Application Note 1109

Rapid Determination of Nitrobenzenes in Drinking Water Using Automated SPE with GC-ECD

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Key Words
AutoTrace 280 SPE, TRACE 1310 GC, U.S. EPA Method 8091, Chinese Ministry of Environmental Protection, Method HJ 648-2013

Introduction

Nitrobenzenes are used as precursors for the production of numerous industrial chemicals including polyurethane, pesticides, azo dyes, explosives, and pharmaceuticals. These compounds may be released into the environment by production plants through wastewater discharge and may contaminate ground and surface waters that surround these plants. Since ground and surface waters serve as sources of drinking water, human exposure to nitrobenzene compounds has raised concerns among regulatory agencies throughout the world. The U.S. Environmental Protection Agency (U.S. EPA) considers nitrobenzene and its derivatives to be likely human carcinogens[1] and the World Health Organization's International Agency for Research on Cancer (IRAC) considers nitrobenzene a Class 2B carcinogen.[2] The Chinese Ministry of Environmental Protection promulgated Hygiene Standards for Domestic Drinking Water (GB 5749-2006)[3] to limit the concentration of nitrobenzene compounds in environmental waters and have recently added a standard method for detection of 15 nitrobenzene compounds in drinking water (HJ 648-2013)[4] for compliance monitoring (Table 1).

Gas chromatography with electron capture detection (GC-ECD) is typically used for sensitive determination of nitrobenzene compounds in environmental waters.[4, 5] Solid-phase extraction (SPE) is often used prior to chromatography to extract and concentrate the analytes by using a vacuum manifold.[4] Vacuum manifolds require the manual addition of sample, elution solvent, and extraction solvent by an analyst. Both analyte recovery and reproducibility may be highly variable when using this technique and analytical results may be compromised due to this variation. The challenges associated with manual SPE steps can be overcome by automating sample and solvent delivery processes to maximize analyte recovery and improve reproducibility of the extraction process. This application note demonstrates the use of automated SPE with GC-ECD for the determination of 15 nitrobenzene compounds in finished drinking water. The automated Thermo Scientific™ Dionex™ AutoTrace 280 Solid Phase-Extraction instrument was used in conjunction with the Thermo Scientific™ TRACE™ 1310 gas chromatograph using an Instant Connect micro ECD. This work optimizes SPE and gas chromatography conditions and the results show high sensitivity (MDL of 0.02 ug/L) for the 15 nitrobenzene compounds specified in HJ 648-2013.

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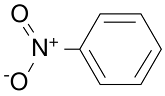
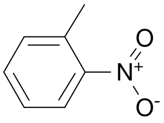
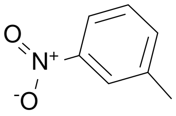
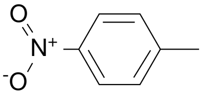
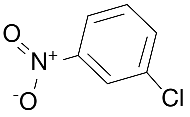
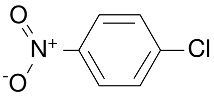
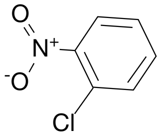
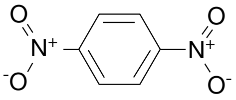
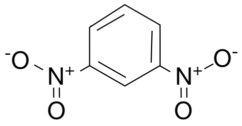
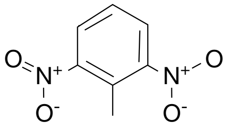
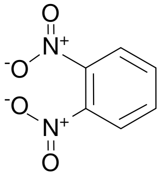
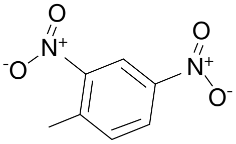
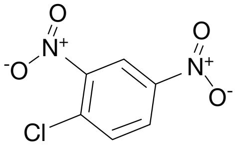
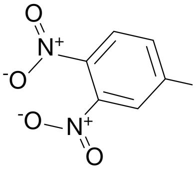
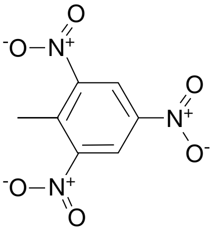
Table 1. The 15 nitrobenzene compounds for Chinese Method HJ648-201.

