# Determination of Pesticide Residues in Drinking Water Using Automated Solid-Phase Extraction and Gas Chromatography with Nitrogen Phosphorus Detection

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**Key Words**
AutoTrace 280, SPE, TRACE 1310 GC, GC-NPD, Organophosphorous Pesticides, Chinese Method GB 5750.9-2006

## Introduction

Organophosphorous pesticides have been widely used to prevent and treat plant diseases and pests in agricultural products and as a result, excessive residual pesticide content is frequently reported in drinking and surface water sources. A number of organophosphorous pesticides are acutely toxic to human and animal life, making the detection of residual organophosphorous pesticides a growing concern.

This application note addresses the detection of trace amounts of organophosphorus pesticides in water and optimizes the conditions for solid-phase extraction (SPE) and chromatographic analysis using the Thermo Scientific™ Dionex™ AutoTrace 280 Solid-Phase Extraction instrument and the Thermo Scientific™ TRACE™ 1300 Series gas chromatograph with a nitrogen-phosphorus detector (NPD). Eight different types of organophosphorus pesticide residues were detected in drinking water. The results show that this method achieves rapid, simple extraction with small quantities of solvent. In addition, the method has high sensitivity to the eight types of organophosphorus pesticides, a high recovery rate and good linear range.

## Summary of the Method

The Chinese Ministry of the Environment Method GB 5750.9-2006 is used to extract and analyze organophosphorous pesticide residues in environmental waters. This method primarily uses liquid–liquid extraction (LLE) to remove the residues but automated SPE can be used in place of LLE to reduce the quantity of organic solvent used. A nitrogen–phosphorus detector (NPD) was used to selectively detect trace amounts of organophosphates in the water.

## Sample Preparation

Methanol (2 mL) and NaCl (5 g) were added to 500 mL of tap water taken from the Beijing laboratory. These samples where then spiked with 50 µL of 10 µg/mL mixed standard solution. The sample was purified on a Thermo Scientific™ HyperSep™ Retain PEP SPE cartridge and eluted with ethyl acetate and dichloromethane. The collected liquid was concentrated to 1 mL by nitrogen blowing and analyzed using gas chromatography.

## Dionex AutoTrace 280 SPE Instrument

| No. | Method | SPE Steps |
|---|---|---|
| 1 | Add 2 mL of methanol and 5 g of NaCl to 500 mL of the sample water | |
| 2 | Spike with 50 µL of 10 µg/mL mixed standard solution | |
| 3 | Purify sample on a HyperSep Retain PEP SPE Cartridge (500 mg/6 mL, P/N 60107-206) | Purify |
| 4 | Elute sample with ethyl acetate and dichloromethane | Elute |
| 5 | Concentrate collected liquid to 1mL by nitrogen blowing and analyzed using gas chromatography | Collect |
| **Process six samples using the following procedure:** | | |
| 1 | Condition cartridge with 5.0 mL methanol (MeOH) into solvent waste | |
| 2 | Condition cartridge with 5.0 mL ethyl acetate (EtOAc) into solvent waste | |
| 3 | Condition cartridge with 5.0 mL dichloromethane (DCM) into solvent waste | |
| 4 | Condition cartridge with 5.0 mL ethyl acetate (EtOAc) into solvent waste | |
| 5 | Condition cartridge with 5.0 mL MeOH into solvent waste | |
| 6 | Load 550.0 mL of sample onto cartridge | |
| 7 | Load 550.0 mL of sample onto cartridge | |
| 8 | Dry cartridge with gas for 10 min | |
| 9 | Collect 4.0 mL fraction into sample tube using EtOAc | |
| 10 | Collect 4.0 mL fraction into sample tube using EtOAc | |
| 11 | Collect 4.0 mL fraction into sample tube using DCM | |

| **Test Conditions** | |
|---|---|
| Solvent 1 | Water (reagent grade) |
| Solvent 2 | $CH_3OH$ (methanol) |
| Solvent 3 | EtOAc (ethyl acetate) |
| Solvent 4 | DCM (dichloromethane) |
| Condition flow | 15.0 mL/min |
| Load flow | 10.0 mL/min |
| Rinse flow | 10.0 mL/min |
| Elute flow | 3.0 mL/min |
| Condition air flow | 15.0 mL/min |
| Rinse air flow | 10.0 mL/min |
| Elute air flow | 3.0 mL/min |

