Gas Chromatographic Determination of Phenol in Fish Tissues as a Phenyl Acetate Derivative Following Solvent Extraction of Acidified Samples

H. Kim¹*, Y. Kim², S. Park², J. Lee³ and J. Choi⁴

¹School of Natural Resources and Environmental Science, Kangwon National University, Chuncheon, Kangwon-do 24341, Republic of Korea
²Department of Environmental Science, Kangwon National University, Chuncheon, Kangwon-do 24341, Republic of Korea
³Natural and Human Co., Ltd., Bugwon-ro, Wonju, Kangwon-do 26424, Republic of Korea
⁴Institute of Environmental Research, Kangwon National University, Chuncheon, Kangwon-do 24341, Republic of Korea

Received: 13 August 2018; accepted: 10 September 2018

This study aimed to develop a chromatographic method to quantitatively determine phenol in fish tissues. This method involves solvent extraction of acidified samples, followed by derivatization to phenyl acetate and analysis with gas chromatography coupled with mass spectrometry (GC-MS). Phenol in a representative tissue sample (belly, gill, or renal tubules), which was homogenized with 2 N sulfuric acid, was extracted with ethyl acetate and derivatized to phenyl acetate using acetic anhydride and K₂CO₃ in water. An n-butyl acetate extract was injected into the GC–MS. The linearity (r²) of the calibration curve was greater than 0.996. The analytical repeatability, which is expressed as the relative standard deviation, was less than 6.14%, and the recovery was greater than 96.3%. The method detection limit and the limit of quantitation were 8.0 μg/kg and 26 μg/kg, respectively. The proposed method is also applicable to the analysis of other biological tissues for phenol and its analogs, such as pentachlorophenol.

Keywords: acetylation, fish, GC–MS, phenol, solvent extraction

Introduction

Phenol is an organic compound of an anthropogenic origin, which is widely used in phenolic resins and plywood adhesives among others, and is also found in petroleum products, such as coal tar and creosote [1]. Additionally, phenol occurs naturally in the environment, where it is formed via the degradation of organic matter, such as benzene. Benzene is commonly found in the environment, including surface water [2].

To date, no toxicological studies have reported phenol to be carcinogenic for humans. However, it may cause acute toxicities, such as cardiovascular diseases or gastrointestinal disorders, which can lead to death in the event of a high dose [1, 3]. Depending on the administered dosage, fish suffer behavioral, respiratory, and lethal effects [4, 5]. Therefore, the concentration of phenol in fish and in the water needs to be measured in order to protect fish and humans from any possible harmful effects.

Most studies on the quantitative determination of phenol are commonly conducted on water, usually using gas chromatography (GC) mostly, following derivatization to more lipophilic compounds, such as phenyl acetate [6–10]. However, the analytical methods for biological tissues, including fish, are highly limited. The United States Environmental Protection Agency (EPA) monitored 268 toxic chemicals, including phenol, in tissues of fish from lakes and reservoirs in the USA [11] using the EPA Method 1625C [12]. This method was essentially developed for analyzing semivolatile organic compounds in water, soil, and municipal sludge, not for biological samples. This method employed ultrasonication with dichloromethane (DCM) for extraction after acidifying samples to a pH of less than 2, cleanup using gel permeation chromatography, and gas chromatography coupled with mass spectrometry (GC–MS) analysis without derivatization. A similar procedure was applied to analyze foods, such as corn, mackerel, and rice, and the limit of quantitation (LOQ) was estimated as 100 or 300 μg/kg [13].

The aim of this study was to lower the limit of quantification and to reduce the analysis time by employing acidified homogenization, centrifugation, solvent extraction, and derivatization.

Experimental

Chemicals. Phenol (5000 μg/mL) was purchased from Supelco (Belleville, PA, USA), and 0.2 mL of the phenol solution was diluted with methanol to prepare 100 mL of a 1 mg/L standard solution. Further, o-cresol, potassium carbonate, and acetic anhydride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sulfuric acid and sodium chloride were purchased from Junsei Chemical (Kyoto, Japan) and Showa (Tokyo, Japan), respectively. DCM, ethyl acetate, and methanol were purchased from Tedia (Fairfield, OH, USA). n-Butyl acetate and sodium sulfate were obtained from Daejung (Suheung, Republic of Korea).

Sample Preparation. One gram of each fish tissue sample (belly, gill, and renal tubules) was placed in a 40-mL glass vial, into which 2 μL of o-cresol (207.2 mg/L) as a surrogate to compensate for the variations in matrix effect and method performance between samples and 5 mL of 2 N H₂SO₄ were added. The mixture was homogenized using a homogenizer (SHG-15A; Scilab, Seoul, Republic of Korea) and then

* Author for correspondence: kimsh@kangwon.ac.kr

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes - if any - are indicated.

