Detection of the Carbaryl in Wastewater by HPLC-DAD

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Abstract. A method for determination of the carbaryl pesticide residue in wastewater by high performance liquid chromatography (HPLC). HPLC using Agilent ZORBAX SB-C18 column as a separate column; the use of volume ratio(55:45) of methanol: water as the mobile phase; the use of a diode array detector (DAD) detection at 268 nm at carbaryl residues; pretreatment of water samples using the extraction method is simple and feasible method of operation; laboratory water samples from wastewater, test results show: accuracy relative standard deviation (RSD) was 0.67%, and the recovery rates were in the range of 102 to 112%.

1. Introduction
Carbaryl, also known as sevin, scientific name 1-Naphthalenyl methyl carbamate, belongs in the category of naphthalene carbamate pesticides. Since carbaryl was discovered in 1953, a sery of carbamates insecticide[1] such as aldicarb, methomyl, carbofuran had been developed in succession. Up to 2011, carbamates insecticide had made up around 15% of insecticide market shares over the world. Because carbamates insecticide has a certain degree of water solubility, while spreading, 40-60% of it lands in soil, gets into the water circulation system by rain wash and transfer of river and atmosphere, only part of it can be degraded, instantaneous high concentration or accumulation of it results in ecological disturbance[2].

Long-term exposure to carbaryl for human body would cause carbaryl chronic neurotoxia, damage nerve center and effect activity of daily living[3]. Besides, carbaryl has an environmental hormonal effect, once it is used massively for a long term, a great damage would be caused for immune system and reproductive endocrinology system of animal and human being[4]. Carbaryl has a wide spreading range, a high transmission efficiency and is hard to decompose, which is a huge hidden danger for human body and environment[5]. In < Environmental quality standards for surface water >, it is stipulated that in the earth surface water source for centralized domestic drinking, a particular item carbaryl limit is 0.05mg/L[6].

The detection for carbaryl usually includes chromatography[7], MS-MS[8], fluorescence method[9], extraction pretreatment[10], direct injection and solid phase extraction (SPE)[11]. High performance liquid chromatography (HPLC) has a high popularizing rate and is efficient for analysis, this paper chooses HPLC as detection method; in recent years SPE has been frequently used in scientific research, but it is inadequate for normal pretreatment. Thus this paper chooses extraction and direct injection for optimizing the pretreatment. This test focuses on carbaryl residual in tap water and municipal sewage, and detects its residual quantity by HPLC-DAD, optimizes the pretreatment method of water sample, thereby gets a higher test efficiency and a higher detection precision.
2. Experimental

2.1. Equipment, reagent and sample
- Agilent HPLC 1100 (equipped with vacuum degasser, automatic sampler, column oven and DAD);
- Agilent chromatographic column: ZORBAX SB-C18(150mm×4.6mm×3.5µm, pH=1-8);
- Rotary evaporator YA RONG RV-211M;
- PHS-3C pH meter: Shanghai Precise Scientific Instrument Limited Company;
- Constant vacuum distillation unit;
- OCEAN microporous filter(aqueous phase0.45µm);
- Carbaryl (chromatographically pure):1000mg·L⁻¹, GBS05-2301-2008-2.

2.2. Chromatographic analysis condition
- Mobile phase:CH₃OH:H₂O(V/V)=55:45;
- Flow rate: 1.0 mL·min⁻¹;
- Injection volume: 10 µL;
- Chromatographic column temperature: 40 ℃;
- DAD(λ=268 nm);
- Analysis time:7.0 min .

2.3. Qualitative analysis
In the 1.2 chromatographic condition, analyse 10.00 mg·L⁻¹ carbaryl standard sample with HPLC, the retention time(tᵣ)is 1.653 min, it is identified by the retention time.

2.4. Quantitative analysis
Take 1.0 mL of 1 000 mg·L⁻¹ carbaryl standard solution into a 10 mL volumetric flask, and add in methanol to obtain 100 mg·L⁻¹ standard solution. Proteinchip for 100 mg·L⁻¹ carbaryl standard solution 50µL, 100µL, 200µL, 500 and 1000µL into a 10 mL volumetric flask, and add in methanol till a certain volume. Prepare a series of 0.50, 1.00, 2.00, 5.00 and 10.00mg·L⁻¹ concentration of carbaryl standard solution, detect them with HPLC (as Table 1 shows).

| c / (mg·L⁻¹) | A₁ | A₂ | A₃ | A₄ | A₅ | \( \bar{A} = \frac{A_1+A_2+A_3+A_4+A_5}{5} \) |
|-------------|----|----|----|----|----|------------------------------------------|
| 0.50        | 58.3 | 65.2 | 62.5 | 65.7 | 63.3 | 63.0 |
| 1.00        | 125.3 | 123.2 | 126.2 | 124.5 | 122.8 | 124.5 |
| 2.00        | 248.5 | 244.3 | 246.4 | 249.2 | 253.1 | 248.3 |
| 5.00        | 615.4 | 619.3 | 622.2 | 624.9 | 618.2 | 620.0 |
| 10.00       | 1276.7 | 1288.1 | 1280.5 | 1277.4 | 1281.8 | 1280.9 |

A method was developed for the determination of carbaryl in waters by HPLC with external standard method(see Figure 1).
2.5. Water sample pretreatment (distillation and concentration)
Inject certain water sample into the 250mL distillation flask, add phosphoric acid to adjust pH=3, in water bath at 80℃, with reduced pressure distillate the sample till nearly dry, add in methyl alcohol till the volume of 1.0mL, after filtering with 0.45µm membrane filtration, analyse it with HPLC.

