



# YOUNG LIN APPLICATION LETTER

Application Note No : YL-APP-20000004hplc

**Subject : Analysis of Carbamate pesticides using HPLC with post-column derivatization system**

**Key Words : Carbamates, Post-column derivatization, Fluorescence, Pickering**

## Introduction

Carbamates, a class of highly effective commercial insecticides, are used worldwide to protect crops from insect pests. Applied directly to food crops such as grains, fruit, and vegetables. Carbamates may seep into drinking water sources through agricultural runoff. The use of carbamate insecticides has created a requirement for a simple, reliable, and sensitive method of residue analysis for these compounds found in vegetable matter, drinking water, and industrial waste-water.

The USEPA Methods 531.1, the AOAC International protocol 29.A05, and Korean Food Code 83 describe a direct-inject method which employs gradient liquid chromatography with fluorescence detection, accomplished by post-column hydrolysis and derivatization of the eluted carbamates.

## Principle

The general structure of the carbamate insecticides is an N-methyl substituted urethane with the variation in the ester moiety. Each unique R. group represents a different commercial product or its metabolite. The separation of the carbamates is achieved with the C18 column maintained at 42 °C. The chromatographic method recommended for this column is a simple linear water/methanol binary gradient. The carbamates elute principally in relation to their relative hydrophobicity.

The separated carbamates are first saponified by sodium hydroxide (NaOH) at 100 °C to release an alcohol, carbonate, and methylamine. In the second post-column reaction, methylamine reacts with o-phthalaldehyde (OPA) and the nucleophilic Thiofluor™ (or 2-mercaptoethanol) to form a highly fluorescent 1-methyl-2-alkylthioisindole derivative (Figure 1). The derivatives are detected with fluorescence detector.

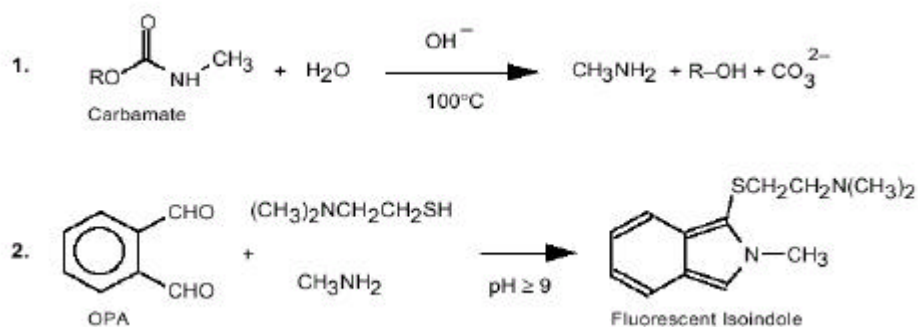


Figure 1. The principle of post-column derivatization reaction.

## Experiment

### Instrument

- Young Lin ACME HPLC (HPG system)
- Waters 474 Fluorescence detector
- Triathlon Autosampler
- Pickering Labs PCX 5200 Post-column reaction system
- Autochro 2000 data system

