Application Note 10451

Determination of Chlorophenols in Water According to U.S. EPA Method 528

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Key Words
AutoTrace 280, Chromeleon Chromatography Data System Software, Environmental, Gas Chromatography, ISQ Mass Spectrometer, SPE Cartridges, SPE Extraction, Organic Water Pollutants, TRACE 1310 Gas Chromatograph, Water Contaminants, Water Quality Analysis

Goal
Development of a complete analytical workflow for the analysis of phenolic compounds in water.

Introduction
Phenolic compounds, either phenol or related substituted species, are involved in many industrial and chemical processes as either reagents or byproducts. Their ubiquitous presence and widespread use makes pollution with phenols very likely. Waters are particularly impacted and careful monitoring is necessary to detect this source of pollution as it can represent a serious threat to environmental and human health.

U.S. EPA method 528 provides guidelines for the GC-MS analysis of phenols in water. The method includes all the steps necessary to collect, prepare, and analyze samples and data. U.S. EPA 528 dictates the use of SPE cartridges for extraction and purification of analytes.

Here, we analyze water samples for chlorophenols using a system comprising a Thermo Scientific™ TRACE™ 1310 series GC and a Thermo Scientific™ ISQ™ LT mass spectrometer, controlled by Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS) software. Injections were handled automatically by a Thermo Scientific™ TriPlus™ RSH autosampler and the Thermo Scientific™ Dionex™ AutoTrace 280 Solid-Phase Extraction (SPE) instrument was used to perform unattended, automated solid phase extraction (SPE). The Dionex AutoTrace 280 SPE system ensures improved reproducibility and throughput of the SPE process and saves time, solvent, and labor. The instrument can process up to six samples in two hours with just a few minutes, generally 10–15, of operator involvement.

Materials
The system used in this analysis includes a TRACE 1310 GC, a TriPlus RSH autosampler, and an ISQ LT mass spectrometer, controlled by the Chromeleon 7.2 CDS software. The purification and extraction was performed by the Dionex AutoTrace 280 SPE instrument. The phenol calibration mix and internal standards were acquired from Restek (Phenols calibration mix EPA 528 p/n 31694 and Internal Standard mix EPA 528 p/n 31696). Solvents and reagents were acquired from Sigma-Aldrich. A Thermo Scientific™ TraceGOLD™ TG-5MS 30 m, 0.25 mm 0.25 um (P/N 26098-1420) column was used for chromatography. The cartridge used for SPE was a Thermo Scientific™ HyperSep™ Retain PEP 500 mg, 6 mL (P/N 60107-206).

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Sample Collection and Preparation

Water samples were collected directly into a 1 L bottle vial without any headspace. Possible residual chlorine was removed by adding 40 mg of sodium sulfite. After dechlorination, the contents of the bottle were acidified to pH 2 by adding hydrochloric acid. Acidification is very important because the acid acts as a preservative for the sample and a pH of 2 is necessary for a good yield from the SPE purification and extraction process.

Sample Extraction and Purification

The Dionex AutoTrace 280 SPE instrument can process six samples simultaneously, storing up to 24 different operating methods. For our extraction purposes, 1 L of water was loaded onto each SPE cartridge. The operating conditions of the Dionex AutoTrace 280 are outlined below.

Table 1. Solvents for extraction.

Solvent No.	Solvent
1	Dichloromethane
2	Methanol
3	HCl 0.05N

Follow the steps below using the solvents listed in Table 1.

1. Rinse cartridge with 3 mL dichloromethane into solvent waste.
2. Rinse cartridge with 3 mL methanol into solvent waste.
3. Rinse cartridge with 3 mL HCl 0.05 N into aqueous waste.
4. Load 1 L of sample onto the cartridge with a flow of 20 mL/min.
5. Dry the cartridge under nitrogen for 15 min.
6. Wash the cartridge with 10 mL water.
7. Dry the cartridge under nitrogen for 5 min.
8. Soak and collect with 3 mL dichloromethane.
9. Collect with 10 mL dichloromethane.

Table 2. Dionex AutoTrace 280 SPE flow rates.

Conditioning Flow	15 mL/min
Load Flow	10 mL/min
Rinse Flow	20 mL/min
Elute Flow	5 mL/min
Conditioning Air Push	15 mL/min
Rinse Air Push	20 mL/min
Elute Air Push	5 mL/min

Table 3. Recommended instrument conditions.

TRACE 1310 GC	
Injection Volume	1 µL
Carrier Gas	He
Column Type	TraceGOLD TG-5MS 30 m, 0.25 mm, 0.25 (P/N 26098-1420)
Column Oven	Initial 40 °C, hold 4 min Ramp 10 °C/min to 250 °C, hold 1 min
SSL Injector	250 °C; carrier gas He in split mode with a split ratio of 33:1
Column Flow	Constant flow at 1.5 mL/min
ISQ LT (EI Mode)	
Source Temperature:	280 °C
Transfer Line Temperature:	250 °C
Operating Mode:	Full Scan 40–350
Dwell Time	0.2

All these operations were handled, unattended, by the Dionex AutoTrace 280 SPE instrument.

The final eluate was concentrated to 0.9 mL under a gentle stream of nitrogen in a lukewarm water bath and the final volume adjusted to 1 mL. The internal standard mix was added to a final concentration of 2 ppm for 1,2-dimethyl 3-nitrobenzene and 5 ppm for 2,3,4,5-tetrachlorophenol.

