

The Determination of N-Methylcarbamate Pesticides by HPLC EPA Method 531.1

Matt Hartz, Ed George, and Jerry Thoma
Environmental Health Labs
John J. Sullivan, Steve Aamodt, and Michael J. Lang
Varian Chromatography Systems

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Introduction:

As a class, the N-methylcarbamates are some of the most widely used pesticides in agriculture. Recent concerns over the appearance of carbamate residues in water and foods have prompted increased awareness and testing for these compounds. Since the carbamates are somewhat polar and thermally unstable, analysis by the more traditional gas chromatographic methods used for most pesticide residues is difficult. Consequently, the U.S. Environmental Protection Agency has established Method 531.1, (1) a high performance liquid chromatography method using fluorescence detection following post column derivatization for enhanced sensitivity and selectivity. This has become the method of choice for the analysis of carbamate residues in both food and water. The HPLC procedure is based on a reversed phase separation of the carbamates with derivatization by o-Phthalaldehyde (OPA) followed by fluorescence detection (2). The PCRS uses a two step reaction in which, first, a strong base is used to cleave off methylamine from the carbamate compound, and then the methylamine is reacted with the OPA and 2-mercaptoethanol. The resulting fluorophore has

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excitation and emission maxima of approximately 330 nm and 450 nm, respectively.

The Varian Carbamate System

Varian has combined the latest in HPLC instrumentation with the most respected name in Post Column Reaction Systems (3) to bring you a Carbamate Analysis System that is unmatched in performance and reliability. All components in the system have been tested and optimized for long-term stability and reliability in automated analysis situations. Among the features of the system:

- Fully automated PC based HPLC system, with single point control of 9012 ternary gradient pump, 9100 AutoSampler and Star Workstation.
- High Sensitivity/selectivity dual grating monochromator 9075 Fluorescence detector.
- Fully integrated PCRS with column heater and advanced safety features including a safety interlock to protect the analytical column and pressure relief valve to prevent over-pressurization of the PCRS.
- HPLC column tested and certified to provide efficient separation of the carbamate pesticides
- Tested supply of all reagents needed for the PCRS.

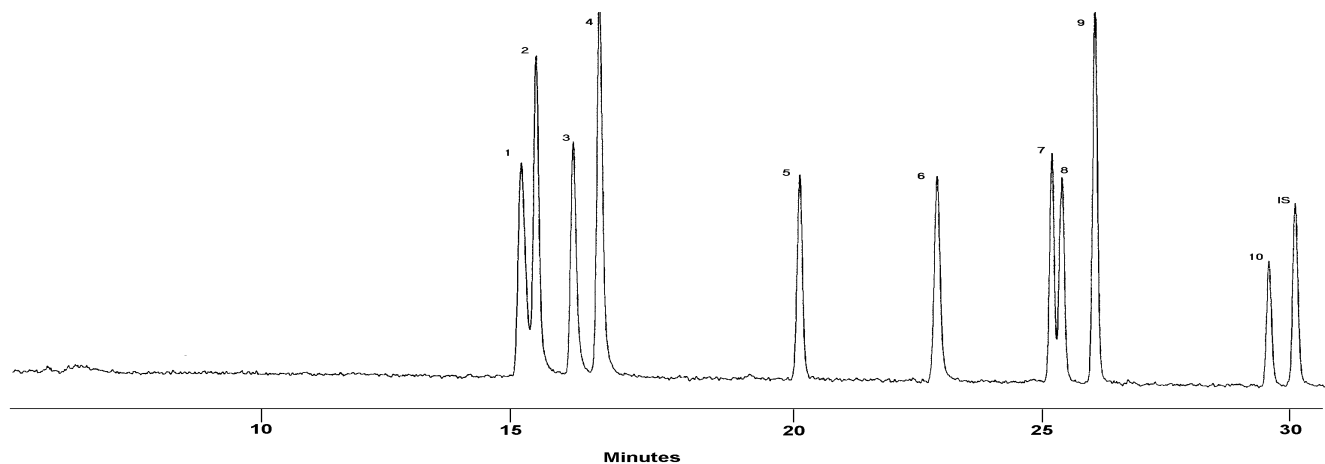


Figure 1. Separation of the Carbamate Pesticides in Water at the 10 ppb Level (400 μ L injection).

