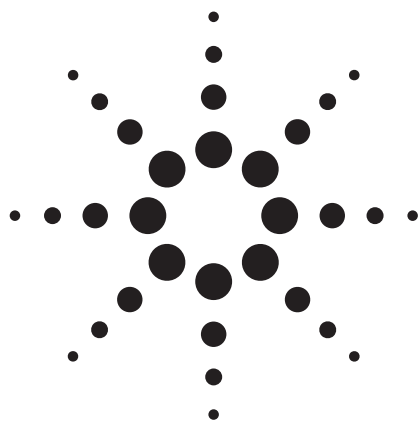




Analysis of Carbaryl and Carbofuran in Drinking Water with Post-Column Derivatization Using Agilent's New LC Column and SampliQ SPE Cartridges

Application Note

Environmental

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Abstract

The herbicides carbaryl and carbofuran were analyzed in drinking water samples using Agilent's new reversed-phase columns, Agilent TC-C18(2), and Agilent's new line of SampliQ C18 SPE cartridges. To meet the requirements of EPA Method 531.1 [1], we tried a 400- μ L direct injection onto the new column. Even with such a large injection volume, the new column provided good resolution from interfering or coeluting compounds, as well as highly symmetrical peaks and a high performance result, which gives high sensitivity. In addition, post-column derivatization and a fluorescence detector were used, making the limit of detection (LOD) less than 0.01 ng on column, a lower LOD than that specified in China's drinking water standards [2]. This method used new Agilent SampliQ C18 cartridges to concentrate the water samples and made it possible to determine carbaryl and carbofuran in drinking water at the ppt level. This total solution is very suitable for trace-level analysis of carbaryl and carbofuran in drinking water.



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Introduction

Carbaryl and carbofuran are N-methyl carbamate pesticides (structure shown in Figure 1), which are classified as broad-spectrum insecticides and are mostly used on rice and corn crops. Excess pesticide can contaminate ground water, surface water, and drinking water. Because this kind of pesticide has been shown to create health problems with blood and with the nervous and reproductive systems, actions have been taken to control these compounds and require that their amounts be measured in drinking water.

Regulatory methods are set in many countries. The China drinking water standards published in 2006 restrict the maximum limit of carbofuran in drinking water to 7 ppb and use liquid-liquid extraction to test the water samples. The U.S. Environmental Protection Agency (EPA) Method 531.1 sets the maximum contamination level (MCL) at 40 ppb and uses direct aqueous injection [1]. Each method has its own disadvantages. Liquid-liquid extraction is laborious, time-consuming work that requires a large amount of organic solvents. Direct aqueous injection is a simple method with less inter-ferential peaks; however, it is not suitable for measuring lower levels of compounds in drinking water.

Solid-phase extraction (SPE) is often used for the enrichment of water samples. This process is typically done using a standard SPE vacuum manifold but can be easily automated for the enhanced sensitivity required to measure low-level organic compounds in drinking water.

This application note describes an improved method for determining low levels of carbaryl and carbofuran in drinking water. The method applies an automated procedure of sample concentration and post-column derivatization to successfully measure carbaryl and carbofuran in drinking water at the ppt level. New Agilent SampliQ C18 SPE cartridges were used for sample enrichment from water. The sample was then analyzed with Agilent TC-C18(2) columns, which provide symmetrical peak shape and high sensitivity. Last, the separated compounds were derivatized before detection with a fluorescence detector. The derivatization reaction is shown in Figure 2. Carbaryl and carbofuran are first hydrolyzed under basic conditions at high temperature into methylamine and then react with o-phthalaldehyde (OPA) and 2-mercaptoethanol into isoindole, which has strong fluorescence. This is a simple, automated, fast, and high recovery method that is fit for quality control of drinking water.

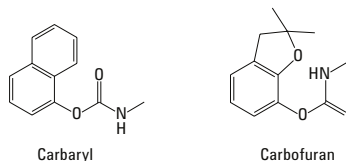


Figure 1. Structures of carbaryl and carbofuran.

