

Method for the Residue Determination of Carbamate Pesticides in Crops

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Carbamate pesticides are important in the field of insect control. The introduction of carbamates has brought a need for the sensitive analytical method of carbamates in agricultural products.

Until recently, the method for the determination of carbamates by colorimetry, combination of thin-layer chromatography and ultraviolet spectrometry, oscillographic polarography and cholinesterase inhibition technique had been studied. Particularly, microquantities of carbamates had been analyzed by the colorimetric method which were depended on hydrolysis with alkali to the phenol, followed by coupling reaction with p-nitrobenzenediazonium fluoroborate⁸⁾.

However, this method was not necessarily satisfactory for residue analysis because there were many problems in respect of stability of reagent, elimination of the impediments which were derived from the vegetable phenols and the coloring matters, and low sensitivity.

The direct application of gas chromatography is generally unsatisfactory because no-column decomposition and the weakness of detector response to carbamates. Therefore, many workers have reported that these difficulties could be overcome by converting carbamates to stable derivatives giving good response to electron-capture or other specific detectors. Carbamates are hydrolyzed to the phenols and the amines in either acidic or basic conditions.

The bromination⁹⁾, monochloroacetylation¹⁾, trichloroacetylation³⁾, or dinitrophenylation⁴⁾

of their phenols became sensitive and suitable derivative for electron-capture detector. Furthermore, there is the investigation which the phosphorylation product of the phenol with dimethyl chlorothiophosphate was sensitive to flame photometric detector²⁾.

A different approach to similar derivatization was described which used 1-fluoro 2,4-dinitrobenzene to form dinitroaniline derivatives with the amines liberated by alkaline hydrolysis of carbamates and determined by electron-capture detector^{5),9)}.

Lau and Marximiller (1970) reported the residue analysis of Landrin (isomer of 2,3,5- and 3,4,5-trimethylphenyl N-methylcarbamate) that converted it to the N-trifluoroacetyl (N-TFA) derivative of carbamic acid by trifluoroacetic anhydride without hydrolysis step and detected by electron-capture gas chromatography⁷⁾.

As a result of the application of this method to the eight carbamate pesticides, we had the good fortune that this reaction was quantitative, reproducible and the high sensitivity of the N-TFA derivatives so we introduced this method for the residue analysis¹⁰⁾.

Analytical procedure of the carbamates from crops were the extraction step with dichloromethane, partition step between acetonitrile and n-hexane, cleanup techniques of Florisil and alumina column chromatography, and trifluoroacetylation.

In Japan, residue tolerance for carbamate pesticides in agricultural commodities is now decided only carbaryl, and the values are

Table 1. Carbamate pesticides cited

Generic names	Chemical names
BPMC	2-sec-butylphenyl N-methylcarbamate
Carbaryl	1-naphthyl N-methylcarbamate
CPMC	2-chlorophenyl N-methylcarbamate
H-22	3-tert-butylphenyl N-methylcarbamate
MPMC	3,4-dimethylphenyl N-methylcarbamate
MTMC	3-methylphenyl N-methylcarbamate
PHC	2-isopropoxyphenyl N-methylcarbamate
XMC	3,5-dimethylphenyl N-methylcarbamate

1.0 ppm on rice, apples, oranges, grapes, Japanese tea, called Kaki, and spinach and 0.1 ppm on potatoes.

General names and chemical names quoted in this article are given in Table 1.

Residue analysis of carbamate pesticides in unpolished rice grain and rice straw

1) Extraction

Ten grams of the unpolished rice powder and five grams of the straw cut in short length were extracted with dichloromethane using the Soxhlet apparatus. The extracts were dried up at 40°C under reduced pressure and the residues were dissolved in 20 ml of n-hexane.

2) Cleanup procedure

The extracts were partitioned between acetonitrile and n-hexane, namely, transferred to 100 ml of separatory funnels, 40 ml of acetonitrile were added and shaken vigorously, and repeated two times. Combined acetonitrile layer was transferred to 500 ml of separatory funnel, diluted by 250 ml of 4% sodium chloride solution, and extracted with 30 ml of dichloromethane two times. Then the extracts were concentrated and carefully poured onto the column packed with five grams of Florisil and eluted with dichloromethane saturated with water. Florisil (60~100 mesh, Floridine Co. U.S.) was activated at 120°C for three hours.

