

Rapid HPLC Determination of Carbofuran and Carbaryl in Tap and Environmental Waters Using On-Line SPE

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Introduction

N-methylcarbamates are widely used agricultural pesticides. Reversed-phase high-performance liquid chromatography (RP-HPLC) with fluorescence detection following postcolumn derivatization, per EPA Methods 531.2 and 8318,^{1,2} is the method typically used for the sensitive determination of carbamates. Thermo Scientific has published a detailed method^{3,4} that is consistent with the EPA methods. When an HPLC with UV absorbance detection method is used, a sample preparation procedure—either liquid-liquid extraction or off-line solid-phase extraction (SPE)—is required to increase the detection sensitivity. However, these procedures are time-consuming, require large volumes of organic solvents, and are deficient in terms of process control.

The work shown here describes an on-line SPE HPLC with UV absorbance detection method to fulfill the rapid determination of carbofuran and carbaryl (structures shown in Figure 1)—two of the most frequently used carbamate pesticides—in tap and environmental waters without the need for postcolumn derivatization. This on-line SPE HPLC system (flow schematic shown in Figure 2) runs automatically on the Thermo Scientific Dionex UltiMate 3000 x2 Dual LC system controlled by Thermo Scientific Dionex Chromeleon chromatography data system (CDS) software, and exhibits the advantages of full automation, absence of operator influence, and strict process control. A Thermo Scientific Dionex SolEx HRP cartridge is used for the enrichment, and a Thermo Scientific Acclaim RSLC 120 C18 column is used for the separation. Under the optimized chromatographic conditions, the complete analysis only requires 5 min, and the resolution between carbofuran and carbaryl was 3.5. Method reproducibility was good with retention time RSDs $\leq 0.1\%$, and peak area RSDs $\leq 1.2\%$.

Method detection limits (MDLs) of the two compounds were both $\leq 0.062 \mu\text{g/L}$, which is lower than the MDLs reported in EPA Method 8318 ($2.0 \mu\text{g/L}$ for carbofuran and $1.7 \mu\text{g/L}$ for carbaryl), and in the standard method enacted by the Chinese government ($7 \mu\text{g/L}$ for carbofuran).⁵ The MDLs were also similar to those reported in EPA Method 531.2 ($0.058 \mu\text{g/L}$ for carbofuran and $0.068 \mu\text{g/L}$ for carbaryl). The MDL for carbofuran is well under the $40 \mu\text{g/L}$ maximum allowable concentration in U.S. drinking water,⁶ and meets the general rule for pesticides in drinking water ($98/83/\text{EC}$) published by the European Union (the maximum admissible concentration of each individual pesticide component is $0.1 \mu\text{g/L}$).⁷ Therefore this method would be universally appropriate for rapid screening of water samples suspected of containing carbamates.

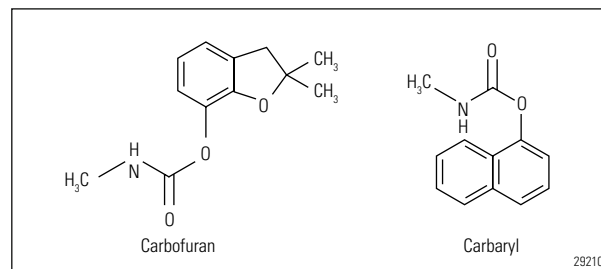


Figure 1. Structures of carbofuran and carbaryl.

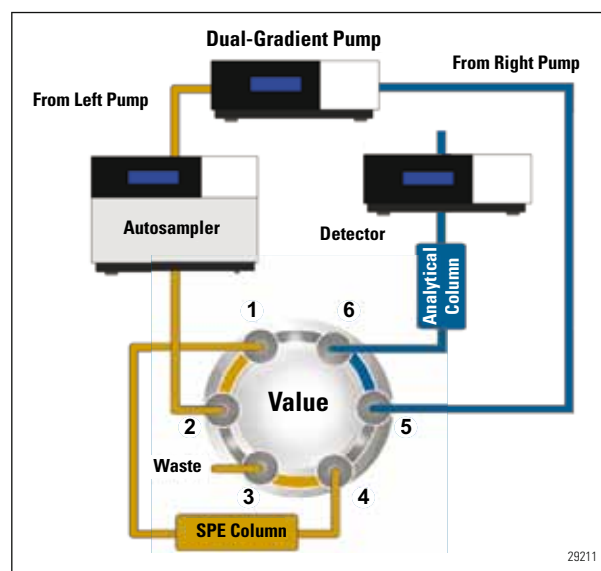


Figure 2. Flow schematic of on-line SPE.