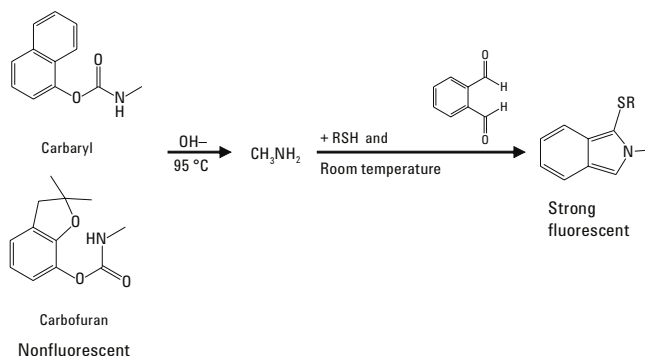


Figure 2. Derivatization reaction of carbaryl and carbofuran.

Experimental

Instruments

HPLC Conditions

Instrument	Agilent 1100 or 1200 with fluorescence detector (FLD): A multidraw upgrade kit (p/n G1313-68711) can be installed when 400-µL injections are needed.
Column	Agilent TC-C18(2) 4.6 × 150 mm, 5 µm (p/n 588935-902)
Mobile phase	A: Water, B: Methanol 0 min 42% B, 5 min 55% B, 12 min 60% B, 13 min 42% B, stop time 15 min, post-run 2 min
Flow rate	1.0 mL/min
Column temperature	30 °C
FLD detector	Ex 339 nm; Em 445 nm
Injection volume	10 µL
Pinnacle PCX derivatization instrument	Pickering Laboratories, Inc.

Post-Column Reaction Conditions

Flow rate of reagents	0.1 mL/min
Reactor temperature	95 °C
Derivatization temperature	Room temperature

Caliper LS Autotrace SPE Workstation

Caliper Life Sciences, Inc.

SPE Cartridges Agilent Technologies, Inc.

SampliQ C18, 6ml/500mg (p/n 5982-1165)

TurboVap II Evaporation System

Caliper Life Sciences, Inc.

Standards for Calibration Curves

Accurately weigh 10 mg of carbofuran and 12.2 mg of carbaryl standards and separately dissolve in methanol to a volume of 10 mL. Make the stock solution by mixing 60 µL of the carbofuran and 40 µL of the carbaryl solutions into a 10 mL volumetric flask. Dilute to volume with methanol to obtain a concentration of 4.88 µg/mL of carbaryl and 6.0 µg/mL of carbofuran. Dilute aliquots of the stock standard solution with methanol into a series of standard solutions shown in Table 1.

Table 1. Standard Solutions for Calibration Curves

Level	1	2	3	4	5
Carbaryl (ppb)	48.8	97.6	244.0	488.0	976.0
Carbofuran (ppb)	60.0	120	300.0	600.0	1200

Derivatization Reagents

Sodium hydroxide	0.05 N – Dissolve 2.0 g of sodium hydroxide (NaOH) in reagent water. Dilute to 1.0 L with reagent water. Filter and degas just before use.
Mercaptoethanol (1+1)	Mix 10.0 mL of 2-mercaptoethanol and 10.0 mL of acetonitrile. Cap. Store in hood.
Sodium borate	0.05 N – Dissolve 19.1 g of sodium borate (Na ₂ B ₄ O ₇ ·10H ₂ O) in reagent water. Dilute to 1.0 L with reagent water. The sodium borate will completely dissolve at room temperature if prepared a day before use.
OPA reaction solution	Dissolve 100 ± 10 mg of o-phthalaldehyde (melting point = 55 to 58 °C) in 10 mL of methanol. Add to 1.0 L of 0.05 N sodium borate. Mix, filter, and degas. Add 100 µL of 2-mercaptoethanol (1 + 1) and mix. Make up fresh solution daily.

Other reagents

Methanol	HPLC grade
Water	Milli-Q water as mobile phase and reagent water; tap water as sample
Acetic acid	AR grade
Sodium thiosulfate	AR grade
Dichloromethane	HPLC grade

Sample Preparation Method

We used an automated SPE workstation for sample preparation instead of traditional liquid-liquid extraction in China drinking water standard. Before extraction with SPE, acetic acid was added to the water sample to a concentration of 0.1%. If tap water is used, sodium thiosulfate should be also added to a concentration of 80 mg/L.

