Bull. Environm. Contam. Toxicol. 26, 789-794 (1981)

A Comparison of Chromatographic and Spectrophotometric Methods for the Determination of Carbaryl Residues in Cabbages

F. J. Bezuidenhout and L. P. van Dyk

Plant Protection Research Institute, Department of Agriculture and Fisheries, Pretoria, South Africa

A number of analytical methods have been published for the determination of carbaryl (1-naphthalenyl methylcarbamate). These include inter alia gas chromatographic methods (HOLDEN 1973, TILDEN & VAN MIDDELEM 1970); high-performance liquid chromatographic methods (LAWRENCE 1977, LAWRENCE & LEDUC 1978); thin-layer chromatographic methods (SHERMA et al. 1978, ERNST et al. 1977, PALMER & BENSON 1968); and a spectrophotometric method (IWATA et al. 1979). The analyst thus is confronted with a bewildering array of methods and usually chooses a method which may seem to be suited to his/her laboratory. The relative speed, precision, accuracy and cost of these methods are usually not taken into consideration. Another important factor is the ease of handling the method. This study was undertaken to compare a number of methods for the above considerations.

EXPERIMENTAL

Sample preparation

Known amounts of analytical-grade carbaryl, in the appropriate solvents, were added to specific masses of cabbage. The added amount depended on the sensitivity of the method used and varied from 0.10 to 100 µg. This was then followed by extraction of the fortified cabbage sample with a solvent.

Methods selected for evaluation

Gas chromatography

a) HOLDEN (1973)
The residue is extracted from crop material with acetonitrile, and the extract is purified by partitioning with petroleum ether and coagulating with phosphoric acid - ammonium chloride solution. Phenolic impurities were largely eliminated by partitioning into a dichloromethane extract with 0.1 N KOH solution. Carbamate residues were then treated with 2,4-dinitro-1-fluorobenzene to form the ether derivative.

b) TILDEN & VAN MIDDELEM (1970)
The residue is extracted from the crop with dichloromethane. The solvent is evaporated and sulfuric acid hydrolysis used to

0007-4861/81/0026-0789 $01.20
© 1981 Springer-Verlag New York Inc.

convert carbaryl simultaneously to a methylamine salt and the crop extractives to water soluble products. Effective separation of the methylamine from the water soluble extractives was accomplished by making the aqueous phase strongly alkaline which also catalyzed the coupling of the free amine with the 4-bromobenzoyl chloride. The derivative 4-bromo-N-methylbenzamide was quantitated by electron-capture gas chromatography.

<u>High-performance liquid chromatography</u> (HPLC)

a) LAWRENCE & LEDUC (1978)
Carbaryl is extracted with acetone. The acetone extract is partitioned into dichloromethane-hexane and concentrated for cleanup on a 5% water-deactivated Florisil column. The fraction containing the carbamate was subjected to HPLC analysis on a 25 cm Lichrosorb Si 60 (5 µm) column by various means. First, the pesticide was analyzed directly with ultraviolet (UV) absorption detection at 254 nm and a mobile phase of trimethylpentane and 2-propanol (96 + 4). After this, a fluorescent derivative was prepared, using dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride), and analyzed by HPLC using trimethylpentane and dioxane (95 + 5) as the mobile phase, with both UV absorption (254 nm) and fluorescence detection. Deviations from the published method were that a 30 cm µ-Porasil (5 µm) column from Waters Associates was used and a fluorescence detector was not available.

b) LAWRENCE (1977)
The pesticide is extracted from the crop with acetone and then partitioned into hexane-dichloromethane. The organic extract was reduced to a small volume and passed through a 2% deactivated Florisil column. The fraction containing the pesticide was evaporated to dryness and dissolved in trimethylpentane for HPLC analysis on a 25 cm x 2.8 mm i.d. column of Lichrosorb Si 60 (5 µm) with UV detection at 254 nm. Deviation was that a 30 cm µ-Porasil (5 µm) column from Waters Associates was used.