DOI: 10.1556/1326.2018.00529
© 2018 The Author(s)

Acta Chromatographica 32(2020), 64–67
First published online: 14 October 2018
ultrasonicated (POWERSONIC 510; Hwashin Instrument, Seoul, Republic of Korea) for 10 min. The homogenate was transferred into a 15-mL glass centrifuge tube and centrifuged at 3600 rpm for 20 min (Centrifuge HA-12; Hanil Science, Daejeon, Republic of Korea). The supernatant was transferred into a 40-mL glass vial, into which 10 mL of ethyl acetate was added, and the resulting mixture was shaken for 15 min using a rotary shaker (MaxiMix III Type 65800; Barnstead Thermolyne, Dubuque, USA) to extract phenol. The upper ethyl acetate layer was transferred into a 40-mL glass vial. This liquid–liquid extraction operation was repeated once more. The extract was dried over ca. 2 g of sodium sulfate. The filtrate was concentrated to ca. 2–3 mL at 40 °C using a rotary evaporator (HS 2000, Hahn Shin S&T, Gimp, Korea). The concentrate was transferred to a 15-mL glass centrifuge tube and dried using a mild nitrogen stream. Phenol and o-cresol in the tube were derivatized to phenyl acetate by adding 100 μL of acetic anhydride and 500 μL of 5% K₂CO₃ into the centrifuge tube containing 1 mL of water (Figure 1). The mixture was vortex-shaken for 20 s and left at room temperature for 10 min, and then 0.5 mg of NaCl and 1 mL of n-butyl acetate were added to the tube, and the mixture was vortex-shaken again for 1 min. Approximately one-half of the 1-mL upper organic layer was transferred into a 2-mL glass vial, and 1 μL was injected into the GC–MS apparatus.

**Instrumental Conditions.** A 7890A gas chromatograph with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used for instrumental analyses (Table 1). A DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies) was used as an analytical column. One microliter of each sample in the vial was injected into the GC–MS apparatus in splitless mode with a 7693A autosampler (Agilent Technologies). The inlet temperature was maintained at 240 °C. The carrier gas was helium, flowing at a rate of 1 mL/min. The gas chromatograph oven temperature was programmed as follows: the temperature was initially maintained at 40 °C for 3 min, then elevated to 150 °C at 10 °C/min and thereafter to 230 °C at 25 °C/min, and finally maintained at 230 °C for 20 min. The mass spectrometer was operated in the electron impact mode for ionization and in the selected ion monitoring mode for quantification. Fragment ions ([m/z] of 66 and 94 were selected for the quantitative determination of phenyl acetate, whereas those of 107 and 108 were selected for o-cresyl acetate.

**Table 1. GC–MS conditions**

| Parameter | Option or condition |
|-----------|---------------------|
| Agilent Technologies | GC (7890A; 5975C; 6890N) |
| Column | Inlet port temperature (40 °C, held for 3 min → raised to 150 °C (10 °C/min) → raised to 230 °C (25 °C/min), held at 230 °C for 20 min) |
| Oven program | 240 °C |
| Injection mode | Splitless mode |
| Carrier gas (flow rate) | He (1 mL/min) |
| Injection volume | 1 μL |
| Transfer line temperature | 240 °C |
| EI mode | 70 eV |
| Ion source temperature | 230 °C |
| Quadrupole temperature | 150 °C |
| Selected ions ([m/z]) | 66, 94 |
| Phenyl acetate | 107, 108 |
| o-Cresyl acetate | |
The LOQ was lower than the value (100 μg/kg), obtained for mackerel, which was analyzed for phenol itself by GC–MS without a derivatization step [13]. The recoveries for 10 μg/kg and 100 μg/kg concentrations were 98.2% and 96.3%, respectively, much higher than those (82.2–86.8%) for fortified 250–1000 μg/kg samples of mackerel [13]. The repeatability, which was represented by relative standard deviations for 10 μg/kg and 100 μg/kg, was 6.24% and 6.14%, respectively (Table 2), indicating a reasonably good accuracy and precision.