Key Words

- Carbamates
- U.S. EPA
- Pesticides
- Drinking Water
- SolEx HRP
- RSLC

Equipment

UltiMate™ 3000 x2 Dual LC system including:

- DGP-3600A Dual-Gradient Analytical Pump with SRD-3600 Solvent Rack and Degasser
- WPS-3000TSL Thermostatted Semipreparative Autosampler with 2500 µL sample loop*
- TCC-3200 Thermostatted Column Compartment equipped with one 2p-6p valve
- DAD-3000RS Rapid Separation Diode Array Detector

Chromeleon™ 6.80 SR9 CDS software or higher

Thermo Scientific Orion 420A+ pH meter

**The analytical version of the WPS-3000TSL autosampler can also be converted to the semipreparative version by installing the Semipreparative Conversion Kit (P/N 6822.2450) for large-volume injections for on-line SPE.*

Reagents

Deionized (DI) water, 18.2 MΩ – resistivity

Methanol (CH₃OH), HPLC grade (Cat. # AC610090040), Fisher Chemical

Acetonitrile (CH₃CN), HPLC grade (Cat. #AC610010040), Fisher Chemical

Ammonium acetate (CH₃COONH₃), analytical grade, SCRC, China

Acetic acid (CH₃COOH), analytical grade, SCRC, China

Sodium thiosulfate (Na₂S₂O₃), 98% Fluka

Potassium dihydrogen citrate (KC₆H₇O₇), 98%, Fluka

Standards

Carbofuran, 98% (CAS 1563-66-2), Aldrich

Carbaryl, PESTANAL®, analytical standard (CAS 63-25-2), Fluka

Preserved Reagent Water

Dissolve 4.6 g of potassium dihydrogen citrate and 40 mg of Na₂S₂O₃ in a 50 mL beaker with reagent water, transfer the solution to a 500 mL volumetric flask, and bring to volume with reagent water. Filter the solution through a 0.45 µm filter prior to use.

Working Standard Solutions for Calibration

Accurately weigh ~25 mg of a standard and dilute in a 25 mL volumetric flask with methanol. The concentration of the standard is 1000 mg/L (stock standard solution 1). Pipet 25 µL of stock standard 1 into a 100 mL volumetric flask and dilute to the mark with methanol. The concentration of the standard is 250 µg/L (stock standard solution 2). Prepare six working standard solutions for the calibration with 0.5, 1.0, 5.0, 10, 50, and 100 µg/L concentrations by adding the proper amount of stock standard solution 2 and making dilutions with preserved reagent water.

Samples

Tap water samples were collected at the Thermo Scientific Applications Lab in Shanghai, China. Pond and river water samples were collected at Zhangjiang High-Tech Park located in the Pudong District of Shanghai. Farmland water samples were collected at Sunqiao, a small town located in the Pudong District of Shanghai. These samples were filtered through a 0.45 µm membrane (Millex®-HN) prior to injection.

Chromatographic Conditions

Solid Phase Extraction (SPE)

Cartridge:	Dionex SolEx™ HRP, 12–14 µm, 2.1 × 20 mm (P/N 074400) with V-3 Cartridge Holder (P/N 074403)
Mobile Phase:	A. Water, B. Methanol
Gradient:	30% B (0–2.0 min), 100% B (3.2–4.4 min), 30% B (4.5–5.0 min)
Flow Rate:	2.0 mL/min
Inj. Volume:	2500 µL
Temperature:	40 °C

Analytical

Column:	Acclaim™ 120 C18, 3 µm Analytical, 3 × 150 mm (P/N 063691)
Mobile Phase:	A. Acetonitrile B. 5 mM Ammonium acetate, pH 5.0 with acetic acid
Gradient:	40% A (0–2.2 min), 45% A (4.0 min), 80% A (4.1–4.7 min), 40% A (4.8–5.0 min)
Flow Rate:	0.8 mL/min
Temperature:	40 °C
Detection:	UV absorbance at 280 nm
Valve Position:	0 min, 1_2; 2.0 min, 6_1; 2.2 min, 1_2