<u>Thin-layer chromatography</u> (TLC)

a) PALMER & BENSON (1968)
The pesticide is extracted from the crop twice with dichloromethane and the extract concentrated on a steam bath. The residue was dissolved in acetone, coagulated and filtered. The precipitate was washed with an acetone - water solution and the filtrate and washings transferred to a volumetric flask. An aliquot was transferred to a separating funnel and extracted with dichloromethane. The extracts were combined and concentrated and then spotted on oven-dried Al_2O_3 TLC plates. The plates were developed with an acetone and benzene solution (1 + 4), air-dried and sprayed with alcoholic KOH followed by the chromogenic solution (saturated diethylene glycol and alcohol solution (1 + 9) with p-nitrobenzenediazonium fluoroborate). Deviations were that, except for Al_2O_3 plates silica gel 60 and nano silica gel 60 SIL 20 plates were also used. No densitometer was used for quantitation.

b) SHERMA et al. (1978)
The same extraction procedure was used as in the Palmer and Benson-method. Chromatographic plates used were Q - 5 silica gel, and Q - 3 Al_2O_3. The plates were also developed with acetone and benzene (1 + 4) and sprayed with alcoholic KOH followed by the chromogenic solution (made up in acetone and not alcohol). Deviations were that alcoholic NaOH was replaced with alcoholic KOH, plates used were silica gel 60, nano silica gel 60 SIL 20, Al_2O_3 and HPTLC silica gel 60. No densitometer was used.

c) ERNST et al. (1977)
The sample is homogenized in dichloromethane, dried and filtered. An aliquot is concentrated and applied to a silica gel G - TLC plate. The chromatogram is developed with acetone - hexane (1:4) and dried. The pesticide is oxidised with bromine vapour, sprayed with enzyme solution and placed in a moist atmosphere at 37°C for 30 min. Then the plate is sprayed with a substrate solution and again placed in a moist atmosphere at 37°C for 15 min. Deviations were that the plates used were HPTLC silica gel plates from Merck. The substrate contained 1-naphthyl acetate instead of 2-naphthyl acetate and the enzyme used was not an extract from bee-heads but a commercial product, namely acetylcholin - esterase from bovine erythrocytes made up in tris buffer (pH 8.5) at 5 mg/20mL. The chromatograms were developed with hexane - chloroform (1:1). No densitometer was used.

Spectrophotometric

IWATA et al. (1979)
The sample is macerated in acetone and filtered through a Büchner funnel. The acetone is removed and a coagulating solution added. The mixture is filtered again, sodium chloride solution added and the mixture extracted with chloroform. The extract is concentrated, hydrolyzed with alcoholic KOH and the resulting 1-naphthol reacted with a colour reagent for colorimetric analysis. Deviations were that filtration was done through filter paper and not through a Büchner funnel, dichloromethane was used for extraction instead of chloroform. The extract was evaporated to just dryness. Sodium chloride addition was omitted.

RESULTS AND DISCUSSION

The accuracy of each method was determined by comparing the concentration found with the concentration added. The precision of each method was determined by comparing the results of three analyses with the particular method.

The results are presented in Tables 1 and 2.

The relative merits or disadvantages of each method are:

(a) Holden
With the Holden method there is a lot of separatory funnel work and the final washing of the trimethylpentane layer is difficult

because of the small volume used. It is a very long and expensive method and not suited for fast routine analysis. The GC response to the derivative is not very good and the method is thus not very sensitive.

TABLE I. Comparison of speed of methods, detection limits, accuracy and precision

Method	Extraction time per sample in minutes	Analysis (a) time per sample in minutes	Limit of detection mg/kg	Accuracy %	Precision %
1) GC					
a) Holden	240	20	0.1	95	± 3
b) Tilden & Van Middelem	210	30	0.004	84	± 2
2) HPLC					
a) Lawrence & Leduc					
i) direct	120	20	0.2	91	±11
ii) fluorescent	105	20	0.04	80	± 5
b) Lawrence	105	20	0.4	112	± 3
3) TLC[(b)]					
a) Palmer & Benson	90	45	1.0	100	±10
b) Sherma et al.	90	45	4.0	100	±10
c) Ernst et al.	30	120	0.2	100	±10
4) Spectrophotometry					
Iwata et al.	90	30	0.03	98	± 2

(a) The times reported are misleading because it may take almost the same time to extract a number of samples. In the case of TLC one can spot a number of samples on the same plate and the analysis times reported were taken as per plate.

