# On the Extraction Methods for the Residues of Carbofuran and its two Metabolites from Fortified Soil.

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# 土壤中의 Carbofuran과 이의 두 代謝物의 殘留成分 抽出方法에 關하여

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## 要 約

現在 우리나라에서 水稻의 멸구類, 매미충類의 防除劑로 使用되고 있는 carbofuran (商品名: 후라단, 큐라텔)과 이의 두가지 중요한 代謝物인 3-keto carbofuran과 3-hydroxy carbofuran의 土壤中 殘留成分의 分析에 관해 서로 다른 3種의 抽出方法으로 比較 檢討하였다.

즉 酸에 의한 加水分解法 (FMC 法)과 水分含量에 따른 acetonitrile을 利用한 blending法 및 混合溶媒를 利用한 blending法 (methylene chloride/methanol: 5/2)으로 토양시료에 첨가하여 준 各 藥劑의 殘留分을 抽出하여 1-fluoro-2,4-dinitrobenzene으로 誘導體를 만들어 ECD를 부착한 gas chromatography法에 의해 수행하였다.

實驗結果를 要約하면 다음과 같다.

1. 各 약제성분의 誘導體는 gas chromatogram 上에서 完全히 分離되었으며, 각각의 檢出最低限界 (LDA)는 carbofuran은 $0.08\times10^{-12}$ g/sec., 3-keto carbofuran은 $0.18\times10^{-12}$g/sec, 그리고 3-hydroxy carbofuran은 $0.28\times10^{-12}$g/sec이었다.

2. 酸에 의한 加水分解法에서는 carbofuran 59.6%, 3-keto carbofuran 40.0% 그리고 3-bydroxy carbofuran 51.4%의 回收率을 보였고,

3. 土壤試料의 水分含量을 달리하여 acetonitrile을 抽出溶媒로해서 blending한 方法은 carbofuran의 回收率이 70% 以上이었으며, 특히 水分含量이 40~60%에서는 72%以上의 回收率을 나타냈다.

4. methylene chloride와 methanol의 混合溶媒로 blending한 方法은 carbofuran의 경우 53.4%, 3-keto carbofuran은 42.0% 이었으며, 특히 3-hydroxy carbofuran의 回收率은 11.4%로 아주 낮았다.

5. 混合溶媒로 抽出한 試料를 지금까지 使用하였던 3% OV-17 column 대신 5% OV-7 column을 利用하여 比較한 결과, carbofuran은 66.0%로 또 3-keto carbofulran은 63.0%로 현저히 높은 수준을 보였으나 3-hydroxy carbofuran은 오히려 8.9%로 더 낮은 水準을 보였다.

따라서 column 充塡物에 따른 藥劑別 감도에 關한 硏究가 수행어야 할 것으로 생각된다.

## Summary

A GLC procedure is described for the residual analysis of carbofuran and its two metabolites, 3-hydroxy carbofuran and 3-keto carbofuran, from fortified soil samples with three different extracting method……acid-hydrolysing, blending with acetonitrile/water and with mixed solvent system (methylene chloride/ methanol: 5/2).

All extracting samples were derivatized with 1-fluoro-2, 4-dinitrobenzene and determined with electron capture dector.

Summaries are as follows:

1. Gas chromatograms of those three chemicals showed fine resolution with high sensitivity.
2. The acid-hydrolysis method was recovered 59.6% for carbofuran, 40.0% for 3-keto carbofuran and 51.4% for 3-hydroxy carbofuran.
3. Blending method with acetonitrile in different water contents of soil samples was appeared higher recoveries(up to 70%) than the other two methods.
4. Blending method with mixed solvent system (methylene chloride/methanol: 5/2) showed the lowest recoveries among three extracting methods……53.4% for carbofuran, 42.0% for 3-keto carbofuran and 11.4% for 3-hydroxy carbofuran.
5. Comparison of column efficiency of 3% OV-17 and 5% OV-7 was indicated much different values for the recoveries of the same sample.