Results and Discussion

Evaluation of Injection Volume

Typically, a larger-volume sample injection improves the detection sensitivity in on-line SPE. However, more interferences will also be bound to the SPE column, which may result in an overload of the SPE column. Therefore, after first finding that 2500 µL was an effective injection volume, a larger injection volume (10,000 µL) was investigated. Comparing a 10,000 µL injection of a tap water sample spiked with 1 µg/L of the two carbamates to a 2500 µL injection of the same sample (four injections each), the peak area ratios for carbofuran and carbaryl were 4.58 and 3.40, respectively. These ratios did not match the theoretical value of 4.0, and for carbaryl suggests that the 10 mL injection volume may be overloading the SPE column.

A comparison of the two injection volumes is shown in Figure 3. The larger injection volume could cause another compound to interfere with detection of carbofuran, as the resolution (R_s) of carbofuran decreased from 1.25 (2500 μL injection volume) to 1.08 (10,000 μL injection volume), and a R_s value of 1.25 is usually regarded as the limit for accurate quantification. This interference is likely the cause of the measured increase recovery (peak area ratio >4) for carbofuran with the 10 mL injection. Therefore, an injection volume of 2500 μL was used. Intermediate injection volumes were not investigated as the benefit to detection limits would be marginal.

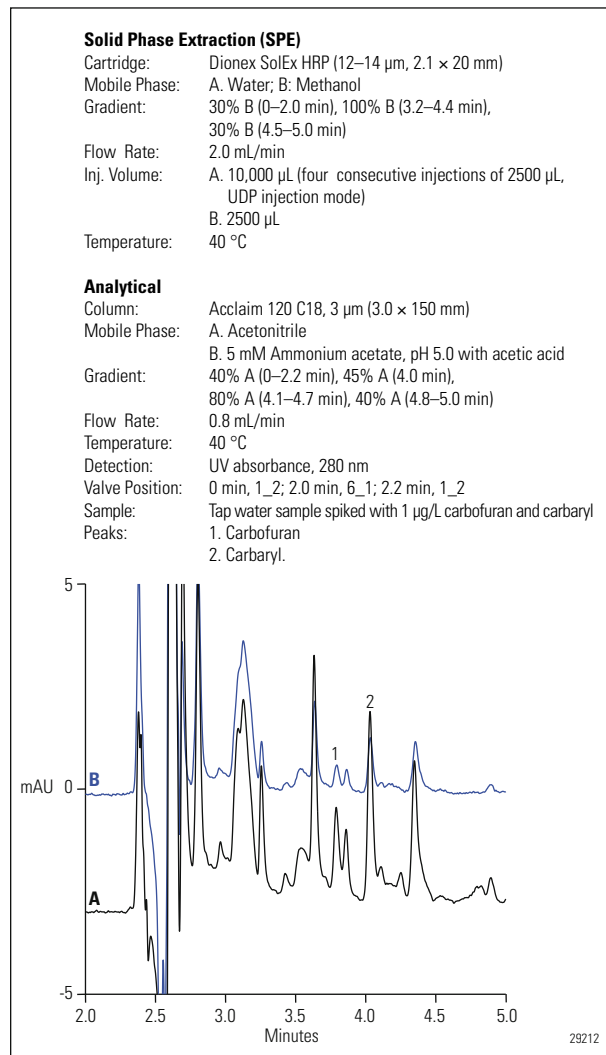


Figure 3. Chromatograms of a tap water sample spiked with 1 $\mu\text{g/L}$ each carbofuran and carbaryl standards: A) 10,000 μL ; and B) 2500 μL injection volumes.