Number	Compound	Retention Time (min)	CAS	Stucture
1	Nitrobenzene	12.493	98-95-3	
2	o-Nitrobenzene	14.948	88-72-2	
3	m-Nitrobenzene	16.652	99-08-1	
4	p-Nitrobenzene	17.482	99-99-0	
5	M-Nitrochlorobenzene	17.603	121-73-3	
6	P-Nitrochlorobenzene	18.085	100-00-5	
7	O-Nitrochlorobenzene	18.972	88-73-3	
8	P-Dinitrobenzene	24.395	100-25-4	
9	M-Dinitrobenzene	24.618	99-65-0	

Number	Compound	Retention Time (min)	CAS	Stucture
10	2,6-Dinitrotoluene	24.907	606-20-2	
11	O-Dinitrobenzene	26.025	528-29-0	
12	2,4-Dinitrotoluene	26.163	121-14-2	
13	2,4-Dinitrochlorobenzene	26.808	97-00-7	
14	3,4-Dinitrotoluene	27.937	610-39-9	
15	Trinitirotoluene (TNT)	29.638	118-96-7	

Equipment, Consumables and Reagents

Dionex AutoTrace 280 SPE Instrument

Thermo Scientific TRACE 1310 GC-ECD

Consumables

- Thermo Scientific™ TraceGOLD™ TG-1701MS GC column 30m x 0.25mm x 0.25um (P/N 26090-1420)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) 7.2.1 (P/N 43280015)
- SPE Cartridges (P/N 60107-206)
  Hypersep Retain PEP or equivalent SolEx HRPHS cartridges (P/N 088127)
  The HRPHS stationary phase is a neutral resin comprised of a high-surface-area, divinylbenzene-based particle grafted with a polyvinylpyrrolidone polymer.

Reagents

- Methanol: chromatographic grade Fisher A452-4
- Acetone: chromatographic grade Fisher A949-4
- Hexane: chromatographic grade Fisher H302-4
- Standards of all the 15 nitrobenzene compounds in this work were purchased from the Chinese Institute of Metrology, concentration: 100 µg/mL
- Anhydrous sodium sulfate: analytical reagent, Beijing Chemical Reagent Factory
- Sodium chloride: analytical reagent, Beijing Chemical Reagent Factory

Conditions

Dionex AutoTrace 280 SPE Instrument

Flow Rates	mL/min
Condition	15
Load	10
Rinse	10
Elute	5
Condition Air Push	15
SPE Parameters	
Push Delay	5 s
Air Factor	1.0
Autowash Volume	1.0 mL

SPE Method

Step	Step Description	Volume Solution
1	Condition cartridge Methanol	5 mL
2	Condition cartridge Ethyl Acetate	5 mL
3	Condition cartridge n-Hexane	5 mL
4	Condition cartridge Ethyl Acetate	5 mL
5	Condition cartridge Methanol	5 mL
6	Condition cartridge Deionized water	5 mL
7	Load Sample	550 mL
8	Rinse Deionized water	5 mL
9	Dry Cartridge Nitrogen Gas	10 min
10	Elute n-Hexane/Acetone (9:1)	10 mL

Clean the sample tubes with 30 mL of methanol for each channel, then flush the sample tubes with 100 mL of DI water to ensure the solvent is flushed away and no air bubbles remain in the sample tube.

TRACE 1310 GC-ECD Conditions

Column	Thermo Scientific™ TraceGOLD™ TG-1701MS 30 m x 0.25 mm x 0.25 um (14% Cyanopropylphenyl Polysiloxane) (P/N: 26090-1420) **OR** TraceGOLD TG-5MS 60 m x 0.25 mm x 0.25 um (5% diphenyl / 95% dimethyl polysiloxane) (P/N 26098-1540) Carrier Gas Nitrogen (99.99% purity)
Column Flow	1.0 mL/min, constant flow
Oven Temperature	TraceGOLD TG-1701MS 50 °C (0 min), 10 °C/min to 100 °C (3 min), 3 °C/min to 125 °C (0 min), 8 °C/min to 250 °C
	TraceGOLD TG-5MS 60 °C (0 min), 15 °C/min to 180 °C (5 min), 4 °C/min to 230 °C (5 min)
Injector Type	Instant Connect Split/Splitless injector
Injector Mode	Splitless (1 min), then split flow of 15 mL/min, constant septum purge
Injector Temperature	280 °C
Detector Temperature	300 °C

Standard and Sample Preparation

Standard Preparation

A stock standard solution was prepared by adding ethyl acetate to 10 mL volumetric flask and diluting to 10 µg/mL. Stock solutions were stored at 5 °C.