The SPE extraction procedure is as follows:

1. Wash syringe with 5.0 mL of CH₂Cl₂.
2. Condition cartridge with 5.0 mL of CH₂Cl₂ into solvent waste.
3. Wash syringe with 5.0 mL of methanol.
4. Condition cartridge with 5.0 mL of methanol into solvent waste.
5. Condition cartridge with 5.0 mL of reagent water into aqueous waste.
6. Load 1,000.0 mL (1 L) of sample onto column.
7. Rinse cartridge with 5.0 mL of reagent water into aqueous waste.
8. Dry cartridge with N₂ for 10 minutes.
9. Wash syringe with 5.0 mL of CH₂Cl₂.
10. Soak for two minutes with 5.0 mL CH₂Cl₂ and collect the eluent.
11. Repeat step 10.
12. Collect 5.0-mL fractions into sample tube using air.
13. Evaporate the eluted sample with a stream of N₂ to a volume of 0.5 mL.
14. Inject 10 µL into HPLC for analysis.

Flow Rates Set

Condition flow	1.0 mL/min
Load flow	5.0 mL/min
Rinse flow	1.0 mL/min
Elute flow	1.0 mL/min
Condition air push	15.0 mL/min
Rinse air push	10.0 mL/min
Elute air push	5.0 mL/min

If you do not have an automated SPE workstation, an Agilent vacuum manifold (p/n 5182-9110 or 5182-9120) is recommended. To automatically pull the water sample through, attach an adapter (p/n 5182-9109) with a pipe onto the SPE cartridge. The water sample should be pulled through the SPE by vacuum at the flow rate of no more than 5 mL/min for good recovery.

Results and Discussion

Separation

The standard solutions were analyzed by injecting 10 μ L of each of the standard solutions onto the Agilent TC-C18(2) column. The chromatograms from the standard injections (Figure 3) show high performance, high resolution, and symmetrical peaks. We have also run this on an HC-C18(2) column. A little longer retention was achieved with almost the same performance as with the TC-C18(2) column. These columns have a different carbon load: 17 percent for the HC-C18(2) column and 12 percent for the TC-C18(2) column. These differences impact retention, with the HC-C18(2) column typically retaining nonpolar and moderately polar compounds more when compared with the TC-C18(2) column. We prefer the column providing a shorter analysis time but resolving all the peaks. Although both columns are suitable for this method, we chose the TC-C18(2) because it provided a slightly shorter analysis time.

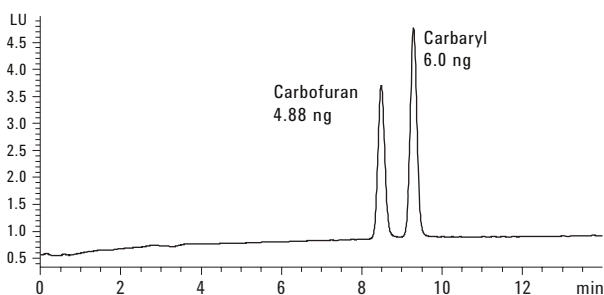


Figure 3. Chromatogram of carbofuran and carbaryl standards on Agilent TC-C18(2), 4.6 $\times$ 150 mm, 5 μ m columns.

Linearity, Reproducibility, and Limit of Detection

The calibration curves resulting from these standard injections on the TC-C18(2) column are shown in Figure 4. The method shows excellent linearity, being very close to 1.0 (0.9997). To evaluate the reproducibility of this method on the TC-C18(2) column, two concentrations of carbofuran and carbaryl were each injected 10 times. The reproducibility of the peak areas is shown in Table 2; the absolute peak area reproducibility is superior. The average relative standard deviation (RSD) is below 3 percent. We calculate the LOD from the level 1 standard with a signal-to-noise ratio of 3. The LOD is 0.066 ng for carbofuran and 0.080 ng for carbaryl, which is three to four times better than the 0.25 ng that is regulated by China's drinking water standard.