Table 1. Instrument parameters and settings.

| | |
|---|---|
| **Instrument model and configuration** | TRACE 1310 GC<br>Instant Connect SSL injector<br>AS 1310 autosampler |
| **chromatography column oven temperature program** | TG-1701 MS, 30 m, 0.25 mm, 0.25 µm<br>80 °C (1 min), 20 °C/min to 250 °C (0 min), 5 °C/min |
| **Detector type and working parameters** | Instant connect NPD: 300 °C<br>Air: 60 mL/min<br>Hydrogen: 2.3 mL/min<br>Make-up gas: Nitrogen<br>Add this info 15 mL/min |
| **Carrier gas type and flow rate** | Nitrogen, constant current mode, flow rate: 1 mL/min |
| **Injection method and volume** | Splitless, for 1 min, Injector temperature of 250 °C; sample volume: 1 mL |

| No. | Extraction Procedure |
|---|---|
| 1 | Activate extraction cartridge with 5.0mL MeOH into the solvent waste channel |
| 2 | Activate extraction cartridge with  5.0 mL EtOAc into the  solvent waste channel |
| 3 | Activate extraction cartridge with 5.0 mL DCM into solvent waste channel |
| 4 | Activate extraction cartridge with 5.0 mL EtOAc into solvent waste channel |
| 5 | Activate extraction cartridge with 5.0 mL MeOH into solvent waste channel |
| 6 | Activate extraction cartridge with 5.0 mL water into solvent waste channel |
| 7 | Load a 550.0 mL (10 mL/min) sample onto the extraction cartridge |
| 8 | Rinse the extraction cartridge with 5.0 mL of water into the waste channel |
| 9 | Dry the small extraction cartridge with nitrogen (15 mL/min for a period of 10 min) |
| 10 | Collect 4.0 mL of eluate from the extraction cartridge with EtOAc in the sample collection tube |
| 11 | Collect 4.0 mL of eluate from the extraction cartridge with EtOAc in the sample collection tube |
| 12 | Collect 4.0 mL of eluate from the extraction cartridge with EtOAc in the sample collection tube |

**Gas Chromatography Conditions**

| | |
|---|---|
| Instrument model and configuration | TRACE 1310 GC<br>Instant Connect SSL injector<br>AS 1310 autosampler |
| Chromatography column | TG-1701 MS, 30 m, 0.25 mm, 0.25 µm |
| Oven temperature program | 80 °C (1 min), 20 °C/min to 250 °C (0 min), 5 °C/min |
| Detector type and working parameters | Instant Connect NPD: 300 °C<br>Air: 60 mL/min<br>Hydrogen: 2.3 mL/min<br>Make-up gas: Nitrogen 15 mL/min |
| Carrier gas type and flow rate | Nitrogen, constant current mode, flow rate: 1 mL/min |
| Injection method and of volume | Splitless, for 1 min, injector temperature 250 °C; sample volume: 1 mL |

## Results and Discussion

### Optimization of Conditions for SPE Device

GB5749-2006 “Hygiene Standards for Domestic Drinking Water” requires that residual amounts of eight types of organophosphorus pesticide in drinking water be monitored. The HyperSep Retain PEP SPE cartridge (6 mL barrel with 500 mg sorbent material, part number 60107-206) was used for this method.

The elution efficiencies of solvents including methanol, ethyl acetate, dichloromethane and n-hexane prepared in different proportions on the organophosphorus pesticides retained on the HyperSep Retain PEP SPE cartridge were tested. The test results showed that carrying out elution with ethyl acetate/ethyl acetate/dichloromethane (4 mL/4 mL/2 mL) produced the highest elution efficiency with the smallest quantity of solvent. The boiling points of demeton and phorate were low and solubility in water was quite high and the recovery rate produced by the direct use of SPE was not ideal. However, adding NaCl to the water increased the recovery rate.