## Introduction

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate) is a wide spectrum insectide-nematocide using wide varieties of crops (Cox, 1966; Pass, 1966), specially controlling hoppers on rice plants. (IRRI, 1972)

This chemical is easily decomposed to several metabolites including 3-hydroxy carbofuran (2, 3-dihydroxy-2, 2-dimethyl-7-benzofuranyl methylcarbamate) and 3-keto carbofuran (2, 3-dihydro-3-keto-2, 2-dimethyl-7-benzofuranyl methylcarbamate) in natural condition. (Stanovic, 1967; 1968; Metcalf et al., 1968)

The radiotrace work of Stanovic (1967) also demonstrated that the conjugated forms of the carbamate residues could be completely released to the aglycone forms. Therefore, the suitable an-alytical methods for determination of carbofuran residues were investigated with many different ways. Apparently almost of the analytical methods of carbofuran residues follow similar technique based on the original of Cook et al. (1969) In addition, it showed the acid-hydrolyzation of sample, extraction of the aqueous phase with methylene chloride and column clean-up to remove possible interferences. Several approaches for the GLC determination of methylcarbamate through the derivatization of the liberated phenol or methylamine caused hydrolytic cleavage.

They were included use of 2, 6-dinitro-4-trifluoromethyl phenyl (Crosby and Bower, 1968), 2, 4-dinitrophenyl (Holden et al., 1969), trichloro acetyl (Butler and McDonough, 1971; Wong et al., 1975) and para-bromobenzoyl (Tilder and Van Middelem, 1970) derivatization.

Also, a few workers introduced non-derivatization methods with nitrogen-specific detectors (Cook et al., 1969; Williams et al. 1973;and Maybury, 1973), but these showed low sensitivity.

Above all things, many researches interested in the extraction methods of carbofuran residues from different types of samples, because of common solvent extractions for the carbofuran residues were not capable of removing the conjugated residues. Except the acid hydrolyzing and methylene chloride extraction, the soxhlet method with mixed solvent system (chloroform/methanol: Wheeler et al., 1973), blending and shaking method with acidic acetone (Cochrane, 1975), and shaking methed with acid ammonium acctate (Care et al., 1973).

Hence, this paper was compared with the efficiency of three different extraction methods such as FMC method (1972), blending with water/acetonitrile and mixed selvent system (methylene chloride/methanol:5/2) from fortified soil sample.

All extracts were derivatized with 1-fluoro-2, 4-dinitrobenzene and determined using ECD by gas liquid chromatography.

Experimental section

All solvents were pesticide grade.

Analytical pure chemicals of carbofuran and its two metabolites were supplied by FMC Co. (New York, U.S.A.)

Cas Chromatography: A Pye gas chromatograph, model 104 was used. A glass column, 3 ft. long and 0.25 inch. I.D., was packed with chromosorb W (80/100 mesh) coated with 3%-OV 17.

1. Derivatization of carbofuran and its two metabolites

1) Ethoxylation

Chemicals dissolved 0.5ppm level in 15ml of methylene chloride to a 100ml of round bottom flask. Add 25ml of absolute ethanol and concentrated to 10ml. And 4 drops of concentrated HCl and reflux attaching water condenser for one-half hour. cooling to room temperature, transfer solution to a 250ml of seperatory funnel and add 100 ml of cold 0.25N HCl. Extract with three 30ml of methylence chloride. Combine extracts into another clean seperatory funnel and wash the solvent portions twice with 50ml of 0.1N NaOH. Dry extracts over anhydrous sodium sulfate.

2) Derivatization

Filter extracts into a 250ml of round bottom flask and concentrated to 20ml. Add 10-15ml of buffer solution A* and 1.0ml of reactant solution B**. Evaporate the solution with Snyder column untill all solvent has been removed. Follow another contineous heating for one-half hour. Cool to room temperature and extract with 5ml portions of isooctaneon mechanical shaker. Wash each portion with distilled water to remove excess reactant. Dry extract over anhydrous sodium sulfate for GLC.

Another derivatization procedure was studied as following:

Transfer the ethoxylated solvent to a 500ml of round bottom flask and concentrate to 20ml. Add 100ml of water, 2ml of 0.5N KOH and 1.0 ml of reactant B. Shake vigorously in 20mins. with stopper. Add 10ml of 5% borax and mix with swirling, heat in steam bath for 20 mins. Cool to room temperature, add 5ml of isooctane and shake vigorouly for 3 mins. Transfer quantitatively to a 500ml seperatory funnel. Discard aqueous layer and wash isooctane portion with distilled water. Dry isooctane portion over anhydrous sodium sulfate.