Calibration standard solutions were diluted from standard stock solutions (10 µg/mL) with ethyl acetate to 5, 10, 20, 50, and 100 µg/L

Sample Preparation

Accurately measure 500 mL of tap water and place in a 1 L vial. Add 1 mL methanol + 1mL ethyl acetate, shake evenly and store ready for testing.

The standard was added to the water samples before loading onto the SPE cartridge (Figure 1).

Results and Discussion

This experiment also investigated the elution efficiency of methanol, ethyl acetate, dichloromethane, n-hexane and acetone in different proportions. The results showed that a preparation of n-hexane and acetone (9:1) produced the highest elution efficiency using the lowest quantity of elution solution.

Optimization of Chromatographic Conditions

Selecting the Chromatographic Column

This experiment investigated the use of both the TraceGOLD TG-1701MS mid-polarity and the TraceGOLD TG-5MS low polarity GC columns. When using an oven temperature ramp up to of 250 °C, all 15 nitrobenzene compounds specific in Chinese Method HJ 648-2013 can be separated in less than 30 minutes. Using the TraceGOLD TG-5MS with an oven temperature ramp to 230 °C will further reduce the run time to 20 min while maintaining good chromatographic resolution.[7] While both columns where able to resolve the 15 nitrobenzene compounds, the TraceGOLD TG-1701MS were used for the remainder of the experiments due to cost considerations.

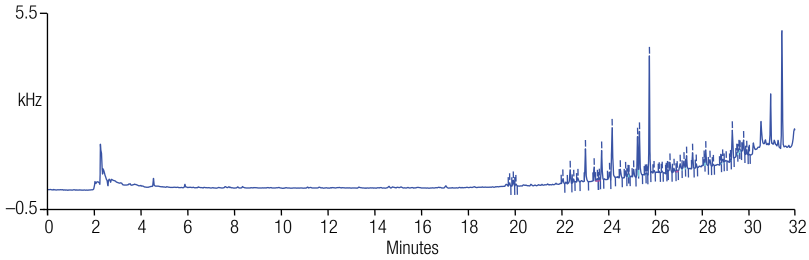


Figure 1. Spiked tap water samples.

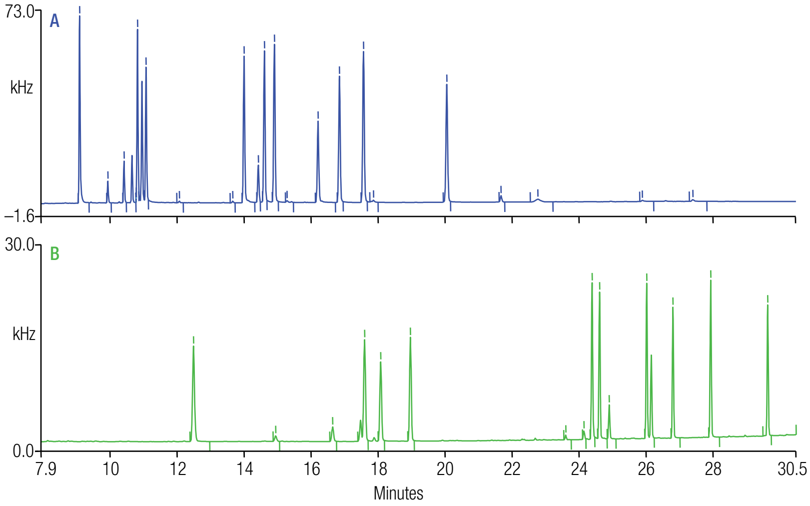


Figure 2. Separation of 15 types of nitrobenzene on TraceGOLD TG-5MS (a) and TraceGOLD TG-1701MS (b) GC Columns.

Standard and Spiked Chromatograms

A chromatogram of the 15 nitrozobenzene compounds in a 0.5 µg/mL standard solution is shown in Figure 3. Figure 4 shows the separation of 15 nitrobenzene compounds in spiked (0.1 µg/L) domestic drinking water. The chromatogram in Figure 4 shows that the 15 nitrobenzene compounds are well resolved and not affected by the background.