Extraction efficiencies were tested by adding 2, 5 and 10 g of NaCl to the water. The recovery rate was highest when adding 5 g NaCl to the water. This is due to the ability of NaCl to reduce the solubility of organic analytes in water, enabling greater retention during the extraction process and resulting in higher recovery rates.

Figure 1 shows the effect of adding different amounts of NaCl on phorate recovery rates. Another reason for low demeton recovery rates is the low boiling point, which makes it degrade easily during testing. Large quantities of demeton are lost easily during the concentration process. Therefore, lowering the the nitrogen blowing temperature to 30 °C during the concentration process and reducing the flow rate to prevent rapid degradation are recommended.

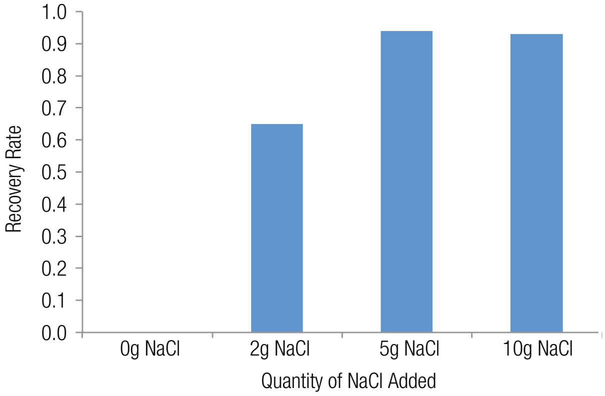

Figure 1. The effect of the quantity of NaCl added on recovery rates.

## Standard Chromatogram and Spiked Chromatogram

See Figure 3 for the standard chromatograms of the eight organophosphate analytes. See Figure 2 for a blank sample that went through the entire preparation process and Figure 4 for chromatogram of the domestic drinking water sample spiked with the eight organophosphate species tested.

## Linearity, Detection Limits and RSD

Calibration solutions were prepared in the following concentrations: 0.05, 0.1, 0.2, 0.5 and 1.0 µg/L and analyzed the solutions using the instrument method specified in Table 1 to investigate linearity for each component within a concentration range of 0.05–1.0 µg/mL. The test results showed a good linear relationship for the eight compounds between 0.05–1.0 µg/mL. The linear correlation coefficients were all greater than 0.99 (see Table 2).

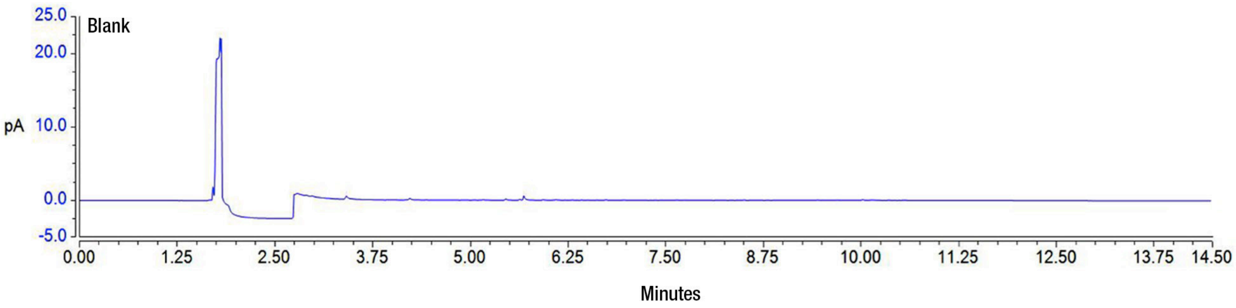

Figure 2. Blank sample chromatogram.