A*; Buffer, PH11.0……mix 8.2ml of 0.1N NaOH and 100ml of 0.05M $Na_2HPO_4$ Adjust the volume to 200ml.

B**; React, dissolve 1gr. of 1-fluoro-2, 4-dinitrobenzeno to 100ml of acetone.

2. Experiments of extracting method with fortified soil.

The soil sample was asilt loam contaning 0.15% water. Carbofuran and its two metabolites were spiked at 0.5ppm level and mixed thoroughly for 18 hours with mixing machine.

1) Acid-hydrolysing method with 0.25N HCL

(FMC method)

Place a 20gr. of soil sample in a 500ml of round bottom flask. Add 100ml of 0.25N HCl and reflux for one hour. Filter hydrolysate through three layers of glass wool into 500ml of Ehrlenmeyer flask. Rinse the flask and glass wool with three 30ml portions of hot 0.25N HCl. Combine and cool in freezer (-15°C) for 1.5 to 2.0 hours. Transfer to a 500ml of seperatory funnel and rinse the flask with three 10ml portions of 0.25N HCl. Add 3ml of 4% aqueous lauryl sulfate and extract with three 100ml portions of methylene chloride. Dry extracts over anhydrous sodium sulfate and transfer to Kuderna-Danish concentrater. Add few drops of acetophenone and concentrat to 15ml on steam bath.

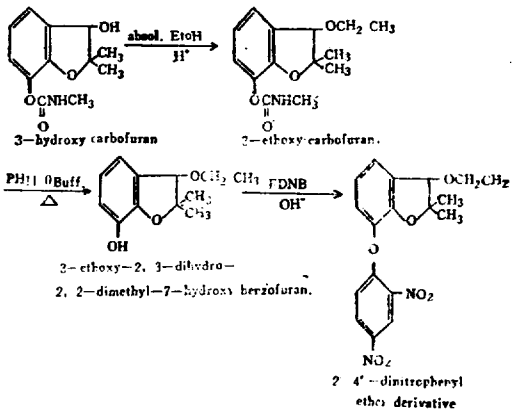
2) Blending methods with acetonitrile/water and mixed solvent (methylene chloride/methanol: 5/2)

Place 20gr. of soil sample into a 250ml Omni-blending jar. Add 100ml of extracting solvent and blend for 5mins. Rest for another 5 mins. and reblend. Filter with suction. Repeat the two same procedures with remains. Combine the filterates and transfer a 500ml of seperatory funnel, and extract with three 60ml portions of methylene chloride. Place the solvent layer into a 500ml of round bottom flask and concentrate. (but mixed solvent was suitable to concentrate before extracting with methylene chloride.)

The ethoxylation and derivatization of sample extracts by each extracting method are the same as described.

Results and Discussion

The reaction of 1-fluoro-2,4-dinitrobenzene with amines originated from Day et al. (1966), but impurities in reagents caused excessive interference and results were not reproducible. Improvement of methodology was capable of detection for the low level of methylcarbamates. Anyway, it is difficult to direct derivatization of 3-hydroxy carbofuran with 1-fluoro-2,4-dinitrobenzene, so ethoxylation is needed to increase the sensitivity of detections. Carbofuran and 3-keto carbofuran derivatize directly with the same method.