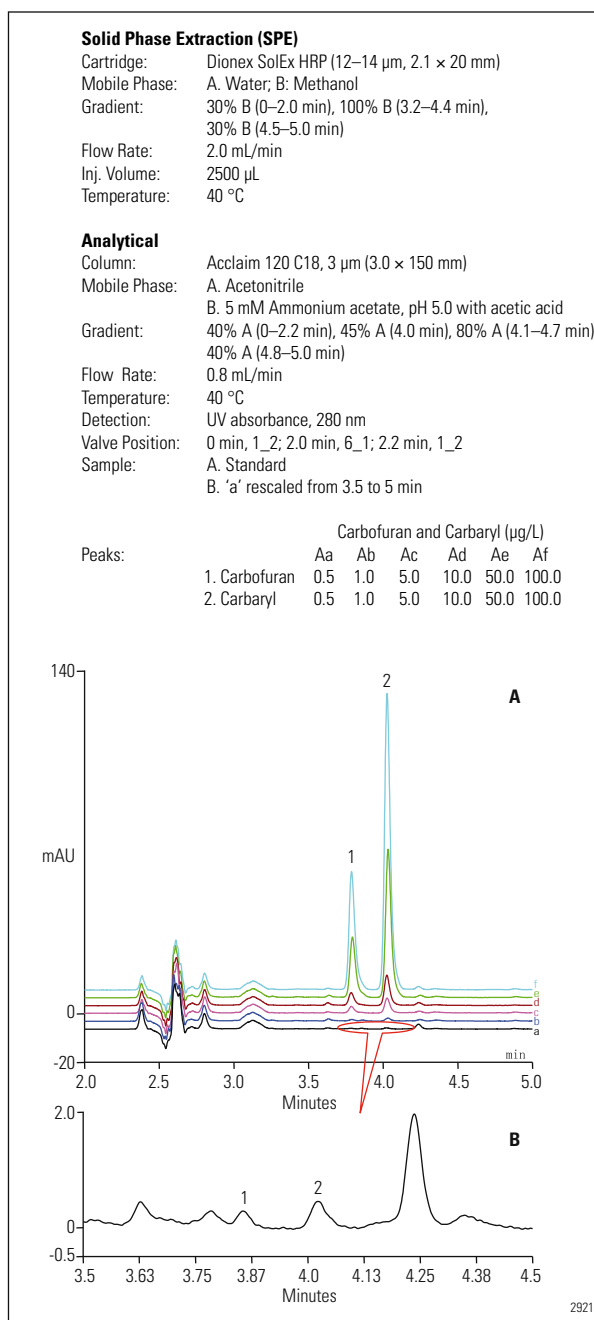


Figure 4. Chromatograms of carbofuran and carbaryl standards with different concentrations.

Reproducibility, Linearity and Detection Limits

Figure 4 illustrates good separation of carbofuran and carbaryl, with concentrations 0.5–100 $\mu\text{g/L}$ following on-line SPE under the specified chromatographic conditions. R_s between carbofuran and carbaryl is 3.5, significantly exceeding the value required by the EPA Methods (≥ 1.0).

Method precision was estimated with UV detection by making five consecutive 2500 µL injections of a calibration standard with concentration of 5 µg/L for each. The retention time and peak area reproducibilities are summarized in Table 1 and show good precision.

Table 1. Reproducibility of peak retention time and area.

Analyte	Retention Time RSD	Peak Area RSD	Standard Concentration (µg/L)
Carbofuran	0.086	1.104	5
Carbaryl	0.086	1.192	

Calibration linearity for UV detection of carbofuran and carbaryl was investigated by making three consecutive 2500 µL injections of a mixed standard prepared at six different concentrations (i.e., 18 total injections). The external standard method was used to establish the calibration curve and quantify carbofuran and carbaryl in the drinking and environmental water samples. Excellent linearity was observed from 0.5 to 100 µg/L when plotting the concentration versus the peak area, and the correlation coefficient was ≥ 0.999 for both (Table 2).

Table 2. Method Linearity Data and Method Detection Limits (MDL).

Analyte	Regression Equation	r	Range of Standards (µg/L)	MDL*, (µg/L)
Carbofuran	$A = 0.0235 c + 0.0003$	0.999981	0.5–100	0.062
Carbaryl	$A = 0.0570 c - 0.0183$	0.999951		0.036

*The single-sided Student's test method (at the 99% confidence limit) was used for determining MDL, where the standard deviation (SD) of the peak area of six injections is multiplied by 4.03 to yield the MDL.

The method detection limits of each carbamate for UV detection calculated by using the equation:

$$\text{Detection Limit} = S_{(n-1, 1-\alpha=0.99)}$$

The symbol S represents standard deviation of replicate analyses, n represents number of replicates, $t_{(n-1, 1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with n – 1 degrees of freedom. Six replicate injections of reagent water spiked with 5 µg/L of carbamate standard mixture was used to determine the minimum detection limits. Table 2 summarizes the MDL data, which show excellent method sensitivity, with detection limits equivalent to those defined in the EPA method 531.2.