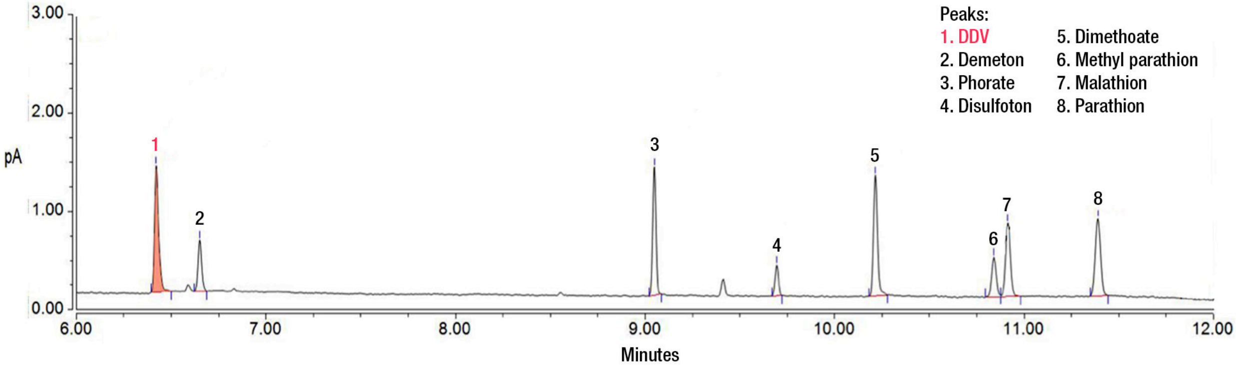

Figure 3. Chromatogram for 0.5 µg/mL mixed standard solution.

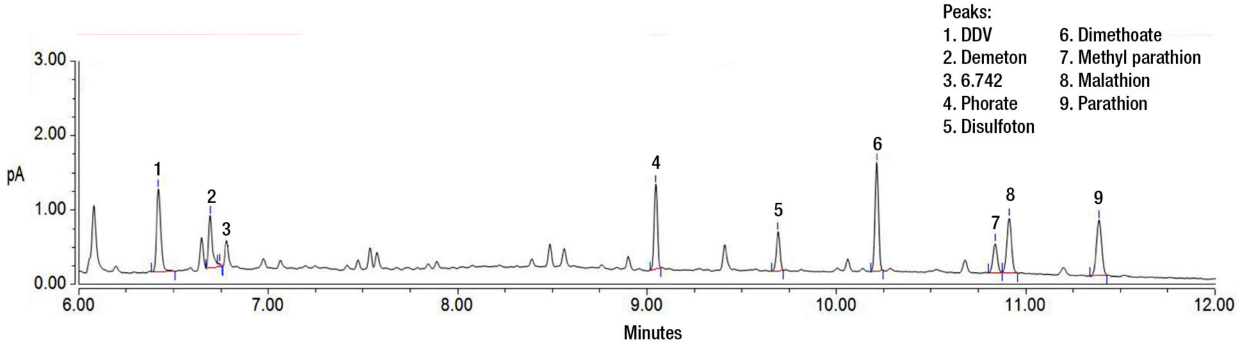

Figure 4. Spiked sample chromatogram (spike volume: 1 µg/L). Peak 3 with a retention time of 6.742 min is an unknown compound.

Table 2. Linearity, detection limits and RSD Data (n=3).