3. Result and discussion

3.1. Sample preparation method optimization

3.1.1. Extraction method from standards[7]
Take 100mL water sample and marked water sample seperately, conduct extraction in 250mL separating funnels. Use 10mL and 5mL dichloromethane for twice extraction, vibration for 5min, after static layering, remove the extraction liquor to 50mL concentrated bottle, in water bath at 40℃, with reduced pressure distillate it nearly dry, add in methyl alcohol till the volume of 1.0mL, after filtering with 0.45µm membrane filtration, analyse it with HPLC.
Because the water sample was not adjusted pH value, extraction was incomplete, extract liquor was not dried, the analyse result showed the marked sample was low in testing (see Figure 2).

3.1.2. Improving extraction method
Take 100mL water sample and marked water sample, add in H3PO4 to adjust pH=3 seperately, conduct extraction in 250mL separating funnels. Use 15mL, 10mL and 5mL dichloromethane for thrice extraction, vibration for 5min, after static layering, desiccate the sample by passing it through a funnel filled with anhydrous sodium sulfate, transfer into 100mL distillation flask, in water bath at 40℃, with reduced pressure distillate it nearly dry, add in methyl alcohol till the volume of 1.0mL, after filtering with 0.45µm membrane filtration, analyse it with HPLC.
In this improved extraction method, the test result of the marked sample was improved, but in HPLC interference peak would cause a larger error in quantitative result (see Figure 3).
3.1.3. Distillation and concentration

Take 100mL water sample and marked water sample separately, put in 250mL distillation flask, add in H$_3$PO$_4$ to adjust pH=3. In water bath at 80℃, with reduced pressure distillate it nearly dry, add in methyl alcohol till the volume of 1.0mL, after filtering with 0.45µm membrane filtration, analyse it with HPLC.

The extraction method can not extract carbaryl in the water completely, thus taking example by direct injection method, concentrate the sample with reduced pressure distillation, the test result of marked sample is better, no interference peak in chromatographic analysis (see Figure 4).

Figure 4. Chromatogram of distillation and concentration($t_R$=1.653min).

Based on the above pretreatment method, direct concentration method is a better sample pretreatment method.

3.2. Water sample analysis result

The water sample is pretreated by distillation and concentration, analyzed by HPLC. The method has been applied to the analysis of carbaryl in industrial wastewater. The residue of carbaryl in wastewater can not be detected, the results of experiment proves that it meets national standards[6].

3.3. Relative standard deviation

Determine the relative standard deviation(RSD) by adding standard sample to wastewater, and continuously analyze 10 times, determine with HPLC-DAD, mark legal quantity outside, calculate the relative standard deviation (as Table 2 shows).The results show that the method has a high precision and meets the detection requirements.

| A/(mAU*s) | 389.8 | 399.9 | 392.8 | 390.4 | 387.1 | 391.2 | 388.5 | 388.8 | 392.7 | 393.6 | RSD/% |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
| c/(mg·L$^{-1}$) | 3.095 | 3.173 | 3.118 | 3.099 | 3.073 | 3.106 | 3.084 | 3.087 | 3.117 | 3.124 | **0.67** |

3.4. Stability test of Carbaryl in water samples

Determining the nature by adding standard sample to actual water. The marked water sample gets pretreatment in direct concentration method, analyse it by HPLC, c=$3.107$mg·L$^{-1}$, freezing storage at 4℃ after 24 hours, c=$3.097$mg·L$^{-1}$, The results show a relative error of -0.33%, thus it concluded that, water sample preserved at low temperature after pretreatment, analyse it in 24h, the error can meet the detection requirement.
3.5. Adding standard recovery
In the 3 levels standard addition test of wastewater, conduct the spiked recovery test, the recovery rate is 102-112% (as table 3 shows), which states the pretreatment recovery rate is high, meanwhile that of extraction method is 92-98%[12] normally.

| Background values (/mg·L⁻¹) | Adding standard mount (/mg·L⁻¹) | Estimated value (/mg·L⁻¹) | Recovery (%) | Average recover (%) |
|-------------------------------|---------------------------------|---------------------------|--------------|---------------------|
| 0                             | 0.50                            | 0.56                      | 112          | 111                 |
|                               | 0.55                            | 110                       |              |                     |
| 1.00                          | 1.11                            | 111                       |              |                     |
|                               | 1.13                            | 113                       |              |                     |
| 2.00                          | 1.96                            | 98                        | 112          | 102                 |
|                               | 2.11                            | 105                       |              |                     |

4. Conclusion
This paper confirmed a HPLC method for testing wastewater, identified by the retention time, quantified in external standard method, with standard curve $R^2=0.9997$, which meets the analyse requirement; by optimizing pretreatment method, treat the water sample in direct concentration method; by analyzing the actual sample, the combined application of pretreatment and HPLC can meet the analyse requirement, RSD of sample, 24h stability test, recovery rate can meet the analyse requirement as well; the method is easy to operate, save the analyze time, which is appropriate for promotion.

5. References
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