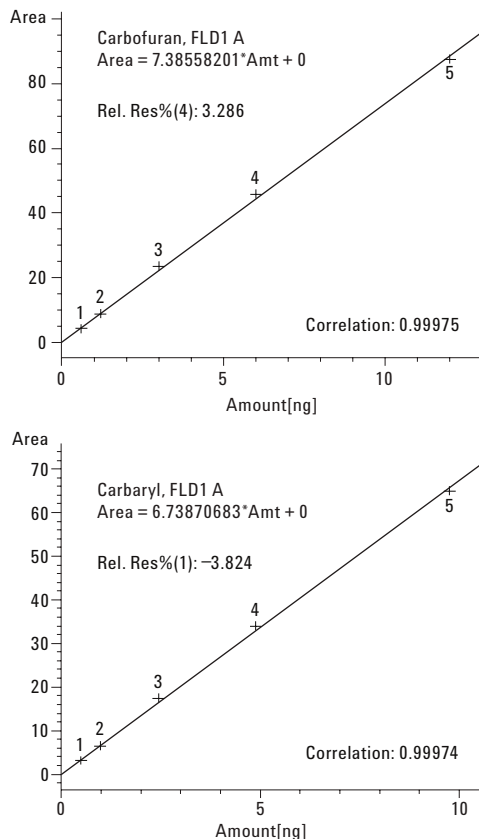


Figure 4. Calibration curves of carbofuran and carbaryl [Agilent TC-C18(2), 4.6 $\times$ 150 mm, 5 μ m columns].

Table 2. Reproducibility of Standards Injections

Analyte	RSD (%) n = 10 Carbofuran 48.8 ppb Carbaryl 60.0 ppb	RSD (%) n = 10 Carbofuran 488 ppb Carbaryl 600 ppb
Carbofuran	2.3	1.6
Carbaryl	1.8	1.5

Recovery

Two different levels were spiked in reagent water and tap water, respectively, and then followed the sample preparation procedure. The recovery data are good, with a typical recovery in the range of 80 to 110 percent (Table 3).

To better match the China drinking water method, in which dichloromethane is used for liquid-liquid extraction, we chose dichloromethane as the SPE cartridge eluent. That provided us clean chromatograms.

It has been reported that the two compounds are not stable under neutral and basic conditions. [1,3] So the water samples were stabilized by the addition of 0.1 percent acetic acid. According to the China drinking water regulatory method, the residual chlorine present in tap water may result in low recovery, thus sodium thiosulfate should be added to the tap water to eliminate the residual chlorine. We therefore compared the recovery of the tap water with and without sodium thiosulfate. The recovery values obtained without sodium thiosulfate were significantly lower than those with sodium thiosulfate. Therefore, to get good recovery values, we added 80 mg/L of sodium thiosulfate to the tap water sample.

Table 3. Recovery for Carbofuran and Carbaryl from Tap Water and Reagent Water

Analyte	Spiked level (ppb)	Tap water R (%) n = 6	Tap water RSD (%) n = 6	Pure water R (%) n = 3	Pure water RSD (%) n = 3
Carbofuran	0.1	107.8	13.5	84.4	9.5
	0.5	98.8	9.2	98.1	6.8
Carbaryl	0.084	87.0	10.1	83.7	6.2
	0.41	91.8	9.6	94.3	3.3

The chromatograms of reagent water and tap water and their spiked samples are shown in Figures 5 and 6. All the potential interfering compounds in reagent and tap water are well separated from the target compounds, demonstrating good method selectivity.