The elution volume of dichloromethane must be previously decided; that is to say, as the

fractions which eluted the carbamates are different respectively, it needs a confirmation test of the eluted fractions by the colorimetric method with p-nitrobenzenediazonium fluoroborate or detection of the N-TFA derivatives by electron-captured gas chromatography. For example, the eluted fractions of MTMC, BPMC and carbaryl were respectively 20~40 ml, 15~40 ml and 30~50 ml of dichloromethane saturated with water.

After Florisil column chromatography, the eluates were concentrated and dissolved in a small amount of n-hexane for alumina column chromatography. Alumina (about 300 mesh for chromatographic adsorption analysis) was deactivated by adding 5% of moisture. Afterwards alumina chromatography was prepared as follows; pack it (from bottom to top) with glass wool, anhydrous sodium sulfate, 10 grams of alumina and anhydrous sodium sulfate, and the extracts were carefully applied to the column and eluted with 10% acetone in n-hexane. The elution volume must be decided beforehand as column chromatography of Florisil. The eluate was evaporated to dryness at 40°C under reduced pressure and the residue dissolved with 0.2 ml of ethyl acetate.

3) Trifluoroacetylation

Procedure for standard:

Trifluoroacetic anhydride reacts with N-methyl carbamates as shown in Fig. 1.

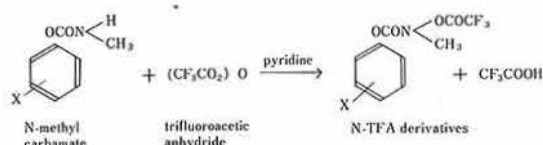


Fig. 1. Trifluoroacetylation

The standard curves for carbamates were developed as follows: 0, 0.05, 0.1, 0.15, and 0.2 ml quantities of each carbamate (10 ppm solution of ethyl acetate) were transferred to a series of 10 ml graduated and grass stoppered test tubes. 0.1 ml of pyridine and 0.2 ml of trifluoroacetic anhydride were added to each tube, the tubes were stoppered, the

contents stirred by shaking, and left at room temperature for 30 min. The derivatives were taken up in 0.3 ml of ethyl ether, and 4.5 ml of n-hexane were added to dilute the organic layer to 5.0 ml total volume. The organic layer was immediately washed with 3~5 ml of distilled water to decompose and remove excess anhydride reagent and the total volume of organic solutions were made up to 5.0 ml with n-hexane. After they were dried over, anhydrous sodium sulfate and 1~5 μ l were injected into the gas chromatograph.

4) Determination

The peak area of the carbamate derivative on gas chromatogram is shown in figure by digital integrator or calculated by measuring the peak height and peak width at $\frac{1}{2}$ height. The amount was counted by calibration curve prepared by plotting weight against peak area of the derivative. Residue amounts in samples were calculated by the following:

$$R = \frac{D \times V / I}{W \times 1000} (\text{ppm.})$$

- R : Residue amounts in samples (ppm)
 D : Detected amounts in injection volume (ng)
 V : Final concentration volume (ml)
 I : Injection volume (μ l)
 W : Picked out sample's volume (g)

Study of trifluoroacetylated reaction and conditions on gas chromatography

1) Examination of trifluoroacetyl reaction

In this reaction, addition of pyridine resulted in contraction of reaction time and

increase of sensitivity to gas chromatography; namely, when reacted with pyridine for 30 min. at room temperature, sensitivity was 4~40 fold as compared with it when pyridine was not added for 3 hours at 50°C or for 16~20 hours at room temperature. Especially, increase of this as CPMC having halogen atom was large.

To examine the influence of different solvents to trifluoroacetylation, BPMT in benzene, ethylacetate, hexane or acetone converted its N-TFA derivative with pyridine and quantified yields of derived products. In this result, only acetone was not fitted because yield of product was low and chromatogram was not clean. It made no great difference among other solvents.

As a result of trifluoroacetylation to make calibration curve, products of all carbamates got a straight line through origin and this reaction was quantitative.

N-TFA derivatives of carbamates were considerably stable, viz., N-TFA derivatives of carbaryl, MTMC and BPMT did not change in the sensitivities to gas chromatography in 20 days at room temperature in an icebox.