Phenol Concentrations in Fish Tissues. In the belly samples, phenol was found at 47.3 (±9.7) μg/kg (n = 4) in crucian carp alone, whereas it was found below the MDL in dace and striped mullet. The concentrations of phenol were 26.5 (±25.5), below the LOQ, and 100 (±34) μg/kg in the gill samples of dace, striped mullet, and crucian carp, respectively. Phenol was found at 216 (±44), 387 (±147), and 121 (±30) μg/kg (Table 2). The LOQ was lower than the value (100 μg/kg), obtained for mackerel, which was analyzed for phenol itself by GC–MS without a derivatization step [13]. The recoveries for 10 μg/kg and 100 μg/kg concentrations were 98.2% and 96.3%, respectively, much higher than those (82.2–86.8%) for fortified 250–1000 μg/kg samples of mackerel [13]. The repeatability, which was represented by relative standard deviations for 10 μg/kg and 100 μg/kg, was 6.24% and 6.14%, respectively (Table 2), indicating a reasonably good accuracy and precision.

Phenol Concentrations in Fish Tissues. In the belly samples, phenol was found at 47.3 (±9.7) μg/kg (n = 4) in crucian carp alone, whereas it was found below the MDL in dace and striped mullet. The concentrations of phenol were 26.5 (±25.5), below the LOQ, and 100 (±34) μg/kg in the gill samples of dace, striped mullet, and crucian carp, respectively. Phenol was found at 216 (±44), 387 (±147), and 121 (±30) μg/kg (Table 2).

Table 2. Method validation results

| Parameter | MDL (μg/kg) | LOQ (μg/kg) | Recovery (%) | RSD (%) |
|-----------|-------------|-------------|--------------|---------|
|           | 10 μg/kg    | 100 μg/kg   | 10 μg/kg     | 100 μg/kg |
| Value     | 8.0         | 26          | 98.2         | 96.3    |
|           | 6.42        | 6.14        |              |         |

Figure 2. Selection of a suitable solvent for extracting phenyl acetate from the derivatization reaction mixture. n-Butyl acetate showed the highest extraction efficiency and was chosen as an extraction solvent (n = 3).

Figure 3. Chromatograms of a 100 μg/kg phenol standard (top) and a belly sample (bottom). o-Cresol was used as a surrogate.
in the renal tubules of dace, striped mullet, and crucian carp, respectively, indicating that phenol is detected at the highest concentration in the renal tubules.

Conclusion

In this paper, an analytical method for the quantitative determination of phenol in fish tissues using solvent extraction and derivatization to a more volatile derivative was developed. This method was demonstrated to have a lower detection limit than previous methods, satisfactory linearity of the calibration curves, high accuracy, and good intraday precision. This method is also applicable to other biological tissues, such as meat, sediments, and soil. Further, the application of this method to other phenol analogs, such as alkylphenols, bisphenols, and chlorinated phenols, can be considered.

References

1. Bruce, R. M.; Santodonato, J.; Neal, M. W. Toxicol. Ind. Health 1987, 3, 535.
2. US EPA Toxicological Review of Phenol (CAS No. 108-95-2) Integrated Risk Information System (IRIS), National Center for Environmental Assessment, Office of Research and Development, Washington, DC, USA, 2002.
3. ATSDR Toxicological Profile for Phenol Public Health Service, U.S. Department of Health Services, Atlanta, GA, USA, 2008.
4. Razani, H.; Nanba, K.; Murachi, S. B. Jpn. Soc. Sci. Fish. 1986, 32, 1547.
5. Saha, N. C.; Bhunia, F.; Kaviraj, A. B. Environ. Contam. Tox. 1999, 63, 195.
6. Ballesteros, E.; Gallego, M.; Valcarcel, M. J. Chromatogr. 1990, 538, 59.
7. Llompart, M.; Lourido, M.; Landin, P.; Garcia-Jares, C.; Cela, R. J Chromatogr. A 2002, 963, 137.
8. Bagheri, H.; Saber, A.; Mousavi, S. R. J. Chromatogr. A 2004, 1046, 27.
9. Faraji, H. J. Chromatogr. A 2005, 1087, 283.
10. Park, S.; Kim, Y.; Jung, S.; Kim, H. Kor. J. Environ. Agric. 2017, 36, 63.
11. Stahl, L. L.; Snyder, B. D.; Olsen, A. R.; Pitt, J. L. Environ. Monit. Assess. 2009, 150, 3.
12. US EPA Method 1625C Semivolatile Organic Compounds by Isotope Dilution GCMS Office of Science and Technology Engineering and Analysis Division, Washington, DC, USA, 1989.
13. Kang, Y. W.; Ahn, J. E.; Suh, J. H.; Park, S. H.; Yoon, H. J. J. Food Hyg. Safety 2014, 29, 312.
14. US EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 Office of Water, Washington, DC, USA, 2016.