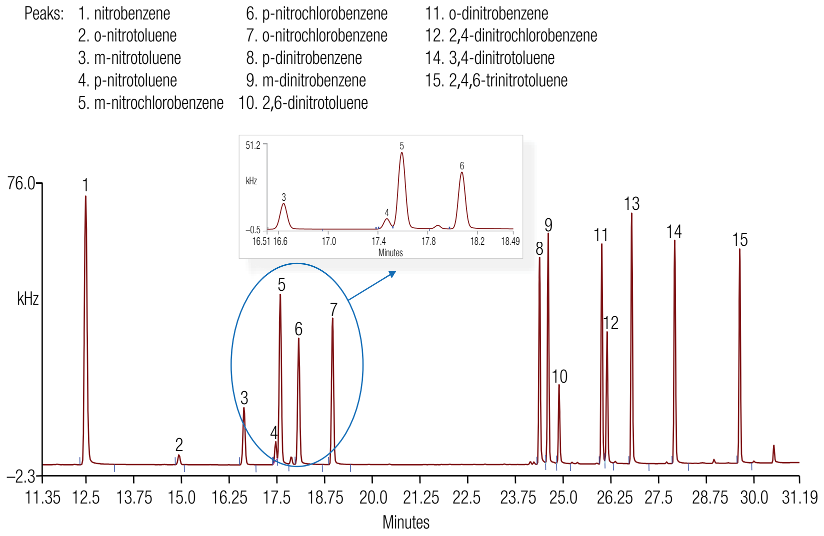


Figure 3. Chromatogram for 0.5 µg/mL mixed standard solution.

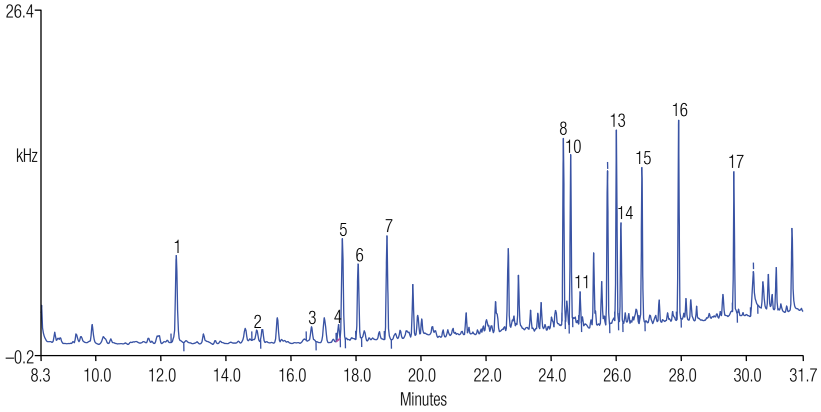


Figure 4. Spiked chromatogram (spiked volume 0.1 µg/L).

Linearity, Spiked Recovery, Reproducibility and Test Limits

Calibration solutions were prepared in the following concentrations: 5, 10, 20, 50 and 100 µg/L and the solutions analyzed using the above method to investigate linearity for each component within a concentration range of 5–100 µg/L. The test results showed a good linear relationship for the 15 components between 5 µg/L and 100 µg/L. The linear correlation coefficients were all greater than 0.99 (Table 2). Mixed standard solution was added to the water samples (spiked concentrations: 0.02, 0.1, and 0.5 µg/L) to investigate the spike recovery rates of the 15 types of nitrobenzene. Results showed that spike recovery rates for each component are between 81.3% and 97.8%, and are in compliance with routine analysis detection requirements. Concentration levels of the spiked samples were measured for 0.02 µg/L, 0.1 µg/L, and 0.5 µg/L three times in parallel. The RSD value was between 2.2% and 8.6%, in compliance with stability requirements. Detection limits were calculated for each component using three times the signal-to-noise ratio with the instrument detection limits for each component 0.1–2 µg/L (Table 2).