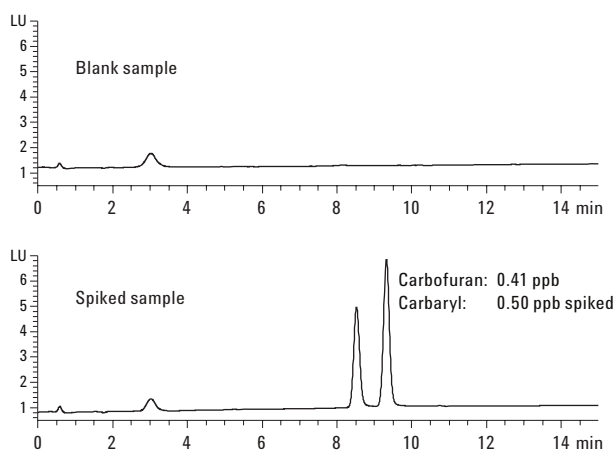


Figure 5. Chromatograms of reagent water and its spiked sample [Agilent TC-C18(2), 4.6 × 150 mm, 5 μm columns].

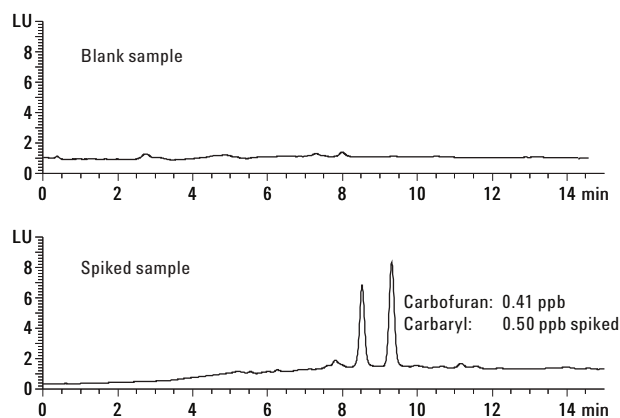


Figure 6. Chromatograms of tap water and its spiked sample [TC C18(2), 4.6 × 150 mm, 5 μm columns].

Direct Injection

A large-volume 400-μL direct injection was applied as described in EPA Method 531.1. We tried a 400-μL injection by using the multidraw attachment on the LC and the TC-C18(2) column. The column has a large surface area (290 m²/g), which allows large-volume injections. The chromatograms in Figure 7 show superior peak shape and high performance. Retention of the two target peaks was excellent and greater-than-baseline resolution was achieved. In addition, the peaks were eluted in a region without any interferences. The column, therefore, can be used for EPA Method 531.1.

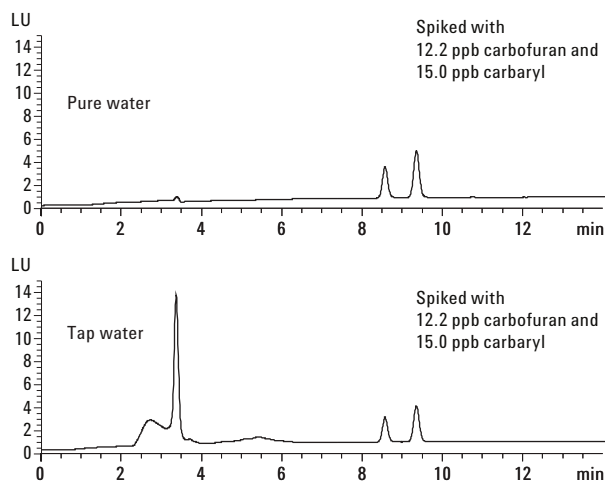


Figure 7. Chromatograms of pure water and tap water spiked with carbofuran and carbaryl.

Conclusions

This total solution enables you to easily analyze drinking water for low levels of the herbicides carbaryl and carbofuran. The Agilent TC-C18(2) column and the new SampliQ C18 SPE cartridge were used for their high sensitivity and low LOD of the two compounds. This method can be used to measure carbaryl and carbofuran in drinking water effectively and quickly.

References

1. United States Environmental Protection Agency, "Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization," Method 531.1, 1989, EPA Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, Ohio 45268.
2. Standards of People's Republic of China, "Standard Examination Methods for Drinking Water – Pesticides, GB/T 5750.9-2006:15.
3. Yuliang Su, et al., "Determination of Carbaryl in Water by HPLC with Direct Injection," *Modern Scientific Instruments*, 2007, 5:125.

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