| Compound | Linear equation | $R^2$ | Spiked sample 0.2 µg/L Recovery rate/% | Spiked sample 0.4 µg/L Recovery rate/% | Spiked sample 1.0 µg/L Recovery rate/% | Detection limit/µg/L | RSD/% (n=6) |
|---|---|---|---|---|---|---|---|
| Dichlorvos | Y = (0.0415 + 6.0597)*X | 0.9904 | 93.2 | 88.4 | 98.6 | 0.01 | 4.1 |
| Demeton | Y = (0.0427 + 18.1974)*X | 0.9948 | 83.3 | 88.5 | 90.7 | 0.05 | 8.7 |
| Phorate | Y = (0.0124 + 6.7178)*X | 0.9936 | 98.7 | 91.5 | 95.4 | 0.05 | 6.3 |
| Disulfoton | Y = (0.0081 + 14.7635)*X | 0.9956 | 95.3 | 89.8 | 95.7 | 0.01 | 7.3 |
| Dimethoate | Y = (0.0419 + 5.2029)*X | 0.9909 | 88.5 | 89.3 | 99.8 | 0.01 | 2.4 |
| Methyl parathion | Y = (0.0258 + 16.4662)*X | 0.9933 | 94.0 | 90.9 | 89.3 | 0.05 | 3.8 |
| Malathion | Y = (0.0266 + 8.1784)*X | 0.9939 | 90.5 | 88.8 | 90.1 | 0.01 | 4.4 |
| Parathion | Y = (0.0193 + 7.2690)*X | 0.9943 | 93.2 | 95.7 | 96.4 | 0.01 | 3.5 |

A mixed standard solution was added to the water samples (spiked concentrations: 0.2, 0.4 and 1 µg/L) to investigate the spike recovery rates of the eight pesticides. The test results showed that spike recovery rates for each component are between 83% and 100% and are in compliance with routine analysis detection requirements. At the same time, water samples were spiked with 0.2 µg/L, 0.4 µg/L and 2.0 µg/L and analyzed six times in parallel. The average RSD value was between 2.4% and 8.7%, in compliance with stability requirements. In the experiment, the detection limit for each component was calculated using three times the signal-to-noise ratio. The detection limits for each component were 0.02–0.1 µg/L (see Table 2).

## Summary

The Dionex AutoTrace 280 SPE instrument can extract organic compounds from water samples ranging from 20 mL to 4 L. The Dionex AutoTrace 280 automatically carries out the activation, loading, rinsing and elution of the chromatography column, greatly reducing preprocessing times and reducing the time needed to manually load the samples (a 500 mL sample of water requires 50 min, so 5 hours are saved when processing six samples). In addition, the Dionex AutoTrace 280 offers excellent reproducibility, making it possible to carry out laboratory analysis and detection on large-volume water samples. Excellent sensitivity, repeatability and reproducibility in the analysis of organophosphate pesticides have been achieved using the innovative TRACE 1310 gas chromatograph. The NPD design uses ceramic beads that maintain excellent peak shape also for phosphorous specified. Its Instant Connect Split/Splitless injector and NPD detector are user-installable and, making the TRACE 1310 GC a versatile system that can be easily switched to other applications. By simply changing injector and detector modules, different methods can be run on the same GC platform in a matter of a few minutes.

## Conclusion

This application note has established a method for determining eight different of organophosphate pesticides in water in accordance with the Chinese Ministry of the Environment Method GB 5750.9-2006 “Hygiene Standards for Domestic Drinking Water”. The sensitivity of the method is low, 0.02–0.1 µg/L for each of the eight organophosphates. Recovery rates for the three spiked levels, 0.2, 0.4 and 1.0 µg/L, were between 83% and 100%. The extraction and analysis methods used here offer many advantages for the detection of organophosphates, including sensitivity, accuracy and a wide linear range, satisfying detection requirements for organophosphates in domestic drinking water.

## References

1. Zhou Fang, Sun Cheng, Zhong Ming et al. Solid-phase extraction and capillary gas chromatography analysis of residual organophosphorus pesticides in water. *Environ. Pollut. Control*. **2005**, 27(2): 151-153.
2. Maciej Tankiewicz, Jolanta Fenik, Marek Biziuk. Determination of organophosphorus and organonitrogen pesticides in water samples. *Trends Anal. Chem.* **2010**, 29(9):1050-1064
3. Yao Chaoying, Yang Lili, Mu Yingfeng. Determination of demeton in water by gas chromatography[J]. *Sichuan Environment*. **2007**, 26(4): 48-50.
4. Hygiene Standards for Domestic Drinking Water, Chinese Ministry of the Environment Method <GB5750-2006>. People’s Republic of China.

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