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

Number	Compound	Linear Equation	R^2	Recovery Rate/%			Instrument Detection Limit/µg/L	RSD/%
				0.02 µg/L	0.1 µg/L	0.5 µg/L		
1	Nitrobenzene	Y = (0.062 + 0.0040)*X	0.99928	88.2	88.9	92.6	1	4.3
2	O-nitrotoluene	Y = (-0.0015 + 0.0003)*X	0.99967	81.3	93.5	95.4	2	8.4
3	M-nitrotoluene	Y = (-0.0052 + 0.0021)*X	0.99392	89.3	89.8	95.8	0.6	6.3
4	P-nitrotoluene	Y = (0.069 + 0.0005)*X	0.99733	88.5	99.3	97.8	1	5.4
5	M-nitrochlorobenzene	Y = (0.0156 + 0.0075 </pt354>)*X	0.99928	86.0	90.6	89.9	0.15	3.8
6	P-nitrochlorobenzene	Y = (0.0163 + 0.0056)*X	0.99803	87.5	98.8	95.1	0.15	3.4
7	O-nitrochlorobenzene	Y = (0.0268 + 0.0064)*X	0.99821	85.2	96.7	92.4	0.15	3.2
8	P-dinitrobenzene	Y = (0.0228 + 0.0065)*X	0.99715	93.2	88.4	98.6	0.12	4.5
9	M-dinitrobenzene	Y = (0.0276 + 0.0074)*X	0.99901	83.3	88.5	90.7	0.1	8.6
10	2,6-dinitrotoluene	Y = (0.0028 + 0.0024)*X	0.99964	95.3	89.8	95.7	0.2	7.3
11	O-dinitrobenzene	Y = (0.0325 + 0.0070)*X	0.99898	88.5	89.3	99.8	0.1	2.2
12	2,4-dinitrotoluene	Y = (0.0150 + 0.0041)*X	0.99744	94.0	90.9	89.3	0.2	3.8
13	2,4-dinitrochlorobenzene	Y = (0.0185 + 0.0062)*X	0.99979	90.5	88.8	90.1	0.1	4.4
14	3,4-dinitrotoluene	Y = (0.0390 + 0.0081)*X	0.99826	93.2	95.7	96.4	0.1	3.5
15	TNT	Y = (0.0177 + 0.0056)*X	0.99914	97.4	95.4	88.9	0.1	4.1

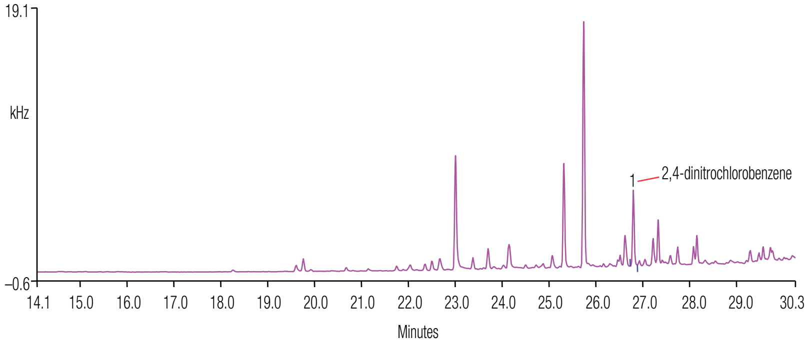


Figure 5. Chromatograph for water samples for a certain area, one of which contained 2,4-dinitrochlorobenzene.

Actual Sample Determination Results

The extraction and chromatographic methods were used to carry out determination on water samples taken from the Beijing Quig River. 2,4-dinitrochlorobenzene was discovered in one of the water samples at a concentration of 0.137 µg/L (Figure 5).

Conclusion

The Dionex AutoTrace 280 SPE instrument can be used to extract and concentrate trace organics in liquid samples such as environmental waters. The extraction method used in this application note used the Dionex AutoTrace 280 SPE to carry out conditioning, rinsing, elution, and collection processes on the SPE Cartridge, reducing manual intervention and the time taken to manually load the samples. The extraction time was calculated to be 50 min per 500 mL water sample, which saves 5 hours over the manual extraction method for six samples. As a result, the Dionex AutoTrace 280 and TRACE 1310 GC with electron capture detection are suitable for carrying out extraction, analysis, and detection of nitrobenzene compounds in environmental water samples. The experimental results show that the method offers high sensitivity, good reproducibility, good linearity, good recovery rates, and reliable results for determining nitrobenzenes and completely satisfies all requirements for detecting nitrobenzenes in drinking water.

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