2) Selection of column packing

As construction of carbamate pesticides is similar, their separation and quantification are difficult. To settle these problems, we tried the separation according to the different retention times on gas chromatography. We measured the retention times of eight carbamate's derivatives on a 5 ft. column packed with Chromosorb W coated with 5% OV-17, 5% OV-25, 2% polyethyleneglycol adipate (PEGA), 2% XE-60 and 2% GF-1+6% OV-17. Tables 2 and 3 illustrate the conditions

Table 2. Conditions of gas chromatography

Conditions	A	B	C	D	E
Column	5% OV-17	5% OV-25	2% PEGA	2% XE-60	2% QF-1 6% OV-17
Column temp. (°C)	130	130	115	110	140
Detector temp. (°C)	199	196	202	199	200
Injector temp. (°C)	210	212	212	210	212
Flow rate (N ₂ , ml/min)	32	35	32	37	40

Table 3. Relative retention times of N-TFA derivatives of eight carbamates

Carbamates	GC conditions				
	A	B	C	D	E
MTMC	1.00 ^{a)}	1.00 ^{b)}	1.00 ^{c)}	1.00 ^{d)}	1.00 ^{e)}
CPMC	1.38	1.44	1.29	1.46	1.29
XMC	1.55	1.54	1.47	1.45	1.41
BPMC	1.76	1.67	1.62	1.77	1.52
MPMC	1.96	1.94	1.59	1.99	1.83
H-22	2.18	2.07	2.11	2.03	1.88
PHC	2.31	2.35	2.34	2.41	2.15
Carbaryl	9.23	10.27	5.79	—	—

Retention times: a) 1.97 min. b) 2.21 min. c) 1.90 min. d) 3.45 min. e) 2.86 min.

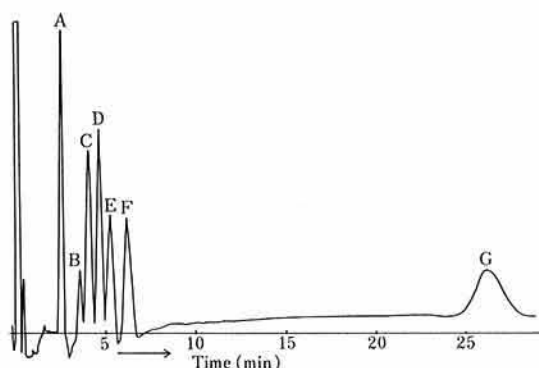


Fig. 2. Chromatogram of trifluoroacetyl derivatives of seven carbamates

A: MTMC 1 ng, B: CPMC 1 ng, C: XMC 1 ng, D: BPMC 1 ng, E: MPMC 1 ng, F: PHC 1 ng G: carbaryl 2 ng,

GC: column: 5 ft x 1/8 in. with 55% OV-17 on Chromosorb W
column temp.: 126°C, detector temp.: 197°C, injector temp.: 208°C
N₂: 32 ml/min.

of gas chromatography and relative retention times of N-TFA derivatives of eight carbamates.

Carbamates could be separated from one another using every column packing. Especially, the separation efficiency was good when the column with 5% OV-17 or 5% OV-25 as stationary phase was used. The chromatogram of seven carbamates trifluoroacetylated in a same test tube is shown in Fig. 2.

Moreover, we measured the sensitivities of peak areas of the trifluoroacetylated carbamates to select column packings of gas chromatography, and Table 4 shows relative sensitivities of peak against MTMC. The sensitivities were different according to each carbamate and the column packings. However when the column of 5% OV-17 was used, the column efficiency was very good. The peak areas' sensitivities of the trifluoroacetylated carbaryl

Table 4. Relative sensitivities of peak areas of each carbamate against MTMC

Carbamates	GC conditions				
	A	B	C	D	E
MTMC	1.00 ^{a)}	1.00 ^{b)}	1.00 ^{c)}	1.00 ^{d)}	1.00 ^{e)}
CPMC	0.26	0.11	0.08	0.10	0.12
XMC	0.73	0.97	0.72	1.17	1.06
BPMC	1.69	1.14	1.66	1.36	3.14
MPMC	1.40	0.76	0.90	0.97	0.89
H-22	1.53	0.94	1.07	1.00	0.91
PHC	1.71	0.97	1.07	1.30	1.41
Carbaryl	0.25	0.26	0.27	—	—

Peak area (cm²/ng): a) 4.00, b) 0.90, c) 1.95, d) 1.54, e) 0.53

and CPMC were lower than the other carbamates.

In case of residue analysis of each carbamate in samples, generally column of OV-17 is apt to be utilized, because the column efficiency is good; however, suitable column packing must be selected in view of separation between carbamates and impediments in samples.

In this method, recoveries were 91.2~98.8% for MTMC in unpolished rice grain and rice straw from 0.1 to 0.4 ppm.

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