(b) The TLC detection limits have been determined visually and can be improved by use of a densitometer.

TABLE 2. Comparison of cost of analysis in S.A. Rand (R = U.S.A. $ 1-34)

	Extraction and (a) Analysis	Reagents	Cost of Instruments
1) GC			9 200
a) Holden	15.15	8.20	"
b) Tilden & Van Middelem	14.00	4.10	"
2) HPLC			8 200
a) Lawrence & Leduc			
i) direct	8.15	7.52	"
ii) fluorescent	15.43	7.82	"
b) Lawrence	7.28	11.00	"
3) TLC			30 000(b)
a) Palmer & Benson	7.87	9.25	"
b) Sherma et al.	7.87	9.25	"
c) Ernst et al.	8.75	6.28	"
4) Spectrophotometric			4 800
Iwata et al.	7.00	2.40	"

(a) R 3.5 per man hour was assumed.
(b) TLC instrumentation includes a densitometer/scanner, spotter and developing tanks.

(b) Tilden & Van Middelem
With this method it was found that the coating of all plant material with the H_2SO_4 is difficult. Although it is also a long method, it is not as expensive as the Holden method and it is a very sensitive method. The Carbowax column tends to deteriorate after a while so it has to be replaced often. Natural amines also forms the same derivative. The long analysis time is necessary due to late eluting peaks. This can be improved by use of a precolumn and backflush system (KAISER 1979) not described in the original method.

(c) Lawrence & Leduc
It was found that cleanup on a Florisil column affected reproducibility and the derivatization in a test tube is done with very small volumes. With direct analysis the extraction time is relatively long and the method is not sensitive. With fluorescence the sample has to go through both methods to get to

the fluorescent derivative. It takes time and is a relatively expensive, although sensitive method.

(d) Lawrence
The method is not sensitive enough, although the extraction is relatively simple.

(e) Palmer & Benson
The extraction procedure is relatively short and easy, but the chromatographic development takes some time. The method is not sensitive enough for residue work.

(f) Sherma et al.
The same as for Palmer & Benson.

(g) Ernst et al.
This method has a short extraction time but long development time on the chromatographic plate. Although it is more sensitive than the previous two methods it is still not sensitive enough for most residue analysis. For colour development the parameters like pH and temperature must be precisely controlled and the use of bromine in routine analysis may be hazardous.

(h) Iwata et al.
It is a relatively short, inexpensive method with high sensitivity. This is originally a Union Carbide method.

CONCLUSION

Of the various methods tested, the spectrophotometric method of IWATA et al. (1979) seems to be most suitable for routine analysis of carbaryl residues in cabbages. It should also be applicable to the analysis of carbaryl in other matrices.

REFERENCES

ERNST, G.F. and C. PIETERSE: J. Chromatogr. 133, 245 (1977).
HOLDEN, E.R.: J. Assoc. Off. Anal. Chem. 56, 713 (1973).
IWATA, Y., M.E. DüSCH, G.E. CARMAN, F.A. GUNTHER: J. Agric. Food Chem. 27, 1141 (1979).
KAISER, R.E.: J. High Res. Chromatogr. and Chromatogr. Comm. 2, 95 (1979).
LAWRENCE, J.F.: J. Agric. Food Chem. 25, 211 (1977).
LAWRENCE, J.F. and R. LEDUC: J. Assoc. Off. Anal. Chem. 61, 872 (1978).
PALMER, N.F. and W.R. BENSON: J. Assoc. Off. Anal. Chem. 61, 679 (1968).
SHERMA, J., A.J. KOVALCHICK, R. MACK: J. Assoc. Off. Anal. Chem. 61, 616 (1978).
TILDEN, R.L. and C.H. VAN MIDDELEM: J. Agric. Food Chem. 18, 154 (1970).

Accepted April 11, 1981