Tap Water and Environmental Water Analysis

Figure 5 compares the chromatograms of tap water and environmental water samples with the same sample fortified with 5 µg/L carbofuran and carbaryl standards. A small peak with retention time near that of carbofuran was found in the tap water sample (Figure 5A). However, comparison of the UV spectra shown in Figures 6A and 6B revealed that the peak was not carbofuran. There were small peaks labeled as carbofuran in the environmental water samples (Figures 5B–D), and their UV spectra were consistent with that of carbofuran standard (Figures 6A and 6C–E), indicating the presence of carbofuran in environmental waters. The analysis results and related data were summarized in Table 3. These data show excellent spike recovery for each carbamate, thereby suggesting method accuracy, and demonstrate that this on-line SPE HPLC method provides good selectivity and suitability for the rapid analysis of carbofuran and carbaryl in tap and environmental water samples.

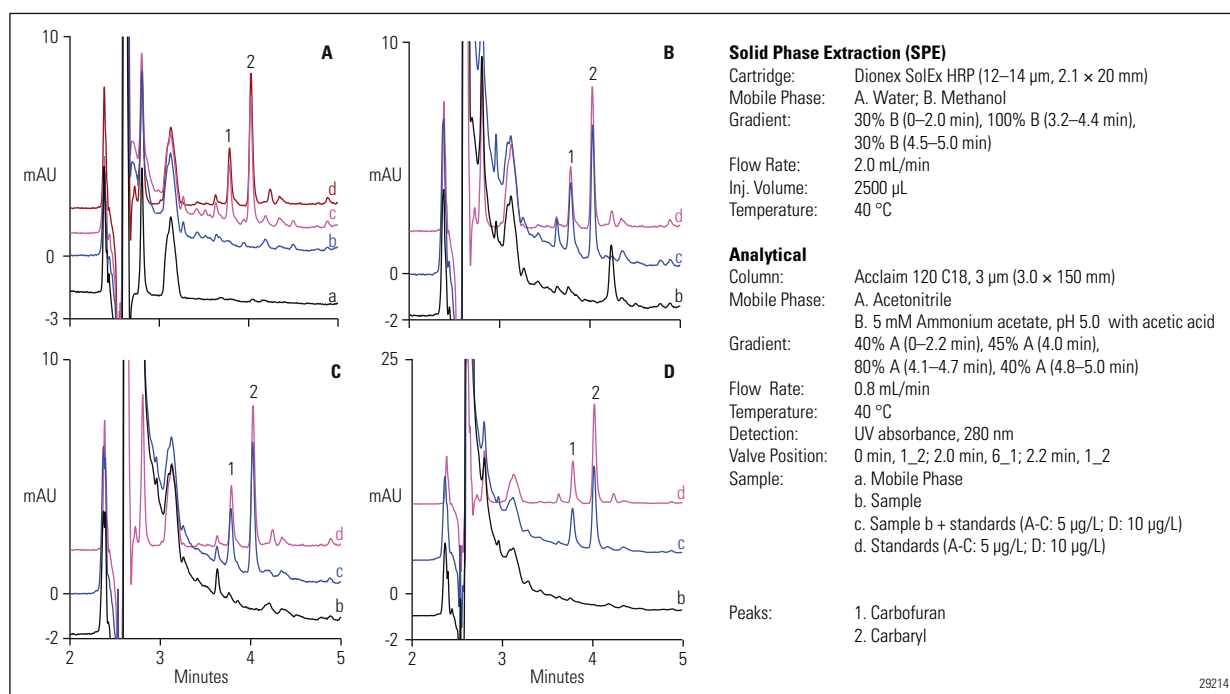


Figure 5. Chromatograms of: A) tap, B) pond, C) river, and D) farmland water samples.

Table 3. Analysis results of carbofuran and carbaryl in water samples.