Table 1. Analytical Conditions

| | |
|-------------------|---|
| Column: | Pickering C18, 4.6 X 150 mm, 5 µm |
| Mobile Phase: | Water to Methanol Binary Gradient |
| Injection Volume: | 200-400 µL water |
| PCRS: | |
| Reagent 1: | 0.05M NaOH Solution @ 0.3 mL/min (Hydrolysis for 20 sec. @ 100°C) |
| Reagent 2: | OPA Solution in 0.05M Borate buffer @ 0.3 mL/min (reaction for 8 sec.) |
| Detector: | 9075 Fluorescence Detector Excitation at 330 nm, Emission at 466nm a slitwidth of 30 nm PMT 600V, Lamp flash 100, rise time 5, range 120, range 210 |

System Performance:

To meet the water testing requirements, the HPLC system must provide efficient separation of the common carbamate pesticide residues with detection limits at or below 1 ppb. Method 531.1 of the EPA defines the requirements for suitability of the HPLC system for this type of testing. Shown in Figure 1 is a chromatogram of the separation of the carbamates in the Varian Carbamate Analysis System. As can be seen, all of the compounds are well resolved within 30 minutes.

Figure 2 shows the calibration curve for one of the carbamates. In this case, aldicarb has a linear correlation coefficient of 0.999922.

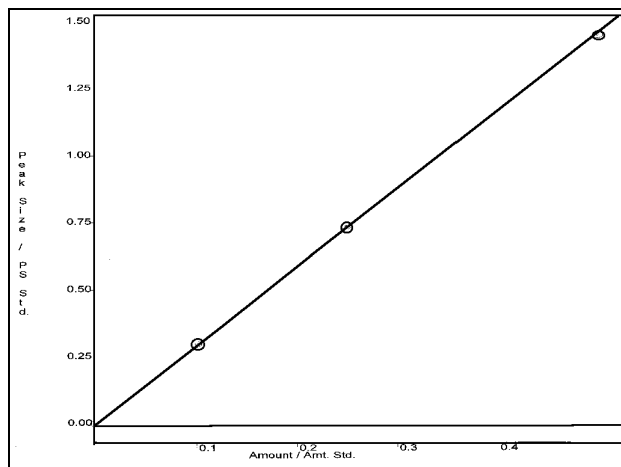
**Figure 2.** Calibration of aldicarb (100 ppb-1 ppb)

Table 2 lists the performance of the Varian system compared to the EPA Method 531.1 requirements. The data represent the periodic analysis of a spiked water sample at the 10 ppb level (400 µL injection) over 12 hours (n=16). For all of the carbamates, the system performance exceeds that of the EPA method. Superior performance coupled with the long-term stability of the Varian Carbamate Analysis System provides an excellent tool for the monitoring of carbamate pesticide residues in water.

Table 2. Comparison of the EPA and Varian Carbamate Methods

| Peak # | Analyte | EPA Method EDL (ppb) | Varian System EDL (ppb) |
|--------|--------------------|----------------------|-------------------------|
| 1 | A. Sulfoxide | 2.0 | 0.28 |
| 2 | A. Sulfone | 2.0 | 0.34 |
| 3 | Oxamyl | 2.0 | 0.28 |
| 4 | Methomyl | 0.5 | 0.20 |
| 5 | 3-OH Carbofuran | 2.0 | 0.28 |
| 6 | Aldicarb | 1.0 | 0.31 |
| 7 | Baygon | 1.0 | 0.36 |
| 8 | Carbofuran | 1.5 | 0.31 |
| 9 | Carbaryl | 2.0 | 0.16 |
| 10 | Methiocarb | 4.0 | 0.40 |

EPA Method: 531.1 (4/15/88)

EDL: Estm. Detection Limit; calculated by multiplying the SD by the student's t value (99%, n-1), or 5 times the noise, whichever is greatest.

Table 3. Analyte-Retention times (min.)

| | | |
|-----|---------------------|------|
| 1. | Aldicarb. Sulfoxide | 15.0 |
| 2. | Aldicarb. Sulfone | 15.3 |
| 3. | Oxamyl | 16.0 |
| 4. | Methomyl | 16.6 |
| 5. | 3-Hydroxycarbofuran | 20.5 |
| 6. | Aldicarb | 23.2 |
| 7. | Baygon | 25.5 |
| 8. | Carbofuran | 25.7 |
| 9. | Carbaryl | 26.4 |
| 10. | Methiocarb | 29.8 |
| 11. | BDMC (is) | 30.3 |

References:

1. EPA Method 531.1, US government (1990)
2. Johnson, Ed and Stevenson, Bob, Basic Liquid Chromatography (1978).
3. Pickering, LC-GC 6(11),994-997(1988)