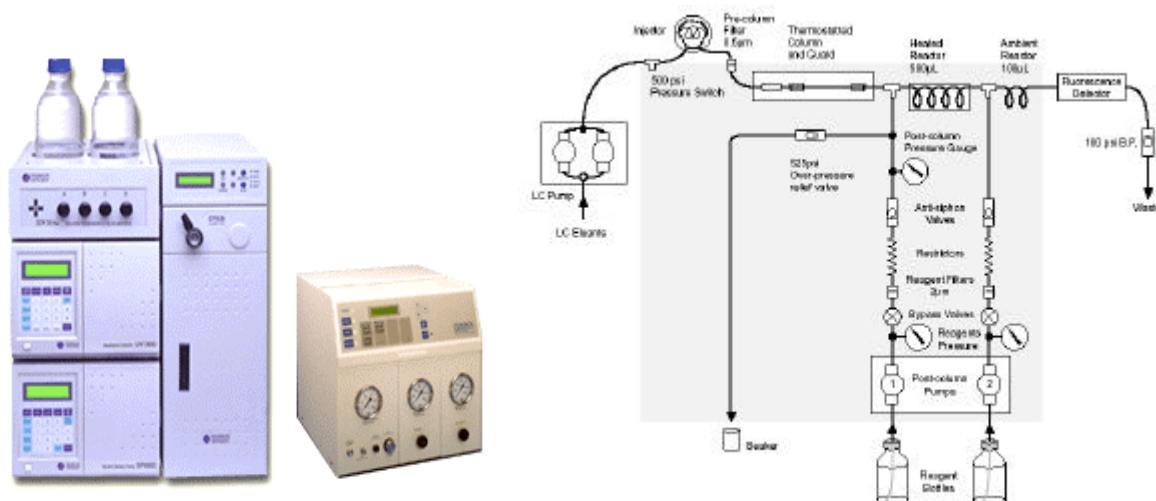


Figure 2. Configuration of the Carbamate analysis system

### Conditions

Parameters	Conditions
Column	Carbamate column (25cm × 4.6mm id × 5.0 µm)
Column temp.	42
Flow rate	1.0 mL/min
Injection volume	20 µL
Run time	33 min
Gradient conditions	A : Water    B : Methanol
	Initial            80            20
	2 min             70            30
	20 min            40            60
	25 min            30            70
	33 min            20            80
D e t e c t i o n wavelength	Exciting : 340 nm Emission : 455 nm Gain : 100
Reagent 1	100 , 0.3 mL/min
Reagent 2	Ambient, 0.3 mL/min

\* Reagent 1 : 0.05M NaOH

\* Reagent 2 : OPA  
(0.05M sodium borate buffer)

## Results and Discussion

Figure 3. a shows 0.1ppm standard mixture chromatogram of 15 carbamates listed in Korean Food Code 83 (Table 1) and b shows good reproducibility of gradient elution. This method can be analyze to ppb level samples. The Propoxur and Bendiocarb is coeluted in this gradient condition.

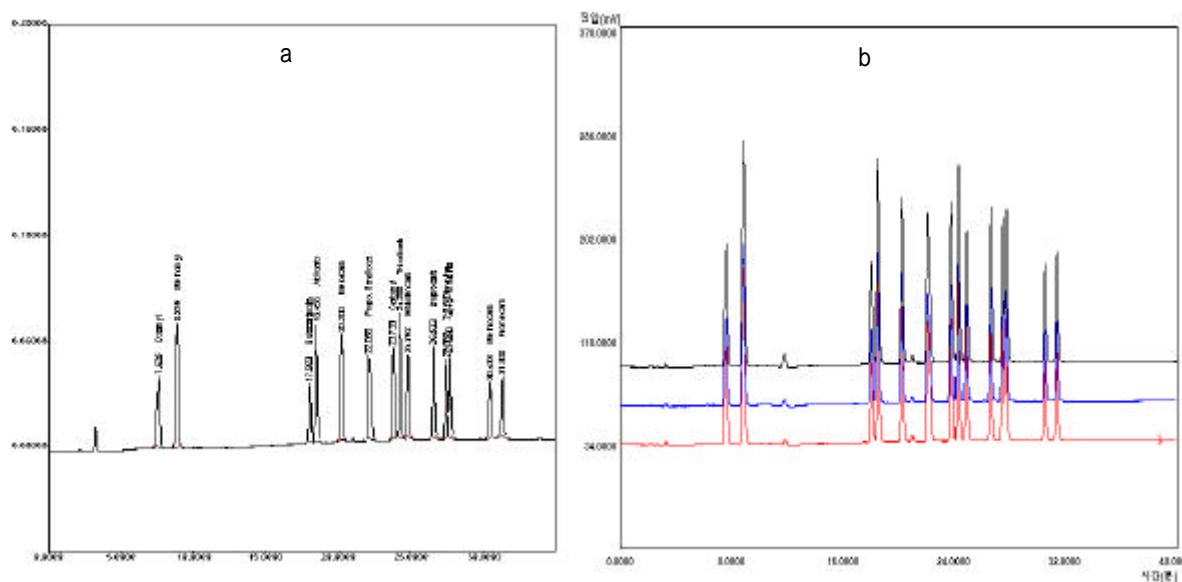


Figure 3. a) 5ppm carbamates standard chromatogram and b) Overlap chromatogram of spiked pear samples.

Table 1. Reproducibility of Gradient elution

#	Compound name	RT(n=3)	%RSD
1	Oxamyl	7.6277	0.06
2	Methomyl	8.8777	0.05
3	Butacarboxim	18.0473	0.10
4	Aldicarb	18.5193	0.11
5	Metolcarb	20.2527	0.13
6	Propoxur, Bendiocarb	22.1083	0.07
7	Carbaryl	23.7973	0.11
8	Thiodicarb	24.2610	0.09
9	Ethiofencarb	24.8447	0.09
10	Isoprocarb	26.6030	0.07
11	2,3,5- Trimethacarb	27.4610	0.07
12	3,4,5- Trimethacarb	27.7220	0.09
13	Methiocarb	30.5220	0.04
14	Promecarb	31.3837	0.05

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