Instrument Method Setup

The method outlined in Table 3 was developed for the TRACE 1310 Gas Chromatograph and ISQ LT Mass Spectrometer.

The calibration curve consisted of eight points at concentrations of 0.1, 0.5, 1, 2, 5, 10, 15, and 25 ppm. The internal standard mix was added to each point at 2 ppm for 1,2-dimethyl 3-nitrobenzene and 5 ppm for 2,3,4,5-tetrachlorophenol.

The 1 L starting volume of the water sample and the 1 mL volume of the final extract, indicate a 1000x concentration factor. This concentration factor allows for the calibration of a concentration range from 0.1 to 25 ppb in the aqueous sample.

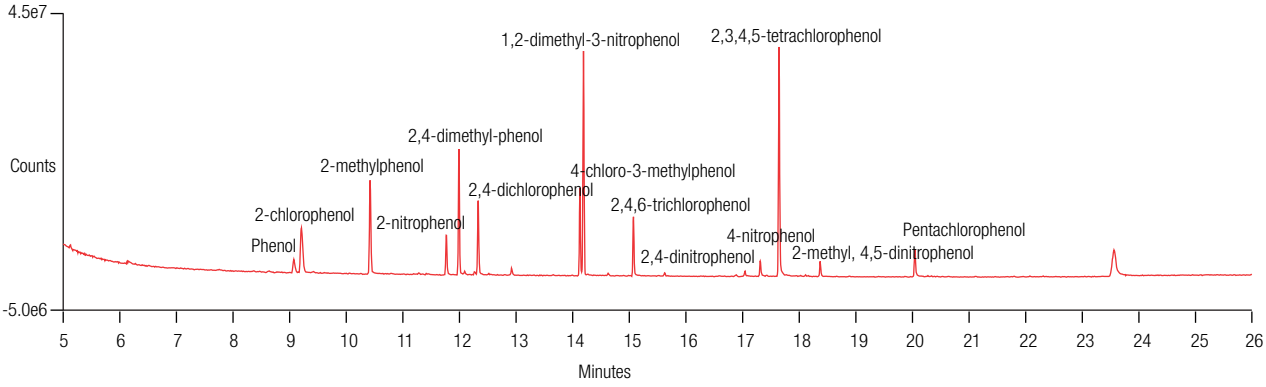

Figure 1. Extracted sample (1 ppm final concentration).

Results and Discussion

A chromatogram of an extracted sample is reported in Figure 1.

The linearity of the calibration curve is shown in Table 4. The results show an r^2 value above 0.99 for each of the tested compounds. The repeatability of the chromatographic system was also tested. The results are reported in Table 5.

The recovery rate was assessed by spiking different water samples at a concentration of 10 ppb and calculating the amount in the final extract.

Recovery rates stated in the official method are 70–130% for all compounds and 50–150% for phenol due to its higher water solubility. The results are reported in Table 6.

Table 4. Calibration results.

Compound	r^2
Phenol	0.999
2-chlorophenol	0.999
2-methylphenol	0.999
2-nitrophenol	0.995
2,4-dimethyl-phenol	0.999
2,4-dichlorophenol	0.998
4-chloro-3-methylphenol	0.998
2,4,6-trichlorophenol	0.998
2,4-dinitrophenol	0.991
4-nitrophenol	0.998
2-methyl, 4,5-dinitrophenol	0.997
Pentachlorophenol	0.999

Table 5. Area repeatability.

Compound (5 injections)	RSD%
Phenol	1.6
2-chlorophenol	1.3
2-methylphenol	1.8
2-nitrophenol	1.9
2,4-dimethyl-phenol	1.7
2,4-dichlorophenol	1.2
4-chloro-3-methylphenol	1.6
2,4,6-trichlorophenol	1.7
2,4-dinitrophenol	3.0
4-nitrophenol	1.9
2-methyl, 4,5-dinitrophenol	2.7
Pentachlorophenol	3.0

Table 6. Average recovery rates.

Compound	Average % Recovery (5 runs)
Phenol	87.92
2-chlorophenol	101.61
2-methylphenol	99.17
2-nitrophenol	97.85
2,4-dimethyl-phenol	99.00
2,4-dichlorophenol	92.57
4-chloro-3-methylphenol	91.23
2,4,6-trichlorophenol	101.12
2,4-dinitrophenol	84.76
4-nitrophenol	72.06
2-methyl, 4,5-dinitrophenol	83.33
Pentachlorophenol	78.63

Conclusion

This application is a viable solution for the determination and quantification of water contamination by phenols. The system used in this analysis greatly reduces operator intervention.

Sample preparation using the Dionex AutoTrace 280 SPE instrument enables automation of the SPE. It allows unattended processing of up to six samples simultaneously, saving time and providing excellent yields and reproducibility. As a result, it may replace techniques such as liquid-liquid extraction or manual SPE with a vacuum manifold, saving time and lowering exposure to potentially harmful solvents.

The injection and analysis of the samples are also highly automated using a self-sufficient autosampler and the Chromeleon CDS software, which offers a powerful integrated system for controlling the chromatographic instrument, injection, sequence handling, and data processing.

This system is capable of handling the total analytical workflow from the raw sample to the final report, with solid and reproducible results and minimum intervention by the operator.

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