 350 nm were taken on all carbaryl peaks at three different locations on the peak to check purity.

RESULTS AND DISCUSSION

A typical chromatogram of a juice sample is shown in Fig. 1. It takes approximately 4 min for carbaryl to elute. Interferences to carbaryl were checked on all juices by taking UV spectral scans from 190 to 350 nm at three points on the peak by employing a photodiode array detector.

Table I lists the results of carbaryl spiked juice study. Juices containing no detectable amount of carbaryl were spiked at concentrations ranging from 12.6 to 157.8 ppb. Samples that contain between 5 and 70 ppb are best analyzed if they are concentrated by C_{18} Sep-Pak cartridges while direct injection works well on samples with more than 70 ppb. Average recoveries ranged from 90.4 to 100.6% with most above 96.6%. These excellent recoveries indicate that for the concentration step there was good binding of carbaryl to the Sep-Pak cartridge with very little if any irreversible binding and that for direct injection there was no juice matrix effect on peak area.

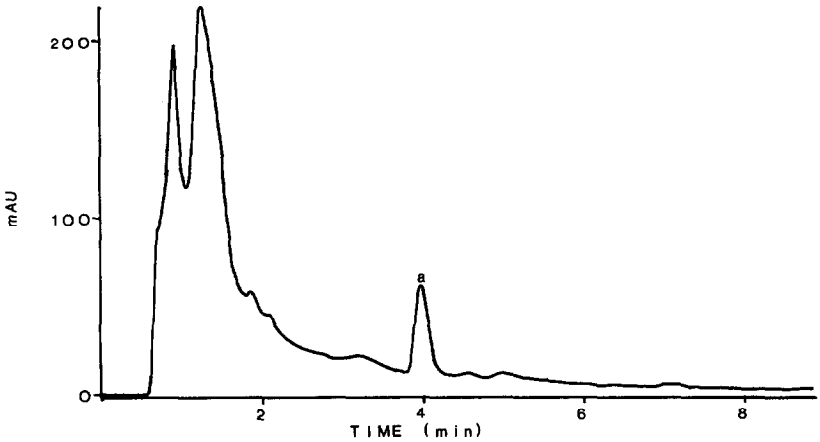

Fig. 1. Chromatogram of apple cherry juice containing 98.3 ppb carbaryl: direct injection. Solvent system, methanol–water–acetonitrile (40:45:15); flow-rate, 1 ml/min; detector sensitivity, 0.04 a.u.f.s.; 224 nm; amount injected 50 μl. Peak: a = carbaryl.

The coefficients of variation (C.V.) ranged from 13.8 to 1.7% which are good repeatable values for a residue method. Especially considering these values were obtained from samples analyzed on different days. Of course the best coefficients of variation were from direct injection which was expected since there were fewer steps.

The break-through amount for carbaryl had been determined previously for C_{18} Sep-Pak cartridges[6] and it was not repeated. However, it was demonstrated that only two 60-ml aliquots of juice could be passed through the cartridges before the recovery of carbaryl began to decrease (about 10% with the third aliquot).

The detector response for area was linear from 1 to 500 ng for carbaryl. α-Naphthol was separated from carbaryl in this method but because of the acidic conditions of the juice one would not expect to find α-naphthol present.

A survey was performed using this method to determine how much carbaryl might be present in fruit juices. Forty-seven samples were analyzed (Table II) of which twenty showed detectable levels of carbaryl (detection limit 5 ppb). Of these twenty the lowest amount found was 6.7 ppb and the highest was 175.0 ppb. Fifteen

TABLE I

CARBARYL ANALYSIS OF SPIKED JUICE SAMPLES BY HPLC

Sample	*n**	*Type of analysis*	*Level spiked (ppb)*	*Average recovery (%)*	*C.V. (%)*
1	7	Sep-Pak	12.6	90.4	13.9
2	19	Sep-Pak	25.2	100.6	10.1
3	3	Sep-Pak	98.0	96.6	1.7
4	3	Direct injection	78.9	96.7	2.3
5	3	Direct injection	157.8	98.3	6.4

* n = Number of samples analyzed.

TABLE II

ANALYSIS OF COMMERCIAL FRUIT JUICES FOR CARBARYL

Sample	*Carbaryl found (ppb)*	*Sample*	*Carbaryl found (ppb)*
Apple cherry 1	ND*	Apple pineapple 2	86.7
Apple cherry 2	ND	Mixed fruit 1	8.3
Apple cherry 3	ND	Mixed fruit 2	6.7
Apple cherry 4	ND	Mixed fruit 3	ND
Apple cherry 5	98.3	Mixed fruit 4	ND
Apple cherry 6	106.7	Mixed fruit 5	ND
Apple cherry 7	118.3	Mixed fruit 6	24.2
Apple cherry 8	ND	Orange apple banana 1	74.0
Apple cherry 9	86.7	Apple apricot 1	136.7
Apple cherry 10	50.0	Apple apricot 2	175.0
Apple cherry 11	ND	Orange 1	ND
Apple cherry 12	ND	Apple 1	ND
Apple cherry 13	115.0	Apple 2	ND
Apple cherry 14	123.0	Apple 3	8.3
Apple cherry 15	113.3	Apple 4	131.7
Apple cherry 16	ND	Apple 5	118.3
Apple cherry 17	ND	Apple 6	8.3
Apple grape 1	ND	Apple 7	ND
Apple grape 2	ND	Apple banana 1	ND
Apple grape 3	ND	Cranberry 1	ND
Apple grape 4	ND	Cranraspberry	ND
Apple grape 5	ND	Fruit blend raspberry 1	ND
Apple grape 6	ND	V-8 1	ND
Apple pineapple 1	70.0		

* ND = None detected at a detection limit of 5 ppb.

had concentrations of 50 ppb or more. The levels of carbaryl found were well below the U.S.A. tolerance of 5 ppm. However, many of these juices were baby food products which may be of some concern; also, U.S.A. tolerances for carbaryl were set several years ago. All twenty juices that had detectable levels of carbaryl had some or all apple juice present. Thus this would tend to indicate that the carbaryl may be coming mostly from treated apples.

CONCLUSION

Because of the complex matrix of fruit juice compared to water, one cannot analyze carbaryl by direct injection or C_{18} Sep-Pak cartridges at as low as a concentration as water. However, carbaryl does have a large enough extinction coefficient at 223 nm that even with a complex matrix like fruit juice one can analyze for carbaryl at rather low levels with only simple clean-up.

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