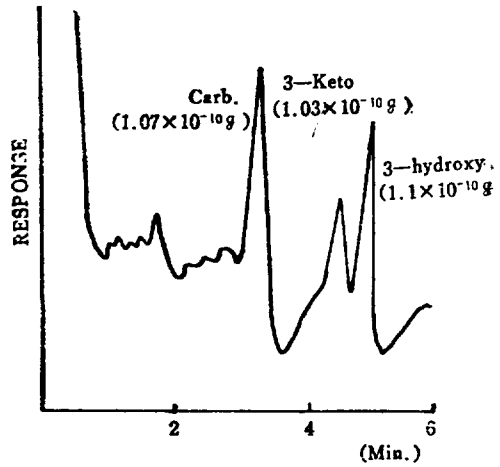



(Fig. 1) Derivatizing steps of 3-hydroxy carbofuran

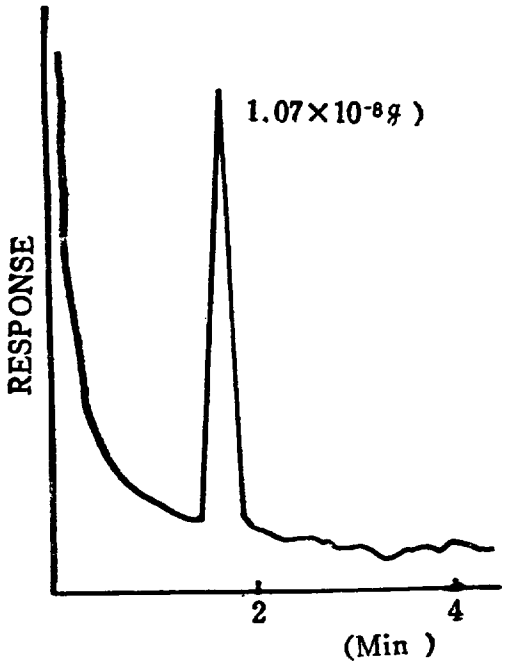
On the other hand, the derivatives of carbofuran and its two metabolites were stable under GLC conditiones employed and standards showed no decrease in peak heights when maintained at room temperature during the course of a working day, but the GLC response of derivatises were decreased after one week storage of working solution.

However, these three derivatized standard chemicals were showed good resolution on GLC chromatogram with 3% OV-17 column, and the least detectable amount (LDA) of these derivatized chemicals was $0.08\times10^{-12}$gr. in carbofuran, $0.18\times10^{-12}$gr. in 3-keto carbofuran, and $0.28\times10^{-12}$ gr. in 3-hydroxy carbofuran, respectively.

Otherwise, it was carried out the derivation with trifluoro acetic anhydride, has a merit of onestep procedure without ethoxylation. But many interfering peaks were appeared onGLC chromatogram in case of using ECD, dissimilarly the clear chromatogram with AFID in accordance with the suggestion of possiblity to that. (Greenhalgh, 1975)

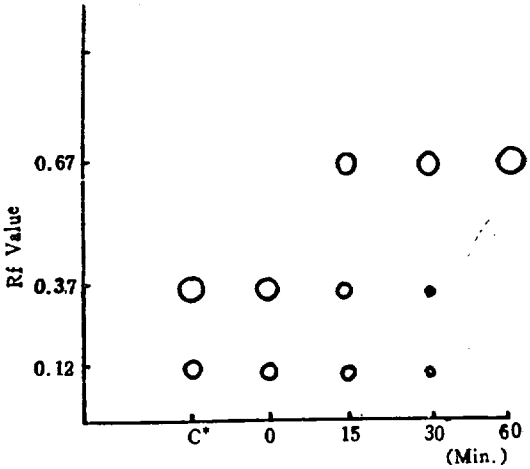



(Fig. 2) Gas chromatograms of derivatized carbofuran and its two metabolites. glass column 3ft, packed with 3% OV-17 on chromosorb W. (80/100mesh) column temp.: 250°C, detector temp.: 300°C,carrier gas (nitrogen) flow rate: 60ml/min., attenuation: $10\times10^2$




(Fig. 3) Gas chromatogram of derivatized carb-ofuran with trifluoroacetic anhydride using AFID. glass column : 3ft, packed with 3% OV-17 on chromosorb W. column temp.: 225°C,$N_2$ flow rate : 60ml/min., attenuation: $2\times10^2$

At the same time, derivatizing ratio with trifluoroacetic anhydride in different reacting time was studied by TLC, too.




(Fig. 4) TLC chromatograms of derivatized in different reacting time.
developing solvent: acetone/hexane (1/3) spot detection: Iodine vapor
C*: non derivatized carbofuran

As shown in Fig. 4) the derivatized carbofuran with trifluoro acetic anhydride started to be visible from 5 mins. after, and the derivatizing ratio was increased in course of time,finally almost of the reaction process was finished in one hour.