Sample	Tap Water				Pond Water			
	Detected µg/L	Added µg/L	Found µg/L	Recovery %	Detected µg/L	Added µg/L	Found µg/L	Recovery %
Carbofuran	0.15*	1.0	0.89	89	0.52	1.0	1.2	120
		5.0	4.8	96		5.0	5.2	104
Carbaryl	ND**	1.0	1.1	110	ND	1.0	1.1	110
		5.0	4.7	94		5.0	4.9	98
Sample	River Water				Farmland Water			
	Detected µg/L	Added µg/L	Found µg/L	Recovery %	Detected µg/L	Added µg/L	Found µg/L	Recovery %
Carbofuran	0.35	—	—	—	0.56	1.0	0.81	81
		5.0	5.1	102		10	10.1	101
Carbaryl	ND	—	—	—	ND	1.0	1.1	110
		5.0	5.1	102		10	0.92	92

* This detected amount was for the compound with retention time near that of carbofuran, but with different UV spectrum.

** ND represents *Not Detected*.

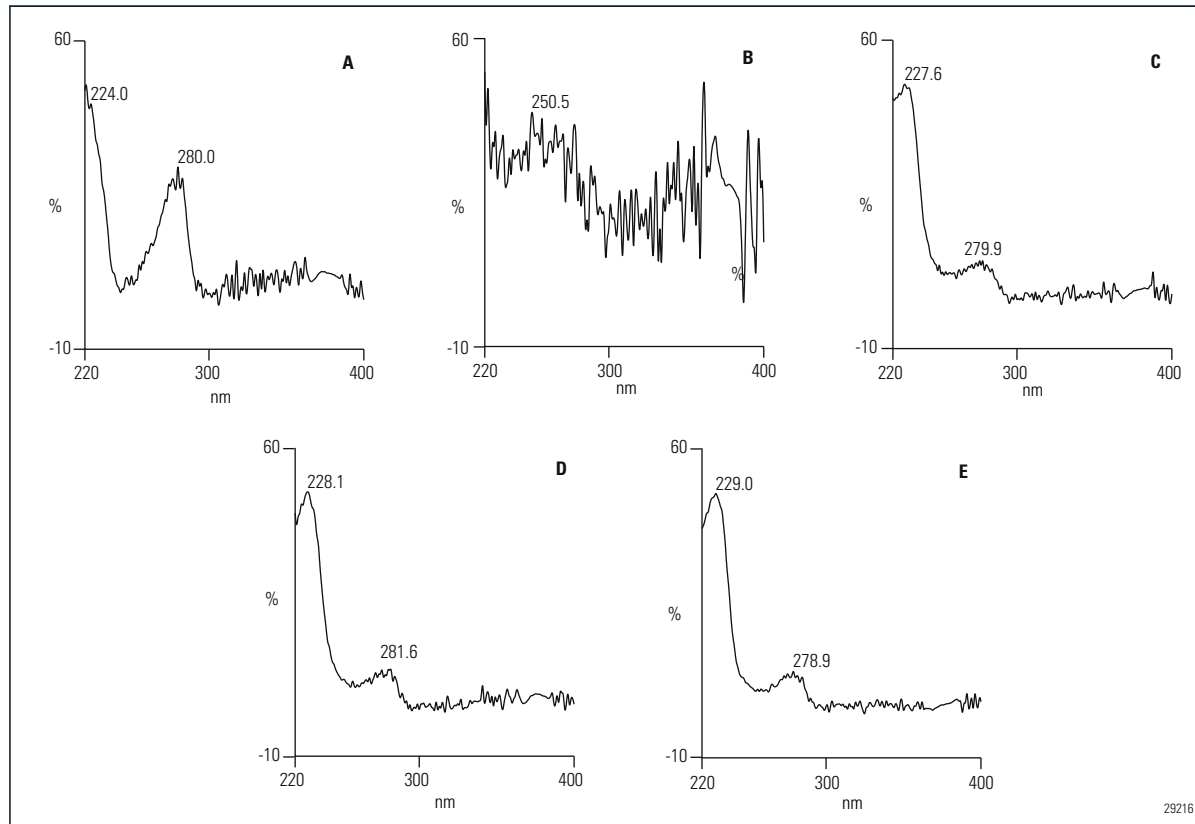


Figure 6. UV spectra of the peak with retention time near A) carbofuran (1 µg/L) in B) tap, C) pond, D) river, and E) farmland water samples.

Conclusion

This work describes an on-line SPE HPLC method with UV absorbance detection for rapidly determining carbofuran and carbaryl in tap and environmental waters. The determination was performed on the UltiMate 3000 LC x2 system controlled by Chromeleon CDS software. The reduced MDLs using UV detection afforded by the on-line SPE created a convenient method for determining these compounds in drinking and environmental waters.

References

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