Also, on the occasion of 3-hydroxy carbofuran the reaction with trifluoroacetic anhydride was appeared as similar as carbofuran, although the TLC chromatogram was not enough to find the complete derivatization.

Now, it discusses to the efficiency of each extracting method from fortified soil sample.

First of all, the acid-hydrolysis method as well known to general look over.

(Table 1) Recoveries of carbofuran and its two metabolites from fortified soil samples by acid-hydrolysis extracting method. (0.5ppm spiking level)

| | sample 1 | sample 2 | averages |
|---|---|---|---|
| carbofuran | 59.8(%) | 59.4(%) | 59.6(%) |
| 3-keto carbofuran | 39.0 | 41.0 | 40.0 |
| 3-hydroxy carbofuran | 51.0 | 51.8 | 51.4 |

Even if it showed lower recoveries than the previous works (Cook et al., 1969; Lee et al., 1976), generally this result was not much differences from those former studies.

Concerning the two blending methods, it has mentioned about blending with acetonitrile in different water contents firstly.

Soil samples for the only carbofuran were added to 20%, 40%, 60% and 80% of water, each sample was determined by the method as the same being described.

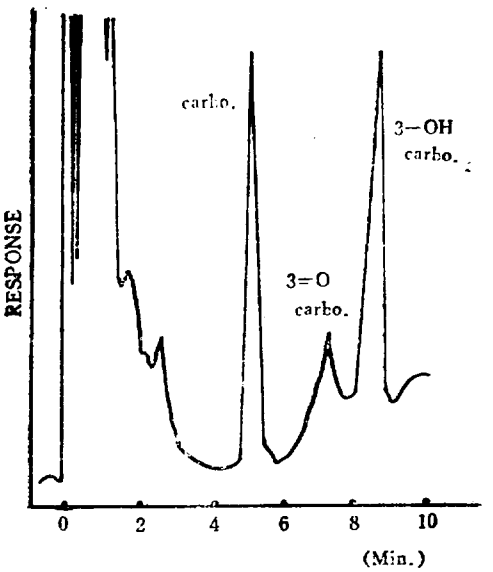
(Table 2) Recoveries of carbofuran from fortified soil samples by blending method with acetonitrile and water. (0.5ppm spiking level)

| water contents (%) | 20 | 40 | 60 | 80 |
|---|---|---|---|---|
| recovery (%) | 70.6 | 72.3 | 72.4 | 70.7 |

As shown in Table 2), the 40% and 60% of water contents appeared nearly same recoveries and they were higher than that of 20% and 80%, but all of them showed above 70% recoveries. Judging from these results, 50% of water contents would be recommended for the determination of carbofuran residues from soil.

The other method, blending with mixed solvent system (methylene chloride/metanol: 5/2), showed the lowest recoveries among those three methods, especially 3-hydroxy carbofuran was appeared very low recoveries.

The derivatizing samples of extracts in this method were compared with column effiency with 3% OV-17 and 5% OV-7.




(Fig. 5) Gas chromatograms of derivatized carbofurn and its two metabolites. glass colum: 3ft, packed with 5% OV-7 on chromosorb W.
columntemp.: 250°C, detector temp.: 300°C, $N_2$ flow rate: 60ml/min.
attenuation: $10\times10^2$
Each sample was $1.0\times10^{-9}$gr., respectively.

Waching these results, 3% OV-17 column for the carbofuran and 3-keto carbofuran. And yet the selection of suitable column materials for carbofuran residues would be examined in the future.

(Table 3) Comparison of column efficiency for the residues of carbofuran and its two metabolites from fortified soil sample by blending with mixed solvent system. (0.5ppm spiking level)

| | carbofuran | 3-keto carbofuran | 3-hydroxy carbofuran |
|---|---|---|---|
| 3% OV-17 column | 55.84(%) | 43.33(%) | 11.18(%) |
| | 51.04 | 40.06 | 10.89 |
| Ave. | 53.44 | 41.97 | 11.39 |
| 5% OV-7 column | 69.18 | 63.64 | 8.72 |
| | 62.89 | 62.27 | 9.17 |
| Ave. | 66.03 | 62.96 | 8.95 |

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